



## Effect of dietary supplementation with live-cell yeast at two dosages on lactation performance, ruminal fermentation, and total-tract nutrient digestibility in dairy cows

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### ABSTRACT

The experimental objective was to determine the effect of dietary supplementation with live-cell yeast (LCY; Procreatin-7, Lesaffre Feed Additives, Milwaukee, WI) at 2 dosages in high-starch (HS) diets [30% starch in dry matter (DM)] on lactation performance, ruminal fermentation, and total-tract nutrient digestibility in dairy cows compared with HS or low-starch (LS; 20% starch in DM) non-LCY diets. Sixty-four multiparous Holstein cows ( $114 \pm 37$  d in milk and  $726 \pm 74$  kg of body weight at trial initiation) were randomly assigned to 32 electronic gate feeders (2 cows per feeder), which were randomly assigned to 1 of 4 treatments in a completely randomized design. A 2-wk covariate adjustment period with cows fed a 50:50 mixture of the HS and LS diets was followed by a 12-wk treatment period with cows fed their assigned treatment diets. The HS diets were fed without (HS0) and with 2 (HS2) or 4 (HS4) g/cow per day of LCY. The LS diet did not contain LCY (LS0) and was formulated by partially replacing dry ground shelled corn with soy hulls. Cows fed LS0 consumed more DM than cows fed HS diets during wk 3, 10, 11, and 12. Yields of actual (44.5 kg/d, on average), fat-, energy-, and solids-corrected milk were unaffected by treatment. Milk fat content tended to be greater for LS0 than for HS0 and HS2 but not different from HS4. Milk urea nitrogen contents were greater for cows fed LS0 than for cows fed the HS diets. Feed conversion (kg of milk/kg of DM intake) was numerically greater for HS diets than for LS0. Ruminal pH was unaffected by treatment. Ruminal molar proportion of acetate was greater, whereas that of propionate was lower, for LS0 compared with HS diets. Dry matter and organic matter digestibilities were greater for HS2 and HS4 than for HS0. Digestibility of neutral detergent fiber was greater for HS4 than for HS0 and HS2. Dry matter, organic matter, and

neutral detergent fiber digestibilities were greater for LS0 than for HS diets; starch digestibility was greater for LS0 than for HS0 and HS4. Feeding LS0 increased DM intake and milk fat content, but reduced feed conversions. The addition of 4 g/cow per day of LCY to HS diets tended to increase milk fat content and increased total-tract fiber digestibility in dairy cows.

**Key words:** lactating cow, rumen, starch, yeast

### INTRODUCTION

Subacute ruminal acidosis has been a significant animal health issue for the dairy industry in recent years (Nocek, 1997). Marden et al. (2008) reported that live-cell yeast (LCY) attenuated the ruminal pH decline after feeding in a similar manner to sodium bicarbonate and reduced mean total ruminal lactate concentrations more than sodium bicarbonate (67 vs. 26% reduction) in early lactation dairy cows fed high corn silage diets. These effects of LCY on ruminal pH and lactate may reduce the incidence of subacute ruminal acidosis and laminitis in dairy cows. The LCY also increased total-tract NDF digestibility compared with both the control and sodium bicarbonate treatment.

Feeding reduced-starch diets is likely to increase ruminal pH and NDF digestibility compared with high-starch diets (Ipharraguerre et al., 2002a,b). Short-term feeding trials suggest that partial replacement of corn grain with high-fiber, low-starch byproduct feedstuffs may be feasible in diets fed to lactating dairy cows (Batajoo and Shaver, 1994; Ipharraguerre and Clark, 2003). However, recent continuous-lactation studies with high-producing cows suggest that reduced-starch diets may reduce milk yield (Ferraretto et al., 2011) and (or) feed conversions (Gencoglu et al., 2010; Ferraretto et al., 2011).

We hypothesized that LCY added to a high-starch diet would improve ruminal fermentation and increase FCM yield compared with the high-starch control without added LCY, and increase milk yield and feed conversions compared with a low-starch control diet without added LCY. The experimental objective was

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to determine the effect of dietary LCY supplementation at 2 dosages on lactation performance, ruminal fermentation, and total-tract nutrient digestibilities in dairy cows.

## MATERIALS AND METHODS

Sixty-four multiparous Holstein cows ( $114 \pm 37$  DIM and  $726 \pm 74$  kg of BW at trial initiation) were randomly assigned to 32 electronic gate feeders (RIC system, Insentec, Marknesse, the Netherlands; 2 cows per gate feeder) in the University of Wisconsin-Madison sand-bedded, freestall barn (Emmons Blaine Dairy Research Center, Arlington, WI). Gate feeders (1.40 m deep, 0.80 m wide, and 0.75 m high) were situated on weigh-cells, and each cow was fitted with an identification transponder to record consumption of each individual cow meal. This electronic feeding system was described by Chapinal et al. (2007). Gate feeders were then randomly assigned to 1 of 4 treatments in a completely randomized design in a continuous-lactation trial; 1 wk for adaptation of cows to gates, a 2-wk covariate adjustment period with cows fed a 50:50 mixture (DM basis) of the high-starch (**HS**) and low-starch (**LS**) diets, and a 12-wk treatment period with cows fed their assigned treatment diets. The HS diets (formulated for 30% starch in DM) were fed without (HS control; **HS0**) and with 2 (**HS2**) or 4 g/cow per d (**HS4**) of LCY. The LS diet did not contain LCY (LS control; **LS0**) and was formulated for 20% starch (DM basis) by partially replacing dry ground shelled corn (**DGSC**) with soy hulls. Ingredient composition of the experimental diets is provided in Table 1. Sodium bicarbonate was not included in the HS diets to increase the negative control aspects of HS0 relative to ruminal pH, but was included in the LS0 diet to increase the positive control aspects of that diet. The HS and LS concentrate mixtures were prepared at the University of Wisconsin Feed Mill (Arlington, WI). Diets were fed as TMR mixed once daily at 1000 h and fed 3 times daily at 1200, 1600, and 0400 h. The LCY (Procreatin-7;  $15 \times 10^9$  cfu/g of *Saccharomyces cerevisiae*) was commercially available from Lesaffre Feed Additives (Milwaukee, WI). The HS2 and HS4 premixes and the HS0 and LS0 placebo premixes were fed at the rate of 56 g/cow per day and added to the TMR separately from the concentrate mixtures. Premixes were prepared at University of Wisconsin-Madison by mixing carrier (calcium carbonate), target yeast dosage, and mineral oil (1% of premix) in a rotating drum mixer (Floor Mixer, Hobart Corp., Troy, OH).

The animal research was conducted under an approved protocol by the Institutional Animal Care and Use Committee of the College of Agricultural and Life

Sciences. All cows were injected with bovine somatotropin (Posilac, Elanco Animal Health, Greenfield, IN) every 14 d commencing on d 1 of the covariate period. The gate feeders were supplied with TMR to allow for 10% refusals, with daily DMI determined on individual cows throughout the 14-wk trial. Feeding behavior was determined using data from the electronic gate feeders. Eating time (min/d) was defined as the time that a cow had her head in the gate feeder. Eating rate (kg of DM/min) was determined by dividing DMI by the time spent eating. Meal frequency (number of daily meals) was computed using a meal criterion of 27.7 min (DeVries et al., 2003) as the minimum time interval between gate visits to be considered a new meal. A meal consisted of eating and interval times or intervals between feeding visits within a meal. Meal duration (min/meal) and size (kg of DM/meal) were determined by dividing eating time and DMI, respectively, by meal frequency.

Body weight and BCS (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded on individual cows on 3 consecutive days at the end of the covariate and treatment periods. Body weight change (**BWC**) for individual cows was calculated as the difference between the average BW at the end of the trial and the end of the covariate period. Milk yield was recorded daily (DairyComp305, Valley Agricultural Software, Tulare, CA) on individual cows milked twice daily in a double-16 parlor (Metatron P21, GEA Farm Technologies, Bakel, the Netherlands) throughout the 14-wk trial and composited by gate before statistical analysis.

**Table 1.** Ingredient composition (% of DM) of the experimental diets

Ingredient	Diet <sup>1</sup>	
	HS	LS
Corn silage	37.5	37.5
Alfalfa silage	12.5	12.5
Dry ground shelled corn	25.1	10.2
Soy hulls	1.9	17.3
Soybean meal (48% CP)	14.6	13.1
Distillers dried grains	5.2	5.2
Energy Booster 100 <sup>2</sup>	1.00	1.00
Calcium carbonate	1.20	1.20
Sodium bicarbonate	—	1.00
Magnesium oxide	0.27	0.27
Mg-K-S <sup>3</sup>	0.10	0.10
Trace mineral salt <sup>4</sup>	0.45	0.45
Vitamin premix <sup>5</sup>	0.18	0.18

<sup>1</sup>HS = high starch, LS = low starch.

<sup>2</sup>Minimum 98% total fatty acids (MSC Company, Dundee, IL).

<sup>3</sup>Dynamate (11% Mg, 18% K, 22% S; The Mosaic Co., Plymouth, MN).

<sup>4</sup>88% NaCl; 0.002% Co; 0.2% Cu; 0.012% I; 0.18% Fe; 0.8% Mn; 0.006% Se; 1.4% Zn.

<sup>5</sup>Vitamin A, 3,300,000 IU/kg; vitamin D, 1,100,000 IU/kg; vitamin E, 11,000 IU/kg.

Milk samples were obtained from all cows weekly on the same 2 consecutive days from the a.m. and p.m. milkings throughout the 14-wk trial and composited by cow by week. Composites were analyzed for fat, true protein, lactose, and MUN concentrations and SCC by infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI) using a Foss FT6000 (Foss Electric, Hillerød, Denmark) with average daily yields of fat and protein calculated from these data for each week. Milk composition data were proportioned by cow milk weight and composited by gate before statistical analysis. Yields of FCM, SCM, and ECM were calculated according to National Research Council (2001) equations. Actual milk, FCM, SCM, and ECM feed conversions were calculated by week using average daily yield and DMI data. Estimated diet energy concentrations was calculated by summing the Mcal of NE<sub>L</sub> from milk production, required for maintenance and in BW change (NRC, 2001), and then dividing the sum by DMI.

Ruminal pH and VFA were determined on samples obtained via rumenocentesis (Garrett et al., 1999; Pereira et al., 1999) from all cows at 6 h after the morning feeding on 1 d at the end of the covariate period, on 1 d during wk 6 of the treatment period, and on 1 d during the last week of the treatment period. Rumen pH was determined immediately using a Cardy Twin pH meter (model #B-213, Spectrum Technologies Inc., Plainfield, IL). Two 1-mL aliquots of rumen fluid were added to microcentrifuge tubes containing 0.02 mL of 50% H<sub>2</sub>SO<sub>4</sub> acid for later VFA analysis. Rumen VFA concentrations were measured by gas-liquid chromatography (Supelco, 1998). Concentrations of individual VFA were measured on a Perkin Elmer Autosystem (Norwalk, CT) using a 4% Carbowax 20 M on 80/120 mesh Carbowax-B-DA, 1.8 mm × 2 mm column (Supelco Inc., Bellefonte, PA).

Locomotion scores, as described by Pereira et al. (1999), were determined for all cows on 1 d at the end of the covariate period, on 1 d during wk 6 of the treatment period, and on 1 d during the last week of the treatment period as cows walked from the freestall pen to the milking parlor. Hoof lesions were scored, as described by Pereira et al. (1999), by a professional hoof trimmer (Karl Burgi, Comfort Hoof Care Inc., Baraboo, WI) on 1 d at the end of the covariate period and on 1 d during the last week of the treatment period.

Samples of TMR, corn silage, alfalfa silage, concentrate mixes, DGSC, distiller dried grains, and soy hulls were obtained weekly and then composited for the covariate period and every 4 wk during the treatment period for analysis. All samples for determination of nutrient composition were dried at 60°C for 48 h in a forced-air oven to determine DM content, ground to

pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen, and composited as described before sending to Dairyland Laboratories Inc. (Arcadia, WI) for analysis. Absolute DM was determined by oven-drying at 105°C for 72 h. All samples were analyzed for DM, OM (method 942.05; AOAC, 2006), CP (method 990.03; AOAC, 2006), ether extract (method 2003.05; AOAC, 2006), NDF using  $\alpha$ -amylase and sodium sulfite (Van Soest et al., 1991), starch (Bach Knudsen, 1997; YSI Biochemistry Analyzer, YSI Inc., Yellow Springs, OH), and particle size. Particle size of TMR, corn silage, and alfalfa silage samples was determined as described by Kononoff et al. (2003). Particle size of the concentrate mixtures, DGSC, and soy hulls were determined by dry sieving using Tyler Ro-Tap Shaker model RX-29 (Mentor, OH) and sieves with 4,760-, 2,380-, 1,191-, 595-, 297-, 149-, and 63- $\mu$ m apertures plus bottom pan with mean particle size calculated using a log normal distribution (Baker and Herrman, 2002). Ruminal in vitro NDF digestibility (30 h) on TMR, alfalfa silage, corn silage, and soy hulls samples, and starch digestibility (7 h) on TMR, DGSC, and corn silage were determined by Dairyland Laboratories Inc. The 30-h in vitro NDF digestibility was performed using an Ankom Daisy Incubator (Ankom Technology Corp., Fairport, NY) as described by Holden (1999). Ruminal in vitro starch digestibility was determined using procedures modified from Richards et al. (1995) for an Ankom Daisy II System (Ankom Technology Corp.).

Total-tract DM, OM, NDF, and starch digestibilities (**DMD**, **OMD**, **NDFD**, and **StarchD**, respectively) were determined using lignin (method 973.18; AOAC, 2006) as an internal marker. Six fecal grab samples were collected from each cow at 8- to 12-h intervals covering every 4-h clock period over 3 consecutive days during wk 4 and 8 of the treatment period. Ort samples were collected daily during the fecal sampling period. Treatment TMR, fecal, and ort samples were composited by period (TMR samples) or gate within period (ort and fecal samples), and the composited samples were analyzed for DM, OM, NDF, starch, and lignin. Total-tract nutrient digestibilities were calculated from lignin and nutrient concentrations in the orts-adjusted diet and feces.

Feed sorting was evaluated during 3 consecutive days during wk 4 and 8 of the treatment period. Individual daily samples (TMR and orts) were analyzed for particle size as described by Kononoff et al. (2003). Dry matter of each fraction was measured after separation by drying at 60°C for 48 h in a forced-air oven. Sorting was calculated as the actual DMI of each fraction expressed as a percentage of the predicted DMI, as described by Leonardi and Armentano (2003); values

<100% indicate selective refusals, >100% indicate preferential consumption, and equal to 100% indicate no sorting.

Data from 2 HS0, 2 HS2, 3 HS4, and 5 LS0 cows were removed from the statistical analysis due to filching or stealing, which resulted in 2 experimental units (gate-feeders) for LS0 being lost from the study. In addition, 2 HS0, 1 HS2, and 3 LS0 cows had truncated records in the later weeks of the trial due to filching, hoof lesions, peritonitis, or toxic mastitis. Stealing was defined as a cow displacing another (victim) cow from the gate feeder without the barrier closing and opening. When stealing occurred, the monitoring system did not record the identity of the stealing cow and instead recorded only a continuous meal for the displaced cow. Stealing cows were instantly removed from the pen upon visual observation of this behavior and were lost from the study. Data from victim cows were omitted the day that stealing occurred. Some cows were able to consume some of the diet by extending over the top of the barrier; this behavior was termed filching. Filching cows remained in the pen and on the gate-feeders, with their intake recorded by the monitoring system. The proportion of intake from nonassigned gates was determined, and cows that consumed more than 15% of their DMI from nonassigned gates had their data removed from the statistical analysis.

Data were analyzed as a completely randomized design with the data from the preliminary period as a covariate using PROC MIXED (SAS Institute, 2004), with week of treatment as repeated measures using the first-order autoregressive covariance structure, which provided the best fit according to Sawa's Bayesian information criterion. The model included treatment, week, and treatment  $\times$  week interaction as fixed effects, and gate-feeder within treatment as a random effect. Degrees of freedom were calculated using the Kenward-Rogers option. Means were determined using the least squares means statement, and treatment means were compared using the Bonferroni *t*-test option after a significant overall treatment *F*-test. The Bonferroni *t*-test is a sequentially rejective test based on the Holm-Bonferroni method (Holm, 1979). Interaction effects were partitioned using the SLICE option (SAS Institute, 2004). Statistical significance and trends were considered at  $P \leq 0.05$  and  $P \geq 0.06$  to  $P < 0.10$ , respectively.

## RESULTS AND DISCUSSION

Nutrient composition and particle size of forages and concentrates are presented in Table 2. The alfalfa and

corn silages were of good quality (NRC, 2001). Diet nutrient composition and particle size are presented in Table 3. The HS diets contained, on average, 10.5 percentage units more starch and 9.6 percentage units less NDF than the LS diet. This was related to the partial replacement of DGSC (approximately 15 percentage units less corn DM) with soy hulls in the LS diet. The HS diets contained 9.1 and 4.3 percentage units, on average, more calculated NFC and TDN at a maintenance level of intake ( $TDN_{1\times}$ ), respectively, than the LS diet. Measurements for other nutrient concentrations were similar across diets. All diets contained 19.8% forage NDF (DM basis).

Treatment effects on covariate-adjusted least squares means for DM and nutrient intakes are presented in Table 4. Dry matter intake did not differ ( $P > 0.10$ ) among the HS treatments. Lack of difference in DMI resulted in similar ( $P > 0.10$ ) nutrient intakes among the HS treatments. The literature is inconsistent with regard to DMI responses to *S. cerevisiae* supplementation of dairy cattle diets, with reports of greater (Desnoyers et al., 2009; Moallem et al., 2009) or similar (Bach et al., 2007) DMI. Supplemental dietary LCY could influence DMI by altering ruminal propionate concentrations (Allen, 1997). However, LCY did not influence ruminal propionate in the current trial (Table 5).

Although no difference ( $P > 0.10$ ) in overall DMI was observed between HS diets and LS0, DMI was 7% greater ( $P < 0.03$ ) on average for LS0 than for HS diets during wk 3, 10, 11, and 12 of treatment (Figure 1). Sodium bicarbonate was added to LS0 and not to the HS diets, which could have influenced the DMI response. However, Erdman (1988), in a literature review, reported that sodium bicarbonate had no effect on DMI when added to diets containing similar proportions of forage and corn silage, as used in our trial. Furthermore, DMI was affected similarly by LS diets in the reports of Gencoglu et al. (2010) and Ferraretto et al. (2011), when sodium bicarbonate was added to both HS and LS diets. Starch intakes were greater and NDF intakes reduced for the HS diets compared with LS0 ( $P < 0.001$ ). Decreased NDF intake as percentage of BW (1.0% on average vs. 1.4% of BW) for HS diets compared with LS0 ( $P < 0.001$ ) suggests that rumen fill did not limit DMI for HS diets. Instead, increased ruminal propionate concentrations (Table 5) with corresponding reduced eating time (mean: 205 vs. 225 min/d for HS and LS0, respectively) at similar eating rate (mean: 0.142 vs. 0.137 kg/min for HS and LS0, respectively) for HS diets compared with LS0 may explain the observed difference in DMI (Allen, 1997; Allen et al., 2009). Least squares means by week on treatment for DMI



**Table 2.** Nutrient composition and particle size of corn silage, alfalfa silage, and concentrates<sup>1</sup>

Item	CS <sup>2</sup>	AS	HSC	LSC	DDGS	SH	DGSC
Nutrient							
DM, % as fed	34.2 ± 2.1	35.3 ± 3.2	90.3 ± 0.3	90.1 ± 0.5	90.6 ± 0.5	87.0 ± 0.7	89.0 ± 0.2
OM, % of DM	95.1 ± 0.3	88.4 ± 0.2	91.4 ± 0.3	90.1 ± 0.4	94.5 ± 0.4	95.0 ± 0.1	98.5 ± 0.4
CP, % of DM	7.9 ± 0.3	22.1 ± 0.8	22.4 ± 1.1	21.9 ± 0.8	28.5 ± 0.1	13.5 ± 2.0	8.1 ± 1.6
NDF, % of DM	36.0 ± 1.6	42.3 ± 1.9	12.7 ± 1.4	32.4 ± 2.0	32.2 ± 1.0	63.7 ± 3.9	7.7 ± 2.5
IVNDFD, <sup>3</sup> % of NDF	54.5 ± 4.3	40.7 ± 2.6	—	—	—	79.9 ± 6.1	—
Starch, % of DM	36.2 ± 1.8	0.3 ± 0.1	35.8 ± 0.7	14.7 ± 0.5	4.1 ± 0.5	0.9 ± 0.7	70.2 ± 6.8
IVStarchD, <sup>4</sup> % of starch	87.3 ± 3.6	—	—	—	—	—	52.3 ± 4.2
Ether extract, % of DM	5.1 ± 0.9	3.9 ± 0.2	5.7 ± 0.6	4.8 ± 0.5	8.5 ± 0.1	2.9 ± 2.2	3.8 ± 0.7
Particle size							
Tyler sieves							
GMPS, <sup>5</sup> μm	—	—	873 ± 113	1,425 ± 298	734 ± 1	3,340 ± 792	615 ± 128
Penn State Sieves, <sup>6</sup> % as-fed retained on sieve							
19 mm	7.1 ± 2.4	33.3 ± 4.3	—	—	—	—	—
8 mm	71.4 ± 2.7	48.9 ± 4.0	—	—	—	—	—
1.18 mm	20.7 ± 3.0	16.5 ± 1.5	—	—	—	—	—

<sup>1</sup>CS = corn silage; AS = alfalfa silage; HSC = high starch concentrate; LSC = low starch concentrate; DDGS = dried distillers grain plus solubles; SH = soy hulls; DGSC = dry ground shelled corn;

<sup>2</sup>Model 1085 forage harvester (Gehl, West Bend, WI) fitted with kernel processor (1.9-cm theoretical length of cut; 2-mm roll clearance).

<sup>3</sup>Ruminal in vitro NDF digestibility at 30 h.

<sup>4</sup>Ruminal in vitro starch digestibility at 7 h.

<sup>5</sup>Geometric mean particle size.

<sup>6</sup>Particle size was measured as described by Kononoff et al. (2003).

are presented in Figure 1; week and week × treatment interactions ( $P < 0.001$ ) were observed.

Treatment effects on covariate-adjusted least squares means for lactation performance measurements are presented in Table 6. Milk yield was unaffected ( $P > 0.10$ ) by treatment. A recent meta-analysis conducted by Desnoyers et al. (2009) found an increase in milk yield with dietary *S. cerevisiae* supplementation or in-

creased yeast dosage. A similar response was reported by Moallem et al. (2009) when cows were fed 6 g/cow per day of LCY ( $1 \times 10^{10}$  cfu/g of *S. cerevisiae*). The LS diets did not influence actual milk, FCM, ECM, or SCM yields in the trials of Batajoo and Shaver (1994), Beckman and Weiss (2005), or Gencoglu et al. (2010). Except for a trend ( $P < 0.06$ ) for greater milk fat content for HS4 than HS0, milk fat content and yield were

**Table 3.** Diet nutrient composition and particle size<sup>1</sup>

Item	Diet <sup>1</sup>		
	COV	HS	LS
Nutrient			
DM, % as fed	50.7 ± 0.9	50.0 ± 1.1	49.0 ± 1.6
OM, % of DM	92.9 ± 0.1	92.2 ± 0.1	91.6 ± 0.3
CP, % of DM	15.7 ± 0.2	16.7 ± 0.3	16.6 ± 0.4
Ether extract, % of DM	4.2 ± 0.1	5.4 ± 0.4	4.9 ± 0.5
NDF, % of DM	35.4 ± 0.2	25.5 ± 0.5	35.1 ± 1.3
NFC, % of DM	38.4 ± 0.2	46.5 ± 0.5	37.4 ± 0.7
Starch, % of DM	23.2 ± 0.2	31.4 ± 0.7	20.9 ± 0.9
TDN <sub>ix</sub> , <sup>2</sup> % of DM	68.4 ± 0.2	76.5 ± 1.5	72.2 ± 1.7
Penn State Separator sieves, <sup>3</sup> % as-fed retained			
19 mm	2.9 ± 0.7	3.8 ± 0.9	5.2 ± 1.2
8 mm	41.3 ± 3.7	37.9 ± 1.9	44.1 ± 3.6
1.18 mm	40.4 ± 2.2	41.8 ± 3.0	37.0 ± 2.0
Bottom pan	15.5 ± 2.2	16.5 ± 2.4	13.7 ± 2.0

<sup>1</sup>COV = covariate period diet formulated to provide 25% starch by mixing the high and low starch concentrate mix; HS = high starch; LS = low starch.

<sup>2</sup>TDN at maintenance; calculated using NRC (2001) summative energy equation.

<sup>3</sup>Particle size was measured as described by Kononoff et al. (2003).

**Table 4.** Effect of treatment on covariate-adjusted least squares means for DM and nutrient intakes

Item	Diet <sup>1</sup>				SEM	<i>P</i> < <sup>2</sup>
	HS0	HS2	HS4	LS0		
DMI, kg/d	28.9	28.0	28.3	30.1	1.0	0.20
OM intake, kg/d	26.7	25.8	26.1	27.6	0.7	0.28
NDF intake, kg/d	7.4 <sup>b</sup>	7.2 <sup>b</sup>	7.2 <sup>b</sup>	10.6 <sup>a</sup>	0.2	0.001
% BW	0.95 <sup>b</sup>	0.94 <sup>b</sup>	1.05 <sup>b</sup>	1.41 <sup>a</sup>	0.4	0.001
Starch intake, kg/d	9.1 <sup>a</sup>	8.8 <sup>a</sup>	8.9 <sup>a</sup>	6.3 <sup>b</sup>	0.2	0.001
CP intake, kg/d	4.8	4.7	4.7	5.0	0.1	0.26

<sup>a,b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and reduced-starch diet with no live-cell yeast added to TMR (LS0).

<sup>2</sup>Week effect ( $P < 0.001$ ) for all parameters; week  $\times$  treatment interaction effect ( $P < 0.001$ ) for all parameters, except OM intake ( $P < 0.01$ ).

unaffected ( $P > 0.10$ ) by LCY supplementation in the current study. Desnoyers et al. (2009) reported a trend for increased milk fat content with *S. cerevisiae* supplementation, whereas Moallem et al. (2009) observed no response. Our results may be related to the similar ruminal acetate and propionate molar proportions among HS diets (Table 5). Milk fat content tended ( $P < 0.06$ ) to be greater for LS0 than for HS0 and HS2. Similar results were reported by others when partially replacing DGSC with mixtures of high-fiber byproducts (Batajoo and Shaver, 1994; Beckman and Weiss, 2005). Increased milk fat content for cows fed the LS0 diet was likely related to both reduced starch and greater NDF intakes (Table 4) and possibly sodium bicarbonate supplementation (Erdman, 1988), which led to a greater ruminal acetate:propionate ratio (Table 5) than was observed for the HS diets. A positive correlation between acetate:propionate ratio and milk fat content was reported by Erdman (1988). Least squares means by week on treatment for milk fat content are presented in Figure 2; week ( $P < 0.001$ ) and week  $\times$  treatment

interactions ( $P < 0.04$ ) were observed. Milk fat content was greater for cows fed HS4 compared with those fed HS0 at wk 3, 4, 6, and 8 and compared with HS2 at wk 8 and 11 of treatment. Because milk fat content was similar for HS4 and LS0, supplemental LCY might be a strategy for reducing milk fat depression with HS diets. Similar milk fat contents for HS4, but not HS0 and HS2, compared with LS0 cannot be explained by ruminal VFA profiles, but greater NDFD for HS4 and LS0 may play a role (Table 7), although the relationship between NDFD and milk fat content was inconsistent in the report of Oba and Allen (1999). More research to elucidate the mechanism by which LCY may increase milk fat with HS diets is warranted.

Milk protein content and yield did not differ ( $P > 0.10$ ) among HS diets. Similar milk protein content has previously been reported with dietary *S. cerevisiae* supplementation (Desnoyers et al., 2009; Moallem et al., 2009). The MUN concentrations did not differ ( $P > 0.10$ ) among HS diets. Milk protein content was numerically greater for cows fed HS diets than for those fed

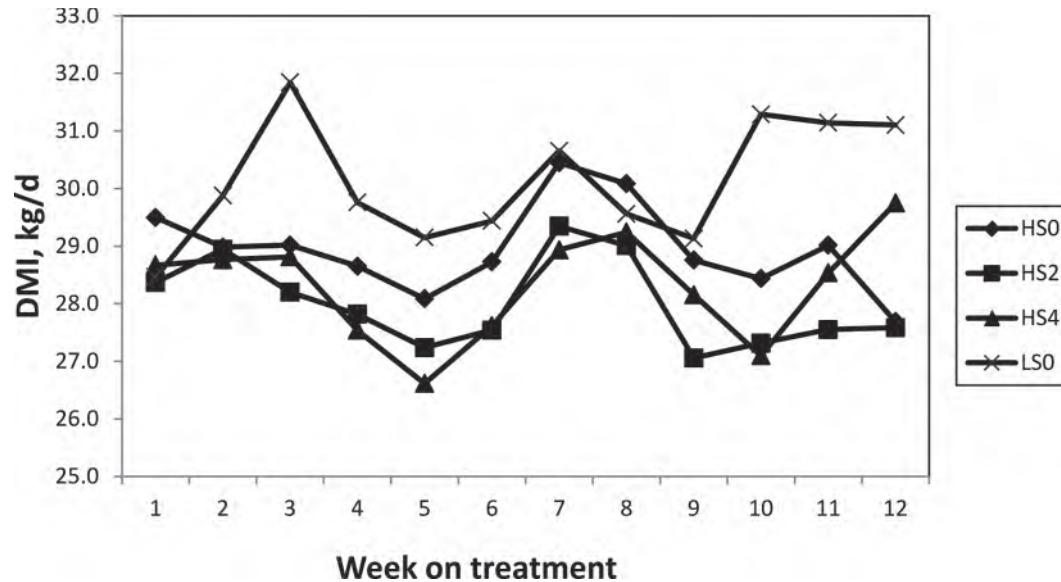
**Table 5.** Effect of treatment on covariate-adjusted least squares means for ruminal pH and VFA

Item	Diet <sup>1</sup>				SEM	<i>P</i> < <sup>2</sup>
	HS0	HS2	HS4	LS0		
pH	6.44	6.51	6.43	6.63	0.10	0.58
Acetate (A), mol/100 mol	59.4 <sup>b</sup>	60.6 <sup>b</sup>	58.8 <sup>b</sup>	64.4 <sup>a</sup>	0.8	0.001
Propionate (P), mol/100 mol	24.5 <sup>a</sup>	23.0 <sup>a</sup>	24.9 <sup>a</sup>	20.5 <sup>b</sup>	0.8	0.01
Butyrate, mol/100 mol	1.3	1.3	1.2	1.3	0.6	0.78
Total VFA, mM	103.4	103.3	107.9	89.6	6.3	0.30
A:P	2.53 <sup>b</sup>	2.78 <sup>b</sup>	2.50 <sup>b</sup>	3.30 <sup>a</sup>	0.12	0.01

<sup>a,b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and reduced-starch diet with no live-cell yeast added to TMR (LS0).

<sup>2</sup>Week trend ( $P < 0.10$ ) for all parameters except acetate and propionate; week  $\times$  treatment interaction effect ( $P < 0.03$ ) for acetate and trend ( $P < 0.09$ ) for butyrate.



**Figure 1.** Effect of treatment on DMI (kg/d) covariate-adjusted least squares means by week on treatment. Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and low-starch diet with no live-cell yeast added to TMR (LS0). Week and week  $\times$  treatment interaction effects ( $P < 0.001$  and  $P < 0.001$ , respectively); SEM = 1.0. Treatment effect ( $P < 0.05$ ) wk 3 and wk 10 to 12.

LS0. Reduced milk protein content was reported when DGSC was partially replaced by soy hulls (Gencoglu et al., 2010) or a mixture of nonforage fiber sources (Batajoo and Shaver, 1994; Ferraretto et al., 2011). The MUN contents were greater for LS0 than HS diets ( $P < 0.01$ ) in the current trial. Similar responses to feeding LS diets have been reported by others (Gencoglu et al.,

2010; Ferraretto et al., 2011). Intraruminal dosing with starch decreased ruminal ammonia concentration more than dosing with NDF (Hristov et al., 2005), which may explain the MUN response to feeding LS diets. Least squares means by week on treatment for MUN are presented in Figure 3; week and week by treatment effects ( $P < 0.001$ ) were observed. In wk 2 to 8, the

**Table 6.** Effect of treatment on covariate-adjusted least squares means for lactation performance

Item	Diet <sup>1</sup>				SEM	$P <^2$
	HS0	HS2	HS4	LS0		
Yield						
Milk, kg/d	45.1	44.2	44.8	44.0	0.87	0.82
4% FCM, kg/d	34.5	34.1	34.6	34.2	0.39	0.86
SCM, kg/d	40.2	40.0	41.7	41.2	0.96	0.59
ECM, kg/d	43.5	43.3	44.9	44.6	0.99	0.54
Milk component						
Fat						
%	3.27	3.34	3.57	3.69	0.05	0.06
kg/d	1.46	1.48	1.55	1.61	0.05	0.17
Protein						
%	3.23	3.21	3.28	3.17	0.03	0.19
kg/d	1.45	1.42	1.45	1.38	0.04	0.52
Lactose, %	4.84	4.83	4.89	4.82	0.04	0.67
MUN, mg/dL	14.6 <sup>b</sup>	14.3 <sup>b</sup>	14.3 <sup>b</sup>	15.6 <sup>a</sup>	0.25	0.01

<sup>a,b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and reduced-starch diet with no live-cell yeast added to TMR (LS0).

<sup>2</sup>Week effect for all parameters ( $P < 0.001$ ); week  $\times$  treatment interaction for all parameters ( $P < 0.001$ ) except for milk yield and fat content ( $P < 0.05$ ).

**Table 7.** Effect of treatment on least squares means for apparent total-tract nutrient digestibilities

Digestibility, <sup>1</sup> %	Diet <sup>2</sup>				SEM	P <
	HS0	HS2	HS4	LS0		
DM	59.8 <sup>c</sup>	64.1 <sup>b</sup>	62.2 <sup>b</sup>	68.3 <sup>a</sup>	0.9	0.001
OM	62.6 <sup>c</sup>	66.5 <sup>b</sup>	64.7 <sup>bc</sup>	70.2 <sup>a</sup>	0.8	0.001
NDF	30.1 <sup>c</sup>	31.5 <sup>c</sup>	37.6 <sup>b</sup>	45.9 <sup>a</sup>	1.9	0.001
Starch	93.2 <sup>b</sup>	94.4 <sup>ab</sup>	93.6 <sup>b</sup>	95.3 <sup>a</sup>	0.5	0.03

<sup>a-c</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Determined using lignin as an internal marker.

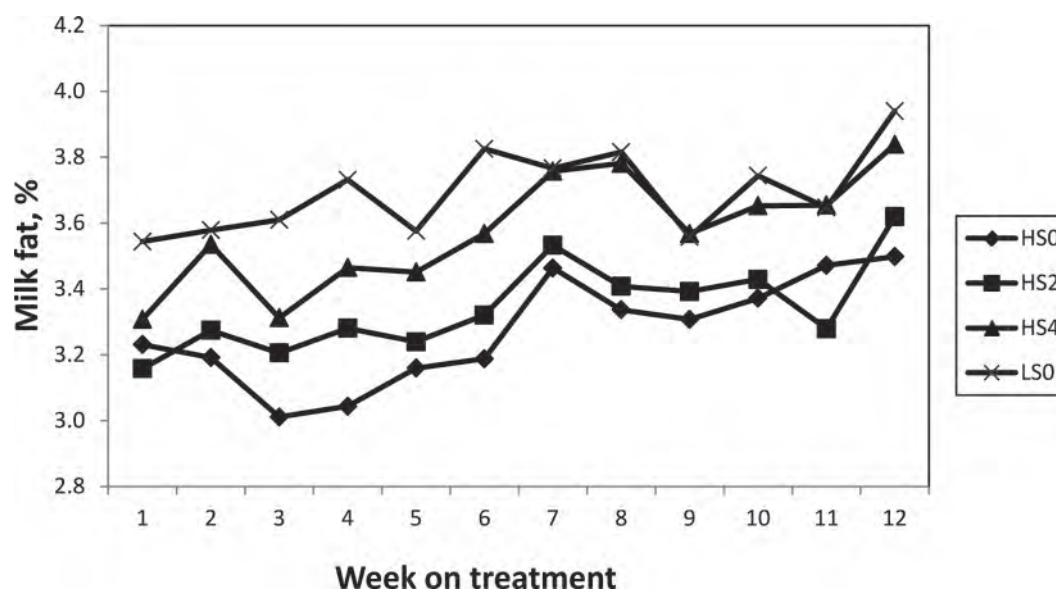
<sup>2</sup>Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and reduced-starch diet with no live-cell yeast added to TMR (LS0).

MUN concentrations were greater for LS0 than for HS diets, in agreement with Gencoglu et al. (2010) and Ferraretto et al. (2011).

Treatment effects on covariate-adjusted least squares means for BW, BCS, and feed conversion, and unadjusted means for BWC and estimated diet energy concentrations are presented in Table 8. Body weight, BWC, and BCS were unaffected by treatment ( $P > 0.10$ ). Feed conversion (kg of milk/kg of DMI) was similar ( $P > 0.10$ ) for the HS diets, and approached a trend to be reduced for cows fed LS0 compared with HS diets. These results are in agreement with reports of Gencoglu et al. (2010) and Ferraretto et al. (2011). Least squares means by week on treatment for feed conversion (kg of milk/kg of DMI) are presented in Figure 4; week and week  $\times$  treatment effects ( $P < 0.001$ ) were

observed. The FCM, SCM, and ECM feed conversions were unaffected ( $P > 0.10$ ) by treatment. Estimated diet energy content (Mcal of NE<sub>L</sub>/kg of DM), calculated using ECM, BW, BWC, and DMI data, did not differ ( $P > 0.10$ ) by treatment. Similar results were reported when DGSC was partially replaced by soy hulls (Gencoglu et al., 2010), but not when a DGSC and soybean meal mixture was partially replaced by wheat middlings and whole cottonseed (Ferraretto et al., 2011). Greater NDF digestibility (Firkins, 1997) for soy hulls than wheat middlings and (or) whole cottonseed might explain this difference in response to LS diets among trials.

Treatment effects on covariate-adjusted least squares means for ruminal fermentation parameters are presented in Table 5. Yeast supplementation did not in-



**Figure 2.** Effect of treatment on milk fat content (%) covariate-adjusted least squares means by week on treatment. Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and low-starch diet with no live-cell yeast added to TMR (LS0). Week and week  $\times$  treatment interaction effects ( $P < 0.001$  and  $P < 0.04$ , respectively); SEM = 0.08. Treatment effect ( $P < 0.02$ ) wk 3, 4, 6 and 8; treatment trend ( $P < 0.10$ ) wk 2 and 12.



**Table 8.** Effect of treatment on covariate-adjusted least squares means for BW, BW change, BCS, feed conversion, and estimated diet energy concentrations

Item	Diet <sup>1</sup>				SEM	P <
	HS0	HS2	HS4	LS0		
BW, kg	767	762	757	758	12.8	0.48
BW change, kg	40.5	47.6	34.0	34.7	12.3	0.50
BCS	2.75	2.70	2.87	2.73	0.06	0.94
Feed conversion <sup>2</sup>						
kg of milk/kg of DMI	1.58	1.60	1.56	1.46	0.04	0.14
kg of 4% FCM/kg of DMI	1.20	1.24	1.21	1.15	0.03	0.27
kg of SCM/kg of DMI	1.39	1.45	1.46	1.38	0.04	0.41
kg of ECM/kg of DMI	1.50	1.56	1.57	1.50	0.04	0.47
Estimated diet energy content, <sup>3</sup> Mcal/kg of DM	1.52	1.57	1.56	1.52	0.32	0.56

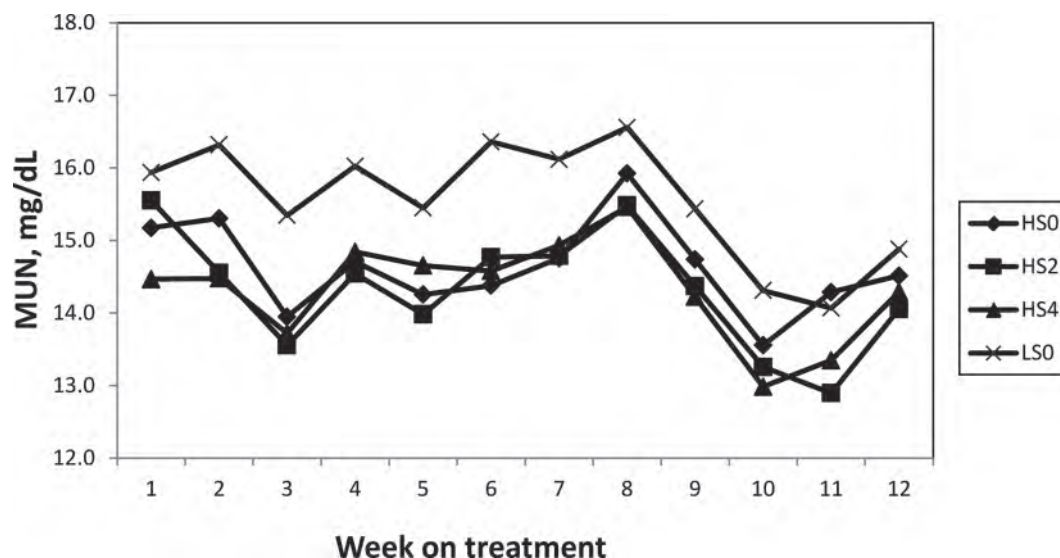
<sup>1</sup>Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and reduced-starch diet with no live-cell yeast added to TMR (LS0).

<sup>2</sup>Week effect ( $P < 0.001$ ); week  $\times$  treatment interaction ( $P < 0.001$ ).

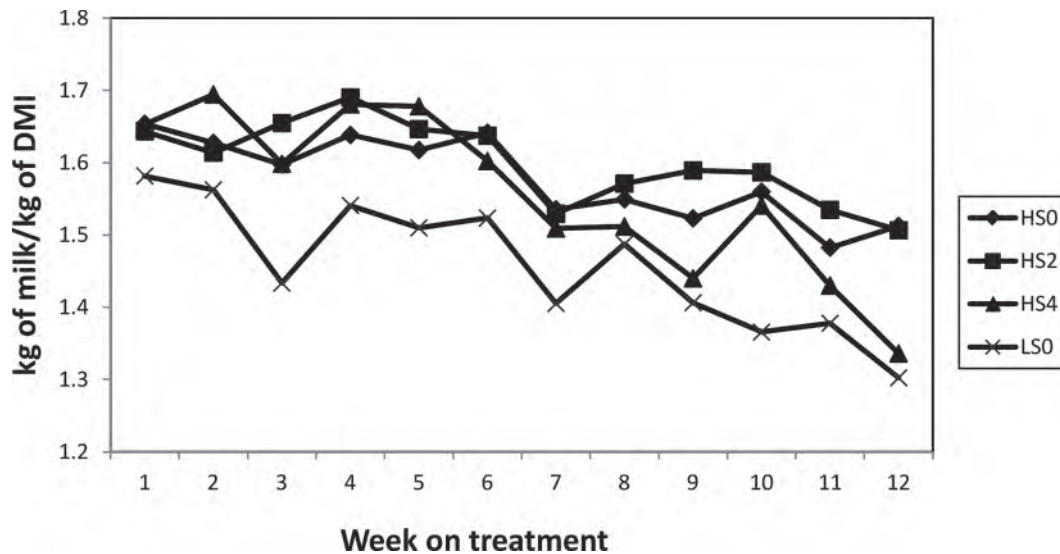
<sup>3</sup>Calculated by summing the Mcal of NE<sub>L</sub> from milk production required for maintenance and in BW change (NRC, 2001) and then dividing the sum by DMI.

fluence ( $P > 0.10$ ) ruminal fermentation parameters. Desnoyers et al. (2009), from a meta-analysis, reported an increase in ruminal pH and total VFA concentration related to *S. cerevisiae* supplementation and dosage. Increased ruminal pH has also been reported by others (Bach et al., 2007; Marden et al., 2008) when cows were fed 5 g/cow per day of LCY ( $1 \times 10^{10}$  cfu/g of *S. cerevisiae*). Sampling at a single time point postfeeding by rumenocentesis might not have been sensitive enough to allow detection of dietary yeast supplemen-

tation effects on ruminal fermentation in our study. Acetate molar percentage was greater ( $P < 0.01$ ) and propionate molar percentage reduced ( $P < 0.05$ ), resulting in an increased ( $P < 0.01$ ) acetate:propionate ratio for LS0 compared with HS diets. Despite these alterations in ruminal VFA profile, ruminal pH was unaffected ( $P > 0.10$ ) by dietary starch content. In a review paper, Ipharraguerre and Clark (2003) reported that diets containing soy hulls consistently increased ruminal acetate:propionate ratio, although ruminal pH



**Figure 3.** Effect of treatment on MUN (mg/dL) covariate-adjusted least squares means by week on treatment. Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and low-starch diet with no live-cell yeast added to TMR (LS0). Week and week  $\times$  treatment interaction effects ( $P < 0.001$  and  $P < 0.01$ , respectively); SEM = 0.3. Treatment effect ( $P < 0.04$ ) wk 1–7 and wk 11; treatment trend ( $P < 0.10$ ) wk 10.



**Figure 4.** Effect of treatment on feed conversion (kg of milk/kg of DMI) covariate-adjusted least squares means by week on treatment. Treatments were high-starch diet with no live-cell yeast added to TMR (HS0), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS2), high-starch diet with 2 g/cow per day live-cell yeast added to TMR (HS4), and low-starch diet with no live-cell yeast added to TMR (LS0). Week and week  $\times$  treatment interaction effects ( $P < 0.001$  and  $P < 0.001$ , respectively); SEM = 0.04. Treatment effect ( $P < 0.05$ ) wk 3, 9, 10, and 12.

was unaffected, in agreement with our results. Furthermore, sodium bicarbonate supplementation increased the acetate:propionate ratio but not ruminal pH in the review by Erdman (1988).

Treatment effects on least squares means for apparent total-tract nutrient digestibilities are presented in Table 7. Digestibility measurements were taken during wk 4 and 8 of the treatment period, and the DMI data for these weeks are provided in Figure 1. The DMD, OMD, and StarchD measurements were greater or tended to be greater for cows fed HS2 than for those fed HS0 ( $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.08$ , respectively). The DMD and OMD tended to be greater and NDFD was greater for HS4 than for HS0 ( $P < 0.06$ ,  $P < 0.09$ ,  $P < 0.01$ , respectively). Marden et al. (2008) reported greater ( $P < 0.03$ ) NDFD when cows were fed 5 g/cow per day of LCY ( $1 \times 10^{10}$  cfu/g of *S. cerevisiae*). *Saccharomyces cerevisiae* supplementation and dosage increased OMD in the meta-analysis report of Desnoyers et al. (2009). The DMD, OMD, and NDFD were greater ( $P < 0.01$ ) for LS0 than for HS diets, and StarchD was greater ( $P < 0.01$ ,  $P < 0.02$ , respectively) for LS0 than for HS0 and HS4. Gencoglu et al. (2010) reported greater total-tract nutrient digestibilities when cows were fed soy hulls in partial replacement of DGSC. Greater total-tract nutrient digestibilities may be related to reduced negative associative effects of starch on ruminal fermentation (Firkins, 1997); however, ruminal pH was unaffected by dietary starch content in the current trial (Table 5). Greater NDFD for the LS0 diet may have

been related to the characteristically high fiber digestibility of soy hulls (Firkins, 1997; in vitro NDFD data in Table 3). The greater StarchD for the LS0 diet may have been related to the greater proportion of dietary starch provided by corn silage (64 vs. 43% on average; data not provided in table) with its relatively high in vitro starch digestibility compared with DGSC (Table 3).

Feeding behavior was unaffected ( $P > 0.10$ ) by LCY supplementation (data not provided in table) in agreement with the report of Bach et al. (2007) and may be explained by the lack of an effect of LCY supplementation on ruminal propionate (Allen et al., 2009). Feeding LS0 (data not provided in table) tended to increase ( $P < 0.06$  and  $P < 0.07$ , respectively) eating time compared with HS0 and HS2 (225 vs. 205 min/d on average). Increased eating time was reported when cows were fed soy hulls and corn gluten feed that partially replaced a mixture of barley, corn, and soybean meal (Miron et al., 2004). Despite trends for increased eating time, similar ( $P > 0.10$ ) eating rates (averaged 0.141 kg of DM/min) and meal frequencies (averaged 6.5 meals/d) were observed among treatments, which may partially explain the greater DMI for LS0. Furthermore, meal duration (averaged 33 min/meal) and meal size (averaged 4.5 kg of DM/meal) were unaffected ( $P > 0.10$ ) by dietary starch content.

Feed sorting was unaffected ( $P > 0.10$ ) by LCY supplementation (data not provided in table). Cows fed HS2 selectively refused ( $P < 0.05$ ) long particles com-

pared with LS0 (94.7 vs. 99.3%). Selective consumption of fine particles was greater ( $P < 0.05$ ) for HS4 than for LS0 (107.0 vs. 104.2%). In contrast, DeVries et al. (2008), with varying forage:concentrate ratios, reported that cows fed HS sorted against short particles, whereas cows fed LS sorted for these particles. However, those authors induced an acidotic rumen environment, whereas similar ruminal pH was observed among treatments in the present study. To our knowledge, this is the first study on the supplementation of LCY to HS diets that evaluated effects on feed sorting.

Locomotion score and white line and toe lesions were similar ( $P > 0.10$ ) among treatments and averaged 1.55, 1.18, and 1.19, respectively (data not shown). Greater sole lesion scores in the front hoof were observed for cows fed HS2 (1.37) compared with HS0 (1.07;  $P < 0.06$ ), HS4 (1.03;  $P < 0.03$ ), and LS0 (1.02;  $P < 0.05$ ). Lower sole lesion scores in the rear hoof were observed for HS2 (1.42) compared with HS0 (2.22;  $P < 0.01$ ) and HS4 (2.02;  $P < 0.04$ ). Similar ruminal pH (Table 5) among treatments, along with the use of sand-bedded freestalls (Cook et al., 2004) and the relatively short period of treatment, may explain the lack of or only minor differences in hoof health measurements.

## CONCLUSIONS

Dietary supplementation of live-cell yeast at a dosage of 4 g/cow per day in a high-starch diet increased total-tract NDF digestibility and tended to increase milk fat content compared with high-starch diets either without or with 2 g/cow per day of live-cell yeast supplementation. Feeding a reduced-starch diet formulated by partially replacing corn grain with soy hulls compared with high-starch diets without or with live-cell yeast supplementation resulted in the following: greater intakes of DM and NDF and reduced intake of starch; greater fat and urea nitrogen concentrations in milk; decreased feed conversion; greater ruminal acetate and reduced propionate molar proportions; and greater total-tract nutrient digestibilities.

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