Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows

A. L. G. Dias,*† J. A. Freitas,*¹ B. Micai,* R. A. Azevedo,* L. F. Greco,* and J. E. P. Santos*‡²

*Department of Animal Sciences, and

†DH Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville 32611

‡Department of Animal Sciences, State University of Maringá, PR, Brazil, 87020-900

ABSTRACT

The objectives of this experiment were to evaluate the effect of feeding a culture of *Saccharomyces cerevisiae* on rumen metabolism and digestibility when cows are fed diets varying in starch content. Four lactating Holstein cows were assigned to a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Treatments were low starch (LS; 23% of diet DM) and no yeast culture (YC; LS-control), LS and 15 g of YC/d (LS-YC), high starch (HS; 29% of diet DM) and no YC (HS-control), and HS and 15 g of YC/d (HS-YC). Periods lasted 28 d, with the last 9 d for data collection. Days 20 to 24 were used to determine production, nutrient flow, and digestibility. On d 25, 3 kg of corn grain DM was placed in the rumen 1 h before the morning feeding, and yields of milk and milk components were measured after the challenge. Blood was sampled −1, 3, 7, and 11 h relative to the morning feeding on d 24 and 25. Rumen pH was measured continuously on d 24 and 25. Rumen papillae were collected on d 24 and 28 to quantify mRNA expression of select genes. Supplementing YC increased yields of milk (26.3 vs. 29.6 kg/d), energy-corrected milk (ECM; 26.5 vs. 30.3 kg/d), fat (0.94 vs. 1.08 kg/d), true protein (0.84 vs. 0.96 kg/d), and ECM/dry matter intake (1.15 vs. 1.30) compared with the control but did not affect dry matter intake (22.6 vs. 22.9 kg/d). Cows fed HS had increased milk true protein percentage (3.18 vs. 3.31%) and yield (0.87 vs. 0.94 kg/d) compared with cows fed LS. Feeding HS-YC increased the proportion of dietary N incorporated into milk true protein from 24.9% in the other 3 treatments to 29.6%. Feeding HS increased the concentration of propionate (21.7 vs. 32.3 mM) and reduced that of NH₃-N (8.3 vs. 6.7 mg/dL) in

rumen fluid compared with the control, and combining HS with YC in HS-YC tended to increase microbial N synthesis compared with LS-YC (275 vs. 322 g/d). Supplementing YC to cows fed HS reduced plasma haptoglobin and rumen lactate concentrations, increased mean rumen pH, reduced the time with pH <6.0, and prevented the decrease in rumen neutral detergent fiber digestion caused by HS. Cows fed HS had less total-tract digestion of organic matter (73.9 vs. 72.4%) because of reduced acid detergent fiber (57.6 vs. 51.7%) and neutral detergent fiber (60.9 vs. 56.7%) digestibility. Production performance after the challenge was similar to that before the challenge, and YC improved yield of ECM. After the challenge, supplementing YC tended to reduce rumen lactate concentration compared with the control and reduced haptoglobin in cows fed HS. Feeding HS but not YC increased expression in rumen papillae of genes for receptors (*FFAR2* and *FFAR3*) and transporter (*SLC16A3*) of short-chain fatty acids but did not affect genes involved in transport of Na⁺/H⁺ or water or in inflammatory response. Supplementing YC to dairy cows improved lactation performance in diets containing low or high starch, and mechanisms might be partially attributed to improvements in rumen pH, digestion of fiber, microbial N synthesis, and reduction in acute phase response.

Key words: digestion, rumen fermentation, starch, yeast culture

INTRODUCTION

Supplementing yeast culture (YC) to dairy cattle diets is a common practice because of the potential beneficial effects on yields of milk and milk components (Poppy et al., 2012). Nevertheless, the effects of YC are heterogeneous and dependent, among other factors, on the stage of lactation when supplementation starts (Poppy et al., 2012). One of the reported benefits of YC is the improvement in efficiency of feed utilization (Schingoethe et al., 2004), presumably because supplementing diets with *Saccharomyces cerevisiae* improves

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¹Present address: Department of Animal Sciences, Federal University of Paraná, Palotina, PR, Brazil, 85950-000.

²Corresponding author: jepsantos@ufl.edu

OM digestion (Desnoyers et al., 2009), results in a more stable rumen pH (Bach et al., 2007), and might increase microbial N yield (Erasmus et al., 1992).

One of the potential sources of heterogeneity in response to YC is the composition of the diet (Williams et al., 1991). The authors observed an interaction between diet composition and supplementation with YC because the benefit in FCM yield was greatest when cows were fed a diet with 60% compared with 50% concentrate content (Williams et al., 1991). Diets with increased concentrate content are more prone to negatively influence rumen function because of the depression in rumination and salivation and the increase in acid load, which can depress pH. It is known that YC and live yeast products attenuate changes in rumen pH (Bach et al., 2007; Desnoyers et al., 2009), in part by reducing lactate accumulation in the rumen fluid because of stimulation of microorganisms that metabolize lactate into short-chain fatty acids (SCFA; Callaway and Martin, 1997). Thus, it is possible that potential improvements of dairy cow performance with YC supplementation to diets containing a large proportion of readily fermentable carbohydrates might be caused by a reduction in lactate concentration and an increase in rumen pH. In fact, one of the benefits reported with YC was that it prevented the reduction in milk fat yield when cows were fed more rumen-fermentable diets (Longuski et al., 2009), which would benefit yield of FCM (Williams et al., 1991). Also, it is possible that the benefits of YC might be greater in diets with increased starch content because such diets result in an increased acid load that demands increased physically effective fiber to maintain a proper rumen environment (Allen, 1997). The more stable rumen environment due to supplementing YC to diets high in starch might result in less need for physically effective fiber and justify increases in DMI that improve yields of milk and milk components.

Yeast culture also benefits rumen fiber digestion because of stimulation of growth of fibrolytic microorganisms (Callaway and Martin, 1997). Because of improved digestion of fiber and OM in the rumen (Williams et al., 1991) and total tract (Desnoyers et al., 2009), it is conceivable that benefits of YC supplementation might increase in diets with a larger forage component and more forage NDF. Yeast culture has been shown to increase DMI when supplemented in early lactation (Poppy et al., 2012), and an increase in rate and extent of forage NDF degradation may stimulate intake and productivity in dairy cows.

One of the issues with diet formulation is the inherent variability in nutrient content either because of changes in ingredient composition or because of mixing

errors (Oelberg and Stone, 2014). Errors in carbohydrate formulation when diets are already high in starch can result in SARA (AlZahal et al., 2014), which often causes milk fat depression (Longuski et al., 2009). The hypothesis of this experiment was that YC influences dairy cow performance by improving digestion and attenuating fluctuations in rumen pH, particularly in diets rich in starch or those prone to rumen acidosis. Therefore, the objectives were to evaluate rumen metabolism, digestion, and performance in response to supplemental YC in Holstein cows fed diets varying in starch content.

MATERIALS AND METHODS

All procedures with experimental cows were approved by the University of Florida Institute of Food and Agriculture Science Animal Research Committee under protocol number 007-13ANS.

Cows, Housing, and Feeding

The experiment was conducted from September to December 2014. Four ruminally cannulated primiparous Holstein cows were used in the experiment. Cows averaged ($\pm$ SD) 176 ± 18 DIM, 564 ± 51 kg of BW, and 26.0 ± 3.6 kg of milk/d at enrollment in the experiment. Cows were housed in a cross-ventilated tiestall barn with individual feed and water troughs. Stalls had gel mats that were bedded with shavings, and bedding material was cleaned and replaced twice daily. Cows were fed twice daily at approximately 0700 and 1330 h. The amounts of feed offered to individual cows were adjusted daily to result in at least 5% refusals, which were weighed once daily before the morning feeding. Twice weekly, diets and ingredients were sampled and dried at 55°C for adjustment of amounts of DM to be offered daily. Samples of dried feeds and orts were frozen and later dried at 105°C for calculation of DMI.

Experimental Design and Treatments

The experimental design was a 4×4 Latin square with a 2×2 factorial arrangement of treatments, resulting in 16 experimental units, with 4 per treatment and 8 per each main effect of level of starch and supplemental YC. The treatment sequence was balanced for carryover, and treatments were 2 levels of dietary starch (23 or 29% of the diet DM) without or with supplemental YC, resulting in 4 combinations: low starch (LS) and no YC (LS-control), LS with YC (LS-YC), high starch (HS) and no YC (HS-control), and HS with YC (HS-YC). Starch in the diet was increased

primarily by replacing a portion of the forage with finely ground corn (Table 1). Diets were isonitrogenous, but the caloric density increased with the additional starch in HS. Diets were formulated to meet or exceed the nutrient requirements of primiparous cows weighing 600 kg of BW and producing 30 kg of 3.5% FCM when intake averages 21 kg/d (NRC, 2001). The YC was an inactivated dry *S. cerevisiae* cultured in sugar cane derivatives (Rumen Yeast, ICC Brazil, São Paulo, SP, Brazil). The YC product was mixed at a 15:85 ratio with finely ground corn, and 100 g of this mixture was top-dressed daily to cows fed the LS-YC and HS-YC diets to result in 15 g of YC/cow per day. The diet of control cows was top-dressed with 100 g of finely ground corn/d.

Each period lasted 28 d; d 1 to 19 were used for adaptation to diets, and d 20 to 24 were used for data collection for intake, production, rumen parameters, and digestibility. On the morning of d 25 of each period, immediately before feeding, cows received a challenge with finely ground corn through the rumen cannula in an attempt to induce changes in rumen fermentation. Production data from d 26 to 28 were collected and analyzed for the postchallenge within each period.

Measurements of Milk Yield and Milk Components

Cows were milked twice daily at approximately 0600 and 1800 h, and individual yield of milk was recorded by the Afikim milking system (Afiflo milk meters, S.A.E. Afikim, Kibbutz Afikim, Israel). Milk was sampled from individual milkings from d 20 to 28 and shipped to the Southeast DHIA laboratory in Belleview, Florida, for analysis of concentrations of fat, true protein, and lactose and for quantification of somatic cells. Milk components were adjusted for the yield of milk in each milking to calculate the daily yields and concentrations of fat, true protein, and lactose and the content of somatic cells. The 3.5% FCM yield was calculated as $(0.4324 \times \text{milk yield}) + (16.218 \times \text{milk fat yield})$; NRC, 2001). The ECM yield was calculated as $(0.3246 \times \text{milk yield}) + (12.86 \times \text{fat yield}) + (7.04 \times \text{protein yield})$; NRC, 2001).

DMI, BW, and Energy Balance

Intake of DM was measured daily from d 20 to 28 in each period. Dry matter intake was calculated after drying TMR and orts of individual cows at 105°C. Immediately after each milking, cows were weighed on a walk-through scale (AfiWeigh, S.A.E. Afikim) located on the exit lane of the milking parlor. The caloric intake was calculated based on daily DMI and the energy

content of diet based on the chemical analysis of dietary ingredients and was adjusted for 21 kg of DMI (NRC, 2001). Energy balance was calculated using daily caloric intake based on the DMI of each cow and the NE_L density of the low- and high-starch diets minus the summation of daily calories as NE_L required for maintenance $(0.08 \times \text{BW}^{0.75})$ and secreted as milk according to yields of fat, protein, and lactose: $\text{milk yield} \times [(0.0929 \times \text{fat } \%) + (0.0563 \times \text{protein } \%) + (0.0395 \times \text{lactose } \%)]$.

Table 1. Ingredient composition and nutrient content of treatment diets

Item	Diet ¹	
	Low starch	High starch
Ingredient, % diet DM		
Corn silage	39.1	32.7
Triticale silage	10.9	9.1
Corn, finely ground	11.7	21.7
Soybean hulls	12.0	11.6
Citrus pulp, dry	4.4	3.6
Whole cottonseed	4.4	3.6
Soybean meal, solvent extracted	8.7	9.7
Soybean meal, cooker processed ²	4.4	3.6
Mineral-vitamin protein mix ³	4.4	4.4
Nutrient content, ⁴ DM basis (mean $\pm$ SD)		
NE _L , ⁵ Mcal/kg	1.59	1.61
OM, %	93.2 $\pm$ 0.3	93.5 $\pm$ 0.3
CP, %	14.6 $\pm$ 0.6	14.6 $\pm$ 0.5
NFC, %	36.6 $\pm$ 1.3	40.3 $\pm$ 1.1
Starch, %	23.2 $\pm$ 1.7	29.0 $\pm$ 1.2
NDF, %	38.1 $\pm$ 1.0	32.9 $\pm$ 0.8
Forage NDF, %	21.0 $\pm$ 1.1	17.0 $\pm$ 0.9
ADF, %	21.7 $\pm$ 0.7	19.5 $\pm$ 0.6
Ether extract, %	3.9 $\pm$ 0.1	3.7 $\pm$ 0.1

¹Within starch levels, diets were supplemented with either 15 g/cow per day of corn meal or 15 g/cow per day of yeast culture (*Saccharomyces cerevisiae*; Rumen Yeast, ICC, São Paulo, Brazil).

²AminoPlus (Ag Processing Inc., Omaha, NE).

³The vitamin-mineral-protein premix added to the concentrate and grain supplement contained (DM basis) 30.0% LysAAmet blood meal (Perdue Agribusiness, Salisbury, MD), 28.5% sodium sesquicarbonate, 13.0% potassium carbonate, 9.0% calcium carbonate, 7.0% dicalcium phosphate, 7.0% magnesium oxide, 3.5% sodium chloride, 1.2% Availa-4 (Zinpro Co., Eden Prairie, MN), 0.33% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.002% calcium iodate, 0.08% premix containing vitamins A, D, and E, and 0.22% Rumensin 90 (Elanco Animal Health, Greenfield, IN). Each kilogram contains 26.4% CP, 5.2% Ca, 1.4% P, 3.9% Mg, 9.5% Na, 2.6% Cl, 652 mg of Zn, 226 mg of Cu, 412 mg of Mn, 6.6 mg of Se, 25 mg of Co, 13 mg of I, 112,000 IU of vitamin A, 34,000 IU of vitamin D, 1,100 IU of vitamin E, and 476 mg of monensin.

⁴Means $\pm$ SD of 4 monthly composite samples of forages and concentrates analyzed.

⁵Calculated based on the chemical composition of the dietary ingredients and adjusted for 21 kg of DMI (NRC, 2001).

Challenge with Corn Grain

On the morning of d 25 of each period, immediately after milking and before feeding, cows were challenged with 3 kg of DM of finely ground corn through the rumen cannula in an attempt to induce changes in rumen fermentation. Approximately 1 h after the challenge, cows were fed their respective diets.

Samples of corn grain were collected the day before the challenge for each period and analyzed for starch (Karkalas, 1985), and particle size was estimated by dry sieving using a 10-sieve shaker with apertures of 3,360, 2,380, 1,680, 841, 594, 420, 297, 212, 150, and 73 μm and pan according to the method described by Baker and Herrman (2002). Corn contained $73.8 \pm 1.2\%$ starch, which resulted in an additional 2.2 kg of starch fed to cows on the day of challenge. The geometric mean particle of the finely ground corn averaged $541.7 \pm 2.7 \mu\text{m}$, with $51.3 \pm 6.3\%$ of the particles retained in the sieve $\geq 841 \mu\text{m}$.

Rumen Papillae Sampling

The rumen of each cow was evacuated at the end of d 24, which preceded the challenge with corn that occurred in the morning on d 25, and again in the afternoon of d 28 of each period. Tissue collection represented the day before and 3 d after the challenge with corn grain. The cannula was removed, and a portion of the rumen contents was placed in a container insulated with plastic bag to return to the rumen immediately after collection. The ventral sac was exteriorized through the incision on the left flank to identify individual papilla. Twenty rumen papillae were removed per cow using a mosquito hemostat clamp placed on the base of the papilla and carefully cutting above the hemostat with scissors without damaging the muscular layer. The papillae were placed on filter paper and thoroughly rinsed 30 times with sterile saline to remove any debris. Papillae were stored in 2 RNase-free vials with 10 papillae per vial, snapped frozen into liquid N_2 , and then stored at -80°C for later analyses. Immediately after tissue collection, the rumen contents were returned to the respective cows.

Papillae Tissue mRNA Extraction and Real-Time Quantitative PCR

Messenger RNA from rumen papillae tissue was extracted using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA) according to the recommendations of the manufacturer, except that Trizol (Molecular Research Center Inc., Cincinnati, OH) and chloroform

(Fisher Scientific, Pittsburgh, PA) were used to obtain the cell lysate. Cells were mechanically disrupted in Trizol using an electrical homogenizer. Two hundred microliters of chloroform was added and the solution was homogenized and incubated for 3 min at room temperature. The tubes were centrifuged at $12,000 \times g$ for 15 min at 4°C . The colorless upper phase was harvested into a new tube, and an equal volume of 70% ethanol was added in solution. The extraction kit protocol was then followed. Concentration of mRNA was determined on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE), and sample quality was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA) before real-time quantitative PCR assays. All samples analyzed had an RNA integrity value ≥ 7.5 .

Ten genes of interest and 2 reference genes were investigated (Table 2). Genes of interest were free fatty acid receptor 2 (*FFAR2*), free fatty acid receptor 3 (*FFAR3*), solute carrier family 16 member 1 (*SLC16A1*), solute carrier family 16 member 3 (*SLC16A3*), solute carrier family 14 member 1 (*SLC14A1*), solute carrier family 9 subfamily A member 1 (*SLC9A1*), solute carrier family 9 subfamily A member 3 (*SLC9A3*), aquaporin 3 (*AQP3*), toll-like receptor 2 (*TLR2*), and toll-like receptor 4 (*TLR4*), and the 2 reference genes were ribosomal protein large P0 (*RPLP0*) and β actin (*ACTB*). The reference genes were included in all plates for all samples. Genes of interest were selected based on previous work that evaluated differences in the transcriptome of rumen tissue (Penner et al., 2009; Wang et al., 2009; Chen et al., 2012; Steele et al., 2013), and the rationale for selected targets was to focus on particular pathways that could be affected by the dietary manipulations such as SCFA receptors (*FFAR2* and *FFAR3*) and transport (*SLC16A1* and *SLC16A3*), water and solute transport (*AQP3*), urea transport (*SLC14A1*), Na^+/H^+ transport (*SLC9A1* and *SLC9A3*), and inflammatory response (*TLR2* and *TLR4*).

Rumen papillae samples had purified mRNA subjected to real-time quantitative PCR for target and reference genes. Briefly, 1 μg of total cellular mRNA was used to synthesize cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc., Foster City, CA) and T100 Thermal Cycler (Bio-Rad, Hercules, CA). Complementary DNA was then used for real-time quantitative PCR using the CFX96 Real-Time System (Bio-Rad). Each reaction consisted of a mixture of 9 μL of a 1:10 dilution of the cDNA in nuclease-free water, 0.5 μL of gene-specific forward and reverse primers at concentrations of 10 μM , and 10 μL of SYBR Green (Applied Biosystems Inc.) in a total reaction volume of 20 μL such that the final

concentration of primer in each reaction was 4.5 pM. Reactions were run in duplicate and consisted of 40 cycles of a 2-step amplification protocol (15 s at 95°C and 30 s at 60°C). Primer efficiency ranged from 89 to 105%. Melting curve analysis and agarose gel of PCR products were performed and demonstrated the amplification of a single product of expected size for all genes investigated. Data are presented using the comparative method developed by Livak and Schmittgen (2001) using the LS-control treatment as the reference for relative expression of mRNA abundance, which was set to the relative value of 1. Because the expression of *ACTB* was not stable according to level of starch, only *RPLP0* was used as a reference gene, and the geometric mean between the two was not calculated. The delta cycle threshold (CT) values for each target gene were obtained after normalization of CT value of the gene with the CT value for *RPLP0*, and the delta CT of each gene obtained for the pre- and postchallenge periods were subjected to statistical analysis.

Blood Sampling and Analyses

Approximately 8 mL of blood was sampled on d 24, which was before the challenge, and on d 25, when cows were challenged in each period. Blood was sampled into evacuated tubes containing K₂ EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) by puncture of the coccygeal blood vessels. Samples were collected 1 h

before the morning feeding and again at 3, 7, and 11 h after feeding on each day. On d 25, cows were challenged with corn grain 1 h before the morning feeding, and the hours of blood sampling corresponded to 0, 4, 8, and 12 h after the challenge.

After sampling, blood tubes were placed on ice and transported to the laboratory within 1 h and then centrifuged at $3,000 \times g$ for 15 min at 4°C for plasma separation. Plasma was frozen at -25°C for later analyses. Concentrations of glucose (Bran and Luebbe industrial method 339-19; Gochman and Schmitz, 1972) and urea N (Bran and Luebbe industrial method 339-01; Marsh et al., 1965) were analyzed using an autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Concentrations of nonesterified fatty acids were analyzed in duplicate according to Johnson and Peters (1993) using a commercial kit (NEFA-C kit; Wako Fine Chemical Industries Inc., Dallas, TX), and haptoglobin was analyzed in triplicate according to Makimura and Suzuki (1982).

Feed Sampling and Analyses

Samples of forages, concentrates, and orts from individual cows were collected daily on d 20 to 28 of each period, dried at 55°C, and ground to pass a 1-mm screen on a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ground samples of forages and concentrates were composited by period and samples of orts were com-

Table 2. Gene, primer orientation, primer sequence (5' to 3'), and National Center for Biotechnology Information (NCBI) accession number and sequence for primers used in real-time quantitative PCR assays

Gene	Primer	Sequence (5' to 3')	NCBI sequence
<i>ACTB</i>	Forward	CTGGACTTCGAGCAGGAGAT	NM_173979
	Reverse	GGATGTCGACGTCACACTTC	
<i>RPLP0</i>	Forward	TGCTTCATTGTGGGAGCAGA	NM_001012682
	Reverse	AGCCGGGTGTGTTTCCAGAT	
<i>FFAR2</i>	Forward	TTTGCTGTGTGAAATGGGCG	NM_001163784
	Reverse	CCGTGAGGATCAAGGAGCTA	
<i>FFAR3</i>	Forward	AACCTCACCTCTCGGATCT	NM_001145233
	Reverse	GCCGAGTCTTGTACCAAAGC	
<i>SLC16A1</i>	Forward	AATGCCACCACAGTGAAGT	NM_001037319
	Reverse	GGAAGCAGCAATCAAGCCAC	
<i>SLC16A3</i>	Forward	GACCGAGCGGCAGCAG	NM_001109980
	Reverse	GTCCTGACAGAGGCTGTTCC	
<i>SLC14A1</i>	Forward	AATCAGGCTCCACAAGGTCG	NM_001008666
	Reverse	TATCCCCCGAAGAACCCAGT	
<i>SLC9A1</i>	Forward	ACGTTTGTTCATCAGCACCT	NM_174833
	Reverse	CCCCCGTAGGCAATGATGAA	
<i>SLC9A3</i>	Forward	CCAGGACCCGTACATCATCG	NM_001192154
	Reverse	GTTGGCGTGAGTGAGAAGGA	
<i>AQP3</i>	Forward	CGACAGAAGGAGCTGGTGAA	NM_001079794
	Reverse	AAGCCAAAGGCCAGGTTGAT	
<i>TLR2</i>	Forward	CTGTGTGCGTCTTCCTCAGA	NM_174197
	Reverse	TCAGGGAGCAGAGTAACCAGA	
<i>TLR4</i>	Forward	AGCCACGGCCATCCTCTCCT	NM_174198
	Reverse	AGCTCAGGTCCAGCATCTTGTT	

posited for each cow in each period and then analyzed in triplicate for DM (105°C for 12 h), OM (512°C for 8 h), sequential analysis of NDF using a heat-stable α -amylase and ADF (Van Soest et al., 1991) with the Ankom Fiber Analyzer system (Ankom Technology, Macedon, NY), N using an automated quantitative combustion digestion method (Elementar Analysensysteme, Elementar Americas Inc., Mt. Laurel, NJ), and starch using an enzymatic digestion method and subsequent quantification of glucose (Karkalas, 1985).

Ruminal and Total Digestibility of Nutrients

Ruminal and total digestibility was determined utilizing Cr_2O_3 as an indigestible external marker to estimate apparent digestibility of DM, OM, CP, ADF, NDF, and starch by the marker ratio technique (Schneider and Flatt, 1975). Cows were dosed with gelatin capsules containing Cr_2O_3 inserted via ruminal cannula from the morning of d 12 until the morning of d 28 of each period. Each cow was dosed with 10 g of Cr_2O_3 twice daily at 12-h intervals, totaling 20 g of Cr_2O_3 /cow per day.

Ruminal, omasal, and fecal samples were collected at 12-h intervals, spaced 2 h each day, from the morning of d 20 until the morning of d 25 before the challenge (e.g., d 20, sampling at 1 and 13 h after the morning feeding; d 21, sampling at 3 and 15 h after the morning feeding, and so on). Samples of rumen content were collected from 5 different locations (dorsal, ventral, cranial, caudal, and central) and then vigorously homogenized. The sample was divided into 2 subsamples. The first sample of approximately 300 g was frozen at -25°C for chemical analyses. The second sample was filtered through cheesecloth, and an aliquot of 10 mL of ruminal fluid was acidified to pH 2.0 with 50 μL of H_2SO_4 50% (vol/vol) and then centrifuged at $3,000 \times g$ for 20 min; the supernatant was separated and frozen at -25°C for later analyses of SCFA, lactate, and $\text{NH}_3\text{-N}$ concentrations.

Digesta flow from the rumen was quantified using the omasal sampling technique as described by Reynal and Broderick (2005). Approximately 300 mL of digesta was collected at each sampling time via rumen cannula using a flexible hose (~ 3 cm i.d.) placed in the reticulum-omasum orifice connected to a vacuum pump, and the content was immediately frozen at -25°C for later analyses. Concurrent fecal samples of approximately 200 g were collected from the rectum via transrectal palpation, and the samples were stored at -25°C for posterior analyses.

Samples of TMR, refusals, omasal content, and feces were dried in a forced-air oven at 55°C for 96 h. Moisture loss was recorded, and then the samples were

ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific) and composited per cow per period. Fecal and omasal samples were analyzed in triplicate to determine Cr concentrations by atomic absorption spectrophotometry using a Perkin Elmer 5000 atomic absorption spectrometer (Perkin Elmer, Wellesley, MA) according to Williams et al. (1962). Samples of TMR, Orts, and omasal content were analyzed for indigestible NDF after in situ incubation for 240 h and posterior analysis of NDF in the residue (Van Soest et al., 1991), which was used as a marker for quantification of omasal flow for calculation of rumen digestion of DM, OM, NDF, and starch. Samples of TMR, refusals, omasum, and feces were also analyzed for content of DM, ash, N, starch, and sequential analysis of NDF and ADF as described previously.

Rumen Fluid pH and Concentrations of SCFA and $\text{NH}_3\text{-N}$

Four indwelling pH meter probes (PHE-7352-15, Omega Engineering Inc., Stamford, CT) were used for continuous measurements of rumen pH. Each probe was assigned to a cow for the entire experiment, and probes were calibrated before each period. Probes were inserted on the bottom of the rumen on d 24 and removed after 24 h of measurement to evaluate rumen pH before the challenge with corn grain. Probes were reinserted on d 25 and removed after 24 h of measurement. Data collected for pH measurements were read using OM-CP Data Logging software (version 2.07.1; Omega Engineering).

Samples of frozen rumen fluid were thawed and centrifuged at $21,500 \times g$ for 20 min at 4°C , and the supernatant was analyzed for concentrations of organic acids using the method of Canale et al. (1984) by HPLC (Hitachi L2400, Tokyo, Japan) coupled to a UV detector set at 210 nm and an Aminex HPX-87H column (Bio-Rad Laboratories) with a 0.015 M sulfuric acid mobile phase and a flow rate of 0.7 mL/min at 45°C , and crotonic acid as the internal standard. The supernatant was also analyzed for concentration of $\text{NH}_3\text{-N}$ according to the procedure of Noel and Hambleton (1976) following colorimetric N quantification using an Autoanalyzer Technicon (Technicon, Tarrytown, NJ).

Collection and Analyses of Urine Samples and Estimation of Microbial N

Urine samples were collected once daily beginning at 0800 h on d 20 and spaced 2 h each day (e.g., d 20, sampling at 1 and 13 h after the morning feeding; d 21, sampling at 3 and 15 h after the morning feeding, and

so on) until the morning of d 25. The perineal area was massaged until a clean and copious stream of urine was obtained. Approximately 50 mL of urine was collected per sample. Samples were acidified to pH <4 using a solution containing 5 M HCl and then frozen at -20°C . Urine samples were thawed, composed within cow by period, and analyzed in triplicate to determine the concentration of total N (Elementar Analysensysteme, Elementar Americas Inc.), urea N (colorimetric method nrea N, colorimetric detection kit no. K024-H1, Arbor Assays, Ann Arbor, MI), creatinine (colorimetric method, kit no. K002-H1, Arbor Assays), allantoin (bovine allantoin ELISA kit no. MBS2603042, MyBioSource Inc., San Diego, CA), and uric acid by fluorescence-based method (kit no. 700320, Cayman Chemical, Ann Arbor, MI). For allantoin ELISA, samples had to undergo dilutions to fall within the standard curve of the assay. An initial assay was run with a sample with serial dilution that demonstrated parallelism of recovery of allantoin. Urinary creatinine was used as a marker to estimate urine volume (Valadares et al., 1999; Chizzotti et al., 2008) considering a daily creatinine excretion of 29 mg/kg of BW (Valadares et al., 1999). Urinary purine derivatives were used to estimate microbial N flow to the duodenum (Gehman and Kononoff, 2010), which was calculated using the estimates of purine derivative excretion according to Chen and Gomes (1992).

Nitrogen Metabolism

Daily intake of N, milk N secretion (milk true protein divided by 6.38), and outputs of urinary and fecal N were calculated for each cow within period. Manure N was calculated as the sum of fecal and urinary N. Retained N was calculated as N intake minus the sum of manure and milk N. Productive N was calculated as the sum of milk and retained N.

Statistical Analysis

Continuous data were evaluated for the distribution of the residuals and homogeneity of variance after fitting the statistical model. Data with deviations from normality were subjected to power transformation before analyses as suggested by the Box-Cox method (Box and Cox, 1964) using the TRANSREG procedure of SAS (SAS/STAT version 9.4, SAS Institute Inc., Cary, NC). The least squares means and standard error of the mean of transformed data were back transformed for presentation according to Jørgensen and Pedersen (1998). Data collected over several days were averaged into a single mean per cow within a period for statistical analysis. Data were analyzed for the prechallenge (d 20–24) and postchallenge (d 25–28) days separately.

Data were analyzed by the MIXED procedure of SAS (SAS/STAT version 9.4). The model included the fixed effects of level of starch (LS vs. HS), supplementation with YC (control vs. YC), interaction between level of starch and supplementation with YC, and period and the random effect of cow. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the *F*-tests in the mixed models. When an interaction between level of starch and supplementation with YC was detected ($P < 0.10$), then comparisons among individual treatments were adjusted by the method of Tukey.

Concentrations of analytes in blood plasma before and after the challenge, rumen pH before and after the challenge, and concentrations of SCFA and lactate in rumen fluid after the challenge were analyzed with repeated measures within cow for a given period. The models included (1) the fixed effects of level of starch; supplementation with YC; interaction between level of starch and supplementation with YC; period; time; and interactions between level of starch and time, supplementation with YC and time, and level of starch and supplementation with YC and time and (2) the random effect of cow nested within level of starch and supplementation with YC. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the *F*-tests in the mixed models. The covariance structure that resulted in best fit based on the smallest corrected Akaike information criterion was chosen. When an interaction between level of starch and supplementation with YC was detected ($P < 0.10$), comparisons among individual treatments were adjusted by the method of Tukey.

Data from quantitative PCR were analyzed using the delta CT for the pre- and postchallenge separately. Data were analyzed by the MIXED procedure of SAS with a model that included the fixed effects of level of starch, supplementation with YC, interaction between level of starch and supplementation with YC, and period and the random effects of cow and PCR plate. The delta-delta CT were obtained from delta CT least squares means differences of pairwise comparisons among treatments and the reference (LS-control) treatment (Yuan et al., 2006). The relative expression of each gene for each treatment was obtained by raising the PCR amplification efficiency ($E = 2$) to the power of delta-delta CT (Yuan et al., 2006) to express the fold change (**FC**) relative to the reference treatment. Confidence limits were computed for graphical representation of relative expression from the lower and upper confidence interval (**CI**) after adjustment by the method of Tukey obtained for delta CT least squares means differences described by Yuan et al. (2006). An additional model for analysis of PCR was conducted

Table 3. Effect of concentration of dietary starch and supplementation with yeast culture (YC) on performance in Holstein cows

Item	Treatment ¹				SEM	P-value ²		
	Low starch		High starch			Starch	Yeast	Starch × yeast
	Control	YC	Control	YC				
DMI, kg/d	22.6	22.9	22.6	22.9	0.7	1.00	0.70	1.00
Milk, kg/d	26.5	28.4	26.2	30.9	1.3	0.28	0.01	0.20
3.5% FCM, ³ kg/d	26.4	30.1	26.8	30.5	1.3	0.77	0.03	0.98
ECM, ⁴ kg/d	26.3	29.7	26.7	30.9	1.2	0.52	0.02	0.76
3.5% FCM/DMI	1.17	1.32	1.19	1.34	0.04	0.63	0.01	0.99
ECM/DMI	1.14	1.27	1.16	1.33	0.05	0.33	<0.01	0.67
Milk fat								
%	3.54 ^b	3.93 ^a	3.65 ^{ab}	3.46 ^b	0.17	0.19	0.45	0.05
kg/d	0.92	1.10	0.95	1.06	0.05	0.92	0.04	0.57
Milk true protein								
%	3.17	3.19	3.27	3.35	0.03	<0.01	0.15	0.29
kg/d	0.83 ^b	0.90 ^b	0.85 ^b	1.02 ^a	0.03	0.02	<0.01	0.08
Lactose								
%	4.79	4.78	4.75	4.79	0.05	0.70	0.74	0.66
kg/d	1.27	1.36	1.25	1.48	0.05	0.38	0.02	0.20
Milk NE ⁵								
Mcal/kg	0.696	0.733	0.711	0.698	0.015	0.37	0.28	0.06
Mcal/d	18.3	20.7	18.6	21.4	0.83	0.54	0.02	0.79
Net energy balance, Mcal/d	8.2	6.1	8.8	6.1	1.3	0.70	0.02	0.70

^{a,b}Means in the same row with different superscripts differ ($P \leq 0.05$) after adjustment by the method of Tukey.

¹Cows were fed diets containing either 23% (low) or 29% (high) starch and not supplemented (control) or supplemented with 15 g of YC/d.

²Starch = effect of level of dietary starch (23 vs. 29%); yeast = effect of YC (control vs. YC); starch × yeast = interaction between starch and yeast.

³3.5% FCM = (0.4324 × kg of milk) + (16.218 × kg of fat yield).

⁴ECM = (0.3246 × kg of milk) + (12.86 × kg of fat yield) + (7.04 × kg of protein yield).

⁵Milk net energy (NE) content = (0.0929 × % milk fat) + (0.0563 × % milk true protein) + (0.0395 × % lactose).

with the pre- and postchallenge responses together to evaluate the effect of challenge on mRNA expression. The models were the same as described above but included (1) the fixed effects of challenge and interactions between starch and challenge, YC and challenge, and starch and YC and challenge and (2) the random effect of cow nested within level of starch and supplementation with YC in place of the random effect of cow. Statistical significance was considered at $P \leq 0.05$, and a tendency was considered at $0.05 < P \leq 0.10$.

RESULTS

Production Performance

Treatments did not influence DMI, and cows averaged 22.8 kg/d (Table 3). Level of dietary starch did not influence yield of milk, but supplementing YC increased ($P < 0.05$) yields of milk (control = 26.3 vs. YC = 29.6 kg/d), 3.5% FCM (control = 26.6 vs. YC = 30.6 kg/d), and ECM (control = 26.5 vs. YC = 30.3 kg/d), which resulted in a 13% increase ($P < 0.01$) in efficiency of feed conversion into ECM (control = 1.18 vs. YC = 1.33). Within cows supplemented with YC, those fed

HS produced milk with less fat content than those fed LS; however, milk fat yield increased ($P = 0.04$) in cows fed YC regardless of level of dietary starch fed (control = 0.94 vs. YC = 1.08 kg/d). Increasing dietary starch or feeding YC increased ($P < 0.02$) true protein yield, and the increment tended ($P = 0.08$) to be greater when both were combined in cows fed HS-YC (Table 3). Lactose content in milk did not differ among treatments, but lactose yield increased in cows fed YC because of greater milk yield than in cows fed the control. A tendency for interaction ($P = 0.06$) between level of dietary starch and supplementation with YC affected milk net energy (NE). Nevertheless, NE secretion in milk increased ($P = 0.02$) by 2.6 Mcal/d with feeding of YC compared with the control, and the response was observed in cows fed LS and HS. Cows were in positive NE balance, but the increased NE secretion in milk in cows fed YC resulted in a less ($P = 0.02$) positive value than in cows fed the control.

Plasma Metabolites

Treatments did not affect concentrations of glucose, nonesterified fatty acids, or urea N in plasma of dairy

Table 4. Effect of concentration of dietary starch and supplementation with yeast culture (YC) on plasma metabolites in Holstein cows

Item	Treatment ¹				SEM	P-value ²		
	Low starch		High starch			Starch	Yeast	Starch × yeast
	Control	YC	Control	YC				
Glucose, mg/dL	62.4	65.3	64.1	67.0	2.0	0.41	0.17	0.99
Nonesterified fatty acids, μM	122.7	120.3	100.6	124.9	19.0	0.66	0.58	0.51
Urea N, mg/dL	8.8	9.5	9.2	10.0	0.6	0.50	0.29	0.89
Haptoglobin, OD ³ × 100	3.95 ^{AB}	3.88 ^{AB}	8.10 ^A	3.51 ^B	1.91	0.19	0.12	0.10

^{A,B}Means in the same row with different superscripts tend to differ ($P = 0.08$) after adjustment by the method of Tukey.

¹Cows were fed diets containing either 23% (low) or 29% (high) starch and not supplemented (control) or supplemented with 15 g of YC/d.

²Starch = effect of level of dietary starch (23 vs. 29%); yeast = effect of YC (control vs. YC); starch × yeast = interaction between starch and yeast.

³OD = optical density.

cows (Table 4). Concentration of haptoglobin in plasma tended ($P = 0.10$) to be influenced by the interaction between level of dietary starch and supplementation with YC because cows fed the HS-YC diet tended ($P = 0.08$) to have smaller concentration of haptoglobin than those fed the HS-control diet.

Ruminal and Total-Tract Apparent Digestibility and Rumen SCFA

Ruminal digestibility of OM was not affected by treatments and averaged 54.9% (Table 5). A tendency for interaction ($P = 0.06$) was detected for rumen

Table 5. Effect of concentration of dietary starch and supplementation with yeast culture (YC) on rumen fermentation and apparent digestibility in Holstein cows

Item	Treatment ¹				SEM	P-value ²		
	Low starch		High starch			Starch	Yeast	Starch × yeast
	Control	YC	Control	YC				
Ruminal digestibility, %								
OM	56.5	53.2	56.0	53.8	2.0	0.96	0.18	0.77
Starch	80.5 ^{AB}	80.6 ^{AB}	84.7 ^A	79.9 ^B	1.2	0.19	0.08	0.06
NDF	60.5 ^a	56.7 ^a	46.2 ^b	56.0 ^a	2.6	0.01	0.21	0.02
Total-tract digestibility, %								
DM	71.7	72.5	70.2	70.7	0.8	0.06	0.38	0.77
OM	73.5	74.3	72.0	72.7	0.5	0.02	0.16	0.96
CP	68.7 ^{ab}	69.3 ^{ab}	67.1 ^b	70.6 ^a	1.1	0.86	0.03	0.10
NDF	60.0	61.8	55.9	57.5	1.8	0.04	0.45	0.92
ADF	57.2	58.0	52.8	50.5	2.3	0.04	0.74	0.50
Starch	95.8	94.8	97.1	95.3	1.1	0.30	0.11	0.65
Rumen								
Total short-chain fatty acids, mM	95.2	106.6	115.6	126.4	10.3	0.10	0.32	0.98
Acetate, mM	66.1	74.3	74.2	80.3	6.9	0.35	0.34	0.88
Propionate, mM	19.6	23.7	30.0	34.6	3.3	0.01	0.20	0.95
Butyrate, mM	9.6	8.6	11.3	11.5	1.3	0.15	0.79	0.71
Acetate:propionate	3.71	3.27	2.75	2.33	0.23	<0.01	0.04	0.98
Lactate, mM	0.78	0.66	2.37	1.56	0.23	<0.01	0.05	0.13
Rumen pH prechallenge 24 h								
Mean pH	6.34 ^a	6.22 ^{ab}	6.02 ^b	6.41 ^a	0.12	0.44	0.17	0.04
Minutes pH <6.00	263 ^b	315 ^b	793 ^a	316 ^b	110	<0.01	0.02	<0.01
Rumen pH postchallenge 24 h								
Mean pH	6.28	6.36	6.04	6.08	0.11	0.03	0.58	0.84
Minutes pH <6.00	434	281	759	582	132	0.04	0.22	0.92

^{a,b}Means in the same row with different superscripts differ ($P \leq 0.05$) after adjustment by the method of Tukey.

^{A,B}Means in the same row with different superscripts tended to differ ($P = 0.07$) after adjustment by the method of Tukey.

¹Cows were fed diets containing either 23% (low) or 29% (high) starch and not supplemented (control) or supplemented with 15 g of YC/d.

²Starch = effect of level of dietary starch (23 vs. 29%); yeast = effect of YC (control vs. YC); starch × yeast = interaction between starch and yeast.

starch digestibility because cows fed the HS-control diet tended ($P = 0.07$) to have greater rumen starch digestion than those fed the HS-YC diet. Increasing dietary starch reduced ($P = 0.01$) ruminal digestibility of NDF, but supplementing YC to the HS diet prevented the decline in rumen NDF digestion.

Total-tract digestibility of DM tended ($P = 0.06$) to be less, and that of OM was less ($P = 0.02$), for cows fed HS than for those fed LS (Table 5). A tendency for interaction ($P = 0.10$) between level of starch and supplemental YC was detected for total-tract CP digestibility because, within cows fed the HS diet, those supplemented with YC had greater digestibility than controls. Total-tract digestibility of NDF (LS = 61.2 vs. HS = 56.7%) and ADF (LS = 57.6 vs. 51.6%) was greater ($P = 0.02$) for cows fed the LS diet than for those fed the HS diet, and supplementing YC did not influence total-tract fiber digestion. Treatment did not affect digestion of starch in the total tract, which averaged 95.7%.

Feeding HS tended ($P = 0.10$) to increase the concentration of total SCFA in rumen fluid (LS = 100.9 vs. HS = 121.0 ± 10.4 mM), but no differences were observed between the control and YC (Table 5). Concentrations of acetate and butyrate in rumen fluid did not differ among treatments; however, those of propionate increased ($P = 0.01$) in cows fed HS compared with LS (LS = 21.6 vs. HS = 32.7 mM). The ratio of acetate to propionate was greater for cows fed LS than for those fed HS (LS = 3.49 vs. HS = 2.54; $P < 0.01$) and for cows fed the control rather than those fed YC (control = 3.23 vs. HS = 2.80; $P = 0.04$). Concentrations of lactate in rumen fluid increased ($P < 0.01$) in cows fed HS compared with cows fed LS (LS = 0.72 vs. HS = 1.96 mM) but decreased ($P = 0.05$) in cows supplemented with YC compared with controls (control = 1.57 vs. YC = 1.11 mM).

Nitrogen Metabolism

Treatment did not affect the estimated daily urinary volume, which averaged 29.9 L/d (Table 6). Feeding HS compared with LS tended ($P = 0.06$) to decrease the concentration of $\text{NH}_3\text{-N}$ in rumen fluid (LS = 8.33 vs. HS = 6.65 mg/dL). Treatment did not influence the flow of N from the omasum, but a tendency for interaction ($P = 0.09$) between starch and YC was observed for purine derivative excretion. Within cows supplemented with YC, feeding HS increased purine derivatives and microbial N compared with cows fed LS. Treatment did not influence the percentage of omasal flow of N that was of microbial origin, which averaged 57.3%. Also, treatment did not affect N intake, fecal N excretion,

urinary urea N, and manure N (Table 6); however, a tendency ($P = 0.10$) for interaction was observed for urinary excretion of non-urea N. Within cows supplemented with YC, those fed the HS diet excreted more urinary non-urea N than those fed the LS diet. Milk N increased with feeding HS or supplementing YC, and it was highest for cows fed the HS-YC diet. Cows fed HS has less ($P = 0.04$) N retention than cows fed LS, but within HS cows, those supplemented with YC had the largest ($P = 0.03$) productive N yield. Relative to N intake, fecal N represented 34%, urinary N represented 40%, and milk N represented 26%. Fecal N relative to N intake increased ($P = 0.05$) in cows fed HS compared with LS (LS = 33.3 vs. HS = 34.7) but decreased ($P = 0.02$) with supplementing YC (control = 34.8 vs. YC = 33.1%). The proportion of N intake excreted as urinary N did not differ with treatment, but feeding the HS-YC diet resulted in the largest ($P < 0.01$) proportion of N intake partitioned as milk N.

Rumen pH Pre- and Postchallenge

Rumen pH was influenced by dietary treatments (Table 5). Prechallenge mean rumen pH was affected by the interaction ($P = 0.04$) between level of dietary starch and supplemental YC because within cows fed HS, supplementing YC increased rumen pH. In fact, YC was capable of reducing ($P < 0.01$) the duration of pH < 6.0 in cows fed HS (Figure 1A and B). On the other hand, feeding HS resulted in smaller ($P = 0.03$) mean rumen pH postchallenge (LS = 6.32 vs. HS = 6.06) and a longer ($P = 0.04$) period with pH < 6.0 compared with feeding LS (LS = 358 vs. HS = 671 min), and supplementing YC did not attenuate the changes in rumen pH postchallenge (Figure 1C and D).

Plasma Metabolites and Rumen SCFA Concentrations Postchallenge

The profiles of plasma glucose, nonesterified fatty acids, and urea N concentrations after challenge did not differ among treatments (Figure 2A–C); however, feeding HS tended ($P = 0.09$) to increase the concentrations of haptoglobin postchallenge, but only in the absence of supplemental YC (Figure 2D). In fact, within HS cows, those supplemented with YC tended ($P = 0.06$) to have a smaller concentration of haptoglobin than cows fed the control (HS-control = 6.4 vs. HS-YC = 3.5 optical density).

Treatments did not influence the concentrations of acetate, butyrate, or total SCFA in rumen fluid after the challenge (Figure 3A, C, and D). However, concentrations of propionate were greater ($P < 0.01$) in

Table 6. Effect of concentration of dietary starch and supplementation with yeast culture (YC) on nitrogen metabolism in Holstein cows

Item	Treatment ¹				SEM	<i>P</i> -value ²		
	Low starch		High starch			Starch	Yeast	Starch × yeast
	Control	YC	Control	YC				
Estimated urine excretion, L/d	32.5	30.1	27.2	29.6	3.5	0.40	0.99	0.47
Purine derivatives excretion, mmol/d	394.2 ^{ab}	361.2 ^b	382.6 ^{ab}	417.5 ^a	22.4	0.24	0.96	0.09
Rumen								
NH ₃ N, mg/dL	8.82	7.84	6.54	6.76	0.74	0.06	0.63	0.45
Total N, g/d	516.8	544.0	524.8	584.0	56.0	0.63	0.39	0.75
Microbial N, g/d	302.4 ^{AB}	275.2 ^B	292.8 ^{AB}	321.6 ^A	16.0	0.24	0.96	0.09
Microbial:total	0.60	0.52	0.58	0.59	0.06	0.67	0.51	0.37
Mass, g/d								
Intake of N	538.4	543.9	539.0	544.6	18.4	0.97	0.75	0.99
Fecal N	181.0	178.6	194.1	181.5	8.1	0.21	0.23	0.41
Urinary urea N	184.9	194.2	188.9	181.6	12.8	0.71	0.93	0.47
Urinary non-urea N	28.4 ^{ab}	26.6 ^b	27.8 ^{ab}	29.9 ^a	1.4	0.22	0.85	0.10
Total urinary N	213.2	220.8	216.7	211.5	14.0	0.81	0.92	0.60
Manure ³ N	394.2	399.4	410.8	393.0	21.3	0.76	0.70	0.49
Milk N	130.1 ^b	141.1 ^b	133.4 ^b	160.6 ^a	5.3	0.02	<0.01	0.08
Retained ⁴ N	14.0	3.4	−5.1	−9.0	8.5	0.04	0.27	0.59
Productive ⁵ N	144.1 ^{AB}	144.5 ^{AB}	128.3 ^B	151.6 ^A	8.3	0.48	0.09	0.10
Fecal N, % of N intake	33.7	32.8	36.0	33.4	0.8	0.05	0.02	0.17
Urinary N, % of N intake	39.6	40.7	40.4	38.9	1.9	0.73	0.90	0.36
Manure N, % of N intake	73.2	73.5	76.3	72.2	1.7	0.46	0.15	0.12
Milk N, % of N intake	24.1 ^b	25.9 ^b	24.8 ^b	29.6 ^a	0.9	0.01	<0.01	0.04

^{a,b}Means in the same row with different superscripts differ ($P \leq 0.05$) after adjustment by the method of Tukey.

^{A,B}Means in the same row with different superscripts tend to differ ($P = 0.08$) after adjustment by the method of Tukey.

¹Cows were fed diets containing either 23% (low) or 29% (high) starch and not supplemented (control) or supplemented with 15 g of YC/d.

²Starch = effect of level of dietary starch (23 vs. 29%); yeast = effect of yeast culture (control vs. YC); starch × yeast = interaction between starch and yeast.

³Manure N = fecal N + urinary N.

⁴Retained N = N intake - (manure N + milk N).

⁵Productive N = milk N + retained N.

cows fed HS than in those fed LS (LS = 21.3 vs. 30.4 ± 1.5 mM) and tended ($P = 0.09$) to be greater in cows supplemented with YC than in controls (control = 23.9 vs. YC = 27.8 ± 1.5 mM), and these differences were more pronounced at 12 and 48 h after the challenge (Figure 3B). The increase in propionate in cows fed HS resulted in a smaller ($P = 0.03$) ratio of acetate to propionate compared with cows fed LS (LS = 3.39 vs. HS = 2.49; Figure 3E). Lactate >0.50 mM was detected in 13 of the 24 samples analyzed after the challenge. Dietary starch level did not influence concentrations of lactate in rumen fluid, but supplementing YC tended ($P = 0.09$) to reduce the concentrations of lactate in rumen fluid from 1.96 to 1.26 mM (Figure 3F).

Rumen Papillae Gene Expression

We investigated the relative changes in mRNA expression for genes that are receptors for SCFA; involved in their transport, Na⁺/H⁺ exchange, and transport of urea or water; and related to inflammatory response (Figure 4). Increasing dietary starch content upregu-

lated ($P < 0.05$) mRNA expression of *FFAR2* pre- and postchallenge 2.2- and 2.4-fold, respectively; that of *FFAR3* pre- and postchallenge 2.4- and 3.1-fold, respectively; and that of *SLC16A3* postchallenge 3-fold. Nevertheless, interactions ($P = 0.05$) existed between starch and supplemental YC for mRNA expression for *FFAR3* and *SLC16A3* postchallenge because cows fed the HS-YC diet had the greatest increment in relative gene expression. Treatment did not affect expression of *SLC16A1*, *AQP3*, *SLC9A1*, and *TLR2* pre- and postchallenge and that of *SLC16A3* prechallenge and *SLC14A1*, *SLC9A3*, and *TLR4* postchallenge. Nevertheless, cows fed HS tended ($P = 0.06$) to have less mRNA for *SLC14A1* prechallenge than cows fed LS (0.7-fold) and had less ($P = 0.05$) mRNA expression prechallenge for *TLR4* (0.6-fold) than cows fed LS. A tendency ($P = 0.09$) for interaction between level of dietary starch and supplemental YC was observed for mRNA expression of *SLC9A3* prechallenge because for cows supplemented with YC, those fed HS tended ($P = 0.07$) to have less mRNA expression than those fed LS (0.4-fold).

When rumen papillae mRNA data were analyzed with the pre- and postchallenge together in the same statistical model, grain challenge affected expression of 3 of the 10 genes investigated. Expression of *FFAR2* tended ($P = 0.09$) to increase with challenge (FC = 1.47; 95% CI = 0.93–2.30), *FFAR3* increased ($P = 0.03$) with challenge (FC = 1.36; 95% CI = 1.04–1.77), and *TLR2* increased ($P = 0.02$) with challenge (FC = 2.14; 95% CI = 1.16–3.98). The expression of all other genes investigated did not differ between before and after the grain challenge.

Production Performance Postchallenge

Production performance of cows after the challenge resembled, for the most part, that of the prechallenge (Table 7). Supplementing YC to cows fed HS increased

($P = 0.05$) DMI compared with the HS-control diet. Level of dietary starch did not influence yields of milk or milk components or concentrations of fat, protein, or lactose in milk. Yeast culture increased ($P < 0.01$) yields of milk (control = 27.3 vs. YC = 30.1 kg/d), 3.5% FCM (control = 27.0 vs. YC = 30.2 kg/d), and ECM (control = 27.0 vs. YC = 30.3 kg/d), which resulted in improved ($P = 0.03$) yield of ECM per kilogram of DMI (control = 1.18 vs. YC = 1.28). Concentrations of milk components did not differ with supplemental YC; however, because of the differences in milk yield, cows fed YC produced ($P < 0.05$) more fat (control = 0.94 vs. YC = 1.06 kg/d), true protein (control = 0.88 vs. YC = 0.98 kg/d), and lactose (control = 1.32 vs. YC = 1.45 kg/d) than cows fed the control. The changes in yields of milk components with feeding YC resulted in increased ($P < 0.01$) NE secretion as milk (control = 18.8 vs. YC = 21.0 Mcal/d).

Figure 1. Rumen pH over a period of 24 h in dairy cows fed diets with low (23%) or high (29%) starch content not supplemented (control) or supplemented with yeast culture (YC). Rumen pH was measured for 24 h before and 24 h after a challenge with 3 kg of finely ground corn. (A) Cows fed low starch before the challenge. (B) Cows fed high starch before the challenge. (C) Cows fed low starch after the challenge. (D) Cows fed high starch after the challenge.

Figure 2. Concentrations of metabolites in plasma after a challenge with 3 kg of corn DM at h 0 in cows fed diets containing either 23% (LS) or 29% (HS) starch and not supplemented (control) or supplemented with 15 g of yeast culture (YC)/d. (A) Plasma glucose concentrations (SEM = 3.6); effects of level of starch ($P = 0.62$), YC ($P = 0.50$), and interaction between level of starch and YC ($P = 0.74$). (B) Plasma nonesterified fatty acid (FA) concentrations (SEM = 11.0); effects of level of starch ($P = 0.76$), YC ($P = 0.96$), and interaction between level of starch and YC ($P = 0.65$). (C) Plasma urea N concentrations (SEM = 0.95); effects of level of starch ($P = 0.62$), YC ($P = 0.96$), and interaction between level of starch and YC ($P = 0.71$). (D) Plasma haptoglobin concentrations (SEM = 1.08; OD = optical density); effects of level of starch ($P = 0.09$), YC ($P = 0.14$), and interaction between level of starch and YC ($P = 0.21$). Error bars represent SEM (A, SEM = 3.6; B, SEM = 11.0; C, SEM = 0.95; D, SEM = 1.08).

DISCUSSION

High-starch diets are more prone to influencing rumen pH and fiber digestion and can depress milk fat content, whereas supplemental yeast products, either as culture or only live yeast cells, have been shown to attenuate shifts in rumen pH and improve fiber digestion and milk fat yield. Therefore, it was hypothesized that the effects of YC would be enhanced in cows fed HS compared with those fed LS. Nevertheless, many of the responses to supplemental YC were independent of dietary starch content. Feeding YC improved yields of milk, 3.5% FCM, ECM, and milk components, and the increments in production were observed in cows fed LS or HS. Feeding *S. cerevisiae* products often has been shown to improve productive performance in dairy cows in numerous experiments, and a meta-analysis of effects of YC showed increases in yields of milk and milk components irrespective of the delivery method of

YC (either mixed with the TMR or fed as top-dress; Poppy et al., 2012). Top-dressing likely results in larger temporal changes during the day in concentrations of the YC products in the rumen compared with mixing with the diet, but either method of delivery is effective in improving lactation performance. The results of Poppy et al. (2012) showed that of the 54 treatment comparisons of supplementation with YC, 19 used top-dressing, and those comparisons resulted in a larger but more variable response in milk yield compared with mixing with the TMR. A companion experiment with the same dietary treatments used herein showed that cows fed YC top-dressed produced 2.1 and 2.0 kg/d more 3.5% FCM and ECM, respectively, than cows fed diets without YC (Dias et al., 2018).

Yeast culture is thought to improve animal performance by influencing the rumen microbiota to support growth and activity of fibrolytic microorganisms and those that metabolize lactate. This might be important

Figure 3. Concentrations of short-chain fatty acids (SCFA) and lactate in rumen fluid after a challenge with 3 kg of corn DM at h 0 in cows fed diets containing either 23% (LS) or 29% (HS) starch and not supplemented (control) or supplemented with 15 g of yeast culture (YC)/d. (A) Rumen acetate concentrations (SEM = 8.6); effects of level of starch ($P = 0.48$), YC ($P = 0.90$), and interaction between level of starch and YC ($P = 0.81$). (B) Rumen propionate concentrations (SEM = 4.0); effects of level of starch ($P < 0.01$), YC ($P = 0.09$), and interaction between level of starch and YC ($P = 0.24$). (C) Rumen butyrate concentrations (SEM = 2.2); effects of level of starch ($P = 0.45$), YC ($P = 0.67$), and interaction between level of starch and YC ($P = 0.95$). (D) Rumen total SCFA concentrations (SEM = 10.4); effects of level of starch ($P = 0.15$), YC ($P = 0.52$), and interaction between level of starch and YC ($P = 0.79$). (E) Rumen acetate to propionate ratio (SEM = 0.48); effects of level of starch ($P = 0.03$), YC ($P = 0.31$), and interaction between level of starch and YC ($P = 0.57$). (F) Rumen lactate concentrations (SEM = 0.51); effects of level of starch ($P = 0.24$), YC ($P = 0.09$), and interaction between level of starch and YC ($P = 0.50$). Error bars represent SEM (A, SEM = 8.6; B, SEM = 4.0; C, SEM = 2.2; D, SEM = 10.4; E, SEM = 0.48; F, SEM = 0.51).

in diets that promote increased SCFA production in the rumen and result in reduced rumen pH. In fact, feeding YC increased rumen NDF digestibility, and the benefit was observed when cows were fed HS. Recent

work using sequencing of bacterial DNA of rumen samples showed that supplementing live yeast to the diet of dairy cows increased the relative abundance of cellulolytic, amylolytic, and lactate-utilizing microor-

Figure 4. Relative mRNA expression in rumen papillae 24 h prechallenge and 72 h postchallenge with 3 kg of corn DM in dairy cows fed diets with low (LS, 23%) or high (HS, 29%) starch content not supplemented (control) or supplemented with yeast culture (YC). Genes investigated were those involved in short-chain fatty acid transport [free fatty acid receptor 2 (*FFAR2*) and 3 (*FFAR3*); solute carrier family 16 member 1 (*SLC16A1*) and 3 (*SLC16A3*)], water and solute transport [aquaporin (*AQP3*)], urea transport [urea transporter 1 (*SLC14A1*)], Na^+/H^+ transport [solute carrier family 9 subfamily A member 1 (*SLC9A1*) and member 3 (*SLC9A3*)], and inflammatory response [toll-like receptor 2 (*TLR2*) and 4 (*TLR4*)]. Data were analyzed for the pre- and postchallenge separately. S = effect of level of dietary starch (LS vs. HS); Y = effect of inclusion of yeast cultures (control vs. YC). Expression of mRNA is represented as relative to treatment LS-control. Error bars represent the 95% CI.

Table 7. Effect of concentration of dietary starch and supplementation with yeast culture (YC) on performance of Holstein cows after a challenge with corn grain

Item	Treatment ¹				SEM	P-value ²		
	Low starch		High starch			Starch	Yeast	Starch × yeast
	Control	YC	Control	YC				
DMI, kg/d	23.3 ^{ab}	23.0 ^{ab}	22.6 ^b	24.5 ^a	0.8	0.51	0.20	0.09
Milk, kg/d	27.4	29.1	27.2	31.1	1.4	0.18	<0.01	0.11
3.5% FCM, ³ kg/d	27.6	29.6	26.4	30.7	1.0	0.88	<0.01	0.15
ECM, ⁴ kg/d	27.5	29.5	26.6	31.0	1.0	0.63	<0.01	0.13
3.5% FCM/DMI	1.19	1.29	1.17	1.25	0.04	0.43	0.02	0.66
ECM/DMI	1.18	1.29	1.18	1.27	0.04	0.74	0.01	0.65
Milk fat								
%	3.58	3.67	3.36	3.43	0.19	0.14	0.55	0.93
kg/d	0.97	1.05	0.90	1.06	0.05	0.43	0.01	0.32
Milk true protein								
%	3.19	3.22	3.26	3.34	0.09	0.21	0.42	0.72
kg/d	0.87	0.93	0.88	1.03	0.04	0.12	0.02	0.21
Lactose								
%	4.85	4.81	4.78	4.84	0.05	0.64	0.77	0.27
kg/d	1.33 ^b	1.40 ^{ab}	1.30 ^b	1.50 ^a	0.08	0.34	<0.01	0.09
Milk NE ⁵								
Mcal/kg	0.70	0.71	0.68	0.70	0.04	0.19	0.37	0.86
Mcal/d	19.2	20.6	18.4	21.6	0.5	0.78	<0.01	0.11

^{a,b}Means in the same row with different superscripts differ ($P \leq 0.05$) after adjustment by the method of Tukey.

¹Cows were fed diets containing either 23% (low) or 29% (high) starch and not supplemented (control) or supplemented with 15 g of YC/d.

²Starch = effect of level of dietary starch (23 vs. 29%); yeast = effect of yeast culture (control vs. YC); starch × yeast = interaction between starch and yeast.

³3.5% FCM = $(0.4324 \times \text{kg of milk}) + (16.218 \times \text{kg of fat yield})$.

⁴ECM = $(0.3246 \times \text{kg of milk}) + (12.86 \times \text{kg of fat yield}) + (7.04 \times \text{kg of protein yield})$.

⁵Milk net energy (NE) content = $(0.0929 \times \% \text{ milk fat}) + (0.0563 \times \% \text{ milk true protein}) + (0.0395 \times \% \text{ lactose})$.

ganisms, whereas supplementing dead yeast increased populations of amylolytic and lactate-utilizing microorganisms (Jiang et al., 2017), confirming earlier findings in vivo (Harrison et al., 1988) and in vitro (Wiedmeier et al., 1987; Callaway and Martin, 1997) that supplementation of YC containing live cells or the culture media with yeast products or their filtrate can influence rumen microbial growth and carbohydrate digestion. In fact, feeding cultures of *S. cerevisiae* increased the total and cellulolytic bacteria in rumen fluid of dairy cows (Wiedmeier et al., 1987; Harrison et al., 1988). The changes in rumen microbial population are expected to stimulate digestion of carbohydrates in the rumen, which likely explains the increased rumen NDF digestion in the present experiment and the total-tract digestion of OM (Desnoyers et al., 2009).

Desnoyers et al. (2009) compiled data from numerous yeast products fed to ruminants and found that *S. cerevisiae* supplementation increased OM digestibility and that the benefits increased as dietary NDF content increased. Although YC increased rumen and total-tract digestion of NDF and total-tract digestion of CP in cows fed the HS diet, the increments were not sufficient to influence digestibility of OM. Part of the inability to detect differences in OM digestion was probably because

cows fed YC tended to have less starch digestion when fed the HS diet and had numerically less total-tract starch digestion. Allen and Ying (2012) suggested that supplementation with YC reduced the rate of starch digestion in the rumen in cows with greater DMI. Cows in the current experiment did not have high DMI, likely because they were primiparous, started at 176 DIM and finished at 288 DIM, and produced an average of 28.5 kg of 3.5% FCM/d. Therefore, the reduction in rate of rumen starch digestion described by Allen and Ying (2012) in cows supplemented with YC might also apply to cows of less production such as those in the present experiment, which might contribute to the effects in maintaining a more stable rumen environment.

Feeding HS reduced rumen and total-tract NDF digestion, increased propionate concentration in rumen fluid, and reduced rumen pH compared with feeding LS. Nevertheless, the decline in rumen pH with feeding HS was attenuated by supplemental YC. It has been shown that supplementing a yeast product to a culture medium provided soluble growth factors that stimulated growth of rumen microorganisms that metabolize lactate (Callaway and Martin, 1997), and increments in the population of microbes that convert lactate into SCFA might help control sudden shifts in rumen pH

after a meal. It is interesting that supplementing YC increased rumen pH, but only when cows were fed HS. Also, the ability of YC to increase rumen pH was not observed when those fed HS were challenged with an extra 3 kg of corn grain DM. In those cows, the extra corn grain resulted in a diet with 34.9% starch on the day of the challenge, which might have been excessive for YC to exert its effects minimizing decreases in rumen pH. Supplementing diets of dairy cows with live yeast products minimized the reduction in rumen pH after a meal (Bach et al., 2007) and minimized the risk of SARA (AlZahal et al., 2014), and Bach et al. (2007) attributed part of the effect to increased meal frequency compared with nonsupplemented cows. Similar to the findings of Bach et al. (2007), cows supplemented with YC tended to have an increased number of meals compared with controls (control = 5.9 vs. YC = 6.4 bouts/12 h; Dias et al., 2018). Furthermore, cows supplemented with YC had reduced concentrations of lactate and increased rumen pH, particularly those fed the HS diet (Dias et al., 2018). The meta-analysis of Desnoyers et al. (2009) also showed an increase in rumen pH and a tendency for a smaller concentration of lactate in the rumen fluid of animals fed diets supplemented with *S. cerevisiae* products. Collectively, results from the current and companion experiments (Dias et al., 2018) indicate that supplementation of diets with YC attenuates changes in rumen pH by reducing rumen lactate concentration and influencing eating behavior.

There is vast literature documenting the benefits of supplementing diets with YC on lactation performance of dairy cows, and current results corroborate with the findings of others (Shaver and Garrett, 1997; Poppy et al., 2012). Nevertheless, what is less clear is whether the benefits to supplemental YC differ according to the structure of the diet, particularly the content of starch and NDF. Williams et al. (1991) observed that supplemental YC increased FCM of cows fed a diet with more concentrates. Longuski et al. (2009) demonstrated that supplementing diets with YC prevented the reduction in milk fat yield when cows were fed more rumen-fermentable diets. Productive performance of cows in the present experiment improved by feeding supplemental YC, and the benefits were observed in cows fed LS or HS. Furthermore, the increments in yields of milk, 3.5% FCM, and ECM and the improved feed efficiency were documented even after cows were challenged with additional dietary starch. In fact, on the day of the challenge, the HS diet contained 34.9% starch, which was expected to depress DMI and milk fat content. Concentration of fat in milk did not differ among treatments after the challenge, although the pattern of content was exactly the same as that observed before the challenge. Although the concentration of starch in the diet on the

day of grain challenge increased 5.9 percentage units, sufficient to extend the duration of rumen pH <6.0 an additional 90 min compared with the day preceding the challenge, it might not have been sufficient to induce a marked milk fat depression. Dias et al. (2018) used the same dietary treatments and challenge with cows in early lactation and showed that the additional starch from the grain challenge induced a decline in milk fat content and yield that lasted for at least 3 d, which was not observed in the present experiment. Cows in Dias et al. (2018) were earlier in lactation and consuming 27 kg of DM on the days preceding the challenge, and it is possible that the additional starch resulted in excessive starch intake to maintain proper rumen fermentation to support milk fat yield. On the other hand, cows in the present experiment were in mid to late lactation and consuming 22.8 kg/d, so the resulting total starch intake on the day of the challenge was substantially less than that in Dias et al. (2018).

Concentrations of SCFA peaked by 12 h after the challenge, which coincided with the nadir rumen pH, and they were slightly greater than those observed in the prechallenge period. Concentrations of total SCFA increased by 9.8%, and concentrations of individual SCFA increased by 5.7% for acetate, 12.0% for propionate, and 33.7% for butyrate at 12 h after the challenge compared with the mean values for the days preceding the challenge. The largest relative increment was lactate, which increased from 1.3 to 2.3 mM from the days before challenge to 12 h after the challenge. Concentrations of lactate in rumen fluid were low and transient, as typically observed for dairy cattle (Aikman et al., 2011), and 46% of the rumen fluid samples collected after the challenge had concentrations of lactate <0.50 mM (11 of 24 samples). Krause and Oetzel (2005) restricted the amount of feed to lactating dairy cows to approximately 11.5 kg of DMI. On the following day, the authors challenged cows with 3.5 to 4.6 kg of DM of 50% finely ground barley and 50% finely ground wheat concurrent with refeeding that resulted in 22.2 kg of DMI, similar to what cows were consuming during the prechallenge period (Krause and Oetzel, 2005). The subacute acidosis challenge resulted in rumen lactate concentrations that increased from approximately 2 mM to 16 mM at peak at 10 to 12 h after the challenge. Therefore, it seems that rumen lactate concentrations increase substantially only when dietary starch intake is extremely high or when cows undergo a period of feed restriction followed by refeeding and a challenge with highly fermentable starch.

Microbial N yield was greatest in cows fed HS-YC, and the concentration of $\text{NH}_3\text{-N}$ decreased in cows fed the HS diet compared with the LS diet. Hristov et al. (2010) observed that cows fed a high-NFC diet had

increased microbial protein yield when supplemented with YC. In some cases, supplementing diets with YC increased the duodenal flow of Met, which changed the profile of AA available for absorption in the small intestine (Erasmus et al., 1992). In other cases, the flow of microbial N did not change, but that of nonmicrobial NAN tended to increase in cows fed diets supplemented with YC (Putnam et al., 1997). The increments in available AA in the small intestine would support increased milk protein yield, which was observed in cows fed YC in the current experiment. In fact, supplementing YC improved the amount of milk N and the partitioning of dietary N into milk N, and the increment was greatest in cows fed the HS diet. It is possible that increments in microbial N improved the quality of AA reaching the intestine when cows were fed YC, particularly combined with the HS diet, which is expected to stimulate yields of milk and milk protein.

Maintenance of a stable rumen environment following digestion of OM and acid production is dependent on absorption of SCFA, buffering of the acid load, and removal of acids with the rumen outflow (Allen, 1997). Allen (1997) suggested that SCFA absorption is responsible for more than 50% of the H^+ removal from the rumen, and the process is mediated by specific proteins present in the rumen epithelium. It was hypothesized that feeding diets that stimulate SCFA production in the rumen would stimulate expression of genes that encode for their receptors and genes involved in their absorption by the rumen epithelium. Indeed, feeding HS increased relative mRNA expression for *FFAR2* and *FFAR3* compared with feeding LS, and feeding the HS-YC diet resulted in expressive upregulation of the *SLC16A3* gene during the postchallenge period. G-protein coupled receptors *FFAR2* and *FFAR3* have been identified in the rumen epithelium of cattle (Wang et al., 2009), and expression has been shown to be stimulated by increased dietary NFC in goats (Lu et al., 2015), presumably because of increased availability of SCFA or induced by the reduction in rumen pH when more fermentable diets are fed. Cows fed HS had reduced rumen pH and increased concentration of propionate in the rumen fluid, which likely explain the increase in expression of *FFAR2* and *FFAR3*. These receptors use SCFA as ligands and act as nutrient-sensing receptors in numerous tissues to regulate energy homeostasis. Their role in the rumen epithelium remains mostly unknown, but it is likely that activation of downstream signaling by binding of SCFA to *FFAR2* and *FFAR3* mediates some of the effects of these fatty acids on epithelium metabolism such as activation of immune cells (Le Poul et al., 2003), nutrient sensing by the gut (Tazoe et al., 2009), and expression of genes related to SCFA and urea transport (Lu et al., 2015).

The genes *SLC16A1* and *SLC16A3*—also known as monocarboxylate transporters 1 and 4, respectively—catalyze the transepithelial transport of SCFA, lactate, and ketones from the rumen (Müller et al., 2002). Removal of protons from the rumen by uptake of SCFA by the epithelium induces cytosolic acidification of epithelial cells (Müller et al., 2002), which is counteracted by exporting protons out of the epithelial cells either into the bloodstream by the monocarboxylate transport system or by exchange with Na by the Na^+/H^+ exchange proteins through the apical membrane back into the rumen or through the basolateral membrane to the bloodstream (Aschenbach et al., 2011). Although level of dietary starch influenced expression of mRNA for receptors and transporters of SCFA by rumen papillae, the expression of Na^+/H^+ exchange genes did not differ with treatments either pre- or postchallenge. Although increasing concentrate intake has been well documented to influence mRNA expression of Na^+/H^+ exchange genes in goats (Yang and Martens, 2012), the same has not been shown in dry dairy cows (Penner et al., 2009). Perhaps the dietary manipulations imposed in the present experiment were not sufficiently large to induce major changes in SCFA and ion flux to influence expression of Na^+/H^+ exchange genes in rumen epithelium. It is important to indicate that supplementing YC had only minor effect on the transcriptome of the rumen papillae, which was observed only when accompanied by the HS diet. The changes in mRNA when YC was supplemented to cows fed HS are likely the result of increased rumen digestion and supply of SCFA.

One of the consequences of feeding diets with increased starch content is the depression in rumen pH and the accumulation of bacterial by-products that can induce inflammation. The levels of starch fed in the current experiment mimic those that would typically be fed to lactating dairy cows, and the increase from 23 to 29% was sufficient to reduce rumen pH and increase haptoglobin concentrations in blood plasma. Changes in haptoglobin suggest that the hepatic tissue was sensitized by proinflammatory mediators resulting from dietary changes. It is interesting that the decline in rumen pH and concurrent increase in plasma haptoglobin concentration prechallenge were observed only in the HS-control group, suggesting that supplementing YC to the HS diet attenuated the inflammatory stimulus likely induced by the changes in rumen microbial metabolism with more fermentable diet (Khafipour et al., 2009). After the challenge, YC was not able to lessen the decline in rumen pH in cows fed the HS diet, but those fed the HS-control diet continued to have greater haptoglobin concentrations than cows in the other treatments. Diets caused only numerical changes in relative expression of *TLR2* and *TLR4*. Both recep-

tors play critical roles during inflammatory responses by recognizing pathogen-associated molecular patterns. In the rumen, the molecules that activate toll-like receptors are likely products of microbial metabolism or disruption of microbial cells that can release LPS (Khafipour et al., 2009), which triggers inflammation. In fact, acute dietary shifts that result in SARA might result in systemic inflammation because of the translocation of inflammatory mediators from rumen to blood (Khafipour et al., 2009).

Dietary treatments did not influence expression of *SLC14A1* in rumen papillae. The *SLC14A1* gene encodes for the urea transporter B, and the protein has been identified in the gastrointestinal tract of dairy heifers (Marini and Van Amburgh, 2003). Expression of *SLC14A1* in the rumen epithelium increases with age in calves, and it has been associated with increases in urea N in blood (Naeem et al., 2012), likely because development of the rumen and microbial metabolism increases the need to transport urea for recycling of N back into the rumen. Expression of *SLC14A1* is influenced by dietary carbohydrate intake, and feeding more rumen-fermentable diets stimulated mRNA expression and increased immunostaining for this protein in the rumen tissue of steers (Simmons et al., 2009) and goats (Lu et al., 2015). Also, protein expression for SLC14A1 in rumen mucosa of dairy heifers was stimulated by diets with high protein content (Marini and Van Amburgh, 2003). Diets in the current experiment had moderate CP content for lactating dairy cows, which resulted in relatively low urea N concentrations in plasma. Moreover, although dietary treatments affected rumen carbohydrate digestion, the OM digestion did not differ with treatments, which coupled with the relatively low urea N concentrations in plasma likely explains the lack of differences in expression of *SLC14A1* in rumen papillae.

In summary, incorporating YC into the diets of dairy cows reduced concentrations of lactate in rumen fluid and increased rumen pH in cows fed the HS diet, and it prevented the depression in NDF digestion in the rumen induced by feeding HS. Combining HS with YC resulted in the greatest microbial N yield and increased the proportion of dietary N incorporated into milk true protein by 18.9%. Increasing the dietary starch content depressed total-tract digestion of OM because of reductions in digestion of ADF and NDF. Supplementing YC improved yields of 3.5% FCM, ECM, and milk components, whereas feeding more starch improved only milk protein yield. Supplementing YC reduced concentrations of haptoglobin in plasma before and after the challenge with corn grain, but this effect was observed only in cows fed the HS diet, likely because it prevented SARA. Feeding the HS diet increased concentrations

of propionate and reduced those of $\text{NH}_3\text{-N}$ in rumen fluid. The HS diet increased mRNA expression in rumen papillae for receptors and transporter of SCFA but did not affect genes involved in transport of Na^+/H^+ or water or genes involved in inflammatory response.

CONCLUSIONS

Supplementing diets of dairy cows with YC minimized the risk of SARA, particularly when cows were fed an HS diet, and improved fiber digestion and microbial N yield, which might explain the improvements in yields of milk and milk components. Manipulating dietary starch influenced rumen concentration of propionate and affected expression of genes involved in SCFA sensing and absorption. The effect of YC minimizing the decline in rumen pH in diets with HS does not seem to be mediated by change in expression of genes in the rumen epithelium involved in sensing or transport of SCFA.

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