Effects of dietary starch allocation on feeding behaviors and production performance of dairy cows under various management scenarios

by

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Abstract

Despite there being a large body of literature evaluating dietary strategies to support the metabolism of lactating cows, optimum dietary starch allocation, and resulting effects on milking performance and feeding behaviour are still unknown. Therefore, the overall objective of this research was to evaluate the effects of starch provision on ruminal fermentation, feeding behavior and performance of lactating dairy cows.

In Chapter 2, 88 primi- and multiparous cows were used to evaluate the effects of feeding a control (CON; 14% starch) or high-starch (HI; 26.1%) prepartum diet fed for 28 ± 3 d prepartum, followed by a high-fiber (HF; 33.8% NDF, 25.1% starch) or high-starch (HS; 27.2% NDF, 32.8% starch) postpartum diet fed for 20 ± 2 d. Cows fed HI had greater DMI, concentrations of insulin and glucose before parturition, and greater plasma NEFA concentration and milk fat yield postpartum. Cows fed HS postpartum had lower plasma NEFA concentrations and serum haptoglobin. Feeding HS postpartum tended to increase milk yield compared to HF for cows fed the CON prepartum diet, but not HI. Overall, feeding a high-starch TMR postpartum may decrease fat mobilization and increase milk production, regardless of prepartum dietary treatment.

In Chapter 3, 8 ruminally-cannulated mid-lactation cows were used to evaluate the effects of feeding a high-fiber (F; 33.2% NDF) or high-starch (S; 56.8% starch) pellet, at a low (L; 1kg) or high (H; 3kg) amount twice per day alongside a complementary partial mixed ration (PMR) in a 4 × 4 Latin square design. By design there was a difference in pellet intake between L and H, and PMR intake was reduced when H was fed; however, total DMI tended to be increased when H was fed. Within 3 h following PMR delivery, cows offered S (with a high fiber PMR) consumed less PMR than those offered F (with a high starch PMR). When S was fed, the duration that ruminal pH was below 5.8 was reduced compared to F. These findings suggest that when cows are fed PMR complementary to pellet, feed intake patterns and ruminal fermentation appear to be affected by nutrient composition of the PMR.

Combining these findings, I aimed to evaluate the effects of concentrate allowance through an AMS immediately postpartum (Chapter 4). Sixty-six primi- and multiparous cows were fed a low starch prepartum diet for 30 ± 3 d and assigned to 1 of 3 pellet allocations through the AMS: low (LP; 3 kg/d) or high allowances (HP; 8 kg/d) increased at a moderate

(HPM; increased from 3 to 8 kg over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate. All cows received the same PMR formulated to meet nutrient requirements at the LP allowance and were offered their dietary treatments to 8 wk of lactation. Cows offered LP reached target intakes by wk 2; however, HP cows, regardless of adaptation did not achieve target intake. Intake of PMR was greater for LP for the first 4 wk of lactation as compared to HP, with no difference in total DMI. Cows offered LP had greater milking frequency, milk yield, and yields of milk fat, protein and lactose as compared to HP, with no difference detected between HPM and HPR. From wk 5 to 8, there was no difference in milking frequency however, milk yield was greater for LP as compared to HP. Offering more of a starch-based pellet to early lactation cows, in excess of what the diet is formulated for, may not improve DMI, milkings per day or milk and milk component yield.

In conclusion, it may be possible to feed high starch post-partum diets when cows are fed a conventional TMR, however, when cows are managed with component feeding and milked with AMS, offering greater amounts of high starch pellets may not result in improved milk production or animal performance.

Preface

This study consists of three studies; the first study yielded Chapter 2 and received research ethics approval from the University of Alberta Animal Care and Use Committee for Livestock (AUP00002342. The second study yielded Chapter 3 and received ethics approval from the University of Alberta Animal Care and Use Committee for Livestock (AUP00002170). The third study yielded Chapter 4 and received research ethics approval from the University of Saskatchewan Research Ethics Board (protocol #20190128).

A version of Chapter 2 of this thesis has been published as Haisan, J., Y. Inabu, W. Shi, and M. Oba. 2021. Effects of pre- and postpartum dietary starch content on productivity, plasma energy metabolites, and serum inflammation indicators of dairy cows. J. Dairy Sci. 104:4362-4374. I was responsible for experimental design, data collection, data analysis and manuscript writing. Y. Inabu and W. Shi assisted with data collection and analysis and M. Oba was the corresponding author and assisted with experimental design, data collection and manuscript writing.

A version of Chapter 3 of this thesis has been published as Haisan, J., and M. Oba. 2020. The effects of feeding a high-fiber or high-starch pellet at two daily allocations on feed intake patterns, rumen fermentation, and milk production of mid-lactation dairy cows. J. Dairy Sci. 103:6135-6144. I was responsible for experimental design, data collection, data analysis and manuscript writing. M. Oba was the corresponding author and assisted with experimental design and manuscript writing.

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List of abbreviations

ADF – acid detergent fiber

AMS – automated milking system

AMT – amount

BCS – body condition score

BHB – beta-hydroxybutyrate

BW – body weight

CHO – carbohydrate

CP – crude protein

CV – coefficient of variance

DDGS – dried distillers grains with solubles

DIM – days in milk

DM – dry matter

DMI – dry matter intake

ECM – energy corrected milk

EE – ether extract

F – high-fiber pellet treatment

FADH – flavin adenine dinucleotide

FH – high-fiber, high amount of pellet

FL – high-fiber, low amount of pellet

GH – growth hormone

GLP-2 – glucagon-like peptide 2

H – high amount of pellet

HF – high fiber

Hp – haptoglobin

HP – high pellet allocation

HPM – high pellet, moderate rate allocation

HPR – high pellet, rapid rate allocation

HS – high starch

IGF-1 – insulin-like growth factor 1

L – low amount of pellet

LP – low pellet allocation

LPS – lipopolysaccharide

ME – metabolizable energy

MG – main group

MP – metabolizable protein

MUN – milk urea nitrogen

NADH – nicotinamide adenine dinucleotide

NDF – neutral detergent fiber

NEB – negative energy balance

NEFA – non-esterified fatty acids

NE_L – net energy lactation

NFC – non-fiber carbohydrates

NSC – non-structural carbohydrates

OM – organic matter

PMR – partial mixed ration

S – high-starch pellet treatment

SAA – serum amyloid A

SARA – subacute rumen acidosis

SCC – somatic cell count

SG – small group

SH – high-starch pellet, high amount of pellet

SL – high-starch pellet, low amount of pellet

TMR – total mixed ration

Trt – treatment

VFA – volatile fatty acid

VLDL – very low density lipoprotein

VMS – voluntary milking system

Wk – week

Chapter 1: Literature Review

1.1 Nutritional management during the calving transition

1.1.1 The transition period

The transition period is commonly defined as the 3 weeks prior to parturition to 3 weeks after parturition (Grummer et al, 1995), and is one of the most critical times in a dairy cows' production cycle. During this period, dairy cows experience vast changes in their metabolic and physiological status alongside environmental, managerial, and dietary changes. It is estimated that 30 to 50% of high producing dairy cows experience metabolic or infectious diseases during this period (Leblanc, 2010) including milk fever, ketosis, retained placenta, metritis, displaced abomasum, and mastitis (Drackley, 1999). As such, the transition from late gestation to early lactation may determine whether a cow will have a productive lactation from health, production, and reproductive perspectives.

1.1.2 Negative energy balance

Nutrient requirements of dairy cows change drastically during the transition period. In the three weeks prior to parturition dairy cows experience an increase in nutritional demand for fetal growth and mammary tissue remodeling (Esposito et al., 2014) as nutrient requirements of the developing fetus reach its maximum level (Bell, 1995). Following parturition, nutrient requirements increase further as milk yield, milk protein, fat, and lactose production increase during the first three weeks of lactation. While nutrient requirements for the physiological changes occurring during these periods have been determined (NRC, 2001; NASEM 2021), dry matter intake (DMI) may be reduced by 10-30% prepartum (Bell, 1995) and nutrient requirements of the cow rapidly exceed that obtained by feed intake postpartum (Bertoni et al., 2009). The decrease in DMI that occurs prepartum can carry into the postpartum period (Ingvartsen and Andersen, 2000), and may be compounded by stress or poor management further reducing DMI, resulting in a state of negative energy balance (NEB; Grummer et al., 2004; Ingvartsen, 2006). In an attempt to attenuate this NEB, and meet requirements for milk production and maintenance, metabolic adaptations in fatty acid and glucose metabolism (Overton and Waldron, 2004), and mobilization of body reserves, primarily fat (Gross et al., 2011) occur.

1.1.2.1 Lipid metabolism

There are innate mammalian mechanisms to provide nutrients to the neonate, resulting in the mobilization of body stores during the postpartum period (Bauman and Currie, 1980). The greatest energy store available is adipose tissue (Bell, 1995) with continual break down (lipolysis) and formation (lipogenesis) occurring (Roche et al., 2009). However, for the modern dairy cow, stringent genetic selection on milk production has resulted in greater mobilization of tissues as compared to many other mammalian species (Roche et al., 2009). Work conducted by McNamara and Hillers (1986 a,b) and Smith and McNamara (1990) found that in early lactation lipolysis is primarily controlled by genetics, while enzymes involved in lipogenesis are regulated by energy intake (Rocco and McNamara, 2013).

Pathways for lipolysis and lipogenesis have been extensively described (Bauman, 2000; Roche et al., 2009; Contreras et al., 2017). Briefly, lipogenesis occurs via de novo synthesis, and uptake of preformed fatty acids from circulation. Whereas with monogastrics, glucose is the primary carbon source, ruminants use acetate and glucose (Bauman, 1976). During lipolysis, triacylglycerol stores are hydrolysed into three molecules of non-esterified fatty acids (NEFA) and one molecule of glycerol (Stich and Berlan, 2004). Circulating NEFA can then be oxidized by the liver and skeletal muscle as an energy source or used in the mammary gland as a source of milk fat (Drackley, 1999; Roche et al., 2009). Fatty acids undergo β-oxidation in the liver producing acetyl Co-A and reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) to generate energy via ATP in the citric acid cycle and electron transport chain, respectively (Stich and Berlan, 2004; Roche et al., 2009). During periods of adipose tissue mobilization, hepatic cells convert excess acetyl CoA into the ketone bodies acetoacetate and beta-hydroxybutyrate (BHB). While this process is energetically less efficient, ketone bodies are important for providing energy to dairy cows in early lactation (Bell, 1995; Drackley 1999). If NEFA do not undergo hepatic β-oxidation they are re-esterified to triglyceride and released as very low density lipoproteins (VLDL). Dairy cows have a low rate of VLDL synthesis and export during negative energy balance (Liu et al., 2014); therefore, increased hepatic NEFA uptake can result in hepatocyte triglyceride accumulation, or “fatty liver”.

There are three main hormones involved in regulation of lipogenesis and lipolysis: growth hormone (GH), insulin, and glucagon. In early lactation, high concentrations of growth hormone and glucagon, with low insulin act to favor lipolysis, and the opposite occurs in late lactation to favor lipogenesis (Contreras et al., 2018). Growth hormone concentrations increase at calving to facilitate release of NEFA from adipose stores (Liesman et al., 1995) through enhancement of the lipolytic response (Etherton and Bauman, 1998) and has a negative effect on fatty acid re-esterification (Liesman et al., 1995). Insulin is a regulator of lipogenesis and increases glucose uptake into adipocytes. During early lactation hypo-insulinemia and increased insulin resistance of skeletal muscle and adipose occur (Bell and Bauman, 1997) to support increased availability of glucose for the mammary gland and greater mobilization of tissues. Glucagon primarily acts to regulate glucose metabolism (discussed below) through increasing the rate of glycogenolysis (breakdown of glycogen to glucose) and gluconeogenesis (formation of glucose from non-hexose precursors) to increase glucose output from the liver (De Boer et al., 1985). However, glucagon acts with insulin to stimulate mobilization of glycogen, promote mobilization of lipid reserves and use of fatty acids as an energy reserve (Garnsworthy et al., 2008).

1.1.2.2 Glucose metabolism

It is estimated that over 75% of metabolizable energy supply for ruminants is derived from ruminal fermentation yielding volatile fatty acid (VFA), primarily acetate, propionate and butyrate (Bergman, 1990) and their subsequent absorption. The primary precursor for glucose is propionate from ruminal fermentation, as well as lactate from Cori recycling, amino acids from protein catabolism, and glycerol released during lipolysis (Reynolds et al., 2003; Overton and Waldron, 2004). The contribution of each differs throughout the stages of lactation depending on feed intake, tissue mobilization, and energy balance (De Koster and Opsomer, 2013).

Onset of lactation results in an increased demand for glucose from milk production whereby glucose production is increased and utilization by peripheral tissues is reduced (Bauman, 2000). Unlike monogastrics, ruminants absorb only a small amount of glucose from the intestines, therefore most circulating glucose originates from hepatic and renal gluconeogenesis (De Koster and Opsomer, 2013). Glucose is partitioned to the mammary gland, and other vital organs that cannot use fatty acids as an energy source (e.g., brain and the immune

system; Roche et al., 2009). The primary adaptation of glucose metabolism during the transition period is to increase hepatic gluconeogenesis while decreasing oxidation of glucose by peripheral tissues (Reynolds et al., 2003). Adipose tissue and skeletal muscle become insulin resistant to minimize their uptake of glucose (De Koster and Opsomer, 2013). This insulin resistance, defined as a state where normal concentrations of insulin result in a decreased biological response (De Koster and Opsomer, 2013), allows for glucose to be directed to the developing fetus and mammary gland for milk production (Reynolds et al., 2003; Overton and Waldron, 2004). Insulin resistance can be attributed to decreased insulin responsiveness (response of insulin to glucose), decreased insulin sensitivity (response of tissue to insulin), or both (De Koster and Opsomer, 2012). During early lactation, insulin resistance favors lipolysis and inhibits lipogenesis, which results in an increase in plasma NEFA available for oxidation. However, excessive mobilization of fatty acids from adipose tissue may predispose dairy cows to metabolic diseases, excessive body condition score loss, or impaired milk production (Ingvartsen and Andersen, 2000; Duffield et al., 2009).

1.1.3 Health disorders during the transition period

When cows are unable to adapt to the state of NEB, they are prone to health complications, with most occurring during the first two weeks of lactation (Drackley, 1999; Goff, 2006). Excessive lipid mobilization associated with NEB causes an increase in plasma NEFA concentrations from fat oxidation, resulting in an excess of acetyl Co-A. Excess acetyl Co-A is converted to ketone bodies. The presence of elevated circulating ketone bodies, referred to as hyperketonemia, has been defined as serum beta-hydroxybutyrate (BHB) concentrations greater than 27 mg/dL (Duffield, 2000) where cows experience clinical signs of ketosis. Subclinical ketosis, or hyperketonemia without clinical symptoms, is generally diagnosed when serum BHB concentrations are between 10.4 and 27 mg/dL (Duffield, 2000). McArt et al. (2015) reported that on average 43% of dairy cows on 4 large dairies in the United States experienced subclinical ketosis between 3 and 16 days following parturition. Similarly, Pinedo et al. (2020) found that 28 to 32% of cows across the United States experience subclinical ketosis following calving.

It has been found that up to 60% of dairy cows experience fatty liver (White, 2015). In the postpartum cow, circulating concentrations of NEFA and blood flow to the liver are increased (Reynolds et al., 2003) resulting in increased hepatic NEFA uptake. If this uptake is not matched

by increased metabolism, an overall accumulation of hepatic fatty acids occurs. Development of fatty liver results in damaged liver function and reduced feed intake. Both ketosis and fatty liver have negative effects on animal health and productivity (Gordon et al., 2013; White, 2015), and may increase incidence of other health problems such as displaced abomasum (McArt et al., 2015; Gordon et al., 2013).

During the transition period, dairy cows may experience other metabolic disorders such as milk fever, displaced abomasum, and retained placenta. Goff (2006) anecdotally reported no reduction in health incidence in dairy cows between the 1995 and 2001 from the National Animal Health Monitoring System surveys in the United States. More recently, Pinedo et al. (2020) found that 0.5 to 3.9% of cows experienced a displaced abomasum, and 3.7 to 9.4% of cows experienced a retained placenta, with variability across parity, season of calving and geographic region. Dairy cows have been found to experience immunosuppression during the transition period (Ingvartsen and Moyes, 2015), and while the exact mechanism is unknown, it is speculated that there is a link with metabolic status (Bradford et al., 2015; Ingvartsen and Moyes, 2015). While maintaining DMI is paramount to reduce the effects of NEB, the immune system and inflammation may play an important role in whether cows are more susceptible to transition cow disorders.

1.1.4 Inflammation during the transition period

Recently, there has been an increased focus on the role inflammation may have during the transition period for dairy cattle. Two processes for inflammation have been identified: acute and subacute. Acute inflammation refers to the classic signs: redness, swelling, heat and pain (Bradford et al., 2015), that are commonly visible and associated with infection or injury. In contrast, subacute inflammation is associated with chronic inflammation resulting in changes in tissue function, leading to tissue malfunction (Bradford et al., 2015). Regardless of type of inflammation, immune responses can be innate and adaptive. The innate response is non-specific and occurs as a response to invading cells and activates an immune response by releasing cytokines that attract more immune cells and stimulate a secondary acute phase protein response (Ceciliani et al., 2012; Tothova et al., 2014). Acute phase proteins are designed to hold the infection until the adaptive immune system response is initiated (Tothova et al., 2014). The adaptive immune response is specialized to recognize specific antigens, generate pathogen-

specific pathways to eliminate pathogens or pathogen-infected cells, and develop an immunogenic memory (Marshall et al., 2018).

Detection of inflammation can be done through various blood markers, with recent focus on acute-phase proteins for ruminants. Acute phase proteins are produced in the liver and include haptoglobin, ceruloplasmin, serum amyloid A, and C-reactive protein (Bradford et al., 2015); with haptoglobin and serum amyloid A being the primary acute phase proteins present in ruminants (Eckersall and Bell, 2010; Tothova et al., 2014). Their concentrations are typically low in the blood, but elevated during systemic infection (Ceciliani et al., 2012). Serum amyloid-A concentrations rise rapidly following stimulation and fall rapidly, while haptoglobin concentrations remain constant after immune activation (Gruys et al., 2005).

Dairy cows experience an inflammatory state postpartum (Bertoni et al., 2008), with acute-phase markers being elevated following parturition, even in the absence of clinical diseases (Bionaz et al., 2007; Bradford et al., 2015). Qu et al. (2014) found that haptoglobin concentrations were elevated in cows experiencing calving difficulty. While damage to uterine tissue during calving leads to inflammation (Bradford et al., 2015), inflammatory states have also been identified in liver tissues (Gessner et al., 2013) and adipose tissue during the first week of lactation (Loor et al., 2005; Bradford et al., 2015). However, acute phase protein concentration has also been associated with systemic inflammation during the postpartum period (Nightingale et al., 2015) and may not be associated with one specific organ or process. Systemic inflammation in the transition dairy cow may be caused by several factors including: social stressors, infectious diseases, dietary stressors, heat stress, infections, tissue damage, dietary changes or excess circulating lipids (Bradford et al., 2015). Regardless of the cause or initial insult, it appears that there is an association with “leaky gut” and bacterial derived endotoxins, such as lipopolysaccharide (LPS; Rodriguez-Jimenez et al., 2019). Lipopolysaccharides are considered endotoxins and cause an immune response in a host (Alexander and Rietschel, 2001). Briefly, leaky gut is defined as an inability of the gastrointestinal tract to prevent unwanted molecules or antigens, such as LPS, from passing through the epithelial barrier into the body. Bacterial LPS are found on the outer membrane of gram-negative bacteria and are shed during growth and released following cell disintegration (Plazier et al., 2012). Under normal conditions, the ruminant digestive tract contains many gram-negative bacteria; however, the epithelium can

prevent entry into the interior of the body. Under epithelial barrier failure (leaky gut), LPS may enter body circulation (eg. blood or lymphatic system) and stimulate an inflammatory response (Plazier et al., 2012).

In the case of dietary changes, it is well established that dairy cows may undergo drastic changes between pre- and postpartum diets. This transition has the potential to cause reductions in ruminal pH causing ruminal acidosis and inflammation due to an increase in fermentable carbohydrates and DMI (Emmanuel et al., 2008; Gott et al., 2015) or feed restriction/withdrawal (Marques et al., 2012) as they transition to lactation. Inflammation caused by LPS translocation may occur due to rumen epithelial damage caused by ruminal acidosis (Gozho et al., 2005), or intestinal epithelial damage in the case of hindgut acidosis (Li et al., 2012). In the case of feed restriction, it is believed that there may also be stress-induced leaky gut whereby intestinal motility and permeability is altered (Rodiño-Janeiro et al., 2015; Rodriguez-Jimenez et al., 2019).

Most recently there has been interest in the interaction between energy balance and inflammation. Research has identified a link between body fat mobilization, oxidative stress, and inflammatory mechanisms (Bradford et al., 2015). In vitro studies have found that increasing concentrations of NEFA increase some inflammatory responses of bovine aortic endothelial cells (Contreras et al., 2012). In vivo studies have shown varying results, Kerwin et al. (2022) found no association between haptoglobin concentrations and occurrence of ketosis though a large field-study including 72 farms in the United States. In contrast, Abuajamieh et al. (2016) proposed that development of ketosis may be from increased intestinal permeability, based off findings indicating that cows experiencing ketosis following parturition had increased concentrations of inflammatory markers both pre and postpartum. These authors proposed that LPS infiltration may affect liver lipid metabolism resulting in cows being more prone to ketosis. Similarly, Horst et al. (2021) proposed that decreased DMI, increased NEFA concentrations and ketosis may be a result of immune activation, rather than causing immunosuppression allowing for disease.

Historically it has been believed that inflammation during the transition period has been the cause of metabolic disorders. Many studies investigating metabolic disorders during the transition period are retrospective in that blood metabolites are linked to a health outcome. In

other words, cows are classified based on exhibiting a metabolic disorder (eg. ketosis), and then determining what blood metabolites were leading up to this event (Horst et al., 2021). From this, the traditional thought was that an increase in NEFA or BHB leads to immunosuppression which in turn will result in a transition disorder such as a retained placenta or mastitis, thus decreasing milk yield (Horst et al., 2021). Recently it has been proposed that inflammation occurs first, which causes a transition disorder, followed by a change in energy status, DMI or milk yield.

A model used to investigate this theory has evaluated calcium homeostasis as it relates to inflammation. Several authors (Waldron et al., 2003; Kvidera et al., 2017a; Al-Qaisi et al., 2020; Chandler et al., 2023) have observed a decrease in blood calcium following LPS administration. Similarly, when acidosis was induced in sheep, blood calcium levels are reduced (Minuti et al., 2014). As immune activation is highly energetic (Kvidera et al., 2017a) and partitions nutrients away from normal pathways, low blood calcium, and cows ultimately experiencing milk fever may be a tactic to cope with inflammation, rather than a result of inflammation; a concept can be applied to other transition disorders. However, in non-induced inflammation models, a limitation to data determining the cause-effect mechanism of NEB and inflammation is that markers of inflammation are not site specific. It is difficult to determine what underlying mechanism may be causing inflammation and establish the true cause.

1.1.5 Carbohydrate nutrition during the prepartum period

It goes without argument that DMI and ultimately energy intake postpartum is a driver of optimal animal performance. Thus, the goal of the prepartum period is to prepare the cow for the transition to lactation, through minimizing negative energy balance, inflammation, and promoting DMI. Dry matter intake declines dramatically prior to calving, in particular within the 7 to 10 days prepartum (Hayirli et al., 2003; Hayirli and Grummer, 2004). The reduction in DMI prepartum may initiate NEB, which is then exacerbated postpartum when milk yield increases at a faster rate than DMI can supply nutrients for (Coppock, 1985). However, it is important to note that it is not solely the decline in intake that negatively affects the cow, but rather the experience of NEB (Hayirli and Grummer, 2004). Agenäs et al. (2003) fed cows 6, 9 and 14.5 kg DM/d prepartum and found that while there was no depression in intake for the 6 kg treatment, cows on that treatment experienced prolonged NEB postpartum, compared to the other treatments. While the factors affecting DMI and decline in DMI during the prepartum period are unknown, it may

be influenced by animal factors (ie. body weight, day of gestation, parity, body condition, health), dietary factors (ie. ingredient and nutrient composition including, forage:concentrate ratio, forage or roughage NDF and inclusion of digestible byproducts), managerial factors (ie. level of production, feeding and housing) and climate animals are exposed to (Ingvartsen and Andersen, 2000; Hayirli et al., 2004; NASEM, 2021).

Historically, the goal of nutritional management during the prepartum period was to maximize DMI and energy intake. The most common method for doing this was to increase the amount of fermentable carbohydrate in the prepartum diet (NRC, 2001), as this was seen to maximize DMI prior to parturition and adapt the rumen microbial population and papillae to a highly fermentable diet (Grummer et al., 1995). Employment of this nutritional strategy has typically been done by adjusting the amount of energy supplied through non-fiber carbohydrates (NFC) such as starch or sugar. Several studies have reported positive responses to feeding high NFC diets during the prepartum period. For example, Minor et al. (1998) fed either a high energy or low energy diet by changing the amount of cracked corn versus forage in the diet. These authors found that cows fed the high energy diet had higher DMI and energy intake as compared to the low energy diet resulting in cows fed the high energy diet being in positive energy balance prepartum. A varying range of NFC concentrations have been studied in prepartum diets; however, feeding a high NFC diet generally results in increased DMI (Holcomb et al., 2001; Keady et al., 2001; Doepel et al., 2002), energy intake and energy balance prepartum (Vandehaar et al., 1999; Dewhurst et al., 2000; Mashek and Beede, 2000).

Despite this improvement in DMI prepartum, cows fed high NFC diets still reduce DMI as they approach calving, often reducing intake more than cows fed low NFC diets (Vandehaar et al., 1999). Feeding high amounts of NFC prepartum has shown to increase BCS of cows, and increase prepartum insulin concentrations (Holtenius et al., 2003; Douglas et al., 2006; Dann et al., 2006) thus intensifying NEB postpartum (Rukkwamsuk et al., 1999) as compared to low NFC prepartum diets. In addition, high NFC diets prepartum result in increased postpartum milk yield in some (Minor et al., 1998; Mashek and Beede, 2000) but not all studies (Dann et al., 2006). As such, in recent decades there has been an increased interest in feeding low NFC diets prepartum to manage lipid accumulation and mobilization during the periparturient period. On commercial dairies, the dietary strategy of feeding pre-partum cows high NFC had minimal

improvement on transition cow diseases (Drackley and Janovick, 2007), and may not result in improved performance postpartum (Mashek and Beede 2000).

Research conducted by Drackley and his colleagues over the years resulted in the concept of feeding dairy cows a controlled energy prepartum diet, or "goldilocks" close-up diet (Drackley and Janovick Guretzky, 2007). The basis for this diet is utilizing low quality feedstuffs such as straw to control energy intake closer to animal requirements, without restricting feed intake. Increasing NDF concentration with inclusion of low-quality grass (Holcomb et al., 2001), or straw (Dann et al., 2006; Janovick et al, 2010; Mann et al., 2015) has resulted in a decrease in DMI prepartum, but reduction in the DMI decline as seen with high energy prepartum diets (Grummer et al., 2004). While there may be concern that controlled energy diets result in reduced intake prepartum, high DMI prepartum has not necessarily been advantageous (Holcomb et al., 2001).

To evaluate energy intake during the dry period, many research studies have been conducted using models that overfeed, meet, or restrict energy intake. Janovick and Drackley (2010) fed three prepartum diets to supply 150, 100, or 80% of NRC (2001) requirements. Cows on the 80% treatment had restricted intake, cows at 100% were fed a diet with chopped wheat straw, and overfed cows were allowed ad libitum intake. In this study, meeting or underfeeding to requirements resulted in cows maintaining intake approaching calving, less gain in BCS prepartum, and less body condition mobilization postpartum. Douglas et al. (2006) evaluated energy intake through inclusion of starch or fat during the prepartum period to restrict (80% of net energy of lactation (NE_L) requirement) or overfeed (160% NE_L requirement) energy and reported that restricting intake resulted in lower concentrations of NEFA and BHB during the dry period. Postpartum concentrations of total lipid and triglycerides were lower in the liver for cows that were feed restricted. Loor et al. (2006) proposed that prepartum dairy cows do not regulate intake to meet energy requirements, and overfeeding may predispose cows to fatty liver and compromised liver health. More recently, Mann et al. (2015) evaluated a high-fiber controlled energy and a high energy diet during the prepartum period and found that when cows were fed the same postpartum diet, cows fed the controlled energy prepartum diet had lower concentrations of BHB and NEFA, with less negative predicted energy balance. These findings further support the use of a controlled energy prepartum diet to minimize NEB.

While feeding a controlled energy diet has shown to be beneficial for liver health, there is still the concern that switching cows from a low NFC diet to high NFC lactating diet may result in reduced ruminal pH and greater risk for ruminal acidosis. Dirksen et al. (1985) reported that ruminal papillae shorten during the dry period, and Steele et al. (2015) found histological differences between pre and post partum. Minuti et al. (2015) reported a change in the rumen microbial population during the transition period, likely due to dietary changes. While it is recognized that the rumen undergoes great changes in rumen papillae size, surface area and mass during the transition period (Dirksen et al., 1985; Reynolds et al., 2004; Steele et al., 2015), data on whether feeding a high-energy prepartum diet reduced reduction in rumen pH postpartum is contradictory. NRC (2001) recommended increasing starch prepartum to adapt rumen microbes to a highly fermentable diet; however, several authors have found no effect of dry cow dietary adaptation on ruminal pH in early lactation cows (Rabelo et al., 2003; Reynolds et al., 2004; Penner et al., 2007 Dieho et al., 2016). Further research is warranted regarding the impact prepartum diets may have on postpartum rumen pH and health.

1.1.6 Carbohydrate nutrition during the postpartum period

During the postpartum transition period, dairy cows are often in a negative energy balance due to the lag in increasing energy intake relative to energy demand (Grummer et al., 2004). Therefore, the goal of this period is to maximize energy intake to support lactation and minimize health disorders. It is possible to increase energy intake by adding more grain to the diet; however, this may reduce DMI by increasing propionate flux according to the hepatic oxidation theory (Allen et al., 2009). Briefly, this theory indicates that increased propionate flux from the rumen result in a decrease in DMI, especially during the transition period when DMI and energy intake is paramount (Allen et al., 2009; Allen and Piantoni, 2013; Kennedy et al., 2020). Excess fat mobilization and oxidation of NEFA in the liver may induce satiety, further depressing feed intake (Allen et al., 2009; Allen and Piantoni, 2013). Alternatively, feeding increased concentrations of readily fermentable carbohydrates may also have negative impacts on rumen pH, exposing cows to ruminal acidosis and a resulting depression in DMI (Krause and Oetzel, 2006; Penner et al., 2007). Cows that experience SARA and inflammation of the rumen wall have been shown to have elevated pro-inflammatory cytokine concentrations (Kuhla, 2020), which may be associated with a reduction in feed intake (Trevisi et al., 2015).

Within the literature, there are varying responses to feeding high-starch diets in the postpartum period. Rabelo et al. (2003, 2005) compared energy density pre- and postpartum and found that regardless of prepartum treatment, cows offered the high-energy diet postpartum had higher concentrations of glucose and insulin, but similar NEFA, increased DMI, and energy intake during the first 20 d of lactation, and faster increase in milk yield. However, cows that received the high-energy density diet had lower ruminal pH compared to the low energy density diet. Similarly, Williams et al. (2015) fed low (21% starch) or high (27% starch) starch fresh cow diets and found high starch to result in lower ruminal pH, and more time with ruminal pH < 5.8. Guo et al. (2007) abruptly changed from a medium energy prepartum diet (1.54 Mcal NE_L/kg, 53% NDF) to a high energy lactating diet postpartum (1.77 Mcal Ne_L/kg, 35% NDF), and found positive effects on energy balance and lipid metabolism, compared with feeding a transition diet for the 2 wk pre- and postpartum (1.71 Mcal NE_L/kg; 35.2% NDF). Similarly, McCarthy et al. (2015) fed high (26.2% starch) or low (21.5% starch) starch diets postpartum and found cows offered high starch had a faster rate of increase in DMI and lost less body condition. In addition, cows offered high had lower BHB concentrations as compared to low suggesting improved energy metabolism.

In contrast, Dieho et al. (2016) found that cows had increased fat and energy corrected milk yields when they had a slower rate of increase in concentrate allowance (0.25 kg of DM/d) compared to faster increase (1.0 kg of DM/d) with no difference in DMI. Dann and Nelson (2011) found improved lactation performance when cows were fed low (21% starch) or transitioned to (21% starch for 21 d followed by 26% starch) a high (26% starch) starch diet as opposed to being fed the high starch diet immediately following calving. In a large study, Shi et al. (2019) fed cows either a low (22.1% starch) or high (28.3% starch) starch diet postpartum for 23 ± 3 d and reported that cows fed the low starch diet had greater milk yield with no difference in DMI. In this study, postpartum diets were formulated to be different in starch, but similar in NFC through the inclusion of beet pulp. As such, the authors hypothesized that the energy from beet pulp may have partitioned more to the mammary gland. Using a subset of animals from the aforementioned study, Shi et al. (2019) found that ruminal pH was lower for cows offered the high starch diet immediately postpartum, but not at 7 or 21 DIM, compared to low. This contrasts with what was reported by Rabelo et al. (2003).

Based off these data, it is unclear as to what the optimal approach for feeding cows immediately postpartum may be. A difference among these studies that may contributing to contrasting outcomes is the prepartum diet fed. Where high starch or high NFC prepartum diets have been fed (McCarthy et al., 2015; Rabelo et al., 2005; Guo et al., 2007) it appears there are benefits to feeding high starch postpartum diets as seen through improved DMI, milk production or reductions in negative energy balance. However, cows may experience decreases in ruminal pH and bouts of SARA. In contrast, when low starch prepartum diets are fed (Dann and Nelson, 2011; Shi et al., 2019; Dieho et al.,2016), it appears cows fed low starch postpartum, or are adapted to high starch lactation diets have increased milk production and components. Therefore, further research is warranted evaluating whether there is an interaction between pre and postpartum dietary strategies to optimize production of lactating dairy cows.

1.2 Nutritional management with automated milking systems

1.2.1 Characteristics of automated milking systems

Automated milking systems (AMS), also referred to as voluntary milking systems (VMS) or robotic milking systems, originated in the Netherlands in the 1980's (Hyde and Engel, 2002) with the concept of creating a machine that would prepare, milk, and apply post milking treatments for dairy cattle without any human interaction. In 1992, the first AMS was installed on a commercial dairy farm, and since then the practice has been widely adopted across the globe (Hyde and Engel, 2002). In 2022, 16% of dairy farms enrolled in the national milk recording program in Canada were using AMS (Canadian Dairy Information Centre, 2022). Adoption of AMS is increasing due to their ability to reduce the labour required for milking (Hansen, 2020), provide a more flexible lifestyle to producers (de Koning, 2010), and potential ability to increase milk yield and cow comfort (Jacobs and Siegford, 2012). In Canada, use of AMS has been most common on smaller farms (less than 2 AMS; Tse et al., 2017), however usage on large farms (greater than 4 AMS) has grown in recent years. A consideration with AMS is the financial expenditure required as compared to traditional parlor milking systems. Some evaluations have found the capital investment of AMS to cost 15 to 46% more than parlor milking systems, depending on herd size (Future Dairy, 2023), and daily operation costs to be greater for AMS, resulting in AMS being less profitable (Salfer et al., 2017). However, the economic impact of AMS would be farm-specific and primarily dependent on labour savings,

additional milk production obtained with AMS milking, and lifespan of the milking unit (Salfer et al., 2017). In addition, most AMS are capable of milking 60-70 cows per unit (Bach and Cabrera, 2017) which may pose challenges for expansion as increasing cow numbers without purchasing additional AMS units, an issue not faced with most parlor milking systems.

Management of cows milked with AMS is different from a parlor or tie-stall as cows are required to enter the AMS voluntarily to minimize labor associated with fetching cows and allow for frequent milkings. There are three general options for barn design when utilizing AMS: free-flow, guided-flow, and forced-flow (Ketelaar-de Lauwere et al., 2000; Bach et al., 2009), with the latter being less common in recent years.

Free-flow barns allow cows to move throughout the barn from the stalls to the feed alley to the robot without restriction (Schewe and Stuart, 2015; Lely, 2022). Guided-flow barns are unidirectional; however, they utilize automated pre-selection gates to direct cows throughout the barn, without denying cows movement to a new area. The two types of guided barns are milk-first and feed-first (DeLaval Inc., 2015). As described by Delaval Inc. (2015) with milk-first, cows from the stalls pass through the pre-selection gate prior to accessing the feed alley. If the cow has milking permissions, she is directed to a holding area to be milked, if she has no milking permissions, she is directed to the feed alley. After milking, cows are released to the feed alley. From the feed alley to return to the stalls, cows pass through a one-way gate. With feed-first, cow traffic is opposite whereas from the stalls, cows go through a one-way gate to the feed alley. To return to the stalls, the cow goes through the pre-selection gate in front of the robot. If the cow has milking permissions, she is directed to the holding area to be milked, if she has no permissions, she is directed to the stalls. After milking, the cow is released to the stalls. Forced-flow barns also direct cows in a unidirectional manner set up in a milk-first or feed-first orientation like guided-flow. However, with forced-flow, if a cow does not have permissions when she goes to a pre-selection gate she is denied access to the either the stalls or feed alley and must remain in her current location until milking permissions are received (Melin et al., 2007; Schewe and Stuart, 2015).

Initial literature with a simulated AMS found that implementation of forced-traffic increased the number of visits to the AMS and increased the amount of time cows spent standing, altering cows behaviour (Ketelaar-de Lauwere et al., 1998). Hermans and colleagues (2003)

evaluated forced vs. semi-forced, where cows had open access to the stalls and feed bunk at one end of the barn but had to attend the AMS for additional concentrate, and found that number of milkings did not differ; however, when cows were managed with forced flow, the number of non-milking visits to the AMS were increased. In this study, it was found that cows managed with semi-forced traffic spent more time eating, less time standing in free-stalls, but more time standing in alleys. Bach et al. (2009) found an increase in milkings per day with forced traffic, and despite no difference in DMI or milk production, cows managed with forced-traffic had a reduction in meals per day with greater meal duration and meal size. The general conclusion from aforementioned authors is that free-flow systems are better for the cow than forced. However, it is important to note that the forced-traffic systems utilized one-way gates to direct cows and do not evaluate a modern guided-traffic facility where cows are not held back in one area of the barn until milking permissions are received.

More recently, Tremblay et al. (2016) surveyed AMS herds across North America and reported that forced and guided traffic facilities had decreased milk production compared with free traffic hypothesizing feed intake as being a potential contributor. However, this survey documented data from only one AMS brand. In a more comprehensive survey across Canada, Matson et al. (2021) surveyed 197 farms, and found 12.2% of herds were utilizing guided-traffic (feed-first, milk-first or a hybrid approach) likely due to the fact that only one brand of AMS provides guided-traffic. These authors did not present benchmarking data by barn design. Robot manufacturers and farmers have strong preferences towards specific traffic systems (Pitkaranta et al., 2019) depending on one's desire to optimize milk production, AMS performance (i.e. milkings per unit), labor, and economic efficiency. Within the literature, it is unclear what the effects of current guided-traffic designs have on cow DMI, milk production, or welfare.

Success of an AMS is dependent on cows voluntarily visiting the AMS to optimize labor requirements, milk production, and economic benefits of AMS (Bach and Cabrera, 2017; Pitkaranta et al., 2019). It has been suggested that cows enjoy the milking process as it relieves the pressure in their udder and simulates the natural process of nursing their calf (Rathore, 1982; Wall and McFadden, 2012). However, this is not a primary motivator for voluntary visits. While some cows may be motivated to voluntarily enter the AMS, Prescott et al (1998) found that most cows were not motivated to enter an AMS without feed, and their response was variable and

changed with stage of lactation. As such, it is common practice to use feed in the AMS to encourage visits.

1.2.2 Feeding management with AMS

1.2.2.1 Component feeding dairy cattle

The concept of component feeding is not new to the dairy industry. Historically, dairy cows were fed their grain and forage separate from one another. As milking parlors became more common and the industry determined it was a beneficial to feed cows concentrates, it became popular to feed cows concentrate in the milking parlor to help draw them in for milking or through concentrate feed stations. In many cases, cows received most, or all of their concentrate separate from forages; however, high producing cows were unable to consume enough concentrate during milking resulting in poor performance and this practice led to digestive upset resulting in the move towards a total mixed ration (TMR; Eastridge, 2006; Schingoethe, 2017). While there is record of researchers evaluating the concept of a TMR in the early 1900's, it isn't until the 1950's and '60's that research on TMR became more prevalent in scientific literature (Schingoethe, 2017).

Since then, there have been great advances in our knowledge of TMR and how to create them to optimize animal production. In 2014, it was estimated that over 85% of dairy farms with 100 cows or more were using a TMR (USDA National Animal Health Monitoring System, 2014). While some areas practice component feeding with parlors, tie-stalls and where cows are managed with grazing, component feeding is becoming more common again as dairies transition to automated milking systems (AMS). Component feeding, in relation to AMS is typically defined as feeding cows concentrate through the AMS, as either a pellet, mash or individual ingredients, and a partial mixed ration (PMR) at a feed bunk (Bach and Cabrera, 2017).

1.2.2.2 Precision Feeding

Feeding management with AMS is different from conventional milking due to using a form of concentrate fed in the AMS as well as a PMR at the feed bunk. This method of feeding has given rise to the idea of precision feeding dairy cattle through the AMS. Precision feeding attempts to improve productivity and efficiency by having nutritional programs meet individual cow requirements (Cerosaletti et al., 2004; Gehman, 2011) rather than delivering feed based on

herd averages. With AMS, cows can be offered different quantities or types of concentrate in the AMS based on their level of production, stage of lactation, or parity (DeLaval Inc., 2015; Bach and Cabrera, 2017; Lely, 2022).

Theoretically, nutrient requirements of individual cows can be met with AMS to potentially improve productivity and production efficiency (Cerosaletti et al., 2004; Gehman, 2011; Bach and Cabrera, 2017). As mentioned previously, unlike TMR feeding scenarios where one diet is formulated to meet the nutrient requirements at one level of production, using feed tables, one is able to assign a specific amount of AMS concentrate relative to a specific level of production. However, this requires the ability to measure or predict milk production responses to assign a specific concentrate supplementation and the ability to evaluate the corresponding change in PMR intake.

Predicting milk yield is a challenge as production changes throughout lactation and is affected by the environment and management cows are exposed to (André et al., 2011). Currently AMS assign feed based off current or previous 7 d average milk productions and lack the ability to assign AMS concentrate based on future predicted milk yield. This poses a challenge for precision feeding as AMS concentrate allowance will exceed or lag current daily milk production.

1.2.2.3 Limitations of precision feeding

Formulating rations for individual cows managed with AMS on commercial farms comes with several challenges including: inability to measure or dispose of refused AMS concentrate resulting in build up of concentrate in the feeder, or consumption by another cow (Bach and Cabrera, 2017), achieving targeted AMS concentrate intake and the inability to measure or predict changes in PMR intake. Within the literature, it has been repeatedly found that actual AMS intake is less than what is targeted. Bach et al. (2007) targeted 3.0 or 8.0 kg/d of AMS concentrate intake and achieved 2.6 and 6.8 kg/d, respectively. Halachmi et al. (2005) targeted 7.0kg/d and achieved 5.2 kg/d. More recent studies have offered more AMS concentrate than what is required to meet target AMS concentrate intakes (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019). In addition, several authors within the past 5 years have conducted research with both free-flow and guided systems and have found that increasing the amount of AMS concentrate offered results in increased variability in AMS intake and inconsistent response

on milk production (Hare et al., 2018; Hendriksen et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019).

It has been found that in controlled studies monitoring both AMS and PMR intake, increasing concentrate allowance does not always stimulate DMI and in fact there is a substitution of PMR for AMS concentrate. Bach et al. (2007), Hare et al. (2018) and Menajovsky (2018) reported that for every 1 kg increase in AMS concentrate, PMR intake was reduced by 1.14, 1.58 and 0.84, respectively, in studies using mid to late lactation cows. Using early lactation cows, Schwanke et al. (2019) reported a substitution rate of 0.63 kg/d, while Henricksen et al. (2019) reported that early lactation cows reduced intake of PMR with increases in AMS concentrate intake resulting in a substitution rate of 5.00 kg/d.

The concept of substitution rate is not novel to AMS and has been of primary interest to cows managed under grazing conditions (Kellaway and Harrington, 2004). Similar to what is seen with AMS, when concentrates are fed to grazing animals, pasture intake can be depressed, and is why variation in milk response may be seen with supplementation. Substitution rate under grazing conditions has been affected by pasture allowance, type of concentrate fed, digestibility of the forage, chemical and physical properties of the concentrate, adaptation to concentrate supplementation, and stage of lactation. Kellaway and Harrington (2004) summarize results of several grazing studies evaluating factors affecting substitution rate; however, note that it is greatest when there is ample pasture availability, pasture is highly digestible and where starch-rich concentrates are fed. Similarly, it is hypothesized with PMR fed dairy cows, energy density of the PMR affects substitution rate (Jensen et al., 2016), with increased substitution with increasing energy density of PMR (Henriksen et al., 2018a). Furthermore, Menajovsky et al. (2018) evaluated a high vs. low forage PMR with either a low (2 kg/d, DM basis) or high (6 kg/d, DM basis) pellet allocation and found that with a low forage PMR the substitution rate was 0.89 while with the high forage PMR, substitution was 0.78. This substitution rate is important to recognize with AMS as it directly affects nutrient intake and the ability to precision feed. Inconsistencies across literature warrant further investigation.

1.2.2.4 Characteristics of AMS concentrate

Physical and chemical characteristics of AMS concentrate have been found to influence milk production and voluntary visits to the AMS. To date it has been recommended to offer a

hard pellet with few fines (Rodenburg et al., 2004) over a mash (Spörndly and Asberg, 2006). It has been found that when offered the different forms of the same feed, cows consumed pellets at a faster rate (250-400 g/min) than texturized or mash (Kertz et al., 1981). This is an important consideration when feeding cows with AMS as cows must be able to consume their concentrate allowance within the time constraint of their milking duration (Bach and Cabrera, 2017). Recently there is more interest in using a mash in AMS to utilize more on-farm ingredients and reduce feed costs (de Jong et al., 2003; Johnson et al., 2022).

Research regarding use of a pellet over a mash is minimal; however, Johnson et al. (2022) observed that cows offered steam-flaked barley had fewer voluntary visits (2.71 vs. 2.90 milkings/d) as compared to offering pelleted barley in a feed-first guided traffic barn. In addition, cows spent more time in the holding area before entering the AMS, indicating that cows were not as motivated to be milked when offered steam-flaked over pelleted barley. In this study, there was no difference in milk or milk component yields. In contrast, Henriksen et al. (2018a) found an increase in AMS visits and milk yield when cows were offered a mixture of pellet and steam-rolled barley as compared to pellet-only concentrate in a free-flow traffic barn.

In terms of nutritional characteristics, it has been found that cows preferentially consume barley-oat blends, or wheat-based concentrate over corn or barley-only based (Madsen et al., 2010), and it is believed that cows are attracted to high-starch concentrates which increase voluntary visits to the AMS (Bach et al., 2007). Composition of concentrate should be considered as high-starch concentrates may negatively affect appetite, NDF digestibility, or ruminal pH which may alter milk or milk component production (Bach and Cabrera, 2017). It has been established that supplementation with highly digestible concentrates, such as high starch, may result in a decline in ruminal pH and cows being susceptible to sub-acute rumen acidosis (SARA; Plaizier et al., 2009; Hills et al., 2015), which negatively impacts NDF digestibility of forages (Plaizier et al., 2009). As such, many research studies have evaluated supplementing dairy cows with high starch or high fiber concentrates, and the resulting impact on DMI and milking performance, with some results varying between component fed where all concentrate is separate from the forage and concentrate + PMR fed studies.

When cows are supplemented with all concentrate separate from forage, use of a starch based concentrate as compared to fiber based has shown to increase milk yield and protein and lactose yield, while having negative effects on milk fat concentrations in early lactation (Jenkins

and McGuire, 2006; Sutton et al., 1993; Higgs et al., 2013; Hills et al., 2015), with milk fat yield responses dependent on the magnitude of change in milk yield vs. milk fat concentration. The findings of these studies are not surprising as it has been well established that starch supplementation increases propionate and decreases acetate production in the rumen which may be associated with increased yield, and decreased milk fat concentrations, respectively (Hills et al., 2015). However, it is important to consider that in these studies all concentrate was fed separate from forage, not fed alongside a PMR. Evaluating concentrate type when fed in TMR's has resulted in contradictory results. Bougouin et al. (2018) fed a 50% isoenergetic forage diet with supplementation with a starch (46% starch; wheat and corn based) or fiber (11.7% starch; beet pulp and soy hull based) concentrate in the TMR. In this study, cows offered the high starch pellet had reduced DMI with no difference in milk production or milk composition.

When concentrate has been offered to cows alongside a PMR containing concentrates, varying results have been found on milk production and feeding behaviours. Where cows have been offered concentrate through feed stations and milked in a parlor, Miron et al. (2004a) evaluated a barley based or soyhull based pellet fed at 8.6 kg per day alongside a common PMR and found no difference in total DMI or feeding behaviour. Cows fed the soyhull based pellet had increased milk fat and protein concentrations; however, there was no difference in milk yield or yields of milk fat or protein. Similarly, Miron et al. (2004b) evaluated a high starch (barley and corn based) and high fiber (soyhull and corn gluten feed) pellet fed at 25% of the diet alongside a common PMR and found no difference in milk yield, however milk fat concentrations were higher when the high fiber pellet was fed, which resulted in increased milk fat yield. In this study, cows fed the high fiber pellet consumed more meals and had longer meals as compared to high starch; however, high starch cows had greater PMR intake per meal and rate of eating as compared to high fiber. This resulted in greater DMI for cows offered the high fiber pellet as compared to high starch. Experimental conditions were similar for both studies, and based on the data presented it is unclear as to why feeding behaviour results differed. However, the data from these studies suggest that supplementation with a high fiber pellet does not compromise milk yield and may be a suitable alternative to feeding a high starch pellet.

Where cows are managed with AMS, Halachmi et al. (2006) evaluated a high starch (barley, corn and sorghum grain based) or high fiber (soy hull and corn gluten feed based) pellet and found no differences in milk or milk component yields when cows were consuming ~5.4kg/d

pellet between the AMS and feed station, and no difference in voluntary milkings. In a broader study, Halachmi et al. (2009) evaluated a high starch or high fiber (47.9 and 40.9% starch and soluble carbohydrate, respectively) pellet across various stages of lactation (averaging 10.2kg/d from 10 to 60, 11.1 kg/d from 61 to 120 and 10.4 kg/d from 121 to 180 DIM) fed through an AMS or feed station, alongside a basal PMR. Cows offered high fiber pellets had increased milk yield across all stages, and increased milk fat and protein yield up to 120 DIM, with no difference in component yield observed beyond 121 DIM. There was no difference in voluntary milkings.

The data mentioned above indicate that use of a high fiber pellet is acceptable to maintain milk production and component yield, without affecting voluntary visits to the AMS. However, in these studies, cows were fed the same PMR regardless of pellet type, and pellets were formulated to contain the same nutrients, only differing in their starch/NDF content. While this approach allows for focus on pellet composition, results are confounded by the nutrient profile of the total diet consumed (concentrate + PMR). Therefore, further research is warranted evaluating the effects that pellet composition and feeding rate may have when the total diet (concentrate + PMR) is formulated to be similar.

Besides use of high starch pellets to attract cows for milking, use of flavoring is also common, with many commercial pellets containing a flavoring agent. However, use of artificial flavoring has shown mixed results across the literature (Bach and Cabrera, 2017). Another consideration is whether AMS concentrates should contain vitamins and minerals. There are concerns from a pellet quality perspective, and because they have poor palatability (Back and Cabrera, 2017). However, when cows are offered high amounts of AMS concentrate, PMR intake may be reduced resulting in insufficient supply of vitamins and minerals from the PMR alone (Bach and Cabrera, 2017). In addition, there may be opportunity to precision-feed vitamins and minerals relative to milk production and stage of lactation if provided through the AMS concentrate.

1.2.2.5 Current AMS feeding recommendations

Literature from the 1970s and 1980s evaluated supplementation of concentrates to grazing or cows fed forage. In these studies, cows were assigned to flat rate feeding (setting one level of daily concentrate for the entirety of lactation) and stepped feeding (offering more concentrate per day in early lactation as compared to mid or late lactation), with the same total

amount of concentrate fed over the entire lactation. Results of several studies found no benefit of stepped feeding on annual milk production over multiple years (Rakes and Davenport, 1971; Ostergaard, 1979; Gordon 1982; Hills et al., 2015), suggesting that there may not be a benefit to precision feeding as a means to drive milk production. Despite this, it has been perceived that providing more concentrate through the AMS will lead to more voluntary visits, thus improving milk production (Tremblay et al., 2016). Many AMS manufacturers have developed AMS feeding strategies using feed tables whereby cows may be offered upwards of 8.0 kg/d (Lely, 2022) or 12.0 kg/d (DeLaval Inc., 2021) depending on the level of production. In contrast, GEA Farm Technologies recommends offering no more than 2.0 kg per milking to avoid drops in ruminal pH. In addition, many industry professionals recommend formulating the PMR to meet requirements that are 6.0 to 8.0 kg of milk production below the herd average, with the remainder of the nutrients coming through the AMS concentrate or formulating the PMR to meet 80 to 90% of the total DMI and milk production (Brouk, 2017).

A survey conducted with 635 North American Lely farms found that cows producing 35 to 45 kg of milk per day were offered 5.6 to 7.1 kg/d of concentrate (Tremblay et al., 2016). Contrary to industry belief, this survey data also found a negative relationship between the amount of concentrate offered and milk production. Another survey conducted by the University of Minnesota found that cows managed in free-flow were offered between 0.9 and 11.36 kg of concentrate in the AMS per day, while cows managed with milk-first guided were offered between 0.91 and 5.45 kg of concentrate per day (Endres and Salfer, 2016)

There are various recommendations available for fresh cow feeding strategies with AMS, primarily based on opinion or survey data of existing practices. It is generally suggested to adapt cows to AMS concentrate by increasing the amount offered per day during the fresh period. Survey data and industry recommendations suggest offering from 2.0 to 4.0 kg/d immediately after calving and increasing at a rate of 0.18 to 0.45 kg/d until target AMS concentrate levels are reached (Rodenburg, 2011; DeLaval Inc, 2021). As previously mentioned, transition dairy cows are at risk of experiencing metabolic disease (Drackley et al., 1999), and reduced ruminal pH leading to ruminal acidosis (Penner et al., 2007; Penner et al., 2009). Utilizing AMS and feed tables offers the unique ability to adapt cows to lactating diet, which is not available with TMR

feeding. Despite this, little research has been conducted on postpartum dairy cows managed with AMS.

When comparing a rapid versus slow increase in concentrate allowance to cows immediately postpartum, there has been a discrepancy in outcomes. Dieho et al. (2016) conducted a study using 12 cows and found that rapidly increasing concentrate offered through the AMS did not improve dry matter intake or milk production. Kokkonen et al. (2004) found an increase in milk yield and reduced milk fat concentrations when cows were rapidly increased, whereas Ingvartsen et al. (2001) found no change in milk yield but a reduction in milk fat concentration. Feeding management with AMS is similar to component feeding, which has been previously reported to cause digestive upset in early lactation (Coppock, 1977). Therefore, there is likely a benefit of reducing the amount of concentrate offered to postpartum transition dairy cows to reduce the likelihood of metabolic diseases (Kokkonen et al., 2004). However, there is the challenge of meeting the requirements of a postpartum transition cow with low concentrate allowances, if the PMR is formulated to meet nutrient requirements significantly under the average production of the herd.

Similarly, data available for mid to late lactation cows would suggest that feeding higher amounts of AMS concentrate may not encourage more visits or increase milk production (Bach et al., 2017; Hare et al., 2018; Paddick et al., 2019). In fact, feeding more concentrate often results in a substitution rate between AMS concentrate and PMR intake, thus reducing PMR intake which may compromise total DMI (Bach et al., 2017; Hare et al., 2018; Henriksen et al., 2019), a finding that is of particular interest to fresh cows.

There are currently two main philosophies when feeding early to peak lactation cows with AMS to encourage increased milk production and peak milk; using feed tables to either "pull" or "push" milk production. When "pulling" milk production, feed tables often lead-feed, whereby the amount of concentrate offered exceeds what is necessary for current milk production (Salfer and Endres, 2018). This is believed to increase peak milk and encourage persistency. Once peak-yield has occurred, cows are fed according to production (Bach and Cabrera, 2017; Salfer and Endres, 2018) and a "push" feeding strategy is employed.

When "pushing" milk production, feed tables are designed to provide AMS concentrate based on days in milk or milk production from the previous 7 d. High producing cows are

generally allocated greater quantities of concentrate than lower production cows (Siewart et al., 2017); however, most survey style data does not evaluate the cause-effect relationship of this. In other words, does offering more concentrate increase milk production, or is a cow offered more concentrate simply because she has higher milk production already?

Feeding with AMS also offers the ability to reduce the amount of concentrate as cows reach the end of their lactation (Bach and Cabrera, 2017). When cows are producing large amounts of milk, the amount of concentrate can be reduced to decrease milk production and prepare for dry-off; however, this may result in increased fetching. Another option is to reduce the number of milkings per day cows are allowed to achieve (Lely, 2022). In doing so, you can reduce the amount of AMS concentrate consumed due to less opportunities for feed consumption, as well as reduce the number of milkings per day which has been found to reduce milk production with no negative impact on udder health (Dingwell et al., 1999).

1.3 Summary

The transition to lactation is a difficult time for dairy cows as they face many physiological, dietary, and managerial changes. Nutritional management during this period should aim to minimize NEB and health concerns while optimizing DMI and milk production. While some previous studies suggest that controlled energy diets prepartum reduce intake, they have been found to reduce negative energy balance postpartum, having beneficial effects on cows. However, within the literature, there is discrepancy as to how cows respond to postpartum diets. Increasing energy density of postpartum diets through starch has shown to be beneficial to increasing DMI, energy intake and milk production, but not in all cases. While it seems that there may be an interaction between pre and postpartum feeding strategies on animal performance, this has not been specifically investigated.

As the dairy industry transitions to automated milking, little controlled research has been conducted to evaluate feeding strategies for cows managed with AMS immediately following calving. There is little information regarding the impact that AMS concentrate composition may have on production and feed intake parameters, nor the rate to which cows should be adapted to AMS concentrate immediately postpartum. Studies evaluating pellet composition have done so by evaluating pellet composition alongside the same basal PMR, confounding the results of pellet type with overall diet (Miron et al., 2004a, b; Halachmi et al., 2006; Halachmi et al.,

2009). Most recommendations for feeding strategies for AMS are opinion based or sourced from survey style publications and contradict what has been determined in controlled research studies (e.g. Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019).

Similarly, studies evaluating pellet quantity often substitute concentrate between the PMR and pellet. These studies fail to evaluate the effect that additional concentrate, in excess of what the diet is formulated for may have, which is of particular interest to industry as cows immediately postpartum may be allocated more pellet than what the diet formulation requires in an attempt to offer increased energy to cows.

Overall, there is a lack of knowledge regarding: whether there is an effect of prepartum dietary strategy on postpartum performance, the rate of, or duration of adaptation to concentrate immediately following calving, nor the impact that concentrate composition may have. It was hypothesized that 1) postpartum performance of dairy cows on high or low starch diets would be influenced by starch content of prepartum diet, 2) feeding a high-starch pellet would decrease rumen pH and have negative implications on animal performance, and 3) offering an increased amount of AMS concentrate postpartum would increase animal performance. Therefore, the objectives of this research were to 1) evaluate the effects of starch content of the pre and postpartum diet during the transition period on productivity, metabolites and indicators of inflammation in early lactation 2) evaluate the effects of a high starch or high fibre pellet fed alongside a complementary PMR whereby the overall diet (PMR + pellet) is the same on feed intake patterns and rumen fermentation characteristics of dairy cows and 3) evaluate the effects of concentrate allowance and rate of increase on animal performance immediately following calving for cows milked with an AMS.

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Chapter 2: Effects of pre- and post-partum dietary starch content on productivity, plasma energy metabolites, and serum inflammation indicators of dairy cows

2.1 Introduction

Dairy NRC (2001) suggested that cows should be fed increased energy or a highly fermentable diet during the pre-partum period to prepare for diets fed during lactation. However, extensive research over the past 20 years showed that feeding controlled energy diets to pre-partum cows could reduce insulin resistance and lipid mobilization (Drackley et al., 2001; Douglas et al., 2006; Janovick et al., 2011), increase DMI (Douglas et al., 2006), and reduce the extent of negative energy balance (Dann et al., 2006) post-partum, indicating that controlling energy intake and feeding less fermentable diets pre-partum appear to be beneficial to the cow.

Following calving, cows must increase feed intake rapidly to support lactation. It has been suggested that limiting starch intake post-partum may increase DMI due to less production of fermentation acids such as propionate (Allen et al., 2009) and reduce the risk for cows to have depressed rumen pH (Penner et al., 2007). However, recent research evaluating post-partum dietary strategies on animal performance showed inconsistent results; some reported positive responses to high starch diets (Rabelo et al., 2003; McCarthy et al., 2015; Piantoni et al., 2015) while others reported negative responses (Dieho et al., 2016; Shi et al., 2019). The discrepancy among the studies, in response of post-partum cows to high starch diets, may be partially attributed to differences in dietary starch content during the pre-partum period. McCarthy et al. (2015) and Piantoni et al. (2015) fed pre-partum diets containing 17.4 and 18.1% starch on a DM basis, respectively, and reported that post-partum cows fed high starch diets had greater DMI and lost less BCS, compared with those fed low starch diets, without differences in milk yield. However, Shi et al. (2019) fed a pre-partum diet containing 13.9% starch on a DM basis, and reported that fresh cows fed high starch diets decreased milk production compared with those fed low starch diets.

In addition, research has indicated that feeding high starch diets (Emmanuel et al., 2008), short-term feed withdrawal (Marques et al., 2012), or feed restriction (Kvidera et al., 2017a), all dietary stressors that are associated with the calving transition period, may increase inflammation

and decrease early lactation performance. A sudden and drastic change in diet fermentability after temporal reductions in feed intake, which is typically observed during the calving transition period, may be a contributing factor to affect fresh cow responses to high starch diets. However, the interaction effects of starch content between pre-partum and post-partum diets on animal performance after calving have not been extensively studied.

Therefore, we hypothesized that animal responses to starch content of post-partum diets would be affected by starch content of pre-partum diets, and cows fed high-starch diets during the pre-partum period would increase their productivity with high-starch post-partum diets as compared to low-starch post-partum diets. The primary objective of this study was to evaluate the effects of starch content of pre- and post-partum diets on inflammation indicators, DMI, and milk production during the 21-d post-partum.

2.2 Materials and methods

All experimental procedures were pre-approved by the University of Alberta Animal Care and Use Committee: Livestock (AUP #2342) and conducted according to the guidelines of the Canadian Council on Animal Care (2009).

2.2.1 Animals, experimental design and diets

One hundred (42 primi- and 58 multi-parous) Holstein dairy cows housed in a tie-stall facility were randomly assigned to pre- and post-partum dietary treatments balanced for parity and pre-trial BCS at d 28 ± 3 prior to expected calving date. Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) pre-partum diet commencing 28 ± 3 d prior to expected calving date. As barley silage from the second pit used in the study was very high in starch content, the High pre-partum diet contained far higher starch than we had intended. Although this was not desirable, we chose to maintain a difference in starch content between the pre-partum diets by feeding a very-high-starch diet and a control diet that was reasonable in starch content rather than feeding a high-starch diet and a control diet containing > 50% straw. Therefore, following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2 % NDF, 32.8% starch, DM basis) post-partum diet for the first 20 ± 2 d following calving. Thus, the dietary treatment

combinations were Control-HF (n = 25), Control-HS (n = 25), High-HF (n = 25) and High-HS (n = 25).

All diets were formulated using dairy NRC (2001) to meet or exceed all requirements for a 650-kg cow producing 33 kg/d of milk with 4.0% milk fat and 3.1% milk protein (Table 1). Cows were fed their experimental diets once daily at 0800 h to allow for 5 to 10% refusals based on actual feed intake (as-fed basis) of the previous day. Feed was fed in individual feed mangers as a TMR and cows had access to it throughout the day. Following calving, cows were milked in their stalls twice daily at 0400 and 1600 h.

2.2.2 Animal management

At enrollment, 28 ± 3 d prior to expected calving date, all animals received a controlled-release intraruminal monensin bolus (Kexxtone, Elanco, Guelph, ON, Canada) and an intramuscular selenium-vitamin E injection containing 5 mg selenium and 50 mg vitamin E per mL (10 mL; MU-SE, Merck Animal Health, Kirkland, Quebec, Canada). All cows were housed in tie-stalls and moved to individual maternity pens 4 ± 3 d prior to calving and were returned to individual tie-stalls following calving. Cows were offered 3 kg/d grass hay for the first 3 d after calving alongside their treatment TMR, and all multiparous cows received two calcium boluses (Bovikalc, Boehringer Ingelheim, Duluth, GA) within the first 24 h following calving.

2.2.3 Data and sample collection

Throughout the study, the amount of feed offered and refused (as-fed basis) was recorded daily. Forage samples were collected weekly and concentrate samples every other week. Dry matter concentration was determined in a forced-air oven at 55°C for 72 h, and the diet formulation adjusted if necessary. Dry matter intake was determined by multiplying the as-fed feed intake by the weekly DM of the total diet fed. The DM of the offered and refused feed was assumed to be the same. The dried weekly forage, and bi-weekly concentrate samples were kept and ground with a Wiley mill (Thomas Scientific, Philadelphia, PA) to pass through a 1-mm screen. Ground forage samples were composited every 2 mo, and concentrate samples composited every 4 mo.

To facilitate routine management of animals and the dairy operation, data and samples were collected on Monday and Thursday, unless otherwise noted. Body weight and BCS were

recorded on two consecutive days before feeding at the beginning of the study (d - 28 ± 3), following calving on d 1 and on two consecutive days at the end of the study (d 20 ± 2). Body condition score was recorded by 2 individuals using a 5-point scale (Wildman et al., 1982). Both BW and BCS were averaged before statistical analysis, and changes in BW and BCS before and after calving were calculated. Milk yield was recorded daily for all cows after calving. Milk samples were collected from all cows from 2 consecutive milkings (p.m. and a.m.) on d 10 ± 2 and 20 ± 2.

Blood was sampled from all cows before feeding (~0730 h) on d 10 ± 3 prior to calving and d 3 ± 1, 10 ± 2 and 20 ± 2, following calving. All blood samples were collected via the coccygeal vein into an evacuated sodium heparin or serum vacutainer (BD Vacutainer, Franklin Lakes, NJ). The sodium heparin vacutainer was immediately placed on ice until centrifugation at 3,000 × *g* for 20 min at 4°C, and plasma was stored at -20°C until analysis. The serum vacutainer was left at room temperature for a minimum of 20 min before centrifugation at 3,000 × *g* for 20 min at 4°C, and serum was stored at -20°C until analysis.

2.2.4 Sample analysis

Feed samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD), and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), ADF (AOAC International, 2000; method 973.18), NDF (Van Soest et al., 1991), starch (Hall, 2009), and CP (AOAC International, 2000; method 990.03), and NFC were calculated as per dairy NRC (2001). Milk samples were sent to the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada), and analyzed for concentrations of fat, CP, lactose, SCC and MUN by infrared spectroscopy (MilkoScan 605, Foss North America, Brampton, ON, Canada).

Plasma glucose was determined using a glucose oxidase/peroxidase enzyme (Sigma Co., St. Louis, MO) and dianisidine dihydrochloride (Sigma Co.) with absorbance at 450 nm determined using a SpectraMax 190 plate reader (Molecular Devices Corp., Sunnyvale, CA). Plasma insulin concentration was determined using a solid-phase competition immunoassay with Eu-labeled bovine insulin and polystyrene microtiter strips coated with anti-guinea pig γ-globin (Takahashi et al., 2006; Inabu et al., 2017).

Plasma free-fatty acid concentration was determined using a commercial kit (NEFA HR2; Wako Chemicals USA Inc., Richmond, VA) and BHB concentration was measured by the enzymatic oxidation of BHB to acetoacetate in the presence of 3-hydrooxybutyrate dehydrogenase (Roche, Mississauga, ON, Canada) and NADH determination at a wavelength of 340 nm.

Plasma GLP-2 concentration was analyzed using time-resolved fluoro-immunoassay techniques as described by Sugino et al. (2004), Elsabagh et al. (2017) and Inabu et al. (2017). Briefly, GLP-2 concentrations were measured using a solid-phase competition immunoassay with Eu-labeled human GLP-2 (Peptide Institute Inc., Osaka, Japan), polyclonal anti-rat GLP-2 (Yanaihara Institute Inc., Shizuoka, Japan), and polystyrene microtiter strips coated with goat-anti-rabbit γ-globulin (Elsabagh et al., 2017).

Serum samples were analyzed for concentrations of serum amyloid A (SAA) and Haptoglobin (Hp) using commercially available kits (Invitrogen SAA Livestock ELISA kit, Fischer Scientific, and PHASE Haptoglobin Assay, Tridelta Development Ltd., respectively). Concentrations of serum IGF-1 were analyzed at Prairie Diagnostic Services (University of Saskatchewan, Saskatoon, SK, Canada) using a solid-phase, enzyme labelled chemiluminescent immunometric assay using a commercially available kit (Immulite 1000 analyzer, Siemens, Oakville, ON, Canada).

2.2.5 Statistical Analysis

Statistical analysis was conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed separately for the pre- and post-partum periods using the following models [1] and [2], respectively

$$Y_{ij} = \mu + A_i + P_j + AP_{ij} + e_{ij} \quad [1]$$

$$Y = \mu + A_i + F_k + AF_{ik} + P_j + D_l + AP_{ij} + FP_{kj} + AD_{il} + FD_{kl} + AFP_{ijk} + AFDP_{ijkl} + e_{ijk} \quad [2]$$

Where Y_{ij} = observations for dependent variables, μ = overall mean, A_i = fixed effect of pre-partum treatment (Control or High), F_k = fixed effect of post-partum treatment (HF or HS), P_j = fixed effect of parity, D_l = effect of day relative to calving as a repeated measure, APij = effect of pre-partum treatment and parity, AF_{ik} = effect of pre- and post-partum treatment

interaction, FP_{kj} = effect of post-partum treatment and parity, AD_{il} = effect of prepartum treatment and day, FD_{kl} = effect of postpartum treatment and day, AFP_{ijk} = effect of pre- and post- partum treatment and parity interaction, $AFDP_{ijkl}$ = effect of pre- and post-partum treatment, day relative to calving and parity interaction; and e_{ijkl} = error.

The repeated measure was used for variables measured over time such as DMI, metabolites and milk yield and components using the REPEATED statement in the MIXED procedure of SAS. The covariance structure with the smallest Akaike and Bayesian criteria was used. When the interaction between treatments or sampling day was significant, least square means were separated using the PDIFF procedure of SAS. Pearson correlation coefficients were determined using the MULTIVARIATE method of JMP 14 (SAS Institute Inc.). Significance was declared when $P \leq 0.05$ and tendencies discussed when $P > 0.05$ but < 0.10.

2.3 Results

Of the 100 cows enrolled in the study at 28 d prior to calving, 8 cows were removed as they calved early and were not fed the pre-partum diet for at least 14 d. Following parturition, 2 Control-HF, 1 Control-HS and 1 High-HF cows were removed due to severe health complications. Therefore, final animal numbers for the data presented are: Control-HF $n = 22$, Control-HS $n = 21$, High-HF $n = 22$, High-HS $n = 23$.

2.3.1 Pre-partum

There was no interaction between parity and treatment during the pre-partum period. During the pre-partum period, cows fed High had greater DMI compared to those fed Control ($P < 0.01$; Table 2). There was no difference in BCS change between Control or High (-0.29 vs. -0.22, respectively) from enrollment to calving. At 10 d prior to calving, feeding High increased plasma concentrations of glucose (68.1 vs. 65.0 mg/dL; $P = 0.03$) and insulin (1.72 vs. 1.42 ng/mL; $P = 0.04$), GLP-2 (0.41 vs. 0.32 ng/mL; $P < 0.001$), and IGF-1 (152 vs. 127 ng/mL; $P < 0.01$) compared with Control.

2.3.2 Post-partum

A summary of health events for cows that remained on the study for its entirety can be found in Table 3. There was no interaction between the pre- and post-partum diets on BW change or BCS change from calving to 21 DIM. However, cows fed the High pre-partum diet lost less

body condition as compared to those fed Control (-0.16 vs. -0.35; $P = 0.02$; Table 4). There was no difference in post-partum DMI among treatments (Figure 1), but a tendency ($P = 0.09$) of interaction between pre-partum and post-partum treatments was observed for milk yield (Figure 2); feeding the HS diet post-partum increased milk yield compared with the HF diet for cows fed the Control diet pre-partum (40.8 vs. 37.9 kg/d; $P < 0.05$) but not for cows fed High diet pre-partum. In addition, cows fed the High pre-partum diet had greater milk fat yield as compared to cows fed the Control pre-partum diet (1.64 vs. 1.48 kg/d; $P = 0.03$).

Plasma concentrations of glucose increased from d 3 to 20 post-partum ($P < 0.01$; Table 5), but there was a tendency for an interaction among day, pre-partum, and post-partum treatment ($P = 0.08$). There were no differences among treatments at d 3 or 20, but feeding the HS diet post-partum increased plasma glucose concentration compared to the HF diet for cows fed High diet pre-partum (61.3 vs. 58.9 mg/dL; $P < 0.05$) but decreased it for cows fed Control diet pre-partum (61.1 vs. 64.9 mg/dL; $P < 0.05$). Similarly, there was a tendency for an interaction among day, pre-partum, and post-partum treatments for plasma BHB concentration ($P = 0.08$). There were no differences in plasma BHB concentrations among treatments at d 3 (9.3 mg/dL), but cows fed the High pre-partum diet had greater plasma BHB concentration than those fed the Control pre-partum diet at d 10 (8.87 vs. 7.18 mg/dL; $P = 0.03$). A tendency of interaction between pre-partum and post-partum treatment effects ($P = 0.06$) was detected for plasma BHB concentration at d 20, showing that cows fed the High pre-partum diet and the HF post-partum diet had the highest BHB concentration than the other cows. There was no interaction between the pre- and post-partum diets on plasma free fatty acid concentrations. Concentrations of free fatty acids were reduced from d 3 to 20, however cows fed High pre-partum had greater free fatty acid concentrations as compared to Control (452 vs. 363 μEq/L; $P = 0.01$) and cows fed HS had greater free fatty acid concentrations as compared to HF (372 vs. 442 μEq/L; $P = 0.05$) during the post-partum period.

Plasma concentrations of GLP-2 increased from d 3 to 20 ($P < 0.01$), but an interaction between day and pre-partum treatment was observed ($P = 0.03$); cows fed the High pre-partum diet tended to have greater GLP-2 concentration than those fed the Control pre-partum diet on d 20 ($P = 0.07$). Serum concentrations of Hp and SAA were reduced from d 3 to 20 of lactation ($P < 0.01$). Although they were not affected by the pre-partum diet, cows fed HS post-partum had

lower serum Hp concentrations during the 3-wk period after calving (0.46 vs. 0.70 mg/mL; P = 0.01) and SAA concentration on d 3 (111 vs. 159 mg/L; P = 0.05) and d 10 (32 vs. 54 mg/L; P = 0.06) as compared to those fed HF post-partum.

2.4 Discussion

Previous studies evaluating dietary strategies for pre-partum cows evaluated diets that met or exceeded energy requirements within the same study (Dann et al., 2005, 2006; Douglas et al., 2006). However, in the current study both pre-partum diets exceeded energy requirements. Cows consumed, on average, 120 and 154% (for Control and High, respectively) of their NE_L requirements during the pre-partum period and responded to increased dietary starch content as expected. Cows fed High had greater DMI and plasma concentrations of IGF-1, insulin and glucose. It is well established that DMI and energy intake in excess of requirements during the pre-partum period increases plasma concentrations of glucose and insulin (Dann et al., 2005, 2006; Douglas et al., 2006; Janovick et al., 2011).

Despite the differences in DMI and plasma metabolites between pre-partum treatments, there was no detectible difference in BCS change from enrollment to parturition. There is discrepancy in the published literature regarding the effect of energy content in dry cow diets on BCS. Dann et al. (2006) found no difference in BCS change during the pre-partum period when cows were fed 80 or 150% of NE_L requirements, whereas Janovick and Drackley (2010) reported that cows gained BCS when fed at 150% NE_L requirement relative to those fed at 100%. In addition, Douglas et al. (2006) fed dry cows targeting an increase in BCS of 0.6 units, and obtained a 0.17 unit increase, despite cows consuming enough DMI to achieve greater BCS gain. Therefore, it has been previously hypothesized that cows may deposit more fat internally rather than subcutaneously, which is assessed during BCS measurement (Drackley et al., 2014).

Previous research showed that the gastrointestinal tract undergoes hypertrophic growth (Bauman and Currie, 1980) in response to increased feed intake during early lactation (Ingvartsen and Andersen, 2000; Reynolds et al., 2004). In addition, the transition to a highly fermentable lactating diet, coupled with increased feed intake, has been associated with an increase in VFA production, reduction in rumen pH and cows experiencing sub-acute ruminal acidosis (Penner et al., 2011; Steele et al., 2016), with the potential for hindgut acidosis (Gressley et al., 2011). These changes may result in damages to the rumen or intestinal

epithelium, thus compromising growth and development of the gastrointestinal tract. As such, pre-partum preparation to enhance the gut adaptation to a highly fermentable diet may be favorable.

Glucagon-like peptide-2 has been investigated in relation to gastrointestinal growth and development. It is a peptide released from mucosal enteroendocrine L-cells of the intestine in response to nutrient intake (Brubaker and Anini, 2003). When mature ruminants were exposed to a feed restriction challenge, infusions of GLP-2 increased intestinal villus height and mucosal surface area (Kvidera et al., 2017b). Additionally, GLP-2 has been associated with enhanced glucose and peptide transporter expression, increased intestinal weight and mucosal development (Drucker and Yusta, 2014; Connor et al., 2015a; Connor et al., 2015b) and increased intestinal epithelial and crypt cell proliferation thus increasing the absorptive capacity of the gut (Burrin et al., 2005);glucagon-like peptide-2 increased mesenteric blood flow and enhanced gut integrity through promoting barrier function and protecting intestinal mucosa from inflammation (Connor et al., 2013; Kvidera et al., 2017b). In the current study, feeding High during the pre-partum period increased concentrations of GLP-2, and to our knowledge, this is the first study to report plasma GLP-2 concentrations during the transition period. While the effect of increased GLP-2 during the pre-partum period is unknown, previous research suggests there is a benefit to the gastrointestinal tract, warranting further investigation into the effect of enhanced plasma GLP-2 on the transition dairy cow.

It is well documented that excess energy intake during the pre-partum period increases the risk of metabolic disorders post-partum. Previous research reported that controlling energy intake pre-partum can reduce the extent of insulin resistance (Drackley et al., 2001), reduce lipid mobilization (Douglas et al., 2006; Janovick et al., 2011), increase post-partum DMI (Douglas et al., 2006), and reduce incidence of ketosis (Vickers et al., 2013). In the current study, there was no difference in post-partum DMI among treatments, however cows fed the High pre-partum diet had increased concentrations of plasma free fatty acids and increased milk fat yield, indicating greater fat mobilization (Palmquist et al., 1993), which is in alignment with previous findings. However, it should be noted that cows in the current study had relatively modest concentrations of free fatty acids indicating that they did not experience great body fat mobilization. In contrast, feeding the Control pre-partum diet, regardless of post-partum diet resulted in cows losing more

BCS from parturition to 21 DIM. This reduction in BCS was not associated with markers of increased fat mobilization, therefore, the post-partum change in BCS may not necessarily indicate the extent of fat mobilization. As mentioned previously, BCS measurement may not be sensitive enough to capture changes in internal fat deposits over a short period of time as it only measures changes in subcutaneous fat (Drackley et al., 2014).

The transition period is characterized by a reduction in feed intake and shift to a highly fermentable lactating diet (Zebeli et al., 2015) leaving cows at risk of developing low rumen pH after calving (Penner et al., 2007, 2011). Abrupt increases in dietary starch content can cause excess fermentation acid production resulting in a reduction in rumen pH, with cows experiencing ruminal acidosis. There is a growing body of literature indicating that poor rumen health, a result of low rumen pH, results in systemic inflammation (Plaizier et al., 2012; Zebeli et al., 2015), and increasing the concentration of grain in the diet has been shown to increase markers of inflammation in blood (Emmanuel et al., 2008). Therefore, we had hypothesized that the transition from the Control pre-partum to HS post-partum diet would increase serum concentrations of Hp and SAA as this diet combination is associated with the biggest change in dietary starch content.

In the current study, there was no difference in DMI post-partum among treatments and cows fed the HS post-partum diet had lower concentrations of Hp and SAA as compared to those fed HF post-partum. These findings are opposite to what was expected as the HS diet contained more highly fermentable feed ingredients as soy hulls were used in the HF diet to replace corn grain. This substitution was not expected to increase fermentability of the diet as it has been previously reported in a comprehensive review by Ipharraguerre and Clark (2003) that soy hulls can be used as a replacement for corn grain without having negative effects on DMI, rumen fermentation or milk production of mid to late lactation cows when fed at moderate amounts. Furthermore, in the current study, regardless of dietary treatment, there was a negative correlation between post-partum DMI and concentrations of Hp ($r = -0.356$; $P < 0.001$; Table 6) and SAA ($r = -0.417$; $P < 0.001$). As greater intake would be associated with greater ruminal fermentation, the negative relationships between DMI and inflammation markers suggest that reduced rumen pH might not have been the primary factor contributing to the increase in Hp and SAA concentrations found in cows fed the HF post-partum diet.

Research conducted using other animal models has reported that fatty acids can modulate and impact the inflammatory response by increasing systemic inflammation (Sordillo et al., 2009) and causing immunosuppression (Contreras et al., 2018). However, the exact cause – effect relationship between lipid mobilization, free fatty acid concentrations and inflammation remains unclear. In the current study, HF cows had greater free fatty acid concentrations post-partum, which is consistent with previous literature evaluating high-fiber (or low-starch) post-partum diets (McCarthy et al., 2015). Several studies have reported increased markers of inflammation and elevated plasma free fatty acid concentrations in lactating dairy cows. Stengärde et al. (2008) reported elevated free fatty acid concentration alongside increased concentrations of Hp in post-partum cows, and it has been suggested that increased Hp may be associated with fatty liver (Yoshino et al., 1992; Katoh and Nakagawa, 1999). Similarly, Kvidera et al. (2017b) found increased free fatty acid concentrations when cows were feed-restricted and exhibited signs of inflammation. Therefore, we speculate that the increased concentrations of Hp and SAA found in cows fed the HF post-partum diet was at least partly attributed to greater fat mobilization indicated by elevated plasma free fatty acid concentrations.

However, it is important to note that inflammation is an energy demanding state (Kvidera et al., 2017a) and in the current study we cannot distinguish whether greater fat mobilization caused inflammation or *vice versa*. Recent research evaluating strategies to mitigate inflammation around parturition reported that administering an anti-inflammatory can reduce Hp concentrations (Pascottini et al., 2020), improve energy status (Pascottini et al., 2020) and increase milk production (Trevisi et al., 2005; Carpenter et al., 2016) indicating that inflammation may have negative impacts on the metabolic status of dairy cows. In addition, while we did not see a difference in clinically diagnosed health events post-partum, inflammation could have been caused from mastitis and poor reproductive tract health during the post-partum period (LeBlanc, 2012; Bradford et al., 2015). Concentrations of Hp have been previously associated with metritis (Huzzey et al., 2009), purulent vaginal discharge and endometritis (Dubuc et al., 2010). Therefore, general animal health should not be overlooked as a potential cause of increased markers of inflammation.

Given the difference in starch content between the pre- and post-partum dietary treatments, we hypothesized that DMI and milk production would be increased with the treatment combinations of High-HS or Control-HF, relative to High-HF or Control-HS,

respectively. Contrary to this hypothesis, we found no difference in DMI post-partum among treatments, and the highest milk yield when cows were fed the Control-HS dietary treatment combination compared with the other diet combinations. This is contrary to findings of Shi et al. (2019) who reported lower milk yield for cows fed high-starch diets compared to those fed low starch diets and attributed their finding to a drastic change in dietary starch content from 13.8 to 28.3% between pre-partum and post-partum, respectively.

This discrepancy might be partly attributed to the source of additional starch for the HS diet. Shi et al. (2019) increased the dietary inclusion of steam rolled barley grain by 9.2% on a DM basis while the dietary inclusion of dry ground corn was increased by 11.2% in the current study. Previous research using dry ground corn to increase the starch content of post-partum diets (Piantoni et al., 2015; McCarthy et al., 2015a) reported positive or no negative animal responses to high starch diets. Albornoz and Allen (2018) reported that fresh cows fed dry ground corn had greater DMI compared with those fed high-moisture corn which is more fermentable in the rumen. Interestingly, Dieho et al. (2016) reported that milk production was lower for fresh cows fed more barley and wheat grains. Previous research has consistently shown that dry ground corn grain ferments slower in the rumen compared to steam rolled barley grain (Overton et al., 1995). Therefore, the response of fresh cows to dietary starch content may be affected by type and processing methods of starch source.

Another possible reason for the discrepancy in responses of post-partum cows to high starch diets between the current study and the study of Shi et al. (2019) is that free-choice hay was offered to cows for the first 3 days after calving in the current study while diets were abruptly changed immediately after calving in the previous study (Shi et al., 2019). With grass hay offered alongside TMR, cows had an opportunity to change starch content of diets that they actually consumed at their own rates, and this might be a possible reason to allow for a smooth transition from the Control pre-partum diet to the HS post-partum diet. It has been previously reported that cows are able to selectively alter their diet to increase intake of long particles (Keunen et al., 2002) and physically effective fiber (Maulfair et al., 2013) to recover from bouts of reduced rumen pH. Offering free-choice hay for the first 3 d post-partum may have helped smooth transition to the HS post-partum diet in the current study.

It should be noted that pre-partum diets affected animal responses to the HS diet post-partum in the current study; the HS cows fed the High diet pre-partum had lower milk yield compared with those fed the Control pre-partum diet. We speculate that the feeding the High diet pre-partum might have induced SARA before calving, and feeding the HS diet post-partum would have exacerbated it. Shi et al. (2020), who concurrently conducted another study with ours, fed the same pre-partum diets to ruminally cannulated cows and reported that cows fed the High diet pre-partum had lower rumen pH before calving. Interestingly, the High cows continued to have lower rumen pH and tended to have higher SAA concentration on d 7 after calving even though all cows were fed a common diet after calving in their study (Shi et al., 2020). Reduced rumen pH pre-partum might have had lasting effects on rumen morphology, putting them at a greater risk for SARA following the transition to a more fermentable diet after calving. This is consistent with Dohme et al. (2008) who reported that previous episodes of SARA increased the future risk of SARA using a repeated SARA challenge model. As such, we cannot exclude a possibility that the pre-partum High diet increased energy requirements of animals for immune functions, leading to lower milk production. However, it should be noted that we failed to detect pre-partum treatment effects on inflammation markers that we measured in this study, and exact reasons for greater milk production for the Control – HS diet combination remains unknown.

In addition, it is important to note that cows in the current study were housed in a tie-stall, consuming the diet in a non-competitive environment. It has been found that when cows are fed individually, they sort against long-particles less, as compared to cows fed at a common bunk (Leonardi and Armentano, 2007). As such, cows in the current study may have consumed adequate amounts of physically effective fiber allowing for adequate rumen buffering and less apparent negative consequences of the high-starch diets on rumen pH.

2.5 Conclusion

Feeding a low-starch pre-partum diet followed by a high-starch post-partum diet increased milk production without decreasing DMI or increasing serum concentrations of Hp and SAA. Diet formulation strategies to minimize abrupt changes in diet fermentability during the calving transition by feeding the High pre-partum diet, the HF post-partum, or both did not increase productivity of dairy cows, but increased fat mobilization after calving. Our findings

suggest that even with a drastic change in dietary starch content between pre-partum and post-partum diets, there may be no negative effects of feeding high-starch diets post-partum.

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2.7 Tables and figures

Table 2.7-1 Ingredient and nutrient composition of the control and high-energy prepartum diets and the highfiber and high-starch postpartum diets[1]

	Pre-Partum		Post-Partum	
Item	Control	High	HF	HS
Ingredient, % DM				
Barley silage	56.9	56.6	39.9	39.9
Straw	23.6	4.4	-	-
Dry ground barley grain	-	22.1	14.3	14.3
Dry ground corn grain	-	-	11.8	23.0
Soy hulls	0.9	2.6	11.4	-
Canola meal	6.6	1.8	8.1	8.1
DDGS	-	-	2.9	2.9
Malt sprouts	7.0	7.0	2.4	2.4
Corn gluten meal	-	-	2.4	2.4
Soybean meal	-	-	1.9	1.9
Mill run	1.8	1.7	-	-
Other[2]	3.2	3.8	4.9	5.1
Nutrient content				
% DM	42.8	42.1	64.8	66.1
CP, % DM	15.0	14.6	17.9	17.8
ADF, % DM	31.5	23.3	22.0	16.4
NDF, % DM	47.7	37.8	33.8	27.2
Starch, % DM	14.0	26.1	25.1	32.8
NFC, % DM	28.1	39.4	40.4	47.1
EE, %DM	3.23	3.33	3.78	3.88
NE_L allowable milk, kg/d[3]	-	-	32.7	33.4
MP allowable milk, kg/d[3]			34.6	35.1

[1]Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date. Following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 20 ± 2 d following calving.

[2] Prepartum contained 10.9 KIU/kg vitamin A, 1.7 KIU/kg vitamin D, 4.3 KIU/kg vitamin E, 0.52 mg/kg Co, 5.51 mg/kg Cu, 22.6 mg/kg Mn, 29.3 mg/kg Zn, and 0.20 mg/kg Se. Postpartum

contained 13.0 KIU/kg vitamin A, 1.2 KIU/kg vitamin D, 4.7 KIU/kg vitamin E, 0.54 mg/kg Co, 36.0 mg/kg Cu, 62.9 mg/kg Mn, 42.3 mg/kg Zn, and 0.08 mg/kg Se.

[3] Estimated using NRC (2001).

Table 2.7-2 Effects of feeding a control or high-energy prepartum diet[1] on body condition change from enrollment to parturition, DMI at wk −3 to −1 relative to parturition, and plasma metabolites at d 10 ± 3 before parturition

Item	Control	High	SEM	*P*- value[2]
BCS Change from d - 28 to calving	-0.29	-0.22	0.05	0.36
DMI, kg/d[3]				
Wk -3	10.5	13.0	0.43	< 0.001
Wk -2	10.5	12.4	0.41	< 0.01
Wk -1	9.7	11.8	0.38	< 0.001
Plasma at d -10 ± 3				
Glucagon-like peptide-2, ng/mL	0.32	0.41	0.02	< 0.001
IGF-1, ng/mL	127	152	6.60	< 0.01
Insulin, ng/mL	1.47	1.72	0.08	0.04
Glucose, mg/mL	65.0	68.1	0.95	0.03

[1] Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date.

[2] P-value for the effect of prepartum treatment.

[3] P-value for Wk < 0.01, Wk × Trt = 0.19.

Table 2.7-3 Health events (no.) incurred when cows were fed a control or high-energy prepartum diet and a high-fiber or high-starch postpartum diet[1]

	Control		High	
Health Condition	HF	HS	HF	HS
Milk fever	1	1	0	3
Ketosis	0	0	0	2
Retained placenta	2	1	3	2
Edema	1	1	3	1

[1]Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date. Following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum for the first 20 ± 2 d following calving.

Table 2.7-4 Effects of feeding a control or high-energy prepartum diet and a high-fiber or high-starch postpartum diet on postpartum BW change, BCS change, DMI, milk yield, and milk components[1]

	Control		High		SEM	*P* – value[2]		
Item	HF	HS	HF	HS		Pre	Post	Pre × Post
BW change, kg/d	-1.8	-2.2	-2.0	-1.9	0.44	0.92	0.70	0.47
BCS change, points	-0.35	-0.34	-0.14	-0.17	0.07	0.02	0.87	0.75
DMI, kg/d	17.7	17.6	18.3	17.2	0.65	0.78	0.59	0.38
Milk yield, kg/d	37.9^{b}	40.8^{a}	39.8^{b}	38.6^{b}	1.16	0.90	0.48	0.09
Fat yield, kg/d	1.51	1.46	1.63	1.64	0.06	0.03	0.78	0.61
Protein yield, kg/d	1.23^{b}	1.27^{a}	1.32^{a}	1.22^{b}	0.04	0.54	0.48	0.09
Lactose yield, kg/d	1.72^{b}	1.88^{a}	1.89^{a}	1.73^{b}	0.06	0.87	0.99	< 0.01
Fat, %	4.03^{b}	3.66^{c}	4.02^{b}	4.40^{a}	0.14	0.01	0.93	< 0.01
Protein, %	3.29	3.15	3.26	3.25	0.04	0.42	0.10	0.12
Lactose, %	4.59^{b}	4.63^{a}	4.64^{a}	4.55^{b}	0.03	0.78	0.39	0.04
Milk urea nitrogen, mg/dL	16.4	15.2	16.1	15.3	5.32	0.97	0.29	0.82
Total solids, %	13.0^{a}	12.5^{b}	13.0^{a}	13.3^{a}	0.21	< 0.01	0.58	< 0.01

[a–c] Means in the same row with different superscripts differ significantly (P < 0.05).

[1] Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date. Following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 20 ± 2 d following calving.

[2] Pre = prepartum treatment; Post = postpartum treatment.

Table 2.7-5 Effects of feeding a control or high-energy prepartum diet and a high-fiber or high-starch postpartum diet on plasma energy metabolites and serum inflammatory markers[1]

	Control		High		SEM			*P*- value[2]			
Item	HF	HS	HF	HS		Pre	Post	Pre × Post	Day	Day × Pre	Day × Post	Day × Pre × Post
Glucose, mg/dL						0.35	0.49	0.69	< 0.01	0.34	0.38	0.08
d3	57.1	60.8	59.1	59.2	1.74	0.88	0.22	0.53				
d10	64.9[a]	61.1[b]	58.9[c]	61.3[b]	1.49	0.08	0.64	0.03				
d20	62.3	63.7	60.9	62.8	1.91	0.57	0.38	0.90				
BHB, mg/dL						0.44	0.15	0.46	0.03	0.21	0.64	0.08
d3	10.5	8.80	8.80	9.24	1.22	0.81	0.35	0.61				
d10	7.12	7.23	9.47	8.26	0.77	0.03	0.49	0.39				
d20	8.24[b]	8.34[b]	10.2[a]	6.98[c]	0.86	0.74	0.08	0.06				
Free fatty acids, µEq/L						0.01	0.05	0.78	< 0.01	0.76	0.76	0.66
d3	472	411	571	449	25.4							
d10	389	336	494	399	24.6							
d20	318	251	410	387	25.1							
GLP-2, ng/mL						0.66	0.56	0.13	< 0.01	0.03	0.62	0.65
d3	0.43	0.39	0.40	0.39	0.03	0.50	0.32	0.39				
d10	0.51[a]	0.42[c]	0.44[c]	0.47[b]	0.04	0.73	0.32	0.08				
d20	0.48	0.44	0.51	0.55	0.04	0.07	0.91	0.23				
Haptoglobin, mg/mL						0.38	0.01	0.28	< 0.01	0.67	0.26	0.86
d3	1.21	0.68	1.25	0.98	0.07							
d10	0.57	0.21	0.47	0.38	0.07							
d20	0.36	0.22	0.36	0.30	0.07							
Serum amyloid A, mg/L						0.59	0.01	0.52	< 0.01	0.31	0.20	0.10
d3	183	101	135	121	23.4	0.57	0.05	0.16				
d10	45.3	24.2	61.7	39.9	13.2	0.15	0.06	0.98				
d20	25.3	31.3	53.7	32.5	11.3	0.26	0.56	0.30				

a–c Means in the same row with different superscripts differ significantly (P < 0.05).

[1]Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date. Following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 20 ± 2 d following calving.

[2] Pre = prepartum treatment; Post = postpartum treatment

Table 2.7-6 Correlation coefficient between DMI, milk yield, free fatty acids, haptoglobin (Hp) and serum amyloid A (SAA) for cows fed Control or High pre-partum, and HF or HS post-partum diets[1]

Item[2]	DMI	Milk yield	Free fatty acids	Hp
DMI				
Milk yield	0.603*			
NEFA	-0.076	0.140*		
Hp	-0.356*	-0.367*	0.156*	
SAA	-0.417*	-0.429*	0.124	0.653*

[1] Cows were fed either a control (Control; 14.0% starch, DM basis) or high-starch (High; 26.1% starch, DM basis) prepartum diet commencing 28 ± 3 d before expected calving date. Following calving, cows were fed either a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 20 ± 2 d following calving.

[2] NEFA = nonesterified fatty acids; Hp = haptoglobin; SAA = serum amyloid A.

*$P < 0.05$.

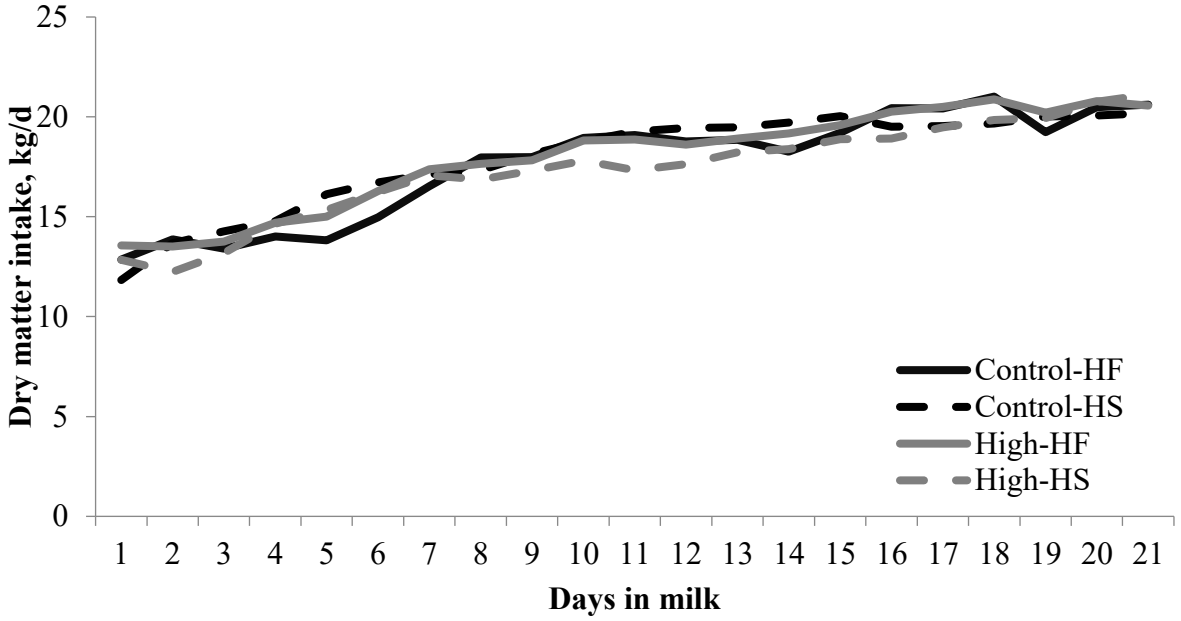

Figure 2.7-1 Postpartum DMI of cows fed a control (Control; 14.0% starch, DM basis) or high-energy (High; 26.1% starch, DM basis) prepartum diet commencing 28 d prior to parturition and a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 21 DIM.

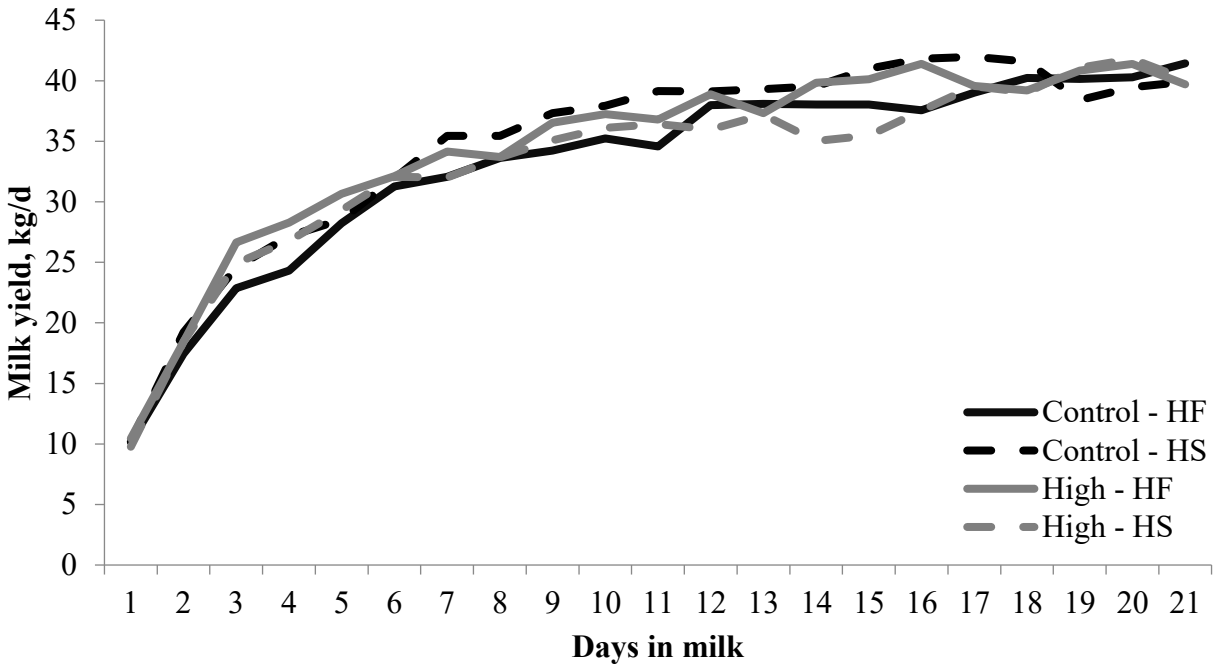

Figure 2.7-2 Milk yield of cows fed a control (Control; 14.0% starch, DM basis) or high-energy (High; 26.1% starch, DM basis) prepartum diet commencing 28 d prior to parturition and a high-fiber (HF; 33.8% NDF, 25.1% starch, DM basis) or high-starch (HS; 27.2% NDF, 32.8% starch, DM basis) postpartum diet for the first 21 DIM.

Chapter 3: The effects of feeding a high-fiber or high-starch pellet at two daily allocations on feed intake patterns, rumen fermentation and milk production of mid-lactation dairy cows

3.1 Introduction

Dairy cattle in North America are typically fed TMR where all feed ingredients are combined to deliver the same nutrients with every mouthful of feed. However, as automated milking systems (AMS) grow in popularity, there is a move back to component feeding, where a portion of the diet is fed as a concentrate in the AMS, and the remainder at the bunk as a partial mixed ration (PMR). With this feeding management, it is common for the concentrate in the AMS to be delivered as a high-starch pellet to encourage voluntary visits to the AMS (Prescott et al., 1998, Bach and Cabrera, 2017).

A concern with offering a high-starch pellet is that its consumption in a short period of time may reduce rumen pH, alter feed intake patterns, and decrease overall DMI. The effect of pellet feeding on PMR intake pattern is of interest for cows managed with AMS as milk production is affected by total DMI. While there is a growing body of research indicating that it is not necessary to feed high amounts of pellet in the AMS to encourage voluntary visits (Hare et al., 2018, Henricksen et al., 2018b; Paddick et al., 2019), little research has evaluated the effects of nutrient composition of pellet and nutrient allocation between the pellet and PMR on feeding behavior and rumen fermentation. Previous research has shown that feeding a high-starch pellet to dairy cows decreases DMI, number of meals and eating duration of cows (Miron et al., 2004a,b) when fed through automatic feed stations, but does not affect milk production or number of milkings per day when implemented with AMS (Halchemi et al., 2006) as compared to a high-fiber pellet. Based on these findings, it would be acceptable to feed a high-fiber pellet in order to maximize DMI while maintaining milk production and number of milkings. However, in these studies, cows were fed the same PMR regardless of pellet type thus cows fed a high-starch pellet would have greater starch intake in their overall diet, in which effects of pellet type on rumen fermentation are confounded by effects of overall diet. Therefore, it is not known what effects a high-starch pellet would have when fed alongside a complementary PMR formulated to create a common overall diet and whether animal response to pellet type may be affected by the amount of pellet fed.

The objective of this experiment was to evaluate the effects of feeding a high-fiber or high-starch pellet at two feeding amounts on rumen fermentation, feeding behaviors, DMI, and milk production when fed alongside a complementary PMR. We hypothesized that feeding a high-starch pellet would decrease rumen pH compared to high-fiber pellet, which would reduce PMR intake following pellet consumption thus reducing overall DMI, and that these effects would be more pronounced when greater amounts of pellet were fed.

3.2 Materials and methods

All procedures were preapproved by the Animal Care and Use Committee for Livestock at the University of Alberta (AUP #2170) and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada).

3.2.1 Experimental design, diet and treatments

All cows were fed a diet as a TMR, ad libitum, once daily at 1200 h, allowing for 5% refusals during a 21-d diet adaptation period. The diet was formulated to provide adequate ME and MP for a 650-kg cow producing 40 kg of milk per day with a DMI of 25 kg/d (NRC, 2001). Following this diet adaptation, a portion of the diet was removed and fed as a high-fiber (**F**; 33.2% NDF, 13.5 % starch on a DM basis) or a high-starch (**S**; 8.3% NDF, 56.8% starch on a DM basis) pellet, fed separately from the rest of the diet at a high (**H**; 3 kg as fed basis) or low (**L**; 1 kg as fed basis) amount twice per day, resulting in four experimental treatments of FH, FL, SH and SL. Four complementary partial mixed rations (PMR) were formulated such that the total diet provided to the cows (pellet + PMR) was the same among all treatments with a target DMI of 25 kg/d. The concentrate portion of each complimentary PMR was fed as a mash, with all ingredients ground. All ingredients in the pellets were ground before pelleting via steam conditioning at 170°F and using a pellet mill (CPM Model 3020; California Pellet Mill, Western Process Equipment, Calgary AB, Canada) with a 9/64 in diameter. Pellets were stored in 20 kg bags and not exposed to augers, staying intact through handling. Each pellet and mash were produced from one batch at the beginning of the study to maintain consistency in particle size and formulation for the entirety of the study.

Pellets were fed twice per day to allow for a large pellet meal to induce more pulsatile rumen fermentation. A conceptual description of the dietary treatments is shown in Table 1, and ingredient and nutrient composition of each dietary treatment are shown in Table 2.

Eight multi-parous Holstein cows fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID) were used in a 4 × 4 Latin square design with 14-d periods consisting of 10 d of experimental treatment adaptation and 4 d of sample collection. Pre-experiment DIM (mean ± SD) was 115 ± 21 d. Cows were housed individually in tie-stalls and milked twice daily at 0500 and 1700 h. Pellet treatments were fed at 0600 and 1800 h and PMR was offered at 1200 h within 2 min after previous day's orts were removed. Cows were fed PMR in individual feed mangers, and their treatment pellets in 8 quart duraflex rubber feed pans (Miller Manufacturing, Eagan, MN) separate from their feed mangers to avoid contamination of the PMR and given 10 min to consume the pellet.

3.2.2 Data and sample collection

The amount of pellet and PMR offered and refused was recorded for individual cows at the time of feeding, and the amount of PMR fed was adjusted daily to maintain 5% refusals. Dietary ingredients were sampled on d 11 to 13 and composited for each period to determine chemical composition of the diet. All ingredient samples were dried for 72 h at 55 °C in a forced air oven and stored until further analysis. One-eighth of refused PMR was kept on d 11 to 13 and composited to yield one sample per cow per period to determine extent of sorting. Disappearance of PMR relative to its delivery was determined by weighing the amount of feed left every 3 h in individual feed mangers; cows were prevented from eating for 1 min while feed mangers were weighed, in front of the stall with feed in them to minimize disruption of feeding behavior.

Milk yield was recorded at every milking and milk samples (approximately 40 mL) were taken from 6 consecutive milkings from d 11 to 13 and stored at 4 °C with 2-bromo-2-nitropropane-1,3-diol until milk composition analysis. Rumen pH was determined every 30 s from d 11 to 13 using the Lethbridge Research Centre Ruminal pH Measurement System (Dascor, Escondido, CA, USA) and millivolt readings were converted to pH units as described by Penner et al. (2006). Duration of pH depression was calculated as the total number of minutes that rumen pH was below 5.8. Severity of pH depression was calculated as the area under the

curve when pH was below 5.8, and acidosis index calculated as the severity of pH depression normalized for DMI (Gao and Oba, 2014).

On d 14, cows were fitted with jugular catheters (0.86 mm I.D. × 1.32 mm O.D; Scientific Commodities Inc., Lake Havasu City, AZ) and blood samples were collected every 90 min for a 24 h period, to account for diurnal variation in blood metabolite concentrations, beginning at PMR delivery. A 5 mL waste sample was removed before sample collection and catheters were flushed with 5mL of heparinized saline (2% solution) following collection. Blood samples were collected using 12-mL syringes and then placed into a sodium heparin vacutainer (BD Vacutainer, Frankin Lakes, NJ). Following collection, samples were immediately placed on ice before centrifugation at 3,000 × *g* for 20 min at 4 ºC; harvested plasma samples were stored at -20 ºC until analysis.

3.2.3 Sample analysis

Dried feed samples were ground through a 1-mm screen with a Wiley mill (Thomas-Wiley, Philadelphia, PA) and sent to Cumberland Valley Analytical Services (Hagerstown, MD) for analysis of DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), NDF (Van Soest et al., 1991), starch (Hall, 2009) and CP (AOAC International, 2000; method 990.03). Milk samples were individually analyzed for concentrations of milk fat, CP, lactose and MUN (AOAC International, 2002; method 972.16; MilkoScan 605, Foss North America, Brampton, ON, Canada) at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada). Particle size distribution of the PMR and orts were determined using a Penn State Particle Separator with 2 sieves (aperture size of 19 and 8 mm) and the bottom pan using the procedure described by Lammers et al., (1996). Sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator, on an as fed basis (Leonardi and Armentano, 2003). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, selective consumption and selective refusals of each particle portion of PMR, respectively.

Plasma samples were analyzed for concentrations of glucose and insulin. Plasma glucose concentration was determined using a glucose oxidase/peroxidase enzyme (No. P7119; Sigma Co., St. Louis, MO) and dianisidine dihydrochloride (No. F5803; Sigma Co.). Absorbance at 450 nm was determined using a SpectraMax 190 plate reader (Molecular Devices Corp., Sunnyvale,

CA). Plasma insulin concentration was determined using a solid-phase competition immunoassay with Eu-labeled bovine insulin and polystyrene microtiter strips coated with anti-guinea pig γ-globin (Takahashi et al., 2006; Inabu et al., 2017). Strips were read using time resolved fluorometry (VictorX multi-plate reader, PerkinElmer, Inc., Waltham, MA)

3.2.4 Statistical analysis

Statistical analysis was completed using the FIT model of JMP (version 14; SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ijkl} = \mu + C_i + A_j + CA_{ij} + P_k + H_l + e_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, C_i is the effects of type of concentrate pellet, A_j is the amount of pellet fed, CA_{ij} is the effect of interaction among type and amount of pellet, P_k is the effect of period, H is the random effect of cow and e_{ijkl} is the residual. Significance was declared when $P < 0.05$ and tendencies were discussed when $P < 0.10$.

3.3 Results

During sampling, all cows were able to consume the pellet within 6 min of delivery. By design, cows fed H had greater pellet DMI than L (5.31 vs. 1.81 kg/d; $P < 0.001$; Table 3). Partial mixed ration DMI was reduced for cows fed H amount of pellet, regardless of the type fed (22.9 vs. 25.3 kg/d; $P < 0.001$), as compared to L. Cows fed the H amount of pellet tended to increase total DMI (28.2 vs. 27.1 kg/d; $P = 0.08$) compared to L. Feed disappearance recorded as the percentage of PMR intake consumed was higher for cows fed the F pellet in the 3h after feed delivery (33.5 vs. 28.6% PMR intake; $P = 0.04$; Figure 1) compared to the S pellet. There was a tendency for an interaction between pellet type and amount in sorting long particles (> 19 mm) of PMR (Table 3), but sorting indexes were not different from 100, indicating that animals did not sort PMR in the current study.

There was no difference in minimum, mean or maximum pH among treatments (Table 4). Feeding the F pellet tended to increase the duration that pH was below 5.8 (196 vs. 126 min/d; $P = 0.08$) and the acidosis index (1.68 vs. 0.78 pH × min/DMI; $P = 0.09$) compared to the S pellet. Feeding the L amount of pellet tended to increase duration that pH was below 5.8 (197 vs. 124 min/d; $P = 0.06$) and severity of rumen pH depression (44.8 vs. 24.2 pH × min/d; $P = 0.06$)

compared to H. Rumen pH over a 24-h period is shown in Figure 2. There was no difference in milk yield (42.6 kg) or components among treatments (Table 5).

Feeding the F pellet increased plasma concentrations of glucose (70.0 vs. 66.0 mg/dL; $P < 0.01$; Table 6) and insulin (2.25 vs. 1.9 ng/mL; $P = 0.02$) compared to S, regardless of amount.

3.4 Discussion

Controlled research studies evaluating feeding strategies for AMS have found that large concentrate allowances in the AMS do not increase voluntary visits or milk production (Bach et al., 2007; Hare et al., 2018; Henricksen et al., 2018a; Paddick et al., 2019). However, survey data from industry indicate that programmed amounts of concentrate fed through the AMS range from 0.9 to 11.3 kg per cow per day (Salfer and Endres, 2014), with 22% of Canadian farms offering more than 5.0 kg per cow per day (de Jong et al., 2003). A possible concern is that cows provided a large meal of a high-starch concentrate during milking in the AMS may return to the stalls and not be motivated to move to the feed bunk as frequently. If this happens, total DMI as well as milk production may decrease. While previous research has shown that it is possible to use a high-fiber pellet to attract cows to an AMS (Halchemi et al., 2006), little is known about the effects of feeding a high-fiber pellet on rumen fermentation and feed intake patterns. Therefore, the objective of this experiment was to evaluate the effects of feeding a high-fiber or high-starch pellet at a high or low amount on rumen fermentation and feed intake pattern when fed alongside a complementary PMR, in a simulated AMS setting.

The current study was conducted in a tie-stall facility with pellet fed separately from the PMR twice per day. By conducting the experiment in a tie-stall facility, we were able to determine the specific effects of pellet type and feeding amount on rumen fermentation and feed intake pattern in an environment where cows can stand, lie or eat whenever they want, thus minimizing confounding factors such as stall design, flooring, stocking density or competition for feed that may be seen in a free-stall facility. While we acknowledge that the current study has limitations as cows are not exposed to the aforementioned variables and group dynamics, and not milked with an AMS, we think that the principles of our findings are applicable to nutritional management for cows managed with an AMS.

3.4.1 High-starch vs. high-fiber pellet

Previous studies evaluating effects of component feeding have shown that as concentrate intake increases, PMR intake is reduced (Bach et al., 2007; Hare et al., 2018; Henriksen et al., 2018a,b; Menajovsky et al., 2018) which is in alignment with our findings. In these studies, the extent to which PMR intake is reduced with an additional unit increase in concentrate allowance is inconsistent, therefore total DMI may or may not be reduced. The reduction in forage or PMR DMI per unit of additional concentrate intake is called the substitution rate (Faverdin et al., 1991). In the current study, the calculated substitution rate was 0.88 and 0.50 for the F and S pellet, respectively, meaning that for every 1-kg increase in F pellet DMI, PMR DMI was reduced by 0.88 kg, while when the S pellet was fed a 1-kg increase in S pellet DMI reduced PMR DMI by 0.50 kg.

The exact mechanism behind how cows substitute intake among concentrate and forage or PMR is unknown (Jensen et al., 2016). Older literature evaluating the substitution rate when dairy cows were fed concentrate separately from forage found that feeding a high-starch concentrate had a higher substitution rate compared to a high-fiber concentrate (Faverdin et al., 1991), which contrasts with the results observed in the current study. However, it is important to note that Faverdin et al. (1991) did not feed concentrate alongside a PMR but rather concentrate separate to silage only, and the amount of concentrate accounted for up to 50% of total DMI. In the current study and other recent studies evaluating substitution rate (Menajovsky et al., 2018; Paddick et al., 2019), the amount of concentrate accounted for less of the total diet and concentrate was fed alongside a PMR containing both forage and concentrate.

In the current study, cows were fed complementary PMR as an effort to make the overall diet similar in nutrient composition among all treatments, which may account for some discrepancies as there were no negative effects of feeding the S pellet on rumen fermentation or feed disappearance following pellet feeding. It has recently been hypothesized that the substitution rate of PMR for concentrate is dependent upon the energy content of the PMR rather than the concentrate itself, with greater substitution when a high energy PMR is fed (Hendriksen et al., 2018b). In the current study, when the F pellet was fed, the complementary PMR was high in starch and when the S pellet fed, the complementary PMR was high-fiber, thus our findings

regarding substitution rate are in alignment with the proposed hypothesis that the energy content of the PMR may influence substitution rate rather than the type of pellet fed.

Prior to this study, we had hypothesized that feeding S pellet would reduce rumen pH following consumption thus supressing feed intake in the period after pellet feeding. Contrary to this hypothesis, feeding S pellet tended to reduce the duration that pH was below 5.8, and severity of pH depression. We observed no difference in feed intake pattern relative to pellet delivery. It is well established that feeding a high-starch TMR increases the risk of ruminal acidosis, and the animal responses observed with the F pellet (fed with high-starch PMR) are consistent to those fed a high-starch TMR. In the first 3-h following PMR delivery, the amount of starch consumed through PMR for FH and FL was 2.1 and 1.8 kg while SH and SL consumed 0.96 and 1.4 kg starch, respectively. The dose of starch from the PMR fed alongside the S pellet is less than the 1.5 kg dose obtained with the SH treatment. Therefore, feeding starch through a controlled dose may act to regulate rumen pH when fed alongside a high fibre PMR.

Cows consumed 28 to 35% of their total PMR intake within the first 3 h after PMR delivery, which is in alignment with previous research. Feed delivery has been shown to be the primary driver of feed intake when feeding a TMR (DeVries et al., 2005; DeVries and von Keyserlingk, 2005), and previous research comparing feeding a TMR versus a PMR plus pellet at 9.5% of DMI found that feeding the pellet 1 h before feed delivery did not influence intake patterns of dairy cows (Niu and Harvatine, 2017).

However, feed intake in the 3 h after PMR delivery was reduced when the S pellet was fed as compared to the F pellet. We speculate that the greater fiber content of the PMR fed with the S pellet increased reticulo-rumen fill thus reducing intake during the first 3 h after feed delivery. While differences were not observed in feed disappearance at each of the other 3-h periods of the day, PMR consumption during the remaining period of the day must have increased for cows fed S compared to F pellet as PMR intake was not affected by pellet type. Extrapolating these findings to an AMS setting, there may be an advantage to feeding a S pellet alongside a high-fiber PMR as there were no negative effects on rumen fermentation and there may be the possibility for an increase in cow movement in the barn as cows may be motivated to consume PMR more evenly throughout the day, and this should be investigated further.

In addition, glucose and insulin concentrations were increased when the F pellet was fed. The high-starch PMR, fed alongside the F pellet, is expected to increase rumen starch fermentation which would increase plasma concentrations of glucose and insulin following consumption (Reynolds, 2006). We speculate that the effects observed in the current study are better attributed to nutrient composition of PMR rather than pellet type as when a S pellet was fed, the PMR was high-fiber, and when a F pellet was fed, a high-starch PMR was fed. It should be noted that the overall diet was relatively low in starch content due to the experimental design, where we needed to use high-fiber feedstuffs for F pellet, as well as feeding low starch barley silage. Therefore, the results observed may be different when an overall diet is higher in starch content.

3.4.2 Low vs. high amount

We hypothesized that feeding H amounts of pellet would reduce rumen pH, modifying feed intake patterns and reducing DMI. In addition, we expected that cows fed H would have lower total DMI as the PMR fed was higher in forage NDF and more filling than the PMR fed to L cows. However, feeding H did not reduce rumen pH or modify intake patterns, but tended to decrease duration and severity rumen pH depression. In addition, cows consumed more PMR than expected with a substitution rate of less than 1.0 regardless of pellet type, and overall DMI tended to be higher for cows fed H.

There are inconsistencies in the literature when cows are fed a high or low amount of concentrate through an AMS and its effect on total DMI. Bach et al. (2007) and Hare et al. (2018) both reported a reduction in PMR intake when a high amount of concentrate was offered to cows housed with AMS. In both studies, PMR intake was reduced and the substitution ratio was greater than 1, resulting in the reduction in total DMI. In both studies, feeding a high amount of concentrate reduced meal size, and Hare et al. (2018) reported a reduction in eating time with high concentrate allowance. While not measured, Hare et al. (2018) speculated that feeding a high amount of concentrate and the modification in feed eating behavior reduced rumen pH thus causing a reduction in DMI. However, Paddick et al. (2019) conducted a similar study and found that increasing AMS concentrate from 0.5 to 5.0 kg/d did not affect DMI and that the substitution ratio was 0.94 as concentrate allocation increased. In this study, the PMR was adjusted such that target nutrient intake was same when the pellet and PMR were considered. While there is no

clear indication as to factors affecting DMI when an increased amount of concentrate is fed, it is likely that there are animal characteristics that influence animal responses such as DIM, availability of feed and animal group dynamics. For example, in the studies conducted by Hare et al. (2018) and Bach et al (2007), cows were in late lactation whereas Paddick et al. (2019) used mid lactation cows which may explain the difference in substitution rate and DMI among the studies.

In the current study, we were able to control factors such as feed availability and group dynamics by using a tie-stall facility. In the current study, feed intake pattern was not affected relative to pellet feeding times or the amount of pellet fed. Feeding an H amount of pellet tended to reduce the duration and severity of ruminal pH depression, likely due to the increased amount of forage NDF in the PMR fed alongside the H treatment. Therefore, it is likely that total DMI was not limited by rumen fill but rather by rumen pH in the current study as feeding the low forage PMR associated with an L amount of pellet tended to increase duration and severity of ruminal pH depression, resulting in a tendency for decreased DMI.

In the current study, despite the greater forage content of PMR with the L treatment, the sorting indexes were not different from 100, indicating that cows did not exhibit sorting behavior. The PMR fed had 9.3 and 7.7% of the diet retained on the top screen for the H and L treatment, respectively, so it is likely that cows were unable to sort the PMR due to uniformity of each PMR and lack of long particles. It should be noted that there are no clear recommendations for particle size distribution of a PMR. While one can deduce that if concentrate is removed from the PMR, there should be a shift in proportions found on each screen, with an increase in longer particles, it is unclear as to the extent this shift should be and the implications it may have. Thus, guidelines for PMR are challenging as a PMR is often formulated to be high in NDF and provide moderate energy and nutrient requirements, without encouraging sorting due to an increase in long particles.

Even though there were detectible treatment effects on rumen pH, blood metabolites, and total DMI, there was no difference in milk yield or components among treatments. The differences might not be large enough to elicit a milk production or component response with the Latin square design used. In addition, caution should be used when evaluating the milk

production data due to the low number of experimental units and insufficient statistical power to detect the differences.

3.5 Conclusion

Feeding a high-starch pellet alongside a complementary high-fiber PMR reduced the duration that rumen pH was below 5.8 and did not affect feed intake patterns or total DMI of mid-lactation dairy cows. In addition, feeding a high amount of pellet, regardless of type, tended to decrease the duration and severity of rumen pH depression, and increase DMI. Results from many of the measured variables indicate that the composition of the PMR may have a greater influence on feeding behaviors and rumen fermentation than the type of pellet fed. However, animal responses may be different if a common PMR were fed and the overall diet were different between pellet types. Nonetheless, based on this experimental design and findings, it would be suitable to feed a high-starch pellet at 6 kg/d, as fed, to mid-lactation dairy cows, and emphasis should be placed on the PMR when formulating diets for lactating dairy cows fed a pellet alongside PMR.

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3.7 Tables and figures

Table 3.7-1 Experimental diets (% diet DM) containing a high-fiber (F) or high-starch pellet (S) fed separate to a partial mixed ration (PMR) at a high (H; 3 kg, as fed basis) or low (L; 1 kg, as fed basis) amount twice per day assuming DMI of 25 kg/d[1].

	Treatment			
Diet component, % diet DM	FH	FL	SH	SL
Pellet				
High-fiber pellet fed outside PMR	21.2	7.2	-	-
High-fiber pellet fed within PMR	-	14.0	-	-
High-starch pellet fed outside PMR	-	-	21.2	7.2
High-starch pellet fed within PMR	-	-	-	14.0
Basal PMR				
Basal PMR for high-fiber pellet treatment	78.8	78.8	-	-
Basal PMR for high-starch pellet treatment	-	-	78.8	78.8

[1] Actual diet component consumed by animals differed for those consuming more or less than 25 kg/d DMI because pellets fed outside PMR were limit-fed, whereas basal PMR and pellets in PMR were fed ad libitum.

Table 3.7-2 Ingredient and nutrient composition of the total diet, fiber (F) and starch (S) pellet, and their complementary partial mixed rations (PMR) when a high (3kg, as fed basis; H) or low (1 kg, as fed basis; L) amount of pellet was fed twice per day.

Item	Total Diet	Pellet F	Pellet S	PMR FH	PMR FL	PMR SH	PMR SL
Ingredient, % DM							
Barley silage	38.7			49.5	41.8	49.4	41.8
Beet pulp	10.8	51.9			7.7	13.7	11.7
Alfalfa meal	7.4	35.5			5.3	9.4	8.0
Barley grain	12.5		61.1	16.1	13.6		8.9
Corn grain	5.4		26.2	6.9	5.8		3.9
Wheat grain	1.8	8.8	8.9		1.3		1.3
Molasses	1.5	2.9	2.9		1.1		1.0
Canola oil	0.8	1.0	1.0	0.5	0.4	0.5	0.4
Canola meal	6.1			7.8	6.6	7.8	6.6
Soybean meal	6.1			7.8	6.6	7.8	6.6
Wheat:corn DDGS[1]	6.1			7.8	6.6	7.8	6.6
Vitamins & mineral mix[2]	2.8			3.6	3.0	3.6	3.0
Analyzed nutrient composition							
% DM	48.8	91.2	89.7	55.8	59.6	47.8	51.9
CP, % DM	17.3	13.7	11.7	17.3	16.4	17.9	17.0
ADF, % DM	20.2	22.4	4.9	20.6	21.1	24.3	21.7
NDF, % DM	32.3	33.2	13.5	32.8	33.3	36.9	33.8
Forage NDF, % DM	21.1			24.3	19.9	24.2	19.9
Starch, % DM	20.4	8.3	56.8	22.5	20.4	11.5	17.8
NFC, % DM	42.3	45.7	71.9	41.8	42.1	35.8	40.6
Particle distribution of PMR, %							
Top > 19 mm				10.3	7.8	8.3	9.2
Middle 8 – 19 mm				35.3	33.4	33.4	31.2
Bottom < 8 mm				54.4	58.8	58.3	59.6

[1] DDGS = distillers dried grains with solubles.

[2] Contained: 13.1 KIU/kg of vitamin A, 1.4 KIU/kg of vitamin D, 40.8 IU/kg of vitamin E, 0.91 mg/kg of Co, 34.0 mg/kg of Cu, 79.4 mg/kg of Mn, 76.9 mg/kg of Zn and 1.03 mg/kg of Se.

Table 3.7-3 The effects of feeding a high-fiber (F) or high-starch (S) pellet at a high (3 kg, as fed basis; H) or low (1 kg, as fed basis; L) quantity twice per day on DMI and sorting behaviors of mid-lactation dairy cows.

	Treatment					*P* – value[1]		
Item	FH	FL	SH	SL	SEM	CHO	AMT	CHO × AMT
Pellet DM intake, kg/d	5.2	1.8	5.4	1.8	0.12	0.62	< 0.001	0.46
Partial mixed ration DM intake, kg/d	22.2	25.2	23.7	25.5	0.85	0.12	< 0.001	0.30
Total dry matter intake, kg/d	27.4	27.0	29.1	27.3	0.89	0.11	0.08	0.25
Sorting index								
Top > 19 mm	103.9	97.4	99.1	104.9	3.3	0.68	0.91	0.08
Middle 8 – 19 mm	95.6	98.8	99.0	97.4	1.6	0.53	0.63	0.15
Bottom < 8 mm	101.8	100.5	100.8	99.8	1.0	0.41	0.26	0.89

[1]CHO = type of carbohydrate (high-fiber or high-starch), AMT = amount of pellet fed (high or low).

Table 3.7-4 The effects of feeding a high-fiber (F) or high-starch (S) pellet at a high (3 kg, as fed basis; H) or low (1 kg, as fed basis; L) quantity twice per day on rumen pH of mid-lactation dairy cows.

	Treatment					*P* – value[1]		
Item	FH	FL	SH	SL	SEM	CHO	AMT	CHO × AMT
Ruminal pH								
Minimum	5.35	5.28	5.44	5.33	0.075	0.26	0.12	0.75
Mean	6.18	6.15	6.22	6.18	0.05	0.39	0.33	0.85
Maximum	6.88	7.06	7.06	7.22	0.11	0.19	0.19	0.93
Duration pH <5.8, min/d	147	245	101	150	60.5	0.08	0.06	0.49
Area pH <5.8, (pH × min/d)	29	57	19	33	13.7	0.12	0.06	0.49
Acidosis index (pH × min/DMI)	1.21	2.14	0.64	0.92	0.521	0.09	0.25	0.53

[1]CHO = type of carbohydrate (high-fiber or high-starch), AMT = amount of pellet fed (high or low)

Table 3.7-5 The effects of feeding a high-fiber (F) or high-starch (S) pellet at a high (3 kg, as fed basis; H) or low (1 kg, as fed basis; L) quantity twice per day on milk production of mid-lactation dairy cows.

	Treatment					*P* – value[1]		
Item	FH	FL	SH	SL	SEM	CHO	AMT	CHO × AMT
Milk, kg/d	42.5	42.9	42.1	42.9	1.0	0.67	0.22	0.59
Fat, %	3.70	3.70	3.62	3.68	0.07	0.29	0.51	0.57
Protein, %	3.25	3.24	3.26	3.24	0.03	0.82	0.16	0.72
Lactose, %	4.59	4.60	4.59	4.60	0.04	0.70	0.66	0.97
Fat, kg/d	1.57	1.59	1.52	1.58	0.07	0.65	0.59	0.59
Protein, kg/d	1.38	1.39	1.37	1.39	0.03	0.76	0.70	0.57
Lactose, kg/d	1.97	1.97	1.94	1.98	0.05	0.78	0.38	0.39
MUN, mg/mL	13.2	13.5	13.6	12.6	0.7	0.60	0.50	0.22

[1]CHO = type of carbohydrate (high-fiber or high-starch), AMT = amount of pellet fed (high or low)

Table 3.7-6 The effects of feeding a high-fiber (F) or high-starch (S) pellet at a high (3 kg, as fed basis; H) or low (1 kg, as fed basis; L) quantity twice per day on plasma glucose and insulin concentrations of mid-lactation dairy cows.

	Treatment					*P* – value[1]		
Item	FH	FL	SH	SL	SEM	CHO	AMT	CHO × AMT
Plasma glucose, mg/dL	69.6	70.3	66.6	65.4	1.35	<0.01	0.86	0.50
Plasma insulin, ng/mL	2.0	2.5	1.9	1.9	0.14	0.02	0.11	0.09

[1]CHO = type of carbohydrate (high-fiber or high-starch), AMT = amount of pellet fed (high or low)

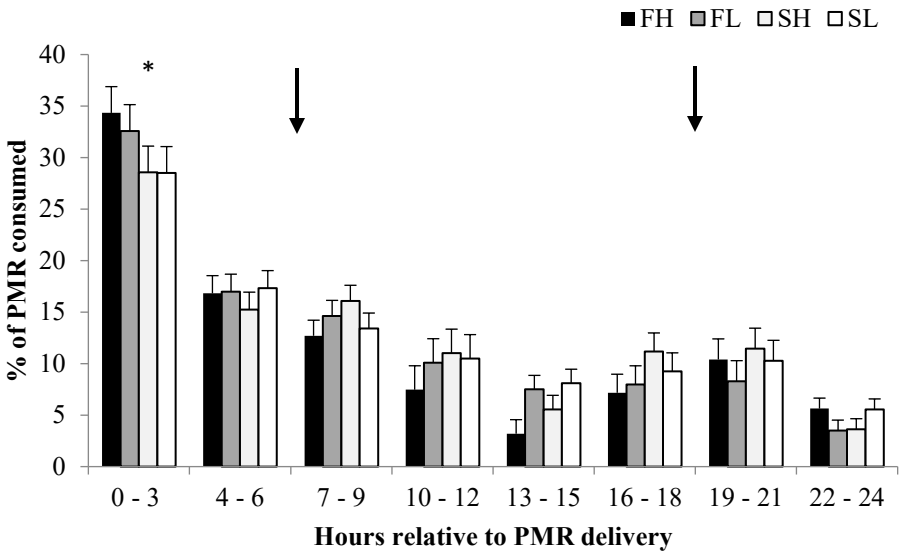

Figure 3.7-1 Feed disappearance pattern of partial mixed ration (PMR) when cows were fed a high-fiber (F) or high-starch (S) pellet at a high (H) or low (L) amount. Arrows indicate when pellet was fed relative to PMR delivery. *Cows fed the S pellet had lower PMR intake in the first 3 h after PMR delivery ($P = 0.04$). Error bars denote SEM.

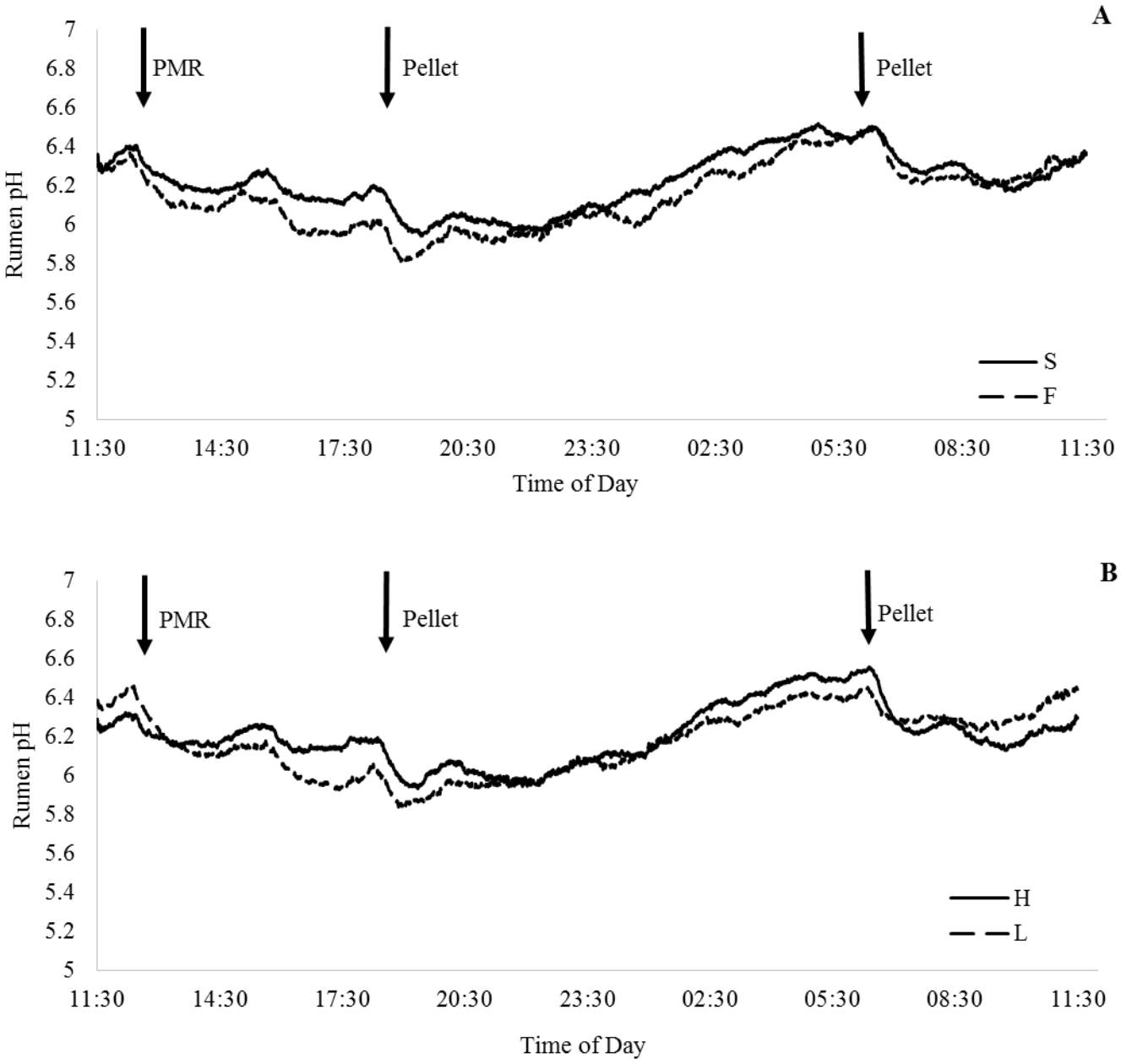

Figure 3.7-2 Rumen pH over a 24-h period beginning 30 min before PMR delivery of a high-fiber (F) or high-starch (S) pellet (panel A) or at a high (H) or low (L) amount (panel B).

Chapter 4: Effects of automated milking system concentrate allowance on milk production, milking parameters, feed intake, and feeding behavior of early lactation dairy cows milked in a guided-traffic automated milking system

4.1 Introduction

As the dairy industry continues to adopt automated milking systems (AMS), knowledge regarding feeding strategies for cows managed with AMS continues to grow. Feeding for cows managed with AMS differs from conventional milking as cows are offered a partial mixed ration (PMR) at the bunk and concentrate in the AMS. Emphasis has been placed on AMS concentrate feeding strategies as it is considered as the primary motivator for cows to voluntarily visit the AMS (Prescott et al., 1998; Bach et al., 2007) and may enable an individualized feeding approach for cows depending on their parity, milk production, and stage of lactation (Bach and Cabrera, 2017). Anecdotally, within the dairy industry there is a belief that feeding more AMS concentrate will enhance voluntary visits, milk production, and DMI. Survey data from commercial farms have reported that on average cows are offered 0.16 kg of concentrate/kg of milk produced (Tremblay et al., 2016), with feed provision in the AMS up to 11.3 kg per day for free-flow traffic and 8.2 kg per day for guided traffic (Salfer and Endres 2014). In addition, increasing AMS concentrate allowance has been suggested as a strategy to support nutrient requirements of early lactation dairy cows by increasing dietary nutrient density.

Despite the notion of precision feeding, the concept has not been supported in research studies as the amount of concentrate programmed to be delivered does not equate to the concentrate consumed by cows in the AMS (Bach and Cabrera, 2017; Henriksen et al., 2018a), and that both the PMR and AMS concentrate should be considered in combination as they affect nutrient intake and production outcomes (Menajovsky et al., 2018; Paddick et al., 2019). Moreover, as a reduction in PMR intake occurs with increasing concentrate provision (Bach et al., 2007; Henriksen et al., 2018a; Paddick et al., 2019) substitution of PMR for AMS pellet challenges the ability to quantify the dietary characteristics at a cow level and consequently might limit application of individualized diets for cows. To date, little research has evaluated the effect of increased concentrate allowance immediately after calving. Henriksen et al. (2019) evaluated two concentrate allowances immediately following calving. In that study, AMS

concentrate offered was increased from 1 to 3 kg per day from 1 to 14 DIM with those cows offered 3 kg thereafter, versus increasing the amount of concentrate on a per cow basis relative to PMR intake throughout lactation with a target of achieving an AMS concentrate provision equal to 30% of the PMR intake. However, Henriksen et al. (2019) reported no difference for AMS concentrate consumption until beyond 15 DIM between treatments. Therefore, it is unknown how providing greater concentrate allowance or the rate of increase for that concentrate allowance may affect voluntary visits, and milk and milk component yields of early lactation cows milked with AMS. To the authors knowledge, no other studies have evaluated feeding strategies for cows milked with AMS immediately in early lactation.

The primary objective of this study was to determine whether increased concentrate allowance and the rate at which concentrate allowance was increased would influence the number of milkings, milk and milk component yield, total DMI, and feeding behavior of early lactation cows. The secondary objective was to characterize how cows adapt to AMS milking given different concentrate allowances. The null hypothesis was that the amount of AMS concentrate would not influence the number of milkings, milk and milk component yields, and would not affect variability in AMS concentrate intake, PMR intake, or change feeding behavior.

4.2 Materials and methods

The study took place at the University of Saskatchewan Rayner Dairy Research and Teaching Facility (Saskatoon, SK, Canada). All experimental procedures were pre-approved by the University of Saskatchewan Research Ethics Board (protocol # 20190128) and general management followed the Code of Practice for the Care and Handling of Dairy Cattle (National Farm Animal Care Council, 2009) and the Canadian Council on Animal Care (Ottawa, ON, Canada). Research facility staff completed all basic animal husbandry and were blinded to treatments.

4.2.1 Prepartum animal management, and data and sample collection

A total of 66 (22 primi- and 44 multi-parous) Holstein dairy cows were used in a randomized complete block design to evaluate the effects of pellet allocation in the AMS on feed intake and feeding behavior. Cows were blocked by expected calving date, parity, and BCS measured 30 ± 3 d prior to their expected calving date before being randomly assigned to 1 of 3

treatments (described below). Forty of the 44 multiparous cows had been milked with the AMS in previous lactations, while four multiparous and all primiparous cows had no exposure to the AMS prior to their first milking.

At 30 ± 3 d prior to calving, all cows and heifers were moved into the barn from an outside pen and housed in a close-up group consisting of 12 free-stalls and 8 Insentec bunks (Hokofarm Group, Marknesse, Flevoland, the Netherlands). At the time of movement, body weight and BCS were measured on two consecutive days by two independent research personnel, blinded to treatments. A maximum of 8 cows were housed in the close-up group at any given time. An individual cow was assigned to 4 of the 8 Insentec bunks. Exposure to multiple bunks was used to enable measurement of DMI without limiting bunk space and to ensure cows were trained to use the bunks during the close-up period. Cows were fed a TMR at 1100 h daily (Table 1). The TMR was formulated to meet the requirements of a 750 kg cow at 235 d of gestation using the Cornell Net Carbohydrate and Protein System (6.55) platform of NDS (The RUM&N Company, Reggio Emilia, Italy). The ration was delivered into Insentec bunks allowing for 5% refusal (as fed basis), and cows had free access to feed and water.

Cows were moved to an individual maternity pen at 7 ± 3 d prior to their expected calving date where they were provided the same close-up diet individually. Cows presenting visual signs of calving were moved to the individual maternity pen regardless of their projected calving date. On average, cows were fed the close-up diet for a total of 24 ± 2 d (free-stall pen + maternity pen). Following calving, multiparous cows received 2 calcium boluses (Bovikalc, Boehringer Ingelheim Animal Health Canada Inc, Burlington, ON, Canada) within 24 hours of calving as a routine prophylactic treatment.

Blood samples were collected on d -7 ± 3 relative to their calving date when cows were moved into an individual maternity pen. While housed in the individual maternity pen, feed intake was manually recorded as the weight fed minus the weight of the refusals measured the following day. Feed intakes obtained from the Insentec bunks and manual measurement were corrected for TMR DM to determine DMI during the prepartum period and averaged by week relative to actual calving date. General characteristics of dry cows can be found in Table 2.

4.2.2 Postpartum facility design and management

The barn design for lactating cows consisted of two groups of cows accessing a single AMS (Classic VMS, DeLaval, Tumba, Sweden) through a common holding area. A small group (**SG**) provided access to 12 free-stalls and 8 Insentec bunks, while the main group (**MG**) contained 49 free stalls and head lockers at the feed alley. The total number of cows milked by the AMS, including study and non-study cows, ranged between 35 to 52, with the AMS averaging (mean ± S.D) 114 ± 13 milkings/d and cows in the AMS produced 43.2 ± 2.2 kg milk/d for the duration of the study. Stalls were fit with rubber mats and bedded with wood shavings. In both groups, cows had access to the AMS using a feed-first guided traffic flow system. From the free stalls, cows passed through a single one-way gate to enter the feed alley where they had access to PMR. Cows in the SG were assigned to an individual Insentec bunk for PMR provision while cows in the MG were provided their PMR at the standard feed bunk with head locks. From the feed alley area, cows were required to pass through a pre-selection gate that either directed them to the holding area if they had milking permission or to the free stall area if permission criteria were not met (described below). From the holding area, cows entered the AMS where they were milked and received their AMS concentrate. Following milking, cows in the SG were directed back to the free-stalls; whereas, cows in the MG were directed back to the MG feed alley and were required to go through the pre-selection gate again to obtain access to stalls. Each pass through the selection gates and upon attendance at the AMS were recorded by the AMS software (DelPro 5.3, DeLaval, Tumba, Sweden). Water was provided in 1 trough (160 × 41 × 23.5 cm) in the free stall area of the SG, and in 2 troughs (179 × 34.3 × 15.25 cm) located in the free stall area of the MG and 3 individual troughs (30.5 × 20.3 × 3.8 cm) located along the feed alley of the MG. No water was available in the holding area.

The pre-selection gates were programmed to allow a maximum of 10 cows (from both the SG and MG combined) into the holding area to avoid overcrowding. If the total number of cows in the holding area exceeded that amount, cows were directed to the free-stall area of their respective group. Milking permissions were set to allow primiparous and multiparous cows less than 100 DIM access to the AMS every 4.5 h or if their expected yield since the last milking was greater than 8 kg for primiparous and 10 kg for multiparous cows. Cows were manually fetched

when their last milking was more than 12-h prior. Fetching times were held static at 0500, 1230, and 2030 h daily and all fetching events were recorded by barn or research staff.

The maximum amount of pellet to dispense at each milking in the AMS was 2.5 kg with a dispensing rate of 0.50 kg/min (as fed basis). All non-study cows were programmed to receive 3.0 kg/d of AMS concentrate, on a dry matter basis to minimize pellet carryover between study and non-study cows. The robot pellet dispenser was calibrated weekly. A custom-built feed manger was used to allow for the collection of pellet refusals. Following milking, when the exit gate of the AMS opened, the base of the manger slid open allowing remaining pellet to fall from the manger. This mechanical action also triggered a vacuum to collect the pellet into a canister placed on a scale with an automated recording system to allow for monitoring of the date and time of pellet collection. The date and time of pellet collection was then related to milking times for cows thereby determining the pellet refusal for individual milkings. When the exit gate of the AMS closed, the base of the manger closed, triggering the vacuum to stop. Due to technical issues with the system to weigh pellet refusals, refusal data was only collected from a subset of animals (n = 33) and time periods throughout the study. Pellet refusals collected from this subset, regardless of treatment, were observed to be less than 5% of the dispensed amount. Therefore, to maintain consistency, the data presented and referenced as pellet intake was the amount of pellet recorded as dispensed to cows, excluding refusal measurements.

All cows were milked on the AMS from their first milking and for the duration of the study. Primi- and multiparous cows were manually moved to the AMS for their first 3 milkings and remained in the maternity pen during this period (approximately the first 24 h following calving), before being moved to the SG and assigned to an indvidual Insentec bunk. If cows were unable to be milked unsupervised by the AMS due to attachment issues or demeanor towards the AMS during the first 7 DIM, they were housed in the SG, but manually taken to the AMS 3 times per day. If cows had not adapted to being milked unsupervised by 8 DIM, they were removed from the study. Milking data, including yield, number of milkings, milking duration, and incomplete milkings were recorded from all cows using the DelPro software. The software also recorded the amount of time cows spent standing in the holding area based on the amount of time between the cows being identified by the pre-selection gate directing them into the holding area and the time when the cow was identified by the AMS.

4.2.3 Experimental design and dietary treatments

From calving to 56 DIM, all cows were offered the same PMR formulated to meet the requirements of a 725 kg cow producing 45 kg of milk with a target milk fat of 4% and milk protein of 3.3% at 90 DIM (Table 1). While this dietary approach was not formulated specifically for fresh cows, the use of a common PMR is a practical reality in AMS herds. The PMR was fed at 1000 h into Insentec bunks to allow for 5% refusals. The previous days refusals were removed from bunks at 0930 h. At calving, cows were allocated to a common AMS pellet (Table 1) to allow for 3 kg/d (DM basis) of intake until 3 DIM when they were offered one of three AMS pellet allowance strategies including a low allocation (3 kg/d; **LP**; n= 22) or one of two high allocation strategies (**HP**; 8 kg/d on a DM basis). Pellet allocations for the HP treatment increased at a moderate (**HPM**; increased from 3 to 8 kg/d over 15 d; n = 22) or rapid (**HPR**; increased from 3 to 8 kg/d over 5 d; n = 22) rate. To ensure that the as fed pellet offered equated to the DM allowance, the DM concentration of the pellet was measured weekly and used to determine the as fed pellet quantity. For each treatment, the amount of pellet available in the AMS was set to exceed the target quantity to aid in achieving target pellet intake (Menajovsky et al., 2019; Paddick et al., 2019). Therefore, cows fed LP were programmed to receive 3.75 kg/d and HP 9.85 kg/d on an as fed basis. The treatment-based pellet allowances were offered until 56 DIM.

4.2.4 Postpartum data and sample collection

Cow BW and BCS were measured between 6 and 24 h following calving, on d 28 and 29, and on d 56 and 57 at 0900 h by two independent research personnel. Blood samples were collected before PMR feeding at 0900 h within 6 and 24 h after calving and on 7, 21, 28, and 56 DIM. Blood was collected via the coccygeal vein into an evacuated container containing sodium heparin (148 IU; BD Vacutainer, Franklin Lakes, NJ) for plasma collection and with no additive (BD Vacutainer) for serum collection. The sodium heparin vacutainer was immediately placed on ice until centrifugation at 3,000 × g for 20 min at 4°C. Plasma was stored at -20°C until analysis. The serum vacutainer was left at room temperature for a minimum of 30 min before centrifugation at 3,000 × g for 20 min at 4°C, and serum was stored at -20°C until analysis.

Milk yield was recorded daily from all cows using the DelPro software. This software provided information including milk yield per milking; number of milkings; milking duration;

and incomplete milkings on each quarter. Milk samples were taken from each milking starting at 1000 h for a 24-h period on 4, 7, 10, 14, 21, 28, 35, 42, 49, and 56 DIM using a sampling system connected to the AMS and a 30-mL daily composite (proportional to yield) was prepared for each cow in containers containing Bronopol Microtab preservative (Dairy Herd Improvement Laboratory, Edmonton, Alberta, Canada). Samples were stored at 4°C for a maximum of 7 d before submission.

For the first 28 d when cows were in the SG, the amount of feed offered and refused (as-fed basis) was recorded from the Insentec bunks daily with the exception being the period immediately following calving when cows were housed in the maternity pens and intake was determined manually. Daily values were averaged to yield one value per week. The Insentec bunks recorded the date, time, duration, and size of each PMR visit for each cow during the experimental period. The raw data was processed to remove visits to the feed bunk where no feed was consumed. The intermeal intervals between each visit were then calculated and $\log_{10}$ transformed (Tolkamp et al. 1998). Transformed data were fit to normal distributions to determine meal criteria for each cow within each period using the MIXDIST package (Macdonald and Green, 1988) of the R Statistical Analysis Software (The R foundation, Adelaide, South Australia, Australia) as explained by DeVries et al (2003). The meal criterion was defined as the minimal time away from the bunk to identify a new meal. These data were then used to determine the number of meals, length of meals, size of meals, and rate of consumption. There was no measurement of PMR intake from 28 to 56 DIM when cows were in the MG. The amount of AMS pellet dispensed per milking was recorded using the AMS software (Delpro 5.3) for the entirety of the study.

All daily feed refusals were collected, mixed, and a representative sample equating to 20% of the daily refusal were kept. Daily samples were composited to yield one sample per cow per week to determine particle size. A representative sample of PMR delivered to cows was collected weekly and used for particle size determination. Particle size distribution of the PMR and feed refusals were determined using a Penn State Particle Separator with 3 sieves (aperture size of 19, 8 and 4 mm) and the bottom pan using the procedure described by Lammers et al. (1996). Sorting index was calculated as the ratio of actual intake relative to the predicted intake for particles retained on each sieve of the separator should no sorting occur (as-fed basis;

Leonardi and Armentano, 2003). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, selective consumption, and selective refusals of each particle portion, respectively.

Dietary forage and AMS pellet samples were collected weekly and concentrate components provided through the PMR were collected every 2 wk. Dry matter concentration of all feeds was determined in a forced-air oven at 55°C for 72 h, and DM coefficients were adjusted to ensure the ingredient inclusion rates of the formulated and mixed diets were similar. In addition, the DM concentration was used to determine the inclusion rate of water added to the PMR to maintain a DM of 50%. Dry matter intake was determined by multiplying the as fed PMR or AMS pellet intake by the DM concentration of the PMR or AMS pellet, respectively. Forage samples were ground through a 5-mm screen with a Christy Turner lab mill (Christy Turner, Ipswich, Suffolk, United Kingdom). Ground forage and dried concentrate samples were combined on an equal DM basis to yield a 4-mo composite and sent for analysis of chemical composition (described below).

4.2.5 Sample analysis

Milk samples were sent to the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada) and analyzed for concentrations of fat, CP, lactose, SCC, MUN and beta-hydroxybutyrate (BHB) by infrared spectroscopy (MilkoScan 605, Foss North America, Brampton, ON, Canada). Milk yield on the day of sample collection was used to calculate milk fat, protein, and lactose yield by multiplying the milk yield (kg) by component value (%) to determine kg of component. Milk samples collected outside of the weekly sample on d 4 and 10 were excluded from this and used for milk BHB reporting only. Energy corrected milk was calculated using the formula: ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

Plasma glucose concentrations were determined using a glucose oxidase/peroxidase enzyme (Sigma Co., St. Louis, MO) and dianisidine dihydrochloride (Sigma Co.) with absorbance at 450 nm determined using an Epoch 2 spectrophotometer (BioTek Instruments, Inc. Winooski, VT). Serum NEFA concentrations were determined using a commercial kit (NEFA HR2; Wako Chemicals USA Inc., Richmond, VA) and serum BHB concentration was measured by the enzymatic oxidation of BHB to acetoacetate in the presence of 3-hydroxybutyrate

dehydrogenase (Roche, Mississauga, ON, Canada) and NADH at a wavelength of 340 nm. For all analysis, samples were run in triplicate and re-analyzed if the intra-assay CV was greater than 5 for any sample. Final inter-assay CV for all plates were 4.41. 1.75 and 3.89 for BHB, glucose, and NEFA analysis, respectively.

Feed ingredient samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD), and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), ADF (AOAC International, 2000; method 973.18) modified to incorporate the use of Whatman 934-AH glass microfibre filters with 1.5 µm particle retention in place of a fritted glass crucible, and NDF using α-amylase and sodium sulfite as described by Van Soest et al. (1991) using the same modifications as the ADF method. Starch was analyzed as per Hall (2009), and CP using method 990.03 (AOAC International, 2000) using a Leco FP-528 nitrogen combustion analyzer (Leco 3000, Lakeview Avenue, St. Joseph, MI, USA).

4.2.6 Statistical Analysis

Of the 66 cows enrolled, 4 primi-parous cows were removed due to temperament and inability to be milked unsupervised within the first week after calving. Three multiparous cows were removed due to: severe milk fever (1 HPM), lameness (1 LP), and mastitis (1 HPR) within the first week of calving. Therefore, final cow numbers for the data presented for wk 1 to 4 are LP $n = 20$, HPM $n = 20$ and HPR $n = 19$. Of the cows that remained in the study, 3 multi-parous cows were treated for: retained placenta (1 LP), ketosis (1 LP), and milk fever (1 HPM), and 1 primi-parous cow was treated for ketosis (HPR). From wk 5 to 8, 4 cows were removed from the study due to mastitis at the end of: wk 4 (2 multiparous cows; 1 HPM and 1 HPR), wk 6 (1 HPR multiparous cow), and wk 7 (1 LP primiparous cow). Data from these cows are included up until the week prior to removal from the study.

Statistical analysis on pre- and post-partum data were performed independently using the MIXED procedure of SAS 9.4 (SAS Institute Inc.). Data were divided into two distinct time periods equating to d 1 to 28 and 29 to 56 reflecting the differing housing conditions. All daily data were averaged by week and analyzed using a model including the fixed effects of treatment, parity, week, and their interactions. Data were analyzed using the REPEATED statement with week as the repeated variable. Calving date was included as a random effect, and pre-partum

DMI was used as a covariate. Covariance error structures were tested to determine which yielded the lowest Akaike and Bayesian criterion values. The covariance structure that suited the data was autoregressive. Least square means were separated using the PDIFF procedure of SAS with the Bonferonni correction. When the interaction of treatment and week was significant, contrast statements were used to evaluate responses within a week. Primary response variables were tested for normality using JMP 16.1 (SAS Institute Inc.) using the model described above and found to be normally distributed. A 2-tailed t-test was used to evaluate if PMR sorting behaviors were different from 100 for each particle length within each week and treatment. Response variables means and SEM reported represent the treatment × week interaction unless otherwise noted. For all analysis, significance was declared when $P \leq 0.05$ and tendencies were discussed when $0.05 < P < 0.10$.

4.3 Results

Characteristics of cows prior to parturition and treatment exposure are presented in Table 2. There were no differences for BW or BCS at enrollment; however, cows assigned to the LP treatment consumed 1.6 kg/d less DMI than HP during the dry period. At -7 ± 3 d relative to calving there were no differences in serum BHB or plasma glucose concentrations; however, serum NEFA concentrations tended to be greater for LP cows as compared to HP (508 vs. 366 mEq/L; $P = 0.06$).

The amount of AMS pellet dispensed was not different during the first wk of lactation among treatments. However, from wk 2 through 4, there was a difference between LP and HP, regardless of rate of adaptation (treatment × week, $P < 0.01$; Table 3). For all treatments there was an increase from wk 1 to 2, but HPM and HPR did not differ during wk 3 and 4. Despite being allocated more pellet through the software, HPM and HPR cows only received a maximum of 57.5 and 55.6% of the target pellet allocation in the AMS. For all treatments, PMR intake increased from wk 1 to wk 4; however, PMR intake increased at a greater magnitude for cows offered LP compared to HP. There was no difference for PMR intake during wk 1; however, LP cows consumed more PMR than HPR on wk 2, 3, and 4, with HPM not different than LP or HPR on wk 2 and 3. Total DMI increased from wk 1 to 4 regardless of treatment; however, DMI (19.2 kg/d; $P \geq 0.22$) was not different among treatments during the first 4 wk of lactation.

The amount of pellet dispensed per milking (Table 4) did not differ among weeks for LP during the first 4 wk of lactation, and was not different from HP on wk 1 (treatment × week, P = 0.003, but the amount of pellet dispensed increased for HPM and HPR from wk 1 to 2 as allocation increased. Variability, reported as the standard deviation in the amount of pellet dispensed, was less during wk 1 to 2 for LP and stayed consistent from wk 2 to 4; while, the HPM and HPR allocations had greater variability than LP (P <0.001), with no difference between HPM or HPR on wk 2 and 3. That said, cows fed HPR had less variability than HPM on wk 4. The number of PMR meals/d was not different for cows fed LP or HP allocations but tended (P = 0.06) to be fewer for cows on HPM than HPR. Additionally, the number of PMR meals/d increased from wk 1 to 2 with no further change thereafter. The inter-meal interval was not different among treatments (45.6 min; data not presented), and not affected by treatments or time (P > 0.10). There was a tendency for cows offered LP to have larger PMR meals as compared to HP (P = 0.08), and HPM cows tended to have larger meals compared to HPR (P = 0.06). The PMR meal size also increased from week 1 to 3 and 4 (P <0.01). Partial mixed ration meal duration (23.9 min) and eating rate (9.9 g/min) were not different among treatments; however, duration was consistent for wk 1 and 2, and was increased thereafter while eating rate was consistent for wk 1 and 2, and wk 3 and 4. There was no difference in the standard deviation of PMR intake among treatments; however, it increased from wk 1 to 3.

There were no effects of dietary treatment on sorting behavior of the PMR (Table 5). In the present study, cows selectively consumed long particles during wk 1 although the sorting index values did not differ from 100%. Moreover, cows increased selective pressure against long particles during wk 2 and 3 and reduced the magnitude of the selective avoidance during wk 4. During wk 1, cows selectively consumed particles retained on the 8-mm sieve, but there was no further preferential selection or avoidance of this particle size fraction from wk 2 to 4. There was limited selection of particles retained on the 4-mm sieve and pan with the exception of during wk 1 where these smaller particles were avoided. For particles on the pan, the selective avoidance diminished from wk 1 to wk 2 and 3, and further for wk 4.

Milking frequency increased as lactation progressed between wk 1 to 2, and 3 to 4, and cows offered LP had greater milking frequency than those offered HP (3.31 vs. 3.05 milkings/d; P = 0.02; Table 6). There was no difference in milking frequency between HPM and HPR (P =

0.96). The milking duration did not differ among treatments and increased from wk 1 to wk 2, and further from wk 2 to wk 3 and 4. The inter-milking interval was shorter for cows provided the LP allocation as compared to HP. Cows offered LP tended ($P = 0.06$) to spend less time in the holding area when reported on a min/milking basis but not when determined as min/d. There were no differences in incomplete milkings or somatic cell count among treatments.

Data for fetched milkings are presented by parity as the interaction of treatment × parity was significant (Table 7). The proportion of fetched milkings tended ($P = 0.07$) to be less for primiparous cows offered LP as compared to HP and was not affected by week of lactation. There was no effect of dietary treatment on fetching for multiparous cows; however, fetching was greater during wk 1 than during wk 2, 3, and 4.

Cows offered LP had greater milk yield ($P <0.01$) and yields of milk fat ($P = 0.02$), protein ($P = 0.04$), and lactose ($P = 0.01$) as compared to HP, with no differences detected between HPM and HPR (Table 8). Concentrations of milk fat and protein were generally reduced as lactation progressed, while concentrations of lactose increased from wk 1 through 3.

Milk BHB measured on d 4, 7, 19, 14, 21 and 28 was not affected by treatment or time or their interactions ($P > 0.57$) with an average milk BHB concentration of 0.08 mmol/L during the first 4 wk of lactation ($P > 0.57$). Primi-parous cows had lower BHB concentrations as compared to multiparous cows (0.06 vs. 0.10 mmol/L; $P = 0.03$) during the first 4 wk of lactation. There were no effects of treatment on BW, BCS, and concentrations of plasma glucose, serum BHB, or serum NEFA (Table 9). Regardless of treatment, BW and BCS were reduced from calving to d 28, and concentrations of glucose and NEFA were reduced from d 7 to d 21.

Cows offered HP were dispensed more AMS pellet than LP (4.15 vs. 2.97; $P < 0.001$) from wk 5 to 8 of lactation (Table 10). There were no differences for milking frequency (2.89), milking duration (8.85 min/milking), or time spent in the holding area per milking (56.8 min) or per day (156 min). There were no differences for the inter-milking interval (496 min) or proportion of fetched milkings (7.0%) among treatments or week. However, cows offered LP produced more milk (49.3 vs. 44.8 kg/d; $P < 0.01$), and had greater yields of fat ($P = 0.04$), protein ($P = 0.02$), and lactose ($P <0.01$) which resulted in greater ECM (46.3 vs. 40.9 kg/d; $P = 0.01$) as compared to HP. There were no differences in milk fat (3.46%), protein (3.04%) or lactose (4.62) concentrations. Milk BHB measured on wk 5, 6, 7, and 8 were not different among

treatments or week and averaged 0.07 mmol/L ($P > 0.32$). For all the aforementioned variables, there was no effect of altering the rate of AMS pellet provision within the HP treatments. When measured on 56 DIM, there were no differences among treatments for BW, BCS, or blood metabolites (Table 11).

4.4 Discussion

While cows in this study were randomly assigned to dietary treatments based on parity, expected calving date, BCS 30-d prior to calving, and fed the same close-up diet, differences in pre-partum intake and a tendency for differences in NEFA concentrations were detected. Cows fed LP had reduced pre-partum DMI and tended to have increased concentrations of NEFA as compared to HP pre-partum. However, there were no differences in glucose, BHB, or NEFA concentrations measured 6 to 24 h postpartum. Reduced pre-partum DMI is associated with increased NEFA concentrations prepartum, and elevated NEFA concentrations may pre-dispose cows to metabolic disorders postpartum (Drackley, 1999; Ospina et al., 2010). Although the number of cows used prevents statistical comparisons of metabolic disorders, the occurrence was low and there did not appear to be difference among treatments for the need to remove cows from the study. Concentrations of NEFA change dramatically around parturition, and there is insufficient evidence to suggest that these findings bias the results of the present study, particularly as the pre-partum data would suggest that LP cows may have been exposed to greater pre-partum transition period challenges. In addition, prepartum DMI was included in the statistical model as a covariate to partially account for these findings.

Within the dairy industry, it is common practice and often recommended to offer more AMS concentrate to early lactation or high producing dairy cows. This practice is promoted to achieve precision feeding, improve milking frequency, and milk production for cows in AMS (Rodenburg, 2011; Salfer and Endres, 2014; Siewert et al., 2017). In the current study, we observed that the target AMS pellet consumption was not met for cows offered the HP allocation, and that offering more AMS concentrate did not improve milking frequency, milk production, or milk components of early lactation dairy cows. In fact, milk and milk component yields were greater for the LP than the HP treatments from wk 1 to 8 of lactation. These findings are in alignment with several studies that have evaluated AMS feeding strategies for mid or late

lactation cows (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019) and partially support findings of Henricksen et al. (2019).

4.4.1 Feed intake and feeding behavior

Cows fed the LP allocation reached target pellet intake by wk 2 of lactation and maintained their intake throughout the study; whereas cows fed either of the HP allocation strategies did not achieve the target intake of 8.0 kg/d with the observed intake averaging 4.08 and 3.98 kg per day for HPM and HPR, respectively throughout the study. With AMS systems, the amount of concentrate dispensed during a milking is a function of many variables including maximum concentrate allowance per day and per milking, dispensing rate of concentrate, and milking duration. It is also important to note that with the DeLaval VMS, cows must have their head down in the manger for concentrate to be dispensed; therefore, the desire to consume the feed offered contributes to the amount dispensed. To improve probability that AMS allocation targets were met, the maximum pellet allowance was set greater than the targeted quantity; a strategy that has been employed previously to meet concentrate allowances (Hare et al., 2018; Paddick et al., 2019; Schwanke et al., 2019). In the current study, we set the maximum amount dispensed per visit to 2.5 kg with a dispensing rate at 0.50 kg/min assuming cows would visit a minimum of 3.2 times per day, with a milking duration of greater than 5 min to allow for delivery of the 8 kg. While cows offered HP did not achieve over 3.1 milkings per day, on average, milking duration was greater than 6 minutes, which still allowed for 2.5 kg dispensed per milking, meaning theoretical intake could have been 7.5 kg/d, much greater than what was observed. As the AMS was set to provide adequate opportunity for cows to receive the target pellet amounts, we do not believe that settings contributed to not achieving target intake.

Others have reported an inability to achieve the target AMS concentrate intake when large allocations of concentrate were offered through the AMS (Bach et al., 2007; Menajovsky et al., 2018; Henriksen et al., 2019), and we hypothesize that cows were not motivated to consume the AMS pellet. Given that pellet was not dispensed, they were not putting their heads down to consume the feed while in the AMS. Palatability is one consideration; however, on average 40, 85, 95, and 95% of cows offered LP consumed their pellet allocation on wks 1, 2, 3, and 4, respectively. In comparison, 4, 20, 25, and 21% of cows offered HPR, and 1, 27, 43, and 36% of cows offered HPM consumed at least 6 kg of robot pellet per day on wks 1, 2, 3, and 4,

respectively. In addition, refusals of AMS pellet were very low suggesting limited concern with palatability. Given that LP cows adequately consumed the AMS pellet and low refusals, the data challenge the concept that providing more pellet in the AMS further stimulates voluntary attendance over smaller allocations.

Regardless of treatment, PMR intake increased as lactation progressed, and at wk 4 cows offered LP consumed more PMR than those offered HP. Despite this, DMI was not different among treatments during the first 4 wk of lactation. This finding supports the concept of substitution whereby the AMS concentrate acts as a substitute for PMR and that offering more concentrate does not always increase total DMI or nutrient intake (Bach and Cabrera, 2017, Henricksen et al., 2018a). Substitution rate has been investigated primarily with dairy cows under grazing conditions where concentrate is provided in addition to pasture and a PMR (Bargo et al., 2002). While energy density of the basal forage or PMR and composition of the concentrate affects the substitution rate (Gill et al., 1988; Bargo et al., 2002), little is known about factors affecting substitution rate for cows fed a PMR and milked with AMS.

In the current study, we observed substitution rates of 1.82, 1.53, 1.34, and 2.71 kg PMR/kg of AMS pellet (DM basis) on wk 1, 2, 3, and 4, respectively. These values are similar to the substitution rate of 1.58 reported by Hare et al. (2018); however, are greater than 0.89 reported by Menajovsky et al. (2018) or 0.97 by Paddick et al. (2019) which were all conducted using the same traffic design as the current study, but with mid to late lactation cows. With free-traffic systems, variability in substitution rates have also been reported with values as high as 5.0 using early lactation cows (Henriksen et al., 2019), and as low as 1.14 (Bach et al., 2007) and 0.53 (Schwanke et al., 2019) with mid-to-late lactation cows. It has been previously hypothesized that stage of lactation (Henricksen et al., 2018a, 2019) and energy density of the PMR has an influence on substitution (Jensen et al., 2016), whereby substitution increases with increasing energy density (Menajovsky et al., 2018; Henriksen et al., 2018a). The use of early lactation cows and relatively high energy density PMR in the present study may help explain the large substitution rates observed in the present study.

This study was designed to represent a practical feeding scenario with the basal diet formulated to meet the ME and MP requirements of a cow producing 45 kg of milk consuming 28 kg of DM, and AMS concentrate intake feeding rate of 3 kg. This strategy results in both the

LP and HP diets receiving a high energy density PMR. The PMR contributed 88.5% of the ME requirement, or 7 kg less milk than what was targeted. This approach is common in industry as Salfer and Endres (2016) found in a survey of AMS users that PMR are formulated for 4 to 9 kg less milk than targeted by the overall diet for guided-traffic barns. In addition, DMI of dairy cows is driven by energy demand and controlled by physical and chemical characteristics of the feed (Allen 2000), with the latter playing a greater role immediately after calving (Allen et al., 2009). It is plausible that offering HP alongside a nutrient dense PMR acted to increase substitution rate; whereas, when cows are grazing or fed high forage diets immediately following calving substitution rates are lower (Dillon et al., 1997; Bargo et al., 2002).

While increased AMS concentrate provision is seen as a strategy to deliver additional energy to fresh or high producing cows, the consumption of both the PMR and AMS pellet along with their composition must be considered. As observed in the current study, despite increased AMS pellet intake, the reduction in PMR intake for HP cows resulted in no increase for total DMI. As the PMR offered had a high nutrient density, the greater PMR intake when coupled with the AMS concentrate intake for LP cows supplied more ME and MP intake than for HP. Consequently, the changes in PMR and AMS intake resulted lesser predicted ME and MP allowable milk by 1.4 and 2.0 kg/d, respectively (Table 12). In contrast, had the substitution rate been less than 1, whereby additional AMS pellet resulted in an increase in total DMI for HP, the additional pellet intake would have increased overall energy and protein intake relative to LP. These data highlight the importance of understanding how AMS pellet intake affects PMR intake and further research is needed to elucidate factors affecting the substitution effects.

Due to increased pellet intake for HP, the amount of pellet dispensed per milking increased during the first 2 wk of lactation; whereas, it remained consistent for LP. We observed that offering HP increased the variability in AMS pellet intake when compared to LP. Others have also reported that increasing the pellet allocation in the AMS increases the day-to-day variation in the amount of pellet dispensed (Menajovsky et al., 2018; Paddick et al., 2019) and consumed (Henriksen et al., 2019). We had hypothesized that increased variability in pellet intake may change feeding behavior and sorting of the PMR, affecting milk and milk component yield due to a reduction in consistency for nutrient intake. However, we did not observe differences in variability in PMR intake or sorting behavior among treatments. That said, cows

offered LP tended to have larger PMR meals compared to HP without differences in the number of meals per day, meal duration, or eating rate were detected between the LP and HP treatments. Several studies have reported that during early lactation cows increase their meal size and meal duration to increase DMI (Grant and Albright, 1995, Schmitz et al., 2018). In the current study, cows, on average, consumed 7.0 meals per day which is less than what has been previously reported when early lactation cows were fed a TMR (8.1 meals/d; Adin et al., 2009; 8.0 meals/d; Azizi et al., 2009), PMR with automated concentrate feeders (8.5 meals/d; Schmitz et al., 2018), or PMR with AMS (8.8 meals/d; Schwanke et al., 2019). However, studies conducted in the same facility as the current, using cows later in lactation found similar meals per day (5.9 meals/d, Menajovsky et al., 2018; 6.8 meals/d, Paddick et al., 2019). It is possible that barn design may affect the number of meals as well as the definition of meal criterion used to define a meal (DeVries et al., 2003), parity (Azizi et al., 2009), or formulation of the PMR.

4.4.2 Milk production and milking characteristics

To date few studies have been conducted evaluating AMS concentrate allowances and the effect on milking frequency. We did not observe any evidence for increased milking frequency with increased AMS pellet allowance as cows provided LP had greater milking frequency compared to HP, and milking frequency increased more rapidly during the first 2 wk of lactation for LP as compared to HP. In fact, there is evidence that cows offered the HP allocations had less motivation to enter the AMS based on increased holding area time from wk 1 to 4 of lactation. A previous study (Johnson et al., 2022) suggested that longer holding area times may be reflective of less motivation to visit the AMS in guided-traffic flow barns; however, that study evaluated form of the AMS concentrate rather than the amount provided as in the present study. The greater milking frequency for LP relative to HP likely aided in maintaining increased milk production beyond the first 4 wk. Greater milking frequency in early lactation (Hale et al., 2003; Patton et al., 2006) results in carryover effects on milk yield for the remainder of lactation. Greater milking frequency in early lactation may have supported the greater milk and milk component yield for LP from wk 5 to 8 of lactation. Given the lack of difference for BW, BCS, and blood metabolites in addition to weekly milk BHB values from wk 5 to 8 suggest that there was no negative impact on energy status.

In the current study, there was a treatment by parity interaction for fetched milkings. For multiparous cows, concentrate allocation through the AMS did not affect number of fetched milkings; however, primiparous cows fed LP tended to require fewer fetched milkings during the first 4 wk as compared to HP. It is not unexpected that multiparous cows required less fetching as they had been exposed to the AMS in previous lactations. Spolders et al. (2004) reported that cows having prior contact with an AMS adapted to voluntary milking quicker than those that had no prior contact. With regard to the quantity of concentrate, Bach et al. (2007) found that offering a high amount of concentrate did not reduce the need to fetch cows that would not otherwise visit voluntarily. In contrast, Schwanke et al. (2019) reported that offering 6.0 vs. 3.0 kg/d of concentrate through the AMS reduced the need to fetch early to mid-lactation primiparous cows in a free-flow system. Research is needed to evaluate factors that affect motivation of cows to enter the AMS and whether factors change during lactation.

Fetching is typically increased in early lactation as compared to mid- and late-lactation (Henriksen et al., 2019) in free-flow and guided-traffic barns, with guided-traffic barns being associated with less overall fetching (Siewert et al., 2019). Previous studies have reported no difference in milkings per day or change in the number of fetched cows when mid to late lactation cows were offered a greater quantity of concentrate managed in a guided-traffic system (Hare et al., 2018; Menajovsky et al., 2018). However, greater concentrate allocation has resulted in greater milkings and less fetching with early- to mid-lactation cows in free-traffic systems in some (Bach et al., 2007; Schwanke et al., 2019) but not all studies (Henricksen et al., 2018a). While it is possible that the traffic system of the barn influenced the number of milkings due to the use of preselection gates (Jacobs and Siegford, 2012), it is important to recognize that cows must be motivated to move through gates for there to be a milking event. All cows in the current study were managed alike, therefore, there was an aspect of the LP treatment that motivated cows to move through the facility more, resulting in more milkings.

Cows offered LP had greater milk and milk component yield, which resulted in greater ECM yield throughout the 8-wk study period. While it has been suggested that offering more concentrate through the AMS to match milk and milk component yields of high producing cows may improve energy balance (King et al., 2018), in the current study there was no difference in BW or BCS loss from calving to 28 or 56 DIM between treatments, and no difference in blood

metabolites measured at 7, 21, 28, or 56 DIM. Moreover, serum BHBA levels did not reach thresholds (> 1.2 mg/L BHB in serum; LeBlanc, 2010) to suggest cows were experiencing subclinical or clinical ketosis for any treatment at any timepoint. It should be noted that blood samples were collected at 0900 h. Supporting blood results, milk samples collected throughout the study were also analyzed for BHB concentration at each sampling point and concentrations did not exceed the threshold for ketosis at any timepoint (> 1.5 mmol/L BHB in milk; Denis-Robichard et al., 2014, Santschi et al., 2016). Thus, the increased milk and milk component yield for cows offered LP did not come at the expense of increased body tissue mobilization. We speculate that greater nutrient consumption due to less substitution of PMR from the AMS pellet and potentially due to greater digestibility as variability in AMS pellet consumption was less helped to support the greater milk component production. As such, the data in the present study are interpreted to challenge previous results suggesting greater AMS concentrate allocations may improve energy balance and milk and milk component yields. It should be recognized that survey-based studies cannot infer cause nor effect and feed tables implemented in AMS often provide greater quantities of concentrate for cows that have greater milk production (Siewert et al., 2017).

While ruminal fermentation parameters were not measured in the current study, previous literature has found a greater risk for subacute ruminal acidosis for cows managed with AMS (Huot et al., 2023). In addition, provision of a gradual adaptation to concentrates as a means to adapt the rumen microbiota and ruminal epithelia to a lactating diet may improve energy intake and production responses (Dieho et al., 2017; Humer et al., 2018). While we found no difference between HPM and HPR treatments, it is possible that the increased variability in AMS pellet intake seen with HP relative to LP negatively affected ruminal pH or fermentation patterns leading to an inflammatory response. Previous literature has shown that inflammatory responses increase energy and amino acid demands (Krogstad and Bradford, 2023; Horst et al., 2021; Horst et al., 2019), and can occur from several reasons including acute and chronic diseases, negative energy balance, or compromised gastrointestinal barrier function. Unfortunately, the data obtained from the current study cannot substantiate, what, if any, implication this may have had. Nonetheless, it is possible that cows experienced a certain level of inflammation such that HP had reduced milk production as compared to LP.

4.5 Conclusion

Increasing the AMS pellet allocation to early lactation dairy cows in a guided traffic flow barn reduced PMR intake, did not alter total DMI, reduced AMS milking frequency, and milk and milk component yields. Increasing concentrate to early lactation cows, beyond 3 kg/d, did not result in the ability to precision feed, and may not be beneficial in promoting improved animal performance. Consideration of the substitution rate, PMR nutrient density and the impact they may have on intake of nutrients for fresh cows is paramount in designing a successful transition program for cows managed on an AMS immediately following calving.

4.6 Literature cited

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4.7 Tables and figures

Table 4.7-1 Ingredient and nutrient composition of the total mixed ration during the close-up period, the partial mixed ration (PMR), and the pellet offered in the automated milking system (AMS) fed for the duration of the study.

Item	Close-up TMR	Lactating PMR	AMS pellet
Ingredient, % DM			
Barley silage	17.4	16.4	-
Corn silage	17.4	20.6	-
Alfalfa hay	-	10.8	-
Straw	33.3	3.1	-
Beet pulp	-	5.3	24.5
Barley grain	-	13.9	46.7
Corn grain	-	5.7	19.1
Dry molasses	-	-	9.7
Soybean meal	4.0	4.5	-
Canola meal	10.1	11.1	-
Vit/min mix[1]	10.3	4.0	-
Palmitic acid[2]	-	1.9	-
Bypass fat[3]	-	2.7	-
Bypass choline[4]	0.4	-	-
Anionic supplement[5]	7.3	-	-
Nutrient Content (mean ± SD)			
% DM[6]	50.1 ± 1.2	50.1 ± 1.2	88.6 ± 0.50
CP, % DM	13.9 ± 0.28	16.2 ± 0.33	11.3 ± 0.57
ADF, % DM	31.3 ± 0.76	20.0 ± 1.25	11.5 ± 0.22
NDF, % DM	45.6 ± 0.56	32.5 ± 1.10	22.2 ± 0.95
Forage NDF, % DM	40.9 ± 0.75	23.5 ± 1.25	-
Starch, % DM	12.8 ± 0.41	21.4 ± 0.58	40.4 ± 1.26
NFC, % DM	25.7 ± 0.39	37.7 ± 1.02	62.1 ± 0.66
NSC, % DM	15.0 ± 0.49	24.8 ± 0.76	46.8 ± 1.29

[1]Prepartum contained: 4.83% Ca, 1.79% P, 0.23% Mg, 0.46% K, 0.73 Na, 852 mg/kg Fe, 121 mg/kg Cu, 52 mg/kg Mn, 87 mg/kg Zn, 2.64 mg/kg Se, 62.4 KIU/kg vitamin A, 17.5 KIU/kg vitamin D and 1,158 IU/kg vitamin E.

Postpartum contained 12.5% Ca, 2.12% P, 2.31% Mg, 2.68% K, 6.01% Na, 1,243 mg/kg Fe, 117 mg/kg Cu as copper sulfate and 120 mg/kg as methionine-copper chelate, 195 mg/kg Mn, 662 mg/kg Zn, 4.43 mg/kg Se, 144.4 KIU/kg vitamin A, 32.1 KIU/kg vitamin D and 779 IU/kg

vitamin E.

[2]Palmitic acid source was Enervive (95% C:16; sourced through Cargill Animal Nutrition, North Battleford, SK).

[3]Bypass fat source was Essentiom (minimum 80% fatty acids; sourced through Cargill Animal Nutrition, North Battleford, SK).

[4]Bypass choline source was ReaShure (Balchem, New Hampton, NY).

[5]Anionic supplement used was SoyChlor (Halchemix, Port Perry, ON).

[6]Based off DM coefficients of each ingredient, water was added to the TMR or PMR daily to maintain 50% DM.

Table 4.7-2 Body weight and body condition score of cows at 28 ± 3 d prior to expected calving date, average dry matter intake (DMI) during the prepartum period and blood metabolites at 7 ± 3 d prior to expected calving date of cows offered a high or low allocation of AMS pellet following calving.

		HP		SEM	*P*- value[1]	
Item	LP	HPM	HPR		LP vs. HP	HPM vs. HPR
n	20	20	19			
Pre-partum period, d	24	23	25	1.0	0.68	0.11
BW at d -28 ± 3[2], kg	740	714	681	18.5	0.06	0.21
BCS at d -28 ± 3[2]	3.42	3.45	3.38	0.08	0.96	0.52
Prepartum DMI[2], kg/d	11.2	13.0	12.2	0.63	0.02	0.22
Blood metabolites at d -7 ± 3						
BHB, mg/dL	5.79	5.79	5.36	0.039	0.68	0.47
Glucose[2], mg/dL	70.6	77.8	73.6	2.49	0.11	0.28
NEFA, mEq/L	508	360	372	56.5	0.06	0.89

[1]Parity × treatment did not reach significance for any variable ($P > 0.46$).

[2]Parity, $P < 0.05$.

Table 4.7-3 Effects of providing a low (LP; 3 kg/d; DM basis) automated milking system (AMS) pellet allowance or adapting cows to a high (HP; 8 kg/d; DM basis) allowance using a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) adaptation rate during the first 4 wk of lactation on AMS pellet intake, partial mixed ration (PMR) intake, and total DMI.

		HP			*P* – value[1]			
Item	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR	Wk	Trt × Wk
AMS pellet [2], kg/d				0.223	<0.01	0.72	<0.01	<0.01
wk 1[y]	2.57[c]	2.85[bc]	2.95[bc]	0.121	0.02	0.65		
wk 2[x]	2.99[b]	4.38[a]	4.16[a]	0.224	<0.01	0.52		
wk 3[x]	3.00[b]	4.60[a]	4.45[a]	0.251	<0.01	0.71		
wk 4[x]	3.02[b]	4.47[a]	4.34[a]	0.273	<0.01	0.75		
PMR intake[2], kg/d				0.788	<0.01	0.74	<0.01	0.04
wk 1[z]	13.0[efg]	12.3[fg]	12.5[g]	0.691	0.44	0.81		
wk 2[y]	15.7[cd]	13.8[de]	13.6[ef]	0.623	0.01	0.74		
wk 3[x]	17.6[b]	16.2[bc]	14.9[cdef]	0.768	0.03	0.21		
wk 4[w]	19.2[a]	15.4[bc]	15.5[bcd]	0.778	<0.01	0.91		
DMI intake[2], kg/d				0.828	0.22	0.65	<0.01	0.57
wk 1[z]	15.7	16.0	15.5					
wk 2[y]	19.4	19.3	18.1					
wk 3[x]	20.9	21.2	19.5					
wk 4[w]	22.5	21.6	20.2					

[1]Trt = treatment, Wk = week of lactation; the interactions of treatment × parity, week × parity, or treatment × week × parity did not reach significance for any variable ($P > 0.12$).

[2]Primi-parous cows consumed less AMS pellet and PMR and had lower DMI intake as compared to multiparous cows (parity, $P < 0.02$).

[a-g]Means with different superscripts differ ($P < 0.05$) for the treatment × week interaction.

[w-z]Means in the same column with different superscripts differ ($P < 0.05$) for the week response.

Table 4.7-4 Partial mixed ration (PMR) feeding, and automated milking (AMS) pellet consumption behavior of cows offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount AMS pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate during the first 4 wk of lactation.

Item	LP	HP		SEM	*P* – value[1]			
		HPM	HPR		LP vs. HP	HPM vs. HPR	Wk	Trt*Wk
AMS pellet dispensed, kg/milking				0.183	0.03	0.70	<0.001	0.003
wk 1[z]	0.97[b]	1.11[b]	1.20[b]	0.122	0.21	0.68		
wk 2[y]	0.97[b]	1.46[a]	1.56[a]	0.151	0.01	0.62		
wk 3[x]	0.97[b]	1.58[a]	1.67[a]	0.208	0.02	0.75		
wk 4[x]	1.00[b]	1.54[a]	1.63[a]	0.215	0.03	0.73		
Standard deviation for daily AMS pellet, kg/d[2,3]				0.066	<0.001	0.68	0.09	0.002
wk 1	0.59[d]	0.87[bc]	0.75[cd]	0.092	0.01	0.22		
wk 2	0.38[e]	0.76[bcd]	0.82[bc]	0.101	<0.001	0.57		
wk 3	0.40[e]	0.78[bcd]	0.92[ab]	0.11	<0.001	0.19		
wk 4	0.40[e]	1.05[a]	0.86[bc]	0.081	<0.001	0.02		
PMR meals, no./d				0.44	0.44	0.06	0.03	0.88
wk 1[y]	6.3	6.3	7.0					
wk 2[x]	6.8	6.6	7.7					
wk 3[x]	7.2	7.1	7.8					
wk 4[x]	6.8	6.5	7.8					
PMR meal size, kg				0.201	0.08	0.06	<0.01	0.59
wk 1[z]	2.25	2.16	1.82					
wk 2[yz]	2.39	2.30	1.85					
wk 3[y]	2.55	2.55	1.99					
wk 4[x]	2.96	2.61	2.08					
PMR meal duration, min/meal				1.71	0.12	0.12	<0.01	0.25
wk 1[z]	20.6	20.3	19.3					
wk 2[z]	23.2	22.9	19.0					
wk 3[y]	26.1	25.6	21.5					
wk 4[x]	30.5	27.1	23.1					
PMR eating rate, g/min				0.074	0.35	0.49	0.05	0.72
wk 1[x]	10.7	10.7	9.7					
wk 2[x]	10.5	9.9	9.9					
wk 3[y]	9.9	9.7	9.3					
wk 4[y]	9.9	9.6	9.0					
Standard deviation for daily PMR intake, kg/d				0.189	0.43	0.92	<0.001	0.21
wk 1[x]	2.36	1.99	1.87					
wk 2[y]	1.54	1.42	1.50					
wk 3[z]	1.42	1.26	1.62					
wk 4[z]	1.36	1.65	1.26					

[1]Trt = treatment, Wk = week of lactation.

[2]Multi-parous cows had lower standard deviation in AMS pellet intake as compared to primi-parous cows (parity, $P < 0.03$).

[3]Cows fed LP had a lower standard deviation in daily AMS pellet as compared to HP. Primi-parous HPM cows had greater deviation than multiparous HPM, but were not different from primi- or multi-parous HPR cows (treatment × parity, $P < 0.04$).

[a-d]Means with different superscripts differ ($P < 0.05$) for the treatment × week response.

[x-z]Means in the same column with different superscripts differ ($P < 0.05$) for the week response.

Table 4.7-5 Sorting index for the partial mixed ration (PMR) when cows were offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate during the first 4 wk of lactation.

		HP			*P* – value[1]			
PMR Sorting Index[2], %	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR	Wk	Trt × Wk
Particles > than 19 mm				3.98	0.51	0.84	<0.01	0.97
wk 1[x]	107.9	103.2	102.7					
wk 2[y]	94.5*	94.1*	94.6[§]					
wk 3[y]	95.8*	94.9*	95.1[§]					
wk 4[z]	96.1*	91.2*	94.5*					
Particles 8 to 19 mm				1.2	0.97	0.33	<0.01	0.69
wk 1[x]	103.9*	101.0	103.4[§]					
wk 2[y]	100.1	100.3	101.1					
wk 3[y]	99.9	100.4	101.2					
wk 4[y]	100.0	100.0	100.2					
Particles 4 to 8 mm				1.54	0.34	0.97	0.06	0.97
wk 1	97.1*	97.0[b]	95.7*					
wk 2	99.8	98.1[b]	99.1					
wk 3	100.2	99.5	98.8					
wk 4	100.7	98.7	99.9					
Pan (<4 mm)				2.17	0.32	0.74	0.02	0.64
wk 1[z]	93.2*	98.3	96.8*					
wk 2[y]	99.8	100.4	99.3					
wk 3[y]	99.1	99.5	99.6					
wk 4[x]	97.5	101.8	100.4					

[1]Trt = treatment, Wk = week of lactation; the effect of parity nor the interactions of week × parity, parity × treatment, or treatment × week × parity did not reach significance for any variable ($P > 0.08$).

[2]Sorting index was calculated using the description by Leonardi and Armentano (2003). Values greater than 100 indicated selective consumption, while those less than 100 indicated selective avoidance.

*Significantly different from a sorting index of 100 ($P < 0.05$).

[§]Tendency ($0.05 < P < 0.10$) for the sorting index to be different from 100.

[x-z]Means in the same column with different superscripts differ ($P < 0.05$) for the week response.

Table 4.7-6 Milking characteristics of cows offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate during the first 4 wk of lactation.

		HP			*P* – value[1]			
Item	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR	Wk	Trt ×Wk
Milkings, no./d[2]				0.118	0.02	0.96	0.02	0.42
wk 1[z]	3.04	2.98	2.97					
wk 2[y]	3.36	3.08	3.04					
wk 3[y]	3.39	3.08	3.10					
wk 4[x]	3.45	3.03	3.10					
Milking duration[2], min/milking				2.562	0.84	0.54	< 0.01	0.27
wk 1[z]	6.85	7.22	6.33					
wk 2[y]	7.97	7.70	7.52					
wk 3[x]	8.40	8.33	8.22					
wk 4[x]	8.15	8.45	8.13					
Milking duration[2], min/d				10.58	0.84	0.54	<0.01	0.27
wk 1[z]	20.8	21.5	18.8					
wk 2[y]	26.8	23.7	22.8					
wk 3[x]	28.5	25.7	25.5					
wk 4[x]	28.1	25.6	25.2					
Milking interval[2], min				18.43	<0.01	0.98	0.10	0.48
wk1	465.8	482.1	492.1					
wk2	434.3	485.5	494.1					
wk3	419.0	471.1	472.7					
wk4	415.9	484.1	465.9					
Time in holding area[2], min/milking				10.54	0.06	0.91	0.22	0.06
wk 1	77.4	89.7	81.2					
wk 2	70.4	75.1	88.8					
wk 3	60.2	76.9	94.7					
wk 4	49.7	88.9	71.1					
Time in holding area[2], min/d				26.32	0.17	0.86	0.07	0.31
wk 1	212	245	216					
wk 2	215	208	239					
wk 3	191	214	272					
wk 4	163	249	209					
Incomplete milkings, %				2.36	0.19	0.76	0.67	0.05
wk1	7.3	5.6	1.2					
wk2	10.0	3.0	4.6					
wk3	7.7	3.0	3.5					

wk4	4.1	6.5	5.4					
SCC, cells × 1,000				81.21	0.72	0.70	0.11	0.96
wk 1	181.7	259.9	219.9					
wk 2	77.0	93.8	116.5					
wk 3	98.9	80.8	192.6					
wk 4	100.9	56.7	94.8					

[1]Trt = treatment, Wk = week of lactation; the interactions of week × parity, parity × treatment, or treatment × week × parity did not reach significance for any variable ($P > 0.24$).

[2]Primi-parous cows had fewer milkings per day and longer milking duration as compared to multi-parous cows. Primi-parous cows had greater milking interval and time in the holding area as compared to multi-parous cows (parity, $P < 0.01$).

[x-z]Means in the same column with different superscripts differ ($P < 0.05$) for the week response.

Table 4.7-7 Effect of dietary treatments on percentage of fetched milkings for primi- and multiparous cows[1] when offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate during the first 4 wk of lactation.

		HP			*P* – value[2]			
Fetched milkings, %	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR	Wk	Trt × Wk
Primi-parous				6.05	0.07	0.63	0.39	0.81
wk 1	15.6	13.8	21.3					
wk 2	5.8	15.9	20.6					
wk 3	2.3	9.3	14.1					
wk 4	0.6	15.6	10.3					
Multi-parous				1.38	0.70	0.92	0.02	0.29
wk 1[x]	5.9	3.0	3.0					
wk 2[y]	2.0	1.8	1.3					
wk 3[y]	1.5	0.7	0.5					
wk 4[y]	0.4	2.8	3.1					

[1]The interaction of treatment × parity was significant ($P = 0.04$) for fetched milkings therefore data is presented by parity.

[2]Trt = treatment, Wk = week of lactation.

[x,y]Means in the same column with different superscripts differ ($P < 0.05$) for the Wk response.

Table 4.7-8 Effects of dietary treatments on milk yield and milk components during the first 4 wk of lactation for cows offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate.

Item	LP	HP		SEM	*P* – value[1]			
		HPM	HPR		LP vs. HP	HPM vs. HPR	Wk	Trt × Wk
Milk yield[2], kg/d				1.44	0.01	0.83	0.65	0.48
wk 1	32.5	29.2	29.8					
wk 2	43.6	39.5	38.9					
wk 3	47.2	43.8	43.1					
wk 4	49.7	44.7	43.8					
Fat, %				0.173	0.21	0.32	<0.01	0.21
wk 1[x]	4.40	4.00	4.50					
wk 2[y]	4.08	4.00	4.00					
wk 3[z]	3.78	3.34	3.70					
wk 4[z]	3.78	3.51	3.38					
Protein[3], %				0.054	0.14	0.44	<0.01	0.81
wk 1[w]	3.73	3.84	3.81					
wk 2[x]	3.34	3.43	3.43					
wk 3[y]	3.08	3.21	3.12					
wk 4[z]	2.99	3.11	3.01					
Lactose, %				0.039	0.81	0.88	<0.01	0.67
wk 1[z]	4.40	4.45	4.39					
wk 2[y]	4.57	4.56	4.55					
wk 3[x]	4.65	4.62	4.63					
wk 4[x]	4.63	4.60	4.40					
MUN, mg/dL				1.03	0.30	0.55	0.01	0.61
wk 1[z]	11.3	10.1	12.2					
wk 2[z]	12.2	10.2	10.6					
wk 3y	13.9	12.3	12.2					
wk 4[x]	13.8	13.3	13.7					
Fat[2], kg				0.098	0.02	0.67	<0.01	0.33
wk 1[z]	1.43	1.12	1.34					
wk 2[y]	1.76	1.58	1.56					
wk 3[xy]	1.78	1.47	1.59					
wk 4[x]	1.86	1.52	1.47					
Protein[2], kg				0.048	0.04	0.80	<0.01	0.32
wk 1[z]	1.21	1.23	1.14					
wk 2[y]	1.45	1.35	1.34					
wk 3[y]	1.45	1.40	1.34					
wk 4[x]	1.50	1.33	1.31					
Lactose[2], kg				0.066	0.01	0.92	<0.01	0.27
wk 1[z]	1.45	1.30	1.32					

wk 2[y]	1.99	1.80	1.78					
wk 3[xy]	2.18	2.02	1.99					
wk 4[x]	2.30	1.96	2.03					
ECM, kg/d				1.93	0.01	0.83	<0.01	0.23
wk 1[y]	37.8	33.0	35.3					
wk 2[x]	47.4	43.0	42.5					
wk 3[x]	48.9	43.4	44.3					
wk 4[x]	51.0	43.3	42.7					
Feed efficiency (kg ECM/ kg DMI)				0.112	0.06	0.19	0.02	0.78
wk 1[xy]	2.46	2.04	2.32					
wk 2[x]	2.49	2.24	2.36					
wk 3[y]	2.38	2.04	2.29					
wk 4[y]	2.30	2.08	2.14					

[1]L = low, H = high pellet allocation; M = moderate and R = rapid robot pellet adaption; Trt = treatment, Wk = week of lactation; the interactions of treatment × parity or treatment × week × parity did not reach significance for any variable ($P > 0.11$).

[2]Primi-parous cows had lower milk yield, and fat, protein, and lactose yields as compared to multi-parous cows (parity, $P < 0.001$).

[3]During wk 1, primi-parous cows had lower milk protein concentration than multi-parous cows (parity × week $P < 0.001$).

[x,y,z]Means in the same column with different superscripts differ ($P < 0.05$) for the week response.

Table 4.7-9 The effects of offering a low (LP; 3 kg/d; DM basis) or high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate during the first 4 wk of lactation on BW, BCS and blood metabolites.

		HP			*P* – value[1]			
Item	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR	Time	Trt × Time
BW[2,3], kg				14.2	0.11	0.17	<0.01	0.95
Calving	702	695	667					
d 28	659	644	618					
BCS[2]				0.082	0.95	0.64	<0.01	0.93
Calving	3.24	3.31	3.23					
d 28	3.05	3.06	3.06					
Glucose[2], mg/dL				1.75	0.33	0.67	<0.01	0.97
d 7	57.9	60.1	58.5					
d 21	59.7	62.4	61.9					
d 28	62.0	63.5	62.9					
BHB, mg/dL				0.103	0.54	0.89	0.99	0.93
d 7	0.71	0.71	0.76					
d 21	0.69	0.81	0.71					
d 28	0.67	0.75	0.76					
NEFA[2], mEq/L				67.7	0.63	0.37	<0.01	0.70
d 7	714	603	629					
d 21	495	502	611					
d 28	501	420	479					

[1]Trt = treatment; the interactions of treatment × parity or treatment × time × parity did not reach significance for any variable ($P > 0.22$).

[2] Primi-parous cows had lower BW but greater BCS as compared to multi-parous cows. Primi-parous cows had higher glucose and lower NEFA concentrations as compared to multi-parous cows (parity, $P < 0.02$).

[3]Parity × Time $P < 0.02$).

Table 4.7-10 The effects of dietary treatment on average pellet dispensed, milking characteristics, milk yield and milk components from wk 5 to 8 when cows were offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d) rate.

Item	LP	HP		SEM	*P* – value[1]			
		HPM	HPR		LP vs. HP	HPM vs. HPR	Wk	Trt×Wk
Pellet dispensed, kg/d	2.97	4.41	3.90	0.27	<0.01	0.19	0.25	0.74
Milkings, no./d	2.96	2.89	2.84	0.118	0.45	0.66	0.49	0.93
Milking duration[2], min/milking	9.05	8.98	8.53	0.352	0.57	0.46	0.18	0.08
Milking duration, min/day	26.8	26.0	23.6	0.92	0.48	0.35	0.22	0.09
Inter-milking interval, min	479.6	499.7	509.7	20.5	0.25	0.70	0.19	0.77
Time in holding area[2], min/milking	49.8	52.7	68.0	7.53	0.19	0.11	0.38	0.98
Time in holding area[2], min/d	140	148	180	20.4	0.24	0.18	0.39	0.99
Fetched milkings[2], %	7.25	5.10	8.70	2.28	0.89	0.13	0.28	0.31
Milk yield[3], kg/d	49.33	45.15	44.62	1.61	<0.01	0.77	0.15	0.96
Fat, %	3.55	3.34	3.49	0.178	0.45	0.50	0.02	0.82
Protein, %	3.01	3.07	3.03	0.052	0.4	0.51	0.82	0.84
Lactose, %	4.64	4.61	4.61	0.05	0.62	0.98	0.77	0.96
Fat yield[3], kg/d	1.73	1.51	1.55	0.103	0.04	0.81	0.01	0.87
Protein yield[3], kg/d	1.48	1.38	1.35	0.052	0.02	0.52	0.07	0.94
Lactose yield[3], kg/d	2.29	2.08	2.06	0.079	<0.01	0.73	0.28	0.96
ECM[3], kg/d	49.4	44.3	44.2	1.57	0.01	0.96	<0.01	0.85
MUN, mg/dL	14.3	13.9	14.9	1.18	0.94	0.53	0.91	0.89
SCC, cells × 1000	100.8	138.8	102.6	51.9	0.68	0.52	0.21	0.03
Incomplete milkings[2], %	4.95	8.03	5.78	2.51	0.4	0.43	0.25	0.14

[1]Trt = treatment, Wk = week of lactation; the interactions of parity × week, treatment × parity, or treatment × week × parity did not reach significance for any variable ($P > 0.14$).

[2] Primi-parous had greater milking duration, time spend in the holding area and fetched and incomplete milkings as compared to multi-parous cows (Parity $P < 0.05$).

[3] Primi-parous had lower milk, milk component yield and energy corrected milk as compared to multi-parous cows (Parity $P < 0.05$)

Table 4.7-11 Effects of dietary treatments on BW, BCS and blood metabolites at 56 DIM when cows were offered a low (LP; 3 kg/d; DM basis) or adapted to a high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet at a moderate (HPM; increased from 3 to 8 kg/d over 15 d) or rapid (HPR; increased from 3 to 8 kg/d over 5 d).

					P – value[1]	
Variable on 56 DIM	LP	HPM	HPR	SEM	LP vs. HP	HPM vs. HPR
BW[2], kg	669	657	632	14.2	0.16	0.22
BCS	2.91	2.92	2.85	0.13	0.81	0.63
Glucose[2], mg/dL	65.5	66.5	67.1	1.06	0.31	0.70
BHB, mg/dL	0.61	0.63	0.61	0.03	0.79	0.58
NEFA, mEq/L	309	325	343	33.4	0.54	0.71

[1]The interactions of parity × week, treatment × parity, or treatment × week × parity did not reach significance for any variable ($P > 0.20$).

[2]Primi-parous cows had lower BW and greater glucose concentrations as compared to multi-parous cows (Parity, $P < 0.04$).

Table 4.7-12 Formulated and observed PMR and AMS concentrate intake, and corresponding predicted ME and MP allowable milk yield for cows offered a low (LP; 3 kg/d; DM basis) or high (HP; 8 kg/d; DM basis) amount of automated milking system (AMS) pellet.

Variable	LP	HP
Formulated		
PMR intake, kg	25.0	20.0
Pellet dispensed, kg	3.00	8.00
Predicted ME allowable milk[1], kg	49.1	49.7
Predicted MP allowable milk[1], kg	45.8	46.4
Observed		
PMR intake, kg	16.4	14.3
Pellet dispensed, kg	2.85	4.03
Predicted ME allowable milk[1], kg	30.5	28.5
Predicted MP allowable milk[1], kg	29.8	28.4
ECM yield, kg	46.3	40.9

[1] Allowable milk predicted by the Cornell Net Carbohydrate and Protein System (6.55) platform of NDS (The RUM&N Company, Reggio Emilia, Italy).

Chapter 5: General Discussion

5.1 Summary

Over the last several decades, transition cow health and management has been a topic of great interest as its importance to lactation performance is paramount. While extensive research has been conducted evaluating prepartum diets, little research has evaluated dietary strategies immediately following calving, or the interaction that may exist between pre- and post-partum diets. In addition, little controlled research has evaluated dietary strategies during the immediate postpartum period when cows are managed with automated milking systems (AMS).

Within this thesis, Chapter 2 evaluated the effects of starch content of pre- and postpartum diets on productivity, metabolites, and markers of inflammation. A high or low starch diet was fed pre- and postpartum, and lactation performance measured until 28 DIM. While I had hypothesized that there would be an interaction between pre- and postpartum diets, that was not the case for many response variables. The results for effect of prepartum dietary strategy align with what has been previously reported whereby feeding a high starch prepartum diet resulted in greater DMI and plasma concentrations of insulin and glucose prepartum as compared to controlled energy diets (Dann et al., 2006; Douglas et al., 2006; Janovick et al., 2011). This was associated with cows fed high starch prepartum having increased free-fatty acids and milk fat yield postpartum indicating greater fat mobilization. In addition, concentrations of GLP-2, a peptide associated with increased intestinal villus height and mucosal surface (Kvidera et al., 2017a), as well as enhanced glucose and peptide transporter expression (Connor et al., 2015a, 2016), was increased when cows were fed a high-starch prepartum diet. While its specific implications are not known, the increased GLP-2 concentrations may be beneficial to gut health during the transition period.

Regardless of starch content of prepartum diets, cows fed a high starch diet postpartum had lower plasma free-fatty acids and serum haptoglobin, suggesting a benefit to feeding high-starch diets postpartum. I had hypothesized that the transition from a low starch prepartum to high starch postpartum diet would increase markers of inflammation due to the potential for reduced rumen pH and cows experiencing acidosis; however, I found this to not be the case. Interestingly, cows fed a high-starch postpartum diet had lower concentrations of haptoglobin

and serum amyloid A. Previous literature has found that fatty acids can influence inflammatory responses (Sordillo et al., 2009), and I believe that the lower inflammatory markers may be attributed to reduced fat mobilization for cows offered a high starch diet postpartum.

When cows are managed with AMS, they are fed a partial mixed ration (PMR) at the bunk and concentrate in the AMS. However, little research has evaluated the effects of different nutrient compositions of the concentrate. Thus, in Chapter 3, I evaluated the effect of pellet type and feeding amount on feeding behavior and rumen fermentation of mid-lactation dairy cows in a 4 × 4 Latin square design study. In this study, a high starch or high fiber pellet was fed at 1 or 3 kg twice per day to mid-lactation cows housed in a tie-stall facility. In this study, a lower substitution rate between concentrate and PMR was observed when a high starch pellet was fed. This was opposite to what was expected, as I hypothesized that the high starch pellet would result in reduced ruminal pH, which was not the case. In fact, feeding a high starch pellet (alongside a high fiber PMR) resulted in a reduction in the duration that ruminal pH was below 5.8 and reduction in severity of pH depression. Feed disappearance following feed delivery was reduced for cows fed the high starch pellet, likely due to the PMR being higher in fiber with this treatment. Cows fed higher amounts of pellet per meal consumed more DMI, with a substitution rate of less than 1 regardless of type of pellet fed. This is believed to be due to the composition of the PMR as when cows were fed a low amount of pellet, a low forage PMR was fed as compared to when the high amount of pellet was offered. The results of this study suggested that feeding starch in a pellet at a set quantity did not cause reductions in rumen pH or changes to feed intake pattern and that the composition of the PMR likely plays a greater role in feed intake pattern and substitution than the pellet.

Further building on the findings of Chapters 2 and 3 in Chapter 4, I aimed to evaluate the effects of concentrate allowance amounts and rate of adaptation to a high amount of concentrate on fresh cow performance when milked with AMS. In this study, cows were managed similarly during the prepartum period and offered either a high (8 kg/d) or low (3 kg/d) amount of concentrate through the AMS postpartum. Cows offered high were adapted to their pellet allowance at either a moderate (over 15 d) or rapid rate (over 5 d). Cows offered the high amount of concentrate did not reach target level of pellet intake; however, did consume more than low cows for the duration of the study. Similarly, there was no difference in pellet intake between the

rapid or moderate treatments. While there are many contributing factors, the inability to achieve target allowance has been previously found in the literature (Bach et al., 2007; Menajovsky et al., 2018; Henricksen et al., 2019), and in the current study cows offered high had increased variability in pellet intake. Cows offered high consumed less PMR as compared to low; however, there was no difference in total DMI, and substitution rate was greater than 1 for the first 4 wk of lactation. This finding supports the concept that offering more AMS concentrate may not be beneficial to increase total DMI or nutrient intake (Bach and Cabrera 2017; Henricksen et al., 2018a). I found no difference in sorting behavior among treatments. Cows offered high AMS concentrate had smaller meals with no difference in meal duration or eating rate, likely due to the reduced PMR intake observed. Offering a high concentrate allowance did not increase number of milkings per day, milk or milk component yield, and there were no detectable difference in BCS, BW change or blood metabolites indicating no difference in energy status among treatments. There were few notable differences between providing the high treatment at a moderate or rapid rate, suggesting that both would be appropriate should offering a high allowance of concentrate be employed. The findings of this study suggest further work is warranted in understanding the mechanisms behind substitution rate to optimize total DMI of cows managed with AMS. However, offering an increased amount of AMS concentrate did not result in improvements in early lactation performance.

5.2 Considerations and future studies

While the findings of Chapter 2 suggest that offering a high starch diet immediately after calving, regardless of prepartum diet, improved energy status of dairy cows, the potential for negative consequences on ruminal pH and ruminal health should not be overlooked. The concept of a controlled energy prepartum diet has been derived from primarily corn-silage based feeding scenarios. While guidelines for what a controlled energy diet should be comprised of from a nutrient perspective (eg. net energy of lactation or starch content) exists, the physical characteristics of corn vs. barley silage must also be considered when formulating diets. Implementation of a controlled energy corn silage based diet is typically done with the inclusion of low quality forages such as straw. While not evaluated within Chapter 2, in practice, I was unable to feed high enough quantities of straw alongside the barley silage to adequately target a true controlled energy diet. Doing so would have potentially limited intake and encouraged

sorting, factors that I wanted to minimize. Therefore, while the study was designed to evaluate high and low energy diets pre-partum through different starch concentrations, both pre-partum diets substantially exceeded energy requirements. In addition, it should be noted that the low starch diet was not as low as what may be seen in industry and could be classified as a moderate starch diet. Because of this, it is important to interpret results cautiously as had the pre-partum diet been lower starch there may be a negative impact on animal performance that measured variables within this study were unable to evaluate. Therefore, changes to energy mobilization and animal performance may be different if a true controlled energy diet were evaluated prior to calving.

It should also be noted that source of starch, both pre and post-partum may play a role in how cows adapt to high-starch postpartum diets. The primary source of starch pre-partum was barley through the barley silage or barley grain, which may have aided in adapting cows to a rapidly fermentable starch prior to the transition to the postpartum diet. Furthermore, the additional starch postpartum between the two diets was provided as corn grain rather than barley grain. As ruminal fermentation of corn is a slower compared to barley (Overton et al., 1995), it is possible that this aided in providing cows additional energy postpartum without any negative consequences to ruminal fermentation dynamics.

In addition, cows were offered free choice hay postpartum, which may have acted as a buffer to minimize any negative effects that cows could have experienced immediately following calving. It is reported that cows may selectively consume longer particles of feed to help buffer rumen pH (Keunen et al., 2002), therefore, it is possible that through consumption of hay, cows did not experience large drops in rumen pH or experience subacute ruminal acidosis associated with the diet change after calving. Similarly, Engelking and Oba (2023) reported a reduction in serum inflammatory markers when cows were offered free-choice hay, separate from the TMR immediately following parturition. Unfortunately, Chapter 2 did not evaluate any metrics to quantify this hypothesis and additional evaluation of the effect that hay consumption may have postpartum is warranted.

Further research evaluating the effect of source of starch on animal performance through the transition, and strategies for feeding cows during the transition period using barley silage as a primary forage are also warranted. In many instances, increased starch content of barley silage is

associated with increased plant maturity at harvest and plant material becoming more straw-like. Inclusion of additional straw in these scenarios is not always practical to achieve the current recommendations for what constitutes a controlled energy prepartum diet. In addition, further evaluation of inflammation around parturition and what impact diet or energy status of the animal may have on inflammation is warranted to enhance our ability to improve animal health and productivity during the transition.

In Chapter 3, I concluded that feeding a high starch pellet at a high inclusion twice daily had no negative impacts on ruminal fermentation or eating patterns. Many response variables indicated that the composition of the PMR may have a greater impact on rumen fermentation and eating behavior than the pellet. However, consideration should be taken as by design the dietary treatments had complementary PMR such that the high starch pellet was fed alongside a high fiber PMR, and visa versa. This may have led to different results than if a high starch pellet were fed alongside a high starch PMR. As such, additional research is warranted investigating the effect of pellet and PMR composition under different feeding scenarios. In addition, this study was conducted in a tie-stall facility. While this management style allowed for controlling potential confounding factors such as feed availability and group dynamics, cows were in a non-competitive environment and results might have been skewed due to this.

In Chapter 4, cows offered the high amount of AMS pellet did not consume the target amounts. While this is an important finding, results may have differed had they consumed what was targeted. It is unknown the specific reasoning as to why, however, it is possible that barn design or diet formulation strategy may have played a role as with a feed-first barn cows have access to PMR prior to going to the AMS for milking and pellet consumption. It may be possible that cows were motivated to consume robot pellet to a lesser extent after PMR consumption. In addition, as cows must go through preselection gates to be milked, the increased number of milkings observed with the low pellet allocation would suggest that cows were more motivated to move through the barn, thus increasing their milkings as compared to those offered a high allocation. Eating behavior was not different between treatments, and the reason they may had more motivation to move through the barn is unknown. Thus, data obtained in this study regarding milking activities may differ from those conducted in free-flow systems due to a

presence of gates guiding cows. Therefore, conducting this study with different barn designs is warranted.

Despite not reaching target levels of AMS pellet consumption on the high treatment, cows on the high treatment received a total diet (PMR + pellet) that was higher starch and lower forage than cows offered the low pellet. This did not increase milk components or production, which is in contradiction to what was found in Chapter 2 with TMR fed cows. Further consideration as to how increased AMS pellet intake, and energy density of the PMR versus pellet affect rumen dynamics and animal performance is warranted, and measurement of rumen pH would be very beneficial.

One major finding from Chapter 4 was substitution rate, such that greater AMS pellet allocation did not lead to greater DMI. While several studies have evaluated substitution rate under grazing scenarios (Faverdin et al., 1991; Jensen et al., 2016), it is still unknown what factors may affect substitution rate for cows managed with AMS. As substitution rate ultimately influences total DMI, further insight into what may influence substitution rate, in particular for postpartum dairy cows, is necessary.

5.3 Industry benefits

The data from the aforementioned studies further build on our knowledge of practical application of feeding strategies for transition dairy cows. Feeding a low starch prepartum diet in Chapter 2 supported the concept that offering a controlled energy diet prepartum is beneficial to metabolism of dairy cows. Chapter 2 also substantiated the concept that feeding high starch postpartum diets may not have negative effects on animal performance, regardless of prepartum diet. These findings suggest that we may be able to feed high starch diets to improve energy balance and performance in early lactation.

Chapter 3 provided insight into composition of pellets, indicating that feeding a high starch pellet may not have negative impacts on animal performance. Offering a high starch pellet did not negatively impact ruminal pH, feed intake patterns nor DMI, and results suggested that the PMR played a greater role in ruminal fermentation.

However, Chapter 4 found that feeding a high starch pellet, more than what the diet was formulated for did not result in improved animal performance, nor that a slow adaptation to a

high allocation of pellet was beneficial. Despite no difference in DMI nor apparent body tissue mobilization, cows offered a high amount of AMS pellet (and consequently a higher starch postpartum diet) had lower milk and milk component yields. While the variables in the study were not able to quantify why this was observed, one potential reason may be an energy expenditure to an inflammatory response if cows offered a high amount of AMS pellet experienced inflammation postpartum. While the findings of Chapter 2 found no increase in inflammatory markers when cows were offered a high starch postpartum diet, the prepartum diet in Chapter 4 was lower in energy and starch content than that fed in Chapter 2. This may have contributed to differences in animal performance between the two studies when offered increased starch postpartum.

While the findings of Chapter 2 and 4 somewhat contradict themselves, it is important to note that there are more factors to consider in Chapter 4 as cows are milked voluntarily, and cows offered the high amount of AMS concentrate had fewer milkings per day which may have influenced milk production more than the diet itself. In addition, success of a feeding strategy for cows managed with AMS immediately postpartum would be dependent on how cows substitute PMR for AMS concentrate. While Chapter 3 found that cows offered increased pellet had increased DMI, this was not the case in Chapter 4. It is likely that PMR formulation and strategy is more impactful than the concentrate or pellet that cows are fed and may vary with stage of lactation. The composition of the PMR likely plays a greater role in eating behaviors and how cows substitute concentrates for PMR, which may influence how cows perform in AMS scenarios. This provides further insight to industry that emphasis should placed on the PMR rather than pellet when making dietary decisions when cows are managed with AMS.

Chapter 4 is one of the first to quantify the effect of concentrate allowance immediately following parturition on a large scale, further contributing to our knowledge, and understanding of the role diet may play on early lactation animal performance. Within industry, anecdotal data obtained through milking systems is available to characterize cows in early lactation, however, within Chapter 4, data regarding how feeding (e.g. meal size, duration and frequency), and milking (e.g. milkings per day, fetching, or time spent in holding areas) behaviors change during early lactation are presented. This data is integral for industry to establish protocols for managing and formulating diets for cows managed with AMS.

5.4 Conclusions

Various diet formulation strategies are employed to improve performance of dairy cows during the transition period. In my studies, strategies to minimize abrupt changes in the diet around parturition did not result in improved productivity of dairy cows. Cows did not exhibit issues with being switched from a low starch prepartum to a high starch postpartum diet. When cows were adapted to a high starch pellet through the AMS at a rapid or moderate rate, I found no differences on animal performance. Overall, the findings of these studies indicate that feeding high starch post-partum diets, regardless of prepartum diet strategy may not negatively affect performance of animals, but that allowing cows to consume more concentrate, in excess of what the diet is formulated for may not be beneficial either.

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