PROGRESS REPORT

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Alberta Mik
SaskMilk
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Evaluating strategies to improve the feeding management of dairy cows housed in automated milking systems

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1. Activities during the reporting period

The project is progressing, generally, as planned. Firstly, 2 M.Sc. students have been recruited as a suitable PhD candidate was not identified.

Activities during the reporting period include:

- Two M.Sc. students have been recruited for this project (Silvia Menajovksy and Keshia Paddick)
- A total of 16 dairy cattle have been fit with ruminal cannula
- Data and sample collection have been completed on the first 2 studies proposed
- The first manuscript is currently under co-author review

STUDY 1. EFFECT OF THE FORAGE-TO-CONCENTRATE RATIO OF THE PARTIAL MIXED RATION (PMR) AND QUANTITY OF CONCENTRATE PROVIDED IN AN AUTOMATIC MILKING SYSTEM (AMS)

ABSTRACT

This study was conducted to evaluate the effects of the forage-to-concentrate ratio of the partial (**PMR**) and the quantity of concentrate offered in the automated milking system (**AMS**) 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. Diets were arranged in a 2×2 factorial 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 provided to achieve a low (2 kg/d; **L-AMS**) or a high (6 kg/d; **H-AMS**) intake. Each period consisted of 28 d with 6 d for dietary transition, 13 d of adaptation, and 9 d for data and sample collection. The first 4 d of the collection were used to evaluate behavioral data (milking frequency, feeding behavior, and standing and lying behavior) and ruminal pH. Subsequently, a rest day was provided and the last 4 days were used to evaluate ruminal fermentation and apparent total tract digestibility. All 8-d were used for measurement of DMI and milk yield. Cows fed the H-AMS consumed 3.5 kg 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, 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), but had greater holding area time. Minimum ruminal pH tended to be lower for cows fed a L-FOR compared to cows fed H-FOR but was not affected by the AMS 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 holding area time relative to feeding a H-FOR PMR.

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 forage in pasture, and a concentrate supplement while in the AMS. Previous research has suggested that provision of the concentrate in the 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 the concentrate to attract cows to the AMS coupled with the ability to provide differing quantities of concentrate for individual cows have resulted in manufactures of AMS 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. These data collectively indicate that concentrate provision among farms and within farms can be both high and variable.

Despite the variable quantities of concentrate provided in the AMS, 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. (2007) 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 and the quantity of concentrate/100 kg milk provided in the AMS, and Hare et al. (2018) reported that lower quantities of concentrate in the AMS, when fed isocaloric diets, 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 allocated 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 of concentrate provision, and maximum meal sizes imposed. The previously stated outcome has been highlighted by Bach et al. (2007) where they targeted 3 or 8 kg/d 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 resulting in an actual consumption of 3.5 and 5 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. (2007) 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 response between the AMS concentrate and PMR warrants further investigation into feeding management when considering the whole diet.

As highlighted previously, both the AMS and PMR contribute towards 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 provide as much as 98% of the dietary DM (Hare et al., 2018). While our 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 to the AMS concentrate, may affect production responses for cows milked using AMS.

Based on the information presented above, we hypothesized that cows provided with lower quantities of concentrate in the AMS will have greater PMR intake, milk and milk component yield, and more stable ruminal fermentation than cows offered more concentrate in the AMS. We further hypothesized that decreasing the forage-to-concentrate ratio of the PMR would increase milk yield without negatively affecting voluntary attendance to the AMS and AMS concentrate intake.

MATERIALS AND METHODS

Experimental Design

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). Eight multiparous Holstein cows were fit with a 9-cm ruminal cannula (Robyn Williams, Melbourne, Victoria, Australia) were used in this experiment. Cows were assigned to one of two squares 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 and 169 ± 9.7 DIM, 708 ± 70 kg, and 41.5 ± 8.35 kg for square 2. Each of the 4×4 Latin squares were designed to balance 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**) forage-to-concentrate ratio (**F:C**) 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 allocation. The AMS concentrate allocation represented 7.12% and 21.35% of the total diet, respectively (Table 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 through the provision of water. The PMR was provided in Insentec feed bunks (Hokofarm Group, Marknesse, The Netherlands) with the daily PMR allocation 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 2). Diets were formulated to be balanced for macro- and micro- nutrient supply and had metabolizable energy and protein allowable milk yield predictions that were similar based on the Nutritional Dynamic System (NDS, RUM&N Sas,

Reggio Emilia, Italy). Predicted 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 to collect data and samples. The diet transition was accomplished by providing 25, 50, and 75% of their 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 periods with 1 d of rest interspacing the two 4-d measurement protocols. The first 4-d was used to evaluate behavioral responses while the second 4-d was used to evaluate metabolic responses.

Throughout the study, cows were housed in a pen with 12 free-stalls bedded with chopped straw. 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 were directed 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.

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.

Dry Matter Intake, Feeding Behaviour, and PMR Sorting Behaviour

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. 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, 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 behavioural and 4-d metabolic collection phases were collected daily. Refusal samples from an individual cow were composited proportionally and stored at -20ºC. The refusal samples collected during the behavioral measurement period 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 respectively dry matter intake of each of the 4-d sampling periods.

Feeding behaviour for the PMR was determined for each cow during the first 4 d of each collection period using the software associated with the Insentec 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 behaviour has been described by Chapinal et al. (2007). Meal criterion for each cow in each period was determined as reported by DeVries et al. (2003).

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). Each composite sample was measured in duplicated and the PSPS had sieves with aperture openings of 19, 8, and 4 mm, along with a bottom pan. 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 Christy Norris 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.

Milk and Milk Component Yield, Milking Activity, and Standing and Lying Time

At each milking, milk yield was recorded 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). The daily milk and milk component yields were subsequently calculated.

In addition, the AMS recorded the number of visits 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 interval, incidence of kickoffs 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 behavioural period (d 20 to 24) using accelerometers (HOBO Pendant G Acceleration data loggers ua-004-64, Onset, Cape Cod, MA), that were attached to the back left leg in an horizontal position, of each cow on d 19 of each experimental period. Based on the positioning of the logger, the X-axis was paralleled to the ground pointing towards the head of the cow, the Y-axis was perpendicular to the ground, pointing dorsally, and Z-axis was perpendicular to the ground pointing away from the cow. The loggers were set to record the position every 30 seconds (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).

Ruminal Fermentation

Ruminal pH was measured during the behavioural 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 for 4 complete days of data collection. 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, with a 3-h offset between days such that the final combined composite (8 samples) was representative of a 24-h 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 NH_3-N. Samples were placed on ice until being stored at −20ºC. Short-chain fatty acid concentrations were determined according Khorasani et al. (1996) and NH_3-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 feces samples were utilized to determine uNDF intake to enable prediction of fecal output and apparent total tract digestibility (Huhtanen et al., 1994).

Statistical Analysis

Data were analyzed as a replicated 4×4 Latin square with a 2×2 factorial treatment arrangement using the mixed model 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 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 $0.10 \geq P > 0.05$.

RESULTS

Body weight, BCS, DMI, PMR and AMS intake

Cow BW and BCS did not differ among treatments (Table 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 intermeal 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 offered 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 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 ($P = 0.65$); 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; $P < 0.01$).

Milking Frequency, Milk and Milk Component Yield, and Milking Behaviour 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). In addition, no differences were observed for milk yield/milking, milking duration/milking, or intermilking interval. The percentage of kick-off milkings and 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. 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 L-FOR cows ($P < 0.01$) and cows provided L-AMS had greater MUN than those fed H-AMS ($P = 0.02$).

Ruminal Fermentation: pH, SCFA and Ammonia Concentration

Minimum pH tended to be greater for H-FOR than L-FOR ($P = 0.09$; Table 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 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 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.

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 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 provision.

Activity Budgets: Gate Passing Events, Times in Areas, Lying and Standing behaviour

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 6). The number of rejections to the holding area did not differ among treatments averaging over 5 rejections/d. Cows fed L-FOR spent 32.38 min/d more time 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 behaviour were not affected by the amount of concentrate offered in the AMS or by the F:C ratio of the PMR.

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. For the primary variables of interest, there were no detected interactions. Thus, the discussion will focus on the main effects independently.

Effects Arising from Increased AMS Concentrate Allocation

Feeding management for cows housed in barns with AMS must consider characteristics of the PMR and the AMS concentrate as contributing components of the diet. Most studies to date have focused exclusively on changing AMS allocation or composition (Halachmi et al., 2005; Migliorati et al., 2005) and have not considered that changes to the AMS concentrate can 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 interpretation of production outcomes. In the present study, the proportional contribution of the AMS concentrate was 7.12% of the dietary DM for L-AMS and 21.35% of the dietary DM for H-AMS. Consistent with previous studies, we observed that increasing the AMS concentrate allocation decreased PMR intake (Bach et al., 2007; Hare et al., 2018). 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, we observed that for every 1 kg increase in AMS concentrate consumed, PMR intake only decreased by 0.83 kg suggesting that increasing the AMS concentrate allocation may increase nutrient intake. In contrast, Bach et al. (2007) reported 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 difference in the substitution ratio among our study and previous studies can likely be attributed to diet formulation strategies. For example, Bach et al. (2007) and Hare et al. (2018) attempted to provide isocaloric diets by shifting concentrate from the PMR to the AMS. In the present study, we purposely increased the nutrient density with the H-AMS vs. L-AMS and with

the L-FOR vs. H-FOR treatments. 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 may have allowed for greater DMI. Additionally, milk production in the present study was markedly greater than that in Bach et al. (2007) and Hare et al. (2018) suggesting that physiological state or nutrient demand may alter responses to increased dietary energy density. In the present study, cows were in mid-lactation with an average of 140.5 ± 13.6 DIM ± SD and 168.5 ± 9.7 DIM ± SD for the respective squares. Bach et al. (2007) utilized 115 cows in mid-lactation; however, the cows in that study were, on average, 191 ± 2.1 DIM ± SD and also incorporated production responses from multiparous and primiparous cows. Hare et al. (2018) also combined responses for primiparous and multiparous and had less milk yield than in the present study.

Although previous studies have assessed increasing the AMS concentrate allocation, cows in previous studies did not consume their full AMS allocation (Halachmi et al., 2005; Migliorati et al., 2005; Bach et al., 2007). For example, Bach et al. (2007) 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 3.5 and 5 kg/d, respectively. In the present study, we 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 standard deviation 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. However, future research is needed to evaluate the magnitude of variation in AMS concentrate allocation as affected by physiological state and the implications on achieving precision feeding strategies and production outcomes.

Recommendations 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 not result in improved milking frequency or milk production (Halachmi et al., 2005; Migliorati et al., 2005; Bach et al., 2007; 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 affected by the amount of concentrate offered within the AMS under free flow cow traffic (Halachmi et al., 2005; Tremblay et al., 2016) or guided cow traffic (Migliorati et al., 2005; Bach et al., 2007; 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. (2007) and Hare et al. (2018) fed iso-caloric 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. In contrast to our findings, Halachmi et al. (2005) and Migliorati et al. (2005) did not observe an increase in milk yield with increasing concentrate allocation. However, those studies did not report PMR intake and hence important information is missing. Moreover, Halachmi et al. (2005) conducted their study encompassing cows varying in days in milk, but did not report whether production responses differed by DIM nor did they report the proportion of cows by stage of lactation. It could be expected that using cows in late lactation would diminish potential effects for milk yield arising from greater energy density.

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, we are unaware of studies characterizing the impact of AMS concentrate allocation on ruminal fermentation. In the current study, we expected to see a reduction in ruminal pH for cows fed H-AMS relative to cows fed L-AMS; however, we 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 small concentrate meals provided. 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 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, we 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 the selection for particles retained on the 4-mm sieve for cows fed H-AMS vs. L-AMS may have modulated the ruminal pH response independent to 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 increases and may further suggest that fermentation responses were evident. Future research is needed to evaluate ruminal fermentation responses for cows managed in AMS.

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 changes in 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, 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 a guided flow system. Clearly, more research is needed to assess activity budget 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. Similarly, to Hare et al. (2018), we did not observe any changes in PMR eating behaviour 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 behaviour remains stable regardless of differences within the 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 high F:C ration, or a PMR with low forage quality, would likely be positively associated providing a greater amount 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.

CONCLUSION

Although we did detect an interaction between the F:C ratio of the PMR and the amount of concentrate offered in the AMS for ruminal SCFA concentration, 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 will reduce PMR intake with only marginal effects on milk and milk component yield. Feeding a greater amount of concentrate in the AMS increases day-to-day variability in AMS concentrate consumption challenging the notion of precision feeding. 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.

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STUDY 2. EFFECT OF THE FORAGE-TO-CONCENTRATE RATIO OF THE PARTIAL MIXED RATION (PMR) AND QUANTITY OF CONCENTRATE PROVIDED IN AN AUTOMATIC MILKING SYSTEM (AMS)

MATERIALS AND METHODS

Animal Husbandry and Experimental Design

This study took place at the University of Saskatchewan's Rayner Dairy Research and Teaching Facility (Saskatoon, SK, Canada). Eight primiparous Holstein cows, previously fit with a ruminal cannula, were used in this study. Animal use was approved by the University of Saskatchewan Research Ethics Board (protocol 20100021). At the start of the study the cows averaged (mean ± SD) 90.6 ± 9.8 DIM and the 7-d milk yield prior to starting the study was (mean ± SD) 37.9 ± 6.0 kg/d. Cows were housed in a free-stall barn with 12 stalls and the barn was designed as a feed-first guided-traffic design with an AMS (DeLaval, Tetra Laval Group, Sweden). All cows had permission to enter the AMS every 4 h or if the predicted milk yield was greater than 9.0 kg. A one-way gate guided cows from the free-stall area towards the feed bunk area that contained 8 Insentec Feed Bunks (Hokofarm Group, Marknesse, The Netherlands). For cows to return to the rest area from the feed bunk area, they had to pass through a pre-selection sort-gate. This gate directed cows either toward the AMS when milking permission was granted or toward the free stall area when milking permission criterion were not met. If cows did not voluntarily enter the milking stall within 12 h, they were fetched and placed in the holding pen to be milked. Fetching times were restricted to 0400, 1030, 1730, and 2230 h daily and fetching activity was recorded.

Within each Latin square, cows were assigned to 1 of 4 treatments with the sequence of treatments balanced to avoid carry-over effects. Periods were designed to consist of 5 d for dietary transition, 14 d for dietary adaptation, a 4-d measurement phase for behavioural data collection, 1 d for device removal, and a 4-d phase for measurement of ruminal fermentation and total tract digestibility. Periods were designed to be 28 d in duration; however, periods 2, 3, and 4 were extended as the AMS required repairs during collection periods. As such, the actual duration of periods 1, 2, 3, and 4 were 28, 30, 32, and 40 d, respectively. Despite the extended periods, all data collected allowed for 4 consecutive days of behavioural measurements and 4 consecutive days for ruminal fermentation and total tract digestibility, as originally planned, with the adaptation phase extended.

Feeding Management and Experimental Treatments

In the present study, diets were formulated to be equal in macro- and micro-nutrient provision allowing for the evaluation of the site of concentrate provision. As such, cows in each treatment received the same total dietary nutrient provision when considering the sum of the PMR and the AMS concentrate. However, treatment groups differed in the amount of concentrate allocated in the AMS with targets of 0.5, 2.0, 3.5 or 5.0 kg/day (DM basis; Table 1). As the AMS concentrate target increased, there was an equal and corresponding reduction in the quantity of concentrate offered in the PMR. To avoid confounding effects, the pellet provided in the AMS was

the same as that offered in the PMR and the forage-to-concentrate ratio (**F:C**) for each treatment (AMS concentrate + PMR) was 50:50. Diets were formulated for a 580 kg cow with an expected milk yield of 36 kg containing 4% fat and 3.2% protein using the CNCPS (6.55) platform of NDS.

Cows were provided their PMR in Insentec Feed Bunks (Hokofarm Group, Marknesse, The Netherlands) with 1 cow assigned to each bunk to allow for measurement of feeding behaviour. The PMR was fed twice daily with 60% of the daily PMR allowance provided at 1100 h and 40% at 2230 h. The quantity of PMR refused was recorded at 1030 h daily and refusals were removed from the feed bunk. The PMR was provided for ad libitum consumption with refusals targeted to be between 5 and 10% (as is basis) of the total PMR offered. The quantity of PMR offered was adjusted every 4th day, based on the previous 3-d average.

To achieve the specified DM provision of the AMS concentrate, the amount of concentrate offered in the AMS was monitored daily and adjustments were made every 4th day based on the average intake of the previous 3 d. The amount of AMS concentrate eligible for each cow exceeded the target to ensure that the target consumption was achieved. The AMS feeder was calibrated weekly (Mondays) to ensure the desired quantity of concentrate (on a DM basis) was dispensed. To calibrate, the feeder was cleaned, and 4 calibration samples were obtained directly from the feeder. The first sample was discarded to ensure material dislodged during the cleaning process did not affect the calibration outcome. The last 3 samples were weighed and an average of the 3 weights were entered into the computer system (Delpro 4.5, DeLaval, Tetra Laval Group, Sweden).

To ensure each treatment contained the targeted F:C, forage components were sampled twice weekly, and concentrate samples were collected weekly. Samples were used for DM determination (described below) and DM coefficients were updated as necessary.

Data and Sample Collection

The BW of each cow was measured on d 0 and d 1 at 0730 h. An average of the 2 BW measurements were used. Body condition score was collected independently by 3 trained personnel on d 1 of each period using the 5-point scale described by Wildman et al. (1982). The individual scores were averaged to yield the value used for statistical analysis.

Feed intake, on an as fed basis, was recorded daily throughout the experiment. Data collected during the 4-d behavioral measurement phase and the 4-d ruminal fermentation and digestibility measurement phases were used for determination of PMR DMI. To determine PMR DMI, individual ingredients were collected daily. In addition, refusals were collected daily for each cow and 20% of the daily refusals were combined to form a composite prior to DM analysis. The silage sampling procedure was accomplished by collecting a representative silage sample (10 grab samples) throughout the face of the silage pit. The sample was mixed, and a 1-kg sub-sample was used for DM determination. Hay samples were collected similarly from a pile of ground hay with grab samples taken from numerous regions of the pile, composited, and sub-sampled. In addition, a 750-g sample was collected from each of the concentrates used in the diets. Samples were stored in a freezer at -20°C. The composites from the behavioural measurements were used to determine DM and particle size distribution (Kononoff et al. 2003). Subsequently, the sorting index was calculated using the PMR only to determine whether cattle were selecting for individual particle sizes (Leonardi and Armentano, 2003). Composited samples from the ruminal fermentation and digestibility phase were analyzed for DM and chemical analysis. Dry matter was determined by placing a 500-g sample into a forced-air oven at 55˚C until the weight was constant. Subsequently, concentrate samples were ground through a 1-mm sieve using an Ultra Centrifugal Mill Type ZM 200 (Retsch GmbH & Co. KG, Germany), while silage and hay samples were ground using a

Christy Norris grinder (Christy Norris Ltd., Chelmsford, England) equipped with a 1-mm sieve. The ground composites from the ruminal fermentation and digestibility phase were sent to Cumberland Valley Analytical for analysis of CP, ADF, NDF, ether extract, starch, $aNDF_{OM}$, ash, Ca, P, Mg, K, Fe, Zn, Mn, Cu, and $iNDF_{OM}$. Analyses were completed as explained below.

Milk and milk component yield. Milk yield was measured using the AMS using DelPro 4.5 (De Laval). The milk yield per visit, number of visits, milking duration, incomplete quarters, quarters with kick-offs, and milkings where the milking machine was unable to find teats. The daily milk yield was recorded during each of the behavioural collection periods. Samples from each milking for each cow were obtained via a sampling system connected to the AMS and a daily 40-mL composite (proportional to yield) was prepared for each cow in containers containing a Bronopol Microtab preservative (Dairy Herd Improvement Laboratory, Edmonton, Alberta). To minimize the duration samples were sitting at barn temperature, samples were retrieved from the sampling device every 4 h and transferred to a refrigerator for storage at 4˚C. After compositing, daily milk samples were sent to the Dairy Herd Improvement Laboratory for analysis of milk urea nitrogen (MUN), protein, fat, lactose, SCC, and total solids. Fat, protein, lactose, solids and MUN were determined using mid-infrared spectroscopy, while SCC was determined using flow cytometry. Samples were stored at 4˚C prior to submission.

Behavioural responses. The Insentec feed bunks that contained the PMR were connected and controlled via computer software (RIC Management Software, The Hokofarm Group, The Netherlands) that recorded the date, time, duration and size of each PMR visit for each cow. These data were processed to remove visits to the feed bunk where no feed was removed. The inter-meal intervals for each visit were then calculated and log transformed. The transformed data were used to determine appropriate meal criteria for each cow for each period (The R Foundation) using descriptions from Slater and Lester (1982). Meal criteria was defined as the length of time between visits to the Insentec Feed Bunks that indicates a new meal. These data were then used to determine the number of meals, size of meals, and rate of consumption for each cow by period (Tolkamp et al., 1998).

Accelerometers were placed on the hind right leg of each cow on d 19, according to the protocol described by Chapinel and Zobel (2013). Devices were removed on d 24 of each period and the data were downloaded onto a computer with data from 4 consecutive days used for analysis. The number of standing and lying bouts and the duration of each bout was determined using SAS (SAS Institute Inc., Cary, NC) as described by Chapinel (2013) with the algorithms of Ledgewood et al. (2010). Data were summarized by cow and period.

Feed sorting behaviour was analyzed during the behavioural measurement period of each treatment period. Sorting behaviour was measured with the Pennsylvania State Particle Separator (**PSPS**) using the same procedure described by Leonardi and Armentano (2003). All particle size measurements were conducted in duplicate (for each ingredient and refusals) for the composited samples according to Kononoff et al. (2003). The PSPS contained aperture openings of 19, 8, and 4 mm with the remaining material caught on a pan.

Ruminal fermentation and total tract digestibility. Ruminal pH was measured during the behavioral measurement periods to ensure that ruminal pH values were not affected by the ruminal digesta sampling protocols (described below). Ruminal pH was measured using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Penner et al., 2006). The LRCpH was inserted through the ruminal cannula into the ventral sac of the rumen, to enable 96-consecutive hours of continuous ruminal pH data collection. The LRCpH was programmed to log data every 1 min. Prior to insertion and following removal the LRCpH was maintained at 39˚C for

standardization in pH buffers 7 (RICCA Chemical Company, USA) and 4 (Fisher Chemical, USA). Following removal from the rumen, data were downloaded from the LRCpH to a computer. The relationship between mV and pH derived from the starting and ending standardizations were used to convert the recorded mV values into pH units assuming a linear offset between the starting and ending regressions. Data were summarized to determine the daily minimum, mean, maximum, duration that pH was less than 5.8 and area when pH was less than 5.8 as described by Penner et al. (2007).

Ruminal digesta and fecal sample collection was initiated on d 25 and was completed with 4 consecutive days of measurement. Samples were collected at 12 h intervals with a 3 h offset over the 4-d period to represent a 24-h cycle. At each time point, 250-mL of ruminal digesta were collected from each the cranial, central, and caudal regions of the rumen fluid/rumen mat interface. The mixed digesta (750 mL) was strained through two layers of cheesecloth, filtrate was mixed, and sub-samples of ruminal fluid filtrate were obtained. One 10-mL sample was added to a 15-mL vial with 2 mL of 25% meta-phosphoric acid for the analysis of short-chain fatty acid (**SCFA**) concentration and the second 10-mL sample was added to a 15-mL vial with 2 mL of sulfuric acid that was subsequently analyzed for ammonia concentration. These samples were sealed and stored at -20˚C until analysis.

Corresponding to the time of ruminal fluid sampling, 200 g of feces was collected directly from the rectum of each cow. Following collection, the fecal sample was thoroughly mixed, and 125 g was added to a plastic container to form a 1000-g composite per cow. The fecal samples were stored at -20˚C until thawed to prepare duplicate 500 g samples. These duplicate samples were placed in a 55˚C forced air oven to determine DM as previously described. Fecal samples were then ground using the Ultra Centrifugal Mill ZM 100 grinder (Retsch GmbH & Co. KG, Germany) through a 1 mm sieve. The ground samples were sent to Cumberland Valley Analytical Services for determination of OM, CP, $aNDF_{OM}$, ADF, starch, ether extract, iNDF, and ethanol soluble carbohydrates (described below).

Sample Analyses

Feed samples collected during the behavioural phase (d 20 to 23) and both feed, refusals, and fecal samples from the metabolic phase (d 25 to 28), were dried and ground (previously described) through a 1-mm sieve. A 200-g sub-sample (feed and refusal) and 75-g sub-sample (feces) was sent to Cumberland Valley Analytical Services for analysis. All samples were analysed according to the Association of Official Analytical Chemists (AOAC, 2000). Crude protein (CP) was analysed by nitrogen combustion (method 990.03, AOAC 2000) with a Leco FP-528 Nitrogen Combustion Analyser (Leco, MI, USA). Acid (ADF) and neutral detergent fibre (NDF) were analysed using Whatman 934-AH glass micro-filters with 1.5 um particle retention (method 973.18, AOAC 2000, edition 17). Ether extract was analysed (method 2003.05, AOAC 2006, edition 18) using a Tecator Soxtec System HT 1043 Extraction unit (Tecator, Foss NA, Eden Prairie, MN). Starch was analysed by using the method described by Hall (2009). Ash was analysed by heating a 1.5-g sample to 550ºC for 4 h (method 942.05, AOAC 2000). Calcium was determined using a drying ash procedure (method 927.02, AOAC 2000), using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, CN, USA). Phosphorus was determined using a drying ash procedure (method 965.17, AOAC 2000) and concentration was read on a spectrometer at 410 nm (Pharmacia, LKB-Ultrasepc®III, Stockholm, Sweeden). Indigestible NDF (iNDF) was determined by measuring the remaining NDF after 240 h. This process used 12 cannulated cows and was run 3 different times on different days to improve accuracy. Samples from the behavioural phase were used to determine diet composition for d 20

to 23, while samples from the metabolic phase were used for diet composition and nutrient digestibility determination.

Ruminal fluid samples preserved with 25% meta-phosphoric acid, were thawed over night at 4˚C and composited (equal volume basis) the following morning to yield 1 sample/cow/period. Sample preparation for GC followed the protocol described by Khorasani et al. (1996). The concentration of SCFA was measured using an Agilent gas chromatographer (6890 series with FID). Samples were injected using a 17:1 split ratio at 170˚C. The column was a Phenom FFAP and the oven and detector temperatures were 120˚C and 250˚C, respectively.

Ruminal fluid samples that were frozen with sulphuric acid, were thawed over night at 4˚C. These samples were then composited as described previously for SCFA samples. Composited samples were then centrifuged at 16,000 × *g* for 10 min at 4ºC. The supernatant was then pipetted into smaller centrifuge tubes and centrifuged again at 1,000 × *g* for 10 min at 4ºC. The supernatant was then transferred in duplicate into glass test tubes with standard solutions (sodium phenate, nitroprusside and hypochlorite) and a standard curve was prepared using distilled water with the standard solutions. After a 1-hour incubation period, these samples were analyzed in a spectrophotometer (SPECTRAmax®PLUS^{384}, Molecular Devices Corporation, USA). The values outputted by the spectrometer were used in calculations to determine the concentration of ammonia. If the duplicate samples had greater than a 7% error, they were prepared and re-run again.

Statistical Analysis

The PROC UNIVARIATE procedure was used to determine if the data was normally, identically and independently distributed prior to further analysis. All statistical analyses were completed using the Mixed Model of SAS 9.4 (SAS Institute Inc.). The model included the fixed effects of treatment, period, and square and the random effects of cow within square. Polynomial contrasts were used to evaluate whether treatments responded in a linear or quadratic manner. Significance was declared when $P < 0.05$ and trends were declared at $0.05 < P < 0.1$.

Data for AMS concentrate intake, PMR intake, and total DMI were analyzed using repeated measures with the day as the repeated variable. Covariance error structures were tested to determine the which one yielded lowest AIC and BIC values. The covariance structure that best suited the data was compound symmetry. The same statistical model was used to evaluate AMS concentrate intake, PMR intake, and total DMI data except that the model included the fixed effects of day and the day by treatment interaction.

The student (Keshia Paddick) is currently preparing the manuscript for this project. Data tables are provided in the abstract and include Tables 7 to 12.

The general conclusion for the studies described above are that feeding more concentrate in the AMS increases daily variability in nutrient intake without affecting voluntary attendance at the AMS and with minor to no effects on milk and milk component yield.

2. Technology Transfer Activities

The following technology transfer activities have been completed. Students involved are in bold and underlined.

G.B. Penner, **S. Menajovsky**, **K. Paddick**. 2017. Optimal feeding programs with automated milking systems (AMS). Advances in Dairy Nutrition and Management.

Menajovsky, S.B. , C.E. Walpole, T.J. DeVries, K.S. Schwartzkopf-Genswein, M. E. Walpole, and **G.B. Penner**. 2017. Does the partial mixed ration (PMR) energy density interact with the amount of concentrate offered in an automated milking system (AMS)? J. Dairy Sci. 100(Suppl. 2): 131.

K.S. Paddick*, **S.B. Menajovsky**, and G.B. Penner. 2017. Is a pelleted feed required in an automated milking system (AMS)? J. Dairy Sci. 100(Suppl. 2): 100.

S.B. Menajovsky, C.E. Walpole, T.J. DeVries, K.S.G. Schwartzkopft-Genswein[3], M.E. Walpole, and **G.B. Penner**. 2017. Evaluating Feeding Management for Dairy Cattle in Automated Milking Systems (AMS). Western Canadian Dairy Seminar, March 7-10th, Red Deer, AB.

K.S. Paddick, **S.B. Menajovsky**, and **G.B. Penner**. 2017. Is a Pelleted Feed Required in an Automated Milking System (AMS)? Western Canadian Dairy Seminar, March 7-10th, Red Deer, AB.

G.B. Penner. 2016. Feeding management for the robotic milking herd. Eastern Nutrition Conference. May 11-12, Guelph, ON, Canada.

3. Changes to Industry Contributions, In-kind Support, Collaborations, or other Resources

No changes have occurred.

Appendix

Table 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
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]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.

Table 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, behaviour associated with PMR intake, PMR sorting behaviour, 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
Intermeal interval, min	207.20	233.22	214.78	230.11	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.

Table 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
Intermilking interval, min	390.23	389.31	411.69	376.81	19.90	0.67	0.10	0.12
Kickoffs, %	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 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 ($P < 0.05$).

Table 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 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.

Table 7. Ingredient and chemical composition for diets used to determine whether increasing dietary concentrate allocation in the AMS affects performance.

	AMS allocation, kg DM/d			
Variable	0.5	2.0	3.5	5.0
Ingredient, % DM				
Barley silage	37	37	37	37
Alfalfa hay	17.4	17.4	17.4	17.4
Palmitic acid	1.3	1.3	1.3	1.3
Barley grain	15.2	15.2	15.2	15.2
PMR pellet	27	20.4	13.9	7.4
AMS pellet	2.2	8.7	15.2	21.7
Chemical composition, % DM				
DM, %	62.3	62.6	62.4	62.2
CP	17.5	17.5	17.5	17.5
ADF	19.8	19.8	19.8	19.8
NDF	30.9	30.9	30.9	30.8
Starch	25.4	25.5	25.5	25.5
Ether extract	4.9	4.9	4.9	4.9

Table 8. Effect of AMS concentrate allocation on AMS and PMR dry matter intake.

	Treatment					*P*-value			
Parameter	0.5	2.0	3.5	5.0	SEM	Treatment	Linear	Quadratic	Cubic
Available AMS concentrate, kg DM/d	0.50[d]	2.00[c]	3.49[b]	4.93[a]	0.08	<0.001	<0.001	0.797	0.929
Allocated AMS concentrate, kg AF/d	0.57[d]	2.26[c]	3.94[b]	5.62[a]	0.001	<0.001	<0.001	0.482	0.443
PMR Intake, kg/d	24.7[a]	23.6[a]	21.3[b]	20.5[b]	1.20	<0.001	<0.001	0.296	0.137
PMR Intake standard deviation	1.60	1.25	1.70	1.25	0.33	0.682	0.690	0.876	0.260
Total DMI, kg/d	25.17	25.58	24.76	25.47	0.60	0.399	0.963	0.655	0.101
Standard deviation in AMS intake, kg/d	0.06[b]	0.42[ab]	0.51[ab]	0.85[a]	0.119	0.001	0.000	0.937	0.322
PMR Meals, no/d	7.1	6.9	6.3	6.6	0.41	0.452	0.231	0.532	0.386
PMR Meal size, kg	3.6	3.4	3.5	3.2	0.18	0.588	0.289	0.868	0.400
PMR Meal duration, min	32.25	34.89	35.70	45.77	4.56	0.211	0.061	0.428	0.595
PMR Consumption rate, g/min	110.01[a]	97.7375[ab]	99.9015[a]	83.7784[b]	6.30	0.001	0.000	0.607	0.062

Table 9. Milking frequency, milk yield, and milk composition for cows fed increasing quantities of AMS concentrate with isocaloric diets.

	Treatment					*P*-value			
Parameter	0.5	2.0	3.5	5.0	SEM	Treatment	Linear	Quadratic	Cubic
Milking frequency, no/d	3.2	3.3	3.1	3.3	0.18	0.31	0.82	0.26	0.14
Yield, kg/d	37.7	37.6	37.3	37.0	2.64	0.96	0.59	0.96	0.97
Fat, kg/d	1.4	1.4	1.5	1.4	0.06	0.721	0.459	0.532	0.563
Protein, kg/d	1.2	1.3	1.2	1.2	0.06	0.568	0.424	0.641	0.295
MUN, mg/dL	17.4	16.9	17.1	16.1	0.55	0.049	0.02	0.38	0.171
Fat, %	3.87	3.89	3.98	3.81	0.187	0.087	0.703	0.039	0.123
Protein, %	3.26[b]	3.38[a]	3.26[b]	3.28[b]	0.087	0.005	0.541	0.040	0.002
Lactose, %	4.71	4.68	4.71	4.68	0.040	0.304	0.275	0.927	0.124
Total Solids, %	12.85	12.96	12.95	12.78	0.289	0.076	0.337	0.016	0.851
Total kickoffs, no/d	0.1	0.1	0.2	0.2	0.06	0.66	0.49	0.61	0.37
Kickoffs, % of milkings/d	4.17	2.60	5.99	4.17	1.972	0.693	0.706	0.948	0.267
Total incomplete milkings, no/d	0.2	0.3	0.3	0.4	0.14	0.58	0.20	0.89	0.66
Incomplete milkings, % of milkings/d	5.21	8.85	9.11	10.94	4.342	0.699	0.280	0.796	0.754
Milkings with teats not found, no/d	0.16	0.09	0.06	0.03	0.07	0.59	0.19	0.82	0.92
Average box time, min/d	7.0	7.2	7.1	6.9	0.34	0.93	0.78	0.58	0.85
Total box time, min/d	22.2	22.9	21.8	22.8	1.71	0.83	0.84	0.84	0.39

Table 10. Rumen fermentation results including rumen pH, ammonia and SCFA concentration.

	Treatment					*P*-value			
Variable	0.5	2.0	3.5	5.0	SEM	Treatment	Linear	Quadratic	Cubic
Minimum pH	5.68	5.67	5.67	5.57	0.060	0.229	0.751	0.173	0.123
Maximum pH	6.69	6.63	6.72	6.69	0.046	0.478	0.645	0.687	0.155
Average pH	6.19	6.11	6.21	6.19	0.055	0.459	0.619	0.514	0.173
Sum of duration pH <5.8 (min)	196.75	269.47	129.06	141.38	65.956	0.112	0.122	0.483	0.068
Sum of area pH <5.8	47.84	45.04	20.92	25.08	20.070	0.421	0.151	0.803	0.431
Rumen ammonia, mg/dL	12.11	11.24	10.29	10.17	0.703	0.051	0.011	0.327	0.539
Acetate, %	62.47	62.58	63.07	62.97	0.730	0.305	0.103	0.678	0.405
Proprionate, %	22.72	22.83	21.87	22.18	0.532	0.226	0.125	0.777	0.163
Iso Butyrate, %	0.79	0.76	0.79	0.75	0.012	0.062	0.138	0.963	0.021
Butyrate, %	11.18	11.06	11.39	11.26	0.405	0.683	0.533	0.982	0.311
Iso Valerate, %	1.21	1.22	1.23	1.16	0.055	0.679	0.486	0.372	0.690
Valerate, %	1.37	1.31	1.34	1.36	0.031	0.290	0.861	0.103	0.327
Caproic acid, %	0.26^{b}	0.25^{b}	0.31^{a}	0.32^{a}	0.029	0.036	0.011	0.480	0.152
Total concentration, μmol/mL	116.94^{a}	116.85^{a}	111.62^{b}	116.10^{a}	2.5757	0.039	0.205	0.103	0.025

Table 11. Apparent total tract digestibility for DM, OM, CP, NDF, ADF, and starch when cows were fed increasing quantities of concentrate in the AMS

	Treatment					*P*-value			
Parameter	0.5	2.0	3.5	5.0	SEM	Treatment	Linear	Quadratic	Cubic
DM, %	69.22	69.38	68.85	68.34	0.813	0.292	0.096	0.406	0.686
CP, %	71.20	71.31	70.41	69.92	1.025	0.587	0.215	0.717	0.699
Starch, %	95.15	95.19	96.10	95.60	0.507	0.185	0.144	0.416	0.139
NDF, %	46.47	47.04	45.71	44.70	1.137	0.156	0.056	0.283	0.491
ADF, %	39.62	41.18	40.00	37.98	1.406	0.122	0.139	0.059	0.628
E.E., %	94.98^{c}	98.11^{b}	99.07^{ab}	99.33^{a}	0.237	<0.001	<0.001	<0.001	0.182
OM, %	70.82	70.82	70.42	70.05	0.752	0.518	0.167	0.671	0.827

Table 12. Cow-time budgets of cows fed differing amounts of concentrates in the AMS.

	Treatment					*P*-value			
Parameter	0.5	2.0	3.5	5.0	SEM	Treatment	Linear	Quadratic	Cubic
Standing time, h/d	12.9	12.7	12.1	12.0	0.62	0.405	0.111	0.890	0.633
Standing bouts, #/d	10.6	9.9	10.7	9.7	0.91	0.532	0.468	0.851	0.210
Standing bout duration, min/d	77.2	85.5	73.5	83.5	10.51	0.615	0.836	0.905	0.198
Lying time, h/d	11.1	11.3	11.9	12.0	0.62	0.405	0.111	0.890	0.633
Lying bouts, #/d	10.8	10.6	9.9	9.9	0.91	0.579	0.473	0.863	0.244
Lying bout duration, min/d	65.1	74.8	66.9	77.8	6.31	0.209	0.173	0.900	0.102