The Effect of Risk for Subacute Ruminal Acidosis, Feeding Frequency, and Photoperiod on the Feeding Behaviour of Lactating Dairy Cows

by

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Abstract

Cows fed the same high-grain diet have a large variation in rumen pH and can be categorized as higher or lower risk for developing subacute ruminal acidosis (SARA). The objective of this research was to determine if differences in feeding behaviour existed between the two categories and if these differences could be managed with feeding frequency or photoperiod. In the preliminary studies, the feeding behaviour of 6 higher risk cows and 10 lower risk cows was observed over 24 hours. Cows at a higher risk for SARA ate for a longer period of time (186 vs. 153 min; $P = 0.01$) soon after feed was delivered once per day in the morning and less time overnight (19 vs. 43 min; $P = 0.01$) before feed delivery the next day than lower risk cows. In the primary experiment of Experiment 1, 4 higher risk and 4 lower risk cows were fed either once or three times daily and feeding behaviour was observed. Cows that were fed 3 times vs. once daily reduced eating time in the morning (103 vs. 145 min; $P < 0.01$) and increased eating time overnight (76 vs. 44 min; $P < 0.01$) which resulted in a decrease in the severity of SARA in higher risk cows (area below a pH of 5.8; 51 vs. 98 pH × min/d; $P = 0.05$) when they were fed 3 times vs. once daily. In Experiment 2, 30 cows were subjected to a long day or short day photoperiod and behaviour was observed. A long day photoperiod tended to increase eating time between 0300 to 0800 h (53 vs. 39 min; $P = 0.06$) and reduced overall daily sorting ($P = 0.07$). These findings suggest that feeding behaviour is a contributing factor to increased risk for SARA, where eating for longer following feed delivery increases the risk. Feeding more often and providing more hours of light increased the daily distribution of eating time, which may reduce the risk for SARA.

Preface

This thesis is an original work by Kira Macmillan. The primary experiment of Chapter 2, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, project name "Rumen acidosis and feeding behaviour", AUP00001555, May 12, 2015. The experiment of chapter 3, of which this thesis is a part, received ethics approval from the University of Alberta Research Ethics Board, project name "Photoperiod Study", AUP00000891, June 12, 2013.

Chapter 2 of this thesis has been submitted for publication as Macmillan, K. A., X. Gao, and M. Oba., "Increased feeding frequency reduces severity of subacute ruminal acidosis in higher risk cows and increases milk fat yield" to the Journal of Dairy Science. I was responsible for the data collection and analysis as well as the manuscript composition. X. Gao designed the preliminary studies and contributed to manuscript edits. M. Oba was the supervisory author and was involved with concept formation and manuscript composition.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADF	Acid Detergent Fibre
AR	Acidosis Resistant
AS	Acidosis Susceptible
BHBA	βa-hydroxybutyrate
BUN	Blood Urea Nitrogen
BW	Body Weight
CCAC	Canadian Council of Animal Care
CLA	Conjugated Linoleic Acid
CP	Crude Protein
CV	Coefficient of Variation
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
F:C	Forage:Concentrate
FA	Fatty Acid
GH	Growth Hormone
Hp	Haptoglobin
HR	Higher-Risk
IGF-1	Insulin-Like Growth Factor – 1
IGFBP	Insulin-Like Growth Factor Binding Protein
LP	Long-Day Photoperiod
LPB	Lipopolysaccharide Binding Protein
LPS	Lipopolysaccharide
LR	Lower-Risk
mRNA	Messenger Ribonucleic Acid
MUN	Milk Urea Nitrogen

NDF	Neutral Detergent Fibre
NEFA	Non-esterified Fatty Acids
NRC	National Research Council
NSC	Non-Structural Carbohydrates
peNDF	Physically Effective Neutral Detergent Fibre
SAA	Serum Amyloid A
SARA	Subacute Ruminal Acidosis
SEM	Standard Error Mean
SP	Short-Day Photoperiod
TMR	Total Mixed Ration
RSPCA	Royal Society for the Prevention of Cruelty to Animals
VFA	Volatile Fatty Acids

1.0 Literature Review

1.1 The Importance of Behaviour in Dairy Production

1.1.1 Introduction

Global population increase has led to an increase in demand for food products, including products from the dairy industry (Geraldo et al., 2012). This increasing demand has put more pressure on the dairy industry to increase production efficiency to increase output (Wirsenius et al., 2010). Dairy production has focused on nutrition, reproduction, genetics, and health care of animals, however animal welfare, specifically behaviour, handling and housing has become more important (Fraser et al., 2013). Behaviour of cattle is an important consideration in production as it is an indicator of an animal's welfare and health (Fraser et al., 2013). Animal behaviour may also identify areas of management that are inadequate as well as the effectiveness of a change in management practices.

1.1.2 Animal Behaviour and Welfare

The importance of behaviour measurements extends beyond just good production to consider animal welfare. Two of the main frameworks for animal welfare are biological function, or an animal's ability to cope with its environment, and affective states, which include the animal's emotional experience (Hemsworth et al., 2015). Behaviour is an indicator for both of these frameworks and is thus essential in determining overall well-being (Hemsworth, 2015). Common behavioural indicators of biological function include eating, ruminating, lying, and activity, all of which have production and health implications. Winckler (2014) also outlined several considerations of 'appropriate behaviour' during a welfare assessment. This included the expression of social behaviour (e.g., allo-grooming, conspecific interaction), expression of

natural behaviours (e.g., ease of movement, access to grazing/outdoors), good human-animal relationship (e.g., avoidance distance to handlers) and a positive emotional state (e.g., play, seeking-behaviour, relaxation). When considering an animal's affective state it is important to not only minimize negative states but also to promote positive ones (Winckler, 2014).

Public awareness of on-farm practices and animal welfare is increasing and many consumers are basing purchasing decisions on how livestock are raised (Webster et al., 2015). Kehlbacher et al. (2012) found an increase in sales of 164% in a U.K. grocery chain of products sold under the Freedom Food scheme, a Royal Society for the Prevention of Cruelty to Animals (RSPCA) program that assures consumers animal products are produced to a specified welfare standard. The authors also found the perception that meat raised to a higher welfare standard was a higher quality product and therefore consumers had an increased willingness to pay. The increase in demand for welfare centered production practices has led to legislated minimum requirements for animal welfare and labelling to indicate how livestock were raised in the food industry as a whole (Verbeke and Viaene, 2000; Kehlbacher at al., 2012). As these new regulations influence animal production practices on-farm it is important for animal researchers and producers to continue to consider the interaction between behaviour and management practices as a welfare indicator.

1.1.3 Animal Behaviour and Health

Animal health is an aspect of welfare with animal behaviour being one indicator of health status. In a review by Sepulveda-Varas et al. (2013) the authors outlined common behavioural changes that occur during illness, such as decreases in exploratory activity, reproductive activity, water intake, feed intake, grooming and other social behaviours. Decreases in activity may serve to conserve energy and redirect it to recovery systems, such as the immune system. Aubert

(1999) hypothesized that immune mediators, such as cytokines, may decrease feed intake to reduce important nutrients that may be used by an invading pathogen, thus facilitating recovery. In a study looking at the severity and incidence of metritis, Huzzey et al. (2007) observed decreases in feeding time and dry matter intake (DMI) pre-partum in cows that experienced severe metritis post-partum. In another experiment, cows that received mammary injections of *Escherichia coli*, mimicking mastitis, showed decreases in feeding time, self-grooming and rumination as well as an increase in standing idly (Fogsgaard et al., 2012). Identifying changes in behaviour that could allow for early intervention of illness is important for both animal welfare, animal health and production.

1.1.4 Animal Behaviour and Production

Behaviour of animals and its relationship with health and production is used to identify problem cows. The individual behaviour of cows' may predispose them or their pen-mates to reduced production and/ or health. For example, the re-direction of sucking behaviour in artificially fed calves, due to deprivation of natural sucking from the dam, can lead to cross-sucking on other calves which may inflame and/ or damage the area being sucked (i.e. udder; Jensen, 2003). Some individuals may continue this behaviour later in life; where a survey conducted on 80 different farms reported 11 % of heifers continue to cross suck after weaning (Keil et al., 2000). Aggressive behaviours for feed, often where dominant cows will push subordinate cows away, can result in injury and limit feeding times for subordinate cows. DeVries et al. (2004) doubled the feeding space, from 0.5 to 1.0 m per cow, and saw a 57% decrease in aggressive interactions. In this way, a behavioural problem can indicate the need for a management solution, and once that solution is applied the reduction in the problem behaviour can indicate the effectiveness of solutions.

This thesis focuses on the feeding behaviour of lactating dairy cows and the relationship with a metabolic disorder; subacute ruminal acidosis (SARA). Certain feeding behaviours are identified as potential casual factors for SARA, e.g., feed sorting and feeing pattern, and these behaviours are used as an indicator of the effectiveness of management strategies. Thus, animal behaviour is a necessary part of animal-husbandry (welfare, health, and production), and is therefore a necessary aspect of animal science research in order to improve the efficiency of production.

1.2 Subacute Ruminal Acidosis

1.2.1 Introduction

Sub-acute ruminal acidosis is a metabolic disorder most often found in high producing dairy cows. Dairy cows, especially around peak lactation, require tremendous energy to sustain milk production, which comes from fermentable carbohydrate ingredients in the diet. A diet high in fermentable carbohydrates will quickly be converted to acids in the rumen, which reduce the ruminal pH and lead to SARA (Plazier et al., 2008). Therefore, producers must balance milk production with rumen health to maintain healthy, productive animals.

Acidosis is a depression in rumen pH that leads to consequences in animal health and production, such as reduced dry matter intake, milk fat depression, laminitis and liver abscess (Plaizier et al., 2008). Acidosis can be divided into 2 categories, acute and subacute acidosis, depending on the pH of rumen fluid, however the exact threshold for each category varies. Acute acidosis has more severe consequences to cow health and is generally determined using a threshold pH of 5.0 (Nagaraja and Letchburg, 2007). Subacute acidosis, a milder form of acidosis, has been defined at several pH thresholds from 5.2 to 5.8, varying amongst experiments and depending on methods used to measure rumen pH. In a research setting, the most common

method to measure pH is to surgically fit a cannula into the side of the cow to allow access to the rumen itself. A pH data logger can then be placed inside the rumen to continuously measure pH for several days (Penner et al., 2006). Several research groups use the duration pH is below 5.6 (greater than 180 min) and the area below 5.6, or extent to which the pH dropped below the threshold, to identify SARA in cows (Krause and Oetzel, 2005; Gozho et al., 2007; Khafipour et al., 2009a). However, Duffield et al. (2004) compared measuring rumen pH through cannulas or by extracting rumen fluid by needle, rumenocentesis, and found that for data collected through rumen cannulas, a pH of 5.8 was accurate to identify SARA. The threshold of pH 5.8 was also used by Beauchemin et al. (2003) as a pH below 5.8 was reported to decrease the growth and activity of cellulolytic bacteria (Russell and Wilson, 1996), indicating that negative effects due to a sub-optimal pH can occur before pH drops below 5.6. For the work completed and described in this thesis, a rumen pH of 5.8 was also used as the threshold for SARA.

1.2.2 Mechanisms of SARA

The mechanism of SARA depends on a balance between acid production, absorption and neutralization in the rumen. When SARA is induced through an increase in highly fermentable carbohydrates in the diet there is an increase in total volatile fatty acid (VFA) produced in the rumen (Guo et al., 2013; Khafipour et al., 2009a; Blanche et al., 2009). Firstly, starch is broken down into free glucose in the rumen, which stimulates the rapid growth of bacteria. Free glucose is converted to pyruvate, which is then converted into VFA by rumen microbes. The primary VFA produced in the rumen are acetate, propionate and butyrate, which are used by the epithelium for energy or absorbed into the blood stream and converted into energy for the cow in peripheral tissues (Owens et al., 1998). The rapid production of VFA reduces the pH of the rumen environment and can cause a shift in microbial populations. At a pH below 5.8,

cellulolytic bacteria have reduced function, thus reducing fibre digestibility in the rumen and further exacerbating the problem (Russell and Wilson, 1996). When rumen pH drops below 5.0, the environment favors lactate producing bacteria over lactate utilizing bacteria, increasing the accumulation of lactic acid which drops the rumen pH further (Owens et al., 1998). Unlike acute acidosis, where the accumulation of lactic acid contributes to a severely decreased pH, the accumulation of VFA is thought to be the main cause of the pH drop in SARA (Goad et al., 1998). Similarly, Blanch et al. (2009) did not see a significant change in lactate producing or utilizing bacteria and no change in lactate concentration in cows experiencing SARA. A bout of SARA, however, shifts concentrations of VFA-producing bacterium (Petri et al., 2013) and is often accompanied by a decrease in acetate and an increase in propionate production (Krause et al., 2002a; Guo et al., 2013; Yang and Beauchemin, 2009). For example, Li et al. (2014) induced SARA in goats and observed a decrease in some cellulolytic bacteria, responsible for acetate production, and an increase in amylolytic bacteria, responsible for propionate production. Increasing starch in the diet increases production of VFA from all rumen microbes but the resulting decrease in pH favors amylolytic bacteria. This shift in microbial population may also contribute to the decrease in fibre digestibility (Li et al., 2014). An increase in acid production and acid accumulation in the rumen is a major contributing factor for SARA.

Acid neutralization largely occurs through the production of saliva, which contains buffering components such as bicarbonate (Plazier et al., 2008). Approximately half the bicarbonate used to neutralize acid in the rumen comes from saliva with the other half transported across the epithelium in exchange for VFA (Owens et al., 1998). Cassida and Stokes (1986) estimated the salivation rate of eating and resting to be 150 ml/min and 177 ml/min, respectively, while rumination produced the greatest rates at 300 ml/min. Although Chibisa et al.

(2016) did not measure ruminating salivation, the authors did find an increase in eating and resting salivation, by 21 and 17 mL/min, respectively, when cow were fed a high forage diet at 70% forage. Feeding sufficient neutral detergent fibre (NDF) to promote chewing, especially rumination, is important for increasing the buffering capacity in the rumen when feeding high concentrate diets. Beauchemin et al. (1994) determined that rumination is correlated with NDF intake, as more chewing is required with slowly digestible carbohydrates, and that NDF provided by forage as opposed to concentrates, promoted rumination. Mertens (1997) defined the term physically effective NDF (peNDF) which indicates how effective a fibre source is at stimulating chewing. This term combines the NDF content of the diet as well as the physical particle size to assess the effect on chewing activities. It is important to consider the particle size as long forage particles, greater than 19-mm, are positively correlated with chewing time (Krause et al., 2002a) and mean ruminal pH (Yang and Beauchemin, 2009). Using peNDF, both Krause et al. (2002a) and Yang and Beauchemin (2009) found a positive correlation or tendency of positive correlation with chewing time. While reducing acid production is essential for preventing SARA, promoting acid neutralization through chewing is just as important.

Finally, it is necessary to consider the absorption of acids and its role in alleviating acid accumulation in the rumen. The absorption of VFA in the rumen is dependent on rumen papillae surface area; papillae surface area increases as acid concentration increases, however capacity for absorption takes several weeks to adapt to a high grain diet, as reviewed by Kleen et al. (2003). Allen (1997) explained that VFA are absorbed in the de-ionized form which results in a net removal of hydrogen atoms. The author estimated 53% of total hydrogen ions in the rumen were removed as a result of VFA absorption. Shurmann et al. (2014) challenged calves with a moderately fermentable diet, 50% forage, and found an increase in butyrate and acetate

absorption, primarily through passive diffusion, in comparison to a less fermentable diet, 91.5 % forage. However, VFA transport out of the rumen can also occur by protein mediated transport, most often via bicarbonate exchange or nitrate-sensitive, bicarbonate independent transport (Aschenbach et al., 2014). Owens et al. (1998) also indicated that increased osmolality of rumen fluid can inhibit VFA absorption, which has been observed with high concentrate diets that increase the concentrations of free glucose, pyruvate and VFA in the rumen. Damage to the rumen epithelium due to low pH, resulting in parakeratosis or hyperkeratosis (the hardening of the epithelium), may also reduce the absorptive capacity (Gonzalez et al., 2012). In order to address the causes of SARA, it is important to understand the balance of acid production, neutralization and absorption in the rumen.

1.2.3 Causes of SARA

There are several different aspects that can increase the risk of cows developing SARA, the main factors discussed here are stage of lactation, diet formulation, and behaviour.

1.2.3.1 Stage of Lactation

The stage of lactation influences the risk for SARA, with cows in early lactation being at the greatest risk (Penner et al., 2007). First, cows in the dry period are fed a low energy diet to prevent excessive body weight gain, but immediately following parturition there is a large increase in energy requirement. The onset of lactation requires more energy than the cow is consuming thus putting her in a negative energy balance. At this time her fat stores are being mobilized to maintain production (Butler and Smith, 1989). In order to reduce the energy deficit, fresh cows are fed a high concentrate diet to provide adequate energy while preventing gut fill caused by high forage diets (DeVries et al., 2008). This sudden change in diet can be a shock to

the rumen microbes and rapidly increased acid production, additionally the rumen papillae are not yet adapted to absorb the extra acid load (Kleen et al., 2003). Dairy cows increase milk production until peak lactation at around 2.5 months after calving and during this time DMI is also increasing (Butler and Smith, 1989). High quantities of intake, especially intake of concentrates, have been associated with lower ruminal pH (Oetzel and Nordlund, 1998). Therefore high producing, early lactation cows being fed a high concentrate diet are at the greatest risk for SARA (DeVries et al. 2008). That being said, mid and late lactation cows can also experience SARA but more likely due to mistakes in nutritional management, such as poor formulation of a high concentrate diet, mistakes in ingredient inclusion in the diet or when feedings are skipped (Kleen et al., 2003).

1.2.3.2 Diet

Dairy cows are often fed a total mixed ration (TMR) that contains all of the dietary ingredients mixed into one ration. This ensures that cows are receiving balanced nutrition in every bite and thus a balanced delivery of nutrients to the rumen. A TMR includes ingredients that make up the major nutrient fractions such as NDF, acid detergent fibre (ADF), non-structural carbohydrates (NSC), crude protein (CP), ether extract (fat), and minerals and vitamins. A common term used to describe the composition of a TMR is forage to concentrate ratio (F:C) which determines the percent inclusion of forage and the percent inclusion of everything else. This term is important in regards to SARA as it gives an idea of how fermentable the diet will be. As mentioned above it is important to consider both the concentrate fraction, as a source of increased acid production, and the fibre fraction, as a source of rumen buffering.

1.2.3.2.1 Concentrate Fraction

The concentrate fraction of a TMR is made up of all the ingredients that are highly fermentable in the diet. The most important ingredients are those that are high in NSC, specifically starch. These ingredients include a variety of different grains. The NRC (2001) recommends 30 – 40 % NSC for lactating dairy cows to maintain optimal milk production and rumen health. However, both the content of NSC and the physical characteristics are both major drivers for fermentation in the rumen (Gonzalez et al., 2012). While some grains contain large amounts of starch, if it is not easily degradable than the starch is released slower and there is less of an impact on fermentation (Huntington et al., 2006). Based on these 2 factors, Huntington et al. (2006) identified the fastest to slowest degradation rate of common grains being oats, wheat, barley, corn and milo. Offner et al. (2003) also outlined the importance of processing of grains and found that particle size reduction, heat treatments, and shear force (extrusion) each increased the degradability of starch. Increased degradability will lead to a large, fast increase in acid production that can overwhelm the rumen's ability to remove it, resulting in SARA (Gonzalez et al., 2012). A common TMR F:C for high producing dairy cows is 50:50, but again the type and processing method of grains may alter the optimum ratio. Endres and Espejo (2010) surveyed 50 farms in Minnesota and found the average forage percent in the high-producing diet was 52%. When SARA is induced in a research setting a common F:C ratio in TMR is 40:60 (Beauchemin et al., 2003; Yang and Beauchemin, 2009; Krause et al., 2002a). When more concentrate is fed, the rumen is subjected to higher starch concentrations and thus higher acid production in addition to a decrease in rumination activities caused by the low NDF content in the diet. (Faleiro et al., 2011; Yang and Beauchemin, 2009). Forage to concentrate ratio and NDF to NSC ratio are negatively correlated so as one increased most often the other decreases. So while excess

concentrate intake is thought to be the main cause of SARA, the resulting insufficient forage intake also plays a major role.

1.2.3.2.2 Fibre Fraction

The amount and characteristics of NDF is an essential consideration to balance acid production. The NRC (2001) recommends 25 % NDF in the diet, with 19 % coming from a forage source. NDF is important in the diet as it promotes chewing (eating and ruminating) and thus saliva production, leading to increased buffering of the rumen (Beauchemin, 1991). Beauchemin (1991) fed cows different dietary concentrations of NDF (31, 34, and 37 %) by adjusting the forage to concentrate ratio. Increasing NDF linearly increased time spent eating and chewing and also increased the milk fat content. The increase in milk fat is likely due to the decrease in acid production from reduced concentrate feeding in addition to the increase in saliva production. Beauchemin et al. (1994) evaluated the effects of different forage to concentrate ratios, and found that with a high forage diet, 65:35, there was 0.63 % increase in milk fat content as well as an increase in time spent eating and ruminating, in comparison to a low forage diet, 40:60. Yang and Beauchemin (2009) tested forage to concentrate ratios on rumen fermentation and found that a low forage diet, 35:65, increased the area and duration below a pH of 5.8, in addition to reducing time spent ruminating and chewing, in comparison to a high forage diet, 60:40. Although the authors found the correlation between dietary NDF and chewing was weak, there was a strong correlation between area below pH 5.8 and forage NDF, indicating that NDF provided by a forage source is important.

While NDF is the favored measurement for total fibre in a diet, Mertens (1997) suggested that the physical characteristics of a fibre source, not just chemical, should be considered. Physically effective NDF takes into account the physical aspects of fibre, primarily particle size,

that influence chewing. Mertens observed that rumen fermentation and milk fat can be negatively impacted when fibre is finely chopped, with no change in the F:C or NDF content. Inadequate amounts of peNDF intake can lead to decreased chewing, reduced saliva, and a lower rumen pH. A Pennsylvania State Particle Separator can be used to determine the particle size distribution, which contains 3 sieves and thus 4 particle size ranges (Kononoff et al., 2003). A sample is placed at the top of the box and shaken down until particles settle into their respective size ranges, >19-mm (long forage particles), > 8-mm (medium forage and concentrate particles), >1.18-mm (small forage and concentrate particles) and the bottom pan (fine particles). In a review by Zebeli et al. (2012), the authors suggested that feeding long forage particles will increase rumination and buffering but may also decrease passage rate and negatively impact milk production. Feeding finely chopped forage, on the other hand, will reduce rumination and increase fermentation, especially in high concentrate diets. A moderate forage particle size (10 to 15-mm) improved degradation of fibre and increased TMR uniformity. The authors found that calculating peNDF by multiplying NDF content by the sum of particles retained on the top 2 sieves, >8-mm, was better able to predict rumen pH, DMI, chewing and rumination activities. Beauchemin et al. (1994) tested 2 different silage particle sizes, 10-mm vs. 5-mm, and found that the longer particle size increased rumination time, and when fed with a high concentrate diet, it increased milk production while maintaining milk fat content and DMI. Yang and Beauchemin (2009) reported that peNDF >8-mm was positively correlated with chewing time and mean ruminal pH. However, the authors found that when peNDF intake was the same, cows fed a low F:C diet still had higher degrees of acidosis, indicating that peNDF does not take into account the fermentatbility of the diet. While peNDF is an important consideration in providing a balanced

diet, the forage to concentrate ratio as well as the fermentability of that concentrate are essential factors in maintaining rumen health.

1.2.3.3 Behaviour

1.2.3.3.1 Sorting

Behavioural factors may also contribute to the risk of developing SARA. One of these factors is sorting, or the preferential selection for or against certain parts of a TMR. Sorting is measured by comparing the actual intake of a particle size range, based on the orts sampled, to the expected intake, based on the TMR sampled. This gives the sorting index, in which an index below 100 indicated sorting against a certain particle size and an index above 100 indicated sorting for a particular particle size (Leonardi and Armentano, 2003). Leonardi and Armentano (2003) found that within a group of 24 Holstein cows, most cows sorted against long particles, >26.9-mm, and for fine particles, <1.18-mm. Within this group only 2 cows did not sort against long particles and one extreme cow did not consume any long particles at all. As mentioned above, the long particles contain the most peNDF and stimulate rumen buffering, whereas the fine particles are made up of 95% of grain mixture and are highly fermentable in the rumen. Cows generally prefer the smaller particles and are better able to sort against the longest particles, therefore cows may be consuming a diet with less NDF and more NSC than was formulated and delivered (Leonardi and Armentano, 2003). As the mean particle length of a TMR increases, Leonardi et al. (2005) found increased sorting against long particles and for short particles, which likely contributed to reduced NDF intake. Although the authors also observed an increase in chewing time as particle length increased, there was no effect on rumen pH. However, the mean rumen pH for all diets was high, > 6.0, which may have minimized the acid neutralizing effect of increased chewing time and particle size. The authors also noted that

for a uniform response across the herd it is better to feed the least amount of particles > 26.9 mm and to feed the greatest amount of particles between 26.9 to 9.0 mm. DeVries et al. (2007) compared the effect of two different F:C, 62:38 vs. 51:49, on sorting behaviour in a crossover design. Cows fed the low forage diet sorted against long particles, > 19 mm, and for short particles, < 8.0 mm, to a greater extent than cows on the high forage diet, leading to increased sorting against peNDF. The authors had hypothesized that a high forage diet would be easier to sort, but they found that the low forage diet motivated cows to select for concentrate and against forage. Cows at a high risk for SARA, early lactation, high producing, or fed a low fibre diet, sorted their TMR to a greater extent than low risk cows, as reported by DeVries et al. (2008). The authors also found that sorting against long particles led to a lower maximum rumen pH and that increased sorting of particle fractions higher in starch and lower in NDF reduced nadir, mean and maximum ruminal pH. Although longer particles in diets contribute to increased peNDF, it is important to consider the length of particle actually consumed by the cow. Feeding a higher proportion of medium particles, retained on the 8-mm sieve of Penn State Particle Separator, will reduce sorting and increase buffering in the rumen, maintaining a healthy rumen pH.

1.2.3.3.2 Feeding Pattern

Another contributing factor for SARA is the feeding pattern of cows. Gonzalez et al. (2012) attributed the amount of VFA production in a certain period of time to meal size and eating rate, where eating larger amounts in a shorter period of time increased VFA production and risk for SARA. The hunger of cows will also alter the feeding pattern; if feed delivery schedules vary or if a feeding is missed it may increase SARA risk. Patterson et al. (1998) reported that DMI rates increased when cows were fasted up to 6 h, for both grazing and silage-fed cows. Similarly, Gregoini et al. (2009) found that as rumen fill was manually reduced by

removing rumen contents at different amounts, there was a linear increase in intake rate. Individual cows within a group may also display varying feeding patterns. Serment and Giger-Reverdin (2012) observed a steeper decrease in rumen pH in goats who were identified as "fast-eaters" (ate 70% of daily feed in the 90 min following feeding) compared to "slow eaters". Finally, the diet provided may also change feeding patterns in cows. When feeding a low forage diet, F:C of 51:49, DeVries et al. (2007) observed an increase in DMI and decrease in eating time. The decreased dietary peNDF concentration contributes to shorter eating time for low forage diets thus increased intake rate, particularly following a feed delivery. Cows fed the low forage diet also tended to have shorter meal times and larger meals. Feeding pattern is something that must be considered in terms of feeding management to reduce SARA risk.

1.2.4 Consequences of SARA

SARA is an important metabolic disorder to the dairy industry due to its impact on production and profitability of dairy farms.

1.2.4.1 Reduced Dry Matter Intake

A common consequence of SARA is a reduction in DMI, which is an issue as DMI is highly and positively correlated with milk yield (Dado and Allen, 1994). Cows directly following parturition are more susceptible to SARA, due to diet change and increased intake to meet energy demands, and thus any decrease in feed intake at this time is detrimental to production (DeVries et al., 2008). Feeding a highly fermentable TMR has been found to decrease DMI (Fulton et al., 1979; Allen, 2000; Blanch et al., 2009), however, there is some inconsistency in the literature where some studies have found no change in DMI (Krause and Oetzel, 2005; Gohzo et al., 2007; and Guo et al., 2013). It is difficult to say why there is discrepancy in DMI

response to SARA as there are several possible causes for DMI depression. One of these factors is the increase in propionate, a volatile fatty acid produced in the rumen, caused by the increased production from amylolytic bacteria that use starch (Li et al., 2014). Oba and Allen (2003) conducted an experiment where lactating Holstein cows were fed either a high or low concentrate diet and infused with a propionate solution at different rates. Increasing the rate of propionate infusion decreased DMI, however, the reduction in DMI was related to plasma glucose concentration. The authors speculated that propionate is initially used by the liver for gluconeogenesis until the glucose demand has been met and after the propionate is oxidized in the liver which signals the brain to reduce intake. When highly fermentable diets are fed, there is an excess of both propionate and available glucose which leads to the signalling for reduced intake (Oba and Allen, 2003). Bradford and Allen (2007) aimed to determine if the variation in intake response to increased starch fermentabiliy could be predicted by metabolic factors. The authors found that plasma insulin concentration, in the period before a highly fermentable diet was fed, was negatively correlated to the change in DMI. Cows that were better able to clear the increase in glucose caused by the challenge diet were better able to maintain their DMI, which relates to propionate then being used for gluconeogenesis.

In a review by Allen (2000), increased osmolality in the rumen was explored as a possible causal factor for DMI depression due to SARA. The increase in VFA production, and thus increase in VFA absorption, may stimulate receptors in the rumen epithelium and signal for a reduction in DMI. There is also some evidence for lipopolysaccharide (LPS), a component of gram negative bacteria, to affect DMI in cattle. According to Waldron et al. (2003) infusing LPS into the blood stream of cattle increased tumor necrosis factor alpha, which is responsible for anorexic behaviour during times of illness. Plasma concentrations of LPS have also been found

to increase in cattle experiencing SARA, and will be discussed in a later section. Whether or not we can identify the specific causal factor, decreasing DMI during bouts of SARA negatively impacts productivity.

1.2.4.2 Milk Fat Depression

Another production loss due to SARA is milk fat depression. Milk fat is an important factor in profitability as dairy farmers in Canada are paid based on the fat content of their milk. When Khafipour et al. (2009a) fed cows a high grain diet, 60 % concentrate, to induce SARA, milk fat concentration was reduced by 0.37% unit and milk fat yield was reduced by 0.12 kg/d. Several studies have recorded a loss in milk fat yield (Krause and Oetzel, 2005), milk fat concentration (Oba and Allen, 2003; Gott at al., 2015), or both (Guo et al., 2013; Khafipour et al., 2009b) due to SARA. On the other hand, Gozho et al. (2007) fed a 68 % concentrate diet and found no change in milk fat concentration or yield. However, the cows fed a control (high forage) diet also experienced a rumen pH below 5.6 for over 3 hours, therefore they may also have experienced milk fat depression and were not a good comparison. Krause et al. (2002b) reported that duration and area below a pH of 5.8 were negatively correlated to milk fat concentration.

A decrease in pH alters the rumen environment and plays a role in altering milk fat synthesis (Bauman and Griinari, 2003). Around 50% of milk fatty acids are synthesized de novo in the mammary gland, primarily medium and short chain fatty acids (FA), while the other half is from circulating lipids made up of dietary fat and fat mobilized from the reserves of the cow's body (Bauman and Griinari, 2003). Dietary fats ingested by the cow are metabolized in the rumen by microbial action, and when rumen pH drops, it leads to altered biohydrogentation of FA and production of unique FA intermediates (Bauman and Griinari, 2003). These intermediates inhibit milk fat synthesis and result in milk fat depression. This form of milk fat depression results in a

higher concentration of long chain fatty acids, incorporated from the diet, and a lower concentration of short chain fatty acids, synthesized by the cow in milk (Bauman and Griinari, 2003). Another review, conducted by Shingfield and Griinari (2007), identified trans–10, cis–12 conjugated linoleic acid (CLA) as a major fatty acid intermediate contributing to inhibition of milk fat synthesis. Odens et al. (2007) fed cows a high amount of rumen inert CLA, 600 g/d, and observed decreases in milk fat concentration and yield as well as a decrease in de novo fatty acid synthesis in comparison with a low CLA diet. In a study looking specifically at different forms of CLA, Perfield et al. (2007) found that trans-9, cis-11 CLA and trans-10, cis-12 reduced milk fat yield by 15 and 27%, respectively. When Gott et al., (2015) induced SARA and milk fat depression in lactating cows, they detected an increase in trans-10, cis-12 CLA but did not measure trans-9, cis-11. Altering fat biohydrogenation pathways in the rumen can lead to fatty acid intermediates that inhibit milk fat synthesis and ultimately and cause milk fat depression and a loss in profitability for the farmer.

1.2.4.3 Compromised Animal Health

Subacute rumen acidosis can also be a concern for animal health, which in turn will reduce production and animal welfare. In a comprehensive review by Nocek (1997), the author indicated that SARA that reduced rumen and systemic pH is a casual factor for laminitis in cattle, which is the inflammation of the dermal layers in the hoof causing lameness. A low pH leads to increased blood flow to the hoof, and along with a release of endotoxins and histamines, can lead to seepage through the vessel walls and edema in the hoof causing tissue damage and lameness. Damage to the rumen wall, due to a low pH, also has production and welfare implications. Krause and Oetzel (2005) reported that consistently low rumen pH can lead to rumenitis, swelling of the rumen wall tissue, and damage to the rumen epithelium. This damage

can result in the leakage of bacteria into the portal vein and eventual liver abscess. This was also supported by Nocek (1997) who attributed the liver damage to the release of the bacteria *Fusiformis necrophorus* from the rumen.

In addition to bacteria translocating into the blood stream, the movement of LPS into the blood can cause problems in cattle. Lipopolysaccharides are released from gram negative bacteria during lysis or rapid growth, and LPS concentrations have been found to increase during instances of low rumen pH (Nagaraja et al., 1978). When LPS finds its way into the bloodstream it triggers an inflammatory response, which is evident by the increase in acute phase proteins, such as haptoglobin (Hp) and serum amyloid A (SAA; Baumann and Gauldie, 1994). Gozho et al. (2005) induced SARA in 3 steers and saw an increase in free LPS in the rumen, which likely translocated into the bloodstream, causing the increase in Hp and SAA. In order to test LPS concentrations in the blood, Gozho et al. (2007) induced SARA in 4 Holstein heifers and found an increase in rumen LPS but were unable to detect LPS in the plasma. The inability to detect LPS in the blood could indicate that LPS translocation is not occurring and that the increase in acute phase proteins is being stimulated by other factors. However, Khafipour et al. (2009a) used a higher-sensitivity assay for plasma LPS and measured lipopolysaccharide binding protein (LPB) in the blood. The LPB facilitates the binding of LPS to the cell membrane which triggers the inflammatory response. The authors induced SARA in lactating Holstein cows and found an increase in plasma LPS from < 0.05 to 0.52 endotoxin units/mL as well as increases in Hp, SAA and LBP concentrations. Although these results provide evidence that free rumen LPS generated by a low pH environment can get into the blood, there is some dispute on whether LPS is able to translocate through the rumen epithelium. Khafipour et al. (2009b) formulated a diet using alfalfa pellets to induce SARA by reducing rumen buffering with no increase in starch content.

The authors hypothesized that rumen LPS does not translocate through the rumen wall but instead starch increased LPS release in the terminal small intestine and large intestine, which then translocate into the blood stream. The experimental diet resulted in more severe SARA than many grain-induced experiments and increased rumen LPS to a greater extent as well. However, there was no change in plasma concentration of LPS, SAA, Hp or LBP. These results support the theory that LPS is less likely to translocate though the rumen epithelium. Although SARA itself may not be responsible for all LPS-induced inflammation, providing a high concentrate diet, as is the cause of most instances of SARA, can increase the risk for systemic inflammation.

1.2.4.4 Detection

SARA is also a significant issue due to the difficulty associated with detection and diagnosis. While some of the consequences of SARA have been outlined above, it is also important to note that these aliments have a multifactorial etiology and may not necessarily indicate SARA (Nocek, 1997, Kleen et al., 2003). For instance, milk fat depression often occurs during incidences of SARA, and thus could be used as a practical, non-invasive method of detection. However, milk fat can change due to a variety of reasons other than low pH, such as the amount and composition of dietary fat (Shingfield and Griinari, 2007) and heat stress (Thatcher, 1974). Additionally, some experiments inducing SARA found a decrease in rumen pH but no effect on milk composition (Krause et al., 2002b; Gozho et al., 2007). Although milk fat depression is a common consequence of SARA it is not a reliable method of detection. Currently, the most widely used way to diagnose SARA is through rumen pH measurement, which is most often done in a research setting using ruminally cannulated cows and pH data loggers. However, this requires an invasive surgery that cannot be used easily on-farm to detect SARA. Rumenocentesis has been used on farm and involves sticking a long needle into the rumen and extracting fluid.

This method is still somewhat invasive and also uses spot samples to measure pH (Garrett et al., 1999), which is risky as rumen pH varies throughout the day and in relation to feeding times. Other studies have looked at less invasive methods to diagnose SARA in the herd. Danscher et al. (2015) found that inducing SARA resulted in decreases in urine and fecal pH with no change in blood pH. Li et al. (2012) also observed a decrease in fecal pH along with an increase in total fecal VFA. While they also looked for LPS concentration in the blood, they were not able to detect differences between treatments. Using rumen temperature and wireless sensing devices has been examined and AlZahal et al. (2008) found the highest correlation between rumen temperatures over 39.4 °C and a rumen pH below 5.8. While there is evidence supporting each of these methods to detect SARA, they are still unreliable and not commercially used. Therefore it is important to understand the causes of SARA and focus on prevention instead.

1.2.4.5 Prevalence

The difficulty in detecting SARA has led to limited research on its prevalence in dairy herds. Garett et al. (1997) surveyed 15 herds in Wisconsin and, using rumenocentesis, found that 19% of early lactation cows and 26% of mid-lactation cows had SARA. Oetzel et al. (1999) also found similar results when surveying 14 herds in Wisconsin with 20.1% of early and peak lactation having SARA. More recently, Morgante et al. (2007) measured rumen pH of early lactation cows in 10 Italian free-stall farms and found that 3 herds had >33 % of cows with a pH below 5.5 and 3 farms had >33% of cows with a pH below 5.8. O'Grady et al. (2008) measured rumen pH in early lactation cows on 12 pasture based farms and determined that 53% of cows had a rumen pH below 5.8. Stone et al. (1999) conducted a study with a dairy farm in New York and determined that the losses due to decreased milk fat and milk protein concentration as a result of SARA equaled around $400 per cow per lactation.

1.2.5 Individual Variation

Another aspect that affects the risk for SARA in cattle is individual variation in response to a high grain diet. Brown et al. (2000) induced rumen acidosis in beef steers by dosing steam flaked corn at 3 % of BW directly into the rumen. Among the 5 steers dosed, 1 was euthanized due to acute acidosis and 1 showed no signs of acidosis (min. rumen pH > 5.6), with the rest falling in between. In a study conducted by Penner et al. (2007), heifers fed the same high grain diet displayed a wide range in severity of SARA, indicated by the area below pH 5.8 or the extent of pH decrease below 5.8, in which the range was larger around parturition than later in lactation. These experiments indicate that not all cattle respond to a high concentrate diet in the same way. The second paper from the experiment conducted by Penner et al., (2007) was published by Mohammed et al. (2012) who organized cows based on severity of SARA as mild (mean pH 5.8 – 5.5), moderate (pH 5.5 – 5.2), and severe (pH < 5.2). Between the separate severity groups the authors found no difference in DMI, total VFA concentration or profile, nor milk fat concentration. This extent of variation in pH response to a high grain diet makes it difficult to manage SARA in a group of cows. However, categorizing cows based on their response allows us to determine what contributes to some cows developing acidosis while others do not. Penner et al. (2009) induced SARA in 6 non-pregnant, non-lactating cows and used an acidosis index (area below pH 5.8/ DMI) to assess the severity of SARA normalized for individual intake. This is more accurate than just measuring the duration and area below pH 5.8 as intake directly impacts the amount of fermentation occurring (Penner et al., 2009), making it easier to compare animals. Cows with a higher acidosis index experienced greater severity of SARA per unit of feed intake. In this study, the authors found no relationship between the index and rate of VFA absorption or gene expression associated with absorption, however the data, including outliers

and tendencies, suggested that there may be a decrease in the genes related to VFA metabolism in higher index cows.

Using the acidosis index, researchers began to define cows as susceptible or tolerant to a high grain diet. Using these extreme cows would ideally make it easier to determine what factors were different between them. Penner et al. (2009) induced acidosis in sheep and found no difference in total VFA concentration or VFA profile between tolerant and susceptible animals. However, the authors found that tolerant animals had increased acetate and butyrate uptake through bicarbonate dependent exchange as well as bicarbonate independent, nitrate sensitive uptake of acetate. Additionally, tolerant animals had increased plasma BHBA, likely due to the increase in butyrate uptake. Chen et al. (2012) identified 3 acidosis resistant (AR) and 3 acidosis susceptible (AS) steers, and induced SARA by force-feeding 60 % of expected DMI of a high grain diet within 30 min. The authors found that AS steers had a higher total VFA concentration, with a higher percentage of propionate and lower percentages of acetate and butyrate. This indicated either increased production or decreased absorption of VFA. The larger density of bacteria in the rumen suggests that increased fermentation occurred in AS steers. The second paper from this experiment was published by Schlau et al. (2012) with the objective of comparing the expression of VFA transporters in the rumen epithelium between AS and AR steers. The only difference detected was an increase in NHE3 in AR steers, a protein transporter that imports sodium into the cell and exports hydrogen ions back into the rumen. This increase may be due to a higher absorption of undissociated VFA into the cell, which then dissociate, leaving H^+ to be transported back out of the cell to maintain pH (Schlau et al., 2012). The authors also measured and detected no difference in plasma glucose, insulin, β-hydroxybutyrate (BHBA), and non-esterified fatty acids (NEFA) between AS and AR steers. Gao and Oba (2014) induced SARA in

late-lactation, average DIM = 282 ± 34, Holstein cows by feeding a TMR with 65 % concentrate. Unlike the studies in steers, there was no difference in total VFA concentration or VFA profile between resistant and tolerant cows. This could be due to feeding a lower concentrate diet (90 % vs. 65 %), or due to the difference in animals used. Gao and Oba (2014) also detected no difference in DMI, milk yield or milk fat, or in plasma concentrations of glucose, insulin, BHBA and NEFA. The authors evaluated feeding behaviour and observed no difference in total daily eating, ruminating, or chewing time and thus it did not contribute to different responses in severity of SARA between the two categories of cows. However, susceptible cows sorted to a greater extent for short particles and against long particles, which may promote a decrease in rumen pH (Gao and Oba, 2014). This study also detected lower milk urea nitrogen (MUN) in susceptible cows, which may be a result of increased fermentation in the rumen.

Another important consideration for studies comparing cows at a higher or lower risk for SARA is determining indicators that could identify AS or AR cows without measuring rumen pH. Gao and Oba (2015) used milk fat and MUN as indicators, selecting late-lactation cows with either the lowest milk fat % and MUN or the highest as higher risk (HR) and lower risk (LR) cows, respectively. In the following lactation, after cannulation surgery, rumen pH was measured, and the results confirmed that milk fat and MUN in late lactation could be used to identify HR cows. Identifying contributing factors for individual variation in response to a high grain diet as well as external indicators are important for reducing the severity of SARA in lactating dairy cows.

1.3 Effects of Feeding Frequency

1.3.1 Introduction

The focus of this thesis is on behaviour of lactating dairy cows and how it can contribute to and be used to manage SARA. As covered previously, there are several aspects of behaviour that can increase the risk for SARA, and may also contribute to individual response to a high grain diet. Ideally, implementing management practices that target cows at a higher risk for SARA would be beneficial for production because a producer could improve the rumen health of HR cows without negatively affecting the production of LR cows. A possible management approach to this end may be frequency of feed delivery. The delivery of feed represents a substantial labor cost for the producer and thus a way to reduce cost is to feed once daily (DeVries et al., 2005). However, feeding more than once per day has been shown to have benefits for rumen health and production.

1.3.2 Feeding Frequency and Rumen pH

The effect of feeding frequency on rumen pH has been variable in past literature. Nocek and Braund (1985) fed a high grain diet to first lactation cows either 1, 2, 4 or 8 times daily and found no effect on mean rumen pH. Although French and Kennelly (1990) also observed no change in mean pH when cows were fed more often, the authors did observe a change in diurnal pH pattern; cows fed twice daily had a steep decline in pH following both feed deliveries whereas cows fed twelve times daily had almost no postprandial changes in pH. The decrease in diurnal variation due to increased feeding frequency is also supported by other studies (Kaufmann et al., 1976; Bragg et al., 1986). In a study changing the feeding frequency of a protein supplement fed with part of the daily concentrate intake, cows fed twice daily had a decreased mean pH and increased propionate production, indicating an increase in rumen

fermentation, compared with cows fed 5 times daily (Robinson and McQueen, 1994). On the other hand, Shabi et al. (1999) found a decrease in mean rumen pH when cows were fed 4 times vs. twice daily, however this treatment also led to a significant increase in DMI likely contributing to the increased VFA production observed. Those authors reported a significant decrease in the variation of rumen pH, when cows were fed four times daily vs. twice. Overall, feeding frequency affects rumen pH, by reducing the variation over 24 hours.

Total VFA concentration has generally not been found to be affected by feeding frequency (Bragg et al., 1986; French and Kennelly, 1990; Robinson and McQueen, 1994) however some studies have reported an increase in VFA concentration (Klusmeyer et al., 1990; Shabi et al., 1999). Tendencies for greater propionate proportion in ruminal fluid have been observed (Shabi et al., 1999; Klusmeyer et al., 1990) as well as tendencies for greater acetate proportion (Bragg et al., 1986; French and Kennelly, 1990) indicating opposing trends to increase or decrease fermentation in the rumen. However, in each of these studies, only 4 cannulated cows were used and so tendencies in the data should be interpreted with caution.

1.3.3 Feeding Frequency and Behaviour

As previously mentioned, behavioural responses due to a change in feeding frequency have been variable among past experiments. Nocek and Braund (1985) did not directly measure feeding behaviour but they noticed that cows fed more often tended to evenly distribute eating over the day with no change in DMI. When Robinson and McQueen (1994) measured feeding behaviour, they found no change in total daily eating, ruminating or chewing time. However, several experiments since then have observed a more uniform pattern of eating time throughout the day when cows are fed more often. Le Liboux et al. (1999) fed cows a high forage diet 4 or 6 times daily and found that cows fed 6 times had a more even distribution of eating time, which is

consistent with observed smaller range of rumen pH. Both Mantysaari et al. (2006) and Phillips and Rind (2001) observed a more uniform eating pattern when cows were fed five and four times daily vs. once daily, which the authors attributed to small peaks in feeding activity following feed delivery or an increase in eating time in the late evening, respectively. DeVries et al. (2005) conducted 2 experiments comparing once vs. twice daily feeding and twice vs. four times daily feeding. In both experiments, when cows were fed more often there was an increase in daily eating time as well as a decrease in time spent eating during the 90 minutes following feed delivery. The increase in eating time came from an increase in time spent eating during the late evening and early morning hours, again displaying a more uniform pattern of daily eating. This alteration in eating pattern has implications for rumen health. If time spent eating following feed delivery is reduced and eating time is spread out throughout the day there will be a reduction in the amount of fermentation occurring at any one point in time. This allows acid production and removal to remain balanced, which is likely a contributing factor to the reduction in diurnal pH variation mentioned above. As noted by Bragg et al. (1986) it would also be better to combine the feeding of a high concentrate diet with more daily feedings. Thus this high concentrate diet is delivered to the rumen in smaller increments throughout the day and not all at once as seen with once a day feedings. Most dairy cattle are fed for ad libitum intake, so feed is always available, even in once a day feeding. Therefore, the change in eating pattern seen with more frequent feeding may not be influenced by feed availability but likely by the delivery of fresh feed itself. DeVries and von Keyserlink (2005) compared delivering feed while cows were being milk or delivering feed 6 hours after milking, and found that the delivery of fresh feed was a larger stimulator for eating activity than returning from milking. As the delivery of fresh feed

stimulates cattle to eat, feeding more often will encourage cows to distribute eating time throughout the day instead of concentrating that time after a once daily feed delivery.

An interaction between feed sorting and feeding frequency has also been observed in past work. DeVries et al. (2005) measured the NDF content at the feed bunk over time and found that the NDF content of the TMR that remained in feed bunk increased in a curvilinear fashion for all treatments, feeding either once, twice or 4 times daily, but this increase was most pronounced when cows were fed once daily. This may indicate that cows were sorting against long particles and for short particles, thus increasing the NDF proportion left in the TMR, and that cows fed once daily sorted to a greater extent. Endres and Espejo (2010) observed 50 free stall barns in Minnesota and found that NDF content of the TMR increased throughout the day, an average of 6.75 percentage units between delivered feed and orts. Their data also showed a significant association between feeding frequency and sorting, where less frequent feedings per day increased the NDF content change in the TMR over a day. In a similar study in Eastern Ontario, Sova et al. (2013) sampled 24 dairies and found that cows, in general, sorted against long particles and for short particles, with a sorting index of 97.3 and 101, respectively. Feeding frequency tended to be associated with sorting long particles, where farms that fed twice daily vs. once daily found a reduction in sorting against long particles by 0.86 % units. While feeding frequency has implications for encouraging a more evenly distributed feed consumption throughout the day, there is also evidence that it reduces sorting of the TMR itself.

1.3.4 Feeding Frequency and Milk Production

Frequency of feeding also has conflicting results in milk yield and composition. Several studies have found that when the frequency of TMR or concentrate feeding is increased there is no effect on DMI, milk yield or milk composition (French et al., 1990; Nocek and Braund, 1985;

Klusmeyer et al., 1990). Nocek and Braund (1985) fed a high concentrate diet 1, 2, 4, or 8 times daily and found no change in DMI or milk production, however cows were adapted to the diet for only 10 days before data were collected, which may not have been enough time to detect treatment effects. In an experiment comparing twice and four times daily feeding of a TMR containing 45% concentrate, Klusmeyer et al. (1990) also found no change in intake, milk yield or composition. Robinson and McQueen (1994) observed similar results, however their experiment was comparing the feeding frequency of a protein supplement, which may have led to different results than if they were feeding a concentrate or TMR at different frequencies. In an experiment feeding concentrates 12 times or twice daily, French et al. (1990) found no effect on milk yield or composition, however the authors attributed this to a lower dietary inclusion of concentrate (<50%). When French and Kennelly (1990) fed concentrate 12 times or twice daily with a total dietary F:C of 40:60 the more frequent feeding treatment increased milk fat concentration by 0.39 % units. If feeding more often leads to even distribution in eating and thus a reduction in rumen pH variation, it can be expected that maintaining a higher rumen pH throughout the day will reduce the amount of negative FA intermediates (i.e. tras-10, cis-12; Bauman and Griinari, 2003), which will increase milk fat yield (Bauman and Griinari, 2003). However, this would be more pronounced in a high concentrate diet that can lower rumen pH drastically. Both Manysaari et al. (2006) and Le Liboux and Peyraud (1999) reported no change in milk composition and fed mid to high forage diets, at 49% and 69% of dietary DM, respectively. However, previous experiments have also fed mid- to high-forage diets and observed a change in milk composition. Shabi et al. (1999) fed a diet containing 49% forage and found an increase in DMI as well as milk fat concentration and yield when cows were fed four vs. twice daily. Rottman et al. (2014) fed a TMR with an F:C of 60:40, however the dietary NSC

concentration was 42%, which is above the recommended content of 40%, and the fermentability of the diet may have been high with ingredients such as cookie meal and sugar. When the diet was fed four times daily vs. once daily, there were increases in DMI (by 2.2 kg/d), milk fat yield (by 0.13 kg/d) and milk fat concentration (by 0.26 %). The authors also found an increase in trans-10 18:1 in the milk when cows were fed once daily, which is associated with milk fat depression, suggesting a lower rumen pH environment. While the results on milk composition and feeding frequency vary, there is a tendency for more fermentable diets to increase milk fat yield when fed more often. Results on DMI and milk yield are still more variable, but overall intake and milk yield do not seem to be strongly affected by feeding frequency.

A few studies have also looked effects of feeding frequency on blood metabolites. When French and Kennelly (1990) fed a moderately fermentable diet and delivered the concentrate more often, they did not find any difference in average or temporal patterns of plasma growth hormone (GH), insulin, or glucose concentrations. Rooke et al. (2008) fed a high- or low-concentrate diet either twice or four times daily and found that when the high concentrate diet was fed more often there was a decrease in plasma insulin concentrations measured after feeding. This study provides more evidence for frequent feeding to prevent spikes in nutrient intake and plasma insulin concentrations, which is more beneficial when a more fermentable diet is fed. Similarly, Rottman et al. (2014) also reported a decrease in the amplitude of peak plasma glucose, insulin, NEFA, and blood urea nitrogen (BUN) concentrations when their diet was fed 4 times vs. twice.

In conclusion, previous literature has suggested that feeding frequency influences eating pattern, rumen pH, milk composition and blood metabolites. Feeding diets even twice vs. once daily may encourage a more even distribution of eating throughout the day and thus a less

variable rumen pH. The effects of frequent feeding also appears to be more pronounced when a highly fermentable diet is fed, which is beneficial for reducing the severity of SARA, especially in high producing dairy cows.

1.4 Photoperiod

1.4.1 Introduction

In addition to feeding frequency as a management approach to alter behaviour and reduce risk for SARA, photoperiod management may be another approach to this end. Photoperiod is the length of daylight in contrast to darkness within a day and is another management tool used in dairy production. A long-day photoperiod generally consists of 16 hours of light while a short day photoperiod is 8 h of light (Crawford et al., 2015). Previous literature has shown that dairy cows will increase DMI by 1 to 1.5 kg/d and increase milk yield by 2 to 2.5 kg/d under a long day photoperiod (Collier et al., 2006). Photoperiod management has larger impacts in areas where photoperiod changes to a greater extent throughout the year, like in Alberta where the shortest day is less than 8 hours of light. Due to the long and harsh winters in Alberta many farms house their cows indoors, which makes the manipulation of photoperiod easier. Using photoperiod to increase milk production may be caused by a variety of factors in the cow, which may also affect other aspects of production, such as feeding behaviour and the distribution of behaviours throughout the day.

1.4.2 Photoperiod and Endocrine Changes in the Cow

According to a review conducted by Dahl and Petitclerc (2003), the majority of studies conducted with dairy cows have shown an increase in milk yield by 2 kg/d on average when cows are exposed to a long day photoperiod in comparison to a short day photoperiod, but there

is generally no effect on milk composition. They also identified that a long day photoperiod treatment can be applied at any stage of lactation with positive effects on milk yield and that the treatment has been evaluated for up to 43 weeks in published studies. Peters et al. (1978) made the first observation that a long day light treatment would increase milk yield and that the increase was observed after 3-4 weeks of treatment, which has been confirmed in more recent studies (Dahl and Petitclerc, 2003).

The primary mediator behind the effects of a long day photoperiod are hormonal. Light is detected by the retina which signals the pineal gland to inhibit an enzyme that synthesizes melatonin. Melatonin is the active mediator of photoperiodic responses, as reviewed by Dahl et al. (2000). Melatonin concentrations increase at night (Hedlund et al., 1977) and thus there is a higher concentration during short day photoperiods. Auldist et al. (2007) implanted 12 of 24 cows with melatonin and found a reduction in prolactin after 4 weeks and a reduction in milk yield after 6 weeks that resulted in a 23 % decrease in production by week 12. Therefore an increase in prolactin is also possibly associated with long day photoperiods. In a review by Tucker (2000), the author stated that estrogen initiates lactation by causing a release of prolactin and an increase in prolactin receptors in the mammary cells. However, prolactin may not have a direct effect on milk yield. Plaut et al. (1987) injected 4 of 8 multiparous cows with exogenous prolactin for 2 weeks before and after peak lactation, and increased plasma prolactin concentrations by 2 to 5 fold. However, there was no effect on milk yield and composition at both stages of lactation. Tucker et al. (2000) also stated that supressing prolactin in lactating cattle had no effect on milk yield.

Another possible factor that increases milk yield for long day photoperiod is growth hormone (GH). In an experiment conducted by Dahl et al. (1991) the authors found that injection of

exogenous GH into lactating dairy cattle increased milk yield and it was mediated by increased insulin-like growth factor 1 (IGF-1) synthesis stimulated by GH. Growth hormone is released by the pituitary gland and binds to hepatocytes to stimulate IGF-1 synthesis which acts on type 1 and 2 receptors in the mammary tissue (Tucker et al., 2000). Prosser et al. (1990) examined direct effect of IGF-1 on milk yield, and found an increase in milk yield when IGF-1 was injected into an artery of the mammary gland. Although IGF-1 synthesis is stimulated by GH, previous experiments have documented an increase in IGF-1 in long day photoperiods independent of GH. Dahl et al. (1997) exposed 40 cows to either a long day or short day photoperiod and found an increase in plasma IGF-1 concentration after 14 days, followed by an increase in milk yield after 28 days for cows exposed to long day photoperiod. There was no effect of treatment on circulating GH as well as IGF binding proteins (IGBP) 2 and 3 which are responsible for IGF-1 clearance. The authors concluded that the increase in IGF-1 occurs at the secretory level, independent of GH, and not due to hormone clearance. Kendall et al. (2003) exposed steer calves to different photoperiods and took liver biopsies, however they found no change in expression of IGF-1 mRNA or GH receptor 1A. This indicated that long day photoperiods did not increase secretion of IGF-1 nor available GH receptors to increase IGF-1 synthesis. Flint et al. (2001) hypothesized that additional IGFBP, other than 2 and 3, could be responsible for increased concentrations of IGF-1. The authors suggested IGFBP 5 as an inhibitor of IFG-1, which is reduced during a long day photoperiod. This binding protein is inhibited by prolactin, which accounts for the reduction during long days, and is a possible factor that increases IGF-1 release. In conclusion, light treatments affect a variety of hormones in the body of cattle which could all influence milk yield. Although there is no definite answer on why

milk yield increases, there is evidence that IGF-1 is the primary factor, which could be mediated by binding proteins and prolactin.

1.4.3 Photoperiod and Behaviour

An early hypothesis explaining the increase in milk production due to a long day photoperiod centered on more hours of light altering the feeding pattern of cows. Dahl et al. (2012) suggested, in their review, that there was an advantage for neonatal survival if parturition was coordinated with environmental and resource cues. If cattle give birth as days grow longer in the spring, the milder temperature and increase in feed resources in the spring and summer allows the cow to sustain milk production and the calf has a better chance of survival. Additionally, cows prefer to eat during light hours as Phillips and Denne (1988) observed, with 52% of grazing occurring during the day (0700 to 1600 h), 54% in the evening (1600 to 2300 h) and 11 % at night (2300 to 0700 h). Therefore, if more hours of light are provided in a day cows would spend a longer time eating, thus increasing DMI and milk yield production, where authors assumed that eating time was directly related to feed intake. Phillips and Schofield (1989) tested this hypothesis in 24 lactating cows by providing 12 cows with a short day photoperiod (SP) and 12 cows with a long day photoperiod (LP) using supplemental lighting. Cows on a SP had light from 0800 to 1600 h and LP cows had supplemental light from 0530 to 0800 h and 1600 to 2330 h. Despite a 3 kg/d increase in milk yield in LP cows, there was no difference in total time spent eating, which indicated that increased DMI and milk production could not be attributed to differences in time spent eating. This provides more evidence that the casual factor behind greater production is likely hormonal, not behavioural. However, the light treatment influences other behaviours in cattle. Although Phillips and Schofield (1989) observed no increase in total eating time, there was an additional peak in eating activity for LP cows during the evening

supplemental light period. The alteration of eating pattern may affect rumen health. For an example, if supplemental light increases the distribution of eating time throughout the day, it may have similar effects as increasing feeding frequency. In addition to changes in eating pattern, Phillips and Schofield (1989) also found that the LP treatment increased lying times and reduced overall activity, in terms of distances walked and frequency of oestrus behaviours performed. Phillips et al. (1997) found similar results when subjecting 48 heifers to SP or LP; no change in total feeding times but increased time spent lying in the LP cows. Increased lying times were also observed in calves, where calves spent more time lying and more time in total on the side of the bedded area that was provided with supplemental light vs. natural light (Weiguo and Phillips, 1991). Based on these experiments there is a general trend for light to promote resting behaviours, i.e. lying down. Leining et al. (1980) measured a significant decrease in plasma glucocorticoid concentration by 29% and 39% when 16 and 20 hours of light were provided to bulls vs. 8 hours of light, respectively. A reduction in plasma glucocorticoid concentration during light hours could promote rest in cattle. A decrease in activity and increase in time spent resting could reduce daily energy requirements in cattle and possibly contribute to increased production. Additionally, lying down has been shown to increase mammary blood flow rate (Rulquin and Caudal, 1992), which can increase metabolism of nutrients in the mammary gland and thus increase milk production. While behaviour may not be the driving force for LP to increase milk production, LP may influence behaviour in a way that could benefit milk production and rumen health.

1.5 Conclusion

Subacute ruminal acidosis is a major concern for the dairy industry as it can negatively impact production and cow health. Cow behaviour is a major contributing factor to the risk for

developing SARA, and individual animal variation plays a role in determining which cows display problem behaviours, such as sorting and eating large meals quickly. There are management factors that may be used to manage these problem behaviours and two potential management approaches are feeding frequency and photoperiod management.

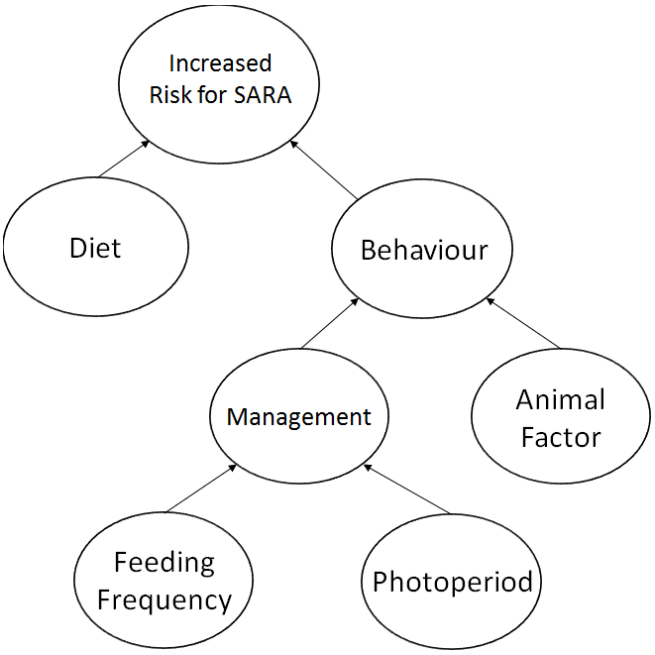

Figure 1-1. The relationship between risk for SARA, behaviour and management strategies

1.6 Knowledge Gaps

Based on the literature reviewed above, 3 knowledge gaps have been identified for thesis research.

1) It has been established that cows all fed the same high grain diet vary in rumen pH response. Categorizing extreme cows as higher or lower risk for SARA allows researchers to identify differences between these two groups and possible casual factors for the difference. One potential factor that has yet to be explored is feeding pattern, specifically distribution of eating

time throughout the day. Distribution of eating time has implications for acid production in the rumen and its health.

2) In addition to identifying possible causal factors for the variable responses to a high grain, it is also important to identify possible management approaches to decrease the severity of SARA experienced by higher risk cows without negatively affecting the production of lower risk cows. Feeding frequency has been shown to reduce the diurnal variation of rumen pH but the effects of feeding frequency on rumen pH of cows with a different risk of SARA has not been explored. If difference in eating pattern is indeed a potential causal factor for cows to have a higher risk for SARA, then altering feeding frequency would change the diurnal eating pattern to reduce risk.

3) Increasing photoperiod length has been shown to influence both production and behaviour. Although behaviour may not cause the increase in production, altered feeding pattern through light treatment may have implications on rumen health. Studies evaluating effects of photoperiod management on feeding patterns are scarce, and the effects of an LP treatment on sorting behaviour has yet to be explored.

2.0 Experiment 1: Increased Feeding Frequency Reduces Severity of Subacute Ruminal Acidosis in Higher Risk Cows and Increases Milk Fat Yield

2.1 Introduction

Subacute ruminal acidosis (SARA) is a metabolic disorder mainly found in high-producing dairy cows fed highly fermentable diets (Plazier, 2008). A prolonged rumen pH below 5.8 can lead to problems in animal health such as laminitis and liver abscess as well as production losses from milk fat depression and reduced DMI (Nocek, 1997). Previous research has established there is a large amount of variation in individual rumen pH response to the same high grain diet in beef steers (Schlau et al., 2012) and dairy cows (Penner et al., 2007), but the causes of the variation have not been clearly identified. In recent studies, cows were categorized as tolerant or susceptible to a high grain diet based on an acidosis index, which is the severity of SARA normalized for intake (area below pH 5.8 / DMI; Penner et al., 2009), and efforts have been made to characterize animals tolerant to a high grain diet. Schlau et al. (2012) observed decreased VFA concentrations in tolerant animals which may have been due to decreased VFA production or increased absorption, while Gao and Oba (2014) reported that tolerant animals sorted against long particles to a lesser extent. However, distribution of feeding behaviour among animals that differ in tolerance to a high grain diet has not yet been evaluated. As eating larger amounts of feed in a short period of time can lead to a rapid decrease in rumen pH, through a spike in nutrient delivery to the rumen and thus a spike in VFA production from microbes

(Gonzalez et al., 2012), it is possible cows more susceptible to SARA exhibit different feeding behaviours or eating patterns throughout the day.

Previous studies have reported that increasing feeding frequency to more than once per day reduced the diurnal variation of rumen pH (French and Kennelly 1990; Shabi et al., 1999). The delivery of fresh feed was shown to stimulate eating in cows (DeVries and von Keyserlingk, 2005) and feeding 5 times vs. once daily led to more frequent peaks in eating activity, corresponding to each feeding (Mantysaari et al., 2006). DeVries et al. (2005) also found that feeding 4 times per day instead of twice or once reduced the time spent eating in the 90 min following each feeding. Therefore, increasing the frequency of feeding may encourage increased distribution of eating throughout the day, reducing large intakes following feed delivery and thus contributing to more stable pH levels. Although effects of feeding frequency have been extensively studied, its effect on rumen pH has not been consistent (Shabi et al., 1999; French and Kennelly, 1990; Robinson and McQueen, 1994), and the relationship between feeding frequency and individual risk for SARA has yet to be evaluated.

The current experiment includes two preliminary studies, the results of which led to the primary experiment. The objective of the preliminary studies was to determine if cows that differ in their risk for SARA also differ in their distribution of feeding behavior throughout the day. We hypothesized cows at a higher risk for SARA fed once daily would eat for a longer period of time in the 8 h after feeding and would reduce eating time later in the day. The objective of the primary experiment was to determine effects of increased feeding frequency from once to 3 times daily on feeding behavior and rumen pH of lactating dairy cows that differ in their risk of developing SARA. We hypothesized that frequent feeding would increase distribution of eating throughout the day and reduce the severity of SARA in higher-risk (HR) cows.

2.2 Materials and Methods

All experimental procedures used in this study were approved by the University of Alberta Animal Care Committee and conducted according to the guidelines of the Canadian Council of Animal Care (CCAC, 2009). All cows were housed individually in a tie-stall barn bedded with wood shavings and had free access to water. Cows were milked twice daily at 0400 and 1500 h. Cows were fed for 5 to 10 % daily orts.

2.2.1 Preliminary Studies

Study 1 was conducted in 2012 (Gao and Oba, 2014) with 10 ruminally cannulated lactating Holstein cows (DIM = 277 ± 37; BW = 600 ± 77 kg) and Study 2 was conducted in 2014 (Gao and Oba, 2015) with 9 ruminally cannulated lactating Holstein cows (DIM = 247 ± 19; BW = 686 ± 42 kg). In the current preliminary studies behaviour data was analyzed to determine the effect of risk for SARA on feeding pattern. In both studies, cows were fed high-grain diets once daily to induce SARA (Table 2-1), at 0800 h for Study 1, and at 0900 h for Study 2. For both studies, cows were fed ad libitum for 21 d with 17 d of diet adaptation and 4 d of sample and data collection. Although the preliminary studies focused solely on the collection of rumen pH and behavior data, Gao and Oba (2014) also measured VFA concentrations from rumen fluid samples, sorting behavior, blood metabolites, and milk yield and components. Three cows were used in both studies so only data from Study 2 was reported, leaving a total of 16 cows used for statistical analysis. Of these 16 cows, 3 were primiparous and 13 were multiparous.

For both studies rumen fluid pH was measured continuously in the ventral sac every 30 s for 24 h on d 18 using the pH measurement system evaluated by Penner et al. (2006). This

system was used to determine mean, minimum and maximum pH, duration below a pH of 5.8 as well as area below pH of 5.8, or the extent of decrease below 5.8, for each cow. The data were then used to determine an acidosis index (area below pH 5.8 / DMI; Penner et al. 2009) which indicates the severity of SARA normalized for intake. Cows below the acidosis index threshold of 1.0 were categorized as lower-risk (LR) and those above were categorized as HR.

For both studies, feeding behavior was recorded over 24 h on d 18, coinciding with rumen pH recording. All behavior observers were trained to standardized behavior definitions and recordings. Cows were observed for eating and ruminating every 5 min and the behavior performed at that time was recorded and assumed to last for the full 5-min period (Beauchemin et al., 2003). Eating was considered as head in the feed bunk and chewing or head above the feed bunk, chewing and with feed on the muzzle, while ruminating was considered as no interaction with the feed bunk, chewing, with no feed present on the muzzle. Feeding behavior was summarized separately for three 8-h time-periods relative to feeding to account for different feeding times between experiments (e.g., if the cow was fed at 0800 h, time-period 1 would be from 0800 to 1600 h, time-period 2 from 1600 to 2400 h and time-period 3 from 2400 to 0800 h). This analysis approach allowed us to determine what part of the day, relative to feed delivery, the cows spent more time eating. Two eating bouts needed to be separated by more than 10 min or else it was assumed to still be the same bout of eating as described by Dado and Allen (1993).

All response variables were evaluated using the Fit Model procedure of JMP (version 11, SAS Institute Inc., Cary, NC). The model included the fixed effects of Experiment (1a vs. 1b), category (higher- vs. lower-risk of SARA), and experiment by category interaction. As the interaction between experiment and category was not significant ($P > 0.10$), the interaction term

was removed from the statistical model. Significance was declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$

2.2.2. Primary Experiment

Eight ruminally-cannulated lactating Holstein cows were used in a cross-over design study with 21-d periods (Table 2-2). Treatments were feeding frequency, where 4 cows were fed once daily (1×) at 0800 h and the other 4 cows were fed three times daily (3×) at 0800, 1500, and 2200 h. Three feedings were chosen as a previous study had found increased treatment effects on increasing distribution of eating time when feeding frequency was greater than twice per day (e.g. 4 times daily; DeVries et al., 2005). A TMR for all cows was mixed once before the 0800-h feeding for all cows and TMR for the 3× cows was separated into thirds and vacuum sealed to minimize spoilage and heating. Cows were weighed after the morning milking over two consecutive days immediately before the start of the study. Four cows in early lactation (DIM = 50 ± 20) and 4 cows in late lactation (DIM = 381 ± 46) were used, with two early and two late lactation cows assigned to each treatment sequence, 1× to 3× or 3× to 1×. All cows were fed the same diet containing 36.5% forage, 17.9% forage NDF and 31.6 % starch to induce SARA (Table 2-1). The DM concentration of the barley silage was determined twice per week and the diet formulation was adjusted if necessary. Each of the 2 periods of the study had 16 d of adaptation to feeding frequency treatment and 5 d of sample and data collection. Samples of TMR and orts for each cow were collected from d 17 to 19 and the orts were combined from all 3 days to yield one sample per cow.

Rumen pH was measured as described for the preliminary studies but over a 72-h period (d 17 to 19), therefore pH variables (min, mean, max, and are and duration below 5.8) were averaged over 3 d to obtain one value per cow per period. The area below pH 5.8 was normalized

for DMI, by dividing the area below 5.8 for each cow by its individual intake, to determine the acidosis index. The acidosis index calculated for 1× treatment was used to categorize all cows as HR or LR for SARA, as this was our baseline measurement.

Feeding behavior was evaluated as described for the preliminary studies, except that behaviour was recorded over a 72-h period (d 17 to 19) to coincide with the pH measurement period. Feeding behavior was recorded as 1) eating, 2) ruminating and standing, 3) ruminating and lying, 4) no activity and standing, or 5) no activity and lying, where no activity is a lack of eating and ruminating. Behavior was analyzed separately for 3 different time periods for all cows, based on the 3× feeding schedule. Time-period 1, 2, and 3 were from 0800 to 1500 h, from 1500 to 2200 h, and from 2200 to 0800 h, respectively. Each time-period started with a feeding for the 3× cows to better determine the effect of feed delivery on their feeding behavior. The minimum interval between bouts of eating was 10 min, and between bouts of ruminating and lying was 5 min. Particle size distribution of the TMR and orts samples was determined using a Penn State Particle Separator with 3 sieves (aperture size of 19.0, 8.0, and 1.18 mm). The particle size distribution for the TMR was 6.2 % on the top screen, 38.9 % on the middle screen, 38.5 % on the bottom screen, and 16.5 % in the pan. Sorting index was calculated as the ratio of actual intake to predicted intake of particles retained on each sieve of the separator. A sorting index above 100 indicates sorting for particles and an index below 100 indicates sorting against particles (Leonardi and Armentano, 2003).

Milk samples were collected at both am and pm milkings from d 17 to 19. Samples were analyzed for fat, CP, lactose and MUN by infrared spectroscopy (AOAC International, 2002; method 972.16; Milkoscan 605, Foss North America, Brampton, ON) at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada).

On d 20, cows were fitted with a jugular catheter to facilitate blood sample collection on d 21. Samples were collected, every 3 h over a 24-h period starting at 0700 h, into tubes containing heparin (Fisher Scientific Company; Nepean, ON, Canada). Samples were centrifuged at 3,000 × *g* at 4°C for 20 min immediately after collection; plasma was harvested and stored at -20°C until further analysis. Plasma samples were analyzed for glucose concentrations using a glucose oxidase and perioxidase enzyme (Sigma, St. Louis, MO) and dianisidine dihydrochloride (Sigma) procedure. Absorbance was determined by a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA) at a wavelength of 450 nm. Commercial kits were used to determine concentrations of plasma fatty acids (Wako Chemicals USA Inc., Richmond, VA) and insulin (Coat-a-Count kit, Diagnostic Products Corp., Los Angeles, CA). These plasma metabolite data were summarized to obtain one value per cow per period prior to statistical analysis.

In addition to blood samples, rumen fluid was collected every 3 h over a 24-h period on d 21, starting at 0700 h. Samples were collected from the cranial, ventral, and dorsal sacs, and then combined and strained through a perforated screen (Peetex, Sefar Canada Inc., Scarborough, ON, Canada). The samples were centrifuged at 3,000 × *g* at 4°C for 20 min and the supernatant was stored at -20°C until further analysis. Rumen fluid samples were combined to form one sample per cow per period for analysis. The VFA profile was analyzed by gas chromatography as described by Khorasani et al. (1996) and rumen ammonia-N concentration was determined as described by Fawcett and Scott (1960) using a plate reader (SpectraMax 190).

All response variables were evaluated using the Fit Model procedure of JMP (version 11, SAS Institute Inc., Cary, NC). The general linear model included the fixed effects of period, treatment, category and category by treatment interaction, as well as the random effect of cow

nested within category. Treatment sequence, 3x to 1x vs. 1x to 3x, had been included as a fixed effect in the initial statistical model, however sequence was removed as its effect was not significant for all response variables. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$. Least square means were separated using the Student T-test.

2.3 Results

2.3.1 Preliminary Studies

In the group of 16 cows, 7 were categorized as HR and 9 as LR. The minimum pH and mean pH were lower ($P < 0.001$; Table 2-3) for HR cows than for LR cows. There was also a tendency ($P = 0.08$) for maximum pH to be lower for HR cows than for LR cows as well. The duration below 5.8 ($P < 0.001$), the area below a pH of 5.8 ($P < 0.001$) and the acidosis index ($P < 0.01$) were both greater for the HR cows then for the LR cows. No differences were observed in milk yield and DMI between HR and LR cows.

In time-period 1, the first 8 h after feeding, higher risk cows ate for a longer period of time ($P = 0.01$; Table 2-3) than lower risk cows; there was no difference observed in ruminating time. No difference in either eating or ruminating time was observed in time-period 2. In time-period 3, higher risk cows ate for less time ($P = 0.01$) and ruminated for a longer period of time ($P = 0.01$) than lower risk cows.

2.3.2 Primary Experiment

Feeding 3× daily decreased ($P < 0.01$; Table 2-4) eating time between 0800 and 1500 and increased ($P < 0.01$) eating time between 2200 and 0800 h for all cows, regardless of risk category, in comparison to 1× feeding. There was also a treatment effect on ruminating while lying between 0800 and 1500 h, in which cows fed 3× increased ($P < 0.01$) time ruminating

while lying in comparison to cows fed 1×. An interaction effect ($P < 0.01$) was observed for ruminating while standing between 2200 and 0800 h, in which LR cows decreased time ruminating while standing when fed 3× with no significant effect on HR cows. There was no observed difference in behavior between 1500 and 2200 h. There was also no difference (Table 2-5) in treatment, category, or an interaction between treatment and category on sorting behavior.

In regards to rumen pH, 4 cows were categorized as HR and 4 as LR and within both groups 2 cows were late lactation and 2 early lactation. There was a treatment by category interaction ($P = 0.05$) effect on area below a pH of 5.8 ($P = 0.05$; Table 2-6), in which 3× feeding reduced the area for HR cows ($P < 0.05$) with no significant difference for LR cows. There was also an interaction ($P = 0.03$) effect on acidosis index, again in which HR cows decreased ($P < 0.05$) their index when fed 3× and no significant difference was observed for LR cows. There was no treatment or interaction between treatment and category on minimum pH but there was a tendency ($P = 0.10$) for HR cows to have a smaller nadir pH in comparison to LR cows. Mean pH was less ($P < 0.05$) for HR than LR cows and there was an interaction ($P < 0.01$) between treatment and category for maximum pH, where HR cows had a lower ($P < 0.05$) maximum pH when fed 3× with no significant effect on LR cows. There was no treatment or interaction between treatment and category effect on DMI, however HR cows ate more than LR cows (28.3 vs. 23.4 kg/d; $P = 0.05$). There was no difference in total VFA or ammonia concentrations in rumen fluid between treatments or between risk categories for SARA (Table 2-7).

There was no difference in plasma glucose or NEFA concentrations observed, but HR cows tended ($P = 0.06$; Table 2-7) to have a higher plasma insulin concentration compared to LR cows, but it was not affected by treatment. There was no difference in milk yield between risk

categories or feeding frequency treatment (Table 2-8), however 3× feeding increased ($P = 0.01$) milk fat yield and tended ($P = 0.06$) to increase milk fat content in comparison to 1× feeding. An interaction ($P = 0.05$) between treatment and category was observed for milk CP yield because responses to feeding frequency varied with risk group for SARA (Table 2-9). There was no difference between category or treatment and MUN concentration.

2.4 Discussion

The objective of the preliminary studies was to determine if any differences in feeding behaviors were observed between cows with a higher or lower risk of developing SARA. The hypothesis was that HR cows would eat for a longer time directly following feed delivery, than LR cows. Cows at a higher risk for SARA ate for a longer period of time in the 8 h following feeding and for less time in the 8 h before feed delivery of the next day than lower risk cows. There was no difference in total DMI between risk categories, therefore the differences in eating time distribution are likely the contributing factor to only certain cows developing SARA, even when all cows were fed the same diet. The amount of VFA produced in a period of time is dependent on meal size and eating rate, in which large intake of fermentable carbohydrates increases the risk for SARA (Owens et al., 1998; Gonzalez et al., 2012). From the results of the preliminary studies, a possible management approach that would encourage HR cows to eat less after feed delivery and distribute eating time more evenly throughout the day was evaluated. The hypothesis was that frequent feeding, 3× vs. 1×, would increase the distribution of eating throughout the day and reduce the severity of SARA experienced by HR cows. Some nutritional approaches to mitigate SARA such as feeding a lower grain diet would improve rumen health of HR cows, but likely reduce milk production of LR cows that do not experience SARA. In the

primary experiment, feeding frequency, 3× vs. 1×, was evaluated as a management approach to reduce the severity of SARA in HR cows without decreasing productivity of LR cows.

In previous experiments, increased feeding frequency has had a variable effect on rumen pH. Shabi et al. (1999) found a decrease in mean rumen pH when cows were fed four times vs. twice daily, however cows fed four times had an increased total DMI and NSC intake. Several experiments have found no effect of frequent feeding on mean rumen pH but a decrease in the diurnal variation of rumen pH was observed, in which feeding more often maintained a more stable pH throughout the day (Bragg et al., 1986; French and Kennelly, 1990; Kaufmann, 1976). Robinson and McQueen (1994) reported an increase in mean rumen pH when cows were fed five times vs. twice daily, which the authors attributed to the dramatic decrease in rumen pH following each of the twice daily feedings. In the current experiment, there was no effect of feeding frequency on rumen pH parameters, except for maximum rumen fluid pH. Nevertheless, once cows were categorized as higher or lower risk of SARA, then 3x daily feeding reduced rumen pH differently for each category, in which feeding more often reduced the severity of SARA based on area of rumen fluid pH below 5.8 for HR cows with no effect on LR cows.

The positive effects of frequent feeding on rumen pH are likely attributed to a change in feeding behavior, specifically eating pattern. When cows were fed more often, regardless of SARA risk category, there was a more even distribution of eating over the three time-periods. Mantysaari et al. (2006) found a similar result when feeding cows five times vs. once daily; frequent feeding resulted in more even eating distribution throughout the day. When DeVries et al. (2005) fed cows four times vs. twice daily, they found that cows fed more often ate more in the late evening and early morning (2000 to 0600 h) and ate less in the 90 min following feeding. The delivery of fresh feed stimulates cattle to eat (DeVries and von Keyserlingk, 2005) and

likely contributes to the even consumption of feed throughout the day. As mentioned earlier, larger meals in a shorter amount of time can increase VFA production and increase the risk for SARA (Gonzalez et al., 2012). Serment et al. (2012) observed a steeper decrease in the rumen pH of goats classified as 'fast-eaters' where 70% of the daily feed was consumed in the 90 minutes following feeding. In the current study, data need to be interpreted with caution as DMI was measured daily but not for each time period. We assumed that a difference in the distribution of eating time led to a similar difference in the distribution of intake and considered it as a possible reason for variable rumen pH among animals fed the same diet, but this still needs to be confirmed. As daily eating time and overall DMI are not strongly correlated (Dado and Allen, 1994), it is also possible that distribution of eating time affects ruminal fermentation directly without changes in distribution of DMI.

In addition to eating pattern, we looked at other behaviors as potential contributing factors to reduced severity of SARA. Feeding 3× increased time spent ruminating while lying during time period 1 (0800 to 1500 h) in comparison to feeding 1×, which was associated with the decrease in time spent eating. This could be another possible causal factor as increased time spent ruminating has been shown to increase rumen pH (Beauchemin et al., 2003).

Overall DMI did not differ between feeding frequency treatments, which has also been observed in previous work (Klusmeyer et al., 1990; DeVries et al., 2005). Shabi et al. (1999) found an increase in DMI when feeding more often, however feed was available for an extra 2 h per day for the cows fed four times vs. twice daily. In the current study, animal responses to 3x feeding in rumen pH cannot be attributed to daily DMI. However, HR cows had greater DMI by 5 kg/d compared to LR cows. Although increased DMI of a highly fermentable diet increases the risk for SARA (Owens et al., 1998), a greater DMI for HR cows was not observed in the

preliminary studies or in past experiments (Gao and Oba, 2014; Gao and Oba 2015). Therefore, we cannot conclude that DMI is always greater in HR cows.

Sorting against long particles in the TMR can reduce NDF intake (Leonardi at al., 2005) and increase risk for SARA (Cook et al., 2004). However, frequent feeding did not affect sorting behavior in the current study, so reduced severity of SARA in the higher-risk cows cannot be attributed to reduced sorting. Similar results were observed by Hart et al. (2014) when cows were fed three times daily, with no change in sorting behavior. However, DeVries et al. (2005) evaluated the effect of feeding frequency once, twice or four times daily on NDF content of the diet over time as a measure of feed sorting, and reported an interaction between treatment and sampling time, which indicated that cows fed once daily sorted more over the course of the day than those fed twice daily. Sova et al. (2013) also observed that group housed cows on 24 different commercial dairies sorted less against long particles when they were fed twice a day as opposed to once per day. Endres and Espejo (2007) sampled TMR from 50 different free stall barns and found a negative relationship between feeding frequency and increase in NDF content of the TMR throughout the day, where feeding once per day resulted in a greater increase in NDF content of the TMR. The differences in experimental design between these studies and the current one (number of cows, group vs. individual sampling, and diet composition) may explain the discrepancies in effects of frequent feeding on sorting behavior.

In addition to 3× feeding reducing the severity of SARA in HR cows, we found positive effects of 3× feeding on milk fat of all cows. Feeding cows 3× did not increase milk yield, which is consistent with previous work (Shabi et al., 1999; Mantysaari et al., 2006; Hart et al., 2014). However, 3× feeding increased milk fat yield and tended to increase milk fat concentration in comparison to 1× feeding. Effects of frequent feeding on milk component production have been

variable with some studies reporting increases in milk fat yield and concentration (Shabi et al., 1999; French and Kennelly, 1990; Sutton et al., 1988) and others reporting no effect (Klusmeyer et al., 1990; Mantysaari et al., 2006; Hart et al., 2014). These discrepancies may be attributed to dietary forage content; the studies that reported greater milk fat yield for cows fed more frequently(Shabi et al., 1999; French and Kennelly, 1990; Sutton et al., 1988), including the current one, fed low forage diets, from 10 to 48.8 % of the diet DM as forage, whereas the studies that did not report effect of increased feeding frequency on milk fat yield fed cows diets with greater forage content, 49 to 55 % of the diet DM (Klusmeyer et al., 1990; Mantysaari et al., 2006; Hart et al., 2014). Providing low-forage diets may have resulted in a lower rumen pH and thus leading to a greater benefit from more frequent feeding. However, the current study reported greater milk fat yield for the 3× treatment for all cows, despite no significant change in severity of SARA for the lower-risk cows. These results indicate that rumen pH is likely not the sole reason why milk fat yield was elevated in cows fed more often. Oetzel (2007) suggested that the milk fat depression seen with SARA may be influenced by factors other than rumen pH. For example, large amounts of poly-unsaturated fatty acids in the diet can contribute to incomplete biohydrogenation of fatty acids in the rumen and milk fat depression (Bauman and Griinari, 2003). In the current experiment vegetable oil was fed at 1 % DM, which when fed 1× vs. 3× may have overwhelmed the rumen microbes ability to complete biohydrogenation, as well as interacting with a low rumen pH, and resulted in milk fat depression. However, neither milk fatty acid composition nor microbial profile were measured in the current experiment, which could have given some insight into the causal factors behind the milk fat increase when cows were fed 3×.

In the current study, there was no effect of treatment on total rumen fluid VFA concentration or molar proportions of VFA, and therefore changes in VFA did not contribute to the treatment effect on milk fat yield. Previous studies have reported variable results for the effect of feeding frequency on rumen VFA parameters. French and Kennelly (1990) also reported no change in total VFA concentrations or molar proportions when cows were fed a concentrate twice compared with twelve times daily or a TMR twelve times daily. A couple of experiments observed no change in the total concentration of VFA but a tendency (P-value) for increase in the molar proportion of propionate with more frequent feeding (Klusmeyer, 1990; Shabi 1999), whereas Robinson and McQueen (1994) found a decrease in total VFA concentration when cows were fed five times compared with twice daily, but a decrease in propionate concentration. Bragg et al. (1986) reported no change in total VFA concentration but an increase in the molar proportion of acetate when cows were fed eight times versus twice daily. All of these studies had drastically different treatments, diets, and feeding frequencies which may have contributed to the variable results. Rumen fluid samples were collected every 3 h in the current study, but studies with at least hourly sampling did not observe the treatment effects on VFA concentration and its profile (French and Kennelly, 1990; Sutton et al., 1988). As such, lack of treatment effects in the current experiment cannot necessarily be attributed to insufficient sampling frequency. No difference was observed in total VFA concentrations between higher and lower risk cows, which was also found by Gao and Oba (2014). Chen et al. (2012) reported an increase in total VFA concentration in higher risk steers as well as increased rumen bacterial density, which indicates an increase in VFA production. In the second paper from this experiment, Schlau et al. (2012) found an increase in VFA absorption in lower risk steers, which may have contributed to the decreased total VFA concentration. The use of beef steers and a

larger amount of concentrate in the diet (95 %) may explain the discrepancies between these experiments and the current experiment.

Increasing feeding frequency did not affect the concentrations of glucose, insulin and NEFA in plasma of dairy cows. Previous studies (Rooke et al., 2008; French et al., 1990) also reported that the average daily concentrations of these metabolites were not affected by feeding frequency. A couple of studies did report a tendency for increased feeding frequency to lower peak and average insulin concentrations (Sutton et al., 1988; French and Kennelly, 1990), however these studies fed cows 8 or 12 times a day which may have contributed to the discrepancies with the current study. Nonetheless, plasma metabolites measured did not explain treatment effects in this experiment.

2.5 Conclusion

The current study showed that cows at a higher risk of developing SARA ate for a longer time soon after feeding compared with those at a lower risk. Feeding three times daily, which reduced the duration of eating following the first feeding and increased eating time in the later hours of the day, reduced the severity of SARA experienced by higher risk cows without negatively affecting productivity of lower risk cows. In addition, increasing feeding frequency resulted in greater milk fat yield regardless of risk categories of developing SARA. Frequent feeding, particularly for high-grain diets, may be a beneficial approach to reduce SARA and increase production of lactating dairy cows.

Table 2-1. Ingredient and chemical composition of diets for Studies 1, 2, and the primary experiment

Item	Study 1 [1]	Study 2 [2]	Primary Experiment
Ingredient, % DM			
Barley silage	30.0	30.0	36.5
Barley grain, dry rolled	25.0	25.5	24.1
Corn grain, ground	20.0	18.9	18.0
Canola meal	7.4	14.5	14.7
Corn gluten meal	5.3	4.7	3.6
Alfalfa hay	5.0	…	…
Beet pulp	4.0	2.9	…
Vegetable oil	1.0	0.9	0.9
Mineral and vitamin mix	2.4 [3]	2.6 [4]	2.3 [5]
Nutrient Composition, %DM			
DM	60.8	54.6	56.2
CP	15.9	16.9	18.5
NDF	25.6	26.8	29.4
Starch	31.1	31.8	31.6
Ether extract	4.0	4.1	4.0
NFC	49.8	47.5	42.7
Forage NDF	14.3	13.9	17.9

[1] Study 1 (Gao and Oba, 2014)

[2] Study 2 (Gao and Oba, 2015)

[3] Contained 15.7% Ca, 3.32% P, 14.1% Na, 21.8% Cl, 5.70% Mg, 0.23% S, 0.06% K, 2867.4 mg/kg Fe, 468.7 mg/kg Cu, 902.8 mg/kg Mn, 11.2 mg/kg Co, 718.0 mg/kg Zn, 7.08 mg/kg Se, 21.0 mg/kg I, 442.8 kIU/kg vitamin A, 45.0 kIU/kg vitamin D, 1449.9 kIU/kg vitamin E

[4] Contained 15.35% Ca, 2.83% P, 12.44% Na, 24.99% Cl, 5.53% Mg, 0.28% S, 6.43% K, 3274.31 mg/kg Fe, 617.15 mg/kg Cu, 1160.00 mg/kg Mn, 13.92 mg/kg Co, 937.69 mg/kg Zn, 9.23 mg/kg Se, 30.25 mg/kg I, 473.1 kIU/kg vitamin A, 48.0 kIU/kg vitamin D, 1547.7 IU/kg vitamin E

[5] Contained 19.1% Ca, 2.99% P, 19.5% Cl, 4.92% Mg, 0.06% K, 12.7% Na, 0.28% S, 3667 mg/kg Fe, 1147 mg/kg Cu, 2115 mg/kg Mn, 24.7 mg/kg Co, 1432 mg/kg Zn, 54.1 mg/kg I, 17.2 mg/kg Se, 536 kIU/kg Vitamin A, 54.9 kIU/kg Vitamin D, 1734 IU/kg Vitamin E

Table 2-2. Characteristics of higher and lower risk cows in the primary experiment

Variable	Higher-risk[1]	Lower-risk[1]	SE	P - value
Parity	3.75	3.00	0.60	0.41
DIM	216	214	97.6	0.99
Weight	718	687	34.2	0.54
BCS	3.13	3.00	0.14	0.54

[1]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

Table 2-3. Comparison of milk yield, DMI, rumen pH, and behavior measurements between risk categories of SARA in preliminary studies

Variable	Higher-risk[3]	SE	Lower-risk[3]	SE	P-value
Milk Yield, kg/day	25.2	2.75	29.9	2.43	0.23
3.5 % FCM[2]	23.7	3.85	28.9	3.27	0.12
DMI, kg/day	21.1	1.30	20.0	1.48	0.59
Ruminal pH					
Nadir	5.20	0.08	5.70	0.07	< 0.001
Mean	5.96	0.06	6.40	0.05	< 0.001
Maximum	6.70	0.06	6.90	0.05	0.08
Duration pH <5.8, min/d	535	60.4	18.4	53.2	< 0.001
Area pH <5.8, pH × min/d	148	24.1	2.20	21.3	< 0.001
Acidosis Index, pH × min/kg	8.03	1.55	0.11	1.37	< 0.01
Eating, min					
Time Period 1	186	7.94	153	7.20	0.01
Time Period 2	62.1	10.1	63.3	9.16	0.78
Time Period 3	19.3	5.95	42.8	5.40	0.01
Ruminating, min					
Time Period 1	129	11.9	151	10.5	0.20
Time Period 2	200	17.2	178	15.2	0.36
Time Period 3	243	12.0	197	10.6	0.01

[1]Each variable was measured in 3 eight hour time periods starting with the hour the cow was fed

[2]Fat corrected milk: FCM = [0.4324 x milk yield (kg)] + [16.126 x fat yield (kg)] (Tyrell and Reid, 1965)

[3]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

Table 2-4. Effect of feeding frequency and risk category on feeding and lying behavior in the primary experiment

	Higher-risk		Lower-risk			P-value		
Variable[1]	**1×**	**3×**	**1×**	**3×**	**SE**	**Treatment[2]**	**Category[3]**	**Treatment×Category**
Eating, min/d								
Time Period 1	150	103	140	103	9.92	<0.01	0.47	0.95
Time Period 2	85.8	93.8	87.3	91.0	8.02	0.38	0.95	0.74
Time Period 3	56.8	81.8	29.5	69.5	12.9	<0.01	0.29	0.31
Ruminating (Standing), min/d								
Time Period 1	49.3	65.8	70.8	78.3	27.4	0.21	0.67	0.62
Time Period 2	68.0	60.5	56.5	53.0	15.5	0.65	0.63	0.87
Time Period 3	110[ab]	114[ab]	158[a]	108[b]	30.7	0.008	0.65	0.005
Ruminating (Lying), min/d								
Time Period 1	74.3	91.5	33.0	73.0	21.1	0.02	0.33	0.25
Time Period 2	92.8	82.5	86.8	88.0	17.8	0.98	0.85	0.65
Time Period 3	179	116	155	150	29.2	0.74	0.42	0.10
No Activity[4] (Standing), min/d								
Time Period 1	41.3	43.3	91.8	105	10.9	0.42	0.005	0.55

Time Period 2	44.8	41.3	82.0	63.5	10.3	0.28	0.04	0.44
Time Period 3	53.0	58.8	106	85.8	8.11	0.49	0.001	0.23
No Activity (Lying), min/d								
Time Period 1	89.5	53.0	102	54.8	17.9	0.56	0.12	0.66
Time Period 2	119	92.5	127	106	12.2	0.36	0.13	0.80
Time Period 3	195	184	184	173	17.2	0.30	0.66	0.98
Total Ruminating, min/d								
Time Period 1	123	158	95.5	151	16.4	<0.01	0.46	0.24
Time Period 2	161	146	139	141	10.8	0.26	0.39	0.17
Time Period 3	290	268	274	257	11.3	0.20	0.21	0.87
Total Lying, min/d								
Time Period 1	164	194	93.3	128	30.5	<0.01	0.16	0.73
Time Period 2	211	214	175	194	22.9	0.64	0.28	0.73
Time Period 3	374	338	300	323	41.4	0.71	0.46	0.12

[1]Each variable was measured in 3 separate time periods; 0800 to 1500 h (Time Period 1), 1500 to 2200 h (Time Period 2), and 2200 – 0800 h (Time Period 3)
[2]Feeding frequency treatment of once daily (1×; 0800 h) or 3 times daily (3×; 0800, 1500, and 2200 h)
[3]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0
[4]No activity represents the absence of eating and ruminating behaviors
*Means within each row with different superscripts are significantly different from each other ($P \leq 0.05$)

Table 2-5. Effect of feeding frequency and risk category on sorting in the primary experiment

	Higher-risk		Lower-risk			P-value		
Variable	**1×**	**3×**	**1×**	**3×**	**SE**	**Treatment[2]**	**Category[3]**	**Treatment×Category**
Sorting Index[1]								
> 19.0 mm	93.1	96.9	95.7	94.5	3.85	0.47	0.98	0.22
19.0 to 8.0 mm	99.7	99.7	99.7	99.4	0.42	0.61	0.72	0.76
8.0 to 1.18 mm	100.7	100.4	100.6	101.7	0.69	0.43	0.50	0.21
< 1.18 mm	101.8	101.3	101.0	103.0	1.03	0.31	0.73	0.11

[1] Sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator. A sorting index above 100 indicates sorting for particles and an index below 100 indicates sorting against particles (Leonardi and Armentano, 2003)

[2]Feeding frequency treatment of once daily (1×; 0800 h) or 3 times daily (3×; 0800, 1500, and 2200 h)

[3]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

Table 2-6. Effect of feeding frequency and risk category on rumen pH measurements in the primary experiment

Variable	Higher-risk		Lower-risk		SE	P-value		
	1×	3×	1×	3×		Treatment[1]	Category[2]	Treatment×Category
Rumen pH								
Nadir	5.34	5.42	5.66	5.54	0.09	0.75	0.10	0.18
Mean	6.08	6.03	6.31	6.31	0.07	0.54	0.03	0.62
Max	6.88[a]	6.68[b]	6.95[a]	7.01[a]	0.05	0.04	0.03	< 0.01
Duration pH <5.8, min/d	375	274	50.5	119	65.7	0.79	0.02	0.20
Area pH <5.8, pH × min/d	97.9[a]	51.2[b]	4.28[c]	20.1[bc]	11.4	0.26	<0.01	0.05
Acidosis Index, pH × min/kg	3.47[a]	1.82[b]	0.19[c]	0.90[bc]	0.41	0.28	<0.01	0.03
DMI, kg/d	28.0	28.6	22.5	24.2	1.59	0.27	0.05	0.59

[1]Feeding frequency treatment of once daily (1×; 0800 h) or 3 times daily (3×; 0800, 1500, and 2200 h)

[2]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

*Means within each row with different superscripts are significantly different from each other ($P \leq 0.05$

Table 2-7. Effects of feeding frequencies and risk categories on total VFA, VFA composition, rumen ammonia, and blood plasma metabolites and hormones in the primary experiment

	Higher-risk		Lower-risk			P-value		
Variable	**1×**	**3×**	**1×**	**3×**	**SE**	**Treatment[1]**	**Category2**	**Treatment×Category**
Rumen VFA Composition,								
mol/ 100 mol VFA								
Acetic Acid	56.1	55.1	57.7	57.4	1.84	0.41	0.46	0.65
Propionic	24.3	25.8	23.1	23.8	2.31	0.13	0.63	0.50
Isobutyric	1.13	1.04	1.13	1.12	0.05	0.33	0.43	0.46
Butyric	14.0	13.8	13.7	13.8	1.26	0.93	0.95	0.89
Isovaleric	1.89	1.61	2.16	2.09	0.15	0.14	0.09	0.35
Valeric	2.10	2.07	1.69	1.47	0.40	0.27	0.39	0.39
Caproic	0.54	0.60	0.47	0.40	0.04	0.92	0.02	0.21
Total VFA, mM	107	114	103	102	6.69	0.56	0.36	0.36
Rumen NH_3, mg/dL	9.24	8.12	6.24	5.91	1.52	0.60	0.18	0.77
Plasma glucose, mg/dL	61.7	63.4	63.2	63.7	1.73	0.34	0.69	0.58
Plasma fatty acids, mEq/L	150	92.9	131	121	30.3	0.23	0.90	0.37
Plasma insulin, µIU/mL	8.51	8.25	4.31	4.54	1.23	0.98	0.06	0.61

[1]Feeding frequency treatment of once daily (1×; 0800 h) or 3 times daily (3×; 0800, 1500, and 2200 h)
[2]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

Table 2-8. Effect of feeding frequency and risk category on milk yield and milk components in the primary experiment

	Higher-risk		Lower-risk			P-value		
Variable	**1×**	**3×**	**1×**	**3×**	**SE**	**Treatment[2]**	**Category[3]**	**Treatment×Category**
Yield, kg/d								
Milk	38.2	37.6	39.5	39.8	10.3	0.78	0.91	0.51
Fat	1.12	1.25	1.04	1.19	0.18	0.01	0.79	0.85
CP	1.26	1.21	1.19	1.23	0.25	0.74	0.95	0.05
Lactose	1.76	1.83	1.70	1.85	0.50	0.35	0.88	0.09
3.5 % FCM[1]	34.6	36.5	33.9	36.3	7.29	0.01	0.97	0.69
Milk Composition								
Fat, %	3.39	3.70	2.89	3.20	0.41	0.06	0.41	0.99
CP, %	3.52	3.37	3.20	3.27	0.26	0.56	0.58	0.20
Lactose, %	4.57	4.40	4.54	4.60	0.13	0.51	0.60	0.21
MUN, mg/dL	12.2	12.3	11.5	11.4	1.22	0.97	0.65	0.90

[1]Fat corrected milk: FCM = [0.4324 x milk yield (kg)] + [16.126 x fat yield (kg)] (Tyrell and Reid, 1965)

[2]Feeding frequency treatment of once daily (1×; 0800 h) or 3 times daily (3×; 0800, 1500, and 2200 h)

[3]Cows were categorized as higher or lower risk for developing SARA using an acidosis index (area below pH 5.8/DMI) and a threshold of 1.0

3.0 Experiment 2: The Effect of Long Day Photoperiod on Feeding Behaviour of Lactating Dairy Cows

3.1 **Introduction**

Providing lactating dairy cows with a long day photoperiod, 16 h of light and 8 h of dark, has been shown to increase milk yield in comparison to a short day photoperiod, 8 h of light and 16 h dark (as reviewed by Dahl et al., 2000). Both behavioural and hormonal mechanisms have been explored as causal factors for the increase in milk yield. For behavioural responses, researchers hypothesized that providing more light would increase time spent eating as cattle prefer to graze during the day (Phillips and Denne, 1988). However, eating time did not increase for cows on a long day photoperiod treatment (Collier et al., 2006). While the hypothesis was not supported, impacts of photoperiod management on other behaviours were observed in several studies. Phillips and Schofield (1989) reported an increase in lying time and a decrease in activity, measured with pedometers as number of steps daily, in cows on a long day photoperiod treatment. An additional peak in eating activity was also observed during the evening time, 1600 to 2330 h, when supplemental light was provided. This indicates that feeding pattern may be altered in cows exposed to different photoperiods. Increasing peaks in feeding activity or increasing the distribution of eating time throughout the day has been shown to reduce the diurnal variation of rumen pH (Shabi et al., 1999). Few studies have looked at feeding pattern in relation to photoperiod management, and none to our knowledge have looked at a possible effect of photoperiod management on feed sorting, another behaviour that increased the risk for SARA. Therefore, the objective of this study was to determine how a long day photoperiod will affect

the feeding behaviour of lactating dairy cows. We hypothesized that providing 16 hours of light will increase time spent eating during supplemental hours in the evening, increasing distribution of eating activity throughout the day and reducing feed sorting.

3.2 Materials and Methods

All experimental procedures used in this study were approved by the University of Alberta Research Center Animal Care Committee and conducted according to the guidelines of the Canadian Council of Animal Care (CCAC, 2009). All cows were housed individually in a tie-stall barn bedded with wood shavings and with free access to water. Cows were milked twice daily at 0400 h and 1500 h. All cows were fed the same mid-lactation diet, formulated to meet requirements to produce 36.5kg/d milk, once daily at 0830 allowing for 5 – 10 % daily orts.

Thirty lactating Holstein cows (days in milk = 115 ± 33, body weight = 617 ± 70 kg) were used in this study. Espinoza (2016) conducted an experiment using 60 cows, 2014 (n = 30) and 2015 (n = 30), and behaviour measurements in the current study were taken during the adaptation to light treatments in 2015. Treatments were a long day photoperiod (LP), 16 hours of light and 8 hours of dark, and a short day photoperiod (SP), 8 hours of light and 16 hours of dark. Before the light treatment was applied, all cows were on a SP. Fifteen cows were assigned to each treatment group and located on separate ends of the barn with a buffer zone of approximately 32 m in-between, where cows not on the study were housed, to minimize light interference from the LP treatment. The study was conducted during winter months (December 9 to January 6) to minimize any interference from external light. All lights were controlled by a timer to ensure that hours of light/dark provided were consistent each day. Light photometers (Extech SDL 400, Extech Instruments, Nashua, NH) were used to measure the light intensity during the experiment and, on average, during the light hours the intensity was 225 lux and 160

lux for the LP and SP groups, respectively, which is above the threshold of 150 lux for cows to respond to light hours (Dahl et al., 2000). During dark hours the light intensity was around 10 lux for both treatment groups. The barn was temperature regulated, with an average temperature of 11.6 °C.

Data were collected for 3 consecutive days before and after a 21-d adaptation to the light treatment to compare treatment effects on sorting and feeding behaviour. TMR and ort samples were collected for 3 consecutive days and ort samples were composited to form 1 sample per cow. Particle size distribution of the TMR and orts samples was determined using a Penn State Particle Separator with 3 sieves (aperture size of 19.0, 8.0, and 1.18 mm). A sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve, where predicted intake was determined using the TMR samples collected (Leonardi and Armentano, 2003). A sorting index of 100, less than 100, and greater than 100 indicates no sorting, selective refusals and selective consumption, respectively. Feeding behaviour was measured by live observation over a 24-h period. Cows were observed for eating, ruminating (while either lying or standing) or no feeding activity (while either lying or standing) every 5 minutes and the behaviour observed was assumed to last for the full five minute period as described by Beauchemin et al. (2003). The 24-h period was analyzed in 4 separate time periods (based on light schedule) to determine if behaviour changed depending on whether or not supplemental light was provided. This consisted of Period 1 (7 pm to 3 am; both treatments had no light), Period 2 (3 am to 8 am; only LP treatment had light), Period 3 (8 am to 4 pm; both treatments had light), and Period 4 (4 pm to 7 pm; only LP treatment had light). Data on milk yield was reported by Espinoza et al. (2016) who observed an increase in milk yield for LP cows after 21 days of adaptation to treatment.

All response variables were analyzed using the fit model procedure of JMP including the fixed effects of treatment (SP vs. LP), time (pre vs. post adaptation period) and treatment by time interaction. As effects of photoperiod treatment were confounded by location in the barn, specific effect of photoperiod treatment was indicated by a significant interaction between treatment and time. Significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

3.3 Results

There was no treatment by time interaction for DMI, total lying time, or overall feeding behaviour ($P > 0.10$, Table 3-1). The only significant interaction between treatment and time was observed in Period 2 (3 am to 8 am) when only LP cows had supplemental light. The LP treatment decreased lying time (85 vs. 113 min; $P = 0.02$) and had a tendency to increase eating time (53 vs. 27 min; $P = 0.06$; Table 3-1). There was also a tendency of the interaction for LP to decrease sorting against long particles (>19-mm; 85.5 vs. 91.4; $P = 0.06$; Table 3-2) and to decrease sorting for fine particles (<1.18mm; 103 vs. 102; $P = 0.08$).

3.4 Discussion

The objective of this study was to determine if photoperiod treatments affected the feeding pattern of lactating dairy cows. In the current study, there was no difference in total eating time between LP and SP treatments. This was also observed in previous studies (Phillips and Schofield, 1989; Weiguo and Phillips, 1991; Phillips et al., 1997) that concluded the increase in DMI and milk yield was not due to an increase in eating time and likely due to hormonal changes occurring during a long day photoperiod. While there was no difference in total eating time we did observe a tendency for increase in eating during the morning supplemental light period for LP cows (3 a.m. to 8 a.m.). Increased eating activity during this time period is likely due to the preference of cows to eat in the light (Phillips and Arab, 1998). However, there was no

increase in eating time during the evening supplemental light period. Hayley et al. (2000) observed an increase in eating time in tie-stall cows immediately following feed delivery and milking. In the current study cows were milked 0400 h, during the morning supplemental light period, and at 1500 h, before the evening supplemental light period. The additive effects of light provision and milking during the light supplement period in the morning may explain greater feeding activity for LP cows. Phillips and Schofield (1989) also observed an additional peak in eating activity during a supplemental light period in LP cows, however the peak was in the evening period at 2000 – 2200 h. Phillips and Schofield (1989) provided an additional 4.5 h of light in the evening and 2.5 less h in the morning than in the current study, and having a longer supplemental light period in the evening may explain the increase in feeding activity during that time. Cows in the study of Phillips and Schofield (1989) were also fed and milked later in the day (1100 h, and around 0700 h and 1600 h, respectively) and group-housed in a shed, all of which could have contributed to a different feeding pattern than was seen in the current study.

There was no difference observed in total lying time between light treatments. Previous studies have found that long photoperiod increased lying time in both cows (Phillips and Schofield, 1989) and heifers (Phillips et al., 1997). These authors attributed increased lying time to less vigilance during light hours. Both studies referenced Leining et al. (1980) who detected a significant decrease in glucocorticoids in pre-pubertal bulls under long days. Reduced glucocorticoids may indicate a reduction in stress and thus more relaxed cattle. In the current study, cows were housed in a tie stall barn, as opposed to group pens, which limited their natural interactions among cows in a group and may have reduced any potential treatment differences in lying time. Phillips and Schofield (1989) speculated that increased lying time would reduce

energy requirements and may contribute to increased milk production. However, in the current study, increases in milk yield (Espinoza, 2016) cannot be attributed to changes in lying time.

Some of the behavioural differences observed in cows exposed to long photoperiod may have implications for rumen health. First, additional eating time before feed delivery could prevent cows from eating the majority of their meal quickly after delivery. Eating larger amounts in shorter periods of time increases VFA production in the rumen and risk for SARA (Gonzalez et al., 2010). Increasing the number of peaks in eating time, as seen in animals fed more frequently, can reduce the time spent eating directly after feed delivery (DeVries et al., 2005). Distributing eating time throughout the day has been shown to decrease the diurnal variation in rumen pH (Shabi et al., 1999). In addition, LP cows tended to sort for short particles and against long particles to a less extent than SP cows. DeVries et al. (2005) suggested that sorting against long particles reduces NDF intake by cows and can lead to the consumption of a more fermentable portion of the diet than was delivered, which increases the risk for SARA. Therefore, reducing sorting in the current study may have contributed to a higher rumen pH. However, rumen pH was not measured in the current study, so any implications of light treatment on rumen health are speculation and require further study.

3.5 Conclusion

Behaviour observations in this study suggest that the provision of supplementary light may reduce sorting and increase number of peaks in feeding activity throughout the day. In addition, the change in feeding pattern may be affected by the time of day that supplementary light is provided.

Table 3-1. Comparison of behaviour responses between long and short day treatments pre and post treatment adaptation over 24 h and for each time period

	Pre-Adaptation		Post-Adaptation			P-Value		
Variable[1](min)	**LP[2]**	**SP[2]**	**LP**	**SP**	**SE**	**Time**	**Treatment**	**Time*Treatment**
Total Daily Behaviour								
Eating	271	292	287	309	11.5	0.15	0.06	0.95
Drinking	59.6	21.3	47.1	21.0	6.15	0.30	0.0001	0.33
No Activity[3] (Standing)	220	227	194	157	17.4	0.01	0.40	0.22
Ruminating (Standing)	135	124	162	95.3	19.7	0.98	0.05	0.16
No Activity (Lying)	408	390	416	452	26.5	0.20	0.74	0.31
Ruminating (Lying)	346	386	334	405	21.5	0.89	0.01	0.47
Total Standing	355	351	356	253	32.0	0.14	0.10	0.13
Total Lying	755	776	750	857	37.5	0.31	0.09	0.26
Total Ruminating	481	510	496	500	14.8	0.86	0.27	0.41
Total No Activity	629	617	610	609	20.6	0.52	0.77	0.79
Time Period 1								
Eating	27.1	11.0	18.9	11.0	3.62	0.26	0.001	0.26
Drinking	100	78.7	77.5	20.0	8.71	0.005	0.007	0.73
No Activity (Standing)	25.4	26.3	27.5	21.3	5.10	0.78	0.61	0.49
Ruminating (Standing)	108	113	141	151	11.6	0.003	0.54	0.86
No Activity (Lying)	58.6	82.3	57.5	81.3	9.72	0.92	0.02	1.00
Ruminating (Lying)	132	285	281	248	15.7	0.03	0.05	0.90
Total Standing	166	195	199	232	15.7	0.03	0.05	0.90
Total Lying	83.9	109	85.0	103	8.41	0.77	0.02	0.68
Total Ruminating	208	192	219	201	8.92	0.27	0.06	0.91
Total No Activity	161	169	157	166	9.09	0.71	0.38	0.96
Time Period 2								
Eating	27.1[b]	32.7[b]	52.5[a]	39.3[ab]	4.90	0.002	0.44	0.06
Drinking	8.20	4.00	8.90	3.00	1.70	0.93	0.004	0.61
No Activity (Standing)	20.7	37.3	26.8	30.7	4.50	0.95	0.03	0.16
Ruminating (Standing)	11.4	16.7	6.80	4.70	3.90	0.04	0.69	0.35
No Activity (Lying)	65.0[a]	48.7[ab]	46.1[b]	53.0[ab]	6.30	0.25	0.46	0.07
Ruminating (Lying)	47.5	40.7	38.9	49.3	6.60	0.99	0.79	0.20

Total Standing	67.5[b]	90.7[a]	95.0[a]	77.7[ab]	81.0	0.37	0.72	0.02
Total Lying	113[a]	89.3[b]	85.0[b]	102[ab]	8.10	0.37	0.72	0.02
Total Ruminating	58.9	57.3	45.7	54.0	6.70	0.22	0.62	0.47
Total No Activity	85.7	86.0	72.9	83.7	5.90	0.20	0.35	0.38
Time Period 3								
Eating	49.3	66.3	53.2	82.7	5.96	0.10	0.0003	0.30
Drinking	15.0	4.30	13.2	5.70	2.15	0.92	<0.0001	0.48
No Activity (Standing)	61.1	58.3	58.2	40.3	7.36	0.16	0.17	0.31
Ruminating (Standing)	58.6	44.3	80.4	40.3	11.5	0.44	0.02	0.27
No Activity (Lying)	139	142	133	148	12.1	0.99	0.45	0.59
Ruminating (Lying)	157	165	143	163	12.9	0.53	0.28	0.63
Total Standing	184	173	205	169	17.8	0.64	0.20	0.48
Total Lying	269	307	275	311	17.8	0.64	0.20	0.48
Total Ruminating	215	209	223	203	9.05	0.93	0.15	0.46
Total No Activity	200	200	191	189	9.81	0.28	0.92	0.92
Time Period 4								
Eating	33.2	24.0	23.9	21.7	3.70	0.12	0.13	0.35
Drinking	9.30	2.00	6.10	1.30	1.35	0.16	<0.0001	0.36
No Activity (Standing)	38.6	53.0	31.4	36.3	4.70	0.01	0.05	0.32
Ruminating (Standing)	39.3	36.3	47.5	29.0	7.16	0.95	0.14	0.28
No Activity (Lying)	96.1	86.0	96.1	100	8.05	0.39	0.71	0.39
Ruminating (Lying)	83.6	98.7	95.0	112	9.00	0.18	0.08	0.93
Total Standing	120	115	109	88.3	8.95	0.04	0.16	0.39
Total Lying	180	185	191	212	8.95	0.04	0.16	0.39
Total Ruminating	123	135	143	141	8.95	0.17	0.57	0.44
Total No Activity	135	139	128	136	8.40	0.56	0.44	0.79

[1]Each variable was measured in 4 separate time periods; 1900 to 0300 h (Time Period 1), 0300 to 0800 h (Time Period 2), 0800 – 1600 h (Time Period 3) and 1600 to 1900 (Time Period 4)

[2]Long day photoperiod cows (16 h light and 8 h dark; LP) went from a short day photoperiod pre-adaptation to a long day photoperiod during the 21 d adaptation, short day photoperiod cows (8 h light and 16 h dark; SP) remained on a short day photoperiod from pre to post-adaptation

[3]No activity represents the absence of eating and ruminating behaviors

Table 3-2. Comparison of sorting behaviour between long and short day treatments pre and post treatment adaptation

	Pre-Adaptation		Post-Adaptation			P-Value		
Variable	**LP[2]**	**SP[2]**	**LP**	**SP**	**SE**	**Time**	**Treatment**	**Time*Treatment**
Sorting Index[1]								
> 19.0 mm	85.5^{ab}	85.9^{ab}	91.4^{a}	78.0^{b}	3.52	0.79	0.08	0.06
19.0 to 8.0 mm	101	99.6	98.1	97.5	0.59	<0.0001	0.06	0.30
8.0 to 1.18 mm	101	102	102	104	0.55	0.02	0.02	0.43
< 1.18 mm	103^{ab}	103^{ab}	102^{b}	104^{a}	0.75	0.70	0.08	0.08

[1] Sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator. A sorting index above 100 indicates sorting for particles and an index below 100 indicates sorting against particles (Leonardi and Armentano, 2003)

[2]Long day photoperiod cows (16 h light and 8 h dark; LP) went from a short day photoperiod pre-adaptation to a long day photoperiod during the 21 d adaptation, short day photoperiod cows (8 h light and 16 h dark; SP) remained on a short day photoperiod from pre to post-adaptation

4.0 General Discussion

4.1 Summary of Findings

The main objective of experiments in Chapters 2 and 3 were to evaluate the feeding behaviour of cows at a high or low risk for SARA and the effect of feeding frequency and photoperiod on feeding behaviour. In Chapter 2, time spent eating and ruminating over a 24 hour period was analyzed in 3 separate time periods to assess when cows spent most of their time eating. In the preliminary studies cows at a higher risk for SARA ate for a longer period of time soon after the once per day feeding, likely contributing to the steeper decrease in rumen pH, than LR cows. The primary experiment in Chapter 2 was conducted to determine if feeding more often would increase the distribution of eating and decrease the severity of SARA in HR cows. When all cows were fed 3x vs. 1x daily it decreased eating time following the morning feeding and increased eating time over night before the morning feeding the next day. The more even daily feeding pattern likely contributed to the decrease in the area below pH 5.8 in higher risk cows. In Chapter 3, time spent eating and ruminating in a 24 hour period was analyzed in 4 separate time periods, following the lighting schedule for the LP cows. Providing supplemental light in the morning before feeding, 3 AM to 8 AM, increased the time spent eating for LP cows. Although this has implications for rumen health, rumen pH was not measured in this experiment.

Chapters 2 and 3 evaluated effects of two different management strategies, feeding frequency and photoperiod on milk production, in addition to feeding behaviour. In Chapter 2, increasing feeding frequency to 3 times daily had no effect on DMI or milk yield, but there was an increase in milk fat yield. Rumen pH is not the sole reason for this increase as the milk fat yield of LR cows increased despite no change in the area below pH 5.8. Blood metabolites and rumen VFA profiles were also unaffected by the feeding treatment. In Chapter 3, there was no

effect of photoperiod on DMI, but there was an increase in milk yield. Milk composition was not measured in this experiment. Chapter 2 presented results that highlight differences in the feeding behaviour between cows at a higher or lower risk for SARA. Both Chapters 2 and 3 provided data on management strategies that may improve rumen health and production in lactating dairy cows.

4.2 Implications

The work presented in both chapters has practical implications for the dairy industry. Firstly, these experiments took into account individual animal variation, where differences in feeding behaviour might have a negative impact on production. In Chapter 2, the preliminary studies identified a behavioural factor, daily eating time distribution, which may affect risk for SARA, while the primary experiment in Chapter 2 and Chapter 3 provide management solutions for this factor. In a commercial setting, producers are feeding groups of animals and not just individuals, therefore it is essential to target cows at a higher risk of metabolic disorders, such as SARA. Identifying the negative impact of increased time spent eating following feed delivery on rumen pH for HR cows allowed us to also test management strategies that would target HR cows without negatively affecting the production of LR cows. Feeding cows more frequently is a practical solution that can be implemented on farm to reduce the severity of SARA experienced by HR cows. When feeding more frequently, producers are able to feed a TMR to optimize milk production, while using a feeding management strategy to reduce the negative impact of a high concentrate diet on rumen pH specifically in cows more susceptible to SARA. Although feeding more frequently would increase labor requirements and thus increase cost, there are additional benefits to this strategy. More frequent feeding has also been shown to reduce sorting (DeVries et al., 2005), competition at the feed bunk (DeVries et al., 2005), and increase time spent

standing after milking, when feeding coincides with milking, thus reducing the chance of bacterial infection to the udder (DeVries and Von Keyserlingk, 2005). Based on the current experiments and past research, feeding more than once a day is a practical and economical recommendation for producers.

Although we were not able to confirm the effects of long day photoperiod treatment on rumen health in Chapter 3, this may still be a beneficial management approach for producers. Providing a long day photoperiod treatment increased the distribution of eating time throughout a 24-h period and tended to reduce sorting, which have both been shown to reduce diurnal variation in rumen pH (Le Liboux et al., 1999; DeVries et al., 2008). In addition, a long day photoperiod also increased milk production with no change in diet. Therefore photoperiod management is still an effective tool to increase production on farm without challenging cows to a more fermentable diet and risking incidences of SARA.

4.3 Limitations

One of the limitations of this study was the use of individual feeding bins as opposed to floor feeding cows, as is used more often in a commercial setting for both tie-stall and free-stall barns. As such, one aspect that was not included in this research was the pushing up of feed. Floor-fed cows tend to push the feed farther away, and feed push-up is required to return the feed within reach and improve feed access. Previous literature has found that feed-push up will stimulate cows to use the feed bunk (Menzi and Chase, 1994), which may imply that feeding more often may not be necessary. However, DeVries et al. (2003) tested the stimulatory effect of push-up on feed activity by providing additional feed push up at 0030 and 0330 h, both times when feeding activity was minimal. The authors observed a large increase in feed bunk attendance in the hour following delivery of fresh feed and return from milking with a much

smaller increase following push-up, and concluded that feed-push up did little to alter diurnal feeding pattern. DeVries and von Keyserlingk (2005) also found that delivery of fresh feed stimulated feeding activity to the greatest extent and that feeding at 2300 h caused cows to alter their diurnal feeding pattern and eat later in the evening as opposed to the majority of time spent eating during the day pre-treatment. Based on these results, the delivery of fresh feed is able to stimulate cows to alter their feeding pattern, which was the objective of the primary experiment in Chapter 2. While feed push up may also stimulate cows to eat, it is not to a great enough extent to alter feeding patterns and therefore the conclusion of the primary experiment in Chapter 2 would not be altered.

Another potential limitation to experiments in both Chapters 2 and 3 is the use of tie-stall housing as opposed to free-stall housing. Free-stalls are used more often commercially and this housing system alters the management practices and behaviour of cows. Therefore, the implication of results may vary when not used in a tie-stall setting. A behavioural factor that may be different based on housing type is feed sorting. In a tie-stall barn, cows are limited to sort only the feed they individually receive, whereas in a free-stall barn cows can move to fresh or unsorted sections of the feed bunk. Leonardi and Armentano (2007) compared sorting between a tie-stall and free-stall setting in a cross-over design and found that cows in a free-stall barn sorted against large particles and for fine particles to a greater extent; 63 vs. 73 % and 108 vs. 106 %, respectively. Although no difference in sorting was observed in the primary experiment of Chapter 2, Gao and Oba (2014) reported that HR cows sorted to a greater extent than LR cows. Previous literature has also used tie-stalls in experiments measuring sorting (Leonardi et al., 2005; Maulfair et al., 2010), which indicated that individual housing is not inappropriate for the sorting measurements taken in Chapters 2 and 3. The primary experiment in Chapter 2 found no

difference in sorting between feeding frequency treatments while previous work in a free-stall barn has observed a reduction in NDF increase in the TMR throughout the day (DeVries et al., 2005; Endres et al., 2010) and a decrease in sorting against large particles (Sova et al., 2013), when cows were fed more often. If the primary experiment in Chapter 2 was conducted in a free-stall setting, the feeding frequency treatment may have affected sorting behaviour.

Another behavioural factor removed by individual feeding is competition for feed, as seen in group housed cows. Competition for feed is increased in free-stalls when the stocking density is increased and the number of headlocks per cow or feeding space is decreased (Huzzey et al., 2006; DeVries and von Keyserlingk, 2009). DeVries et al. (2004) found that when feeding space was increased and aggressive interactions were reduced by 57%, there was an increase in time spent eating, especially in the 90 minutes following feeding (24 % increase). The increase in feeding activity was even more significant for subordinate cows. Huzzey et al. (2006) also observed that increased competition reduced feeding time, increased idle standing time and aggressive interactions. Competition influences feeding time which may also impact the effectiveness of feeding frequency treatment in a free-stall setting. That being said, DeVries et al. (2005) found an increase in daily eating time and a decrease in the displacement of subordinate cows from the bunk when feeding more frequently. This indicates that feeding more often may also have a positive effect on eating time and equal access to feed when competition is present. Although the use of tie-stall housing may have altered the behaviour of cows, in comparison to free-stall housing, the experiments in Chapters 2 and 3 were able to meet their objectives of determining the change in feeding behaviour due to the respective treatments.

An additional limitation of the primary experiment in Chapter 2 was the use of late lactation cows to study the effects of SARA. Early lactation cows are at a higher risk for SARA

due to the drastic change in diet and increasing DMI (Penner et al., 2007), making them a better animal model for the objective in the primary experiment of Chapter 2. Due to the availability of cannulated cows, 4 late lactation animals were used to increase the number of cows included in the experiment. We did not have the funding to cannulate additional cows in early lactation. Although the use of late lactation cows was not ideal, the dietary challenge applied was still effective in inducing SARA in these animals, as indicated by a drop in rumen pH below the threshold of 5.8 (Beauchemin et al., 1994). Based on the acidosis index the 4 HR and 4 LR cows consisted of 2 late and 2 early lactation cows each. The balance in categorization gives further merit to the use of animals in this experiment to meet the objective.

A limitation in the animal model in Chapter 3 was the lack of cannulated cows. The main objective was to determine the changes in feeding behaviour due to photoperiod treatment, which was accomplished. Although increased distribution in daily eating time has been shown to reduce the variation in rumen pH (Shabi et al., 1999), being able to measure rumen pH in all or some of the cows in each treatment would have given more complete data. This experiment was designed and conducted as a part of another research project (Espinoza, 2016) where rumen pH measurement was not necessary. An oversight, in terms of relating the behaviour data to rumen health, was lack of milk composition data. Milk fat data would have allowed speculation on rumen pH as a decrease in milk fat yield and or concentration has been observed during SARA (Khafipour et al., 2009a). Although the main objective of measuring changes in feeding behaviour between photoperiod treatments was met, more data could have been collected to create a more complete picture in regards to rumen health.

Another limitation occurred in the design of both Chapters 2 and 3, which was not measuring the DMI at the end of each time period in which behaviour data was separately

analyzed over 24 hours. These experiments were designed to evaluate feeding behaviour pattern throughout the day and not to provide insight on actual intake pattern. The assumption on time spent eating was that it would reflect DMI, where eating for a longer time following feed delivery would translate into increased intake, greater fermentation in the rumen, and thus a decreased rumen pH. However, Dado and Allen (1994) did not find a correlation between daily eating time and daily DMI among cows. As differences in eating time is a likely reason for the decrease in severity of SARA in HR cows when fed more often, Chapter 2, eating time could have an indirect effect on rumen pH through intake or a direct effect through rumen buffering. Maekawa (2002) measured salivation rate during eating, resting and rumination and estimated that the salivation rate was equal during eating and ruminating. Increases in time spent both eating and ruminating, or total chewing time, increases saliva production and thus increases rumen buffering. Supporting evidence was reported by Yang and Beauchemin (2009) who found a positive correlation between chewing time and mean rumen pH, where chewing time was increased through both a greater time spent ruminating and eating. Measuring DMI in each period would have had additional benefits in the primary experiment of Chapter 2. The decrease in time spent eating during the first time period (0800 to 1500) was assumed to be caused by the 2200 feeding, which would stimulate eating and reduce hunger in the morning. However, reduced time spent eating may have also been due to feed restriction during the first time period. As the cows ate for longer following the morning feeding when fed 1x, they may have tried to do the same in the 3x treatment but ran out of feed, which shortened eating time. Cows were fed 1/3 of their daily TMR at each feeding, which may have prevented them from consuming feed ad lib during the first time period. Although treatment and category differences in time spent eating

were observed in Chapters 2 and 3, the relationship between eating time and DMI in smaller time periods throughout the day has yet to be determined.

4.4 Future Research

Future research in this area should consider some of the limitations for the current experiments as well as longer term implications. Conducting a project similar to the primary experiment in Chapter 2, with the addition of DMI measurements at the end of each period would help to determine if eating time influences DMI and thus rumen pH indirectly or if eating time has a direct effect on rumen health. In addition, conducting an experiment on the interaction between feeding frequency and severity of SARA in both HR and LR cows in a free-stall setting would increase the applicability of this concept to commercial dairies. Although Chapter 3 met the objective of determining behavioural differences between a long and a short day photoperiod, it could have been conducted more effectively to determine its influences on rumen health. Measuring rumen pH and milk fat would determine if this management strategy has implications on the severity of SARA. Based on the results that time of day in which supplemental light is provided alters feeding pattern, it would also be interesting to determine if distribution of eating time could be spread out further, depending on the light schedule. For instance, keeping the lights off during feed delivery in the morning may reduce time spent eating, and then keeping lights on in the evening and night may encourage eating.

In addition, there is also an opportunity to determine longer-term effects of the work conducted. As discussed, cows in earlier stage of lactation likely have greater risk for SARA possibly due to high DMI. Therefore it would be interesting to see how and if distribution of daily eating time changed throughout lactation in cows differing in their risk for SARA. Similarly, a longer term experiment is necessary to determine if feeding more often is beneficial

for HR cows throughout the entire lactation. In addition, although HR cows have a greater area below pH 5.8 and acidosis index, it has yet to be determined how this affects production and health in the long term. In Chapter 2, none of the negative consequences associated with SARA, i.e. reduced DMI and milk fat depression, were observed in the HR cows. Gao and Oba (2014 and 2015), also did not observe reduced DMI or milk fat depression in HR cows. A numerical decrease in milk fat concentration was observed, but it was not significant likely due to large amount of individual variation. A long-term experiment or increased number of cows in each category may be needed to detect effects of SARA on milk fat in HR cows. Among the studies that evaluated the differences between higher and lower risk cows, the longest experimental period was 21 d, which may not be long enough to observe the consequences of SARA in HR cows. The rumen pH in HR cows is more likely to drop below 5.8, but further studies are needed to determine the effects on animal health and production in the long-term. Conducting future studies in this area should confirm the long term consequences of SARA, as well as the long term effects of frequent feeding on feeding behaviour.

4.5 Conclusion

The current thesis research showed that feeding behaviour of lactating dairy cows contributes to the risk for SARA and should be managed on farm. Cows that spend more time eating directly following feed delivery are at greater risk for SARA. Feeding cows three times versus once daily can increase the distribution of eating time throughout the day. Providing supplemental light can also alter the daily feeding pattern. Increasing the distribution of eating time more evenly throughout the day, through more frequent feeding, can reduce the severity of SARA in higher risk cows and increase the milk fat yield. Feeding three times a day is a practical approach for producers to improve rumen health and production of milk fat.

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