EFFECT OF THE FORAGE-TO-CONCENTRATE RATIO OF THE PARTIAL MIXED RATION AND THE QUANTITY OF CONCENTRATE ALLOCATED IN AN AUTOMATIC MILKING SYSTEM ON DRY MATTER INTAKE, MILK YIELD AND COMPOSITION, RUMINAL FERMENTATION, TOTAL TRACT DIGESTIBILITY, AND ACTIVITY OF MULTIPAROUS HOLSTEIN COWS

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ABSTRACT

This study was conducted to evaluate the effects of the forage-to-concentrate ratio of the partial mixed ration (PMR) and the quantity of concentrate offered in an automated milking system (AMS), in a feed-first guided-flow barn, on the behavior and performance of dairy cows. Eight ruminally-cannulated multiparous Holstein cows were used in a replicated 4×4 Latin square balanced for carry-over effects. Treatments were arranged in a 2×2 consisting of a PMR that contained (DM basis) either a low (54:46; L-FOR) or a high (64:36; H-FOR) forage-to-concentrate ratio and AMS concentrate provision to achieve low (2 kg/d; L-AMS) or high (6 kg/d; H-AMS) intake. Each period consisted of 28 d with 6 d for dietary transition, 13 d for adaptation, and 9 d of collection. The first 4 d of data and sample collection were used to evaluate behavioral data (milking frequency, feeding behavior, and standing and lying behavior) and ruminal pH. Subsequently, a sampling device removal day was provided, and the last 4 d were used to evaluate ruminal fermentation and apparent total tract digestibility. All 9-d were used for milk yield measurement, while the 8-d were used for DMI measurement. Cows fed the H-AMS consumed 3.5 kg/d less PMR while consuming 4.2 kg/d more AMS concentrate, but total DMI (PMR+AMS) was not affected by treatments averaging 27.3 kg/d. Although cows fed H-AMS had greater concentrate intake, they also had greater variability for AMS concentrate intake among days (0.85 vs. 0.25 kg/d, respectively). The number of PMR meals and PMR eating behavior were not affected by the PMR or AMS treatments. Feeding H-AMS did not affect milking frequency averaging 3.63 milkings/d, but tended to increase milk yield by 1.25 kg/d relative to L-AMS. Likewise, cows fed the L-FOR tended to have greater milk yield relative to H-FOR (39.3 vs 37.9 kg/d, respectively), but had greater holding area time. Minimum ruminal pH tended to be lower for cows fed L-FOR compared to cows fed H-FOR but was not affected by the AMS concentrate treatment. When fed the L-FOR, feeding the H-AMS increased total short-chain fatty acid concentration in the rumen relative to cows fed L-AMS, while the response for H-FOR was not affected by the AMS concentrate. These data suggest that feeding H-AMS may improve milk yield, but also increases the day-to-day variability in AMS concentrate consumption. Feeding a L-FOR PMR may increase milk yield without affecting variability in AMS concentrate consumption; however, it may reduce ruminal pH and increase the time spent in the holding area compared to cows fed a H-FOR PMR.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
AMS	Automatic milking system
aNDFom	Ash-free neutral detergent fiber
BCS	Body condition score
BTSCC	Bulk tank somatic cell count
BW	Body weight
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
F:C	Forage-to-concentrate ratio
FAS	Flavor-appetizing substances
FFA	Free fatty acids
H-AMS	High concentrate provision in the automatic milking system
H-FOR	High forage in the partial mixed ration
L-AMS	Low concentrate provision in the automatic milking system
L-FOR	Low forage in the partial mixed ration
LRCpH	Lethbridge Research Centre Ruminal pH Measurement System
MUN	Milk urea nitrogen
NDF	Neutral detergent fiber
NE_L	Net energy of lactation
NFC	Non-fiber carbohydrates
NH_3-N	Ammonia nitrogen
NRC	National Research Council
OM	Organic matter
peNDF	Physically effective fiber
PMR	Partial mixed ration

PSPS	Penn-State Particle Size Separator
SARA	Subacute ruminal acidosis
SCC	Somatic cell count
SCFA	Short chain fatty acid
SD	Standard deviation
SEM	Standard error of mean
TBC	Total bacterial count
TMR	Total mixed ration
uNDF	Undegradable neutral detergent fiber
VMS	Voluntary milking system

1.0 GENERAL INTRODUCTION

Since the development of the first automatic milking system (AMS) in 1992, the adoption of this technology continues globally. Difficulty finding qualified labor (Rotz et al., 2003), the rise in labour cost, and particularly the potential for increased milk production (de Koning et al., 2002) are some of the reasons that dairy producers may adopt AMS. Apart from potentially improving the lifestyle of dairy producers by reducing the amount of intense working hours with milking related activities (Reinemann and Smith, 2001) and allowing for a more flexible working schedule (Reinemann, 2008), AMS may positively impact the health and welfare of the cows. Moreover, when compared to conventional twice-daily milking, AMS may increase milk production due to the expected increase in the frequency of daily milking events. Research has suggested that milk yield may increase by up to 12% (Holloway et al., 2014; Jacobs and Seigford, 2012). Past studies support the improvement in milk production not only in primiparous but also in multiparous cows regardless of the initial yield being produced (Erdman and Varner, 1995; Svennersten-Sjaunja and Pettersson, 2008).

Automatic milking systems require a completely different management system compared to conventional milking systems (tie-stall or parlors), integrating technology and relying on strategies to motivate cows to enter the AMS (Svennersten-Sjaunja and Pettersson, 2008). Since cows are not kept in a structural and uniform milking and feeding routine (Bach and Cabrera, 2017), cow traffic systems and factors enticing cows to enter the AMS are two relevant aspects producers consider to increase the amount of milking events per day or milking frequency. Therefore, a well-functioning of AMS presumes a maximum number of voluntary milking events per day as it is considered a major factor affecting the capacity of the AMS. Maximizing the capacity of the AMS, referred to as the amount of milkings or yield of milk the equipment is capable to handle within the available time period (Rotz, 2003), is an important aspect to consider in AMS management.

Due to the negative effects associated with forced cow traffic in terms of lying behavior and feeding, free cow traffic and guided cow traffic with its possible alternative of milk-first or feed-first, are being the most recommended cow traffic designs (Bach et al., 2009). Both alternatives cow traffic systems rely primarily on voluntary visits to the AMS and although milking frequency is lower than forced cow traffic (Bach et al., 2009; Munksgaard et al., 2011), milk production per cow and per robot per day is greater in free cow traffic compared with forced cow traffic or guided traffic systems (Tremblay et al., 2016). Rodenburg (2017) indicates that the only clear benefit of a forced cow traffic design is the less labor required to fetch cows to the AMS; free cow traffic accounts not only for greater milk production and feed intake but also for longer resting times suggesting that this type of cow traffic should be preferred over the forced cow traffic.

Regardless of the cow traffic within the barn, the primary goals of the feeding program in AMS are to meet the nutrient requirement while encouraging cows to voluntary enter the AMS, promoting more frequent milking events, greater milk cow/h, and lower labour associated with fetching cows. Thus, regarding motivation to enter the AMS, manufacturers usually recommend feeding concentrates in the AMS, as it has been suggested that the concentrate provided within the AMS is the primary stimulus for the cow to visit the AMS (Rodenburg, 2011) and shown to be a greater motivation than the milking process itself (Prescott et al., 1998). However, partial mixed ration (PMR) characteristics are seldom considered other than the general recommendation of formulating a PMR that provides 85-90% of the nutrients required to achieve the targeted milk yield. Neither the role of the partial mixed ration (PMR) to encourage cows to enter the AMS, nor the possible interactions between the AMS concentrate and the forage-to-concentrate ratio within the PMR are contemplated in this recommendation.

The concept of using AMS concentrate to attract cows to the AMS with the possibility to offer differing amount of it for individual cows has been used by manufactures to support the idea of offering greater amount of concentrates to improve milking frequency, achieve a greater milk yield, and allow for precision feeding (Rodenburg, 2011; Bach and Cabrera, 2017). However, other than an attractant, there is no scientific evidence to support that recommendation. On the contrary, several studies (Migliorati et al., 2005; Bach et al., 2007a; Hare et al., 2018) reject that recommendation by indicating that greater amount of concentrate offered in the AMS does not improve milking frequency or milk yield. A more recent study (Tremblay et al., 2016) reported that increasing the amount of concentrate offered in the AMS is positively correlated to a decrease in milk yield.

While most studies have focused on AMS program feeding strategies, there is a lack of information regarding PMR feeding strategies. Apart from the concentrate given in the AMS, cows are provided PMR at a feed bunk. Therefore, the PMR should be considered as part of the total diet as it represents from 60% (Salfer and Endres, 2014) up to 98% of the dietary DM (Hare et al., 2018). In addition, past studies (Bach et al., 2007a; Hare et al., 2018) indicate that providing greater amount of concentrate in the AMS affects PMR intake; thus, ignoring the role of the PMR could lead to misinterpretation of the performance outcomes.

Feeding management for cows housed in a barn and milked with an AMS still requires improvements (Jacobs and Siegford, 2012). One of the barriers for AMS adoption is the lack of guidance regarding feeding strategies that optimize performance and maximize profitability. Much of the recommendations are based on empirical evidence or single-farm cases studies. No studies have systematically compared the amount of concentrate provided within the AMS, and the energy density of the PMR. In fact, there are only a few studies that have evaluated PMR composition and intake as part of the feeding program in an AMS. Understanding how the PMR feeding strategy may impact cow performance when different amount of concentrate is used in the AMS provides an opportunity to develop feeding strategies towards precision feeding to optimize performance and maximize profitability. Therefore, the overall objective of this study was to evaluate the effects of the forage-to-concentrate ratio of the PMR, the amount of concentrate provided in the AMS, and their interaction to affect DMI, milk and milk component yield, ruminal fermentation, and behavior of lactating dairy cows.

2.0 LITERATURE REVIEW

2.1 History of Automatic Milking Systems

The concept of fully automated milking machines gained interest in the 1970's but it was not until the 1980's when researchers began developing automated milking systems (AMS) (Hyde and Engel, 2002). The first AMS was installed in the Netherlands in 1992 (Hyde and Engel, 2002; de Koning and Rodenburg, 2004; Svennersten-Sjaunja and Pettersson., 2008) and since then, adoption of this new technology has grown. Initial adoption occurred in north-western European countries, with the Netherlands having the greatest proportion (Reinemann, 2008) with AMS systems now installed throughout the world. Despite more broad adoption, more than 90% of the dairy farms that currently use AMS are in European countries (de Koning and Rodenburg, 2004). In North America, the first commercial installation of an AMS occurred in Ontario, Canada in March of 1999 (Rodenburg, 2002). Adoption of AMS have continued since that time. As of 2017, 6% of the herds in Canada used AMS with a greater adoption in the western provinces with 15, 7, and 8% of the herds utilizing AMS in Manitoba, Saskatchewan, and Alberta, respectively (Tse et al., 2017). There is an anecdotal indication for a continued adoption of this technology (Barkema et al., 2015). As such, there is no doubt that AMS have become very popular and the increase in abundance supports the notion for research addressing AMS-specific opportunities and challenges.

2.2 Reasons Producers Adopt Automated Milking Systems

Originally, AMS were designed to satisfy the needs and the social conditions of the single-family owners in Europe and dairy farms in North America (Rodenburg, 2011) where high land prices, high price of labour, and demand for high milk production provided an economic incentive for AMS (Reinemann, 2008). Automated milking systems were developed to milk cows without human intervention (Hyde and Engel, 2002) as an approach to reduce labour cost (de Koning and Rodenburg, 2004). According to a survey conducted by de Jong et al. (2003) on 10 farms in the USA and 15 farms in Canada that have AMS, the level of satisfaction was high to very high. In the same year, Karttunen (2003) indicated that, in general, all AMS users in Finland were very satisfied with the system. As such, complete automation of the milking process has been reported to improve the lifestyle for dairy producers, improve milk yield, and milk quality (de Koning and Rodenburg, 2004).

A reduction of the physical work from routine milking activity is an advantage of using AMS (Reinemann and Smith, 2001). Studies in the past support up to 40% reductions in physical labour with milking-related activities when switching to an AMS (de Koning and Rodenburg, 2004). This in turn also decreases potential for negative human-cattle interactions (Holloway et al., 2014). By adopting AMS, producers have more time to spend on activities rather than milking (Rotz et al., 2003), According to one survey conducted by de Jong et al. (2003), 84% of the producers interviewed indicated that the most appreciated benefit from AMS was the possibility of a more flexible working hours, more time for themselves and their families. In addition, Reinemann (2008) reported that having a more flexible work schedule is the most important benefit from AMS. This does; however, change the nature of the labour when compared with the conventional dairy farms (de Koning and Rodenburg, 2004).

The second reason for AMS adoption is the potential for increased milk yield due to the more frequent milkings/day that could be expected (Erdman and Varner, 1995; Rotz et al., 2003; de Koning and Rodenburg, 2004). Increasing milking frequency is known to increase milk yield (Wagner-Storch and Palmer, 2003; Løvendahl and Chagunda, 2011). Erdman and Varner (1995) reported 3.5 kg/d more milk produced when increasing milking frequency from 2 to 3 times/d regardless of the milk yield for both primiparous and multiparous cows (Wagner-Storch and Palmer, 2003). Introduction of AMS within farms where cows are milked more than twice daily may still result in an increased milk yield between 3 and 11% (de Koning et al., 2002; Baines, 2002). French data reported an average increase of 3 to 9% for milk yield when AMS is utilized for more than 2 years (Veysset et al., 2001), and more experiences in Europe and Canada suggest that production from AMS is 4 to 6% greater than twice a day parlor milking (Rodenburg, 2002) Hence, AMS has the potential to enhance milk production compared with twice-daily milking system (Rasmussen et al., 2001; Melin et al., 2005). Herds without AMS but milked more than 2 times a day had a greater milk production than when adopting AMS (de Koning and Rodenburg, 2004; Svennersten-Sjaunja and Pettersson, 2008)

The more frequent milkings/day expected with AMS increases milk production, especially during early lactation (Svennersten-Sjaunja and Pettersson, 2005). Part of the response for greater milk yield is likely due to increased milking frequency (≥3 times/day) as greater frequencies enhance lactation persistency (Wall and McFadden, 2012). The increased milk yield response and persistency due to an increased milking frequency was not only seen in multiparous but also in primiparous cows, even though primiparous cows showed a lower milk yield compared to multiparous cows (Wright et al., 2013). In that study (Wright et al., 2013), twice a day milking was compared to 4 times/day milking frequency using a half-udder model, which apart from being a useful design by evaluating the effects on individual glands (Wall and McFadden, 2007), it reduces the possible variations due to genetics, environment and nutrition (Wright et al., 2013). The biological explanation for the increased milk yield and persistency response is likely attributed to a greater proliferation of the cells within the mammary gland (Hale et al., 2003), and a reduction of mammary cell death by apoptosis (Stefanon et al., 2002). Moreover, since blood flow to the mammary gland is shown to decrease with long intervals between milking, a greater milking frequency likely enhances nutrient supply to the mammary gland, therefore increasing the potential for the mammary gland to extract nutrients from the blood (Delamaire and Guinard-Flament, 2006a, 2006b). This supports that increasing milking frequency reduces the negative feedback on blood flow and improves milk yield.

Apart from the increased milk production and better lifestyle for farmers, welfare for cows and animal health gain attention from the modern society. Wenzel et al. (2003) showed that animals milked through AMS had greater cortisol levels, higher heart rate prior and during milking, and more often step behavior than cows milked in a parlor, but no differences were found regarding kick behaviour between the two systems, suggesting that when adapting to the new milking system cows may express a greater stress compared to cows in a conventional milking parlor. Automatic milking systems may affect behavior and physiological responses but the reasons for those changes still remain unclear.

With the introduction of AMS cows have more freedom and autonomy and they are described as relaxed, happy, and quiet compared to their peers in conventional dairy barns (Holloway et al., 2014). Due to a more consistent milking process in AMS, cows may improve milk production (Rasmussen et al., 1990) since the milker's behaviour affects milk production (Seabrook, 1994). Although there is not a consensus on health benefits, in a well-managed AMS is possible to see welfare benefits for the cows (Rotz et al., 2003). For example, a positive reaction like increasing lying times is seen in cows' behaviour with more frequent milking events (Ipema et al., 1997), which may be due to the less pressure in the udder; thus, more comfort to lie down (Rossing et al., 1997). Farmers also note less hierarchical fights among cows in AMS (Rotz et al., 2003). In addition, based on the vast available information farmers can gain access to, health problems such as lame animals or mastitis can be prevented or detected more rapidly with AMS relative to conventional milking systems. When cows are lame or have mastitis, milking frequency is reduced and the change in their voluntary attendance may serve as an alert method. Additionally, clinical mastitis could be detected by electrical conductivity measured along with the milk color sensor (Rodenburg, 2002).

Adopting AMS requires a large initial capital investment. According to Rotz et al. (2003), the equipment could be twice or three times that required for a conventional milking system, and although multi-stall units appeared to be more profitable than single-stall units, most of the AMS in Canada are single-stall units and cost between $125,000 to $150,000 US (Hyde and Engel. 2002). According to one survey published by the Iowa State University in 2012, the average cost per AMS was $185,000 US without building costs, while two AMS are valued at $270,000 US (Rodenburg, 2002). While AMS is not a new milking system, it does require a completely different management system that integrates technology. Therefore, successful adoption of the AMS depends on the management skills and adaptability of the farmer to optimize the investment in automation (Reinemann, 2008).

2.3 Transitioning to an Automated Milking System from a Conventional Milking System

2.3.1 General considerations and expectations when transitioning to an AMS

Moving from conventional milking systems, either tie-stall or parlor, to an AMS implies not only a change in the equipment but also in the way labor is used. Nevertheless, while routine activities like udder preparation, visual control of the cow, udder health, and even separation of abnormal milk are partially replaced by the milking machine (de Koning and Rodenburg, 2004), monitoring and maintaining equipment, and managing and observing cattle still must occur. Thus, AMS maintenance requires different management skills compared to maintaining conventional systems as AMS relies on more sophisticated technology, suggesting that a more skilled operators needed for daily routines (Svennersten-Sjaunja and Pettersson, 2008).

Some dairy producers welcome this shift in technology while others initially accept the technology but then reject it by returning to conventional milking systems (Rotz et al., 2003). Overall, adoption of AMS is well accepted as indicated by the vast number of farms adopting and utilizing AMS since 1998 (Wagner-Storch and Palmer, 2003; de Jong et al., 2003; de Koning and Rodenburg, 2004). It should be noted that successful implementation of AMS depends on the expectations of the dairy producers (Hogeveen et al., 2001, de Koning et al., 2002, Ouweltjes, 2004), and according to de Koning and Rodenburg (2004) at the end of 2003, around 2200 farms worldwide were using AMS technology. However, approximately 5 to 10% of farms that installed AMS had them subsequently removed indicating some level of dissatisfaction with AMS (de Koning and Rodenburg, 2004). This suggests that realistic expectations, skilled management before, during, and after implementation, ability to work with computers and data, consistency in checking the system and the cows, good technical support of the AMS, and routine maintenance are all important to implement AMS (de Koning and Rodenburg, 2004).

Automatic milking systems can be implemented by switching from an existing conventional milking system (retrofitting a barn) or by building a new barn. Cows require consistent training at the beginning of the transition from a conventional milking system to an AMS, allowing them to learn to voluntarily visit the AMS. The recommended training process consists of moving cows to the AMS when time since the last visit is > 8 hours (Rodenburg, 2002). Fresh cows may need more attention as udder shape may change in the first days of lactation. After this training, cows are commonly fetched after 12 h have passed since the previous milking event (Van der Vorst and Ouweltjes, 2003). According to Rodenburg (2002) cows in an existing barn need 3 to 4 wk to achieve 80 to 90% of the cows using the AMS voluntarily; while, Salfer and Endres (2011) suggest that up to 3 mo may be required to get the majority of the cows trained, with younger and aggressive cows adapted more easily (Rodenburg, 2002; Salfer and Endres, 2011).

Implementation of different patterns for movement throughout the barn (also known as cow traffic systems) can be used to guide cows within a barn (Holloway et al., 2014) encouraging voluntary visits to the AMS. While most AMS farms initially utilized forced cow traffic, guided-traffic and free cow traffic barns are now used (de Jong et al., 2003). Regardless of the system, increasing productivity by allowing cow more frequent milkings is a common expectation (Holloway et al., 2014). However, the previous expectation is not expected to be accurate for herds that implement more than 2 milkings/d.

It is important to note that not all cows are suitable for AMS. Cows with poor udder shape and teat position will result in failed attachments and high rates of incomplete milkings. As such, the initial culling rate may increase due to conformation or because cows fail to adapt to voluntarily enter the AMS (Rodenburg, 2002). Lame cows are also less willing to visit the AMS and the feeding area (Rotz et al., 2003) and thereby require more fetching labour (Borderas et al., 2008) likely leading to greater culling. Although lameness is considered a multi-factorial problem, the strong negative relationship identified between lameness and AMS visits suggests that lameness may be one of the most important factors affecting AMS voluntary attendance and efficiency (Borderas et al., 2008). According to de Koning and Rodenburg (2004), the number of reported cows that were unsuitable for AMS ranged between 5 and 10%. In addition, if there are too many cows per milking unit (the AMS capacity is undersized), younger and submissive cows may have fewer visits to the AMS and feeding area (Rotz et al., 2003). According to one survey (de Jong et al., 2003), the optimal number of cows to begin per AMS is under 55, but this number is dependent on the proportion of cows in early lactation.

The initial capital invested in AMS is relatively expensive compared with conventional milking systems, thus, AMS utilization must be high (Halachmi, 2000). Therefore, capacity, occupancy (hours of use relative to the available hours), and efficiency (milk or milk component yield per AMS) of the AMS are important to consider. Capacity could be considered as the number of milkings per day that the AMS can facilitate (Rotz et al., 2003). Factors such as milking frequency, milking duration, occupancy of the AMS (Ipema, 1997), herd size, and cow traffic system (de Koning and Rodenburg, 2004) affect that capacity. In theory, within the 22.5 h of eligible (after accounting for three 30-min wash cycles interspersed throughout the day), Rossing et al. (1997) showed that theoretically 7.1, 7.5, and 8.6 milkings per hour are possible at 2, 3, and 4 milking per day, respectively with a 100% of occupancy, with assumed time per milking of 8.5, 8.1 and 7.0 minutes per milking at 2, 3 and 4 milkings per day, respectively (Rossing et al., 1997). However, Salfer and Endres (2011) reported average milking time to range from 7.5 to 8.5 min/cow. Additionally, the occupancy rate more commonly ranges between 60 to 90% (Ipema, 1997). As such, a more realistic expectation may be 6 milkings/h with an 80% occupancy rate (Devir et al., 1995).

Another general expectation is improved cow welfare in part due to potential for increased milk frequency and freedom and due to the vast amount of individual information collected and summarized by the hardware and software associated with AMS (Reinemann, 2008). Infections, such as mastitis, can be prevented by allowing the robot to sample milk; then, farmers can be alerted in advance if they noticed some unusual parameter (Holloway et al., 2014); illness or estrous can also be detected by measuring the temperature through sensors that are integrated with the AMS (Rossing et al., 1997), allowing a promising way to prevent future problems.

2.3.2 Milk yield and quality with Automated milking systems

Shifting from a conventional milking system (parlor or tie-stall) where cows are milked twice daily to an AMS could be expected to increase milk yield (Klei et al., 1997; Rotz, 2003; Österman and Bertilson, 2003; Woodford et al., 2015). A recent survey conducted through the University of Calgary indicated that after adopting AMS, 81% of the reported producers experienced an increase in milk yield (Tse et al., 2018). The increase in milk yield is likely attributed to increased milking frequency (De Jong et al., 2003) as many studies show that increasing the milking frequency to three times daily has positive effects on milk production (Rossing et al., 1997; Lovendahl and Chagunda, 2011, Pettersson et al., 2011). Studies in the past confirm that when milking frequency is decreased to once a day milking, milk yield decreases by up to 40% (McNamara et al., 2008; Remond et al., 2009). However, reduced direct interaction among cattle and people in farms using AMS may also support greater milk yield as cows are treated the same way at each milking, improving consistency in the milking routine, stimulating oxytocin release (Dzidic et al., 2004), and allowing more consistent milk ejection (Rasmussen et al., 1990). It should be noted that handling procedures that induce stress immediately prior or during the milking process can reduce milk ejection by 70% (Rushen et al., 1999) thereby affecting milk yield. This suggests that both good and consistent handling and even milking frequency may positively affect production responses (Rotz et al., 2003; Dzidic et al., 2004; Svennersten-Sjaunja and Pettersson, 2008).

As revenue, in Canada, is affected by the amount of milk and milk quality, increasing milk production and component yield while ensuring quality standards are achieved are critical. Milk quality refers to the compositional aspects and hygienic aspects (de Koning and Rodenburg, 2004). Milk component yield responds positively to increasing milking frequency. According to Klei et al. (1997) cows that were milked three times daily produced fat and protein yield that were 4.7 and 7.3% greater than cows milked twice a day. Regarding hygienic aspects, there have been studies reporting that AMS may negatively affect milk quality, at least upon initial introduction (Rossing et al., 1997, Van der Vorst and Hogeveen, 2000; Pomies and Bony, 2001; Van der Vorst et al., 2002; Reinemann, 2008). Increased free fatty acids (FFA), increased total bacterial count (TBC), and somatic cell count (SCC) have been observed as negative aspects at the beginning of AMS introduction (Jepsen and Rasmussen, 2000; Klungel et al., 2000; Rasmussen et al., 2002; Svennersten-Sjaunja and Pettersson, 2008). However, other studies have reported a decrease in bulk tank somatic cell count (BTSCC) (Shoshani and Chaffer, 2002; Tousova et al., 2014). A recent survey study reported that BTSCC varied among farms adopting AMS for more than 24 mo, with greater proportion of producers reporting a decrease or no difference in BTSCC than those who indicated an increase in BTSCC (Tse et al., 2018). This suggest that after introducing AMS, negative outcomes may be transient, improving after some time Moreover, AMS implementation and management may have been improved since AMS appearance to allow a better hygienic condition (Tse et al., 2018).

The available research indicates that initially AMS increased the concentration of FFA in milk relative to conventional systems (Ipema and Schuiling, 1992, Jellema, 1986; Klei et al., 1997; Justesen and Rasmussen, 2000; Klungel et al., 2000; de Koning et al., 2003). Free fatty acids are known to be responsible for rancid flavours in dairy products (Tuckey and Stadhouders, 1967) and therefore, for a decreased ability to convert milk into processed dairy products (Sapru et al., 1997). The increased FFA concentrations were detected after 24 h in the bulk milk tank when held at 4ºC. Possible explanations for the increased FFA are related to the increased milking frequency expected in AMS and the poor handling of the milk. While the exact reasons for elevated concentrations of FFA are still not fully understood, it appears that the short inter-milking intervals (less than 4 or 6 h) may be important.

Both positive and negative effects on udder health have been associated with AMS adoption (Lind et al., 2000). Positive aspects may be related to detachment of the milking unit that is regulated on a quarter level. More frequent milking events may also reduce udder pressure and challenges for udder ligaments to maintain udder structure (Rotz et al., 2003). However, more frequent milking events may challenge teat canal ends and increase risk for mastitis (Rotz et al., 2003). Indeed, an increase in SCC levels have been reported when shifting from conventional milking (parlor or tie-stall) to AMS (Klungel et al., 2000; Rasmussen et al., 2002). However, after an adjustment period (3 mo), SCC have been reported to decline resulting in no difference for SCC between measurements made prior and 1 yr following AMS adoption (Rasmussen et al., 2001). In another study, SCC of herds milked with AMS was similar to conventional milking systems 8-mo after installation (Rodenburg, 2002).

With the installation of AMS, TBC levels were elevated relative to conventional milking systems (Klungel et al., 2000; Rasmussen et al., 2002). Increased TBC suggests that udder preparation, hygiene with the milking system, and the cooling system were probably insufficient enabling microorganisms to originate and multiply (Rodenburg, 2002; Rotz et al., 2003;

Svennersten-Sjaunja and Pettersson, 2008). Automatic cleaning of the teats is an important practice to reduce bacterial spores and dirt on the teats. In addition, a positive association was also found between increased frequency of milk filter changes and reduced bacteria count of the bulk tank suggesting milking system hygiene plays an important role in regulating milk quality. Additionally, in AMS, there is a relatively small amount of milk that goes to the bulk tank. Precooling the milk in-line attempts to reduce potential for bacterial growth (Rossing et al., 1997). Helgren and Reinemann, (2003), indicated that SCC and bacteria counts levels were similar in AMS relative to conventionally milked herds in the USA. A survey conducted by de Jong et al. (2003) indicated that AMS users do not consider milk quality a problem, but more attention is required when switching from conventional milking systems to AMS (Rasmussen et al., 2001). This suggests that the variation in SCC and TBC are probably due to the initial stress in the first few months after AMS installation (Rodenburg, 2002). According to Helgren and Reinemann (2006), the farms that were using AMS for more than 3 years decreased both SCC and TBC compared to conventional milking systems

2.4 Attendance to AMS (milking frequency) and cow traffic

Milking frequency is referred to as the number of voluntary milking events per cow per day. As the number of cows per AMS increases, the potential number of visits to the AMS for each cow declines, while the occupancy of the AMS increases (Artmann, 2002). It should be noted that the AMS may exert some control of milking frequency. The use of milking permission criteria can allow for more or less frequent milking events. Andersson et al. (2013) indicated that the average number of milkings/cow/d was 2.4 when using DeLaval Voluntary Milking Systems (VMS), and 2.8 visits/cow/d for cows with Lely AMS. While this might be interpreted to compare brands, many other factors including barn design, producer management style, stocking densities, herd days in milk, and milking permission settings may affect these responses. In another study (Priekulis and Laurs, 2012), it was reported that the average milkings/cow/d was 2.7 with feed-first or milk-first cow traffic and this was similar to the 2.69 milkings/cow/d reported by Castro et al (2012) with cows in a free cow traffic.

The location of the AMS relative to other areas, such as feeding and resting areas defines the layout of the barn (Holloway et al., 2014). While all barns provide spaces for eating, resting, and milking activity, the cow traffic system within the barn may affect milking frequency (Ipema, 1997; Ketelaar de Lauwere et al., 2000). There are three main types of cow traffic systems: forced-traffic, guided-traffic, and free-traffic.

With guided-traffic, one-way cow routing is imposed. As such, frequent milkings can be ensured (Ketelaar-de Lauwere, 1992; Winter and Hillerton, 1995; Ketelaar-de Lauwere et al., 1998) as cows must pass through the AMS when exiting the feeding area (feed-first) or when exiting the free stall area (milk-first; Ipema, 1997; Rossing et al., 1997). Forced cow traffic prevents the problem of cows that do not visit the AMS at the desired milking frequency (Winter and Hillerton, 1995; Ketelaar de Lauwere et al., 1998; Ketelaar de Lauwere et al., 2000, Bach et al., 2009), minimizes fetching rate (Hogeveen et al., 1998), thereby decreasing the labor. In addition, forced cow traffic is thought to decrease the need to provide a highly palatable concentrate in the AMS while milking (Bach et al., 2009).

Nevertheless, many studies showed negative aspects as well. For example, Ketelaar-de Lauwere et al. (1998) found that cows spent less time standing in the feeding gate compared to cows in a free cow traffic. Forsberg et al. (2002) and Harms (2004) compared free cow traffic and forced cow traffic and indicated that free cow traffic had less visits to the AMS compared to forced cow traffic, but the number of PMR meals were lower for cows with forced cow traffic than cows in free cow traffic conditions. Melin et al. (2007) also reported that cows housed in forced cow traffic barns had less feed intake and an impaired animal welfare, in terms of time budgets and behavior. Ketelaar de Lauwere et al. (2000) indicated that total time standing in the lying area was the highest in forced cow traffic than in free cow traffic barns, and the time spent in the feeding area and eating forage was the lowest in forced cow traffic than in free cow traffic, suggesting that in forced cow traffic cows may require extra behavioral efforts. In addition, forced cow traffic had the greatest number of non-milking visits resulting in an unnecessary occupation of the robot (Rossing et al., 1997; Munksgaard et al., 2011). Bach et al. (2009) reported reduced resting time and a longer waiting time, especially for timid or low-ranked cows. This supports data from Winter and Hillerton, (1995), indicating that time spent in the holding area for cows under forced cow traffic is longer, and the time cows spend for resting is lower relative to cows in a free cow traffic. De Koning and Rodenburg (2004) also showed that cows under forced cow traffic spend a greater time in the holding area than cows under free cow traffic, particularly with high occupancy rate of the AMS. It is likely that increased time spent in the holding area accounts for the reduction in the number of visits to the feeding area and the fewer PMR meals. In addition, the added time in the holding area could be aggravated if subordinate cows are present along with dominant cows, creating negative social interactions that may reduce motivation for subordinate cows to return to the AMS (Jacobs et al., 2012). Collectively, the information presented above may suggest that forced cow traffic may have greater social repercussions and can reduce resting time of cows relative to free-traffic settings (Thune et al., 2002)

Not only can behaviour responses may be negatively affected by forced cow traffic, but milk production may also decrease. Forsberg et al. (2002) and Harms (2004) highlighted that cows under free cow traffic tended to produce more milk than cows in forced cow traffic, which may be due to the more frequent meals and a better feed utilization under free cow traffic, suggesting that while milking frequency is influenced by the cow traffic system, feeding pattern must be considered as well. Bach et al. (2009) also showed that milk production was not greater with forced traffic compared to cows milked under free cow traffic, even though cows under a forced situation had greater milking events. In addition, a more recent study conducted by Tremblay et al. (2016) over 635 North American dairy farms reported that free cow traffic was associated with greater milk production, either per cow per day and per robot per day compared with forced cow traffic systems.

Forsberg (2008) introduced another possible variant within cow traffic systems by including selection gates. This traffic design can be further classified as milk-first guided cow traffic or a feed-first guided cow traffic, depending on the location of the selection gate in reference to the PMR and AMS. In a milk-first situation, all cows must pass through a selection gate situated prior to the AMS in order to reach the feeding area. If cows meet the milking criteria, they can be directed to the holding area located in front of the AMS. If cows do not meet the milking criteria, they are directed to the feeding area. From the feeding area, they can access the lying area by passing through a one-way gate. In a feed-first situation, the selection gate controls the lying area. Cows have free access to the feeding area by passing through a one-way gate from the resting area; however, to reach the lying area again, cows must pass through the selection gate that ether directs movement to the AMS or lying area. According to Endres and Salfer (2014, 2017) milk-first guided traffic systems work better than feed-first guided traffic systems in the USA since, after consuming the PMR, cows in feed-first cow traffic systems tend to spend more time ruminating in the holding area or standing in the alley without visiting the AMS. Current research does not provide an ideal type of traffic system.

The final cow traffic design is free cow barns. With free cow barns, cows can decide whether or not visit the AMS. As such, voluntary attendance can be variable (Rotz et al., 2003). Reduced milking frequency decreases milk yield (Jacobs and Seigford, 2012) because mammary blood flow is decreased (Delamaire and Guinard-Flament, 2006a). In addition, under the previous scenario, milk quality may be affected (Ouweltjes, 1998). Irregular milking intervals and failure of cup attachment may also be plausible reasons for the lack of increased milk production in some farms using free cow traffic (Bach and Busto, 2005). While recent studies have reported greater milk yield with free-cow traffic (Tremblay et al., 2016), previous experiments conducted by Forsberg et al. (2002) and Harms (2004) reported that free traffic systems had the lowest milking frequency and the highest fetching rate due to long milking intervals. In addition, if the AMS is occupied, the intention to visit the AMS by other cows may be abandoned. While this problem could be solved by placing a mentioned holding area (Uetake et al., 1997) some studies showed negative effects of using a holding area in free cow barns. For instance, Ketelaar de Lauwere et al. (2000) showed that cows under free cow traffic with a holding area spent less time in the feeding area. Harms et al. (2002) observed that the holding area reduced the passing through the AMS, increasing the irregular milkings, with negative effects on animal welfare if they are waiting for milking too long (Harms et al., 2002), and animal health (Blowey, 2005; Barker et al., 2008).

Regardless of the cow traffic system within the barn, unnecessary visits to the AMS reduce the capacity of the AMS (Rossing et al., 1997; Swierstra and Smits, 1989; Devir et al., 1993, 1996a). In addition, the type of AMS visit may influence cow's subsequent behavior (Stefanowska et al., 1999). For example, after non-milking visits or failure with milking system attachment, cows may return to the AMS resulting in a shorting inter-visit interval thereby affecting other activities such as resting, feeding, or even social interactions among cows (Ketelaar de Lauwere et al., 2000). Nevertheless, a selection unit, placed before the AMS is a useful tool to prevent unnecessary visits to the AMS (Rossing et al., 1997; Melin, 2005). The selection unit is a control gate that identifies whether a cow meets the milking criteria and can enter AMS (Ipema, 1997; Ketelaar de Lauwere et al., 1998). Selecting cows for milking before entering the AMS represents an optimization of the whole system by enhancing the number of effective visits to the AMS (Wenzel et al., 2003) and reducing waiting times (Devir et al., 1996a) by avoiding ineffective queuing due to repeated undesired visits of some cows. A further decision is whether or not combine the pre-selection gate with a holding area, placed between pre-selection unit and the entrance of the AMS. The purpose of this holding area is to avoid cows to postpone their milking if, by the time they were selected for milking, there is another cow occupying the AMS. (Uetake et al., 1997). Therefore, the selection gate with the holding area aim to increase the capacity of the AMS (Rossing et al., 1997).

2.5 Feeding management in AMS

Regardless of the design of cow traffic system within the barn, the feeding management strategy implemented in the AMS is vital for success. The primary goals of the feeding program in AMS are to meet nutrient requirements while encouraging cows to voluntarily enter the AMS to promote frequent milking events, greater milk/h, and lower labor associated with fetching cows. Data from past studies have led to the conclusion that the motivating reason for cows to enter the AMS more regularly and to have a voluntary cow movement (de Jong et al., 2003) is not related to undesirable effects arising from udder pressure or sensory signals provided during milking; rather, the desire to eat the concentrate provided while milking seems to be the primary motivating factor (Prescott et al., 1998; Ketelaar-de Lauwere et al., 1999; Halachmi et al., 2000; Rodenburg, 2011). This implies that the number of visits to the AMS is positively associated with desire to consume concentrate. Hence, the logic behind using concentrate in the milking box is primarily for enticing cows to visit the AMS voluntarily. Incorporating AMS within a confined system (commonly used in the USA and Canada), or pasture-based systems (as seen in some European countries, and New Zealand, and Australia; Van der Vorst and Ouweltjes, 2003), use concentrate in the AMS as a strategy to ensure frequent milkings occur (Rodenburg, 2017).

In North America, feeding management has been traditionally based on mixing all the components of the diet and providing it as a total mixed ration (**TMR**). However, for cattle milked with AMS, concentrate is also provided in the AMS. As such, it is preferable to distinguish between the two different diet forms including the partial mixed ration (**PMR**) provided at the feed bunk, and the AMS concentrate (Endres and Salfer, 2017). Since, the bulk majority of the diet is provided as a PMR, the concentrate provided in the AMS is used partially as a means to attract cows to the AMS (Bach and Cabrera, 2017). This suggests that the feeding management takes a more complex role in farms using AMS where understanding the total amount of feed consumed in each location is critical.

Generally, current AMS concentrate feeding recommendations provided by the AMS manufacturers promote feeding substantial quantities of concentrate within the milking stall to motivate cows to enter the AMS, and consequently, achieve greater milking frequency and milk production (Rodenburg, 2013). According to Van Mourik et al. (2008), the standard recommendation from Lely was to balance the PMR for a production level approximately 7 kg below the average production for a group of cows that have more than 4 weeks in lactation and to allocate concentrate in the AMS ranging from 2.26 to 7.7 kg/d. The range in concentrate is suggested to provide a mechanism to increase nutrient density of the diet such that energy demand can be matched with energy intake. Futcher (2011) reported that DeLaval recommended a value of 3.63 kg/cow per day by default as a starting value and encouraged greater quantities of concentrate following adaptation.

As stated above, the concentrate fed in the AMS is partially used as an attractant to visit the AMS (Prescott et al., 1998). A logical extension of this concept is the notion that greater quantities of concentrate in the AMS should further enhance voluntary visits, reduce the inter-milking interval, and even to decrease the variation in milking interval (De Koning and Rodenburg, 2004). Should larger quantities of concentrate stimulate voluntary attendance, this strategy could also enhance milk yield and decrease the requirement to fetch cows (De Koning and Rodenburg, 2004). However, the optimal quantities of concentrate to be offered in the AMS has not been clearly assessed (Bach and Cabrera, 2017). Moreover, neither the upper level of concentrate that can be provided in an individual meal nor the minimal amount of concentrate to assure milking visits occur have been determined. These gaps are clearly a challenge that warrants investigation.

Practically, producers need to decide how much concentrate to feed in the AMS and how much to feed in the PMR. Field data shows that there is a high variability regarding the amount of concentrate fed in the AMS. According to one survey conducted using 10 farms in the USA and 15 farms in Canada with AMS, 78% of farms reported fed less than 5 kg/cow/d of concentrate in the AMS and 22% of farms reported feeding more than 5 kg/cow/d (de Jong et al., 2003). Another survey indicated that the minimum of concentrate that producers were feeding was 0.9 kg/cow/d while the maximum amount was 8.6 kg/cow/d (Salfer and Endres, 2011). A later survey conducted by the previous authors reported that the upper level of concentrate being offered in the AMS is 11.3 kg/cow/d (Salfer and Endres, 2014). Assuming cows achieve 11.3 kg/d intake of AMS concentrate and a concentrate DM of 90%, it could be assumed that cows may be provided as much as 40% of their total diet, considering a cow consuming 26 kg of DMI/day. Despite the range in concentrate provided on farm, there is a surprising lack of published studies evaluating feeding strategies in the AMS. The lack of peer-reviewed experiments is problematic and has led to many recommendations for feeding management based on survey data. Moreover, the effect of such feeding strategies and their relationship with voluntary visits to the AMS, milk yield, and component yield are mostly based on retrospective case analysis or from single stall units without adequate control treatments.

The practical decision of how much concentrate offer in the AMS will not only make an impact on how to deliver vitamins, minerals, and feed additives but also it will affect the forage-to-concentrate ratio of the total diet, as well as feed cost and utilization of the protein and starch sources of the commonly pelleted feed used (Bently et al., 2013). Moreover, it is reasonable to expect that the type of cow traffic system may impose a different feeding strategy to incite voluntary entries to the AMS. However, it is also not clear if the feeding management strategies need to differ based on the cow traffic cow within the barn. Based on a survey, the amount of AMS concentrate that producers with guided and forced cow traffic systems are using is less than the amount used in free cow traffic barns (Salfer and Endres, 2014). That said, the study conducted by Scott et al. (2014) showed that cows on a pasture-based system with a forced-traffic design, had a higher milking frequency and milk yield when provided with 300 g of concentrate/milking when compared to no concentrate.

In contrast to the recommendation to feed large quantities of concentrate in the AMS, there is evidence demonstrating that providing high amounts of concentrate in the AMS does not positively affect milking performance. For instance, Migliorati et al. (2005) compared treatments where the actual concentrate consumption was 3.65 kg/cow/d for a high provision of concentrate and 1.4 kg/cow/d for a low provision of concentrate in the AMS, and again no responses were detected of these treatments on milk performance and milking frequency. Bach et al. (2007a) reported no differences in milking frequency when comparing 3 vs. 8 kg/d per cow, and a more recent study, conducted by Tremblay et al. (2016) on 635 North American dairy farms using AMS, indicated that providing more concentrate in the AMS was negatively correlated to milk yield, and negatively associated with the number of milkings per day and milking speed. In addition, increased boxtime, defined as the total time in the AMS, refusals (non-milking visits) and connection attempts were greater when more concentrate was provided in the AMS (Tremblay et al., 2016).

Regarding the minimal amount of concentrate to be offered in the AMS, Halachmi et al. (2005) fed 1.2 kg per visit as the minimal amount of concentrate needed to attract cows to the AMS. In the study of Halachmi et al. (2005), the provision of 1.2 kg of concentrate per visit to the AMS resulted in 3.85 kg/d of concentrate allocated per day. The use of 1.2 kg/d was based on other findings (Wierenga and Hopster, 1991 and Livshin et al., 1995) that 1 kg of concentrate/visit was the minimum amount that needed to be fed. However, the 1 kg/visit was based on computerized self-feeders and while still addressing a component feeding system, results required for self-feeders may not translate well with AMS. Migliorati et al. (2005) reported that the minimal amount of concentrate allowable per milking was 0.55 kg and, as mentioned before, no differences were found in milking frequency (2.52 milking/cow/d). Thus, 1.2 kg/visit suggested by Halachmi et al. (2005) may not be the real minimal amount of concentrate needed to provide. In a study conducted by Scott et al. (2014), in a pasture-based system, they compared feeding a small amount of concentrate in the AMS (300 g/milking/cow) and feeding no concentrate in the AMS (0 g/milking/cow) and they conclude that 300 g/milking was sufficient to act as an incentive for milking. A more recent study (Hare et al., 2018) used 0.5 vs 5.0 kg as targeted amounts of concentrate in the AMS/cow/day and concluded that greater provision of concentrate, in isocaloric diets, does not improve milking frequency or milk production. This data suggests that there is little consensus about the minimal amount of concentrate that should be provided to assure voluntary milking visits.

The maximal quantity that could be offered in each milking requires attention to determine an appropriate maximum meal size provided for each cow. Precision feeding incorporates the provision of concentrate allocation to individual cows during each milking based on set criteria, typically based on milk production. Important factors to consider are milking frequency, the concentrate dispensing rate (the amount of concentrate that could be delivered per minute), milking duration, the number of hours elapsed since last milking visit, and the amount of concentrate offered in the previous milking. While the success of the system to allocate the concentrate in the AMS was validated by Devir et al. (1996b), reporting that 90% of the planned allocation occurred, high producing cows may not get enough concentrate in the AMS to satisfy their requirements based on the number and available milking time (Ketelaar-de Lauwere et al., 1999) or may not have enough time to eat all of their daily concentrate allotment, requiring part of the concentrate to be fed the next day or to be fed within the PMR.

Offering high quantities of concentrate in the AMS, such as that reported by Salfer and Endres (2014) may exceed the ability of cows to consume concentrate in the AMS, since there are several reasons that may explain a limited concentrate intake in the AMS. Ipema et al. (1997) indicated that, for physiological reasons, the upper limit of concentrate that cows can consume per visit is 3.5 kg. Thus, the possible amount of concentrate consumed per day should be 7, 10.5, or 14 kg if cows have 2, 3, or 4 daily milkings, respectively. However, the concentrate intake on each visit may be limited to the milking duration (Bach and Cabrera, 2017). Considering an intake of 3.5 kg per milking, cows would need 12 min of milking time to be able to consume that amount at an intake rate of 300 g/min (Osîtis, 2005) or 10 min of milking time if they can consume the pellet at 350 g/min (Kertz et al., 1981) . However, milking duration is on average 5 to 8 min (Castro et al., 2012) and as such, cows may only be able to eat 1.5 to 2.5 kg of concentrate. Secondly, many cows go to the AMS less than 3 times a day (Wagner-Storch et al., 2003; Bach et al., 2009; Deming et al., 2013); hence, allowing cows to eat more than 8 kg/d may not be practically achievable. Moreover, as pelleted feed alters the rate and the extend of the starch ruminal degradation (Russell et al., 1992), high amount of concentrate offered as a pelleted source may represent a challenge in optimizing dietary formulation.

In addition to the lack of information regarding the optimal amount of concentrate to be offered in the AMS, there are several factors that may cause variability in AMS concentrate consumption. Inherent in some AMS is the ability to carry-over concentrate that was assigned but not delivered to a cow and hence carried over and added to the allocation for the subsequent day. This carry-over concentrate may create a considerable variation in concentrate consumption among days; thus, altering the targeted composition of the diet. There is lack of research regarding how carry-over concentrate strategies should be implemented and how they impact on performance outcomes. Secondly, variation in milking frequency and milking intervals also affect meal size, number of meals, and the total amount of AMS concentrate consumed. Aside from causing a reduction in PMR intake, it is unclear how concentrate feeding strategies affect PMR consumption.

Another possible cause of variation in the concentrate consumed can be imposed by the AMS is that while programmed quantities are entered into the software, the AMS delivers the concentrate using a metered approach rather than a direct weight, suggesting that inconsistency in the concentrate (e.g. density or particle size) could generate discrepancies regarding the provided quantity. Frequent calibration of the concentrate delivery is proposed to ensure equality between the metered volume and the targeted amount.

Another factor that creates variation in the amount of AMS consumed is related to the differences between the amount of concentrate programmed to be offered per day and the actual amount of concentrate delivered, and then consumed per day. This is not often mentioned; however, it is a fact that these discrepancies represent a challenge in the feeding strategy for AMS. For example, Halachmi et al. (2005) offered either 1.2 kg/visit or 7 kg/d to cows but reported that cows offered 1.2 kg/visit consumed 3.85 kg/d and the ones offered 7 kg/d only consumed 5.2 kg. In Bach et al. (2007a), they allocated either 3 or 8 kg/d in the AMS and reported that only 2.6 and 6.8 kg/d were consumed, respectively. Both studies show that cows did not consume the final concentrate targeted of their diet, creating a variability in their final nutrient composition consumed. According to Bach and Cabrera (2017), cows do not eat the whole amount of concentrate in the AMS when the allowance is higher than 4 kg/d, and that variability seems to be increased as the eligible amount increases. To avoid under-consumption of the target AMS concentrate, Hare et al. (2018) purposely entered a computer programmed value that exceeded the ration formulated value. This may be one approach to ensure that cows consume the target concentrate consumption.

While there are no studies to date reporting variability in AMS concentrate consumption across days, Dieho et al. (2016) evaluated how the fermentability of the diets before calving was affected by changing the rate of concentrate inclusion in the diet with a final of 45% DM of concentrate inclusion. They found that a more gradual inclusion (0.25 kg/d DM increase of the supplement) resulted in cows with greater DMI and milk yield than when using a more rapid concentrate inclusion (1 kg/d DM increase of the supplement). In contrast to DMI, fermentable organic matter intake in that study was higher for rapidly adapted cows (1 kg/d DM) than for gradually (0.25 kg/d DM) adapted cows. These results suggest that cows under a higher rate of concentrate allowance, due to a variation allowance among days, may not improve total DMI or milk production. These findings are supported by recommendations in a recent review (Bach and Cabrera, 2017) in which it was suggested to restrict concentrate provision to a maximum of 4 kg/d as a strategy to avoid risk for subacute ruminal acidosis (SARA), reduce variation in nutrient intake, reduce feed cost, and improve consistency in milkings.

While the concentrate fed in the AMS plays an important role as part of the diet for AMS, there is a lack of information regarding how the AMS concentrate feeding strategy interacts with the PMR feeding strategy (Hare et al., 2018). One common recommendation is to provide a balanced PMR for a cow producing around 7 to 8 kg less milk than the average milk yield, and to combine the PMR with different amounts of concentrate in the AMS according to milk production (Rodenburg, 2017). That said, the idea of feeding large concentrates in the AMS may partially stem from the belief that an energy-dense PMR may decrease cow traffic and reduce voluntary milking events, especially for late lactation cows (Rodenburg, 2017). Rodenburg and Wheeler (2002) came to the previously stated conclusion based on information provided by a single farm in Ontario. In fact, Rodenburg (2002, 2011) suggested that the presence of "lazy cows" is due to high energy dense PMR, and the study by Hauspie (2008) is provided as evidence for an increased milk production when the concentrate level in the PMR was reduced by 30% and the level of concentrate in the AMS was increased by 12%. However, according to Tremblay et al. (2016), there may be other possible explanations for feeding large amount of concentrates in the AMS such as poor forage quality or high variability in milk production within the herd.

While past studies (Migliorati et al., 2005; Bach et al., 2007a) have not reported greater milk production when offering greater amounts of concentrate in the AMS, only Bach et al. (2007a) and Hare et al. (2018) have evaluated PMR consumption. Interestingly, results of Bach et al. (2007a) indicate that cows do not eat more DM when they receive a greater quantity of concentrate in the AMS; rather cows decrease PMR intake. The reduction in PMR in response to increasing concentrate is referred to as a substitution effect. This substitution effect may result in a potentially large shifts in the forage-to-concentrate ratio of the total diet. The substitution of PMR for a pelleted concentrate may cause a reduction in the physically effective fiber (peNDF) content in the diets consumed (Humer et al., 2018). Such effects may increase risk for SARA. However, there are no studies to date that have evaluated ruminal fermentation for cows managed in AMS.

Characteristics of the total diet may have an influence in the substituion effect. In the study conducted by Bach et al. (2007a) feeding 3 versus 8 kg/d of concentrate in the AMS under isocaloric diets, resulted in no differences in milking frequency, milk yield, and no improvements regarding the labor associated with fetching cows by the greater concentrate allowance, but they found that for every 1 kg DM increase in concentrate consumption, PMR DMI decreased by 1.14 kg. Another more recent study conducted by Hare et al. (2018), using iso-caloric diets (PMR +

AMS), also noted a substitution effect of 1.58 kg. At the present time, it is unclear what factors cause increased or decreased substitution effects. Thus, further research is needed to assess the influence dietary factors (PMR formulation strategies, AMS concentrate composition) and how they may influence the substitution effect.

Cow behavior regarding PMR intake and AMS intake are additionally important to consider. With AMS, a limited supply of PMR at the bunk may increase a synchrony in feeding behavior for the PMR and resting resulting in many cows trying to occupy the AMS at a single time (Svennersten-Sjaunja and Pettersson, 2008; Forsberg, 2008). In addition, a more even consumption of PMR within the day will contribute to a better rumination and fermentation, improving the ruminal environment for a better nutrient digestibility (Livshin et al., 1995). Unfortunately, there are no studies showing the effects of providing different amount of AMS concentrates in combination with different energy density of the PMR on eating behavior and cow activity patterns.

2.6 Conclusion

Since AMS technology appeared commercially in 1992 farms utilizing AMS increased rapidly. There have been lots of research conducted referring to different areas in order to elucidate this milking system and support that expansion. Implementation of new management strategies are needed to support that expansion as well at an efficient and cost effective milk production. While AMS allow the opportunity for a more precise feeding strategy, feeding management in cows milked in AMS is still one of the most challenging and vital factors for AMS success. Those challenges are due to the completely different management system required in AMS compared to conventional milking systems. Cows housed in AMS are not longer in a structured and consistent milking and eating routine as cows housed in conventional milking systems; therefore, changes in the assumed activities of the cows create a natural domino effect over other activities, altering performance responses as well. The most challenging factor is to be able to balance their diet as cows are under a more dynamic system in which complex interactions among feeding management, voluntary visits and milk production occur. Most of the strategies have been focused on AMS feeding strategies, however a more detailed evaluation of PMR feeding strategy and their relationship is lacking. Evaluating different scenarios by changing the forage-to-concentrate ratio within the PMR in addition to evaluate the interaction between the PMR and the amount of concentrate provided in the AMS is important to improve the understanding of the feeding management of cows housed in AMS.

2.7 Hypothesis

I hypothesized that cows offered 2 kg of concentrate in the AMS will consume a greater quantity of PMR and have greater milk and milk component yield with a more stable ruminal fermentation than cows offered 6 kg of concentrate allowance in the AMS. In addition, it was hypothesized that allowing cows to consume a more energy dense PMR (low forage PMR) would increase milk yield without negative effects on voluntary milking visits to the AMS, AMS concentrate intake, but would have a greater reduction in PMR intake as AMS concentrate allocation increases.

2.8 Objective

The overall objective of this study was to evaluate the effects of the forage-to-concentrate ratio of the PMR, the amount of concentrate provided in the AMS, and their interaction to affect DMI, milk and milk component yield, ruminal fermentation, and behavior of lactating dairy cows.

3.0 EFFECT OF THE FORAGE-TO-CONCENTRATE RATIO OF THE PARTIAL MIXED RATION AND THE QUANTITY OF CONCENTRATE ALLOCATED IN AN AUTOMATIC MILKING SYSTEM ON DRY MATTER INTAKE, MILK YIELD AND COMPOSITION, RUMINAL FERMENTATION, TOTAL TRACT DIGESTIBILITY, AND ACTIVITY OF MULTIPAROUS HOLSTEIN COWS[1]

3.1 Introduction

Feeding management for cows milked using AMS differs from conventional parlor-milked cows, as they are provided a PMR at a feed bunk (or pasture), and a concentrate supplement while in the AMS. Previous research has suggested that provision of concentrate in an AMS is a motivating factor encouraging cows to voluntarily enter the AMS (Prescott et al., 1998; Melin et al., 2005; Bava et al., 2012). As a result, the concept of using concentrate to attract cows to the AMS, coupled with the ability to provide differing quantities of concentrate for individual cows, has resulted in AMS manufactures suggesting that concentrate quantity can be used to minimize fetching and allow for precision feeding (Rodenburg, 2011; Bach and Cabrera, 2017). Producers have apparently implemented these strategies and, in some cases, provide large quantities of concentrate (up to 11.3 kg/cow/d; Salfer and Endres, 2014). Based on a large-scale study, Tremblay et al. (2016) reported a mean AMS concentrate provision of 5.07 kg/d, with a standard deviation of 1.75 kg/d. The data presented above collectively indicate that concentrate provision among farms and within farms can be both high and variable.

Despite the variable quantity of concentrate provided in the AMS among herds and among cows, there is little evidence to support that increasing the quantity of concentrate provided in the AMS may improve production outcomes. For example, Halachmi et al. (2005) reported no differences in milking frequency or milk yield when cows were provided either 1.2 kg of concentrate/milking or 7 kg/d. Migliorati et al. (2005) and Bach et al. (2007a) also reported no improvement in milking frequency or milk yield with increasing AMS concentrate allocation. Additionally, Tremblay et al. (2016) reported a negative association between milk production/cow

[1] A version of this chapter has been accepted for publication. Menajovsky, S.B., C.E. Walpole, T.J. DeVries, K.S. Schwartzkopf-Genswein, M.E. Walpole, and G.B. Penner. In review. The Effect of the Forage-to-Concentrate Ratio of the Partial Mixed Ration (PMR) and the Quantity of Concentrate in an Automatic Milking System (AMS) for Lactating Holstein Cows. J. Dairy Sci.

and the quantity of concentrate/100 kg milk provided in the AMS, and Hare et al. (2018) reported that a low quantity (0.5 vs. 5.0 kg) of concentrate in the AMS, when fed isocaloric diets (PMR and AMS concentrate), tended to improve milk production responses.

Explanations for why additional concentrate in the AMS does not increase milk yield have not been well established. One potential explanation is that the deviation in the quantity of concentrate eligible relative to that delivered increases as the total quantity of concentrate eligible increases (Tremblay et al., 2016). Thus, while cows have more concentrate potentially available, the quantity delivered lags behind due to infrequent milking events, the rate and constraints for concentrate provision, and the maximum meal size imposed. The previously stated outcome has been highlighted by Bach et al. (2007a) where they targeted 3 or 8 kg/d of concentrate, with cows consuming 2.6 or 6.9 kg/d (DM basis), respectively, and by Halachmi et al. (2005), where they targeted 1.2 kg/visit or 7 kg/d of concentrate, resulting in an actual consumption of 3.85 and 5.2 kg/d, respectively. Thus, the diet consumed could be substantially different than the diet formulated. Another potential explanation may be that the AMS allocation also affects consumption of the PMR. Unfortunately, most previous studies have not reported PMR composition or intake (Halachmi et al., 2005; Migliorati et al., 2005; Tremblay et al., 2016). That said, Bach et al. (2007a) reported that for every 1 kg increase in AMS concentrate consumed, cows decreased PMR intake by 1.14 kg, demonstrating an inadvertent consequence of providing more concentrate in the AMS. A more recent study noted a 1.58 kg reduction in PMR intake for every 1 kg increase in AMS concentrate (Hare et al., 2018). The substitution of PMR for AMS concentrate warrants further investigation into feeding management when considering the whole diet.

Both the AMS concentrate and the PMR contribute to meeting the nutrient requirements of dairy cattle. Depending on the quantity of concentrate provided in the AMS, it can be surmised that the PMR could account for at least 60% of the total dietary DM supply (Salfer and Endres, 2014) and may, in some situations, provide as much as 98% of the dietary DM (Hare et al., 2018). While the knowledge regarding AMS concentrate feeding strategies is increasing, little value can be obtained without understanding corresponding changes in PMR composition and PMR intake. To our knowledge, there are no studies evaluating how PMR formulation strategies, independent of the AMS concentrate, may affect production responses for cows milked using AMS.

Based on the information presented above, I hypothesized that cows provided with less concentrate in the AMS will have greater PMR intake, milk and milk component yields, and more stable ruminal fermentation than cows offered more concentrate in the AMS. I further hypothesized that decreasing the forage-to-concentrate ratio (F:C) of the PMR would increase milk yield without negatively affecting voluntary attendance to the AMS, AMS concentrate intake, but would increase the reduction in PMR intake arising from increased AMS concentrate intake.

3.2 Materials and methods

This study was conducted at the Rayner Dairy Research and Teaching Facility at the University of Saskatchewan (Saskatoon, Saskatchewan, Canada). All procedures were pre-approved by the University of Saskatchewan Research Ethics Board (protocol 20100021).

3.2.1 Experimental design

Eight 2nd lactation Holstein cows were fit with a 9-cm ruminal cannula (Robyn Williams, Melbourne, Victoria, Australia) and assigned to 1 of 2 squares in a replicated 4×4 Latin square design based on DIM. At the start of the study the average ± SD for DIM, BW, and milk yield were 141 ± 13.6 DIM, 685 ± 29.9 kg and 47.0 ± 3.74 kg for square 1, respectively, and 169 ± 9.7 DIM, 708 ± 70 kg and 41.5 ± 8.35 kg for square 2, respectively. Each of the 4×4 Latin squares were balanced for carry-over effects and differed in the treatment sequence.

Treatments were arranged in a 2×2 factorial design consisting of a PMR with a low (L-FOR) or high (H-FOR) F:C ratio and either a low (2 kg/d on a DM basis; L-AMS) or high AMS (6 kg/d on a DM basis; H-AMS) concentrate target. The AMS concentrate target represented 7.12% and 21.35% of the total diet, respectively (Table 3.1). The L-FOR PMR contained a F:C ratio of 54:46 compared to a ratio of 64:36 for the H-FOR PMR. All PMR were adjusted to 50% DM by adding water at the time of mixing. The PMR was provided in Insentec (Hokofarm Group, Marknesse, The Netherlands) feed bunks, to which cows were individually trained to consume from their own bunk. Daily PMR provision was distributed amongst 2 feedings: 60% of the daily allocation was offered at 1000 h and 40% offered at 2200 h.

All dietary ingredients were common among treatments, as the pellet provided in the AMS was the same pellet that was used in the PMR (Table 3.1). Diets were formulated to be balanced for macro- and micro- nutrient supply and had metabolizable energy and protein allowable milk yield

Table 3.1. Ingredient composition, chemical composition and, particle size distribution of the PMR for treatments that consisted on a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation.

	L-FOR		H-FOR	
	H-AMS	L-AMS	H-AMS	L-AMS
Ingredient composition, % DM				
Barley silage	16.55	19.57	19.57	23.13
Corn silage	10.50	12.46	12.46	14.70
Alfalfa hay	15.02	17.79	17.79	21.07
Barley grain	26.62	18.86	18.86	9.79
PMR supplement[1]	9.25	23.49	9.25	23.49
AMS supplement[1]	21.35	7.12	21.35	7.12
Palmitic acid[3]	0.71	0.71	0.71	0.71
Chemical composition[4]				
OM, % DM	92.76 ± 0.17	92.27 ± 0.18	92.30 ± 0.20	91.74 ± 0.21
CP, % DM	16.27 ± 0.11	16.55 ± 0.14	16.41 ± 0.10	16.72 ± 0.14
aNDFom, % DM	27.65 ± 0.41	29.54 ± 0.46	29.40 ± 0.38	31.58 ± 0.44
ADF, % DM	17.57 ± 0.10	19.28 ± 0.06	19.24 ± 0.09	21.24 ± 0.07
Starch, % DM	33.86 ± 0.76	30.21 ± 0.55	30.43 ± 0.66	26.18 ± 0.51
NFC, % DM	44.16 ± 0.25	41.25 ± 0.33	41.71 ± 0.21	38.38 ± 0.29
Ether extract, % DM	3.71 ± 0.09	3.77 ± 0.10	3.73 ± 0.10	3.79 ± 0.10
NE_l, Mcal/kg DM	1.71 ± 0.01	1.68 ± 0.01	1.68 ± 0.01	1.64 ± 0.01
Particle size distribution of the PMR[5]				
19 mm, %	3.49 ± 0.18	3.50 ± 0.18	4.13 ± 0.22	4.14 ± 0.22
8 mm, %	26.41 ± 0.39	26.47 ± 0.41	31.17 ± 0.48	31.18 ± 0.51
4 mm, %	51.96 ± 1.46	48.95 ± 3.07	44.35 ± 1.51	41.41 ± 3.12
Pan, %	18.15 ± 1.33	21.08 ± 2.96	20.35 ± 1.37	23.27 ± 2.99

[1]The pellet provided in the AMS (AMS supplement) was the same pellet used in the PMR (PMR supplement) and contained 24.3% barley grain, 27.9% canola meal, 10.4% corn grain, 10.1% soybean meal, 6.2% peas, 2.1% corn DDG with solubles, 4.10% wheat, 2.0% corn gluten meal, 2.2% palmitic acid[3], 1.0% Acid Buf AB Vista, 1.6% Sodium Bicarbonate, 0.11% Calcium Phosphate Mono, 2.5% Limestone Ground, 1.8% Tallow, 2.8% Premix[2], 0.9% Salt White.
[2]Premix contained 3.7% of sulfur, 5.55% of Vitamin D (2,280 IU/g), 2.72% of sel plex 1000, 42.67% of Magnesium sulfate 7H2O (Epsom Salts), 3.70% of Vitamin A (12,500,000 IU/kg), 1.26% of Zn, 0.56% of Vitamin E (500,000 IU/kg), 0.37% of Biotin (DSM 20,000 g/kg), 1.26% of Mn, 0.64% of Cu, 37.01% of Wheat Midds, 0.56% of Chromium Propionate 0.4%, and 0.01% of EDDI.
[3]Energizer RP10 (Scothorn Nutrition, Grand Pré, NS).
[4]Period composites were analyzed and values indicate the mean ± SEM. Water was added to the PMR to achieve a final DM concentration of 50%.
[5]Values indicate the mean ± SEM of the period composites.

predictions that were similar based on the Nutritional Dynamic System (NDS, RUM&N Sas, Reggio Emilia, Italy). Predicted total intake (28 kg DM), cow BW, and pre-study milk yield and composition were utilized to formulate the diets. To ensure that the targeted F:C ratio of the PMR was achieved throughout the experiment, samples of forages (barley silage, corn silage, and grass hay) were collected twice weekly and concentrate samples were collected once weekly. Samples were used to determine the DM concentration by placing them in a forced air oven at 55ºC until achieving a constant weight. The AMS was calibrated once weekly with the calibration procedure conducted in triplicate. To ensure the amount of AMS concentrate targeted was achieved for each treatment, the eligible quantity available exceeded the target quantity. Thus, to achieve 2 and 6 kg/d of concentrate (DM basis), a total of 2.07 and 6.55 kg/d was eligible.

Each period of the Latin square consisted of 28 d. The first 6 d of each period were used to transition cows to their respective diet followed by a 13-d diet adaptation. The last 9 d were used for data and sample collection. The diet transition was accomplished by providing 25, 50, and 75% of the final diet starting on d 1, 3, and 5, with cows receiving 100% of their final diet on d 7. The 9-d sampling period was divided into two 4-d phases with 1 d of rest interspacing the two 4-d measurement protocols. The first 4 d were used to evaluate behavioral responses while the second 4-d period was used to evaluate metabolic responses.

Throughout the study, cows were housed in a pen with 12 free-stalls bedded with chopped straw over mattresses (Eva Cow Mats, BSM Canarm, Ontario, Canada). The free-stall area was divided by a one-way gate that cows passed through to enter the feed bunk area. To leave the feed bunk area, cows passed through a selection gate that either directed them to the holding area for the AMS (De Laval International, Tumba, Sweden), or directed them back to the free-stall area. Cows were granted access to the AMS when the time since the last visit exceeded 4 h or the predicted milk yield exceeded 9 kg. The AMS concentrate allocation at each milking was based on a linear accrual over time with a minimum concentrate provision of 50 g and a maximum of 2.50 kg. Water was available ad libitum in the free-stall area.

3.2.2 Data and sample collection

3.2.2.1 Body weight and body condition score.

Cow BW was measured at the start and at the end of each period on 2 consecutive days and the average BW was calculated. Body condition score was assessed independently by 3 trained observers using a 5-point scale according to Wildman et al. (1982) on d 1 of each experimental period. The average BCS was calculated and used for data analysis.

3.2.2.2 Dry matter intake, feeding behavior, and PMR sorting behavior.

The amounts of PMR offered and refused (as fed basis) were recorded daily. Cows were fed the PMR ad libitum targeting a 5 to 10% refusal rate on an as fed basis. In addition, the amount of concentrate offered in the AMS was recorded daily by the AMS software. The PMR and AMS concentrate consumed were summed to determine total intake. During each of the 4-d sampling periods, feed ingredient samples were collected daily (1 kg for forages and 500 g for concentrates), composited on an equal weight basis, and stored in a freezer (-20ºC) until analysis. Composited feed ingredients were mixed thoroughly, and a representative sample was utilized for DM determination in duplicate. To determine DM, samples were placed in a forced-air oven held at 55°C until achieving a constant weight. A representative sample of the PMR refusals (20% of the refusal weight) from each the 4-d behavioral and 4-d metabolic collection phases were collected daily. Refusal samples from an individual cow were composited proportionally over the sampling period and stored at -20ºC. The refusal samples collected during the behavioral measurement phase were used for DM and particle size separation (described below), and refusal samples collected during the metabolic measurement period were used for DM determination and chemical analysis (described below). Dry matter intake of the PMR and the AMS were determined separately by summing the respective DMI of each of the 4-d sampling periods. The substitution rate was calculated as described by Bargo et al. (2003) with the exception that PMR intake was used instead of pasture intake.

Feeding behavior for the PMR was determined for each cow during the 4-d behavioural collection phase using the software associated with the automated feed bunks (Roughage Intake Control System, Insentec, Marknesse, Netherlands). Feed bunks were recalibrated when the empty feed bunk weight deviated by ± 0.2 kg. The method used to determine feeding behavior has been described by Chapinal et al. (2007). A meal criterion for each cow in each period was determined, as reported by DeVries et al. (2003), with an average of 44.10 ± 49.48 min (mean ± SD).

Particle size distribution of the PMR and refusals were determined using the Penn-State Particle Size Separator (PSPS; Nasco, Modesto, CA) as described by Kononoff et al. (2003) except that the aperture openings were 19, 8, and 4 mm, along with a bottom pan (Maulfair et al. 2011).

Each composite sample was measured in duplicate. After particle size separation, the sorting index was calculated as described by Leonardi and Armentano (2003). Values equal to 100% indicate no sorting, values < 100% indicate sorting against a specific particle size, and values > 100% indicate sorting for a specific particle size.

The dried feed and refusal samples collected during the metabolic collection phase were ground to pass through a 1-mm sieve using a hammer mill (Christy and Norris, Christy Turner Ltd., Chelmsford, UK). Ground samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD, USA) and analyzed for (DM, OM, CP, aNDFom, ADF, starch, ether extract, and undegradable NDF (uNDF) following 240 h of in vitro fermentation. The NDF was analyzed using amylase and sodium sulfite and corrected for ash content.

3.2.2.3 Milk and milk component yield, milking activity, and standing and lying time. Milk yield was recorded at each milking by the AMS. During the 9-d collection period, milk samples were collected from each cow (20 mL) using an automated sampling device. Samples were preserved with potassium dichromate and stored at 4ºC. Milk samples were then mixed and composited proportionally based on the yield at each milking to form a daily composite sample of 40 mL. The daily composite samples for each cow were analyzed for CP, fat, lactose, MUN, and SCC at the Dairy Herd Improvement Laboratory (Edmonton, AB, Canada). Daily milk and milk component yields were subsequently calculated.

In addition, the AMS recorded the number of visits for each cow to the AMS (milking frequency), time of milking, kick-offs during milking, incomplete milking events, and the time and date that cows passed through selection gates. These data were used to calculate milking duration, inter-milking intervals, incidence of kick-offs during milking, and incomplete milking events. These data were also used to calculate the amount of time spent in the holding area prior to milking along with the number of times cows passed through the sort gates but were not provided permission to enter the holding pen.

The lying time, standing time, and lying and standing bouts were measured during the behavioral phase (d 20 to 24) using accelerometers (HOBO Pendant G Acceleration Data Loggers, Onset Computer Corp., Bourne, MA) that were attached horizontally to the left hind leg of each cow, using a flexible bandage, on d 19 of each experimental period. The loggers were set to record the position of the leg every 30 s (Ledgerwood et al., 2010). After removal, the measurements were used to calculate total daily standing and lying time (min/d), the frequency of each bout (no./d), and their duration (min/bout) for the experimental period according to Zobel and Chapinal (2013).

3.2.2.4 Ruminal fermentation.

Ruminal pH was measured during the behavioral period (d 20 to 24) using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA) as described by Penner et al. (2006). The indwelling pH systems were standardized in pH buffer solutions 7 and 4 (Fisher Chemical, Ottawa, ON, Canada) at 39ºC, and set to record mV values every 5 min. The pH systems were inserted in the ventral sac of the rumen on d 19 of each experimental period and were maintained in the ventral sac using two 1-kg weights attached to the electrode shroud. Although inserted on d 19, only data obtained from d 20 to d 24 of each experimental period were used enabling data collection for 4 complete d. Upon removal from the rumen, the pH systems were cleaned, standardized, and the data were downloaded. Data from the starting and ending standardizations were used to derive 2 linear relationships between mV readings and pH. Using the linear relationships, the mV data were converted to pH values using a linear offset between the starting and ending slopes and intercepts. The daily ruminal pH values were then summarized as minimum, mean, and maximum pH for each cow. The duration and area that ruminal pH was below 5.8 were calculated according to Penner et al. (2007).

During the last 4 d of each experimental period (d 25 to 28) samples of ruminal digesta and feces were collected every 12 h over a 96-h period, with a 3-h offset between days such that the final combined composite (8 samples) was representative of a 24-h feeding cycle. For ruminal fluid, 250 mL of mixed digesta from each the cranial, ventral, and the caudal sac of the rumen were collected and combined. The digesta was then strained through 2 layers of cheesecloth and 10 mL of the strained ruminal fluid was added to a tube containing 2 mL of 25% (wt/v) meta-phosphoric acid and analyzed for short-chain fatty acid (SCFA) concentrations. An additional 10-mL sample was added to 2 mL of sulfuric acid for analysis of NH3-N. Samples were placed on ice until being stored at −20ºC. Short-chain fatty acid concentrations were determined according to Khorasani et al. (1996) and NH3-N was determined according to Fawcett and Scott (1960).

At each fecal sampling time, a minimum of 200 g of feces were collected directly from the rectum. Subsequently, 125 g of the collected fecal sample from each sampling point was used to prepare a composite and was stored at −20ºC. Duplicate samples (500 g) of feces were dried in a forced-air oven at 55ºC to determine DM concentration. Fecal samples were ground and sent to Cumberland Valley Analytical Services (Hagerstown, MD, USA) for chemical analysis as previously described. The concentration of uNDF in the feed, refusals, and fecal samples were utilized to determine uNDF intake to enable prediction of fecal output and apparent total tract digestibility (Huhtanen et al., 1994).

3.3 Statistical analysis

Data were analyzed as a replicated 4×4 Latin square with a 2×2 factorial treatment arrangement using the MIXED procedure of SAS (9.4, SAS Institute Inc., Cary, NC). The model included the fixed effects of square, period, PMR, AMS, and the interaction of PMR × AMS, and the random effect of cow nested in square. For variables that incorporated repeated measures (AMS concentrate and PMR intake), day was included in the model and covariance error structures were tested. The covariance error structure for each variable that yielded the lowest Akaike's information criterion (AIC) and Bayesian information criterion (BIC) were utilized. When the F-test for the interaction was significant, means were separated and analyzed using a Bonferroni mean separation test. To determine whether the means for sorting index analysis were different from 100%, a 2-tailed t-test analysis was used. Statistical significance was declared when $P \leq 0.05$ and tendencies are discussed when $0.10 \geq P > 0.05$.

3.4 Results

3.4.1 Body weight, BCS, DMI, PMR and AMS intake

Cow BW and BCS did not differ among treatments (Table 3.2). In addition, total DMI (PMR + AMS) was not affected by PMR or AMS treatments with an average DMI of 27.3 kg/d. While PMR intake was not affected by the F:C ratio of the PMR, feeding a greater quantity of concentrate in the AMS reduced PMR intake (24.9 vs. 21.4 kg/d; $P < 0.01$). As a result, for every 1 kg increase in concentrate allocation in the AMS, cows decreased PMR intake by 0.83 kg. The number of PMR meals/d, meal size, eating rate, and inter-meal interval were not affected by the amount of concentrate offered in the AMS or by the F:C ratio of the PMR ($P > 0.10$). Partial mixed ration eating time (min/meal) was not affected but total PMR eating time was greater when cows were fed the L-AMS compared to cows fed the H-AMS (205.39 vs. 177.24 min/d; $P < 0.01$). Cows consuming L-FOR selected against particles retained on the 8-mm sieve of the PSPS to a greater

Table 3.2. Effect of feeding a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation on BW, BCS, DMI, behavior associated with PMR intake, PMR sorting behavior, and AMS intake.

	L-FOR		H-FOR			*P* value		
Variable	L-AMS	H-AMS	L-AMS	H-AMS	SEM[1]	PMR	AMS	PMR × AMS
BW, kg	707	710	706	705	19.5	0.45	0.80	0.56
BCS[2]	3.13	3.19	3.13	3.13	0.14	0.57	0.57	0.57
Total DMI[3], kg/d	27.2	27.7	26.7	27.5	0.86	0.46	0.18	0.68
PMR eating characteristics								
PMR DMI, kg/d	25.2	21.6	24.6	21.3	0.8	0.39	< 0.01	0.85
Minimum PMR intake, kg/d	22.3	19.3	21.3	18.8	1.1	0.27	< 0.01	0.66
Maximum PMR intake, kg/d	27.4	24.1	27.5	23.9	0.86	0.86	< 0.01	0.81
Daily SD, kg/d	1.70	1.58	2.05	1.71	0.28	0.21	0.22	0.55
Meals, no./d	6.22	5.59	5.94	5.60	0.72	0.80	0.37	0.78
Meal size, kg DM/meal	4.07	4.17	4.15	3.84	0.54	0.78	0.81	0.65
Eating rate, kg DM/min	0.10	0.11	0.11	0.09	0.01	0.66	0.77	0.20
Inter-meal interval, min	207.2	233.2	214.8	230.1	24.65	0.90	0.26	0.77
Eating time, min/meal	34.79	35.64	38.28	36.84	5.02	0.57	0.94	0.78
Eating time, min/d	198.77	174.11	212.01	180.37	11.42	0.26	< 0.01	0.68
PMR Sorting index[4], %								
19-mm sieve	93.90z	95.77z	96.73z	94.63z	3.07	0.74	0.96	0.44
8-mm sieve	99.42z	95.39z	100.53z	97.34z	0.60	0.02	< 0.01	0.50
4-mm sieve	103.79z	106.26z	103.61z	106.59z	0.93	0.93	< 0.01	0.78
Pan	92.64z	95.37z	92.32z	93.12z	1.78	0.43	0.28	0.55
AMS eating characteristics								
AMS DMI, kg/d	2.04	6.09	2.03	6.27	0.22	0.65	< 0.01	0.59
Minimum AMS intake, kg/d	1.69	4.71	1.61	5.01	0.27	0.60	< 0.01	0.37
Maximum AMS intake, kg/d	2.41	7.25	2.36	7.49	0.21	0.62	< 0.01	0.47
Daily standard deviation, kg/d	0.23	0.84	0.27	0.86	0.07	0.64	< 0.01	0.86

[1]SEM for the interaction is reported.
[2]Body condition score (BCS) was assessed using a 5-point scale according to Wildman et al. (1982).
[3]Total DMI was calculated as the sum of PMR intake and the AMS intake.
[4]The sorting index was calculated as described by Leonardi and Armentano (2003).
[Z]Indicates that means differ from 100% using a 2-tailed t-test

extent than cows consuming H-FOR (97.41 vs. 98.94%; P = 0.02). In addition, cows offered the H-AMS selected against particles retained on the 8-mm sieve (P < 0.01) and selected for particles retained on the 4-mm sieve (P < 0.01) to a greater extent than cows fed L-AMS.

The F:C ratio of the PMR did not affect AMS concentrate intake (P = 0.65; Table 3.2); however, by design, H-AMS cows consumed more (6.18 vs. 2.04 kg) than L-AMS cows (P < 0.01). Although the offered levels of concentrate in the AMS were as targeted (6 and 2 kg for H-AMS and L-AMS, respectively), the amount of concentrate that was potentially available for delivery in the AMS exceeded the amount offered as a requirement to ensure target AMS concentrate delivery was achieved. As a result, greater variability in daily AMS concentrate consumption was observed when cows were provided H-AMS compared to L-AMS (0.85 vs. 0.25 kg/d; P < 0.01).

3.4.2 Milking frequency, milk and milk component yield, and milking behavior in the AMS

Milking frequency was not affected by the F:C ratio of the PMR or by the amount of concentrate provided in the AMS (Table 3.3). In addition, no differences were observed for milk yield/milking, milking duration/milking, or inter-milking interval. The percentage of milkings that the milker was kicked-off and percentage of incomplete milkings did not differ among treatments. However, daily milk yield tended to be greater for cows fed L-FOR than H-FOR (39.3 kg/d vs. 37.9 kg/d; P = 0.10) and tended to be greater when fed H-AMS compared to L-AMS (39.2 kg/d vs. 38.0 kg/d; P = 0.10). Crude protein yield followed the same pattern as daily milk yield, while fat yield was not affected by the AMS or PMR treatments. Crude protein concentration was not affected by the F:C ratio of the PMR, but it was greater for cows fed H-AMS than L-AMS (3.24 vs. 3.20%; P = 0.04). Milk fat concentration was not affected by the F:C ratio of the PMR, but tended to be greater for cows fed L-AMS than H-AMS (3.63 vs. 3.51%; P = 0.09). For MUN, cows provided H-FOR had greater MUN than cows provided L-FOR (P < 0.01) and cows provided L-AMS had greater MUN than those fed H-AMS (P = 0.02).

3.4.3 Ruminal fermentation

Minimum pH tended to be greater for H-FOR than L-FOR (P = 0.09; Table 3.4), but mean and maximum pH did not differ. Cows fed H-AMS did not differ from cows fed L-AMS for minimum, mean, or maximum ruminal pH. The duration that pH was < 5.8 was not affected by F:C ratio of

Table 3.3. Effect of feeding a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation on milking activity, milk, milk component yield, and AMS performance.

	L-FOR		H-FOR			*P* value		
Variable	L-AMS	H-AMS	L-AMS	H-AMS	SEM[1]	PMR	AMS	PMR × AMS
Milking frequency, no./d	3.66	3.66	3.47	3.72	0.16	0.41	0.11	0.11
Milk yield, kg/milking	10.54	11.01	10.76	10.07	0.97	0.42	0.80	0.20
Milking duration, min/milking	7.06	7.28	7.13	7.39	0.86	0.68	0.28	0.94
Inter-milking interval, min	390.2	389.3	411.7	376.8	19.90	0.67	0.10	0.12
Kick-offs, %	8.51	7.06	13.14	8.91	5.16	0.19	0.24	0.56
Incomplete milking, %	6.25	8.54	6.01	12.03	5.35	0.64	0.24	0.60
Yield, kg/d								
Milk	38.5	40.0	37.4	38.4	2.13	0.10	0.10	0.73
Crude protein	1.23	1.30	1.19	1.24	0.07	0.10	0.07	0.74
Fat	1.36	1.36	1.38	1.35	0.07	0.93	0.76	0.54
Milk composition, %								
Crude protein	3.21	3.25	3.19	3.24	0.05	0.47	0.04	0.97
Fat	3.57	3.46	3.70	3.55	0.17	0.11	0.09	0.78
Lactose	4.59	4.61	4.54	4.57	0.06	< 0.01	0.04	0.44
MUN, mg/dl	13.30	12.31	14.41	13.66	0.53	< 0.01	0.02	0.74

[1]SEM for the interaction is reported.

Table 3.4. Effect of feeding a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation on ruminal fermentation: pH, SCFA, and Ammonia concentration.

	L-FOR		H-FOR			*P* value		
Variable	L-AMS	H-AMS	L-AMS	H-AMS	SEM[1]	PMR	AMS	PMR × AMS
Ruminal pH								
Minimum pH	5.37	5.21	5.40	5.43	0.10	0.09	0.36	0.15
Mean pH	6.15	5.94	6.14	6.12	0.09	0.23	0.14	0.20
Maximum pH	6.93	6.70	6.81	6.86	0.10	0.85	0.35	0.15
Duration < 5.8, min/d	233	516	209	219	132	0.13	0.18	0.21
Area < 5.8, pH × min/d	68.34	170.24	45.98	50.98	48.24	0.07	0.17	0.22
Total SCFA, m*M*	102.37[b]	109.35[a]	104.67[ab]	106.54[ab]	2.92	0.84	< 0.01	0.05
Acetic, mol/100 mol	59.09	56.53	60.51	58.51	1.03	< 0.01	< 0.01	0.56
Propionic, mol/100 mol	25.66	29.90	23.75	26.78	1.50	< 0.01	< 0.01	0.43
Isobutyric, mol/100 mol	0.72	0.62	0.75	0.69	0.03	0.03	< 0.01	0.27
Butyric, mol/100 mol	11.59	10.23	12.05	11.26	0.57	0.07	0.01	0.47
Isovaleric, mol/100 mol	1.05	0.91	1.05	1.02	0.05	0.22	0.05	0.21
Valeric, mol/100 mol	1.51	1.50	1.48	1.47	0.06	0.46	0.74	0.90
Caproic, mol/100 mol	0.38	0.31	0.41	0.28	0.05	0.97	0.05	0.45
NH_3-N, mg/dL	13.62	12.25	13.64	13.54	1.00	0.38	0.33	0.39

[1]SEM for the interaction is reported.

[ab]Means within a row with uncommon superscripts differ ($P < 0.05$).

the PMR or quantity of AMS concentrate. However, cows fed L-FOR tended to have greater area that pH was < 5.8 than cows fed H-FOR (P = 0.07).

When fed a L-FOR diet, feeding H-AMS increased total SCFA concentration relative to L-AMS, but no differences were detected for cows fed H-FOR regardless of the quantity of concentrate offered in the AMS (PMR × AMS, P = 0.05; Table 3.4). Cows fed L-FOR had less acetate and isobutyrate, greater propionate, and tended to have less butyrate as a molar proportion relative to cows fed H-FOR. While the concentration of acetate was less, propionate was greater, and the concentration of isobutyrate, butyrate, isovalerate, and caproate were less for cows fed H-AMS than L-AMS. Neither the F:C ratio of the PMR nor the amount of concentrate provided in the AMS affected ruminal ammonia concentration.

3.4.4 Total tract digestibility

Digestibility of DM and OM were greater for cows fed L-FOR relative to H-FOR and greater for cows fed H-AMS than L-AMS (P < 0.01; Table 3.5). Digestibility of ADF was greater for cows offered H-FOR compared to L-FOR (P = 0.01) and was also greater for cows fed L-AMS than H-AMS (P = 0.02). Neutral detergent fiber, CP, starch and ether extract digestibility were not affected by the F:C ratio of the PMR or by the amount of AMS concentrate provided.

3.4.5 Activity budgets: gate passing events, times in areas, lying and standing behavior

Cows offered H-FOR in combination with H-AMS tended to pass through the selection gate more than cows fed the other treatments (P = 0.08; Table 3.6). The number of rejections to the holding area did not differ among treatments, averaging over 5 rejections/d. Cows fed L-FOR spent 32.4 min/d more in the holding area than cows fed H-FOR (P = 0.04). However, when evaluated as holding area time/visit to the AMS, there was only a tendency for a greater duration of time in the holding area for cows fed L-FOR relative to cows fed H-FOR (P = 0.06). Cows fed H-AMS spent more time in the AMS relative to cows fed L-AMS (P = 0.05). In contrast, cows fed L-AMS spent more time consuming the PMR than cows fed H-AMS (P < 0.01). Standing and lying behavior were not affected by the amount of concentrate offered in the AMS or by the F:C ratio of the PMR.

Table 3.5. Effect of feeding a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation on total tract digestibility.

	L-FOR		H-FOR			*P* value		
Digestibility, % DM	L-AMS	H-AMS	L-AMS	H-AMS	SEM[1]	PMR	AMS	PMR × AMS
DM	64.18	65.37	62.23	63.91	0.62	< 0.01	< 0.01	0.60
OM	65.74	66.95	63.84	65.39	0.63	< 0.01	< 0.01	0.71
NDF	37.56	36.93	38.13	37.15	1.03	0.58	0.27	0.81
ADF	31.61	29.59	33.84	31.67	1.10	0.01	0.02	0.92
CP	66.86	66.38	64.43	66.13	0.87	0.14	0.49	0.22
Starch	91.65	92.47	91.75	91.64	0.79	0.56	0.58	0.47
Ether extract	82.28	82.05	81.77	82.84	0.62	0.82	0.48	0.28

[1]SEM for the interaction is reported.

Table 3.6. Effect of feeding a L-FOR (forage-to-concentrate ratio of the PMR of 54:46) or H-FOR (forage-to-concentrate ratio of the PMR of 64:36) in combination with a L-AMS (2 kg/d) or H-AMS (6 kg/d) AMS concentrate allocation on gate passing events, times in areas, and time expenditure by standing and lying.

	L-FOR		H-FOR			*P* value		
Variable	L-AMS	H-AMS	L-AMS	H-AMS	SEM[1]	PMR	AMS	PMR × AMS
Passes through the sort gate, no./d	8.59	8.22	7.91	10.06	0.93	0.41	0.21	0.08
Rejections to holding area, no./d	4.94	4.56	4.44	6.34	0.82	0.35	0.27	0.11
Time in holding area, min/d	124.7	101.3	85.4	75.8	20.6	0.04	0.28	0.65
Time in holding area, min/visit	34.2	27.7	25.5	19.4	6.9	0.06	0.16	0.96
Time in AMS, min/d	25.4	26.3	24.1	27.1	2.4	0.79	0.05	0.26
PMR eating time[2], min/d	198.8	174.1	212.0	180.4	11.4	0.26	< 0.01	0.68
Standing time, min/d	808.3	768.3	730.1	754.8	40.8	0.23	0.84	0.39
Standing bouts, no./d	4.38	4.80	4.75	4.77	0.46	0.60	0.51	0.53
Mean standing bout duration, min/bout	102.7	94.3	86.9	87.1	14.6	0.29	0.70	0.68
Lying time, min/d	631.7	671.8	709.9	685.2	40.8	0.23	0.84	0.39
Lying bouts, no./d	5.80	7.22	7.84	6.41	1.58	0.61	0.99	0.24
Lying bout duration, min/bout	68.6	59.1	60.8	69.2	6.1	0.84	0.93	0.14

[1]SEM for the interaction is reported.
[2]Data previously reported in Table 2 but included to provide a complete representation of behavioral responses.

3.5 Discussion

The focus of this study was to evaluate the effect of the F:C ratio of the PMR, the amount of AMS concentrate offered, and their interaction. The only detected interaction among treatments was for the ruminal SCFA concentration. This interaction suggests that when feeding a L-FOR PMR, increasing the concentrate allocation in the AMS results in detectable increases in the SCFA concentration in the rumen; while, with a H-FOR PMR, the amount of concentrate offered in the AMS does not affect SCFA concentration. While other fermentation parameters were not affected by the interaction, the increase in SCFA concentration can be expected as the F:C ratio was the least when L-FOR was fed with H-AMS (Penner et al., 2009).

The general lack of treatment interactions in this experiment is interesting given the marked changes in the dietary chemical composition, particularly the dietary aNDFOM and starch. As such, it appears that while considering both the PMR and AMS concentrate are necessary for dietary formulation and that increasing AMS concentrate reduces PMR intake, altering the F:C ratio of the PMR and the amount of concentrate in the AMS generally impose effects on production responses and feeding behaviour independently.

3.5.1 Effects arising from increased AMS concentrate allocation

To date, most studies have focused exclusively on changing the AMS allocation or composition (Halachmi et al., 2005; Migliorati et al., 2005) and only a few have considered that changes to the AMS concentrate may also affect consumption of the PMR (Bach et al., 2007; Hare et al., 2018). Based on survey data, the proportional contribution of the AMS concentrate likely does not exceed 40% of the total diet (Salfer and Endres, 2014). Thus, ignoring PMR intake when manipulating the AMS concentrate will likely preclude accurate prediction or interpretation of production outcomes. In the present study, the proportional contribution of the AMS concentrate was 7.12% of the diet (DM basis) for L-AMS and 21.35% of the diet for H-AMS. Consistent with previous studies in AMS, I observed that increasing the AMS concentrate allocation decreased PMR intake (Bach et al., 2007; Hare et al., 2018). Providing supplemental concentrate to grazing dairy cows has also been reported to reduce pasture DMI (Bargo et al., 2003; Tozer et al., 2004) or forage organic matter intake (Macoon et al., 2011). In addition, while cows fed H-AMS spent more time eating the concentrate in the AMS compared to cows fed L-AMS, PMR eating time (min/d) was greater for cows offered L-AMS than cows offered H-AMS.

In the present study, when the data from the L-FOR and H-FOR treatments were pooled, every 1 kg DM increase in AMS concentrate consumed decreased PMR DMI by 0.83 kg, suggesting that increasing the AMS concentrate allocation may increase nutrient intake (Bargo et al., 2003). However, the substitution response may differ based on F:C ratio of the PMR as the calculated substitution rates were 0.89 when fed the L-FOR PMR and 0.78 when fed the H-FOR PMR. While I am unable to compare these values statistically, these data indicate that feeding a more energy-dense PMR increases the substitution rate. The difference in substitution rate between the L-FOR and H-FOR PMR supports previous research with grazing cattle where improved forage quality increases the substitution rate (Fieser and Vanzant, 2004).

In contrast to the substitution effects that were less than 1 in the present study, Bach et al. (2007a) reported (DM basis) that for every 1 kg increase in AMS concentrate there was a 1.14 kg reduction in PMR consumed and Hare et al. (2018) reported a reduction of 1.58 kg of PMR intake for every 1 kg increase in AMS concentrate. The differences observed in the substitution rates between the present study and previous studies may be due to dietary formulation strategies and physiological state of cattle in the study. For example, Bach et al. (2007a) and Hare et al. (2018) attempted to provide isocaloric diets by shifting concentrate from the PMR to the AMS. In the present study, I purposely increased the nutrient density with the H-AMS vs. L-AMS and with the L-FOR vs. H-FOR treatments. Furthermore, given that the cattle in the present study were in mid-lactation, it is likely that rumen fill was limiting DMI (Allen et al., 2009) and decreasing the F:C ratio by offering H-AMS may have allowed for numerically greater DMI. Additionally, milk production in the present study was markedly greater than that in Bach et al. (2007a) and Hare et al. (2018), suggesting that physiological state or nutrient demand may alter responses to increased dietary energy density. For example, in the present study, cows were in mid-lactation, with an average DIM of 140.5 ± 13.6 (mean ± SD) and 168.5 ± 9.7 for the respective squares. Bach et al. (2007a) utilized 115 cows in mid-lactation; however, the cows in that study were, on average, 191 ± 2.1 DIM and also incorporated production responses from multiparous and primiparous cows. Hare et al. (2018) also combined responses for primiparous and multiparous cows that had lower milk yield than in the present study. While I cannot determine the cause for the substitution effect in the present study, total PMR eating time and ruminal pH were not affected by the F:C ratio of the PMR suggesting that factors other than those specified for grazing cows (Bargo et al., 2003) may influence the substitution rate for cows in AMS.

Although previous studies have assessed increasing the AMS concentrate allocation, cows in those studies did not consume their full AMS allocation (Halachmi et al., 2005; Migliorati et al., 2005; Bach et al., 2007a). For example, Bach et al. (2007a) targeted consumption of 3 or 8 kg/d (DM basis) of AMS concentrate, but cows only consumed 2.6 or 6.9 kg/d (DM basis), respectively. Halachmi et al. (2005) targeted either 1.2 kg/visit or 7 kg/d but achieved consumption of 3.85 and 5.2 kg/d, respectively. In the present study, I ensured that consumption of the AMS concentrate was equal to the target by adjusting the potentially eligible concentrate such that it was in excess of the target. For example, to achieve target AMS concentrate intake, cows fed L-AMS were eligible to receive 2.07 kg DM/d and cows fed H-AMS were eligible to receive 6.55 kg DM/d. As a consequence, feeding a greater quantity of concentrate in the AMS resulted in a greater SD in concentrate intake among days for individual cows. To our knowledge, there are no previous studies that have evaluated the variability in concentrate intake among days as affected by the quantity offered in the AMS. Despite greater variation among days for cows fed H-AMS than L-AMS, cows did not increase their variability for PMR intake regardless of the F:C ratio within the PMR. The greater variability in AMS concentrate intake with increasing AMS provision diminishes the ability to impose precision feeding strategies when using a single pellet. However, precision feeding strategies could also integrate the use of more than one pellet allowing for dietary compositional changes without markedly increasing the amount of pellet offered in the AMS (Bach and Cabrera, 2017). Future research is needed to evaluate the magnitude of variation in AMS concentrate allocation as affected by pellet composition (starch vs. fiber vs. protein), physiological state, and the implications on achieving precision feeding strategies and production outcomes.

Recommendations for AMS feeding management suggest that providing a greater quantity of concentrate within the AMS will increase voluntary visits and milk yield (Rodenburg, 2011). In our study, increasing the amount of AMS concentrate from 2 to 6 kg (DM basis) did not affect milking frequency, but tended to increase daily milk yield by 1.25 kg. Others have reported that increasing the quantity of concentrate offered in the AMS did not result in improved milking frequency or milk production (Migliorati et al., 2005; Bach et al., 2007a; Hare et al., 2018). Even in free-traffic flow conditions, increasing the AMS concentrate allocation was correlated with reduced milk yield (Tremblay et al., 2016). As such, motivation to enter the AMS seems not to be primarily affected by the amount of concentrate offered within the AMS under free-flow cow traffic (Halachmi et al., 2005; Bach et al., 2007a; Tremblay et al., 2016) or guided cow traffic

(Migliorati et al., 2005; Hare et al., 2018). In addition to the lack of a stimulatory effect on milking frequency with greater concentrate provision, time spent in the holding area prior to milking was not affected by the amount of concentrate provided in the AMS. This may suggest that increasing the amount of AMS concentrate allocation above 2 kg/d may not improve motivation to enter the AMS.

In our study, increasing the AMS concentrate allocation increased dietary energy density, likely accounting for the tendency for increased milk yield despite no changes in milking frequency. In fact, using calculations from NRC (2001), cows fed H-AMS consumed 47.6 Mcal of NE_L vs. 45.0 Mcal of NE_L (data not shown) consumed by cows fed L-AMS. The studies of Bach et al. (2007a) and Hare et al. (2018) fed isocaloric diets when comparing low and high concentrate allocations in the AMS and reported no treatment effect, likely due to the lack of change in predicted energy intake. By increasing the amount of concentrate offered in the AMS, digestibility of OM and DM was increased, providing further explanation for the trend for greater milk yield for cows fed H-AMS relative to L-AMS.

Along with a tendency for greater milk yield, milk CP yield tended to be greater, CP concentration was greater, and fat concentration tended to be less for H-AMS cows than L-AMS cows. The greater CP concentration for cows fed H-AMS compared with cows under L-AMS treatments is not surprising when considering the greater fermentable energy intake and the expected increase in metabolizable protein supply as previously mentioned (Grant and Kononoff, 2007). While milk fat concentration tended to be lower for cows fed H-AMS relative to cows fed L-AMS, the response was likely due to dilution when considering that milk fat yield was not affected. Miron et al. (2004) also observed a reduction in milk fat percentage when feeding greater quantities of concentrate in the AMS.

To date, I am unaware of studies characterizing the impact of AMS concentrate allocation on ruminal fermentation. In the current study, I expected to see a reduction in ruminal pH for cows fed H-AMS relative to cows fed L-AMS; however, I did not observe such a response. The lack of a ruminal pH response may be partially due to variable AMS concentrate intake among days for cows fed greater quantities of AMS concentrate, variable timing of AMS concentrate provision, altered PMR eating and sorting characteristics, and the relatively small concentrate meals provided in the AMS. In particular, substantial day-to-day variation in AMS concentrate intake may have disguised the direct effect of providing a greater amount of concentrate in the AMS on ruminal pH. In addition to the varying day-to-day AMS concentrate consumption, cows offered H-AMS, on average, visited the robot 3.69 times/d and consumed 1.67 kg (DM basis) of AMS concentrate/milking with a maximum of 2.5 kg/visit, regardless of the AMS concentrate treatment. Thus, small amounts (< 2.5 kg) of concentrate provided in a single meal may simply not have a marked effect on ruminal pH. That said, I cannot eliminate the possibility that PMR eating and sorting characteristics may have also impacted the outcome as cows in the present study were required to access the PMR feeding area prior to entering the selection gate that provided access to the AMS. Thus, the consumption of the PMR and the reduction in selection against particles retained on the 8-mm sieve and a reduction in selection for particles retained on the 4-mm sieve for cows fed L-AMS vs. H-AMS may have modulated the ruminal pH response (DeVries et al., 2008), independent of the AMS concentrate allocation. Although ruminal pH was not affected by the amount of concentrate provided in the AMS, there was an interaction for SCFA concentration where cows fed L-FOR had a greater SCFA concentration while being fed greater quantities of concentrate in the AMS. Although there are no studies showing SCFA responses in AMS, this result potentiates the greater energy supply as AMS concentrate increased and may further suggest that fermentation responses were evident. Future research is needed to evaluate ruminal fermentation responses for cows managed in AMS.

3.5.2 Effects arising from increased concentrate allocation in the PMR

To our knowledge, no studies have evaluated the impact of different F:C ratios within the PMR on performance outcomes for cows milked using AMS. In the present study, the PMR accounted for 92.9 and 78.7% of the dietary DM for the L-AMS and H-AMS treatments, respectively. Increasing the proportion of concentrate within the PMR from 36 to 46% tended to increase daily milk yield without changing milking frequency. Hare et al. (2018) reported a tendency for increased milk yield when the PMR contained a greater energy density; however, in that study, the diets were isocaloric. This may suggest that PMR energy density is unlikely to negatively affect milk yield and voluntary visits to the AMS. The greater milk yield response for cows fed L-FOR compared to H-FOR in the present study is likely due to a greater energy supply with a predicted 1 Mcal/d greater energy intake for cows fed L-FOR. Moreover, feeding L-FOR resulted in greater digestibility of DM and OM compared to H-FOR, further suggesting greater nutrient availability for cows fed L-FOR.

Despite a tendency for greater milk yield, feeding the L-FOR PMR may have reduced the motivation to enter the AMS based on greater time spent in the holding area relative to cows fed a H-FOR. The lack of motivation highlights the potential impact that PMR formulation may have on cow activity budgets and may challenge the use of a L-FOR feeding strategy for cows housed in free-traffic flow barns. However, the number of visits to the AMS were not reduced when feeding L-FOR compared to H-FOR, likely due to the selection gate and milking criteria settings in the guided-flow system utilized in this study. Clearly, more research is needed to assess activity budgets of cows and implications on performance outcomes when altering the PMR energy density and to determine whether recommendations should consider cow-traffic flow design.

Altering the F:C ratio of the PMR did not affect PMR intake or AMS intake. This suggests that PMR consumption is more affected by the amount of concentrate offered in the AMS than the energy density of the PMR itself. Moreover, the F:C ratio of the PMR did not affect variability in PMR consumption among days. Similar to Hare et al. (2018), I did not observe any changes in PMR eating behavior and only minimal changes in PMR sorting characteristics when altering the PMR F:C ratio. These results suggest that although PMR intake is impaired by the greater amount of concentrate provided in the AMS, PMR eating behavior remains stable regardless of differences within the PMR. That said, both studies were conducted in a guided-flow system, which may also impose differences in eating behavior pattern relative to other layout designs. More research is needed since past studies, such as DeVries et al. (2007), have reported differences in eating behaviour when changing the F:C ratio; however, previous studies have been conducted with total mixed ration feeding scenarios rather than with PMR.

With respect to ruminal pH, minimum pH tended to be greater for cows fed H-FOR than L-FOR and the area that pH < 5.8 tended to be greater for cows fed L-FOR than H-FOR. The response in ruminal pH is not surprising given the greater proportion of concentrate in the PMR and greater digestibility. In addition, the reduction in the proportion of acetate and increase in propionate concentrations observed in this study are consistent with a decreased F:C ratio diets (Kljak et al., 2017).

While there are numerous studies evaluating the F:C ratio of the TMR on performance outcomes for dairy cows (Voelker et al., 2002; Mäntysaari et al., 2003; Kargar et al., 2010), there are no studies providing such information for cows using AMS. This research is needed as altering the PMR has been previously suggested to affect the AMS concentrate feeding strategy. In a recent study, Tremblay et al. (2016) suggested that the use of a PMR with a high F:C ratio or a PMR with low forage quality would likely require the provision of greater quantities of concentrate in the AMS. In fact, the authors of that study rationalized that low-quality forages, in particular, would require greater AMS concentrate provision and that under such situations, AMS concentrate provision was negatively correlated with milk production. However, no information was provided on PMR composition or PMR intake in that study. Results from the present study suggest that the PMR and AMS concentrate allocation independently affect production responses, thereby providing further justification that the PMR composition and intake must be considered.

3.6 Conclusion

The data in the present study are interpreted to suggest that the F:C ratio of the PMR and the quantity of concentrate offered in the AMS act independently on performance outcomes. Our results indicate that the quantity of AMS concentrate offered reduces PMR intake with only marginal effects on milk and milk component yield, but feeding a greater amount of concentrate in the AMS increases day-to-day variability in AMS concentrate consumption challenging the notion of precision feeding when simply increasing the amount of pellet offered in the AMS. In addition, providing a greater proportion of concentrate in the PMR may improve milk yield without increasing variability in PMR or AMS concentrate intake, but may result in reduced ruminal pH.

4.0 GENERAL DISCUSSION

The use of AMS is continuously increasing around the globe (de Koning, 2010; Haan et al., 2012); but, lack of information and guidance regarding feeding management, in an attempt to optimize performance and maximize profitability in herds using this technology, is still a weakness that may be a barrier for producers that have already adopted this technology or for future AMS adoption. The general objective of this study was then to provide a better understanding of feeding management for cows housed in AMS by evaluating the effects and the interactions between the F:C ratio within the PMR and the amount of concentrate provided in the AMS.

4.1 Importance of PMR and AMS Concentrate when Evaluating AMS

Feeding management for cows under AMS differs from conventional milking systems where a TMR is provided. In order to assure an accurate interpretation of the outcomes in farms using AMS, the feeding management must account for the PMR and AMS characteristics as contributing components of the total diet. Most of the studies in the past have primarily focused on the effect of different AMS concentrate feeding strategies on performance outcomes while ignoring or not reporting PMR characteristics (Migliorati et al., 2005; Halachmi et al., 2005; Tremblay et al., 2016). However, based on survey data, PMR contributes at least 60% of the total diet, implying that PMR it is the major component of the diet for dairy cattle within AMS. While I did not observe the expected interactions between the main components of the total diet in the majority of the variables evaluated under this study, the results of the research conducted within this thesis reinforce the importance of the AMS concentrate and PMR when analyzing production and behaviour outcomes as both the PMR and AMS concentrate independently affected production and behavioral outcomes.

To my knowledge, all the studies that have considered PMR characteristics, have seen a substitution effect, implying less PMR DMI as the amount of concentrate allocated in the AMS increases (Bach et al., 2007a; Hare et al., 2018). According to Bach et al. (2007a) for every 1 kg of AMS concentrate consumed, PMR intake was reduced by 1.14 kg, while Hare et al. (2018) reported 1.58 less PMR DMI for every 1 kg DM increase in AMS concentrate. Past studies have also reported a substitution effect in pasture-based systems (Bargo et al., 2003; Tozer et al., 2004).

In the present study a substitution effect of 0.83 kg reduction in PMR intake was observed for every 1 kg increase in AMS concentrate intake. While part of the differences in the substitution ratio may be due to differences in dietary formulation and physiological state of the animals, all the studies indicate that increasing the amount of concentrate offered in the AMS alter the amount of PMR consumed from the feed bunk; thus, altering the final F:C of the desired whole diet, with potentially negative impacts, such as lameness and digestive upsets (Bach et al., 2007b), with no improvements of milking visits. The substitution effect may partially explain why only marginal improvements for milk yield are observed with increased AMS concentrate as substitution shifts in the F:C ratio of the final diet consumed and the reduction in PMR intake is not predictable.

In this study I further hypothesized that altering the F:C ratio of the PMR would affect the substitution rate. Indeed, feeding a L-FOR may increase the reduction in PMR intake, implying that, although I couldn't compare these values statistically, PMR consumption may be affected by both the AMS feeding strategy and the PMR energy density.

Regarding performance responses, offering a PMR with a 54:46% F:C tended to improve milk yield compared to a PMR with 64:36% F:C that suggests that improving the energy supply within the PMR may also improve milk yield. Interestingly, cows fed L-FOR PMR spent more time in the holding area, which it may be interpreted as less motivation to enter the AMS or "lazy" cows; however, cows did not modify their milking frequency when altering the F:C ratio of the PMR, suggesting that cows under a greater PMR energy density may improve milk yield without negatively affect milking visits. That said, it should be valuable to evaluate whether cow-traffic design may impose a different activity budgets and production responses when changing the F:C ratio of the PMR.

4.2 Developing Precision Feeding Strategies in AMS

A more precise feeding approach seems to be a plausible opportunity with the use of AMS as producers may have a closer control of what the cows are consuming within the two different locations where feed is available. In addition, the possibility of varying the quantity of concentrate offered and type of concentrate offered (assuming more than 1 feed can be provided) in the AMS seems to allow cows to more accurate meet their requirements according to their potential milk production. The general recommendation to allow for precision feeding is to allocate cows with greater milk yield a greater quantity of concentrate in the AMS. The latter strategy is believed to improve milking frequency and to diminish fetching activity (Bach and Cabrera, 2017). However, based on the results of this study, the idea of increasing concentrate in the AMS to improve milking frequency and milk yield are challenged.

To begin with, precision feeding may not be possible without accounting for responses at the PMR. The research within this thesis has provided more evidence supporting that the provision of concentrate in the AMS particularly affects PMR intake. Similar to previous studies (Bach et al., 2007a; Hare et al., 2018), increasing the amount of concentrate in the AMS does not stimulate DMI, rather cows substitute PMR for the concentrate delivered in the AMS. Moreover, since most past studies have ignored PMR intake cows are more at risk to suffer the consequences from an inadvertent shift in the F:C ratio caused by changes in the substitution rate. Presently it is not clear whether, but could be expected that it does, differ with stage of lactation.

Moreover, the research conducted in this thesis showed that AMS may be adjusted to ensure the amount of concentrate delivered and the targeted amount consumed are equivalent. To my knowledge, only one previous study (Hare et al., 2018) purposely increased the targeted concentrate offered as an approach to avoid under-consumption of the AMS concentrate. In past studies conducted with AMS, the targeted amount of concentrate to be consumed was not achieved, with greater discrepancies seen through a greater concentrate provision (Halachmi et al., 2005; Migliorati et al., 2005; Bach et al., 2007a). Concentrate consumption less than the formulated value should be considered as a major concern. In this study, I was successful assuring that the amount of concentrate consumed was according to our treatment targeted valued; however, the approach implemented was to increase the eligible amount of concentrate in the AMS such that it was greater than the target consumed value. This approach coupled with the calibration procedure conducted every week assured that the amount delivered was in accordance with the eligible amount, and helped to assure that the target AMS concentrate amount was achieved for each treatment.

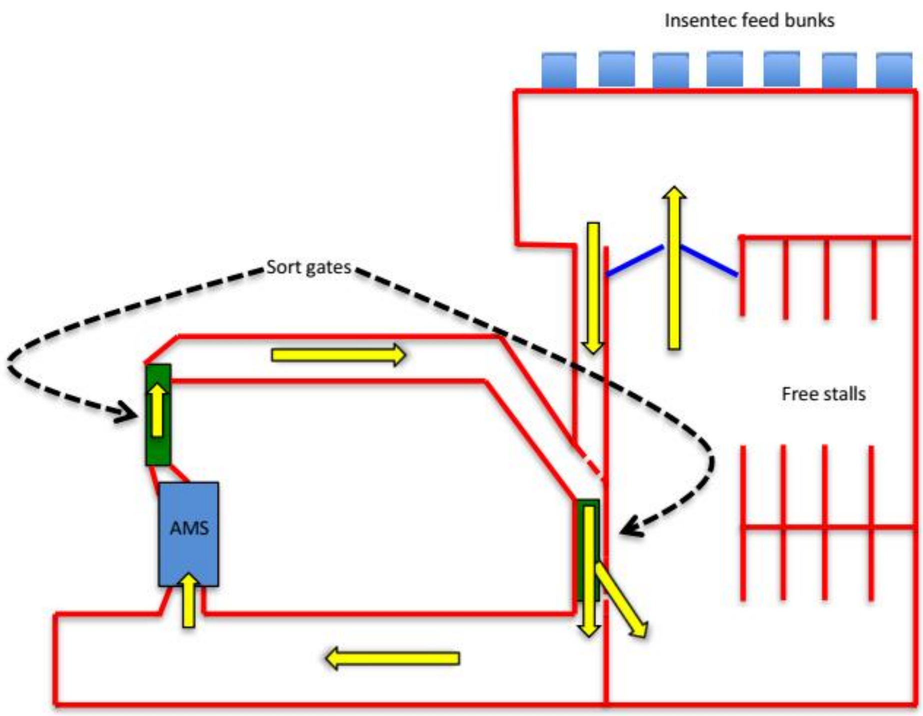
Interestingly, I found that feeding a greater quantity of concentrate in the AMS (6 vs. 2 kg) in, resulted in a greater variability in concentrate intake across days. This is, as far as I am aware, the first study providing evidence of variability in concentrate intake and its implications. Our results showed a greater SD in concentrate consumed with increased provision, challenging the ability of AMS to achieve precision feeding and reducing predictability in production responses. To our surprise, the variability seen in concentrate intake did not correspond with a variability in PMR intake. Ruminal pH was also not affected by the variability in concentrate intake. The results observed in this study implies that precision feeding may be challenged when the approach relies on feeding a greater quantity of concentrate.

4.3 Implications in Other Types of Cow Traffic Systems

While the goals of the feeding program in AMS do not vary regarding the differences in cow traffic systems, the strategies used to achieve those goals may differ. To my knowledge, there are no studies so far that have evaluated the influence of cow traffic systems on feeding management. It appears that the amount of concentrate that producers are offering in the AMS are greater in free cow traffic barns than in guided and forced traffic barns (Salfer and Endres, 2014). However, the available research, conducted in a variety of cow traffic scenarios, suggests that providing more concentrate in the AMS may not increase motivation to enter the AMS or may only subtly improve motivation, milk yield, and milk component yield (Halachmi et al., 2005, Migliorati et al., 2005; Bach et al., 2007a; Tremblay et al., 2016; Hare et al., 2018).

Both studies by Migliorati et al (2005) and Hare et al. (2018) were applied on guided traffic systems. However, in Migliorati et al. (2005), cows were managed in a milk-first guided traffic system, while Hare et al. (2018) evaluated feeding management under a feed-first guided traffic system. Despite the differences imposed by these two types of guided traffic systems, production responses showed similar trends. Neither of those studies reported an improvement in milk yield nor in milking frequency through incrementing the concentrate provision in the AMS. On the other hand, Halachmi et al. (2005), and Bach et al. (2007a), used a free-cow traffic design, again showing similar responses in terms of increasing the concentrate allocation in the AMS.

The current study was conducted in the same facility as Hare et al. (2018) and as such utilized a feed-first guided traffic system (Figure 4.1). However, the specific objective pursued



Courtesy of Koryn Hare.

Figure 4.1. Diagram of the feed-first guided cow traffic system at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, SK, Canada).

Cows could move freely from the lying to the feeding area through a one-way gate. The selection gate was placed on route of the return to the lying area from the feeding area. Only the cows that met the milking criteria were directed towards the holding area in front of the AMS.

was to evaluate the effects of providing different F:C ratio within the PMR coupled with different amount of concentrate in the AMS along with their possible interactions. Thus, the diets obtained were not all isoenergetic as was the case in Hare et al. (2018). Results from both studies showed that regardless of the dietary formulation, cows with greater AMS concentrate may not improve the motivation to attend the AMS more frequently. Hare et al. (2018) reported a tendency for an increased milk yield when lower amount of concentrate was offered in the AMS and greater PMR energy density was offered at the feed bunks (isocaloric diets). This current study demonstrates that providing a L-FOR PMR tended to increase milk yield, and feeding greater amount of concentrate may also improve milk yield likely due to an increase in total dietary energy supply (PMR + AMS).

In addition to production responses, PMR eating behaviour, ruminal pH, and activity budgets showed interesting responses. Our current study showed that PMR eating behaviour was not influenced by the F:C ratio within the PMR, while DeVries et al. (2007) showed differences in eating behavior while changing the F:C ratio of a TMR. Similar to my findings, Hare et al. (2018) did not show changes in PMR eating behavior but both studies were conducted on guided traffic systems. Even though the diet in DeVries et al. (2007) was a TMR, the lack of response in the present study may be partially due to the guided traffic system.

Furthermore, I expected to see a reduction in ruminal pH when offering H-AMS relative to L-AMS (6 vs. 2 kg DM/cow/d), but results suggest that ruminal pH was not affected by the amount of concentrate offered in the AMS, while it was affected by the F:C ratio of the PMR. One of the possible explanations may be imposed by the design, where cows under feed-first guided cow traffic may visit first the feed bunk with the PMR provision prior to the AMS. However, this assumption requires more research as cows may not follow the assumed pattern of feed behaviour even in a guided cow traffic. Another explanation is that with maximum AMS delivery/milking constrained to 2.5 kg as fed, the quantity of AMS concentrate consumed in an individual meal is not large enough to induce changes in ruminal pH. Moreover, considering that the PMR occupied the bulk majority (78.65 and 92.88%, respectively) of the diet for both the L-AMS and H-AMS treatments, it is perhaps not that surprising that the PMR had a greater effect.

Finally, cows under L-FOR PMR spent more time in the holding area, suggesting less motivation to enter the AMS. However, it is not clear whether this response is due to PMR energy density or whether the guided traffic system may have an impact on time budget of cows, affecting performance outcomes in return.

4.4 Motivation to Enter the AMS

Feeding management for cows housed in AMS relies on an understanding of both nutrition and behavior, and it is clear that eating in the AMS represents a greater motivating factor relative to the reward of the milking process itself (Prescott et al., 1998). Since most of the AMS farms use pelleted feed in the AMS (Bently et al., 2013), part of the objective was to evaluate the effect of offering differential levels of concentrate offered as a pelleted source on milking performance. It should be noted that milking permission for each cow was conceded after 4 h had elapsed since the previous milking event or if predicted milk yield was greater than 9 kg. With these unrestricted milking criteria, I attempted to observe behavioural responses, such as milking visits with minimal system limitations. This study showed that varying the quantity of concentrate in the AMS (6 vs 2 kg DM/cow/day) did not affect milking frequency. Similarly, past studies have reported a similar response to increasing AMS concentrate allocation (Halachmi et al., 2005; Migliorati et al., 2005; Bach et al., 2007a; Hare et al., 2018). In addition, Tremblay et al. (2016) reported that positive effects on the number of successful milking events diminish as the amount of concentrates provided in the AMS increase. Therefore, this study helps to demystify the general rationalized idea that greater concentrate allocation equates greater voluntary visits to the AMS and thus milk production.

While the amount of pellet offered in the AMS may not be the primarily motivator to enter the AMS, the physical form and the palatability of the of ingredients used as concentrate sources may be other important factors to consider in terms of intake and milking visit. It seems that hard (Rodenburg et al., 2004) and palatable pellets are preferable than other forms (Spörndly and Asberg, 2006). Rodenburg et al. (2004) indicated that DMI of cows diminish as percentage of fines and crumbled pellets increases. In addition, Madsen et al. (2010) evaluated pellet containing a variety of ingredients, such as barley, barley-oat mix, maize, wheat, artificially dried grass, or pellet with added fat, with a single PMR fed to all the cows and they concluded that cows would prefer a mixture of barley and oats concentrates rather than concentrate based on single ingredients. In addition, they indicated that wheat was preferred than barley or corn. The preference for these types of concentrates was observed through greater milking visits when these types of concentrate were provided relative to a standard pelleted concentrate.

In addition, external substances known as flavor-appetizing substances (FAS) can be incorporated into the concentrate to increase the voluntary attractiveness of the milking stall (Migliorati et al., 2005, 2009). Migliorati et al. (2009) showed an increase in milking frequency when FAS were included. While Migliorati et al. (2005) found no differences in milking frequency when testing the addition of FAS vs. no addition of FAS to the concentrate, the effect of FAS on the concentrate may have influenced the attractiveness towards the AMS as cows that were provided FAS had higher number of visit to the pre-selection gate. Harper et al. (2016) showed no differences in milking frequency and DMI when offering flavored concentrates with fenugreek or vanilla. Suggesting that more research may be needed to clarify whether FAS make a difference in terms of enhancing voluntary visits and number of milkings per day.

The chemical composition of the concentrate is another factor to consider. Feeding concentrate with a high amount of starch, particularly when a high amount of concentrate is included in the AMS, increases the rate of fermentation in the rumen and may affect ruminal pH (Dieho et al.,2016), putting cows at higher risk to develop SARA (Humer et al., 2018) and lameness (Oba and Wertz-Lutz, 2011), which may in turn affect milking frequency, milk production and composition. For example, Miron et al. (2004) reported an increase in milk protein content when a concentrate high in starch was used, and feeding concentrate low in starch and high in digestible fiber, like soybean hulls, increased milk fat content. Halachmi et al. (2006) reported no differences in milking frequency, milk yield and components when feeding lower level of starch (49 vs 25% of starch). In addition, Halachmi et al. (2009) concluded that feeding pellets high in digestible NDF do not impair DMI, milking frequency, milk yield or composition. These results suggest that an alternative pellet with low starch can be utilized without negative effects in milking frequency.

It should be noted that a lower amount of concentrate provision in the AMS may lead to other opportunities, such as the use of simple feeds instead of pellets. While the present study did not evaluate palatability of the pellet, the results suggest that lower quantities of AMS concentrate can be provided. Extrapolating this data, it could be suggested that with low AMS concentrate allocation, other options other than pellets as an AMS concentrate (e.g. flaked cereal grains) may have potential. Although research is needed to confirm these results, overall these data implies that there is more flexibility in feeding strategies when low amount of concentrate is supply in the AMS.

4.5 Future Research Needs

With the expansion in herds utilizing AMS, there has been an increased focus in feeding management due to the implications on productivity and animal health. Feeding management for cows managed in AMS is still an area increasing in importance to support efficient and cost-effective milk production. The present study contributes to improve our understanding of feeding management for cows under AMS and expands the possibilities to further evaluate circumstances and combinations that could be applicable to AMS. That said, there appears to be many future opportunities for research to promote optimal performance and maximize profitability in these systems.

As eluded previously, this is the first study where variability in concentrate intake has been reported. As the provision of concentrate in the AMS increases, variability in concentrate intake across days also does. However, subsequent studies are needed to determine the effect of either physiological state of the cows or stage of lactation on the magnitude of the variation. Further, more research is needed to assess the effect of the variability in AMS concentrate intake on PMR intake across days when using cows on different stage of lactation and when PMR formulation strategies and AMS formulation strategies vary. Variability in PMR intake and AMS concentrate intake is a major concern that should be taken into consideration to assure precision feeding.

While precision feeding strategies could be implemented in farms using AMS, there is limited work using different types of concentrates and in different amounts instead of using one type of concentrate in different amounts. As milk yield varies across the lactation, cows may not get a balanced nutrient supply through it. In addition, the variability in concentrate intake would aggravate this problem precluding precision in feeding management. Therefore, it seems necessary to evaluate the use of different types of concentrates to attempt a more accurate feeding strategy. As far as I am aware, Bach and Cabrera, (2017) is the only peer-reviewed study suggesting the use of multiple concentrates in order to make the AMS more efficient. However, Bach and Cabrera (2017) was a literature review and concrete evidence for this suggestion is lacking. For example, to evaluate the effect of different types of feed used as AMS supplement at different stages of lactation, and the interaction between their fermentability and the energy density of the PMR that may affect production and behavior responses. In addition, further research is needed to evaluate the effect of the physical form of the concentrate provided to enhance motivation, improve milk yield and composition.

Future research is needed to assess factors that may affect the substitution effect. While both Bach et al. (2007a) and Hare et al., (2018) utilized isocaloric diets, they differed in DIM and parity, and they ended up in different substitution rate. On the other hand, diets in the study described in this thesis were not isocaloric. However, cows used also differed in DIM and parity when compared to previous studies, and again, the substitution ratio was different. This may suggest that cow factors, such as DIM and parity may influence that substitution.

Precision feeding strategies commonly relies on concentrate provision in the AMS accordingly to milk yield, but it may be questionable whether milk yield is the most accurate factor to make that balance. Body weight measurement coupled with milk yield or energy output, and stage of lactation may be useful for precision feeding strategies.

Subsequent studies are also needed to determine the effect of PMR energy density on activity budgets and implications on performance outcomes on different cow traffic systems. Feeding a L-FOR may decrease motivation to enter in the AMS, as cows in this study spent more time in the holding area; however, these cows were managed in a guided-cow system. This result could suggest that activity budgets may challenge the use of L-FOR PMR feeding strategy when cows are housed in free cow traffic barns.

Moreover, ruminal fermentation responses should be assessed in future research using AMS, as it may be influenced by feeding behaviour and dietary strategies. In our study, ruminal pH was not affected by the provision of concentrate, but it was affected by the F:C ratio within the PMR. I personally believe that cow traffic design may not be such an influential factor over this response; however, more research is needed to elucidate this concern.

Although no interactions were detected for most of the variables evaluated in the present study, AMS programs and PMR programs should not be evaluated separately. It should be noted that the implication of the combinations of the treatments used are important. That said, more research is needed to confirm these results and contribute with more sample base.

5.0 CONCLUSIONS

This thesis contributes to a better understanding of the feeding management for cows under AMS. The results of this current study demonstrate that the F:C ratio of the PMR and the amount of concentrate offered in the AMS are important factors that independently affect performance and behaviour responses of lactating cows. Feeding a greater amount of concentrate in the AMS marginally affects milk yield and composition but reduces PMR consumption. At the same time, feeding a greater amount of concentrate in the AMS increases variability in AMS concentrate consumption among days challenging the ability of precision feeding strategies. On the other hand, reducing the F:C ratio of the PMR may increase the substitution effect, but does not increase variability in PMR intake or AMS intake, while potentially improving milk yield; however minimal pH may be reduced. Our results imply that providing greater energy supply, either by offering a reduced PMR F:C ratio or by increasing the amount of AMS concentrate, improves milk production. Therefore, this study provides evidence of the complexity of the feeding management in AMS, requiring more research in this area and to consider PMR feeding programs in conjunction with AMS feeding programs to support an efficient and cost-effective milk production.

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