Effects of supplementing a *Saccharomyces cerevisiae* fermentation product during the transition period on rumen fermentation of dairy cows fed fresh diets differing in starch content

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ABSTRACT

The objective of this study was to evaluate the effects of supplementing a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V, Cedar Rapids, IA) during the transition period (d -28 ± 3 to 23 ± 3 relative to calving) on rumen fermentation and mRNA abundance of genes in the rumen epithelium of fresh cows (d 1 to 23 ± 3 after calving) fed diets differing in starch content. Eighteen ruminally cannulated multiparous Holstein cows were fed diets with SCFP (n = 9) or without (CON; n = 9) throughout the experiment. All cows were fed a common basal controlled-energy close-up diet (1.43 Mcal/kg, net energy for lactation; 13.8% starch) before calving. Cows within each treatment (CON or SCFP) were fed either a low-starch (LS; 22.1% starch) or high-starch (HS; 28.3% starch) diet during the fresh period. Cows were assigned to treatment after balancing for parity, body condition score, and expected calving date. Rumen pH was measured continuously for 72 h starting on d -10 , -3 , 1, 7, and 21 relative to calving date. Rumen papillae were collected on d -10 and 21 relative to calving. Supplementation of SCFP had no effect on rumen pH during d -10 to -8 , but mean rumen pH tended to be higher (6.64 vs. 6.49) for SCFP cows than for CON cows during d -3 to -1 . Feeding SCFP decreased the range of rumen pH variation compared with CON within the HS group during both d 7 to 9 (1.08 vs. 1.38) and d 21 to 23 (1.03 vs. 1.30) after calving. In addition, nadir rumen pH tended to be higher (5.64 vs. 5.44) and duration of pH below 5.8 tended to be shorter (116 vs. 323 min/d) for the SCFP group than for the CON group during d 21 to 23 after calving. Supplementation of SCFP increased the mRNA abundance of insulin-like growth factor-6 (1.10 vs. 0.69)

before calving and decreased the mRNA abundance of putative anion transporter isoform 1 (1.12 vs. 2.27) after calving. Nadir rumen pH tended to be higher during d 1 to 3 (5.63 vs. 5.41) for LS cows than for HS cows, but rumen pH was not affected by dietary starch content during other time periods. Dietary starch content had no effect on mRNA abundance of genes in the rumen epithelium after calving. These results suggest that supplementation of SCFP may reduce the range of variation in rumen pH in fresh cows fed HS diets and the duration of subacute ruminal acidosis by the end of the fresh period regardless of dietary starch content and that decreasing dietary starch content during the fresh period may reduce the decrease in rumen pH immediately after parturition.

Key words: calving transition, dietary starch, *Saccharomyces cerevisiae* fermentation product, rumen fermentation

INTRODUCTION

Dairy cows often experience negative energy balance in early lactation because the energy intake lags behind the sudden increase in nutrient demands for milk production (Esposito et al., 2014). High-starch diets are usually fed to early-lactation dairy cows to reduce the energy deficit. However, the large increase in dietary fermentable carbohydrates during the first several weeks after calving decreases rumen pH and further increases the risk of SARA (Penner et al., 2007). The occurrence of SARA is associated with variable DMI, production losses, and impaired animal health (Stone, 2004; Krause and Oetzel, 2006) and thus may exacerbate the negative energy balance. Therefore, reducing the duration and severity of SARA without compromising energy intake should be taken into account for the nutritional management of transition dairy cows. Reducing dietary starch content by replacing grain with nonforage fiber sources has shown positive effects on rumen fermentation of dairy cows without impairing production performance, such as a tendency for

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higher rumen pH (Zhang et al., 2010) and lower pH range (Voelker and Allen, 2003). However, these studies were conducted using dairy cows after peak lactation. A recent study reported that decreasing dietary starch content during the early postpartum period prevented reductions in DMI of cows fed a highly fermentable starch source, but rumen pH was not measured (Albornoz and Allen, 2018).

Saccharomyces cerevisiae fermentation products (SCFP) have been widely used in dairy cow rations to increase production performance and stabilize the rumen environment (Erasmus et al., 2005). The beneficial effects of SCFP on rumen fermentation include probable stimulation of growth of cellulolytic (Harrison et al., 1988; Zhu et al., 2017) and lactate-utilizing bacteria (Callaway and Martin, 1997), fungi (Mao et al., 2013; Zhu et al., 2017), and protozoa (Arakaki et al., 2000; Shen et al., 2018). Thus, SCFP may contribute to greater fiber digestion, reduced lactate concentration of rumen fluid, and increased microbial protein synthesis (Hristov et al., 2010). In addition, the supplementation of SCFP to beef heifers fed a high-grain diet (52.8% starch) increased the nadir ruminal pH and reduced the duration of pH <5.6 by 6 h compared with control heifers and increased ruminal and total-tract NDF digestibility (Shen et al., 2018). Supplementation of SCFP can reduce the decrease in rumen pH induced by feeding a high-starch diet (>50%). However, the interaction effects of dietary starch content and SCFP supplementation on rumen fermentation have not been extensively studied, particularly for the immediate postpartum period.

Therefore, the objective of the current study was to evaluate the effects of SCFP supplementation during the periparturient period on rumen pH, VFA profile, and the mRNA abundance of genes associated with rumen epithelial growth, VFA transporters, and VFA metabolism in the rumen epithelium in fresh cows fed diets differing in starch content. We hypothesized that feeding low-starch diets during the fresh period and supplementing SCFP during the periparturient period would reduce the decrease in rumen pH after calving and that the benefits of SCFP supplementation would be greater for dairy cows fed high-starch diets.

MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Alberta Animal Care and Use Committee: Livestock (AUP#1915) and conducted according to the guidelines of the Canadian Council on Animal Care (2009). Two companion papers reported the effects of starch content and SCFP on production

performance (Shi et al., 2019) and immune function (Knoblock et al., 2019).

Animals, Diets, and Experimental Design

Eighteen multiparous, ruminally cannulated Holstein cows, as a subset of 117 dairy cows used in the companion paper (Shi et al., 2019), were blocked by expected calving date and assigned to treatment with efforts to achieve similar pretrial BCS and BW among treatments. These 18 cows were also used in the companion study by Knoblock et al. (2019), where animal information at enrollment can be found. Complete details of the experimental design and animal management are described in Shi et al. (2019). Animal enrollment and data and sample collection were conducted on certain days of the week (e.g., Tuesday) to facilitate routine management of the dairy operation. This protocol allowed deviations of up to 3 d from the target dates (± 3) except for data and sample collection on d -3 to 3 relative to calving. Briefly, cows were enrolled in the study at 28 d before expected calving date and fed diets supplemented with SCFP (NutriTek, Diamond V, Cedar Rapids, IA; n = 9) or without SCFP (CON; n = 9) until d 23 after calving. The SCFP was a dried culture product produced through anaerobic fermentation of *Saccharomyces cerevisiae* strains with cereal grain and selected liquids (e.g., molasses) as raw ingredients (Shen et al., 2018). The SCFP was supplemented at 0.173 and 0.095% of dietary DM in pre- and postpartum diets, respectively, to meet the target inclusion rate of 19 g/cow per day (as-fed basis) based on expected DMI of the cows (prepartum, 11 kg/cow per day; postpartum, 20 kg/cow per day on a DM basis). All cows were fed a common basal controlled-energy diet before calving, containing 13.8% starch and 1.43 Mcal/kg of DM. Cows within each treatment (CON or SCFP) were fed either a low-starch (LS; 22.1%) or high-starch (HS; 28.3%) diet from d 1 to 23 after calving, resulting in 4 treatment combinations: LS-CON (n = 4), LS-SCFP (n = 5), HS-CON (n = 5), and HS-SCFP (n = 4). Diet ingredients and nutrient composition are shown in Table 1. Details of feed sampling and analysis are reported in Shi et al. (2019).

Rumen pH and Rumen Fermentation

Rumen pH data were recorded every 30 s continuously for 72 h (starting with feeding at 0730 h) in the ventral sac of the rumen during 5 periods, including d -10 to -8 relative to expected calving date and d -3 to -1 , 1 to 3, 7 to 9, and 21 to 23 relative to actual calving date, using the pH measurement system developed by

Table 1. Ingredient and chemical composition of the experimental diets¹

Item	Prepartum		Postpartum			
	CON	SCFP	LS		HS	
			CON	SCFP	CON	SCFP
Ingredient, % of DM						
Barley silage	46.9	46.9	46.5	46.5	46.5	46.5
Alfalfa hay	—	—	2.8	2.8	2.8	2.8
Barley straw	29.1	29.1	—	—	—	—
Corn grain, ground, dry	—	—	12.5	12.5	12.5	12.5
Barley grain, rolled	7.5	7.5	8.9	8.9	18.1	18.1
Beet pulp	—	—	8.3	8.3	—	—
Canola meal	8.2	8.2	10.2	10.2	8.0	8.0
Soybean meal, 44% CP	—	—	3.0	3.0	3.0	3.0
Corn gluten meal	0.5	0.5	0.9	0.9	2.0	2.0
Amino plus	1.8	1.8	2.0	2.0	2.0	2.0
Urea	0.5	0.5	—	—	—	—
F-100, ² dairy fat	—	—	1.1	1.1	1.1	1.1
Limestone	0.7	0.7	1.4	1.4	1.6	1.6
Potassium carbonate	—	—	—	—	0.1	0.1
Premix-CON ³	1.9	—	1.1	—	1.1	—
Premix-SCFP ⁴	—	1.9	—	1.1	—	1.1
Rumensin premix ⁵	0.8	0.8	0.6	0.6	0.6	0.6
Mineral and vitamin mix ⁶	2.1	2.1	0.8	0.8	0.8	0.8
Nutrient content						
DM, %	50.2	50.2	50.2	49.9	50.2	50.1
CP, % of DM	15.3	15.3	17.3	17.0	17.5	16.9
ADF, % of DM	31.6	31.5	20.9	20.3	19.3	19.2
NDF, % of DM	49.5	49.4	33.3	32.7	31.7	31.5
Forage NDF, % of DM	45.1	45.1	24.0	24.0	24.0	24.0
NFC, % of DM	25.6	25.6	39.3	41.3	41.1	42.8
Starch, % of DM	13.8	13.9	21.6	22.5	27.0	29.5
Ether extract, % of DM	2.5	2.5	3.6	3.6	3.7	3.7
NE _L , ⁷ Mcal/kg of DM	1.43	1.43	1.60	1.62	1.63	1.64
DCAD, mEq/kg	26.0	25.0	192	164	170	138
NE _L allowable milk, ⁷ kg/d	—	—	31.5	31.5	32.1	32.1
MP allowable milk, ⁷ kg/d	—	—	32.7	32.7	32.7	32.7

¹Adapted from Shi et al. (2019). CON = control diet with no *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation; Premix-CON was used in the diet. SCFP = diet supplemented with SCFP; Premix-SCFP was used in the diet. LS = low-starch diets; HS = high-starch diets.

²F-100 Dairy Fat (Pro-Ag, Winnipeg, MB, Canada).

³Contained 99.0% dry ground corn grain and 1.0% canola oil, providing 0 g of SCFP/d.

⁴Contained 91.1% dry ground corn grain, 7.9% SCFP, and 1.0% canola oil, providing 19 g of SCFP/cow per day (as-fed basis) based on expected DMI of cows (11 kg/cow per day before calving; 20 kg/cow per day after calving).

⁵Contained 1.2% Rumensin (Elanco Animal Health, Greenfield, IN), 97.5% dry ground barley grain, and 1.3% canola oil, providing 1,210 mg of Rumensin/cow per day (as-fed basis) before calving and 1,600 mg of Rumensin/cow per day after calving based on expected DMI (11 kg/cow per day before calving; 20 kg/cow per day after calving).

⁶Contained 11.0% Ca, 2.78% Na, 14.2% Cl, 11.8% Mg, 7.68% S, 16.2 mg/kg of Mn, 4.88 mg/kg of I, 1,212 kIU/kg of vitamin A, 121 kIU/kg of vitamin D, and 4,040 IU/kg of vitamin E for close-up diet. Contained 0.71% Ca, 24.3% Na, 39.4% Cl, 13.0% Mg, 0.13% S, 2,427 mg/kg of Cu, 4,244 mg/kg of Mn, 174 mg/kg of Co, 4,583 mg/kg of Zn, 10.0 mg/kg of Se, 85.0 mg/kg of I, 543 kIU/kg of vitamin A, 155 kIU/kg of vitamin D, and 5,118 IU/kg of vitamin E for postpartum diets.

⁷Estimated from NRC (2001) using an expected DMI of 20 kg/d.

Penner et al. (2006). Daily nadir, mean, and maximum rumen pH and duration and area below rumen pH 5.8 as well as acidosis index (area of pH below 5.8/DMI) were calculated daily for each cow and averaged for each period. In addition, pH range was calculated as the difference between nadir and maximum rumen pH for each cow.

Rumen fluid samples were collected every 9 h over a 72-h period on d −10 to −8, 7 to 9, and 21 to 23 relative to calving. Briefly, samples were collected from cranial dorsal, cranial ventral, central, caudal dorsal, and caudal ventral sacs and then combined and strained through a perforated material (Peetex, pore size = 355 µm; Sefar Canada Inc., Scarborough, ON, Canada).

Samples were centrifuged at 4°C at $3,000 \times g$ for 20 min immediately after collection and stored at -20°C until analysis. Eight rumen fluid samples taken from each period were composited by equal volume to form 1 representative sample for each cow. Gas chromatography was used to determine rumen fluid VFA profile as described by Khorasani et al. (1996).

Rumen Papillae Collection

Detailed procedures of rumen papillae collection were reported in Knoblock et al. (2019). Briefly, approximately 20 to 30 pieces of rumen papillae were biopsied from the ventral sac of the rumen before morning feeding on d -10 and 21 and were rinsed with ice-cold PBS (pH 7.4) to remove adherent rumen content. After washing, samples were placed into RNeasy lysis buffer (cat. no. AM7020, Thermo Fisher Scientific, Waltham, MA) and stored at -20°C until RNA extraction.

RNA Extraction and Reverse Transcription

The RNA extraction from rumen papillae samples was conducted according to the method described by Chomczynski and Sacchi (1987). The concentration of RNA was measured at absorbance 260 and 280 nm using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The absorbance ratio (260:280) of all the samples was between 1.96 and 2.08, suggesting high RNA purity. Single-strand cDNA was synthesized using the iScript Reverse Transcription Supermix (Bio-Rad, Montreal, QC, Canada). Samples of cDNA were diluted $20\times$ using nuclease-free water and stored at -80°C until use.

Quantitative Real-Time PCR

Before quantitative real-time PCR, primers were tested to ensure the presence of desired genes (Table 2) in all samples. The quantitative real-time PCR was then conducted to measure the relative expression of genes associated with rumen epithelial growth, VFA absorption, and VFA metabolism using the StepOne-Plus Real-Time PCR system (Applied Biosystems, Foster City, CA) using the Fast SYBR Green kits. The reactions were performed according to the following conditions: 20 s at 50°C , 40 cycles of 3 s at 95°C and 30 s at 60 or 63°C . All samples were run in triplicate.

Three housekeeping genes were used in this study, including β -actin (*ACTB*), glyceraldehyde-3-phosphate dehydrogenase (*GADPH*), and ribosomal protein large, P0 (*RPLP0*). These housekeeping genes were expressed in all samples and not affected by treatment. The rela-

tive changes in gene expression were calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). One cow in the LS-SCFP group with the lowest mean rumen pH during the d -10 sampling period was chosen as the calibrator. The ΔC_T was calculated as the difference between the cycle threshold (C_T) for each cow and gene and the corresponding geometric mean of 3 housekeeping genes. The $\Delta\Delta C_T$ for each cow and gene was then calculated by subtracting ΔC_T of the calibrator from the ΔC_T .

Statistical Analysis

Statistical analyses were conducted using the Fit Model procedure of JMP 13 (SAS Institute Inc., Cary, NC). Parturition and postpartum data were analyzed using models 1 and 2, respectively:

$$Y_i = \mu + T_i + e_i \quad [1]$$

$$Y_{ij} = \mu + T_i + S_j + TS_{ij} + e_{ij} \quad [2]$$

where Y_i or Y_{ij} = observations for dependent variables, μ = overall mean, T_i = fixed effect of SCFP supplementation (CON or SCFP), S_j = fixed effects of starch content of fresh diets (LS or HS), TS_{ij} = effect of SCFP and starch content interaction, and e_i or e_{ij} = residuals. Comparisons among treatments were conducted using Student's t -test when an interaction between SCFP supplementation and starch content was detected. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

RESULTS

There were no treatment effects on DMI during periods of rumen pH measurement (Tables 3, 4, 5, 6, and 7), and DMI data were presented solely to show the components of the acidosis index calculations. Dry matter intake data for a full set of 117 dairy cows are shown in the companion paper (Shi et al., 2019).

Rumen pH and VFA Profile

During the d -10 sampling period, no differences were observed in nadir, mean, and maximum rumen pH between SCFP and CON groups or in duration and area of rumen pH below 5.8 (Table 3). Total VFA concentration and molar proportions of butyrate and propionate were not different between SCFP and CON groups, but molar proportion of acetate tended to be greater for SCFP cows than for CON cows ($P = 0.10$). During the d -3 sampling period, nadir rumen pH was

Table 2. Gene name and primer sequences for quantitative real-time PCR analysis

Gene name	Gene ID	Primer ¹	Annealing temperature, °C	Efficiency, %	Source
IGF-binding protein 2 (<i>IGFBP2</i>)	AF074854	F: GCATGGCCTGTACAACCTCAA R: TCCCGGTGTAGGGTTTCACA	63	98.0	Rhoads et al. (2008)
IGF-binding protein 3 (<i>IGFBP3</i>)	NM_001075549	F: GCGACAAGAAAGGGCTTTTACAA R: TATCCACACACCCAGCAAAACC	60	95.7	Steele et al. (2015)
IGF-binding protein 5 (<i>IGFBP5</i>)	BC102850	F: CTACAAGAGAAAGCAGTGCAAAACC R: TCCAGGCACACGACAGATG	60	94.6	Steele et al. (2015)
IGF-binding protein 6 (<i>IGFBP6</i>)	NM_001105613	F: CGCAGAGACCAACAGAGAACT R: GGAGCCCATCTCAGTGCTTG	63	91.5	Steele et al. (2012)
Transforming growth factor β 1 (<i>TGFB1</i>)	NM_177497	F: TGAGCCAGAGGCGGACTACT R: TGCCGTATTCCACCATTAGCA	63	101.2	Steele et al. (2015)
Transforming growth factor β 1 receptor (<i>TGFB1R</i>)	NM_174621.2	F: CAGAGTGGGAACA AAAGGTACATG R: CATTGCATAGATGTCAGCAGTT	60	100.0	Minutti et al. (2015)
Epidermal growth factor receptor (<i>EGFR</i>)	NM_001037319.1	F: TGAAAACAGTGCAAGGCCG R: ACGACTGAAGTTCTGGCAGG	60	93.9	Steele et al. (2015)
Epiregulin (<i>EREG</i>)	NM_001075549	F: GCGACAAGAAAGGGCTTTTACAA R: TATCCACACACCCAGCAAAACC	60	97.6	Steele et al. (2015)
Monocarboxylate cotransporter, isoform 1 (<i>MCT1</i>)	NM_001037319	F: AACACTGTGCAGGAATTACTTTTC R: TGCCAGCGGTCTCTCTTAT	60	90.0	Dieho et al. (2017)
Monocarboxylic acid transporter 4 (<i>MCT4</i>)	NM_001109980	F: GACCGAGCGGACGAG R: GTCTGTACAGAGGCTGTCC	63	92.0	Dias et al. (2018)
Downregulated in adenoma (<i>DRA</i>)	NM_001083676	F: CCTAGCAGGCAGACTTTCCA R: GCGATCAACTCCTGATGTCG	60	95.0	Dieho et al. (2017)
Putative anion transporter, isoform 1 (<i>PAT1</i>)	NM_001076852	F: TACCACAGGGCCTTGCCCTAT R: CTGCCACCATCACAGACAT	60	94.0	Dieho et al. (2017)
Peroxisome proliferator-activated receptor- α (<i>PPAR4</i>)	NM_001034036	F: GGATGTCCCATACGCGATT R: GGTCTATGTCACACGTAAGGATT	60	93.0	Dieho et al. (2017)
3-Hydroxybutyrate dehydrogenase, type 1 (<i>BDH1</i>)	NM_001034600	F: GCAAAGGCGCGTTGTTA R: GTCAGAGAAAGCCTCCACC	60	91.0	Dieho et al. (2017)
3-Hydroxybutyrate dehydrogenase, type 2 (<i>BDH2</i>)	NM_001034488	F: TGGCTCAGAAATCTGGCAAC R: AACTGTTCCTGGACACACACA	60	92.0	Dieho et al. (2017)
3-Hydroxy-3-methylglutaryl-CoA synthase 1 (<i>HMGCS-1</i>)	NM_001206578	F: CAGCTCTTTGAGGAGTCTGGG R: GGCTACCGTCCATCCCAAG	60	98.0	Dieho et al. (2017)
3-Hydroxy-3-methylglutaryl-CoA synthase 2 (<i>HMGCS-2</i>)	NM_001045883	F: GCAACTGACATGAGGGG R: ACCAGTGCATAGCGACATC	60	99.0	Dieho et al. (2017)
Bibosomal protein large, P0 (<i>RPLP0</i>)	AF013214	F: CAACCTGAAAGTCTTGACAT R: AGGCAGATGGATCAGCCA	60	92.9	Robinson et al. (2007)
<i>GAPDH</i>	AF022183	F: GCGGTGAACACAGAAAGTATAA R: CCTCCACGATGCCAAAGT	60	96.4	Robinson et al. (2007)
β -Actin (<i>ACTB</i>)	AF191490	F: CTAGGCACGAGGCGGTAATG R: CCACACGGAGCTCGTTGTAG	60	96.1	Malmuthuge et al. (2013)

¹F = forward; R = reverse.²Calculated using the equation $E = -1 + 10^{(-1/\text{slope})} \times 100$.

Table 3. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on rumen fermentation during d –10 to –8 before calving

Variable	Treatment ¹		SE	P-value
	CON	SCFP		
DMI, kg/d	12.3	11.2	0.78	0.32
Rumen pH				
Nadir	5.99	6.08	0.10	0.53
Mean	6.50	6.54	0.07	0.73
Maximum	6.95	6.99	0.04	0.55
Range	0.97	0.91	0.10	0.68
Duration pH <5.8, min/d	20.1	56.3	46.1	0.59
Area pH <5.8, pH × min/d	2.79	17.8	14.6	0.48
Acidosis index, pH × min/kg	0.27	2.78	2.29	0.45
Rumen VFA				
Total VFA, mM	136	133	5.67	0.64
Acetate, mol/100 mol	64.9	66.1	0.45	0.10
Propionate, mol/100 mol	21.7	20.6	0.76	0.33
Butyrate, mol/100 mol	9.14	8.98	0.31	0.72
Isobutyrate, mol/100 mol	1.12	1.10	0.02	0.63
Valerate, mol/100 mol	1.24	1.26	0.07	0.85
Isovalerate, mol/100 mol	1.48	1.53	0.16	0.81
Caproate, mol/100 mol	0.37	0.40	0.07	0.76
Acetate:propionate	3.02	3.22	0.12	0.24

¹CON = control diet with no SCFP supplementation; SCFP = SCFP-supplemented diet.

Table 4. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on rumen fermentation during d –3 to –1 before calving

Variable	Treatment ¹		SE	P-value
	CON	SCFP		
DMI, kg/d	11.5	10.4	0.90	0.43
Rumen pH				
Nadir	6.03	6.18	0.09	0.22
Mean	6.49	6.64	0.06	0.09
Maximum	6.97	7.12	0.05	0.08
Range	0.95	0.94	0.10	0.93
Duration pH <5.8, min/d	22.1	13.3	14.0	0.67
Area pH <5.8, pH × min/d	2.18	4.66	3.44	0.62
Acidosis index, pH × min/kg	0.27	0.66	0.49	0.58

¹CON = control diet with no SCFP supplementation; SCFP = SCFP-supplemented diet.

Table 5. Effects of starch content of fresh diets (ST) and supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on rumen fermentation during d 1 to 3 after calving

Variable	Treatment ¹				SE	P-value		
	LS		HS			ST	SCFP	ST × SCFP
	CON	SCFP	CON	SCFP				
DMI, kg/d	12.0	12.9	15.4	13.1	1.98	0.39	0.73	0.43
Rumen pH								
Nadir	5.68	5.58	5.44	5.37	0.12	0.09	0.47	0.92
Mean	6.15	6.05	5.96	5.89	0.14	0.25	0.56	0.94
Maximum	6.69	6.66	6.56	6.47	0.13	0.23	0.66	0.84
Range	1.01	1.08	1.11	1.11	0.08	0.45	0.69	0.62
Duration pH <5.8, min/d	291	437	452	575	195	0.46	0.51	0.96
Area pH <5.8, pH × min/d	40.5	68.9	133	131	58.5	0.21	0.83	0.80
Acidosis index, pH × min/kg	4.84	4.69	13.1	10.8	6.19	0.27	0.85	0.86

¹LS = low-starch fresh diets; HS = high-starch fresh diets; CON = control diet with no SCFP supplementation; SCFP = diet with SCFP supplementation.

Table 6. Effects of starch content of fresh diets (ST) and supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on rumen fermentation during d 7 to 9 after calving

Variable	Treatment ¹				SE	P-value		
	LS		HS			ST	SCFP	ST × SCFP
	CON	SCFP	CON	SCFP				
DMI, kg/d	15.4	18.6	17.3	17.3	2.03	0.90	0.45	0.43
Rumen pH								
Nadir	5.61	5.50	5.62	5.69	0.12	0.41	0.87	0.45
Mean	6.14	6.09	6.23	6.24	0.10	0.26	0.82	0.81
Maximum	6.67	6.71	7.00	6.77	0.09	0.04	0.29	0.14
Range	1.06 ^b	1.21 ^b	1.38 ^a	1.08 ^b	0.06	0.10	0.19	<0.01
Duration pH <5.8, min/d	157	282	226	69.1	115	0.54	0.89	0.24
Area pH <5.8, pH × min/d	18.2	50.5	86.2	11.8	43.8	0.74	0.64	0.24
Acidosis index, pH × min/kg	1.60	2.43	7.24	0.60	3.67	0.61	0.44	0.33
Rumen VFA								
Total VFA, mM	157	154	140	145	10.4	0.23	0.91	0.68
Acetate, mol/100 mol	59.5	57.8	55.7	57.1	1.40	0.12	0.93	0.30
Propionate, mol/100 mol	24.8	26.5	27.8	25.9	1.46	0.43	0.91	0.24
Butyrate, mol/100 mol	10.5	11.3	11.6	11.8	0.47	0.12	0.29	0.50
Isobutyrate, mol/100 mol	0.91	0.82	0.96	0.99	0.05	0.04	0.59	0.24
Valerate, mol/100 mol	1.80	1.63	1.85	1.86	0.09	0.16	0.40	0.39
Isovalerate, mol/100 mol	1.77	1.52	1.67	1.79	0.21	0.69	0.77	0.40
Caproate, mol/100 mol	0.72 ^a	0.39 ^b	0.44 ^{ab}	0.65 ^{ab}	0.10	0.92	0.57	0.02
Acetate:propionate	2.40	2.23	2.03	2.24	0.16	0.29	0.87	0.26

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$) when an interaction of main treatment effect was observed.

¹LS = low-starch fresh diets; HS = high-starch fresh diets; CON = control diet with no SCFP supplementation; SCFP = diet with SCFP supplementation.

Table 7. Effects of starch content of fresh diets (ST) and supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on rumen fermentation during d 21 to 23 ± 3 after calving

Variable	Treatment ¹				SE	P-value		
	LS		HS			ST	SCFP	ST × SCFP
	CON	SCFP	CON	SCFP				
DMI, kg/d	21.8	20.8	20.0	20.1	1.73	0.49	0.82	0.76
Rumen pH								
Nadir	5.41	5.58	5.47	5.70	0.10	0.40	0.07	0.77
Mean	6.00	6.21	6.13	6.25	0.10	0.38	0.11	0.67
Maximum	6.53	6.75	6.77	6.72	0.08	0.20	0.26	0.11
Range	1.11 ^{ab}	1.17 ^{ab}	1.30 ^a	1.03 ^b	0.06	0.76	0.11	0.02
Duration pH <5.8, min/d	372	184	274	48.1	119	0.34	0.10	0.88
Area pH <5.8, pH × min/d	87.1	37.4	56.7	9.90	33.1	0.40	0.17	0.97
Acidosis index, pH × min/kg	4.46	1.67	3.07	0.47	1.74	0.47	0.14	0.96
Rumen VFA								
Total VFA, mM	164	155	169	142	9.72	0.67	0.09	0.38
Acetate, mol/100 mol	60.1	61.2	57.4	59.0	1.28	0.08	0.31	0.87
Propionate, mol/100 mol	23.5	22.7	26.4	24.0	1.20	0.10	0.19	0.53
Butyrate, mol/100 mol	11.3	10.9	10.9	11.2	0.45	0.92	0.87	0.45
Isobutyrate, mol/100 mol	0.89	0.98	0.95	1.06	0.06	0.27	0.12	0.85
Valerate, mol/100 mol	1.72	1.86	1.77	1.86	0.20	0.88	0.59	0.91
Isovalerate, mol/100 mol	1.70	1.88	2.06	2.11	0.17	0.11	0.50	0.71
Caproate, mol/100 mol	0.77	0.54	0.46	0.78	0.12	0.75	0.70	0.04
Acetate:propionate	2.57	2.73	2.21	2.48	0.16	0.07	0.19	0.73

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$) when an interaction of main treatment effect was observed.

¹LS = low-starch fresh diets; HS = high-starch fresh diets; CON = control diet with no SCFP supplementation; SCFP = diet with SCFP supplementation.

not affected by SCFP supplementation, but mean rumen pH tended to be higher for SCFP cows than for CON cows (Table 4; $P = 0.09$). The duration and area of rumen pH below 5.8 did not differ between the SCFP and CON groups.

After calving, no interactions were observed for SCFP supplementation and dietary starch content for most of the rumen pH parameters and VFA profile (Tables 5, 6, and 7), except for rumen pH range during the d 7 ($P < 0.01$) and 21 ($P = 0.02$) sampling periods. Within HS group, SCFP supplementation decreased rumen pH range compared with CON during both the d 7 and d 21 sampling period. During both the d 1 and d 7 sampling periods, SCFP supplementation had no effects on nadir, mean, and maximum rumen pH or on duration and area of rumen pH below 5.8. Total VFA concentration and VFA profiles were not different between SCFP and CON cows during the d 7 sampling period. During the d 21 sampling period, nadir rumen pH tended to be higher (5.64 vs. 5.44; $P = 0.07$) and duration of pH below 5.8 tended to be lower (116 vs. 323 min/d; $P = 0.10$) for the cows fed SCFP than the CON cows. However, there were no differences in mean rumen pH and area of rumen pH below 5.8 between the SCFP and CON groups. Total VFA concentration tended to be lower (149 vs. 166 mM; $P = 0.09$) for the SCFP group than for the CON group, whereas VFA profiles were not affected by SCFP supplementation.

Dietary starch content had no effect on mean rumen pH and duration and area of rumen pH below 5.8 during the d 1 sampling period, but nadir ruminal pH tended to be higher for LS cows than for HS cows (5.63 vs. 5.41; $P = 0.09$). During the d 7 sampling period, only maximum rumen pH was affected by dietary starch content and was lower for LS cows compared with HS cows (6.69 vs. 6.89; $P = 0.04$). Total VFA and VFA profiles were not affected by dietary starch content. During the d 21 sampling period, none of the rumen pH parameters were affected by starch content. Total VFA concentration was not different between the LS and HS groups. However, molar proportion of acetate tended to be higher (60.6 vs. 58.2 mol/100 mol; $P = 0.08$) and molar proportion of propionate tended to be lower (23.1 vs. 25.2 mol/100 mol; $P = 0.10$) for LS cows compared with the HS cows, resulting in a tendency of higher acetate:propionate ratio (2.65 vs. 2.34; $P = 0.07$) for the cows fed the LS diet compared with the cows fed the HS diet.

Rumen Epithelium Gene Expression

Before calving, the relative mRNA abundance of genes encoding for VFA transporters and epithelial cell VFA metabolism were not different between the

SCFP and CON groups (Table 8). In addition, among the genes encoding for rumen epithelium growth, the relative mRNA abundance of transforming growth factor β 1 (*TGFB1*) and its receptor (*TGFBR1*), epidermal growth factor receptor (*EGFR*), and epiregulin (*EREG*) did not differ between SCFP and CON cows. However, among those IGF-binding proteins (*IGFBP*), the relative mRNA abundance of *IGFBP6* was higher for the cows fed SCFP compared with the CON group, whereas the relative mRNA abundance of *IGFBP2*, *IGFBP3*, and *IGFBP5* was not different between the SCFP and CON groups.

After calving, the interaction effects of SCFP supplementation and dietary starch content were observed only for the expression of 3-hydroxybutyrate dehydrogenase, type 2 (*BDH2*; Table 9). The majority of the genes evaluated in this study were not affected by SCFP supplementation, but the relative mRNA abundance of putative anion transporter isoform 1 (*PAT1*) was lower for the SCFP group compared with the CON group (1.12 vs. 2.27; $P = 0.01$). Dietary starch content had no effect on the expression of the genes evaluated in this study.

DISCUSSION

Effects of SCFP Supplementation

In the companion paper (Shi et al., 2019), it was found that supplementation of SCFP transiently increased DMI on d 1 and 5 after calving, whereas overall DMI during both the close-up and fresh periods was not different between the SCFP and CON cows. In addition, SCFP supplementation reduced serum concentrations of haptoglobin on d 7 after calving compared with CON (Knoblock et al., 2019).

In the current study, we found that SCFP supplementation tended to increase molar proportion of acetate during the d -10 sampling period and mean rumen pH during the d -3 sampling period. These may indicate increased fiber digestion. Previous studies have shown that SCFP supplementation increased the growth and activity of certain fiber-digesting bacteria (Harrison et al., 1988; Callaway and Martin, 1997). More recently, it has been reported that there was a linear increase in the population of certain cellulolytic bacteria in response to an increasing amount of SCFP in mid-lactation dairy cows fed low-quality forage (Zhu et al., 2017).

In addition to the changes in rumen fermentation, increased mRNA abundance of *IGFBP6* was observed for SCFP-supplemented cows. As a member of the *IGFBP* family, *IGFBP6* binds IGF-2 preferentially over IGF-1 and inhibits IGF-2 actions, including proliferation, differentiation, and survival of many cell types (Bach,

Table 8. Effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on relative mRNA abundance in rumen papillae on d -10 ± 3 relative to expected calving date

Gene ¹	Treatment ²		SE	<i>P</i> -value
	CON	SCFP		
Rumen epithelial growth and differentiation				
<i>IGFBP2</i>	1.52	3.49	0.95	0.17
<i>IGFBP3</i>	0.47	0.62	0.13	0.46
<i>IGFBP5</i>	0.83	0.75	0.17	0.75
<i>IGFBP6</i>	0.69	1.10	0.09	0.01
<i>TGFB1</i>	1.02	1.12	0.11	0.55
<i>TGFBR1</i>	0.91	0.82	0.12	0.59
<i>EGFR</i>	1.37	1.63	0.57	0.75
<i>EREG</i>	62.9	83.0	21.7	0.53
VFA transporter				
<i>MCT1</i>	1.28	1.10	0.33	0.70
<i>MCT4</i>	1.99	1.77	0.30	0.62
<i>PAT1</i>	1.73	1.64	0.35	0.86
<i>DRA</i>	0.89	1.07	0.27	0.66
VFA metabolism				
<i>PPARA</i>	1.34	1.39	0.34	0.91
<i>BDH1</i>	1.05	0.86	1.35	0.33
<i>BDH2</i>	0.63	0.66	0.14	0.87
<i>HMGCS1</i>	0.77	1.15	0.28	0.37
<i>HMGCS2</i>	1.60	0.83	0.33	0.12

¹*IGFBP2* = IGF-binding protein 2; *IGFBP3* = IGF-binding protein 3; *IGFBP5* = IGF-binding protein 5; *IGFBP6* = IGF-binding protein 6; *TGFB1* = transforming growth factor β 1; *TGFBR1* = transforming growth factor β receptor 1; *EGFR* = epidermal growth factor receptor; *EREG* = epiregulin; *MCT1* = monocarboxylate cotransporter, isoform 1; *MCT4* = monocarboxylate cotransporter, isoform 4; *PAT1* = putative anion transporter, isoform 1; *DRA* = downregulated in adenoma; *PPARA* = peroxisome proliferator-activated receptor- α ; *BDH1* = β -118 hydroxybutyrate dehydrogenase, isoform 1; *BDH2* = β -118 hydroxybutyrate dehydrogenase, isoform 2; *HMGCS1* = 3-hydroxy-3-methylglutaryl-CoA synthase 1; *HMGCS2* = 3-hydroxy-3-methylglutaryl-CoA synthase 2.

²CON = control diet with no SCFP supplementation; SCFP = SCFP-supplemented diet.

2016). It has been reported that the expression of *IGFBP6* was downregulated during high-grain feeding (Steele et al., 2011) and was upregulated with increased NDF:starch ratio (Ma et al., 2017), suggesting that the expression of *IGFBP6* may be related to the dietary NDF content. However, NDF content was similar between the SCFP and CON diets in our study; therefore, the greater mRNA abundance of *IGFBP6* for the SCFP group may be related to the tendency of higher molar proportion of acetate.

In this study, we hypothesized that supplementation of SCFP would reduce the decrease in rumen pH associated with the sudden change in diet composition after calving, particularly to a greater extent for dairy cows fed a high-starch fresh diet compared with those fed a low-starch fresh diet. Nadir rumen pH tended to be greater and duration of rumen pH below 5.8 tended to be shorter for SCFP cows compared with CON cows during the d 21 sampling period, but SCFP supplementation did not affect rumen pH during the d 1 and d 7 sampling periods; duration of rumen pH below 5.8 and acidosis index were numerically 3- and 12-fold higher for HS-CON cows than for HS-SCFP cows during the d 7 sampling period. The low number of animals per

treatment along with substantial inherent variation among cows immediately after calving, as indicated by the large standard error values, may have masked treatment differences.

In addition, the lack of treatment effect in rumen pH response to SCFP supplementation may be related to the lower SCFP intake relative to the target. The postpartum diets in our study were formulated based on an expected DMI of 20 kg/d and to provide SCFP at 19 g/d (as fed) according to the manufacturer's recommendation. However, the actual SCFP intakes, calculated from the average DMI during d 1 (13.4 kg/d) and d 7 (17.2 kg/d), were 12.7 and 16.3 g/d, respectively. This was 33 and 14% less than the target amount, respectively; therefore, the unexpected lower SCFP intake during the immediate postpartum period may account for the differences in rumen pH response to SCFP supplementation during the different sampling periods. In support of this speculation, in our companion study (Shi et al., 2019), the positive effects of SCFP supplementation were greater during the post-fresh early-lactation period (d 24–44) than during the fresh period (d 1–23). For example, the increase in feed efficiency was observed for the dairy cows in the SCFP

group during the post-fresh early-lactation period but not during the fresh period.

However, we observed reduced variation ranges of rumen pH for SCFP cows compared with CON cows fed HS fresh diets during both the d 7 and 21 sampling periods. These findings indicate that SCFP supplementation can reduce diurnal variation of rumen pH and stabilize rumen fermentation, particularly under high-starch feeding conditions, probably by maintaining the rumen microbial population (Guo et al., 2018). It was reported that SCFP supplementation prevented the reduction in rumen microbial diversity in dairy cows during grain-induced SARA challenges at wk 5 and 8 after calving (Guo et al., 2018), which help maintain a stable rumen environment. In addition, SCFP supplementation has been reported to facilitate the growth of lactic acid-utilizing bacteria (Callaway and Martin, 1997) and increase the protozoa count in the rumen (Arakaki et al., 2000; Shen et al., 2018), contributing to a more stable rumen environment, particularly for cows fed high-starch diets. Rumen protozoa have been proposed to prevent the decrease in rumen pH by en-

gulfing starch granules and reducing the starch degradation by amylolytic bacteria (Williams and Coleman, 1997), decreasing VFA production rate. Therefore, we speculated that reduced variation ranges in rumen pH for HS-SCFP cows compared with HS-CON cows may be related to an increase in ruminal protozoa count.

Besides rumen pH, we also observed a tendency of lower total VFA concentration and lower mRNA abundance of *PAT1* for the cows in the SCFP group compared with the CON group. The *PAT1* is a transporter protein that is involved in the bicarbonate-dependent transport pathway, which transports dissociated VFA into rumen epithelial cells and moves bicarbonate ions back to the ruminal lumen (Connor et al., 2010). It has been reported that incubation of rumen epithelial cells isolated from goats with VFA increased the mRNA expression of *PAT1*, but decreasing the pH of the incubation medium had no effect on *PAT1* expression (Yan et al., 2014). This suggested that the lower mRNA abundance of *PAT1* for the cows fed SCFP may be associated with the tendency for lower total VFA concentration compared with the CON group. In ad-

Table 9. Effects of starch content of fresh diets (ST) and supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on relative mRNA abundance in rumen papillae on d 21 ± 3 after calving

Gene ¹	Treatment ²					P-value		
	LS		HS		SE	ST	SCFP	ST × SCFP
	CON	SCFP	CON	SCFP				
Rumen epithelial growth and differentiation								
<i>IGFBP2</i>	3.87	3.52	3.57	3.26	1.33	0.84	0.81	0.99
<i>IGFBP3</i>	0.22	0.45	0.27	0.41	0.16	0.98	0.24	0.79
<i>IGFBP5</i>	0.55	0.69	0.29	0.67	0.18	0.47	0.17	0.54
<i>IGFBP6</i>	0.69	0.96	1.16	1.13	0.32	0.34	0.72	0.64
<i>TGFB1</i>	1.10	1.10	1.25	1.52	0.34	0.42	0.70	0.71
<i>TGFBR1</i>	0.73	0.69	1.42	0.78	0.27	0.17	0.22	0.27
<i>EGFR</i>	0.91	1.14	2.08	1.20	0.39	0.14	0.42	0.18
<i>EREG</i>	33.1	55.8	39.2	61.1	22.3	0.79	0.32	0.99
VFA transporter								
<i>MCT1</i>	1.31	1.12	1.01	0.94	0.42	0.58	0.76	0.90
<i>MCT4</i>	1.15	1.29	1.34	1.90	0.41	0.35	0.41	0.62
<i>PAT1</i>	2.70	1.15	1.85	1.08	0.41	0.28	0.01	0.35
<i>DRA</i>	1.23	0.89	1.03	1.28	0.29	0.74	0.89	0.32
VFA metabolism								
<i>PPARA</i>	1.42	1.45	1.94	1.29	0.41	0.67	0.46	0.42
<i>BDH1</i>	1.15	0.89	0.79	1.00	0.17	0.81	0.45	0.36
<i>BDH2</i>	0.88	0.60	0.34	0.85	0.18	0.42	0.54	0.05
<i>HMGCS1</i>	0.85	0.85	1.14	0.80	0.28	0.67	0.55	0.56
<i>HMGCS2</i>	1.31	1.09	1.51	1.39	0.32	0.45	0.61	0.88

¹*IGFBP2* = IGF-binding protein 2; *IGFBP3* = IGF-binding protein 3; *IGFBP5* = IGF-binding protein 5; *IGFBP6* = IGF-binding protein 6; *TGFB1* = transforming growth factor β 1; *TGFBR1* = transforming growth factor β receptor 1; *EGFR* = epidermal growth factor receptor; *EREG* = epiregulin; *MCT1* = monocarboxylate cotransporter, isoform 1; *MCT4* = monocarboxylate cotransporter, isoform 4; *PAT1* = putative anion transporter, isoform 1; *DRA* = downregulated in adenoma; *PPARA* = peroxisome proliferator-activated receptor-α; *BDH1* = β-118 hydroxybutyrate dehydrogenase, isoform 1; *BDH2* = β-118 hydroxybutyrate dehydrogenase, isoform 2; *HMGCS1* = 3-hydroxy-3-methylglutaryl-CoA synthase 1; *HMGCS2* = 3-hydroxy-3-methylglutaryl-CoA synthase 2.

²LS = low-starch fresh diets; HS = high-starch fresh diets; CON = control diet with no SCFP supplementation; SCFP = diet with SCFP supplementation.

dition, β -glucan and mannan oligosaccharides, as the main cell wall components of *Saccharomyces cerevisiae*, may increase rumen epithelium health by preventing the colonization of pathogens (Heinrichs et al., 2003; Ruiz-Herrera, 2016). Supplementing SCFP to lactating cows reduced plasma concentration of LPS (Zhang et al., 2013). Therefore, it is possible that SCFP cows had less rumen epithelial damage compared with CON cows, contributing to greater VFA absorption and less VFA accumulation in the rumen.

Effects of Starch Content During the Fresh Period

In our companion papers, we found that feeding LS diets during the fresh period increased milk production without affecting DMI (Shi et al., 2019) and reduced immune activation in rumen tissue at d 21 after calving as indicated by lower mRNA abundance of IL receptor associated kinase-1 in LS cows compared with HS cows (Knoblock et al., 2019).

In several studies, the rapid change from a high-forage diet to a high-grain diet decreased rumen pH and induced SARA in Holstein dairy cows immediately after switching the diet (Steele et al., 2011; Li et al., 2012; AlZahal et al., 2014). Therefore, it was expected that the change from a controlled-energy close-up diet (25.6% NFC) with 29% inclusion of straw (DM basis) to fresh diets that contained a large amount of grain (41.1% NFC) would largely reduce rumen pH immediately after parturition. In our study, rumen pH decreased and the duration and area of rumen pH below 5.8 increased by approximately 24-fold and 27-fold, respectively, immediately after calving as observed during the d 1 sampling period compared with the d -3 sampling period. Similar changes in rumen pH around parturition have been reported by Penner et al. (2007), in which rumen pH decreased substantially immediately after calving during d 1 to 5.

As rumen pH decreases with excessive amounts of NFC, we hypothesized that feeding an LS diet during the fresh period would reduce the decrease in rumen pH compared with feeding an HS diet. As expected, feeding LS diets tended to increase nadir ruminal pH compared with feeding HS diets during the d 1 sampling period. Although duration and area of pH below 5.8 were not different between the LS and HS groups, high variation existed among animals as indicated by the high standard error. The substantial variation accounted for approximately 44 and 66% of the mean for duration and area of pH below 5.8, respectively, which probably masked any treatment differences (Penner et al., 2007). The increase in rumen pH associated with feeding an LS diet was also observed in other studies for dairy cows after peak lactation, such as a tendency of

higher nadir rumen pH (Zhang et al., 2010) and a tendency of reduced pH range (Voelker and Allen, 2003). However, dietary starch content had no effect on rumen pH during both the d 7 and 21 sampling periods. In contrast to our results, Rabelo et al. (2003) reported that the cows fed a high-NFC diet (47.2% NFC) during the fresh period had lower ruminal pH compared with those fed a low-NFC diet (41.1% NFC) by the end of the fresh period (17 ± 2.0 d). The decreased rumen pH in their study may be a result of a tendency for greater DMI for the cows fed a high-NFC diet compared with those fed a low-NFC diet during the fresh period (d 1 to 20), whereas DMI was not affected by starch content in our study. In addition, rumen fluid samples were taken only once in the study of Rabelo et al. (2003) at 5 to 6 h after feeding for pH measurement; therefore, the rumen pH data in their study were not fully representative of the changes in rumen fermentation.

We speculated that the lack of difference in rumen pH in response to different dietary starch content during the d 7 and 21 sampling periods may be associated with the rapid adaptation of the rumen epithelium in dairy cows fed the HS diets, which allows a faster absorption of VFA from the rumen. The absorption rate of VFA has been shown to be affected by rumen papillae surface area (Melo et al., 2013), and VFA are commonly considered the stimulatory factor of the proliferation of the rumen epithelium (Bannink et al., 2012). Studies have reported that increasing the energy intake of dairy cows increased the absorptive surface of rumen epithelium (Melo et al., 2013). More recently, Dieho et al. (2016) found that rumen papillae respond to a rapid increase in concentrate allowance after calving by increasing growth rate, resulting in increased rumen papillae surface area. Similar to our results, total VFA concentration and rumen pH were not affected by the rate of increase in concentrate allowance in the study by Dieho et al. (2016). Therefore, it is likely that the cows fed HS diets in our study had greater rumen epithelial surface area compared with those fed LS diets, contributing to higher VFA absorption in HS cows and preventing the decrease in rumen pH from additional starch fermentation. In addition, DMI of cows during the fresh period was relatively low; therefore, the difference in starch intake between LS and HS cows might not be large enough to affect rumen pH, and HS cows may have been able to buffer the extra acid load. Furthermore, the high variation within individual treatments as mentioned previously may also contribute to the lack of response during the d 7 and 21 sampling periods.

In addition to the rumen pH during the d 21 sampling period, the expression of genes encoding for rumen epithelial growth, VFA transporters, and VFA metabolism

was not affected by dietary starch content. We noticed that in studies where differential expression of genes involved in the IGF axis (Steele et al., 2011, 2012) and VFA absorption (Yan et al., 2014) were observed, there were larger differences in dietary starch or NFC content. The difference in dietary starch content between the high-forage and high-grain diets was 34 percentage units (DM basis) in Steele et al. (2011) and 11 percentage units (DM basis) in Steele et al. (2012). In addition, the difference in dietary NFC content was 16 percentage units in the study of Yan et al. (2014). However, the difference in starch content between LS and HS diets in our study was approximately 6 percentage units, which was lower than in those studies mentioned above. Therefore, the lack of response in expression of genes evaluated in our study may be partially attributed to the relatively lower difference in dietary starch content.

CONCLUSIONS

The sudden change in diet composition around calving decreases rumen pH immediately after calving. Supplementation of SCFP during the calving transition may reduce the range of rumen pH after calving and the duration of SARA by the end of the calving transition, and feeding a low-starch diet during the fresh period may help increase rumen pH immediately after parturition.

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