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Modification of the feeding behavior of dairy cows through live yeast supplementation

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ABSTRACT

The objective of this study was to determine if the feeding behavior of dairy cows is modified through live yeast supplementation. Twelve lactating Holstein dairy cows (2 primiparous and 10 multiparous) were individually exposed, in a replicated crossover design, to each of 2 treatment diets (over 35-d periods): (1) a control TMR and (2) a control TMR plus 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada). Milk production, feeding, and rumination behavior were electronically monitored for each animal for the last 7 d of each treatment period. Milk samples were collected for the last 6 d of each period for milk component analysis. Dry matter intake (28.3 kg/d), eating time (229.3 min/d), and rate (0.14 kg of dry matter/min) were similar between treatments. With yeast supplementation, meal criteria (minimum intermeal interval) were shorter (20.0 vs. 25.8 min), translating to cows tending to have more meals (9.0 vs. 7.8 meals/d), which tended to be smaller in size (3.4 vs. 3.8 kg/meal). Yeast-supplemented cows also tended to ruminate longer (570.3 vs. 544.9 min/d). Milk yield (45.8 kg/d) and efficiency of production (1.64 kg of milk/kg of dry matter intake) were similar between treatments. A tendency for higher milk fat percent (3.71 vs. 3.55%) and yield (1.70 vs. 1.63 kg/d) was observed when cows were supplemented with yeast. No differences in milk fatty acid composition were observed, with the exception of a tendency for a greater concentration of 18:2 *cis*-9,*cis*-12 fatty acid (2.71 vs. 2.48% of total fatty acids) with yeast supplementation. Yeast-supplemented cows had lower mean ruminal temperature (38.4 vs. 38.5°C) and spent less time with rumen temperature above 39.0°C (353.1 vs. 366.9 min/d), potentially indicating improved rumen pH conditions. Overall, the results show that live yeast supplementation tended to improve meal patterns and rumination, rumen temperature, and milk fat production.
Key words: live yeast, rumination, meal pattern, behavior

INTRODUCTION

Live yeast supplementation to the diet has been associated with increased potential to enhance fiber digestion in the rumen and prevention of a decline in rumen pH. These effects have been typically attributed to decreased lactic acid production, increased utilization of lactic acid by some bacteria [creating more favorable conditions for fiber-degrading (cellulolytic) bacteria within the rumen], and overall greater microbial synthesis in the rumen (Chaucheyras et al., 1996; Newbold et al., 1996; Chaucheyras-Durand et al., 2008). This improved rumen environment may lead to increased feed efficiency of dairy (de Ondarza et al., 2010) and beef cattle (Erasmus et al., 2009).

Some evidence suggests that the provision of live yeast (*Saccharomyces cerevisiae*) may have the potential to modify dairy cow feeding behavior patterns. In a study by Bach et al. (2007), supplementation of active dry yeast not only improved ruminal pH in a small sample of loose-housed lactating cows, but also affected cow eating behavior. Cows supplemented with active dry yeast had a shorter interval between meals (3.32 h) than nonsupplemented cows (4.03 h), suggesting that they ate more frequently. It could be hypothesized that the greater fiber digestibility typically associated with live yeast supplementation may help speed up the passage of feed and, thus, increase appetite and feed intake. This could also explain the reduced interval between meals observed by Bach et al. (2007), which, if translated into greater meal frequency, may also help control rumen pH (Allen, 1997). Some recent evidence has been reported for beef cattle that active dry yeast increases eating frequency (Loncke et al., 2012). Thus, even though live yeasts are well documented to interact with lactic acid-producing and lactic acid-consuming bacteria (Chaucheyras-Durand et al., 2008), Bach et al. (2007) suggested that, among the mechanisms involved

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in the reduction of subclinical acidosis associated with live yeast supplementation, meal frequency may be an important factor to consider. Further research is required using a more powerful design and greater treatment adaptation period to determine if an effect of live yeast supplementation on meal patterning in lactating dairy cows truly exists. In addition to feeding and meal patterns, rumination activity would also be of interest to capture. A more favorable rumen fermentation environment would also be predicted to result in greater rumination activity (DeVries et al., 2009), which would further contribute to stabilization of rumen pH.

Interestingly, in a recent study by Desnoyers et al. (2009b), goats that were live yeast supplemented sorted their ration more (against fiber) than unsupplemented goats. Those researchers suggested that the live yeast-supplemented goats were able to cope with higher concentrate diets because they chose to eat a less-fibrous diet than the offered one. Even though feed sorting in dairy cattle has typically been viewed to contribute to depressions in rumen pH (DeVries et al., 2008), various reports have been published of lactating dairy cows decreasing their selection against long fibrous forage particles in attempt to mediate the effect of low rumen pH (Keunen et al., 2002; Yang and Beauchemin, 2006; DeVries et al., 2008). Given these findings, it would be interesting to also determine if dairy cattle, when supplemented with a live yeast product that has the potential to increase rumen pH, also change their selective consumption (sorting) patterns.

The objective of the current study was to determine if the feeding and rumination behavior patterns of lactating dairy cows can be modified through the supplementation of a feed additive that alters rumen fermentation. The secondary objective of the current research was to determine if the selective consumption patterns of dairy cows also change in response to that altered fermentation. We hypothesized that altering rumen fermentation through adding a direct-fed microbial (*S. cerevisiae*) to the TMR of lactating dairy cows would result in greater meal frequency and rumination activity. Further, we hypothesized that the sorting in favor of long fibrous particles in the ration may be less prevalent in cows supplemented with live yeast.

MATERIALS AND METHODS

Animals and Housing

Twelve lactating Holstein dairy cows, including 2 primiparous and 10 multiparous (parity = 2.2 ± 0.4; mean ± SD), were used in our study. The animals were 48.6 ± 16.5 DIM and were producing 48.5 ± 7.6 kg of milk at the beginning of the trial. The cows were housed 6 at a time in a freestall research pen located at the University of Guelph, Kemptville Campus Dairy Education and Innovation Centre (Kemptville, ON, Canada). Cows had access to 6 freestalls with waterbeds (DCC Waterbeds, Advanced Comfort Technology Inc., Reedsburg, WI). The waterbeds were topped with wood shavings; bedding was replaced as needed. Manure was manually scraped to within reach of the alley scrapers 2× per day at 0600 and 1800 h. Cows were milked 3× per day (at 0700, 1400, and 2100 h) using a robotic milking system (Lely A3 Next, Lely Industries N.V., Maassluis, the Netherlands). At the specified milking times, cows were moved from the research pen into a small holding area adjacent to the robotic milker, where they were milked individually and sequentially. Cows did not receive any supplemental feed from the robotic milking system while being milked. The experiment was conducted from December 29, 2012, to May 30, 2013. The average environmental temperature during the experimental period was 0.2 ± 10.3°C. Use of cows and experimental procedures were approved by the University of Guelph Animal Care Committee. Cows were managed according to the guidelines set forth by the Canadian Council on Animal Care (CCAC, 2009).

Experimental Design

The number of animals required per treatment was determined through sample size and power analysis (Morris, 1999) to detect a 10% level of observed difference for the primary outcome variables, including feeding behavior and feed sorting. Estimates of variation for these variables were based on previously reported values (Leonardi and Armentano, 2003; Bach et al., 2007; Ferraretto et al., 2012). Cows were divided into 2 groups of 6, which were balanced according to DIM, milk production, and average parity. Within each group, cows were randomly exposed to each of 2 treatment diets in a replicated crossover design (with groups replicated over time), with 35-d treatment periods. The treatment diets were (1) a control TMR (Tables 1 and 2) and (2) a control TMR plus 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada). The control diet was formulated to meet the nutrient requirements of lactating dairy cows at 90 DIM producing 45 kg/d (NRC, 2001). Cows received 28 d of adaptation to each treatment followed by 7 d of data collection.

Feeding Procedure

Cows were individually assigned to 1 roughage intake feed bin (Insentec B.V., Marknesse, the Netherlands) to

Table 1. Ingredient and chemical composition of the experimental diet

Composition	Diet
Ingredient, % of DM	
Corn silage[1]	25.9
Oat and red clover silage[2]	12.4
Red clover and timothy or orchard grass haylage[3]	19.4
High-moisture corn	13.1
Protein concentrate pellet[4]	14.4
Grain supplement pellet[5]	14.8
Chemical composition[6]	
DM, %	54.0 ± 1.90
OM, % of DM	92.2 ± 0.41
CP, % of DM	17.9 ± 0.61
ADF, % of DM	21.1 ± 0.58
NDF, % of DM	34.4 ± 1.35
Fat, % of DM	3.7 ± 0.25
NFC, % of DM	36.2 ± 1.44
Ca, % of DM	1.05 ± 0.11
P, % of DM	0.49 ± 0.027
NE_L, Mcal/kg of DM	1.66 ± 0.019

[1]Corn silage had a DM of 45.1 ± 2.7% and chemical composition (DM basis) of 8.2 ± 0.32% CP, 19.0 ± 0.37% ADF, and 32.7 ± 0.55% NDF.

[2]Oat (40%) and red clover (60%) silage had a DM of 50.4 ± 2.3% and chemical composition (DM basis) of 14.9 ± 3.14% CP, 39.4 ± 1.08% ADF, and 54.5 ± 4.90% NDF.

[3]Red clover (70%) and timothy or orchard grass (30%) haylage had a DM of 37.9 ± 5.8% and chemical composition (DM basis) of 19.0 ± 1.08% CP, 34.5 ± 0.82% ADF, and 48.3 ± 1.03% NDF.

[4]Supplied by Dundas Feed & Seed Ltd. (Winchester, Ontario, Canada) including the ingredients (as is): 40% corn dried distillers grains, 25% soybean meal, 16% canola meal, 6.8% calcium carbonate, 4.5% feather meal, 2.8% salt, 2.0% sodium bicarbonate, 1.5% tallow, 0.8% dicalcium phosphate, 0.4% magnesium oxide, 0.15% trace minerals, and 0.05% vitamins.

[5]Supplied by Dundas Feed & Seed Ltd. including the ingredients (as is): 35% wheat shorts, 18% canola meal, 15% corn dried distillers grains, 13.5% wheat, 10% barley, 2.8% calcium carbonate, 2% cane molasses, 2% corn gluten meal, 1% pelleting agent, 0.62% salt, 0.04% trace minerals, 0.025% flavor, and 0.015% vitamins.

[6]Values were obtained from chemical analysis of TMR samples. OM = 100 − % ash. NFC = 100 – (% CP + % NDF + % fat + % ash). NE_L was calculated based on NRC (2001) equations.

Table 2. Particle size distribution[1] and NDF content of the particle fractions of the experimental diet (mean ± SD)

Particle	Diet
DM retained on screen, %	
Long	7.2 ± 2.47
Medium	45.7 ± 2.76
Short	31.9 ± 3.04
Fine	15.3 ± 2.74
NDF, % of screen DM	
Long	52.3 ± 2.10
Medium	38.7 ± 1.61
Short	27.0 ± 1.97
Fine	23.8 ± 0.68
CP, % of screen DM	
Long	11.6 ± 0.87
Medium	15.6 ± 0.73
Short	17.4 ± 0.95
Fine	25.2 ± 0.86

[1]Particle size determined by Penn State Particle Separator (Nasco, Fort Atkinson, WI), which has a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine).

measure individual feed intake and feeding behavior, as validated by Chapinal et al. (2007). Cows received 3 d of training before the start of the experimental period to learn to access their own unique feed bin.

The TMR (without the grain supplement; Table 1) was mixed once daily in a TMR mixer wagon (Jaylor 4425, Jaylor Fabricating, Orton, ON, Canada) and delivered via conveyor into a motorized feed cart (WIC RTM-55, WIC Inc., Wickham, QC, Canada) at 1200 h daily. The grain supplement was weighed on a scale (model 2020, Mettler-Toledo, Columbus, OH) and mixed into the TMR for approximately 4 min using the motorized feed cart. Cows were denied access to the feed bins beginning at 1400 h daily (when they left for milking), at which time feed refusals were removed and sampled as needed and fresh feed was manually delivered into each feed bin. For those cows on the yeast treatment, 0.5 g/head per day of Levucell SC20 (i.e., 1×10^{10} cfu) was provided through a diluted version (diluted at 5% with calcium carbonate and dried distillers grains with solubles), top-dressed at a rate of 10 g/head per day on each cow's TMR.

The total amount of feed offered was adjusted daily to ensure approximately 10% feed refusal per cow. Actual feed refusal averaged 15.0 ± 10.9% (mean ± SD) of the feed offered as fed over the course of the experiment and did not vary by treatment ($P = 0.4$). Cows were given access to the feed bins beginning at 1430 h daily; this time point served as the start of each data collection day.

Behavioral Data Collection

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

Lying behavior patterns of the cows were automatically collected using data loggers (HOBO Pendant G Logger, Onset Computer Corporation, Pocasset, MA) for the last 7 d of each treatment period. These devices measured leg orientation at 1-min intervals and allowed all the standing and lying behavior data to be collected electronically (Ledgerwood et al., 2010). On d 28 of each period, data loggers were placed on the hind leg of each cow using veterinary bandaging tape

(Vetrap Bandaging Tape, 3M, London, ON, Canada) while the cow was restrained in a stall. Data loggers were removed from the cows on d 1 of the following experimental period to ensure a complete 7-d recording period. Data collected were used to calculate standing and lying duration (min/d), bout frequency (n/d), and bout length (min/bout).

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

Ruminal Temperature Data Collection

Ruminal temperature was selected as a measurement for rumen health, as the association between rumen temperature and pH has been previously validated (Alzahal et al., 2008; 2009). Ruminal temperature was recorded for the last 7 d of each treatment period using a telemetric acquisition system (SmartStock LLC, Pawnee, OK), which was composed of the following: a telemetric ruminal bolus (3 cm in diameter and 8.5 cm in height, 120 g in weight), an antenna, a barn receiver unit, a base receiver unit, and a personal computer equipped with a software program for data logging (as validated by Alzahal et al., 2009). Ruminal temperature measurements were broadcasted through a radio frequency (0.3–3.0 GHz) from the bolus to the barn receiver unit through the antenna that was within 20 m of the heifers. The signal was then transmitted (0.9 GHz) from the barn receiver unit to the base receiver unit (located within 10 m), which was connected via cable to a personal computer. The bolus was administered, using a bolus gun, to each cow before the start of the study. The bolus was customized to transmit automatically every minute and each transmission included 12 recordings (i.e., the current and previous 11 recordings) to minimize loss of data resulting from lost transmissions. Data collected were used to calculate daily minimum, maximum, and mean ruminal temperature. The durations (min/d) that ruminal temperature was above given thresholds (38.0 and 39.0°C) were computed to describe the magnitude of elevation in temperature in the rumen (Alzahal et al., 2008, 2009).

Feed Sampling and Analysis

For the last 7 d of each experimental period, duplicate samples of fresh feed were collected at feeding time for the determination of DM, nutrient content, and particle size distribution of the TMR. Duplicate samples of feed refusal for each cow were collected for determination of DM and for particle size separation to determine feed sorting. On d 3, 17, and 31 of each treatment period, duplicate samples of dietary components were collected for DM, chemical, and particle size analysis. All samples were immediately frozen at −20°C until they were further analyzed.

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

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

Milk Production and Components

Milk yield was automatically recorded at each milking for the last 7 d of each treatment period by the robotic milking system (Lely A3 Next, Lely Industries N.V.). Milk samples were collected from each milking for the last 6 d (d 30–35) of each experimental period using the Lely Shuttle Sampling Device (Lely Industries N.V.). Samples taken on d 30, 32, and 34 were sent to a DHI testing laboratory (CanWest DHI, Guelph, ON, Canada) for analysis of milk fat and protein percentage, SCC, and MUN using a near-infrared analyzer (FOSS System 4000 Infrared Transmission Analyzer, Foss, Hillerød, Denmark). Samples taken on d 31, 33, and 35 were sent to the University of Guelph (Guelph, ON, Canada) and analyzed for FA profile.

Milk fat was extracted and methylated and samples were dissolved in pentane using protocols adapted from

Christie (1982) and Chouinard et al. (1997). Fatty acid analysis was performed by injecting FAME into a gas chromatograph (model 7890B, Agilent Technologies, New Castle, DE) equipped with an automatic on-column injector (G4513A, Agilent Technologies) and a flame-ionization detector using a CP-Sil88 fused silica capillary column (100 m × 0.25 mm × 0.2 μm film thickness, CP 7489, Agilent Technologies). Helium was used as the carrier gas at flow rate of 1 mL/min. One microliter of sample was injected directly, cold, on-column at an oven temperature of 50°C. After initiation, the column temperature remained at 50°C for 5 min, then increased by 14°C/min to 165°C, then increased by 2°C/min to 220°C, and subsequently retained at that temperature for 17 min. Identification of FAME peaks were based on retention time of FAME mix C4-C24 FA standard (Supelco, Bellefonte, PA).

For those days where milk components were measured, the yield of 4% FCM (kg/d) was calculated (NRC, 2001) as 0.4 × milk yield (kg/d) + 15.0 × fat yield (kg/d). Energy-corrected milk was calculated using the following equation: ECM = (0.327 × kg of milk) + (12.95 × kg of fat) + (7.2 × kg of protein) (Tyrrell and Reid, 1965). Efficiency of milk production was determined by calculating the kilograms of milk, 4% FCM yield, or ECM yield per kilogram of DMI for each treatment period.

Calculations and Statistical Analysis

Individual feeding bouts were separated into meals using an individual meal criterion (minimum intermeal interval) for each cow on each treatment. Meal criteria were determined, as described by DeVries et al. (2003), using a software package (MIX 3.1.3; MacDonald and Green, 1988) to fit a mixture of normal distributions to the distributions of $\log_{10}$-transformed time intervals between moments of feeding (across all 7 d of data recorded per treatment period). The calculated meal criteria were used to calculate meal frequency (meals/d) by counting the number of intervals that exceeded the criterion and adding one. Meal duration (min/meal) was calculated as the time from the start of the first feeding bout until the end of the last feeding bout at which time the meal criterion was exceeded. Meal size (kg/meal) was calculated by dividing DMI by meal frequency.

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

Data for feeding, ruminating, and lying behavior, DMI, sorting activity, rumen temperature, milk yield, milk composition, and production efficiency were summarized for each cow by treatment period. Prior to analyses, all data were screened for normality using the UNIVARIATE procedure of SAS (SAS Institute, 2013). Data for SCC were right-skewed and, thus, were transformed by taking the natural logarithm.

To test whether sorting of the diets occurred, sorting activity for each fraction of the PSPS was summarized by treatment and tested for a difference from 100 using *t*-tests. All data were then analyzed using the MIXED procedure of SAS. The final model included the fixed effects of period, order of treatment exposure, and treatment. The random effects were group and cow within order of treatment exposure and group. Degrees of freedom for fixed effects were estimated using the Kenward-Roger option in the MODEL statement.

Data for DMI, feeding time, and feeding rate were also summarized on an hourly basis, whereas ruminating time was summarized on a 2-h basis, for each animal on each treatment. Differences among treatments in the distribution of these variables over a 24-h period were analyzed using the MIXED procedure of SAS, treating hour as a repeated measure. The final model included the fixed effects of period, order of treatment exposure, hour, treatment, and hour × treatment interaction. Other interactions of the fixed effects were tested in the initial model and were not significant; therefore, they were removed from the final model. The random effects were group and cow within order of treatment exposure and group. Cow within order of treatment exposure and group was included in the model as the subject of the repeated statement. Compound symmetry was selected as the covariance structure on the basis of best fit according to Schwarz's Bayesian information criterion. Degrees of freedom for fixed effects were estimated using the Kenward-Roger option in the MODEL statement. All values reported are least squares means. Significance was declared at $P \leq 0.05$, and trends reported if $0.05 < P \leq 0.10$. One cow, on the live yeast treatment, developed clinical mastitis on d 32 of the last treatment period of the study. Therefore, only data from d 29 to 31 of that period for that cow were used in the analysis.

Table 3. Effect of treatment diets on DMI, feeding behavior, rumination, and lying behavior[1]

	Diet[2]			
Item	Control	Yeast	SED[3]	*P*-value
DMI, kg/d	28.0	28.5	0.44	0.22
Feeding time, min/d	226.6	232.0	11.47	0.65
Feeding rate, kg/min	0.14	0.13	0.0089	0.54
Meal criterion, min	25.8	20.0	2.31	0.04
Meal frequency, meals/d	7.8	9.0	0.57	0.07
Interval between meals, min	160.3	142.1	9.85	0.09
Meal size, kg of DM/meal	3.8	3.4	0.21	0.09
Meal duration, min/meal	35.3	32.5	3.02	0.39
Rumination, min/d	544.9	570.3	13.17	0.08
Lying bouts, no./d	9.6	9.5	0.43	0.83
Lying time, min/d	697.5	671.1	38.19	0.51

[1]Data are averaged over 7 d for 12 cows on each treatment.

[2]Control = control TMR; yeast = control TMR with 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada).

[3]Standard error of the difference.

RESULTS

Live yeast supplementation had no effect on DMI (Table 3; Figure 1a). No effect of treatment on the amount of time spent consuming feed (Table 3; Figure 1b) or rate (Table 3; Figure 1c) at which it was consumed was observed. Despite no differences in the time course of eating, when cows were supplemented with live yeast, they did modify their meal patterning (Table 3). Meal criteria were 20% (5 min) shorter, which translated into those cows tending to have 1.2 more meals per day, which also tended to be spaced closer together in time and smaller in size, but not different in duration. When cows were fed yeast, they tended to spend 25.4 min more time ruminating across the day (Table 3; Figure 2). The amount of lying time and frequency of lying bouts were similar between treatments (Table 3).

Cows sorted against long particles when supplemented with yeast, but did not on the control ration (Table 4). No sorting for or against medium particles occurred on either treatment diet. Cows sorted against short and fine particles on both treatment diets. The extent of sorting against the short particles was greater when cows were supplemented with yeast. The extent of sorting against the fine particles tended to be greater when cows were fed the control ration.

No effect of yeast supplementation was observed on milk yield or efficiency of production (Table 5). A tendency was noted for milk fat content to be 0.16 percentage points higher and milk fat yield to be 0.07 kg/d higher when cows were supplemented yeast. No treatment effect was seen for protein content or yield. Milk urea nitrogen and SCC were also similar between treatments. No differences in milk FA composition were seen, with the exception of a tendency for a greater concentration of 18:2 *cis*-9,*cis*-12 FA when cows were yeast supplemented (Table 6). Yeast-supplemented cows had lower mean ruminal temperature (by 0.1°C), spent 14 min/d less time with rumen temperature above 39.0°C, and tended to spend 86 min/d less time with rumen temperature above 38.0°C (Table 7).

DISCUSSION

The primary aim of the current study was to investigate the effect of live yeast supplementation on the feeding behavior of lactating dairy cows. Whereas yeast supplementation did not affect the amount of time spent eating or the rate at which feed was consumed, in support of our hypothesis, it did affect the meal patterning of cows. Meal criteria were 20% shorter when cows had yeast; this corresponded to cows tending to have 1.2 more meals per day when supplemented than when they had no yeast supplementation. These meals also tended to occur closer in time to each other. These results very are similar to Bach et al. (2007), who found that meal criteria were numerically shorter with live yeast supplementation than with no yeast (11.5 vs. 14.5 min), and that interval between meals in yeast-supplemented cows was shorter than in nonsupplemented cows. These results combined suggest that when cows are provided live yeast, they pattern their meals closer in time together and have more frequent meals. Similar to Bach et al. (2007), in the present study meal length was not affected by yeast supplementation. However, meal size in the present study tended to be 10% smaller when cows were yeast supplemented. This is similar to that reported by Loncke et al. (2012), who found that growing dairy bull calves supplemented with live yeast had a greater frequency of visits to the feed trough, consuming smaller amounts per visit.

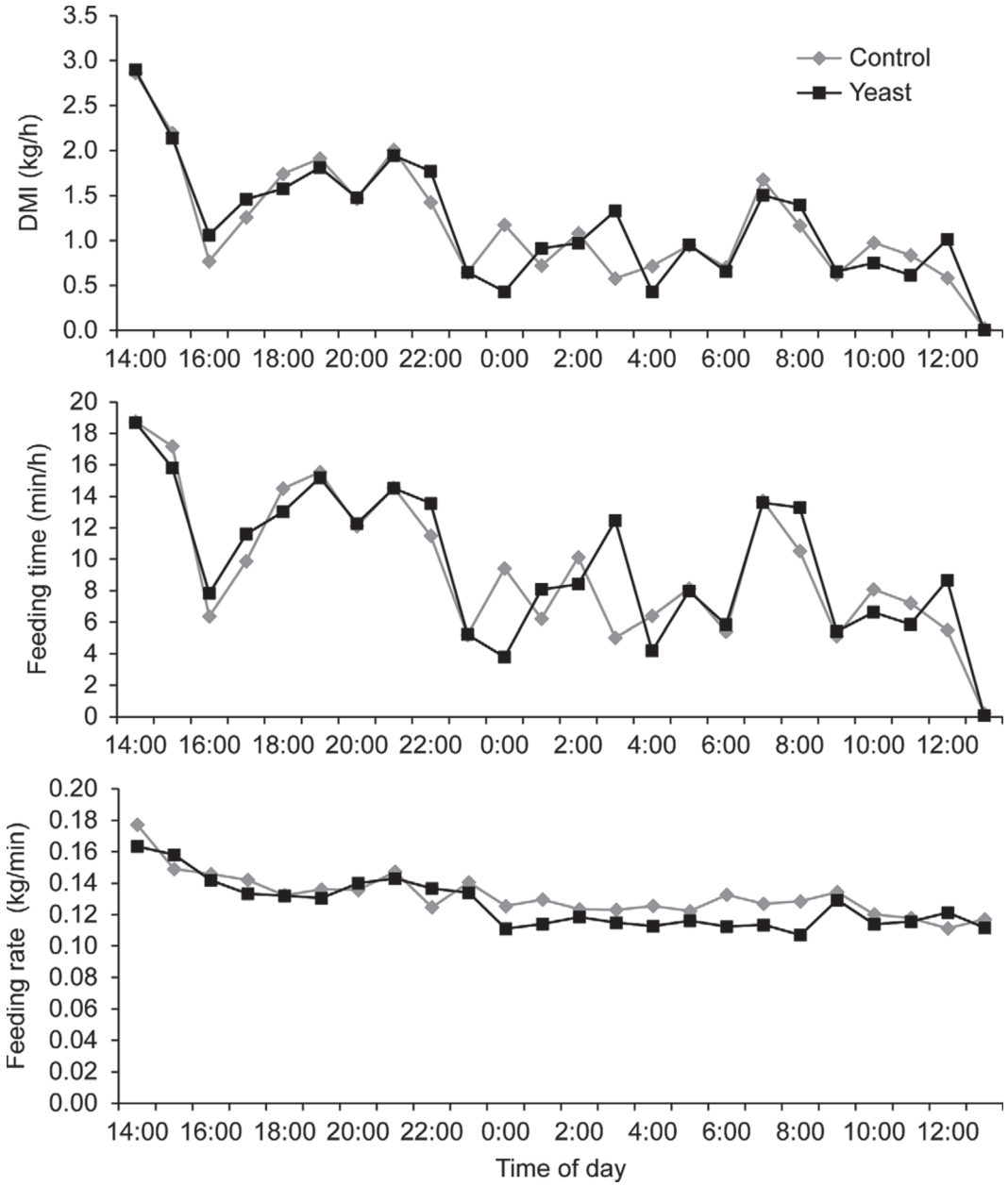

Figure 1. Hourly average of DMI (kg/h; SE = 0.48), feeding time (min/h; SE = 3.97), and feeding rate (kg of DMI/min; SE = 0.014) of lactating dairy cows on a control TMR (Control) and a control TMR with 1×10^{10} cfu/head per day of live yeast (Yeast; *Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada). Cows were fed at 1400 h and milked 3× per day at 1400, 2100, and 0700 h. Data are averaged over 7 d for 12 cows on each treatment.

It is noteworthy that, using similar feeding technology and methodology to calculate meals, Ferraretto et al. (2012) found no differences in meal patterning when cows were supplemented with live yeast, suggesting this was due to a lack of an effect on ruminal propionate production. A potentially contributing factor to this difference in results from the present was that Ferraretto et al. (2012) used a common meal criterion (27.7 min; DeVries et al., 2003) for their meal calculations. As described by DeVries et al. (2003), calculating meal criteria for individual cows for each treatment may be more appropriate than using a common criterion in cases where specific predictions concerning the treatment response of the criterion-based measures are used. Results of the current study and those of Bach et al. (2007) demonstrated that yeast supplementation

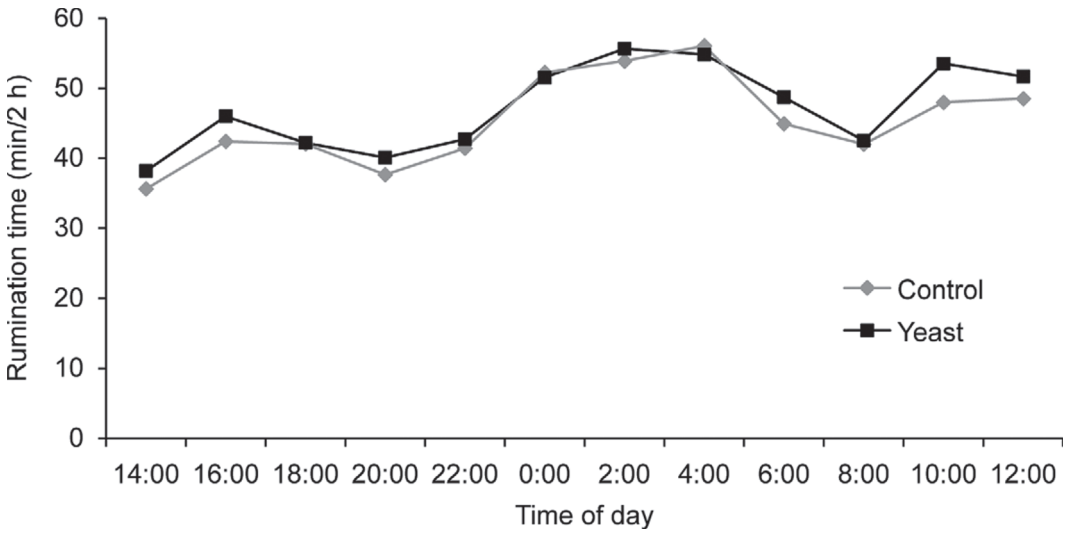

Figure 2. Bihourly average rumination time (min/2 h; SE = 2.75) of lactating dairy cows on a control TMR (Control) and a control TMR with 1×10^{10} cfu/head per day of live yeast (Yeast; *Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada). Cows were fed at 1400 h and milked 3× per day at 1400, 2100, and 0700 h. Data are averaged over 7 d for 12 cows on each treatment.

may affect the intervals between bouts, both within and between meals, and thus affect the calculated meal criteria for individual cows.

A modification of meal patterning in response to a rumen modifier is not unique to yeast supplementation. Evidence exists that providing monensin to dairy cattle (Lunn et al., 2005) and feedlot beef cattle (Erickson et al., 2003) subjected to SARA resulted in an increase in meal frequency. Similarly, Mullins et al. (2012) found that monensin increased meal frequency and decreased the time between meals in the first few days after dairy cows were transitioned to a lactation ration. These authors associated these changes in meal patterns to a reduction in ruminal pH variation. Whereas meal patterning may, in itself, affect ruminal pH (Allen, 1997), it is likely that rumen modifiers, such as live yeast, that have the potential to stabilize ruminal pH and fermentation, will then affect meal patterning as a secondary effect. Specifically, a more consistent fermentation pattern may result in less variation in VFA production, improved fiber digestibility, and quicker return to eating.

Though we were unable to directly measure rumen fermentation end products or kinetics, results of the current study suggest that live yeast supplementation had a positive effect on the rumen environment. A ten-

Table 4. Effect of dietary treatments on the sorting (%)[1] of long, medium, short, and fine particles[2]

	Diet[3]			
Item	Control	Yeast	SED[4]	*P*-value
Sorting of particle fractions, %				
Long	98.3	94.2*	1.83	0.04
Medium	101.8	102.2	0.38	0.24
Short	98.6*	96.8*	0.63	0.02
Fine	97.9*	98.8*	0.46	0.08

[1]Sorting % = 100 × (n DMI/n predicted DMI), where n = particle fraction (long, medium, short, or fine). Sorting values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for). Data are averaged over 7 d for 12 cows on each treatment.

[2]Particle size determined by Penn State Particle Separator (Nasco, Fort Atkinson, WI), which has a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine).

[3]Control = control TMR; Yeast = control TMR with 1×10^{10} cfu/head/d live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada).

[4]Standard error of the difference.

*$P < 0.05$, all other values are $P > 0.05$.

Table 5. Effect of dietary treatments on milk yield, milk composition, and milk component yield, efficiency of production, MUN, and SCC

	Diet[1]			
Item	Control	Yeast	SED[2]	*P*-value
Milk yield, kg/d				
Milk[3]	45.8	45.7	0.65	0.97
4% FCM[4]	42.9	43.1	1.17	0.87
ECM[4]	45.8	45.6	1.22	0.86
Milk composition, %[4]				
Fat	3.55	3.71	0.083	0.09
Protein	2.91	2.89	0.024	0.50
Milk component yield, kg/d[4]				
Fat	1.63	1.70	0.037	0.10
Protein	1.34	1.31	0.042	0.54
Efficiency of milk production, kg/kg (unless otherwise noted)				
Milk/DMI[3]	1.65	1.63	0.022	0.35
4% FCM/DMI[4]	1.52	1.55	0.061	0.66
ECM/DMI[4]	1.63	1.65	0.065	0.73
MUN, mg/dL	12.8	12.6	0.55	0.79
Log SCC[5]	10.91	11.03	0.28	0.67
Retransformed SCC (×1,000 cells/mL)	54.7	61.7	—	—

[1]Control = control TMR; yeast = control TMR with 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada).

[2]Standard error of the difference.

[3]Data are averaged over 7 d for 12 cows on each treatment.

[4]Data are averaged over 3 d for 12 cows on each treatment.

[5]Somatic cell counts (cells/mL) were log-transformed, given that they did not meet the assumption of normality.

Table 6. Effect of treatment diets on milk FA composition (g/100 g of total FA)[1]

	Diet[2]			
Item	Control	Yeast	SED[3]	*P*-value
4:0	3.23	3.30	0.141	0.70
6:0	2.55	2.55	0.153	0.99
8:0	1.49	1.45	0.086	0.62
10:0	3.41	3.36	1.132	0.96
11:0	0.41	0.38	0.033	0.39
12:0	3.85	3.84	0.208	0.95
13:0	0.22	0.20	0.026	0.56
14:0	13.29	13.01	0.391	0.48
14:1	0.97	0.93	0.074	0.58
15:0 *iso*	0.58	0.53	0.059	0.33
15:0	1.49	1.43	0.089	0.47
16:0 *iso*	0.31	0.29	0.046	0.61
16:0	27.67	27.30	0.788	0.65
16:1	1.81	1.70	0.089	0.25
17:0 *iso*	0.05	0.29	0.201	0.23
17:0	0.93	0.95	0.052	0.68
18:0	11.32	11.56	0.547	0.69
18:1 *trans*-9	2.15	2.20	0.145	0.73
18:1 *cis*-9	20.68	20.94	1.037	0.81
18:2 *cis*-9,*cis*-12	2.48	2.71	0.126	0.08
18:3 *cis*-9,*cis*-12,*cis*-15	0.42	0.44	0.029	0.58
CLA	0.69	0.65	0.062	0.58
Summation				
Total SFA	70.81	70.43	1.246	0.76
Total MUFA	25.60	25.77	1.149	0.89
Total PUFA	3.59	3.80	0.167	0.21

[1]Data are averaged over 7 d for 12 cows on each treatment.

[2]Control = control TMR; yeast = control TMR with 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada).

[3]Standard error of the difference.

Table 7. Effect of treatment diets on ruminal temperature characteristics obtained by a telemetric monitoring system[1]

	Diet[2]			
Item	Control	Yeast	SED[3]	*P*-value
Mean, °C	38.5	38.4	0.010	0.02
Maximum, °C	39.6	39.6	0.054	0.44
Minimum, °C	34.2	34.1	0.22	0.61
Duration, min/d				
>39.0°C	366.9	353.1	5.50	0.001
>38.0°C	780.0	693.9	29.07	0.06

[1]Data are averaged over 7 d for 12 cows on each treatment.

[2]Control = control TMR; yeast = control TMR with 1×10^{10} cfu/head per day of live yeast (*Saccharomyces cerevisiae* CNCM I-1077; Levucell SC20; Lallemand Animal Nutrition, Montreal, QC, Canada).

[3]Standard error of the difference.

dency for cows to spend 25 min/d less time ruminating was observed when they were not supplemented with yeast. Cows experiencing lower rumen pH have been shown to have decreased rumination activity (DeVries et al., 2009). Furthermore, in the current study, cows supplemented with live yeast experienced lower mean ruminal temperature and less time with rumen temperature above a threshold of 39°C. In work by AlZahal et al. (2008, 2009), it was shown that duration of time above 39°C is negatively associated with ruminal pH and positively associated with duration of time with ruminal pH <6.0. Given the tendency for increased meal frequency with yeast supplementation, it is possible that this translated into more frequent drinking bouts per day. Thus, it is possible that the mean change in ruminal temperature with yeast supplement may partially reflect increased depressions in temperature associated with water consumption. Water consumption behavior was not recorded; thus, these drops in temperature could not be identified with any level of confidence. Regardless, the difference in duration of time ruminal temperature was greater than 39°C would be irrespective of any water-related drops in ruminal temperature, thus providing evidence that the yeast supplementation was having some effect on the rumen environment. The improvement in rumen environment in dairy cows, particularly rumen pH, with live yeast supplementation is consistent with observations reported elsewhere (Michalet-Doreau and Morand, 1996; Nocek et al., 2002; Bach et al., 2007). Similar to the temperature results, Thrune et al. (2009) reported greater mean ruminal pH and less time spent under a pH threshold of 5.6 with live yeast supplementation. Similarly, Chung et al. (2011) reported that time spent below pH of 5.8 was numerically reduced using the same live yeast strain as used in the present study. *Saccharomyces cerevisiae* has been shown to create a more anaerobic environment through oxygen scavenging (Newbold et al., 1996), as well as provide growth factors (including organic acids, B vitamins, and AA) that stimulate microbial growth, particularly lactate utilizers (Chaucheyras-Durand et al., 2008). Moreover, *S. cerevisiae* has been demonstrated to be more competitive for utilization of sugars than lactate-producing bacteria (Chaucheyras et al., 1996). Thus, it is believed that *S. cerevisiae* supplementation can stabilize rumen pH by promoting more continuous rumen microbial growth and less lactic acid production, thus resulting in more consistent microbial protein synthesis across the day and lesser chance of periods of VFA accumulation (Chaucheyras-Durand et al., 2008).

As demonstrated in several previous studies (Wohlt et al., 1998; Bach et al., 2007; Thrune et al., 2009; Al Ibrahim et al., 2010; de Ondarza et al., 2010), live yeast supplementation had no significant effect on DMI. This is contrary to results of a meta-analysis by Desnoyers et al. (2009a), who reported that, across ruminant species, yeast supplementation increased DMI by 0.44 g/kg of BW. Several researchers have reported productive improvements with live yeast supplementation, including milk yield, FCM yield, milk fat content, and efficiency of production (Desnoyers et al., 2009a; Moallem et al., 2009; de Ondarza et al., 2010). In the current study, no effect of yeast supplementation on milk yield or efficiency of production was seen; this was not surprising, as these production outcomes were secondary to our primary study outcomes (behavior measures), upon which our statistical power was based. Despite this, a tendency was noted for milk fat content and yield to each be 4.5% higher when cows were supplemented live yeast. Improvements in milk fat production have been observed in several studies investigating live yeast supplementation. For example, Desnoyers et al. (2009a) reported a trend for increased milk fat content (0.05 percentage points) in their meta-analysis on the effect of yeast supplementation. Al Ibrahim et al. (2010) reported greater milk fat content as result of live yeast supplementation in early lactation cows (wk 2 and 3 postpartum). More recently, Ferraretto et al. (2012)

reported that supplementing dairy cows with live yeast at a dosage of 4 g/cow per day (6×10^{10} cfu) in a high-starch diet tended to increase milk fat content compared with high-starch diets (31.4%) either without or with 2 g/cow per day (3×10^{10} cfu) of supplementation. These more consistent increases in milk fat content with yeast supplementation are likely attributable to the previously mentioned improvements in rumen fermentation, as higher rumen pH will prevent shifts in the ruminal biohydrogenation pathways, reducing production of the *trans*-10,*cis*-12 isomer of CLA (Choi et al., 2005), thus promoting greater milk fat synthesis (Bauman and Griinari, 2001). Unfortunately, given the methods used in our gas chromatography identification of FA, we were unable to differentiate CLA isomers. Thus, we are unable to make any conclusions on the effect of live yeast supplementation on production of biohydrogenation intermediates and recommend this be investigated in future research. Nevertheless, our results do provide further evidence that live yeast supplementation may be particularly useful in situations where the risk of milk fat depression is high, such as with high-starch diets (as suggested by Ferraretto et al., 2012), or in early lactation cows, who are greater risk of experiencing low rumen pH (Penner et al., 2007).

It is noteworthy that a tendency for more linoleic acid (18:2 *cis*-9,*cis*-12) in milk was observed when cows were supplemented live yeast. This is plausibly explained by the feed sorting data. When cows were supplemented with live yeast they consumed less long forage particles as a proportion of their total intake; those long forage particles would be lower in linoleic acid compared with the smaller particles containing more plant oils (i.e., from corn and soy). Increased dietary selection with yeast supplementation is not unique to our study. Desnoyers et al. (2009b) reported that goats that were live yeast supplemented sorted their ration more against fiber than unsupplemented goats. Even though Ferraretto et al. (2012) reported no overall effect of live yeast supplementation on feed sorting of dairy cows, they did report that supplemented cows fed a high-starch diet selectively refused long particles and consumed fine particles to a greater extent compared with unsupplemented cows fed a low-starch diet. As suggested by Desnoyers et al. (2009b), it is apparent from these results that supplementation with live yeast allows ruminants to cope with lower intake of fiber than predicted while maintaining production.

CONCLUSIONS

Supplementing lactating dairy cows with live yeast resulted in improvements in meal patterning, including more frequent meals that tended to be smaller and occur closer in time together. Cows supplemented with live yeast also tended to ruminate longer and have less periods of elevated rumen temperature. Despite sorting more against the longest, most fibrous ration particles, yeast-supplemented cows tended to have higher milk fat content and yield.

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