Effect of Frequency of Milking and Feed Delivery on the Behavioural Patterns and Productivity of Lactating Dairy Cows

by

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A Thesis
Presented to
The Faculty of Graduate Studies
of
The University of Guelph

In partial fulfillment of requirements for the degree of
Master of Science
in
Animal and Poultry Science

Guelph, Ontario, Canada
ABSTRACT

EFFECT OF FREQUENCY OF MILKING AND FEED DELIVERY ON THE BEHAVIOURAL PATTERNS AND PRODUCTIVITY OF LACTATING DAIRY COWS

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University of Guelph, 2013

The objective of this thesis was to determine the effects of frequency of milking and feed delivery on the behavioural patterns and productivity of lactating dairy cows. In two independent experiments, twelve free-stall housed, lactating Holstein dairy cows were exposed to either varying milking frequency or varying feed delivery frequency over 21-d periods. In the first study, cows milked three times per day and multiparous cows produced more milk than those milked twice per day and primiparous cows. Milking three times per day altered the distribution of feeding activity throughout the day. Multiparous cows had longer, and larger meals, while primiparous cows had smaller, more frequent meals throughout the day when milked three times per day. In the second study, cows delivered feed three times per day consumed more DM than those fed once per day and twice per day. Feed delivery frequency had little effect on feeding behaviour and the distribution of feeding activity, but altered the magnitude of DMI following feed delivery. Cows delivered feed more frequently achieve greater daily DMI by consuming more feed following the return from milking and the delivery of feed.
ACKNOWLEDGEMENTS

Firstly, I would like to thank my academic advisor, Dr. Trevor DeVries, without whom this work would not have been possible. He gave me the opportunity and responsibility to conduct research in a brand new facility, but was always available to help me through the bumps along the road. I would like to thank Trevor for all of his advice and encouragement throughout my graduate experience, and for always helping me look on the bright side of things. I would also like to thank the members of my advisory committee, Dr. Todd Duffield for his guidance through the writing process, and Dr. Brian McBride, for his enthusiastic encouragement in pursuing graduate studies and his never failing passion for academia.

I would also like to extend a heartfelt thank you to all of the staff of the Kemptville Campus Research Station for the memories and laughter they provided. I would like to thank everyone for helping me feel at home, and making my time at Kemptville unforgettable. I would especially like to thank the staff of the Kemptville Campus Dairy Education and Innovation Center including Albert Koekkoek, Jessica Carrier, Brian McIntosh and Gord Black for putting up with me in the barn every day, and for all the laughs they provided. I would especially like to thank Albert for his genuine caring, and for providing me with irreplaceable experiences and memories of Kemptville Campus. I will always have a soft spot for Kemptville and the time I spent with these wonderful people.

I would next like to thank my lab mates, without whom I never would have made it through this journey. Specifically, I would like to thank Amy Sova and Alex Watters not only for their research assistance, but for their unwavering friendship through some trying times and for helping me see the light at the end of the tunnel.

I would also like to sincerely thank my family for all their love and support throughout this whirlwind experience and for always providing words of encouragement when I needed it the most. Finally, I would like to thank my “second family”, the MacLeods, for without them I never would have discovered my love and passion for dairy cows, and may never have started this journey.
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CHAPTER 1: INTRODUCTION

Feeding behaviour is considered any behaviour directed towards the procurement of nutrients (McFarland, 1981) and is of great interest to dairy producers to ensure optimal nutrient intake to meet production demands and ensure cow health. Traditionally, cattle have been thought to exhibit crepuscular activity, meaning that they are most active at sunrise and again at sunset (Albright, 1993; Shabi et al., 2005). Further, cattle have been reported to exhibit diurnal patterns in feeding and grazing behaviour, with greatest feeding activity occurring during the daytime and early evening hours (Albright, 1993; DeVries et al., 2003a).

Grant and Albright (2000) described feed intake to be a function of the number of meals consumed daily, the length of each meal and the rate of feed intake. At pasture, dairy cattle have been observed to spend upwards of 9 h grazing, distributed among an average of 5 meals (Phillips and Denne, 1988); whereas intensively housed dairy cattle fed silage based rations will spend 3 - 7 h/d feeding, distributed among 6 - 14 meals (Phillips, 1993; Grant and Albright, 2000).

Physiological, social, and management factors that alter feed intake may result in changes in the distribution of feeding behaviour throughout the day. Recently, there has been a growing body of research examining feeding behaviour and its influence on voluntary feed intake and cow productivity. Therefore, this review will examine some factors that influence feeding motivation and feeding behaviour in cattle, with emphasis on the influence of management practices (milking and feed delivery) on feeding behaviour and the distribution of behavioural activities of lactating dairy cows.
1.1 Physiological Impacts on Feeding Behaviour

The feeding behaviour patterns of lactating dairy cows are largely influenced by internal factors acting within an individual cow. Hunger is a physiological and psychological state resulting in the initiation of feeding, and satiety, the opposite state, results in termination of feeding (Baile and Della-Fera, 1981). It is generally considered that feed intake or feeding motivation is largely controlled by a series of negative feedback mechanisms from the digestive tract, liver, and other organs in response to the presence of nutrients that modify hunger and satiety levels within the cow (Forbes, 2000). There is evidence that volatile fatty acids produced from ruminal fermentation, such as acetate and propionate, regulate feeding motivation through a negative feedback system controlled by ruminal receptors (Baile and Della-Fera, 1981). Feed intake and feeding motivation is also partially controlled by rumen fill. Stretch receptors in the wall of the rumen signal the degree of ruminal distension and also modify feed intake through a negative feedback mechanism (Forbes, 2000). Voluntary feed intake is therefore a function of both physical and metabolic signal pathways; therefore, factors that alter these control mechanisms within an individual cow will have a significant effect on feeding behaviour.

Level of milk production greatly influences feeding behaviour, as increased milk production is positively correlated with dry matter intake (DMI; Dado and Allen, 1994). High producing dairy cows allowed continuous access to a total mixed ration (TMR) consume 9 - 14 meals daily, while lower producing cows consume only 7 - 9 meals daily (Grant and Albright, 1995). Cows with greater milk yields achieve greater DMI by increasing meal size (Dado and Allen, 1994), while also spending less time eating per day and therefore consuming feed at a faster rate (Azizi et al., 2009).
Furthermore, age, which corresponds with parity, has a significant effect on the nutrient requirements and potential energy balance of an individual cow, particularly since primiparous (PP) cows have not achieved mature body weight at the onset of first lactation. It is considered that the average gut fill in dairy cows is approximately 15% of body weight (NRC, 2001); therefore, it is not unexpected that PP cows have a lower daily DMI than multiparous (MP) cows (Dado and Allen, 1994; Nikkhah et al., 2008; Azizi et al., 2009; DeVries et al., 2011), and exhibit differences in feeding behaviour patterns. Primiparous cows have a slower rate of increase in DMI during the first 5 weeks of lactation, and beyond 5 weeks into lactation, PP cows maintain a 15% lower DMI than MP cows in non-competitive environments (Kertz et al., 1991). Similarly, production for MP cows is positively correlated with meal size and length of eating bout, whereas production for PP cows is positively correlated with meal number and eating rate in non-competitive environments (Dado and Allen, 1994). These results suggest that differences in feeding behaviour, between cows of different parity, in non-competitive environments, may be as a result of the mechanisms controlling individual meals, such as ruminal capacity.

Furthermore, differences in feeding behaviour observed between parities may also be related to social status, associated with age and parity, when animals are grouped in competitive situations, as in free-stall environments. Both age and body size are believed to be related with social rank and position within the dominance hierarchy of the herd (Grant and Albright, 1995); therefore PP cows are often socially subordinate. In competitive environments, PP cows spend less total time feeding throughout the day, consume less dry matter (DM) at each meal, and consume feed at a slower rate than MP cows (Azizi et al., 2009). It is evident that parity affects
the distribution of behavioural activities throughout the day. Therefore, cows of varying parity may respond differently to various management strategies.

Stage of lactation, which usually dictates the energy balance of an individual cow, can be a determinant of feeding behaviour patterns. The onset of lactation dramatically increases energy and nutrient requirements. Milk production usually peaks between 5 - 7 weeks into lactation, while, DMI does not peak until 8 - 22 weeks into lactation (Ingvartsen and Andersen, 2000). Therefore, dairy cattle experience significant negative energy balance during early lactation at which point their feed intake does not meet their nutritional requirements for milk production. Since DMI continuously increases during early lactation (Kertz et al., 1991), feeding behaviour patterns are inherently altered during this period. Totally daily mealtime, meal frequency and meal duration increase during the first 8 weeks of lactation; however, between 8 - 13 weeks of lactation, feeding behaviour patterns stabilize (DeVries et al., 2003b). Therefore, stage of lactation must be considered when evaluating the feeding behaviour of dairy cattle.

Body condition score, which is an estimate of the energy and nutrient reserves stored within the body, is a key factor to consider when evaluating energy balance, and thus potential feeding behaviour. In early lactation, cows rely on body stores to meet nutrient requirements. Mobilization of body stores in the form of β-hydroxybutyrate can have a significant effect on cow health as it increases the risk of ketosis in dairy cattle. Ketosis is characterized by a significant decrease in feed intake, by as much as 10 kg of fresh matter, as well as reduced feeding time and reduced feeding rate, all of which can have effects that carry throughout lactation (Gonzalez et al., 2008). Similarly, a decrease in DMI, time spent at the feed bunk and visits to the feed bunk during the week before calving have been associated with an increased risk of transition diseases (Urton et al., 2005; Huzzey et al., 2007; Goldhawk et al., 2009).
general, health status of the cow can have a significant effect on feeding behaviour and feed intake as both clinical and subclinical infections are known to substantially reduce appetite and performance (Ingvartsen and Andersen, 2000). Typically, sickness behaviour in dairy cattle is characterized by a significant decrease in feed intake and irregular feeding behaviour can increase the risk of metabolic disorders (Grant and Albright, 2000). Therefore, health status may greatly alter or reduce feeding activity in lactating dairy cattle.

1.2 Environmental Impacts on Feeding Behaviour

It is obvious that there is no single factor responsible for the control of voluntary feed intake in lactating dairy cattle. Feeding behaviour is a function of the combined effect of metabolic and physical endogenous stimuli. While much previous research has focused on the effect of such variables on the feeding behaviour patterns of dairy cows, there are many other external factors acting on the dairy cow that need be considered when evaluating feeding behaviour.

External factors influencing the feeding behaviour of lactating dairy cows vary from farm to farm, but can generally be considered as a function of the social environment, the physical environment, or the management practices imposed upon the cows. The social environment is very influential in determining the feeding behaviour patterns of individual cows, and when dairy cows are grouped, social constraints can modify feeding behaviour and productivity (Grant and Albright, 2000). Cows are often more motivated to feed as a group whether an individual cow is hungry or not, which is known as social facilitation (Curtis and Houpt, 1983), and when cows are fed in groups they often consume more feed than when fed separately (Albright and Arave, 1997). However, dominance hierarchies and competition for resources can also modify feeding
behaviour and elicit aggressive interactions that may result in decreased cow productivity, health, or welfare.

Overstocking and limited bunk space availability are large contributors towards aggression among dairy cattle and can, therefore, cause changes to feeding patterns. The percentage of cows at the feed bunk during peak feeding times is negatively correlated with increased stocking density (Huzzey et al., 2006). Overstocked cows consume 11% less feed in the 2 h following the delivery of fresh feed, and they compensate for this by consuming more feed in the hours following (Collings et al., 2011). Overstocking also causes increased displacements from the feed bunk, decreased feeding time and therefore increased feeding rate (Proudfoot et al., 2009; Collings et al., 2011). When a competitive situation exists at the feed bunk, socially subordinate cows are displaced from the feed bunk more frequently (Huzzey et al., 2006), and dominant cows will spend more total time eating and consume greater DM than subordinate cows (Manson and Appleby, 1990). However, feeding activity is drastically increased for subordinate cows when bunk space availability is increased (DeVries et al., 2004). Since it is evident that competition for resources affects the distribution of feeding behaviour patterns, particularly between cows of differing social rank, management practices that alter the social environment are liable to affect feeding behaviour patterns.

The design of the feeding environment may also impact feeding behaviour and has significant impacts on feed availability and competition within the herd. Feed bunk design is particularly important in examining competitive interactions and the resulting feeding behaviour patterns. Most commercial free-stall facilities utilize either a post-and-rail feed barrier system or a head lock feed barrier system. Endres et al. (2005) found that 21% fewer displacements occur at the feed bunk with a head lock feed barrier at 100% stocking density. Likewise, Huzzey et al.
(2006) found that displacements from the feed bunk increased as stocking density increased, particularly for post-and-rail feed barrier systems. While feed barrier design does not alter overall feeding time, the headlock system allows for more equal access to feed during peak feeding times (Endres et al., 2005). Likewise, the presence of a feed stall reduces competition at the feed bunk, thus improving feed access for subordinate cows during peak feeding times (DeVries and von Keyserlingk, 2006). For these reasons, it is suggested that a feed barrier design that provides a physical separation between cows can reduce aggression and competition at the feed bunk and therefore potentially improve feed access and the distribution of feeding behaviour activity.

1.2.1 Milking Management

In conventional production systems, dairy cattle are subject to rigid production schedules; however, this practise does not well represent the natural behaviour patterns of a calf suckling a cow. Newborn calves exhibit 5 - 8 suckling bouts per day, and as the calf grows older, this decreases to 3 - 5 bouts per day (Phillips, 1993). For this reason, the milking constraints of dairy production systems can greatly impact the behavioural patterns of lactating dairy cows.

While traditionally dairy cattle are milked 2x/d, many producers are transitioning to more frequent milking schedules to maximize parlour efficiency, as well as automated milking systems to improve labour efficiency. Thus, there has grown a need to understand the effect that increased milking frequency has upon feeding behaviour in dairy cattle to maintain production efficiency. Milking cows 3x/d can increase milk production by as much as 3.5 kg/d compared to 2x/d milking frequencies (Erdman and Varner, 1995). Likewise, increasing milking frequency from 3x/d to 6x/d increases milk production by 7.3 kg/d (21 %) during the first 6 weeks of
lactation (Bar-Peled et al., 1995). Studies also suggest that frequent milking, appropriately timed within the lactation cycle, can have persistency effects that carry throughout lactation. Bar-Peled et al. (1995) indicated that cows milked 6x/d for the first 6 weeks of lactation produced 5.1 kg/d (13.6%) more milk following the return to 3x/d milking than cows milked 3x/d from parturition. Similarly, Dahl et al. (2004) indicated that cows milked 6x/d for the first 21 days of lactation produce 1118 kg more milk over the 305-d lactation than cows milked 3x/d throughout lactation. These results indicate that more frequent milking, for as little as the first 21 days of lactation, may produce carry-over effects on production later in lactation while also reducing labour inputs required for more frequent milking of the entire herd. However, it remains unknown as to the optimal milking frequency and duration of increased milking frequency to elicit such persistent benefits. Furthermore, it remains unclear as to the effect of increased milking frequency on the behavioural patterns of dairy cows.

The increased milk production achieved by greater milking frequency must be supported by a higher plane of nutrition to meet production demands (Varner et al., 2002). Since the return from milking is a stimulus for cows to feed (DeVries et al., 2003a), and increased milk production has a significant energy cost, it is not surprising that cows milked 6x/d exhibit significantly greatly DMI compared to those milked 3x/d (Bar-Peled et al., 1995). However, in this study, the increased DMI for cows milked 6x/d did not compensate for the increased energy demands and therefore these cows had lower body condition score during early lactation due to greater negative energy balance. On the other hand, research has indicated that 1x/d milking results in a more positive energy balance during the first 3 weeks of lactation compared to 3x/d milking (Patton et al., 2006); however, 1x/d milking frequency is not a practical management strategy for commercial production systems.
While it is generally considered that increased milking frequency increases nutrient demand and therefore requires a higher plane of nutrition, the time required for extra milkings can greatly reduce the time available for feeding, rumination, and lying behaviours which are critical for the maintenance of energy balance, efficient digestion and cow health, and to allow the cow to meet her production demands. In modern production facilities, large group sizes and a disproportion in parlour size and herd size can result in cows spending a significant amount of time away from their home pen for milking. In commercial production facilities, cows milked 2x/d will spend on average 0.5 - 6.0 h/d milking, while cows milked 3x/d will spend on average 1.2 - 5.7 h/d milking (Gomez and Cook, 2010). During these extended periods, when cows are away from their home pen, they cannot access critical resources and this creates a disparity between the behavioural priorities of the cows when they return from milking, and may therefore greatly alter behavioural patterns, and negatively affect the cow.

Reduction in the time available for feeding and lying can significantly alter feeding patterns as lying has a greater priority over feeding when dairy cows have been simultaneously deprived of the opportunity to do both (Metz, 1985; Munksgaard et al., 2005). Since greater time spent milking is associated with less time spent feeding (Gomez and Cook, 2010), and time spent feeding is also correlated with milk production (Shabi et al., 2005), restricted time budgets may negatively affect productivity. However, cows are partially able to compensate for reduced feeding time by consuming feed at a faster rate, thus, feed intake is reduced to a lesser degree than is feeding time (Munksgaard et al., 2005). This provides further evidence that dairy cows may alter the magnitude and distribution of feeding activity when their time budget is restricted. Unfortunately, there has been little research thus far examining the effect that increased milking
frequency has upon feeding behaviour patterns and how dairy cows alter feeding patterns to compensate for time restrictions and production requirements of such management strategies.

1.2.2 Feeding Management

In modern dairy production facilities, the delivery of feed is an event that greatly affects the feeding behaviour patterns of lactating dairy cows. DeVries et al. (2003a) observed a dramatic increase in the number of animals present at the feed bunk immediately following the delivery of fresh feed and coinciding with the return from milking. A follow up study concluded that when dairy cows are fed 6 h after milking, they increase their total daily feeding time by 12.5 % (DeVries and von Keyserlingk, 2005). This change in feeding activity was largely driven by a significant increase in feeding time during the 60 min following the delivery of fresh feed, and a reduction in feeding time during the 60 min following the return from milking (DeVries and von Keyserlingk, 2005). From these results, those authors concluded that the delivery of fresh feed is a stronger stimulus to feed than is the return from milking, and that the daily feeding patterns of dairy cattle in intensive, indoor housing facilities are largely influenced by the delivery of fresh feed rather than the time of sunrise and sunset. For these reasons, management strategies, such as the frequency and timing of feed delivery, can inherently alter the feeding behaviour patterns of lactating dairy cows, and can potentially be manipulated in such a way to optimize cow health and productivity.

Traditionally dairy cattle are fed 2x/d, but many farms are transitioning to 1x/d feeding schedules as a means to reduce labour requirements. It has been observed that cows fed 1x/d may consume more DM than those fed more frequently (Phillips and Rind, 2001; Mantysaari et al., 2006). However, as cattle are highly motivated to feed following the delivery of fresh feed
(DeVries et al., 2003a), reducing feeding frequency can result in undesirable feeding patterns that elicit dramatic peaks in feeding activity (slug feeding) upon the delivery of fresh feed (Philips and Rind, 2001; Mantysaari et al., 2006). When feed is delivered only 1x/d, cows spend less total time feeding, but more time feeding during the 90 min following feed delivery than cows delivered feed more frequently (DeVries et al., 2005). Since ruminal pH declines following meals, and the rate of decline increases with meal size (Allen, 1997), such undesirable feeding patterns result in increased variation in ruminal pH (Shabi et al., 1999) and an increased risk of sub-acute ruminal acidosis (SARA; Shaver, 2002). By eliminating major fluctuations in the supply of energy and nitrogen to ruminal bacteria, efficiency of fermentation may be enhanced (French and Kennelly, 1990) and thus, more frequent delivery of feed may be beneficial to cow health and productivity.

While some research has indicated that 1x/d feed delivery results in greater DMI (Nocek and Braund, 1985; Phillips and Rind, 2001; Mantysaari et al., 2006), there are also reports that feed delivery frequency has no effect on DMI (Robinson and Sniffen, 1985, DeVries et al., 2005) and that cows delivered feed more frequently have greater DMI (Shabi et al., 1999). These varying reports may be attributed to differences in experimental design; therefore, it is difficult to conclude the effect of feed delivery frequency on voluntary feed intake.

Increasing the frequency of feed delivery from 1x/d to 2x/d and 2x/d to 4x/d increases total daily feeding time by increasing feeding activity during the late evening and early morning hours (DeVries et al., 2005). A similar distribution in feeding time was observed by Philips and Rind (2001) such that cows delivered feed more frequently spend less time feeding in the morning, and more time feeding in the evening. In addition, cows fed more frequently (4x/d and 5x/d) tend to consume feed more evenly after each feed delivery and throughout the day (Philips
and Rind, 2001; Mantysaari et al., 2006). More frequent delivery of fresh feed can result in cows having a more even distribution of feed intake throughout the day and more equal access to feed, as socially subordinate cows are displaced from the feed bunk less frequently when feed is delivered more often (DeVries et al., 2005). Such feeding patterns are conducive to a more consistent rumen pH as post-feeding variation in rumen pH is less pronounced with greater frequency of feed delivery (French and Kennelly, 1990; Le Liboux and Peyraud, 1999; Shabi et al., 1999) and potentially reduce the risk for sub-acute ruminal acidosis (DeVries et al., 2005). Decreased diurnal variation in ruminal pH may therefore contribute to improved milk fat (Rottman et al., 2011), fibre digestibility (Dhiman et al., 2002), and production efficiency (Mantysaari et al., 2006).

As frequency of feed delivery has the potential to greatly alter feeding patterns in dairy cows, it has also been suggested that the frequency of feed delivery may affect the quality of the TMR available throughout the course of the day, with potential implications for rumen microbial stability. Dairy cattle preferentially sort for the grain component of a TMR and leave the longer forage components, which results in increased fibre content and therefore decreased quality of the remaining feed throughout the day (Leonardi and Armentano, 2003); this is particularly evident when feeding occurs only once daily (DeVries et al., 2005). DeVries et al. (2005) found that the neutral detergent fibre (NDF) component of total mixed rations increased in a curvilinear manner throughout the course of the day and the amount of feed sorting was reduced with increased frequency of fresh feed delivery. Similarly, Endres and Espejo, (2010) indicated greater changes in NDF content of the TMR for 1x/d feeding compared to 2x/d and 3x/d feeding. Thus, sorting behaviour reduces the intake of physically effective NDF and increases the intake
of the non-structural carbohydrate fraction, therefore increasing the risk of ruminal upset and sub-acute ruminal acidosis (DeVries et al., 2008).

Despite some positive associations of increased feed delivery frequency, Mantysaari et al. (2006) indicated that feed delivery 5x/d may be too frequent and therefore detrimental to cow productivity, due to increased restlessness and decreased lying time for cows delivered feed 5x/d. Similarly, Phillips and Rind (2001) concluded that for cows delivered feed 4x/d, the disturbance following feed delivery reduced DMI and milk production. For these reasons, further research needs to examine the effect of such management strategies on the daily time budget of lactating dairy cows in conjunction with the distribution of feeding behaviour variables.

Unfortunately, the methods employed in previous research have not been conducive for evaluating individual feed intake patterns. Furthermore, much of this research has only evaluated the effect of feed delivery frequency on cows milked 2x/d. Research evaluating the effect of feed delivery frequency on the distribution of individual meals throughout the day for cows managed under varying time budget constraint, may provide greater insight into the shift in the distribution of feeding activity throughout the day and aid in the determination of an optimal feed delivery frequency to achieve greatest production efficiency.

1.3 Objective and Hypothesis

There are many factors that influence feeding behaviour in lactating dairy cows, many of which are as a result of the physiological state of an individual cow, and are therefore difficult to manage. It is evident however, that feeding behaviour patterns are significantly impacted by management factors, such as milking and feed delivery frequency, and therefore these factors play an important role in managing feeding behaviour in commercial dairies to increase
productivity and profitability. Since both feed delivery frequency and milking frequency may have a significant effect on behaviour, and can put constraints on the time budget of the dairy cow, it is critical to consider the combined effect of both of these management practises. However, as of yet, there has been no study of the effect of increased feed delivery frequency for cows managed under a 3x/d milking schedule.

Therefore, the objective of this dissertation was to determine the effect of frequency of milking and feed delivery on the behavioural patterns and productivity of lactating dairy cows. It was hypothesized that increasing milking frequency from 2x/d to 3x/d would result in increased feeding activity, particularly after milking, resulting in a more uniform distribution of feeding activity throughout the day. It was also hypothesized that for cows milked 3x/d, an increased frequency of feed delivery will result in increased feeding activity, particularly after fresh feed delivery, resulting in a more uniform distribution of feeding activity throughout the day, thus providing the required plane of nutrition to meet the demand for increased milk production under 3x/d milking schedules. It was also hypothesized that changes in the time available for other critical behavioural activities (lying and ruminating), as a result of 3x/d milking and feed delivery frequency, may alter the distribution and amount of time devoted to lying and ruminating throughout the day.
CHAPTER 2: EFFECT OF MILKING FREQUENCY ON THE BEHAVIOUR AND PRODUCTIVITY OF LACTATING DAIRY COWS

2.1 Introduction

Increased milk yield and improved production efficiency can be achieved by milking cows more frequently (Erdman and Varner; 1995, Cabrera et al., 2010). Milking frequencies greater than 2x/d can increase milk yield by 10.4 to 21 % (Bar-Peled et al., 1995; Klei et al., 1997; Smith et al., 2002), and greater milking frequencies in early lactation can increase milk yield persistency (Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al., 2004). Cows milked 3x/d have shown significantly decreased milk fat percentages compared to those milked 2x/d (Sapru et al., 1997; Smith et al., 2002), however, milk fat yield is not affected by milking frequency (Barnes et al., 1990). Milking 3x/d is associated with small increases in fat corrected milk (FCM) and energy corrected milk (ECM; Barnes et al., 1990; Smith et al., 2002), and greater technical efficiency on farm (Cabrera et al., 2010). Increased milking frequency has also shown a reduction in SCC score and a tendency for reduced SCC throughout lactation (Smith et al., 2002; Dahl et al., 2004).

Increased milk production comes at a significant energy cost (Bar-Peled et al., 2005), therefore, increased milking frequency must be supported by a higher plane of nutrition (Varner et al., 2002). Unfortunately, the time required for extra milkings to occur at higher milking frequencies can greatly reduce the time available for other activities, such as, feeding, rumination and lying behaviours. These behaviours are critical for the maintenance of energy balance, efficient digestion, cow health and welfare, and to allow the cow to meet her production demands. Since lying has been shown to have priority over feeding when cows have been
simultaneously deprived of the ability to do both (Metz, 1985), and return from milking is a significant stimulus for cows to feed (DeVries et al., 2003a; DeVries and von Keyserlingk, 2005), feeding behaviour patterns are likely to be altered when milking frequency is increased. Greater time spent milking has been associated with less time feeding (Gomez and Cook, 2010), but there has been little research directly examining the effect that milking frequency has on feeding behaviour patterns and how dairy cows alter their behavioural patterns to compensate for the restrictions imposed by such a management practice. Thus, the objective of this study was to determine the effect of milking frequency on the behavioural patterns and productivity of lactating dairy cows. It was hypothesized that increasing milking frequency from 2x to 3x/d will result in increased feeding activity, particularly after milking, resulting in a more uniform distribution of feeding activity throughout the day, and thus alter the distribution of lying and ruminating behaviour patterns throughout the day. Given reports of greater production response in PP cows to increased milking frequency (Gisi et al., 1986; Barnes et al., 1990), it was also hypothesized that these behavioural impacts would be magnified in PP cows.

2.2 Materials and Methods

2.2.1 Animals and Housing

Twelve lactating Holstein dairy cows, including 7 PP and 5 MP (parity = 3.0 ± 1.0; mean ± SD), were used in this study. The animals were 149.5 ± 31.3 days in milk (DIM) and were producing 37.6 ± 8.1 kg milk at the beginning of the trial. The cows were housed 6 at a time in a free-stall research pen located at the University of Guelph, Kemptville Campus Dairy Education and Innovation Centre (Kemptville, ON, Canada). Cows had access to 6 free-stalls with waterbeds (DCC Waterbeds, Advanced Comfort Technology Inc., Reedsburg, WI, USA). The
waterbeds were topped with wood shavings; bedding was replaced as needed. Manure was manually scraped to within reach of the alley scrapers 2x/d at 0600 and 1800 h. The experiment was conducted from January 27 to April 27, 2012. The average environmental temperature during the experimental period was 1.5 ± 7.3°C. Use of cows and experimental procedures were approved by the University of Guelph’s Animal Care Committee. Cows were managed according to the guidelines set forth by the Canadian Council on Animal Care (CCAC, 2009).

2.2.2 Experimental Design

The number of animals required per treatment was determined through sample size and power analysis (Morris, 1999) to detect a 10 % level of difference for the primary outcome variables, including behaviour, DMI, sorting, and milk production and composition. Cows were divided into 2 groups of 6, which were balanced according to DIM, milk production, and average parity. Within each group, cows were randomly exposed to each of 2 treatments in a replicated crossover design, with 21-d treatment periods. The treatments were milking frequency: 1) 2x/d (at 0600 and 1800 h), 2) 3x/d (at 0600, 1400, and 2200 h). Cows were milked using a robotic milking system (Lely A3 Next, Lely Industries N.V., Maassluis, The Netherlands). At the specified milking times, cows were moved from the research pen into a small holding area adjacent to the robotic milker, from where they were milked individually and sequentially. Only the cows that were scheduled for milking, according to treatment, were moved into the holding area. Cows did not receive any supplemental feed from the robotic milking system while being milked. Cows received 14 d of adaptation to each treatment followed by 7 d of data collection.
2.2.3 Feeding Procedure

Cows were individually assigned to one roughage intake feed bin (Insentec B.V., Marknesse, The Netherlands) to measure individual feed intake and feeding behaviour, as validated by Chapinal et al. (2007). Cows received 3 d of training prior to the start of the experimental period to learn to access their own unique feed bin. Cows were fed a base TMR formulated to meet the nutrient requirements of a cow producing 39 kg of milk according to the NRC (2001) nutrient recommendations for high-producing lactating dairy cows. The TMR consisted of 30.4 % grass/legume silage, 30.0 % corn silage, 3.6 % grass/alfalfa hay, 10.5 % high moisture corn, 11.3 % protein concentrate, and 14.3 % robotic pellet supplement on a DM basis (Table 2.1).

The TMR (without the robotic pellet supplement) was mixed once daily in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada) and delivered via conveyor into a motorized feed cart (WIC RTM-55, WIC Inc., Wickham, QC, Canada) between 1000 and 1100 h. The robotic pellet supplement was weighed on a scale (Model 2020, Toledo Scale Corporation, Brazil) and mixed into the TMR, for approximately 4 min, using the motorized feed cart.

Cows were denied access to the feed bins beginning at 1100 h daily, at which time feed refusals were removed and sampled as needed, and fresh feed was manually delivered into each feed bin. The total amount of feed offered was adjusted daily to ensure approximately 10% feed refusal per cow. Actual feed refusal averaged 10.5 ± 6.1 % (mean ± SD) of the feed offered as fed over the course of the experiment and did not vary by treatment ($P = 0.8$). Cows were given
access to the feed bins beginning at 1200 h daily; this time point served as the start of each data collection day.

2.2.4 Behavioural Data Collection

Feeding behaviour was automatically monitored for each cow for the last 7 d of each experimental period using the Insentec system. From the recorded data, we were able to determine the duration of each visit to the feed bin, the amount of feed consumed (start weight - end weight) during each visit, and the rate of consumption for each visit. These data were then summarized to calculate daily DMI (kg/d), daily time spent feeding (min/d), and average feeding rate (kg/min).

Lying behaviour patterns of the cows were automatically collected using data loggers (HOBO Pendant G Logger, Onset Computer Corporation, Pocasset, MA, USA) for the last 7 d of each treatment period. These devices measured leg orientation at 1 min intervals, and allowed all the standing and lying behaviour data to be collected electronically (Ledgerwood et al., 2010). On d 14 of each period, data loggers were placed on the hind leg of each cow using veterinary bandaging tape (Vetrap Bandaging Tape, 3M, London, ON, Canada) while the cow was restrained in a stall. Data loggers were removed from the cows on d 1 of the following experimental period to ensure a complete 7 d recording period. Data collected were used to calculate standing and lying duration (min/d), bout frequency (#/d), and bout length (min/bout). Duration of post-milking standing (min) was calculated as the difference in time between the end of milking and the first recorded instance when the cow lay down following milking.

Rumination behaviour was electronically monitored for the last 7 d of each treatment period using automatic rumination detection devices (Lely Qwes-HR collars, Lely Industries
N.V., Maassluis, The Netherlands). The rumination logger, placed on the neck collar of the cow, continuously records the time spent ruminating within 24 h in 2-h intervals, as validated by Schirmann et al. (2009). Data was transferred at each milking using an automatic reader located within the robotic milking system.

2.2.5 Feed Sampling Analysis

For the last 7 d of each experimental period, duplicate samples of fresh feed were collected at feeding time for the determination of DM, nutrient content, and particle size distribution of the TMR. Duplicate samples of feed refusal for each cow were collected for determination of DM and for particle size separation to determine feed sorting. On d 1, 8 and 15 of each treatment period, duplicate samples of dietary components were collected for DM, chemical, and particle size analysis. All samples were immediately frozen at -20°C until they were further analyzed.

Samples collected for particle size separation were separated using the 3-screen (19, 8, 1.8 mm) Penn State Particle Separator (PSPS; Kononoff et al., 2003). This separated the particles into 4 fractions; long (>19mm), medium (<19, >8 mm), short (<8, >1.18 mm) and fine (<1.18 mm) particles. After separation, the DM of each separated fraction was determined by oven drying at 55°C for 48 h. The particle fractions of the fresh TMR samples were ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA, USA) and were analyzed for NDF using an Ankom® Fiber Analyzer (Ankom Technology, Macedon, NY, USA) with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991).

Samples collected for DM and chemical analysis were oven-dried at 55°C for 48 h and then ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia,
PA, USA). These samples were sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000; method 930.15), ash (535°C; AOAC, 2000; method 942.05), ADF (AOAC, 2000; method 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), and CP (N × 6.25; AOAC 2000; method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI, USA).

2.2.6 Milk Production and Components

Milk yield was automatically recorded at each milking for the last 7 d of each treatment period by the robotic milking system (Lely A3 Next, Lely Industries N.V., Maassluis, The Netherlands). Milk samples were collected from each milking for the last 3 d of each experimental period using the Lely Shuttle Sampling Device (Lely Industries N.V., Maassluis, The Netherlands). These samples were sent to the DHI testing laboratory (CanWest DHI, Guelph, Ontario, Canada) for analysis. Milk samples were analyzed for milk fat and protein percentage using a near-infrared analyzer (FOSS System 4000 Infrared Transmission Analyzer, Foss, Hillerød, Denmark). For those days where milk components were measured, the yield of 4% FCM (kg/d) was calculated (NRC, 2001) as \((0.4 \times \text{milk yield (kg/d)}) + (15.0 \times \text{fat yield (kg/d)})\). Energy-corrected milk yield was calculated using the following equation: \(\text{ECM} = (0.327 \times \text{milk yield (kg/d)}) + (12.95 \times \text{fat yield (kg/d)}) + (7.2 \times \text{protein yield (kg/d)})\) (Tyrrell and Reid, 1965). Efficiency of milk production was determined by calculating the kilograms of milk, 4% FCM yield, or ECM yield per kilogram of DMI for each treatment period.

2.2.7 Calculations and Statistical Analysis

Individual feeding bouts were separated into meals using an individual meal criterion for each cow on each treatment. Meal criteria were determined, as described by DeVries et al.
(2003b), using a software package (MIX 3.1.3; MacDonald and Green, 1988) to fit a mixture of normal distributions to the distributions of log_{10}-transformed time intervals between moments of feeding (across all 7 d of data recorded per treatment period). The average meal criterion was 40.7 ± 27.7 min (mean ± SD) and did not vary by treatment (P=0.6). The calculated meal criteria were used to calculate meal frequency (meals/d), by counting the number of intervals that exceeded the criterion and adding one. Meal duration (min/meal) was calculated as the time from the start of the first feeding bout until the end of the last feeding bout at which time the meal criterion was exceeded. Meal size (kg/meal) was calculated by dividing DMI by meal frequency.

After an infinite number of iterations, we were unable to fit the individual inter-meal distributions for one cow on 3x/d treatment, resulting in no meal criterion for this animal for this period, and exclusion of meal data from the statistical analysis.

Feed sorting was calculated as the actual DMI of each fraction of PSPS expressed as a percentage of the predicted DMI of that fraction (Leonardi and Armentano, 2003). The actual intake of each individual fraction was calculated as the difference between the DM amount of each fraction in the offered feed and that in the refused feed. The predicted intake for each individual fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the offered diet. Values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for).

Data collected for each day of each treatment data collection period for each cow were summarized for each cow by treatment period. To test whether feed sorting occurred, sorting activity for each fraction of the PSPS was summarized by treatment and tested for a difference from 100 using t-tests. All data were then analyzed using the MIXED procedure of SAS (SAS
Institute, 2009). The final model included the fixed effects of parity, period, order of treatment exposure, treatment, and parity × treatment interaction. Other interactions of the fixed effects were tested in the initial model and were not significant; therefore, they were removed from the final model. The random effects were group and cow within order and group.

Data for DMI, feeding time, and feeding rate were also summarized on an hourly basis, while ruminating time was summarized on a 2-h basis, for each animal on each treatment. Differences among treatments in the distribution of these variables over a 24-h period were analyzed using the MIXED procedure of SAS treating hour as a repeated measure. The model included the fixed effects of period, order of treatment exposure, hour, treatment, and hour × treatment interaction. The random effects were group and cow within order and group. Cow within square was included in the model as the subject of the repeated statement. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz’s Bayesian information criterion (SAS Institute, 2009).

All values reported are least squares means. Significance was declared at $P \leq 0.05$, and trends reported if $0.05 < P \leq 0.10$.

2.3 Results

Results for the effect of treatment on milk yield, composition, and efficiency are presented in Table 2.2. Cows milked 3x/d increased their average milk yield by 2.9 kg/d (8.3 %) compared to cows milked 2x/d. Multiparous cows produced 8.2 kg/d (25.5 %) more milk over the course of the experiment than did PP cows. Primiparous cows and cows milked 3x/d tended to have lower milk fat percentage than MP cows and cows milked 2x/d. Milk fat yield was lower for PP cows than for MP cows as a result of lower milk yield and fat percentage produced by PP
cows. There was no effect of treatment or parity on milk protein percentage. Cows milked 2x/d had 0.1 kg/d lower protein yield as a result of reduced milk yield. Primiparous cows produced 0.3 kg/d less protein than MP cows. As a result, PP cows and cows milked 2x/d had lower 4% FCM and ECM than MP cows and cows milked 3x/d, respectively. Cows milked 3x/d had greater milk production efficiency. There tended to be a treatment × parity interaction such that MP cows had greater ECM efficiency when milked 3x/d compared to when milked 2x/d. There were no further interactions between parity and treatment for any other milk yield, composition, or efficiency variables.

Primiparous cows consumed 3.8 kg/d (15.7 %) less DM than MP cows; there tended to be a treatment × parity interaction such that PP cows milked 3x/d had greater DMI compared to PP cows milked 2x/d (Table 2.3). There was also a tendency for cows milked 2x/d to spend less time feeding and, thus, consume their feed at a faster rate than cows milked 3x/d. There was, however, no effect of parity or treatment × parity interaction on total feeding time (min/d).

The analysis of diurnal feeding activity indicated there were differences in the distribution of feeding activity throughout the day as observed by a treatment × hour interaction for DMI ($P = 0.008$; Figure 2.1), feeding time ($P = 0.009$; Figure 2.2), and feeding rate ($P = 0.04$; Figure 2.3). Between 2100 h and 1200 h the following day, all cows, regardless of treatment, demonstrated very similar patterns in DMI and feeding time. Cows milked 3x/d showed a secondary peak in feeding activity following the 1400 h milking, whereas feeding activity of cows milked 2x/d decreased more steadily following fresh feed delivery at 1200 h. At the time of the 1800 h and 2200 h milking, all cows showed an increase in feeding activity. However, DMI and feeding rate of cows that were not milked, particularly around the 1800 h
milking, was lower than those that were milked. Cows milked 3x/d showed an additional peak in feeding activity around 2000 h.

Results for the effect of treatment on meal patterns are presented in Table 2.3. A treatment × parity interaction for meal frequency indicated that PP cows were most affected by increased milking frequency, resulting in them consuming 1.4 more meals/d when milked 3x/d. A treatment × parity interaction was also observed for meal size and meal duration; PP cows milked 3x/d consumed more frequent, shorter, and smaller meals throughout the day.

There was no effect of treatment or parity on the total rumination time (Table 2.3), and there was no treatment × hour interaction for rumination activity ($P = 0.6$; Figure 2.4). There was also no effect of treatment or parity on the lying behaviour or post milking standing time of the cows (Table 2.3).

There was no sorting for, or against long particles (>19.0 mm) or fine particles (<1.18 mm) (Table 2.4). There was a treatment × parity interaction for sorting for medium particles, indicating that PP cows milked 2x/d sorted for medium particles more than PP cows milked 3x/d. Multiparous cows tended to sort against short particles (<8.0, >1.18 mm) on both treatments, while PP cows showed no significant sorting of this fraction.

2.4 Discussion

Many dairy producers milk on a 3x/d schedule to maximize parlour efficiency and productivity. Previous research in this area has been focused on the impact of milking frequency on milk production and lactation persistency. There is much empirical evidence to conclude that increased milking frequency can increase milk production. In the present study, cows milked 3x/d increased their average milk yield by 2.9 kg/d compared to cows milked 2x/d. These results
are fairly consistent with a review of previous research which indicated that, on average, 3x/d milking results in a fixed yield increase of 3.5 kg/d (Erdman and Varner, 1995). However, the magnitude of milk yield response in the current study (8.3%) was lower than previously reported. Previous research report an increase in milk yield of 10.4 to 19.9 % (Amos et al., 1985; Klei et al., 1997; Gisi et al., 1986; Smith et al., 2002). It is possible that the stage of lactation of the cows used in this study (149.5 ± 31.3 DIM at the onset) impacted their sensitivity to treatment, resulting in a lesser milk yield response. Klei et al. (1997) demonstrated that, relative to 2x/d milking, cows milked 3x/d from parturition increased milk yield by 10.4 % compared to 7.8 % and 0.06 % increases in cows switched to 3x/d milking at 100 and 200 DIM, respectively. Thus, as cows get further into lactation, the magnitude of milk yield response is diminished when milking frequency increases.

In the current study, cows milked 3x/d tended to produce milk that was 0.3 percentage points lower in milk fat than cows milked 2x/d, which is consistent with previous research (Klei et al., 1997; Smith et al., 2002). However, despite the greater milk yield for cows milked 3x/d, there was no treatment effect on milk fat yield, which disagrees with Klei et al. (1997) who found that cows milked 3x/d yield 4.7 % more milk fat. Contrary to that reported by Klei et al. (1997) and Smith et al. (2002), milking frequency did not impact milk protein % in the current study. Cows milked 3x/d, in the present study, did have a 7.8 % greater milk protein yield, which is consistent with the findings of Klei et al. (1997). Thus, even though the response of milk composition and milk component yield to milking frequency is typically consistent (Erdman and Varner, 1995), there is variation across studies in these responses. This variation may be attributed to differences in methodology (e.g., cow genetics, diet composition, experimental procedures). Despite the less consistent response in milk composition and milk component yield
in the current study, when accounting for milk composition, milking 3x/d did improve both FCM and ECM yield; this is consistent with previous research (Barnes et al., 1990; Smith et al., 2002).

Multiparous cows had 25.5% greater milk yield, 36.7% greater fat yield and 25.5% greater protein yield and, therefore, greater FCM and ECM than PP cows. Higher milk component yield of MP cows is consistent with previous research (Klei et al., 1997). The response to milking frequency was similar for both PP and MP cows in the present study. While some research has indicated that PP cows have greater milk yield when milked 3x/d (Gisi et al., 1986; Barnes et al., 1990), as demonstrated by Erdman and Varner (1995) and in the current study, this is not a consistent response. Further research is required to understand what conditions may cause PP cows to be more influenced by milking frequency than MP cows.

Despite the production benefits of increased milking frequency, the time required for extra milking could potentially reduce the time available for feeding, rumination, and lying behaviours. For this reason, there is interest to understand the effect milking frequency has on the behavioural patterns of lactating dairy cows. Contrary to the hypothesis above, cows tended to spend more time feeding, at a slower rate, when milked 3x/d. This increase in feeding activity was notable around the times when cows were milked (Figure 2.2 and 2.3), and provides further evidence that cows are motivated to feed around the time of milking (DeVries et al., 2003a), albeit not the same level of motivation as seen at the time of feed delivery (Figure 2.1 and 2.2; DeVries and von Keyserlingk, 2005). For MP cows, the increase in feeding activity was facilitated through having longer, and slightly larger meals when milked 3x/d. Alternatively, PP cows consumed smaller, more frequent meals throughout the day when milked 3x/d. For the PP cows, this translated into a tendency for them to have greater DMI when milked 3x/d. The lack of impact of milking frequency on DMI of MP cows is not surprising, as this effect is common
It has been concluded that cows milked 3x/d utilize feed nutrients and experience a higher rate of tissue catabolism to achieve greater production efficiency (Amos et al., 1985), which is in agreement with the MP cows in the present study. Lower-producing cows, such as the PP, typically do not convert feed to milk as efficiently as higher-producing cows (DePeters et al., 1985); this was also confirmed by the present study as PP cows had lower 4 % FCM and ECM efficiency. Thus, to achieve the same increase in milk yield at greater milking frequency, as done in the current study, the PP cows had to consume more DM, in a manner more conducive to stable and efficient rumen fermentation (Shabi et al., 1999).

As seen in Figure 2.4, periods when cows spend more time ruminating are associated with lower feeding activity (Figure 2.2) and, thus, lower DMI (Figure 2.1), as cows are unable to consume feed and ruminate simultaneously (Schirmann et al., 2012). In the present study, 3 large peaks in rumination activity were observed throughout the day, coinciding with the periods of lowest feeding activity. Norring et al. (2012) observed that as a cow’s milk yield increases, lying time decreases and time spent ruminating while standing increases; this is likely due to increased time spent feeding to meet increased metabolic demands required to support that yield. In the current study, the increase in feeding time associated with milking more frequently, was not substantial enough, however, to elicit any reduction in ruminating time or lying time. A reduction in lying time was expected in the current study, not only due to possible trade-offs with feeding time, but also as the time required for an extra milking would increase the time cows spend away from their home pen. Contrary to this expectation, no effect of milking frequency was found on total lying time in the present study; this agrees with the results of Tucker et al, (2007) for cows milked 1x/d and 2x/d. This result provides further evidence that cows have a
strong motivation for lying (Metz, 1985). In previous research, lying has been demonstrated to be a higher priority over feeding after cows have been simultaneously deprived of the opportunity to do both (Metz, 1985; Munksgaard et al., 2005). Thus, management practices, such as greater milking frequency, which may result in cows spending more time standing (waiting to be milked and feeding at the feed bunk), do not necessarily result in a negative impact on lying time.

It has previously been observed that increased time spent milking was associated with a decrease in other behaviours, such as feeding and lying (Gomez and Cook, 2010). Therefore, it was hypothesized that the extra time required for milking 3x/d may cause cows to lie down sooner after milking. Lying down immediately after milking has been related to higher herd SCC (Barnouin et al., 2004), as well as greater incidence of subclinical (DeVries et al., 2010) and clinical mastitis (Peeler et al., 2000). In the present study, milking frequency had no impact on post milking standing time. The average post milking standing time (68 ± 24 min; mean ± SD) is greater than reports from other studies involving free stall housed cows (35 min: Tyler et al., 1997; 55 min: DeVries and von Keyserlingk, 2005; 62 min: DeVries et al, 2005; 33 min: Fregonesi et al., 2007); this variability may be attributed to differences in methodology (e.g., stocking density, stage of lactation, experimental design). No treatment effect in the present study may be a result of the size of the group of cows tested in each replicate and the facility design. At the 600 h milking, all 6 cows were milked; therefore, the last cow to be milked would have stood in the holding area for up to 1 h. At all subsequent milkings, when only 3 cows were milked, the last cow to be milked was only in the holding area for a maximum of 20 - 30 min. These times may not be fully representative of a commercial situation, where cows milked 3x/d spend on average, 1.2 - 5.7 h/d out of their home pen (Gomez and Cook, 2010). It is possible, as
cows spend longer periods of time standing in holding areas, they may be more motivated to lie sooner after milking. This is speculative, thus further research is required to determine how much time cows need to be deprived from feeding and lying (i.e. time spent in the holding area) to cause change in their post milking standing time.

When one animal eats, another may be stimulated to do likewise, whether it is hungry or not, which is known as social facilitation (Curtis and Houpt, 1983). Around the time of the 1800 and 2200 h milkings all cows showed an increase in feeding activity, regardless of treatment, indicating that social facilitation influenced feeding patterns at these times. Dairy cattle are motivated to feed following the return from milking (DeVries et al., 2003a), therefore, the act of cows on one treatment returning from milking could have potentially motivated the other cows to seek feed as well. This is obviously a limitation of the experimental setup. However, conducting this experiment in identical, isolated facilities would introduce greater sources of error (i.e. confounding location with treatment). The benefit of the present design was that cows on both treatments were subject to identical environmental conditions; thus there is confidence that any differences observed overcame any social facilitation and, thus, can be directly attributed to the treatments imposed.

2.5 Conclusions

Cows milked 3x/d and MP cows produced more milk than those milked 2x/d and PP cows, respectively. The extra time required for milking 3x/d altered the distribution of cow behavioural activity throughout the day. While this did not impact total daily lying or rumination time, feeding time did tend to increase. For MP cows, the increase in feeding activity was facilitated through having longer, and slightly larger meals when milked 3x/d. Alternatively, PP
cows consumed smaller, more frequent meals throughout the day when milked 3x/d, resulting in a tendency for greater DMI. These results indicate that under 3x/d milking schedules, PP cows will positively adjust their feeding behaviour to achieve similar production increases as MP cows.

2.6 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Innovation Centre (Kemptville, Ontario, Canada). In particular we thank Albert Koekkoek, Megan Bruce, Alex Watters, and Ryan Garner of the University of Guelph, Kemptville Campus for their technical help. This project was financially supported by a Dairy Farmers of Ontario (Mississauga, Ontario, Canada) research grant and an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA; Guelph, Ontario, Canada)/University of Guelph Production Systems research grant. Additional project support was received from the Canadian Foundation for Innovation (CFI; Ottawa, Ontario, Canada) and the Ontario Research Fund (Toronto, Ontario, Canada).
Table 2.1 Chemical composition and particle size distribution of the experimental ration and components (mean ± SD).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Corn silage</th>
<th>Grass/alfalfa hay</th>
<th>Grass/legume silage</th>
<th>High-moisture corn</th>
<th>Protein concentrate</th>
<th>Robotic supplement</th>
<th>TMR³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical Composition</strong>⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>41.7 ± 1.5</td>
<td>86.2 ± 3.8</td>
<td>37.8 ± 3.4</td>
<td>80.9 ± 1.0</td>
<td>92.1 ± 0.4</td>
<td>90.1 ± 0.5</td>
<td>48.9 ± 1.0</td>
</tr>
<tr>
<td>OM, % of DM</td>
<td>96.7 ± 0.5</td>
<td>92.4 ± 0.3</td>
<td>89.1 ± 0.8</td>
<td>98.2 ± 0.2</td>
<td>78.1 ± 2.5</td>
<td>93.6 ± 0.3</td>
<td>92.0 ± 0.5</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>7.6 ± 0.2</td>
<td>13.7 ± 1.3</td>
<td>18.6 ± 0.7</td>
<td>9.1 ± 0.1</td>
<td>39.5 ± 0.8</td>
<td>19.7 ± 0.5</td>
<td>16.7 ± 0.7</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>18.8 ± 1.3</td>
<td>45.1 ± 2.1</td>
<td>36.9 ± 3.3</td>
<td>4.5 ± 0.7</td>
<td>6.8 ± 0.9</td>
<td>6.8 ± 0.6</td>
<td>21.6 ± 1.0</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>31.2 ± 1.8</td>
<td>56.2 ± 3.0</td>
<td>49.9 ± 1.6</td>
<td>11.1 ± 0.4</td>
<td>12.9 ± 1.2</td>
<td>26.5 ± 1.4</td>
<td>33.0 ± 1.4</td>
</tr>
<tr>
<td>Calcium, % of DM</td>
<td>0.2 ± 0.0</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>4.9 ± 0.7</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Phosphorus, % of DM</td>
<td>0.2 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>3.3 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.0</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Magnesium, % of DM</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.8 ± 0.2</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Potassium, % of DM</td>
<td>0.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>3.0 ± 0.5</td>
<td>0.5 ± 0.0</td>
<td>1.7 ± 0.1</td>
<td>1.1 ± 0.0</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Sodium, % of DM</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.6 ± 0.5</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Iron, PPM</td>
<td>50.5 ± 3.4</td>
<td>100.5 ± 34.2</td>
<td>731.3 ± 51.5</td>
<td>37.0 ± 0.8</td>
<td>784.8 ± 174.3</td>
<td>147.3 ± 5.7</td>
<td>466.2 ± 7.2</td>
</tr>
<tr>
<td>Manganese, PPM</td>
<td>13.8 ± 2.2</td>
<td>19.0 ± 3.7</td>
<td>55.0 ± 6.2</td>
<td>6.0 ± 0.0</td>
<td>336.8 ± 1075.5</td>
<td>107.5 ± 11.7</td>
<td>82.3 ± 7.2</td>
</tr>
<tr>
<td>Zinc, PPM</td>
<td>22.3 ± 1.7</td>
<td>21.3 ± 1.0</td>
<td>29.5 ± 1.9</td>
<td>24.3 ± 1.0</td>
<td>407.5 ± 78.4</td>
<td>110.3 ± 7.9</td>
<td>80.6 ± 9.1</td>
</tr>
<tr>
<td>Copper, PPM</td>
<td>5.3 ± 0.5</td>
<td>11.0 ± 0.8</td>
<td>11.5 ± 1.7</td>
<td>1.0 ± 0.0</td>
<td>135.0 ± 23.3</td>
<td>23.3 ± 0.5</td>
<td>25.0 ± 3.2</td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>77.0 ± 1.0</td>
<td>53.3 ± 1.6</td>
<td>58.7 ± 1.0</td>
<td>87.2 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>71.7 ± 1.0</td>
</tr>
<tr>
<td>NeL, mcal/kg</td>
<td>1.8 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.7 ± 0.0</td>
</tr>
<tr>
<td>NeM, mcal/kg</td>
<td>1.9 ± 0.0</td>
<td>1.1 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>2.2 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.7 ± 0.0</td>
</tr>
<tr>
<td>NeG, mcal/kg</td>
<td>1.2 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>1.5 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td>NFC, % of DM</td>
<td>55.3 ± 2.1</td>
<td>22.1 ± 4.0</td>
<td>18.9 ± 1.2</td>
<td>73.4 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>38.9 ± 1.3</td>
</tr>
<tr>
<td><strong>Particle Size</strong>₅ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>2.9 ± 1.3</td>
<td>76.3 ± 12.9</td>
<td>30.8 ± 8.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.7 ± 6.6</td>
</tr>
<tr>
<td>Medium</td>
<td>62.8 ± 4.1</td>
<td>10.4 ± 4.6</td>
<td>44.2 ± 5.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>44.6 ± 3.7</td>
</tr>
<tr>
<td>Short</td>
<td>32.4 ± 4.8</td>
<td>12.0 ± 7.2</td>
<td>21.4 ± 4.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31.5 ± 3.6</td>
</tr>
<tr>
<td>Fine</td>
<td>1.8 ± 0.6</td>
<td>4.3 ± 3.0</td>
<td>3.7 ± 1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.1 ± 2.0</td>
</tr>
</tbody>
</table>

¹Supplied by Dundas Feed & Seed Ltd (Winchester, Ontario, Canada) including the ingredients; corn distillers, soybean meal, corn gluten meal, feather meal, blood meal, cane molasses, salt, calcium bicarbonate, dicalcium phosphate, magnesium oxide, sodium sesquicarbontte, magnesium potassium sulfate, animal fat, vitamins, and trace minerals.

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2 Supplied by Dundas Feed & Seed Ltd (Winchester, Ontario, Canada) including the ingredients; ground wheat, wheat shorts, corn distillers, soybean meal, canola meal, cane molasses, salt, calcium bicarbonate, dicalcium phosphate, magnesium oxide, energy booster, vitamins, trace minerals, and flavour.

3 NDF composition (DM basis) of the long, medium, short and fine particle fractions was 40.2 ± 5.4%, 36.5 ± 4.8%, 23.3 ± 1.4, and 20.4% ± 1.4%, respectively.

4 Values were obtained from chemical analysis of TMR and components. OM = 100 - % ash.

5 Particle size determined by Penn State Particle Separator, which has a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine).
Table 2.2 Effect of milking frequency (F) and parity (P) on milk yield, milk composition, milk component yield, and efficiency of production.

<table>
<thead>
<tr>
<th>Item</th>
<th>2x$^1$</th>
<th>3x$^1$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>PP$^2$</td>
<td>MP$^3$</td>
<td>PP</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk$^3$</td>
<td>30.9</td>
<td>38.7</td>
<td>33.4</td>
</tr>
<tr>
<td>4% FCM$^{4,5}$</td>
<td>29.6</td>
<td>39.1</td>
<td>30.4</td>
</tr>
<tr>
<td>ECM$^{4,6}$</td>
<td>32.4</td>
<td>42.3</td>
<td>33.5</td>
</tr>
<tr>
<td>Milk composition$^4$, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.73</td>
<td>4.13</td>
<td>3.50</td>
</tr>
<tr>
<td>Protein</td>
<td>3.35</td>
<td>3.30</td>
<td>3.32</td>
</tr>
<tr>
<td>Milk component yield$^4$, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>1.15</td>
<td>1.57</td>
<td>1.15</td>
</tr>
<tr>
<td>Protein</td>
<td>1.03</td>
<td>1.28</td>
<td>1.10</td>
</tr>
<tr>
<td>Efficiency of milk production, kg/kg$^4$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.31</td>
<td>1.38</td>
<td>1.37</td>
</tr>
<tr>
<td>4% FCM/DMI</td>
<td>1.25</td>
<td>1.39</td>
<td>1.23</td>
</tr>
<tr>
<td>ECM/DMI</td>
<td>1.37</td>
<td>1.50</td>
<td>1.36</td>
</tr>
</tbody>
</table>

$^1$2x = 2x/d milking frequency; 3x = 3x/d milking frequency.

$^2$PP = primiparous; MP = multiparous.

$^3$Data averaged over 7 d for 12 cows on each treatment.

$^4$Data averaged over 3 d for 12 cows on each treatment.

$^5$4% FCM = 4% fat correct milk.

$^6$ECM = energy corrected milk.
Table 2.3 Effect of milking frequency\(^1\) (F) and parity\(^2\) (P) on DMI, feeding behaviour, rumination time, lying behaviour and post milking standing time\(^3\).

<table>
<thead>
<tr>
<th>Item</th>
<th>2x(^1)</th>
<th>3x(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP(^2)</td>
<td>MP(^2)</td>
<td>PP</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>23.6</td>
<td>28.2</td>
<td>24.7</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>221.6</td>
<td>227.6</td>
<td>227.1</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td>0.12</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Meal frequency, meals/d</td>
<td>7.7</td>
<td>6.7</td>
<td>9.1</td>
</tr>
<tr>
<td>Meal size, kg/meal</td>
<td>3.2</td>
<td>4.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Meal duration, min/meal</td>
<td>29.3</td>
<td>36.8</td>
<td>25.8</td>
</tr>
<tr>
<td>Rumination time, min/d</td>
<td>473.5</td>
<td>529.1</td>
<td>468.5</td>
</tr>
<tr>
<td>Lying bouts, #/d</td>
<td>10.3</td>
<td>10.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Lying time, min/d</td>
<td>702.7</td>
<td>705.7</td>
<td>681.6</td>
</tr>
<tr>
<td>Post-milking standing, min</td>
<td>59.8</td>
<td>83.1</td>
<td>65.6</td>
</tr>
</tbody>
</table>

\(^1\)2x = 2x/d milking frequency; 3x = 3x/d milking frequency.

\(^2\)PP = primiparous; MP = multiparous.

\(^3\)Data averaged over 7 d for 12 cows on each treatment.
Table 2.4 Effect of milking frequency\(^1\) (F) and parity\(^2\) (P) on the sorting (%) of long, medium, short, and fine particles\(^3\).

<table>
<thead>
<tr>
<th>Particle Size(^4)</th>
<th>(2x^1) PP(^2)</th>
<th>(2x^1) MP(^2)</th>
<th>(3x^1) PP</th>
<th>(3x^1) MP</th>
<th>SE</th>
<th>P</th>
<th>F</th>
<th>P x F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>97.5</td>
<td>100.5</td>
<td>99.4</td>
<td>100.6</td>
<td>2.6</td>
<td>0.45</td>
<td>0.27</td>
<td>0.35</td>
</tr>
<tr>
<td>Medium</td>
<td>101.7(^*)</td>
<td>101.2(^*)</td>
<td>101.1(^*)</td>
<td>101.4(^*)</td>
<td>0.3</td>
<td>0.77</td>
<td>0.57</td>
<td>0.090</td>
</tr>
<tr>
<td>Short</td>
<td>99.4</td>
<td>98.6(^†)</td>
<td>99.0</td>
<td>98.2(^†)</td>
<td>0.8</td>
<td>0.28</td>
<td>0.067</td>
<td>0.77</td>
</tr>
<tr>
<td>Fine</td>
<td>95.0</td>
<td>93.1</td>
<td>95.9</td>
<td>93.3</td>
<td>1.9</td>
<td>0.043</td>
<td>0.58</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\(^1\)2x = 2x/d milking frequency; 3x = 3x/d milking frequency.  
\(^2\)PP = primiparous; MP = multiparous.  
\(^3\)Sorting % = 100 x (particle size n DM intake/particle size n predicted DM intake), where n = long, medium, short, or fine particle fraction. Sorting values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for). Data are averaged over 7 d for 12 cows on each treatment.  
\(^4\)Particle size determined by a Penn State Particle Separator, which has a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine).  
\(^*\)\(^†\)Difference in sorting values from 100% expressed as: \(^*\)\(P<0.05\), \(^†\)\(P<0.10\).
**Figure 2.1.** Hourly average DMI (kg) of lactating dairy cows milked 1) 2x/d or 2) 3x/d. Data are averaged over 7 days for 12 cows on each treatment.
Figure 2.2. Hourly average feeding time (min) of lactating dairy milked 1) 2x/d or 2) 3x/d. Data are averaged over 7 days for 12 cows on each treatment.
Figure 2.3. Hourly average feeding rate (kg/min) of lactating dairy cows milked 1) 2x/d or 2) 3x/d. Data are averaged over 7 days for 12 cows on each treatment.
**Figure 2.4.** Bihourly average rumination time (min) of lactating dairy cows milked 1) 2x/d or 2) 3x/d. Data are averaged over 7 days for 12 cows on each treatment.
CHAPTER 3: EFFECT OF FEED DELIVERY FREQUENCY ON THE BEHAVIOUR AND PRODUCTIVITY OF LACTATING DAIRY COWS

3.1 Introduction

The delivery of fresh feed and the act of returning from milking stimulate feeding activity in lactating dairy cattle group-housed and -fed indoors (DeVries et al., 2003a). Since the delivery of fresh feed has been demonstrated to be a stronger stimulus to initiate feeding activity (DeVries and von Keyserlingk, 2005), increased frequency of feed delivery has the potential to influence feeding behaviour, health, and productivity. Cows fed more frequently consume feed more evenly after each feed delivery and throughout the day (DeVries et al., 2005; Mantysaari et al., 2006) and, therefore, exhibit more desirable feeding patterns to support rumen health. Such desirable feeding patterns are conducive to more consistent rumen pH (French and Kennelly, 1990), which may contribute to improved milk fat (Rottman et al., 2011); fibre digestibility (Dhiman et al., 2002), and production efficiency (Mantysaari et al., 2006) observed when cows are fed more frequently than 1x/d. In contrast, 1x/d feed delivery frequency results in significant peaks in feeding activity in the immediate time period following feed delivery (DeVries et al., 2005), resulting in slug feeding patterns which predispose cows to SARA (Shaver, 2002) due to large diurnal fluctuations in ruminal pH (Shabi et al., 1999).

Some reports indicate that cows fed more frequently spend more time feeding and show no difference in DMI (DeVries et al., 2005), while others have shown no difference in feeding time, but lower DMI for cows delivered feed more frequently compared to 1x/d feed delivery (Phillips and Rind, 2001, Mantysaari et al, 2006). From this latter research, it was concluded that the disturbances caused by increased feed delivery frequency may have detrimental effects.
on behaviour patterns and thus productivity of dairy cattle. Previous research has demonstrated
no impact of frequency of feed delivery on daily lying time of cows milked 2x/d (DeVries et al.,
2005). It is possible that for cows milked 3x/d, the time required for an extra milking, in
conjunction with greater feed delivery frequency, may alter the amount of time devoted to
behavioural activities such as lying, feeding and rumination, which are all critical for milk
production, maintenance of energy balance, efficient digestion, cow health, and welfare.

Thus, the objective of this study was to determine the effect of feed delivery frequency on
the behaviour and productivity of lactating dairy cows milked 3x/d. It was hypothesized that
under a 3x/d milking schedule, an increased frequency of feed delivery will result in increased
feeding activity, particularly after fresh feed delivery, resulting in a more uniform distribution of
feeding activity throughout the day, thus providing the required plane of nutrition for the
increased milk production demand of 3x/d milking schedules. It was also hypothesized that
changes in the time available for other critical behavioural activities (lying and ruminating) as
result of greater frequency of feed delivery may alter the amount of time devoted to lying and
ruminating behaviour throughout the day.

3.2 Materials and Methods

3.2.1 Animals and Housing

Twelve lactating Holstein dairy cows, including 6 PP and 6 MP (parity = 2.5 ± 0.8; mean
± SD), were used in this study. The animals were 79.1 ± 32.4 DIM and were producing 39.6 ±
5.0 kg milk at the beginning of the trial. Cows were housed 6 at a time in a free stall research pen
located at the University of Guelph, Kemptville Campus Dairy Education and Innovation Centre
(Kemptville, ON, Canada). Cows had access to 6 free-stalls with waterbeds (DCC Waterbeds,
Advanced Comfort Technology Inc., Reedsburg, WI, USA). Waterbeds were topped with wood shavings; bedding was replaced as needed. Manure was manually scraped to within reach of the alley scrapers 3x daily, at 0600, 1400 and 2200 h. Cows were milked 3x/d (at 0600, 1400, and 2200 h) using a robotic milking system (Lely A3 Next, Lely Industries N.V., Maassluis, The Netherlands). At the specified milking times, cows were moved from the research pen into a small holding area adjacent to the robotic milker, from where they were milked individually and sequentially. Cows did not receive any supplemental feed from the robotic milking system while being milked. The experiment was conducted from May 16 to September 26, 2012. The average environmental temperature during the data collection period was 18.8 ± 4.4°C. Use of cows and experimental procedures were approved by the University of Guelph’s Animal Care Committee. Cows were managed according to the guidelines set forth by the Canadian Council on Animal Care (CCAC, 2009).

3.2.2 Experimental Design

The number of animals required per treatment was determined through sample size and power analysis (Morris, 1999) to detect a 10% level of observed difference for the primary outcome variables, including behaviour, DMI, sorting, and milk production and composition. Cows were divided into 2 groups of 6, which were balanced according to DIM, milk production and average parity. Within each group of 6, cows were randomly exposed to each of 3 treatments using a replicated 3x3 Latin square design, with 21-d treatment periods. The treatments were the delivery of feed: 1) 1x/d (at 1400 h), 2) 2x/d (at 1400 and 2200 h), and 3) 3x/d (at 1400, 2200, and 0600 h). The cows received 14 d of adaptation to each treatment followed by 7 d of data collection.
3.2.3 Feeding Procedure

Cows were individually assigned to one roughage intake feed bin (Insentec RIC, Marknesse, The Netherlands) to measure individual feed intake and feeding behaviour, as validated by Chapinal et al. (2007). Cows received 3 d of training prior to the start of the experimental period to learn to access their own unique feed bin. Cows were fed a TMR formulated to meet the nutrient requirements of a cow producing 40 kg of milk according to the NRC (2001) nutrient recommendations for high-producing lactating dairy cows. The TMR consisted of 24.1 % grass/legume silage, 28.3 % corn silage, 23.4 % high moisture corn, 14.3 % protein concentrate, and 9.9 % robotic pellet supplement on a DM basis (Table 3.1).

The TMR (without the robotic pellet supplement) was mixed once daily in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada) and delivered via conveyor into a motorized feed cart (WIC RTM-55, WIC Inc., Wickham, QC, Canada) between 1100 and 1200 h. The robotic pellet supplement was included at a rate of 0.05 kg of pellet to 1 kg of TMR based on the diet formulation for 40 kg/d milk production for the milking herd. The robotic pellet supplement was weighed on a scale (Model 2020, Toledo Scale Corporation, Brazil) and mixed into the TMR, for approximately 4 min, using the motorized feed cart.

Cows were denied access from the feed bins beginning at 1330 h daily, at which time feed refusals were removed and sampled as needed, and fresh feed was manually delivered into each feed bin. The total amount of feed offered was adjusted daily to ensure approximately 10 % feed refusal per cow. The actual feed refusal averaged 10.0 ± 7.1 % (mean ± SD) of the feed offered as fed over the course of the experiment and did not vary by treatment ($P = 0.8$). Cows received approximately 50 % of their daily allotment of feed at 1400 h. The approximate amount
of feed required for subsequent feedings was weighed into 80 L containers using a scale (Model 2020, Toledo Scale Corporation, Brazil), and stored without lids in front of the feeders. At 1400 h, all cows were moved into the holding area and were milked individually; this time point served as the start of each data collection day. Upon return from milking, cows had full feed access. At the specified 2x and 3x feeding times, according to treatment, feed stored in the 80 L containers was added to the feed bins after the cows had been moved into the holding area.

3.2.4 Behavioural Data Collection

Feeding behaviour was automatically monitored for each cow for the last 7 d of each experimental period using the Insentec system. From the recorded data, we were able to determine the duration of each visit to the feed bin, the amount of feed consumed (start weight-end weight) during each visit, and the rate of consumption for each visit. These data were then summarized to calculate daily DMI (kg/d), daily time spent feeding (min/d), and average feeding rate (kg/min).

Lying behaviour patterns of the cows were automatically collected using data loggers (HOBO Pendant G Logger, Onset Computer Corporation, Pocasset, MA, USA) for the last 7 d of each experimental period. These devices measured leg orientation at 1 min intervals, and allowed all the standing and lying behaviour data to be collected electronically (Ledgerwood et al., 2010). On d 14 of each period, data loggers were placed on the hind leg of each cow using veterinary bandaging tape (Vetrap Bandaging Tape, 3M, London, ON, Canada) while the cow was restrained in a stall. Data loggers were removed from the cows on d 1 of the following experimental period to ensure a complete 7 d recording period. Data collected were used to calculate standing and lying duration (min/d), bout frequency (#/d), and bout length (min/bout).
Duration of post-milking standing (min) was calculated as the difference in time between the end of milking and the first recorded instance when the cow lay down following milking.

Rumination behaviour was electronically monitored for the last 7 d of each treatment period using automatic rumination detection devices (Lely Qwes-HR collars, Lely Industries N.V., Maassluis, The Netherlands). The rumination logger, placed on the neck collar of the cow, continuously records the time spent ruminating within 24 h in 2-h intervals, as validated by Schirmann et al. (2009). Data was transferred at each milking using an automatic reader located within the robotic milking system.

### 3.2.5 Feed Sampling Analysis

For the last 7 d of each experimental period, samples of fresh feed were collected at each feeding (1400, 2200 and 0600 h) for the determination of DM and nutrient content. An additional sample was collected at the first feeding (1400 h) for determination of particle size distribution of that TMR. Samples of feed refusal from each cow were collected at 1330 h for particle size separation to determine feed sorting. On d 1, 8 and 15 of each treatment period, duplicate samples of dietary components were collected for DM, chemical, and particle size analysis. All samples were immediately frozen at -20°C until they were further analyzed.

Samples collected for particle size separation were separated using the 3-screen (19, 8, 1.8 mm) Penn State Particle Separator (Kononoff et al., 2003). This separated the particles into 4 fractions; long (>19mm), medium (<19, >8 mm), short (<8, >1.18 mm) and fine (<1.18 mm) particles. After separation, the DM of each separated fraction was determined by oven drying at 55°C for 48 h. The particle fractions of the fresh TMR samples were ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA, USA), and were analyzed for
NDF using an Ankom\textsuperscript{2000} Fiber Analyzer (Ankom Technology, Macedon, NY, USA) with heat-stable \(\alpha\)-amylase and sodium sulfite (Van Soest et al., 1991).

Samples collected for DM and chemical analysis were oven-dried at 55°C for 48 h and then ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA, USA). These samples were sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD, USA) for analysis of DM (135°C; AOAC, 2000; method 930.15), ash (535°C; AOAC, 2000; method 942.05), ADF (AOAC, 2000; method 973.18), NDF with heat-stable \(\alpha\)-amylase and sodium sulfite (Van Soest et al., 1991), CP (N \times 6.25; AOAC 2000; method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

\textbf{3.2.6 Milk Production and Components}

Milk yield was automatically recorded at each milking for the last 7 d of each treatment period by the robotic milking system (Lely A3 Next, Lely Industries N.V., Maassluis, The Netherlands). Milk samples were collected from each milking for the last 3 d of each experimental period using the Lely Shuttle Sampling Device (Lely Industries N.V., Maassluis, The Netherlands). These samples were sent to the DHI testing laboratory (Ontario Dairy Herd Improvement Corp., Guelph, Ontario, Canada) for analysis. Milk samples were analyzed for milk fat and protein percentage using a near-infrared analyzer (FOSS System 4000 Infrared Transmission Analyzer, Foss, Hillerød, Denmark). For those days where milk components were measured, the yield of 3.5 \% FCM (kg/d) was calculated (NRC, 2001) as \((0.432 \times \text{milk yield (kg/d)}) + (16.23 \times \text{fat yield (kg/d)})\). Energy-corrected milk was calculated using the following equation: \(\text{ECM} = (0.327 \times \text{milk yield (kg/d)}) + (12.95 \times \text{fat yield (kg/d)}) + (7.2 \times \text{protein yield (kg/d)})\) (Tyrrell and Reid, 1965). Efficiency of milk production was determined by calculating
the kilograms of milk, 3.5 % FCM yield, or ECM yield per kilogram of DMI for each treatment period.

3.2.7 Calculations and Statistical Analysis

Individual feeding bouts were separated into meals using an individual meal criterion for each cow on each treatment. Meal criteria were determined, as described by DeVries et al. (2003b), using a software package (MIX 3.1.3; MacDonald and Green, 1988) to fit a mixture of normal distributions to the distributions of log_{10}-transformed time intervals between moments of feeding (across all 7 d of data recorded per treatment period). The average meal criterion was 25.5 ± 11.5 min and did not vary by treatment (P = 0.9). The calculated meal criteria were used to calculate meal frequency (meals/d), by counting the number of intervals that exceeded the criterion and adding one. Meal duration (min/meal) was calculated as the time from the start of the first feeding bout until the end of the last feeding bout at which time the meal criterion was exceeded. Meal size (kg/meal) was calculated by dividing DMI by meal frequency. Meal duration and meal size were also calculated for the first meal consumed after each milking based on the calculated meal criteria for each cow and period.

Feed sorting was calculated as the actual DMI of each fraction of PSPS expressed as a percentage of the predicted DMI of that fraction (Leonardi and Armentano, 2003). The actual intake of each individual fraction was calculated as the difference between the DM amount of each fraction in the offered feed and that in the refused feed. The predicted intake for each individual fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the offered diet. Values equal to 100% indicate no sorting,
<100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for).

Data collected for each day of each treatment data collection period for each cow were summarized for each cow by treatment period. To test whether feed sorting occurred, sorting activity for each fraction of the PSPS was summarized by treatment and tested for a difference from 100 using t-tests. All data was analyzed using the MIXED procedure of SAS (SAS Institute, 2009). The final model included the fixed effects of parity, period, treatment, parity × treatment interaction, and period × treatment interaction. Other interactions of the fixed effects were tested in the initial model and were not significant, therefore, they were removed from the final model. The random effects were replicate, square within replicate, cow within square × replicate. Linear and quadratic orthogonal contrasts of treatment effects were tested using the CONTRAST statement of SAS when the overall treatment effect was significant.

Data for DMI, feeding time, and feeding rate were also summarized on an hourly basis, while ruminating time was summarized on a 2-h basis, for each animal on each treatment. Differences among treatments in the distribution of these variables over a 24-h period were analyzed using the MIXED procedure of SAS treating hour as a repeated measure. The model included the fixed effects of hour, treatment, and hour × treatment interaction. The random effects were group and cow within order and group. Cow within square was included in the model as the subject of the repeated statement. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz’s Bayesian information criterion (SAS Institute, 2009).

All values reported are least squares means. Significance was declared at $P \leq 0.05$. 

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3.3 Results

Results for the effect of treatment on milk yield, composition, and efficiency are presented in Table 3.2. There was no effect of treatment on milk yield, however, MP cows produced 6.9 kg/d (18.0 %) more milk, 8.5 kg/d (23.0 %) more 3.5% FCM and 8.4 kg/d (22.4 %) more ECM than PP cows. Milk fat percentage did not differ by treatment or parity, resulting in MP cows having a greater fat yield than PP cows due to greater milk yield for MP cows. There was no discernible effect of treatment on milk protein percentage as there was a significant period × treatment interaction ($P = 0.02$) for this variable. Milk protein yield varied by parity, resulting in MP cows having the greatest protein yield (1.45 kg/d). There was no effect of treatment, parity, or parity × treatment interaction for milk production efficiency (Table 3.2).

Dry matter intake varied with feed delivery frequency (Table 3.3), with greater DMI observed ($P_{quadratic} = 0.027$) in cows fed 3x/d (27.8 kg/d) compared to when fed 2x/d (27.0 kg/d) or 1x/d (27.4 kg/d). There was no effect of treatment or parity on total feeding time (Table 3.3), but PP cows consumed feed more slowly, thus resulting in lower DMI, compared to MP cows.

Primiparous cows consumed smaller meals than MP cows, but there was no effect of treatment, parity, or parity × treatment interaction on meal frequency or meal duration (Table 3.3). However, the size of the first meal consumed after each milking was affected by parity (Table 3.3). PP cows consumed 2.0 kg (50.1 %) less DM during the first meal following the first milking than MP cows. There was also a parity × treatment interaction such that MP cows fed 1x/d consumed the largest meal following the first milking (at 1400 h). Primiparous cows consumed 0.8 kg (26.1 %) less DM during the first meal following the second milking (at 2200 h) than MP cows. There was no difference in the size of the first meal following the third
milking (at 0600 h), and there was no effect of treatment or parity on the length of the first meal following each milking.

The analysis of diurnal feeding activity indicated no treatment or treatment × hour interactions, but there were differences in the magnitude of feeding activity at specific points throughout the day. There was no difference in DMI, feeding time or feeding rate between treatments following the first milking (1400 h). Following the second milking (2200 h), cows fed 1x/d consumed less DM than cows fed 3x/d ($P = 0.036$; Figure 3.1), but there was no difference in feeding time (Figure 3.2) or feeding rate (Figure 3.3). Following the third milking (0600 h), cows that did not receive fresh feed (i.e. those fed 1x/d and 2x/d) consumed less DM ($P < 0.01$; Figure 3.1) than cows that received fresh feed at all milking times (3x/d). Cows fed 1x/d spent less time feeding ($P = 0.017$; Figure 3.2) than cows fed 3x/d following the third milking. There was no difference in feeding rate following the second or third milkings (Figure 3.3). There was no effect of treatment or parity on total rumination time (Table 3.3), but there was a treatment × hour interaction for rumination activity ($P = 0.042$; Figure 3.4).

There was no effect of treatment, parity or parity × treatment interaction on the total lying bouts throughout the day. There was no effect of treatment on total lying time; however, PP cow spent 121.9 min/d (24.5 %) longer lying down than MP cows (Table 3.4). There was no effect of treatment, parity or parity × treatment interaction on the daily average post-milking standing time (Table 3.4). There were no differences in average post-milking standing time following each milking (Table 3.4). However, MP cows spent the greatest amount of time standing following the second milking (Table 3.4).
There was no sorting for or against long particles (100.2 %), medium particles (100.6 %), or fine particles (98.7 %). There was a treatment effect for sorting of short particles (<8.0, >1.18 mm), indicating that cows fed 1x/d sorted against short particles (98.9 %; SE = 0.3; P = 0.03), and cows fed 2x/d tended to sort against short particles (99.2 %; SE = 0.3; P = 0.059). Cows fed 3x/d showed no significant sorting of this fraction (P = 0.2).

3.4 Discussion

Many dairy producers have opted to deliver feed only 1x/d as a means to minimize labour costs. Previous research in this area has been focused on the impact of feed delivery frequency on milk yield and composition, and feed intake. A review of 35 studies, conducted over 30 years ago, reported that increased frequency of feed delivery could lead to increased milk yield, milk fat yield, milk fat %, and milk protein yield, but apparently had little or no effect upon milk protein % (Gibson, 1984). More recent research shows no effect of feed delivery frequency on milk fat and protein % or milk yield (Dhiman et al., 2002; Mantysaari et al., 2006). Phillips and Rind (2001) observed an increase in milk protein % for cows delivered feed more frequently, and attributed this result to a concentration effect due to decreased milk yield for cows fed more frequently. Shabi et al. (1999) observed a similar increase in milk protein % for cows fed 4x/d, but attributed this to an improvement in postruminal digestion of CP and NSC for cows fed more frequently. While there was a numerical increase in milk protein % with greater frequency of feed delivery in the present study, it was not possible to quantify this effect, as there was a period × treatment interaction for this variable. This interaction was driven by an increase in milk protein % over the course of the study. There was no effect of treatment on milk yield, but there was an effect of period on milk yield (P = 0.05); thus, milk protein percentage increased later in lactation as milk yield decreased. In the present study, frequency of feed delivery had no effect
on ECM yields, which is consistent with previous research (Dhiman et al., 2002; Mantysaari et al., 2006).

Since feed delivery is a significant stimulus to initiate feeding activity in dairy cows (DeVries and von Keyserlingk, 2005), and feeding is the second greatest priority for dairy cows next to lying (Munksgaard et al., 2005), there is interest to understand the effect feed delivery frequency has on the behavioural patterns of lactating dairy cows. In the present study, cows fed 3x/d consumed more DM than those fed 1x/d and 2x/d, which is consistent with research reported by Shabi et al. (1999). However, other research has shown no effect of feed delivery frequency on DMI (Robinson and Sniffen, 1985; DeVries et al., 2005), and some research has found that cows fed more frequently had a lower DMI than those fed 1x/d (Nocek and Braund, 1985; Phillips and Rind, 2001; Mantysaari et al., 2006). These varying results may be attributed to differences in experimental design (i.e. sample size, pen level measurements of DMI, housing system, cow genetics, stage of lactation, parity, length of observation period). However, the greater DMI achieved by cows delivered feed 3x/d had no effect on milk yield, composition, or milk production efficiency. It is known that the relationship of milk energy output to increments of feed intake is curvilinear, and this has been attributed to greater partitioning of nutrients towards body tissue synthesis as feed intake increases (Kirkland and Gordon, 2001); therefore, in the present study, the nutrients consumed by cows achieving greater DMI when delivered feed 3x/d may have been metabolized for body stores as opposed to being directed towards milk synthesis.

Over the course of the study period, MP cows had greater DMI and milk yield than PP cows, which is consistent with previous reports (Beauchemin et al., 2002; Azizi et al., 2009; DeVries et al., 2011). Dry matter intake and milk production were proportionally lower for PP
cows than for MP cows, therefore, it is not surprising that the efficiency of milk production did not differ by parity.

The present study found no effect of feed delivery frequency on daily feeding time, which is in agreement with previous work reporting no difference in daily feeding time for group-housed cattle fed 1x/d and 4x/d (Phillips and Rind, 2001). However, these results contradict those of DeVries et al. (2005) and Shabi et al. (1999) who found that increased feed delivery frequency is associated with increased time spent feeding. The average rate of feed intake in the present study (0.12 ± 0.02 kg DM/min; mean ± SD) was greater than that reported by Beauchemin et al. (2002; 0.09 ± 0.007 kg DM/min; mean ± SD). However, Beauchemin et al. (2002) also reported that rate of feed intake did not vary by parity, whereas the present study observed that MP cows consume feed more quickly than PP cows. These results may be explained by differences in experimental design. In the study by Beauchemin et al. (2002) cows were housed in individual tie-stalls, where there was no competition between cows. In the present study, while cows were assigned to individual feed bins, there was still potential for aggressive interactions to occur and for cows to be displaced from their feeder by neighbouring cows. When cows are fed in groups they tend to consume more feed, at a faster rate, than when fed separately (Albright, 1993); thus, the social environment may have had a greater impact on rate of feed intake in the present study, specifically for PP cows which are often of lower standing in the dominance hierarchy.

While there was no effect of treatment on meal patterns, the average values for meal frequency (9.4 ± 1.5 meals; mean ± SD), duration (25.5 ± 5.1 min) and size (2.9 ± 0.5 kg), are in agreement with other reports of meal patterns (Beauchemin et al., 2002; DeVries et al., 2003b; Azizi et al., 2009). The results of the present study indicate that parity had an effect on the size of
the first meal consumed after each milking, but no effect on the length of this meal. Multiparous cows consumed 2.0 kg more DM than PP cows following the first milking. These results may be attributed to differences in rumen capacity between MP and PP cows. More specifically, MP cows fed 1x/d consumed the greatest DM while PP cows fed 1x/d consumed the least DM following the first milking. Evidently, the delivery of fresh feed at 1400 h had the greatest effect on MP cows fed 1x/d as it had been 24 h since their last delivery of fresh feed, and these cows may have had the greatest rumen capacity at this time. It was also observed that MP cows consumed more feed following the second milking, but there was no difference in meal size following the third milking, suggesting that feeding motivation may have decreased throughout the day, potentially due to rumen fill.

The distribution of feeding activity throughout the day was not dramatically impacted by the frequency of feed delivery in the present study. This contradicts previous research reporting that frequent feed delivery results in cows consuming feed more evenly after each feed delivery (Mantysaari et al., 2006), and having a more even distribution of feeding time over the course of the day (DeVries et al., 2005). However, some researchers have concluded that the disturbance caused by frequent feed delivery can have detrimental effects on cow productivity such as reduced milk yield (Phillips and Rind, 2001) and decreased lying times (Mantysaari et al., 2006). Similar results may not have been observed in the present study because the most frequent feed delivery occurred 3x/d compared to 4x/d (DeVries et al., 2005) and 5x/d (Mantysaari et al., 2006).

Also, feed delivery occurred in conjunction with milking times in the present study, whereas in the report by Mantysaari et al. (2006), feed delivery occurred independently of milking times. It is known that the delivery of fresh feed and the return from milking are a
significant stimulus to initiate feeding activity (DeVries et al., 2003a). In the present study, there were only 3 management events occurring per day known to stimulate feeding, whereas in the study by Mantysaari et al. (2006), when cows were delivered feed more frequently, there were 7 events occurring per day known to stimulate feeding. While it is often suggested that it is beneficial for cows to consume smaller, more frequent meals throughout the day, as this is associated with reduced diurnal fluctuations in ruminal pH (Shabi et al., 1999), some research has actually observed that cows that consume the greatest number of meals throughout the day have the lowest daily DMI (Beauchemin et al., 2002). These varying results emphasize that there are multiple factors influencing a cow’s feeding behaviour patterns. While Beauchemin et al. (2002) observed that DMI was lower for cows that consume a greater number of meals throughout the day; efficiency of milk production may be a better measure for comparison, however production variables were not reported in that study.

Since feed delivery occurred while the cows were isolated in the holding area for milking, the visual and auditory cues associated with feed delivery were also likely reduced. Therefore, it is possible that feed delivery occurring while cows are within the vicinity of the feed bunk may be a greater stimulus to initiate feeding activity and may have resulted in a greater response to treatments, but further research is required to confirm this.

Similarly, when one animal eats, another may be stimulated to do likewise, whether it is hungry or not, which is known as social facilitation (Curtis and Houpt, 1983). Since the behavioural response to treatment was less than what was expected, the effect of social facilitation may have had a greater effect on feeding behaviour patterns than did the treatments themselves (i.e. stimulating cows fed 1x/d and 2x/d when they did not receive new feed). These are obviously limitations of the facility design and experimental setup. However, conducting this
experiment in identical, isolated facilities would introduce greater sources of error (i.e. confounding location with treatment). The benefit of the present design was that cows on all treatments were subject to identical environmental conditions; thus there is confidence that any differences observed overcame any social facilitation effects, and can be directly attributed to the treatments imposed.

Despite some comparable results with previous research, the free stall environment and management strategies employed in commercial production systems introduce greater constraints on the amount of time cows allocate to critical behavioural activities (feeding, lying, ruminating), due to the time spent away from the home pen for milking. For this reason, the results of previous studies may not be comparable to the current results, and applicable to all production scenarios. Most notably, all of the research previously conducted in this topic area has examined cows milked 2x/d. It was hypothesized that greater frequency of feed delivery and the higher plane of nutrition required to meet the increased production demands of 3x/d milking schedules may alter the amount of time available for critical behavioural activities, and specifically the amount of time devoted to lying. However, in the present study, feed delivery frequency had no impact on lying behaviour for cows milked 3x/d, which is not surprising as it has previously be demonstrated that lying has a higher priority over feeding (Metz, 1985; Munksgaard et al., 2005). The average lying bouts (9.76 ± 2.64; mean ± SD) and lying time (570 ±121 min/d) were less than previously reported (Munksgaard et al., 2005; DeVries and von Keyserlingk, 2005). The average post-milking standing time (98 ± 49 min) was not affected by treatment, but was highly variable and significantly greater than results previously reported (35 min: Tyler et al., 1997; 55 min: DeVries and von Keyserlingk, 2005; 62 min: DeVries et al, 2005; 33 min: Fregonesi et al., 2007). For this reason, we caution the interpretation of the results for lying behaviour and post
milking standing time. This study was conducted during summer months, therefore it is possible that environmental temperature may have attributed to the variation observed for these results.

Lying has a greater priority over feeding and cows are able to partially compensate for time restrictions by increasing the rate of feed intake, thus feed intake is reduced to a lesser degree than eating time under time budget constraints (Munksgaard et al., 2005). Therefore, we would caution the interpretation of feeding behaviour results for cows managed under varying milking frequencies as the elasticity of behavioural priorities may differ under such time constraints. It is possible that the lack of response observed between treatments in the current study may be attributed to a more inelastic response of feeding behaviour variables when cows are under the time constraints and production demands of 3x/d milking schedules.

3.5 Conclusions

Cows delivered feed 3x/d consumed more DM than those fed 1x/d and 2x/d. Feed delivery frequency had no effect on milk yield, composition, or milk production efficiency. Therefore, the nutrients consumed by cows achieving greater DMI when delivered feed 3x/d may have been metabolized for body stores as opposed to being directed towards milk synthesis. Feed delivery frequency had little effect on feeding behaviour, but altered the magnitude of DMI following feed delivery. The distribution of feeding activity was also not dramatically impacted by feed delivery frequency, but did vary by parity. These results indicate that under 3x/d milking schedules, greater feed delivery frequency resulted in greater DMI as a function of increased DMI following the return from milking and the delivery of fresh feed.
### 3.6 Acknowledgements

We thank the staff and students at the University of Guelph, Kemptville Campus Dairy Education and Innovation Centre (Kemptville, Ontario, Canada). In particular we thank Albert Koekkoek, Amy Sova, Megan Bruce, Morgan Overvest, and John Wynands of the University of Guelph, Kemptville Campus for their technical help. This project was financially supported by a Dairy Farmers of Ontario (Mississauga, Ontario, Canada) research grant and an Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA; Guelph, Ontario, Canada)/University of Guelph Production Systems research grant. Additional project support was received from the Canadian Foundation for Innovation (CFI; Ottawa, Ontario, Canada) and the Ontario Research Fund (Toronto, Ontario, Canada).
Table 3.1. Chemical composition and particle size distribution of the experimental ration and components (mean ± SD).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Corn silage</th>
<th>Grass/legume silage</th>
<th>High moisture corn</th>
<th>Protein concentrate</th>
<th>Robotic supplement</th>
<th>TMR³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical Composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>45.6 ± 11.2</td>
<td>35.5 ± 2.7</td>
<td>81.0 ± 1.4</td>
<td>94.0 ± 0.8</td>
<td>91.9 ± 0.9</td>
<td>50.4 ± 2.1</td>
</tr>
<tr>
<td>OM, % of DM</td>
<td>97.1 ± 0.1</td>
<td>89.6 ± 0.6</td>
<td>98.5 ± 0.1</td>
<td>78.0 ± 3.9</td>
<td>93.0 ± 0.9</td>
<td>92.4 ± 0.8</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>8.1 ± 0.3</td>
<td>19.2 ± 1.0</td>
<td>9.3 ± 0.3</td>
<td>40.7 ± 1.1</td>
<td>20.1 ± 0.6</td>
<td>17.1 ± 0.7</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>20.1 ± 1.3</td>
<td>37.3 ± 2.4</td>
<td>3.9 ± 0.6</td>
<td>10.8 ± 4.1</td>
<td>8.9 ± 0.7</td>
<td>20.4 ± 1.5</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>33.2 ± 1.3</td>
<td>52.2 ± 4.2</td>
<td>10.8 ± 0.7</td>
<td>17.1 ± 4.0</td>
<td>23.9 ± 5.0</td>
<td>31.7 ± 1.7</td>
</tr>
<tr>
<td>NFC, % of DM</td>
<td>52.7 ± 1.3</td>
<td>16.8 ± 3.7</td>
<td>74.2 ± 0.9</td>
<td>-</td>
<td>-</td>
<td>41.1 ± 2.6</td>
</tr>
<tr>
<td>Calcium, % of DM</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>4.4 ± 0.7</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Phosphorus, % of DM</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Magnesium, % of DM</td>
<td>0.2 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>Potassium, % of DM</td>
<td>0.6 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>0.4 ± 0.0</td>
<td>1.6 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Sodium, % of DM</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.7 ± 1.5</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Iron, PPM</td>
<td>48.2 ± 5.6</td>
<td>899.5±325.1</td>
<td>41.2 ± 6.8</td>
<td>462.0 ± 203.6</td>
<td>141.3±15.1</td>
<td>433.7 ± 59.9</td>
</tr>
<tr>
<td>Manganese, PPM</td>
<td>13.2 ± 1.2</td>
<td>48.8 ± 5.7</td>
<td>5.0 ± 0.0</td>
<td>166.0 ± 114.0</td>
<td>93 ± 24.1</td>
<td>55.0 ± 28.1</td>
</tr>
<tr>
<td>Zinc, PPM</td>
<td>25.3 ± 3.6</td>
<td>38 ± 9.9</td>
<td>25.8 ± 3.6</td>
<td>248.5 ± 165.2</td>
<td>134.0±11.1</td>
<td>67.3 ± 29.2</td>
</tr>
<tr>
<td>Copper, PPM</td>
<td>6.0 ± 0.0</td>
<td>12.2 ± 0.8</td>
<td>2.0 ± 0.0</td>
<td>74.7 ± 57.2</td>
<td>29.0±3.0</td>
<td>18.9 ± 9.6</td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>77.0 ± 0.4</td>
<td>58.4 ± 0.9</td>
<td>87.6 ± 0.4</td>
<td>-</td>
<td>-</td>
<td>71.3 ± 1.7</td>
</tr>
<tr>
<td>NeL, mcal/kg</td>
<td>1.8 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.5 ± 0.0</td>
</tr>
<tr>
<td>NeM, mcal/kg</td>
<td>1.8 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>2.2 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.8 ± 0.0</td>
</tr>
<tr>
<td>NeG, mcal/kg</td>
<td>1.3 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>1.5 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td><strong>Particle Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>4.0 ± 2.4</td>
<td>37.2 ± 7.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.6 ± 7.0</td>
</tr>
<tr>
<td>Medium</td>
<td>59.4 ± 7.2</td>
<td>44.1 ± 6.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>46.5 ± 2.2</td>
</tr>
<tr>
<td>Short</td>
<td>34.1 ± 7.1</td>
<td>15.7 ± 2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31.9 ± 4.7</td>
</tr>
<tr>
<td>Fine</td>
<td>2.5 ± 1.1</td>
<td>3.0 ± 1.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.0 ± 2.4</td>
</tr>
</tbody>
</table>

¹Supplied by Ritchie Feed & Seed Ltd (Winchester, Ontario, Canada) contains 38.0% canola, 30.0% cotton seed meal, 9.0% soy meal, 6.6% gluten feed, 3.0% 60% gluten meal, 1.8% Optigen, 6.8% limestone, 3.0% salt, 0.9% magnesium oxide, 0.7% dicalcium phosphate, and 0.2% power punch flavour.
2 Supplied by Ritchie Feed & Seed Ltd (Winchester, Ontario, Canada) contains 27.5% wheat, 26.0% gluten feed, 22.0% barley, 9.5% citrus pulp, 8.3% cotton seed meal, 3.5% limestone, 1.2% salt, 0.6% vitamin ADE, 0.1% Ritchie dairy Pac, 0.2% power punch flavour, 1.2% Ameri-Bond 2x.

3 NDF composition (DM basis) of the long, medium, short and fine particle fractions was 41.6 ± 3.0%, 32.0 ± 1.9%, 21.7 ± 1.7, and 19.7% ± 1.4% respectively.

4 Values were obtained from chemical analysis of TMR and components. OM = 100 - % ash.

5 Particle size determined by Penn State Particle Separator, which has a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine).
Table 3.2. Effect of frequency of feed delivery (F) and parity (P) on milk yield, milk composition, milk component yield, and efficiency of production.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1x&lt;sup&gt;1&lt;/sup&gt;</th>
<th>2x&lt;sup&gt;1&lt;/sup&gt;</th>
<th>3x&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SE</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, kg/d</td>
<td>PP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>MP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>PP</td>
<td>MP</td>
<td></td>
</tr>
<tr>
<td>Milk&lt;sup&gt;3&lt;/sup&gt;</td>
<td>38.4</td>
<td>45.4</td>
<td>38.8</td>
<td>45.2</td>
<td>1.6</td>
</tr>
<tr>
<td>3.5% FCM&lt;sup&gt;4,5&lt;/sup&gt;</td>
<td>37.7</td>
<td>46.8</td>
<td>37.0</td>
<td>44.1</td>
<td>2.8</td>
</tr>
<tr>
<td>ECM&lt;sup&gt;6&lt;/sup&gt;</td>
<td>38.0</td>
<td>47.0</td>
<td>37.6</td>
<td>44.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Milk composition&lt;sup&gt;4&lt;/sup&gt;, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.37</td>
<td>3.71</td>
<td>3.19</td>
<td>3.49</td>
<td>0.31</td>
</tr>
<tr>
<td>Protein</td>
<td>3.09</td>
<td>3.21</td>
<td>3.16</td>
<td>3.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Milk component yield&lt;sup&gt;4&lt;/sup&gt;, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>1.29</td>
<td>1.67</td>
<td>1.24</td>
<td>1.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Protein</td>
<td>1.19</td>
<td>1.45</td>
<td>1.23</td>
<td>1.40</td>
<td>0.05</td>
</tr>
<tr>
<td>Efficiency of milk production, kg/kg&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.59</td>
<td>1.50</td>
<td>1.60</td>
<td>1.54</td>
<td>0.08</td>
</tr>
<tr>
<td>3.5% FCM/DMI</td>
<td>1.55</td>
<td>1.53</td>
<td>1.52</td>
<td>1.49</td>
<td>0.09</td>
</tr>
<tr>
<td>ECM/DMI</td>
<td>1.57</td>
<td>1.54</td>
<td>1.55</td>
<td>1.50</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>1</sup>1x = 1x/d feed delivery frequency, 2x = 2x/d feed delivery frequency, 3x = 3x/d feed delivery frequency.
<sup>2</sup>PP = primiparous; MP = multiparous.
<sup>3</sup>Data averaged over 7 d for 12 cows on each treatment.
<sup>4</sup>Data averaged over 3 d for 12 cows on each treatment.
<sup>5</sup>3.5% FCM = 3.5% fat corrected milk.
<sup>6</sup>ECM = energy corrected milk.
Table 3.3. Effect of feed delivery frequency on DMI, feeding behaviour, and rumination time³.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1x¹</th>
<th>2x¹</th>
<th>3x¹</th>
<th>SE</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP²</td>
<td>MP²</td>
<td>PP</td>
<td>MP</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>24.3</td>
<td>30.6</td>
<td>24.3</td>
<td>29.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feeding time, min/d</td>
<td>238.6</td>
<td>219.9</td>
<td>247.0</td>
<td>220.6</td>
<td>16.0</td>
</tr>
<tr>
<td>Feeding rate, kg/min</td>
<td>0.11</td>
<td>0.15</td>
<td>0.10</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Meal frequency, meals/d</td>
<td>9.3</td>
<td>9.5</td>
<td>9.3</td>
<td>9.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Meal size, kg/meal</td>
<td>2.7</td>
<td>3.3</td>
<td>2.7</td>
<td>3.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Meal duration, min/meal</td>
<td>26.7</td>
<td>23.6</td>
<td>27.9</td>
<td>23.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Post milking meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length at 1400 h, min⁴</td>
<td>44.0</td>
<td>48.5</td>
<td>57.0</td>
<td>45.9</td>
<td>6.0</td>
</tr>
<tr>
<td>Size at 1400 h, kg⁴</td>
<td>3.6</td>
<td>6.3</td>
<td>4.4</td>
<td>5.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Length at 2200 h, min⁴</td>
<td>35.4</td>
<td>31.1</td>
<td>38.0</td>
<td>34.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Size at 2200 h, kg⁴</td>
<td>3.0</td>
<td>3.8</td>
<td>3.3</td>
<td>3.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Length at 0600 h, min⁴</td>
<td>36.2</td>
<td>27.4</td>
<td>44.7</td>
<td>33.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Size at 0600 h, kg⁴</td>
<td>2.8</td>
<td>2.9</td>
<td>2.9</td>
<td>3.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Rumination time, min/d</td>
<td>431.6</td>
<td>522.6</td>
<td>451.8</td>
<td>533.0</td>
<td>52.7</td>
</tr>
</tbody>
</table>

¹1x = 1x/d feed delivery frequency, 2x = 2x/d feed delivery frequency; 3x = 3x/d feed delivery frequency.
²PP = primiparous; MP = multiparous.
³Data averaged over 7 d for 12 cows on each treatment.
⁴Length (min) and size (kg) of the first meal consumed following the return from milking based on individual cow meal criteria.
Table 3.4. Effect of feed delivery frequency on lying behaviour and post-milking standing time.  

<table>
<thead>
<tr>
<th>Variable</th>
<th>1x(^1)</th>
<th>2x(^1)</th>
<th>3x(^1)</th>
<th>SE</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP(^2)</td>
<td>MP(^2)</td>
<td>PP</td>
<td>MP</td>
<td>PP</td>
</tr>
<tr>
<td>Lying bouts, #/d</td>
<td>9.7</td>
<td>9.2</td>
<td>9.8</td>
<td>9.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Lying time, min/d</td>
<td>633.1</td>
<td>507.2</td>
<td>587.4</td>
<td>499.0</td>
<td>635.0</td>
</tr>
<tr>
<td>Post-milking standing, min/d</td>
<td>104.2</td>
<td>91.2</td>
<td>93.6</td>
<td>115.0</td>
<td>75.6</td>
</tr>
<tr>
<td>PMST at 1400 h, min(^4)</td>
<td>116.6</td>
<td>121.0</td>
<td>151.6</td>
<td>137.9</td>
<td>118.3</td>
</tr>
<tr>
<td>PMST at 2200 h, min(^4)</td>
<td>58.0</td>
<td>73.3</td>
<td>53.8</td>
<td>96.6</td>
<td>52.4</td>
</tr>
<tr>
<td>PMST at 0600 h, min(^4)</td>
<td>68.2</td>
<td>87.5</td>
<td>70.1</td>
<td>129.3</td>
<td>52.7</td>
</tr>
</tbody>
</table>

\(^1\)1x = 1x/d feed delivery frequency, 2x = 2x/d feed delivery frequency; 3x = 3x/d feed delivery frequency.  
\(^2\)PP = primiparous; MP = multiparous.  
\(^3\)Data averaged over 7 d for 12 cows on each treatment.  
\(^4\)PMST = post-milking standing time (min) calculated from the time each cow finished milking until the first instance of lying at each milking time throughout the day (1400, 2200, and 0600 h).
Figure 3.1. Hourly average DMI (kg) of lactating dairy cows having received feed delivery 1) 1x/d (at 1400 h), 2) 2x/d (at 1400 and 2200 h), or 3) 3x/d (at 1400, 2200, and 0600 h). Data are averaged over 7 days for 12 cows on each treatment.
Figure 3.2. Hourly average feeding time (min) of lactating dairy cows having received feed delivery 1) 1x/d (at 1400 h), 2) 2x/d (at 1400 and 2200 h), or 3) 3x/d (at 1400, 2200, and 0600 h). Data are averaged over 7 days for 12 cows on each treatment.
Figure 3.3. Hourly average feeding rate (kg/min) of lactating dairy cows having received feed delivery 1) 1x/d (at 1400 h), 2) 2x/d (at 1400 and 2200 h), or 3) 3x/d (at 1400, 2200, and 0600 h). Data are averaged over 7 days for 12 cows on each treatment.
Figure 3.4. Bihourly average rumination time (min) of lactating dairy cows having received feed delivery 1) 1x/d (at 1400 h), 2) 2x/d (at 1400 and 2200 h), or 3) 3x/d (at 1400, 2200, and 0600 h). Data are averaged over 7 days for 12 cows on each treatment.
CHAPTER 4: GENERAL DISCUSSION

4.1 Important Findings

Much previous research has examined the effect of various management strategies on dairy cow productivity, but less research has been focused on evaluating the effect of management practices on cow behaviour. The research presented in this thesis is some of the first to report the effect of management practices on dairy cows using a methodology conducive to evaluating individual feed intake and meal patterns throughout the course of the day.

The results of the first study (Chapter 2) indicate that cows milked 3x/d achieved greater milk yield, FCM yield and ECM yield, but tended to have a lower milk fat percentage than cows milked 2x/d. Primiparous cows produced less milk and had lower milk fat percentage, thus resulting in PP cows having a lower milk fat yield than MP cows. Cows milked 3x/d had the greatest milk production efficiency, and there was a tendency for MP cows milked 3x/d to have the greatest ECM efficiency. Primiparous cows consumed less DM than MP cows; however, PP increased DMI when milked 3x/d, while milking frequency had no impact on the DMI of MP cows. Daily lying time, post-milking standing time, and rumination time were not affected by milking frequency, but there was a tendency for cows milked 2x/d to spend less time feeding and, thus, consume their feed at a faster rate than cows milked 3x/d. It is evident that the extra time required for milking 3x/d altered the distribution of feeding behaviour and meal patterns throughout the day. It was concluded that PP cows were most affected by increased milking frequency, resulting in them consuming more frequent, shorter, and smaller meals throughout the day when milked 3x/d. Alternatively, MP cows increased feeding activity through consuming longer, and slightly larger meals when milked 3x/d. These results indicate that PP cows, that
inherently have lower production efficiency than MP cows, will positively adjust their feeding behaviour when milked 3x/d to achieve similar production increases as MP cows, thus, exhibiting desirable feeding patterns conducive to stable and efficient rumen fermentation.

The results of the second study (Chapter 3) indicate that DMI varied with feed delivery frequency, with greatest DMI observed for cows delivered feed 3x/d. There was no effect of treatment on milk yield or efficiency of production as the nutrients consumed by cows achieving greater DMI when delivered feed 3x/d may have been metabolized for body stores as opposed to being directed towards milk synthesis. Total feeding time, meal frequency, size, and duration were not affected by frequency of feed delivery, but PP cows consumed smaller meals at a slower rate, thus resulting in lower DMI, compared to MP cows. There was no effect of feed delivery frequency on the distribution of feeding activity throughout the day, but the magnitude of feeding activity at peak feeding times (return from milking and delivery of feed) did vary. Cows that did not receive a delivery of feed at a given milking consumed less DM during these peak feeding times. Similarly, PP cows consumed less DM than MP cows following the first and second milkings, likely due to difference in rumen fill. Rumination time, post-milking standing time, and lying time did not vary by treatment, but PP cows spent more time lying than MP cows. It was concluded that under 3x/d milking schedules, greater feed delivery frequency resulted in greater DMI as a function of increased DMI following the return from milking and the delivery of feed.

4.2 Future Research

The first study (Chapter 2) indicated that a management practice, such as greater milking frequency, which may result in cows spending more time away from their home pen for milking,
does not always have a negative impact on lying patterns and daily time budgets. However, given the methodology used in Chapter 2, for cows milked 3x/d, the time spent milking may not have been representative of commercial situations. Matzke (2003) observed that keeping cows away from the pen for extended periods of time around milking (6 vs 3 h) resulted in less lying time and lower production. Therefore, further research is required to determine the milking time threshold at which point the time budget of the cow is restricted to the extent of reducing cow productivity or welfare. Such information would be critical for dairy producers to determine the optimum group size, proportional to parlour size, to maximize cow productivity as well as labour efficiency.

In the research described in this thesis, much focus was placed on the impact of milking and feed delivery frequency on the meal patterns of dairy cows. Previous research has established relationships between meal patterns and rumen conditions (Allen, 1997). It was not possible to measure rumen pH as part of these projects, and therefore the effect of the treatments on rumen function was speculated based on the intake patterns of feed nutrients. Therefore, further research comparing meal patterns and fluctuations in rumen pH may provide a more accurate assessment of the effects of management strategies on rumen health and fermentation efficiency.

Previous research has indicated that both the delivery of feed and the return from milking are significant stimuli to initiate feeding activity in lactating dairy cows (DeVries et al., 2003a). In Chapter 3, feed delivery coincided with the return from milking, however, previous research has indicated that the distribution of feed intake can be significantly altered when feed delivery occurs independently of milking time (DeVries and von Keyserlingk, 2005). For these reasons, further research is required to determine the effect of timing of feed delivery, in relation to
milking time, on the behavioural patterns of dairy cows. Determining the optimal interval between feed delivery and milking that elicits an increased feeding activity response, may promote more desirable feeding patterns, and may also promote cows to remain standing after milking for a sufficient period of time to reduce the risk of mastitis (Peeler et al., 2000; DeVries et al., 2010). However, care must be taken to ensure that the timing of feed delivery does not negatively affect the time budget of the cow.

Finally, it is evident that the behavioural responses observed in the research described in this thesis may have been limited by social facilitation. Furthermore, in this research, treatments were applied at the cow level, and cows were assigned to individual feed bins, therefore altering the social environment and limiting interactions at the feed bunk. Further research examining the effect of feed delivery frequency on larger groups of conventionally housed cows milked 2x/d and 3x/d could provide information that could be used at the farm level to determine the optimum milking and feed delivery frequency to maximize production efficiency as well as labour efficiency, based on the characteristics of an individual farm (i.e. herd size, parlour size, labour, and producer goals).

4.3 Implications

Based on these results, it is recommended that dairy producers milk cows 3x/d to achieve greater milk yield and production efficiency. If herd size and facility design permit, it is recommended that producers employ a management and grouping strategy based on parity. Grouping PP cows independently of MP cows may provide opportunities for improved management efficiency. Milking the PP group 3x/d, will result in greater milk yield and enable these animals to distribute their feeding activity more evenly throughout the day, thus promoting
efficient rumen fermentation. Selecting only the PP group to be milked 3x/d may help to optimize labour efficiency as it does not require the entire herd to be milked 3x/d, but provides the benefit of increased milk production and rumen fermentation efficiency. While milking the MP group 3x/d will also improve milk production, the meal patterns exhibited by this group may lead to compromised rumen fermentation efficiency; therefore, it is also recommended that dairy producers increase the frequency of feed delivery to cows milked 3x/d, particularly for MP cows. This management strategy results in greater DMI, specifically following the return from milking and the delivery of fresh feed, thus improving the supply of nutrients for increased milk production for cows milked 3x/d.

The most important finding of the first study (Chapter 2) indicates that the patterns of feed intake are significantly different between PP and MP cows, and these feeding patterns are significantly altered by milking frequency; this is largely attributed to differences in rumen capacity and nutrient requirements between mature cows and PP cows that are still growing. Furthermore, the social environment of a free-stall housing system may exacerbate these differences, as PP cows are often socially subordinate. The most important finding of the second study (Chapter 3) indicates that the frequency of feed delivery has minimal impact on the behavioural patterns and biological productivity of high yielding lactating dairy cows milked 3x/d.
CHAPTER 5: REFERENCES


CCAC. 2009. Guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON, Canada.


