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# Effects of *Saccharomyces cerevisiae* fermentation products and subacute ruminal acidosis on feed intake, fermentation, and nutrient digestibilities in lactating dairy cows

H. Khalouei, V. Seranatne, K. Fehr, J. Guo, I. Yoon, E. Khafipour, and J.C. Plaizier

**Abstract:** Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) and subacute ruminal acidosis (SARA) on rumen and hindgut fermentation, feed intake, and total tract nutrient digestibilities were determined in 32 lactating Holstein cows between weeks 4 and 9 of lactation. Treatments included control, 14 g·d<sup>-1</sup> Diamond V Original XPC™ (SCFPa; Diamond V, Cedar Rapids, IA, USA), 19 g·d<sup>-1</sup> NutriTek® (SCFPb-1X; Diamond V), and 38 g·d<sup>-1</sup> NutriTek® (SCFPb-2X; Diamond V). During weeks 5 and 8, SARA challenges were conducted by switching from a 18.6% to a 27.9% dry matter (DM) starch diet. This reduced the rumen and feces pH. The durations of the rumen pH below 5.6 during these challenges averaged 175.0, 233.8, 246.9, and 79.3 min·d<sup>-1</sup> for the control, SCFPa, SCFPb-1X, and SCFPb-2X treatments, respectively. Hence, SARA was not induced under the SCFPb-2X treatment. The feces pH during the SARA challenges was lowest during SCFPb-2X, suggesting this treatment shifted fermentation from the rumen to the hindgut. The SARA challenges reduced the total tract digestibility of DM, neutral detergent fiber digestibility (NDFd), and phosphorus, but tended to increase that of starch. The SCFPb-2X treatment increased the NDFd from 52.7% to 61.8% ( $P < 0.05$ ). The SCFPb-2X treatment attenuated impacts of SARA.

**Key words:** dairy cows, SARA, *Saccharomyces cerevisiae* fermentation products, digestibility.

**Résumé :** Les effets des produits de fermentation de *Saccharomyces cerevisiae* (SCFP — « *Saccharomyces cerevisiae* fermentation products ») et de l'acidose subaigüe du rumen (SARA — « subacute ruminal acidosis ») sur la fermentation du rumen et de l'intestin postérieur, sur la consommation, et sur la digestibilité d'éléments nutritifs du tractus complet ont été déterminés chez 32 vaches holsteins en lactation entre les semaines 4 et 9 de la lactation. Les traitements comprennent le témoin, 14 g·j<sup>-1</sup> Diamond V Original XPC™ (SCFPa; Diamond V, Cedar Rapids, IA, USA), 19 g·j<sup>-1</sup> NutriTek® (SCFPb-1X; Diamond V), et 38 g·j<sup>-1</sup> NutriTek® (SCFPb-2X; Diamond V). Durant les semaines 5 et 8, les épreuves SARA ont été effectuées en changeant la diète de 18,6 % à 27,9 % d'amidon (selon les matières sèches [DM — « dry matter »]). Ceci a réduit le pH du rumen et des fèces. Les durées du pH du rumen en dessous de 5,6 pendant les épreuves étaient, en moyenne, 175,0, 233,8, 246,9, et 79,3 min·j<sup>-1</sup> pour les traitements témoin, SCFPa, SCFPb-1X, et SCFPb-2X, respectivement. Donc, le SARA n'a pas été induit sous le traitement SCFPb-2X. Le pH des fèces lors des épreuves SARA était le plus faible durant SCFPb-2X, suggérant que ce traitement transférait la fermentation du rumen à l'intestin postérieur. Les épreuves SARA ont réduit la digestibilité du tractus complet des DM, la digestibilité des fibres détergentes neutres (NDFd — « neutral detergent fiber digestibility »), et du phosphore, mais tendait à augmenter celle de l'amidon. Le traitement SCFPb-2X augmente le NDFd de 52,7 % à 61,8 % ( $P < 0,05$ ). Le traitement SCFPb-2X a eu un effet atténuant sur les impacts du SARA. [Traduit par la Rédaction]

**Mots-clés :** vaches laitières, SARA, produits de fermentation de *Saccharomyces cerevisiae*, digestibilité.

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## Introduction

To support their high potential for milk production, dairy cows need to receive energy-rich diets with high-starch and low-fiber contents (NRC 2001; Plaizier et al. 2008). Feeding these diets alters conditions in the rumen by decreasing the rumen pH and changing the rumen volatile fatty acid (VFA) profile towards increased concentrations of total VFA, propionate, butyrate, and lactate and a reduced ratio of acetate to propionate (Li et al. 2012; Plaizier et al. 2018). Such a decrease of the rumen pH may lead to subacute ruminal acidosis (SARA), which has been defined as a moderate and reversible depression of this pH (Krause and Oetzel 2006; Plaizier et al. 2008, 2018). This disorder affects the production and health of dairy cows by decreasing milk fat production, nutrient utilizations, the functionality of the rumen epithelium, and feed intake, as well as by causing inflammation, laminitis, and diarrhea (Callaway and Martin 1997; Li et al. 2016; Plaizier et al. 2018). In addition, SARA can reduce microbial digestion (especially that of fiber) in the rumen and hindgut, as many rumen and hindgut microorganisms, and their enzymes are sensitive to a low rumen pH (Russell and Dombrowski 1980; Shi and Weimer 1992; Russell and Wilson 1996). In agreement, Plaizier et al. (2001) and Krajcarski-Hunt et al. (2002) observed that experimental induction of SARA by high grain feeding reduced the in situ 24 h neutral detergent fiber digestibility (NDFd) of forages by between 19.6% and 20.5%. However, this does not imply that total tract NDFd is also reduced, as increased hindgut fermentation may compensate the reduced fiber digestibility in the rumen (Demeyer 1991). Nevertheless, as SARA may increase fermentation and acidity in the hindgut (Gressley et al. 2011; Li et al. 2012), this type of SARA may also reduce fiber digestion in the hindgut. Supplementation with phytase can reduce the excretion of phosphorus (P) by cows, suggesting that the microbial breakdown of phytate P can be incomplete (Jarrett et al. 2014). Hence, a reduction in breakdown of phytate P in the rumen during SARA may reduce the bioavailability of dietary P.

*Saccharomyces cerevisiae* fermentation products (SCFP) are produced by anaerobic fermentation with *S. cerevisiae*. These products differ from live yeast as the latter have not gone through a fermentation process. These products include Original XPC (Diamond V, Cedar Rapids, IA, USA) and NutriTek® (Diamond V), which contain beneficial compounds for cows and their gut microorganisms, such as amino acids, polyphenols, antioxidants, and B vitamins (Schingoethe et al. 2004). Supplementation with SCFP has been shown to improve feed efficiency and reduce the impact of high grain feeding on the rumen pH (Williams et al. 1991; White et al. 2008; Allen and Ying 2012). This stabilization may, in part, result from lowering the digestion rate of starch in the rumen, and, thereby, reduce the accumulation of

fermentation acids and limit the increase in acidity in the rumen (Allen and Ying 2012; Shen et al. 2018). In addition, SCFP may also stabilize the rumen of grain-fed cows by increasing the utilization of lactate by rumen bacteria (Nisbet and Martin 1991). Hence, administration of SCFP can result in reduced lactate concentrations in the rumen, increased functionality of fiber-digesting bacteria (Callaway and Martin 1997; Tun et al. 2020), increased fiber digestibility (White et al. 2008), and improved feed intake (Poppy et al. 2012). Li et al. (2016) observed that supplementation with XPC (Diamond V) reduced the variation in rumen pH and the inflammation resulting from grain-induced SARA. NutriTek® contains more antioxidants and polyphenols than XPC (Diamond V 2020). Hence, it can be assumed that the SARA-mitigating effects of NutriTek® are greater than those of XPC.

Reports on the effect of supplementation of live yeast and yeast culture fermentation products on the dry matter intake (DMI) of dairy cows vary among studies (Desnoyers et al. 2009; Poppy et al. 2012). A meta-analysis conducted by Desnoyers et al. (2009) on the results of 157 experiments showed that, on average, the supplementation with live yeast increased dry matter digestibility (DMD) and DMI. Poppy et al. (2012) conducted a meta-analysis using 61 research publications on SCFP supplementation of lactating dairy cows, and reported that early lactation supplementation with SCFP increased DMI by 0.62 kg·d<sup>-1</sup>, whereas it decreased DMI by 0.78 kg·d<sup>-1</sup> thereafter. The variation of the impact of SCFP supplementation could be due to various factors, including differences in diet composition, experimental design, dose of supplementation, duration of supplementation, and stage of lactation (Robinson and Erasmus 2009; Allen and Ying 2012; Poppy et al. 2012). Aspects of the experimental design that could affect the outcome of such studies include insufficient statistical power and carry-over effects (Poppy et al. 2012; Li et al. 2016). It is expected that SCFP have more benefits when the cows experience nutritional challenges, such as SARA (Li et al. 2016; Plaizier et al. 2018).

We hypothesized that grain-induced SARA would reduce the pH and acetate-to-propionate ratio and would increase the concentrations of propionate and butyrate in the rumen and feces, as well as reduce feed intake, the DMD, NDFd as well as the total tract digestibilities of starch and P, and that a second SARA challenge would result in a more severe rumen pH depression than the first. We also hypothesized that SCFP Original XPC™ (Diamond V) and NutriTek® (Diamond V) supplementation would reduce the impacts of SARA, but that NutriTek® would have the largest effect. We further hypothesized that these reductions would depend on the dose of NutriTek® administered. The main objectives of our study were to compare the effects of supplementation with 14 g·d<sup>-1</sup> XPC (SCFPa), 19 g·d<sup>-1</sup> NutriTek® (SCFPb-1X), and 38 g·d<sup>-1</sup> NutriTek® (SCFPb-2X) on the pH and fermentation acids in the rumen and feces, feed intake, and on total tract nutrient

**Table 1.** Ingredient compositions of experimental diets.

Ingredient compositions (% of DM)	Lactating TMR	SARA TMR	Close-up dry cow TMR
Round bale mixed alfalfa/grass silage	35	28	20
Alfalfa first cut haylage	—	—	8
Corn silage	—	—	37
Barley silage	20	16	—
Straw	—	—	13
Ground corn	20	16	—
Dairy Aide <sup>a</sup>	25	20	—
Close-Up Dry Cow supplement <sup>b</sup>	—	—	22
Wheat–barley pellets	—	20	—

**Note:** TMR, total mixed ration; SARA, subacute ruminal acidosis; DM, dry matter.

<sup>a</sup>Dairy Aide contained flaked corn (65.2%), corn distillers grain (7.1%), APF Fat Plus (3.6%), feather meal (3.6%), porcine meat meal (2.9%), soybean meal (2.9%), canola meal (2.9%), AV Fat Rothsay Feeders Choice (2.2%), sodium sesquicarbonate (SQ 810, 1.9%), dicalcium phosphate (1.5%), potassium chloride (DYNA K Red, 1.5%), ground limestone (1.5%), potash (1.5%), dairy LMK Ultra Micro (0.6%), magnesium oxide (0.3%), and methionine analogue (Novus, 0.3%).

<sup>b</sup>Dry Cow supplement contained Landmark Close-Up Dry Cow pellets (50.0%)<sup>c</sup>, flaked corn (20.0%), beet pulp pellets (15.0%), rolled barley (12.5%), liquid molasses (2%), soy oil (0.5%), and liquid caramel (0.01%).

<sup>c</sup>Landmark Close-Up Dry Cow pellets contained: barley (4.0%), limestone (7.0%), corn distillers grain (34.9%), dicalcium phosphate (1.8%), canola meal (23.0%), soybean meal (8.5%), wheat (15.0%), niacin (0.3%), biopowder SXC (0.05%), magnesium oxide (1.6%), transition VB 25K (2.9%), and dry cow microPX premix (1%).

digestibilities during normal feeding and grain-based SARA challenges. We also sought to determine if these effects of SCFP differed between two sequential SARA challenges.

## Materials and Methods

### Animals, diets, and experimental design

The study was conducted at the Glenlea Research Station, University of Manitoba. It was pre-approved by the University of Manitoba Animal Care Committee, and followed the guidelines of the Canadian Council for Animal Care (CCAC 1993). Thirty-two rumen-cannulated multiparous Holstein dairy cows were assigned to a randomised complete block design with eight blocks to avoid carry-over effects of SCFP treatments. Cows were blocked based on parity, expected calving date, and milk production during the previous lactation. The average parity and milk production in the previous lactation of the experimental cows were  $2.67 \pm 0.91$  and  $10\,499 \pm 1619$  L (mean  $\pm$  standard deviation), respectively.

Within each block, cows were randomly assigned to four treatments, i.e., control (no SCFP) and three different SCFP supplementations, and they were monitored between weeks 4 and 9 after calving. Cows in the SCFP groups received  $14\text{ g}\cdot\text{d}^{-1}$  Diamond V Original XPC<sup>TM</sup> (SCFPa),  $19\text{ g}\cdot\text{d}^{-1}$  NutriTek<sup>®</sup> (SCFPb-1X), or  $38\text{ g}\cdot\text{d}^{-1}$  NutriTek<sup>®</sup> (SCFPb-2X) mixed with 126, 121, and  $102\text{ g}\cdot\text{d}^{-1}$  ground corn, respectively. The cows under the control treatment received only  $140\text{ g}\cdot\text{d}^{-1}$  ground corn. These supplements were top dressed once daily immediately after the morning delivery of the diet at 0900. Cows were

housed in individual stalls and had unlimited access to fresh water. The experiment was run between June 2016 and October 2017.

Cows were fed a close-up total mixed ration (TMR) from 4 wk pre-calving up to the calving date (Tables 1 and 2). After calving, cows were switched to a lactation TMR except for the fifth and eighth weeks after calving, during which SARA challenges were conducted. These challenges consisted of substituting TMR with pellets containing 50% ground wheat and 50% ground barley gradually over 3 d to reach a 20% grain inclusion of the total ration dry matter (DM) (Tables 1 and 2) (Khafipour et al. 2009). Stages of the SARA challenge were pre-SARA1 (week 4 of lactation), SARA1 (week 5 of lactation), post-SARA1 (weeks 6 and 7 of lactation), SARA2 (week 8 of lactation), and post-SARA2 (week 9 of lactation). The objective of the SARA challenge was to reduce the rumen pH to below pH 5.6 for more than  $180\text{ min}\cdot\text{d}^{-1}$ . During the 2 wk recovery period between the SARA challenges, the lactation TMR was fed. Diets were provided ad libitum, allowing for between 5% and 10% orts, twice daily at 0900 and 1500. Weights of the feed and orts were recorded each morning before the first feed delivery.

### Rumen, feces, and urine pH

Rumen pH was monitored at 1 min intervals using indwelling pH data loggers (T7-1 LRCpH; DASCOR, Escondido, CA, USA), which were placed in the ventral sac of the rumen of all cows, as described by Li et al. (2016). The data loggers were removed from the rumen every 2 wk for cleaning and calibration, as described by

**Table 2.** Chemical compositions of experimental diets.

	Lactating TMR	SARA TMR	Close-up dry cow TMR
DM (%)	51	60	48
CP (% DM)	17.9	17.2	15.5
Fat (% DM)	4.3	3.6	2.8
NDF (% DM)	34.9	28.2	38.7
ADF (% DM)	26.0	19.9	26.7
Starch (% DM)	18.6	27.9	17.6
Ca (% DM)	1.32	1.06	1.26
P (% DM)	0.45	0.47	0.37
Mg (% DM)	0.37	0.34	0.41
Na (% DM)	0.33	0.27	0.06
K (% DM)	2.57	2.02	2.37

**Note:** TMR, total mixed ration; SARA, subacute ruminal acidosis; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; K, potassium.

Penner et al. (2006). Cleaning was conducted by using a Terg-A-Zyme cleaning solution (Alconox, White Plains, NY, USA). The standardization was performed using pH 4 and 7 buffers at 39 °C. If a drift in pH recording was detected over time, then the recorded pH data were adjusted accordingly.

A rumen pH depression below 5.6 for more than 180 min·d<sup>-1</sup> was used as a threshold for defining SARA for dairy cows (Gozho et al. 2007). However, daily durations of rumen pH below 6.0, 5.8, and 5.6 were also determined and assessed in our study. Rumen liquid digesta was sampled twice weekly at 6 h after the 0900-feed delivery. Approximately 500 mL of whole rumen contents were collected through the canula from five sites (cranial, caudal, dorsal, caudal, and ventral), mixed thoroughly, and the solid and liquid digesta were separated using a Bodum French Press Coffee Plunger. Subsequently, 12 mL of the liquid digesta was centrifuged at 3000g min<sup>-1</sup> for 15 min, and 6 mL of the supernatant was collected. The subsample was mixed with 1.2 mL of 25% meta-phosphoric acid and stored at -20 °C until further analysis. After thawing at room temperature, these samples were analyzed for VFA and lactate using gas chromatography (Model 3900 Star; Varian, Walnut Creek, CA, USA) as described by Bhandari et al. (2007) and for ammonia-nitrogen using a colorimetric assay as described by Novozamsky et al. (1974).

Approximately 250 g of feces were collected daily at 6 h after the 0900-feed delivery from the rectum after cleaning the perianal area and mixed thoroughly. The pH of these samples was determined immediately after sampling using an Accumet Basic 15 pH meter (Fisher Scientific, Fairlawn, NJ, USA) equipped with a Sensorex 450C Flat Surface Combination pH/Reference Electrode (Sensorex, Stanton, CA, USA).

Subsequently, samples were stored at -20 °C until further analyses. After thawing at room temperature, 20 g of subsample were mixed with equal amount of 0.9% physiological saline and analyzed for VFA, lactate, and ammonia-nitrogen as previously described for rumen fluid samples.

Urine was collected twice weekly at 6 h after the 0900-feed delivery. Approximately 50 mL of mid-stream urine samples were collected by stimulating perineal area to initiate urination as described by Li et al. (2012). The urine samples were mixed thoroughly and pH was measured immediately using the same equipment as used for the fecal pH measurement.

#### Chemical analysis and calculations

Feed, Orts, and fecal samples were analyzed for DM by drying at 60 °C for 48 h in a forced-air oven. The DMI was calculated daily by subtracting amount of feed left from the amount of feed delivered per day on a DM basis. Dried samples were ground with a Wiley Mill using a 1 mm screen (Thomas-Wiley, Philadelphia, PA, USA) and kept in sealed bags for further analyses. Ground feed and fecal samples were pooled by stage relative to SARA induction for each cow. Analytical DM for pooled samples for each stage was determined (method 934.01; AOAC 1990). All feed and feces samples were analyzed for neutral detergent fiber (NDF) according to Van Soest et al. (1991) using  $\alpha$ -amylase (Sigma No. A3306; Sigma Chemical Co., St. Louis, MO, USA) and sodium sulfite, and corrected for ash concentration using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY, USA), P (methods 968.08 and 935.13A; AOAC 2005), acid-insoluble ash (AIA; method 920.08; AOAC 2005), and starch using the UV method (method 996.11; AOAC 2005). Feed samples were also analyzed for crude protein using the CuSO<sub>4</sub>/TiO<sub>2</sub> mixed catalyst Kjeldahl procedure (method 988.05; AOAC 1990). Analyses of acid detergent fiber, ether extract, and ash in feed samples were conducted using AOAC method 973.18 (AOAC 1990), AOAC method 920.39 (AOAC 1990), and AOAC method 923.03 (AOAC 2005), respectively. Calcium, P, potassium, magnesium, and sodium in feed samples were measured by inductively coupled plasma emission spectroscopy (method 968.08; AOAC 1990) using an Atom Scan 25 Plasma Spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO, USA) after acid digestion.

The DMD was determined using AIA as an internal marker (Van Keulen and Young 1977; Sales and Janssens 2003; McGeough et al. 2010) by stage relative to the SARA challenge and by cow as  $DMD = 100 \times [(1/M_{feed}) - (1/M_{feces})]/(1/M_{feed})$ , where  $M_{feed}$  is the AIA concentration in the feed and  $M_{feces}$  is the AIA concentration in the feces. The apparent digestibility coefficient (ADC) of nutrient (%) was determined by stage relative to SARA challenge and by cow as  $ND = 100 \times (N_{feed}/M_{feed}) - (N_{feces}/M_{feces})/(N_{feed}/M_{feed})$ ; where  $M_{feed}$  is the AIA concentration

in the feed;  $M_{\text{feces}}$  is the AIA concentration in the feces;  $N_{\text{feed}}$  is the concentration of the nutrient in the feed; and  $N_{\text{feces}}$  is the concentration of the nutrient in the feces.

### Statistical analysis

Data were analyzed with the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) using the following model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + B_k + e_{ijk}$$

where  $Y_{ijk}$  is the observations for dependent variables;  $\mu$  is the overall mean;  $T_i$  is the fixed effect of SCFP supplementation (control, SCFPa, SCFPb-1X, and SCFPb-2X);  $S_j$  is the fixed effect of stage of SARA (pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2);  $TS_{ij}$  is the effect of SCFP supplementation and stage of SARA interaction;  $B_k$  is the random effect of block (1–8); and  $e_{ijk}$  is the residuals. Stage of SARA was considered a repeated measure for the subject of cow within block with the AR(1) covariance structure. Normality of distributions of residuals was tested by the Shapiro–Wilk's statistics using Proc UNIVARIATE of SAS version 9.3. If needed, the data were transformed by the natural logarithm or raising the variable to the power of lambda to alleviate heterogeneity of residual variances. The required lambda value was the lambda calculated using a Box-Cox transformation analysis using TRANSREG procedure of SAS version 9.3. Homogeneity of variance tested by combining stage of SARA and SCFP treatment into a single factor and analyzed as a one-way analysis of variance with Levene's test in PROC generalized linear model of SAS version 9.3. If the assumption of equal variances was not met, then in the following analysis with PROC MIXED of SAS version 9.3. The GROUP = group in the REPEATED statement was included to allow the variances to be estimated separately, and the DDFM = SATTERTHWAITTE option was added in the MODEL statement to adjust the degrees of freedom for the unequal variances. The PDIF option was applied to evaluate pairwise comparisons between treatments and stages. The significant effects of SCFP treatment, stage of SARA, and their interactions were discussed at  $P < 0.05$ , and tendencies were reported at  $0.05 \geq P < 0.10$ .

## Results

### Rumen digesta, urine, and feces

The average daily rumen pH and the durations of the rumen pH below pH 6, 5.8, and 5.6 were affected by the interaction of SCFP treatment and stage of SARA (Table 3). This interaction tended to be significant for the urine pH but was not significant for the fecal pH. Due to the interaction of the effects of SCFP and SARA stage on rumen pH variables, the effects of these factors were evaluated within classes of the other factor. The effects on the duration of the rumen pH below 5.6 were compared within stage of SARA and within SCFP

treatment, as this duration was used as the threshold of SARA (Gozho et al. 2007). The effect of SCFP treatment on this duration was significant during the SARA1 and SARA2 stages, but not during the pre- and post-SARA stages (Table 4). During SARA1 and SARA2, the SCFPb-2X treatment reduced ( $P < 0.05$ ) this duration, but the SCFPa and SCFPb-1X treatments did not have such an effect. During the SARA1 and SARA2 stages, the durations of the rumen depression below 5.6 were higher than the threshold of  $180 \text{ min} \cdot \text{d}^{-1}$  for the SCFPa and SCFPb-1X treatments. However, for the SCFPb-2X treatment, this duration ( $79.3 \text{ min} \cdot \text{d}^{-1}$ ) did not exceed the threshold. Across all SCFP treatments, the durations of the rumen pH below 5.6 were equal during the pre-SARA1, post-SARA1, and post-SARA2 stages (Table 5). In cows on the control and SCFPb-2X treatments, this duration was longer during the SARA1 stage than during the SARA2 stage ( $228.4$  vs.  $121.6 \text{ min} \cdot \text{d}^{-1}$  and  $104.6$  vs.  $53.9 \text{ min} \cdot \text{d}^{-1}$ , respectively,  $P < 0.05$ ). However, during the other SCFP treatments, this duration did not differ between the SARA1 and SARA2 stages.

The urine pH only ranged significantly, but not substantially, among SCFP treatments and stages of SARA from 7.99 to 8.09. The fecal pH during the SARA1 and SARA2 stage was 6.61 and 6.63, respectively, and was lower ( $P < 0.05$ ) than those during the pre-SARA1, post-SARA1, and post-SARA2 stages, which were 6.71, 6.70, and 6.84, respectively (Table 3). Across stages of SARA, the fecal pH of the SCFPb-2X treatment (6.55) was lower ( $P = 0.02$ ) than those of the control, SCFPa, and SCFPb-1X treatments, which were 6.74, 6.75, and 6.74, respectively.

The SARA challenges increased the rumen concentrations of total VFA, propionate, butyrate, and lactate, whereas they reduced those of acetate, other VFA, and ammonia-nitrogen, and reduced the acetate-to-propionate ratio (Table 6). These concentrations and this ratio did not differ between the first and the second SARA challenge. The SCFPb-1X treatment had a higher ( $P < 0.05$ ) rumen propionate concentration than the SCFPb-2X treatment, and the SCFPb-2X treatment tended ( $P < 0.10$ ) to have a lower rumen total VFA concentration than the control and SCFPa treatments. The SCFPa treatment had a lower rumen ammonia-nitrogen concentration than the control and SCFPb-2X treatments. The effect of the interaction between the SCFP treatment and the stage of SARA induction was not significant for any of these rumen variables.

The concentration of propionate in feces was lower during the post-SARA1 stage, than during the SARA1 and SARA2 stages (Table 7). In addition, the fecal acetate to propionate ratio was lower during the SARA1 and SARA2 stages, than during the pre-SARA1 and post-SARA1 stages. Other VFA, lactate, and ammonia-nitrogen variables in feces were not affected by the stage of SARA. The fecal concentrations of acetate and propionate were higher ( $P < 0.05$ ) for the SCFPb-2X treatment, than for the

**Table 3.** Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) treatment (control, SCFPa, SCFPb-1X, and SCFPb-2X) and stage of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on rumen, fecal, and urine pH variables.

	Effects, P												
	Treatment					Stage							
	Control	SCFPa	SCFPb-1X	SCFPb-2X	Pre-SARA1	SARA1	Post-SARA1	SARA1	Post-SARA1	Post-SARA2	Treatment × Stage		
Average rumen pH	6.28	6.25	6.26	6.36	6.35	6.04	6.46	6.09	6.5	0.02	<0.001	<0.001	0.004
Time < pH 6.0 (min·d <sup>-1</sup> )	331.4	356.0	330.5	223.3	139.1	627.3	105.2	590.9	89.1	38.9	<0.001	<0.001	0.003
Time < pH 5.8 (min·d <sup>-1</sup> )	182.1	196.3	199.0	98.5	40.8	355.5	52.1	363.0	33.3	32.0	<0.001	<0.001	<0.001
Time < pH 5.6 (min·d <sup>-1</sup> )	82.3	99.1	106.1	33.2	13.6	179.3	19.0	178.0	10.8	22.1	<0.001	<0.001	<0.001
Fecal pH	6.74a	6.75a	6.74a	6.55b	6.71a	6.61b	6.70a	6.63b	6.84a	0.05	0.01	0.02	0.87
Urine pH	8.08	7.99	8.06	8.06	8.00	8.06	8.09	8.06	8.02	0.03	0.01	0.07	0.08

**Note:** Means with different lowercase letters (a and b) within SCFP treatments and SARA stages differ ( $P < 0.05$ ). SEM, standard error of mean.

other SCFP treatments. The concentration of butyrate in feces was higher in the SCFPb-2X treatment than in the SCFPb-1X treatment, whereas the fecal concentration of total VFA was higher for the SCFPb-2X than for the SCFPa and SCFPb-1X treatments. The fecal concentrations of ammonia-nitrogen were lower in the SCFPa and SCFPb-1X treatments than in the control treatment.

#### Dry matter intake and total tract digestibilities

The DMI did not differ among SCFP treatments (Table 8). However, across SCFP treatments, the DMI was higher during post-SARA1, SARA2, and post-SARA2 stages than during the pre-SARA1 and SARA1 stages.

The DMD was lower during SARA2, than during the other stages of SARA induction (59.6% vs. 71.2%,  $P = 0.05$ ) (Table 8). The NDFd was lower during the SARA1 and SARA2 stages compared with the other SARA induction stages (49.0% vs. 60.0%,  $P < 0.001$ ) (Table 8). The ADC of P was lower ( $P = 0.02$ ) during the post-SARA2 stage than during the pre-SARA1 stage, whereas the ADC of starch tended ( $P = 0.06$ ) to be higher during the SARA1 and SARA2 stages compared with the other SARA induction stages. Across these stages, the NDFd was higher during the SCFPb-2X treatment than during the control and SCFPa treatments (61.8% vs. 52.5%,  $P < 0.05$ ). The DMD and the ADC of P and starch were not affected by the SCFP treatments.

## Discussion

### Effects of SARA challenges

The rumen pH depression during SARA is not well defined, and studies have used different threshold values. Cooper et al. (1999) used a threshold of rumen pH between pH 5.2 and 5.6, whereas Beauchemin et al. (2003) used a rumen pH threshold of 5.8. In this study, a rumen pH depression below 5.6 for more than 180 min·d<sup>-1</sup> was used as the threshold for SARA, as only equal or greater rumen depressions reduced feed intake and caused an inflammatory response in the study of Gozho et al. (2007). This threshold was also chosen as it allowed comparisons with our previous studies on SARA. The threshold was barely exceeded during the SARA challenges in cows on the control, SCFPa, and SCFPb-1X treatments, and it was not exceeded during the SCFPb-2X treatment. Based on rumen pH depression, the SARA induced in our study can, therefore, be considered as mild. The SARA challenges also increased the rumen concentrations of propionate, butyrate, total VFA, and lactate, and reduced that of acetate and the acetate-to-propionate ratio. These changes are commonly observed during high grain feeding and grain-induced SARA (Gozho et al. 2007; Li et al. 2016; Pourazad et al. 2016), and reflect that fermentation of non-fiber carbohydrates produce more VFA and lactate than fermentation of fiber, and that bacteria switch their metabolic pathways to produce more propionate and less acetate in order to reduce rumen pH depression. (Russell and Rychlik 2001; Plaizier et al. 2017, 2018).

**Table 4.** Effect of *Saccharomyces cerevisiae* fermentation products (SCFP) treatments (control, SCFPa, SCFPb-1X, and SCFPb-2X) by stages of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on rumen time < pH 5.6.

Stage	Treatment				SEM	Effects, P
	Control	SCFPa	SCFPb-1X	SCFPb-2X		
Pre-SARA1	7.1	3.2	2.9	6.3	3.6	0.60
SARA1	228.4a	183.1ab	241.0a	104.6b	64.1	0.01
Post-SARA1	26.58	6.28	24.08	4.26	10.1	0.10
SARA2	121.6b	284.4a	252.8a	53.9b	45.4	<0.001
Post-SARA2	14.6	27.6	3.1	21.3	9.7	0.32

**Note:** Means with different lowercase letters (a and b) within SARA stage differ ( $P < 0.05$ ). SEM, standard error of mean.

**Table 5.** Effect of stages of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on rumen time < pH 5.6 by *Saccharomyces cerevisiae* fermentation products (SCFP) treatment (control, SCFPa, SCFPb-1X, and SCFPb-2X).

Treatment	Stage					SEM	Effects, P
	Pre-SARA1	SARA1	Post-SARA1	SARA2	Post-SARA2		
Control	7.1c	228.4a	26.5c	121.6b	14.6c	48.9	<0.001
SCFPa	3.2b	183.1a	6.28b	284.4a	27.6b	59.8	<0.001
SCFPb-1X	2.9b	241.0a	24.0b	252.8a	3.1b	57.4	<0.001
SCFPb-2X	6.3c	104.6a	4.26c	53.9b	21.3c	14.3	<0.001

**Note:** Means with different lowercase letters (a, b, and c) within SCFP treatment differ ( $P < 0.05$ ). SEM, standard error of mean.

The SARA challenges also reduced the fecal pH. This suggests that the SARA challenges increased fermentation in the hindgut, most likely by increasing the starch content of hindgut digesta (Li et al. 2012, 2016; Plaizier et al. 2017). The latter increase can also explain why the SARA challenges increased the propionate content of the feces (Russell and Rychlik 2001; Plaizier et al. 2018). The size of the depression of the fecal pH during the SARA challenges, however, does not indicate that hindgut acidosis was induced (Gressley et al. 2011; Plaizier et al. 2018).

In our study, two successive SARA challenges were conducted to determine if repeated SARA challenges have a more severe impact than a single SARA challenge. This was done as Dohme et al. (2008) and Pourazad et al. (2016) reported that repeated SARA challenges resulted in more severe rumen pH depressions than the initial SARA challenge. These studies contrast with our findings as, for cows on the SCFPa and SCFPb-1X treatments, the rumen pH depression below pH 5.6 did not differ between the first and second SARA challenge and, for cows on the control treatment, the rumen pH depression during the second challenge was lower than that during the first SARA challenge. In addition, the duration below rumen pH 5.6 did not differ among the pre-SARA1, post-SARA1, and post-SARA2 stages, suggesting that, based on the rumen pH depression,

cows recovered from the SARA challenges very quickly. Based on the rumen VFA concentrations, this recovery was also quick. The recovery from a SARA challenge may be long when this challenge decreased the absorptive capacity of the ruminal epithelium and altered the composition and functionality of the rumen microbiota (Dohme et al. 2008). Based on rumen pH values, the SARA induced by Dohme et al. (2008) and Pourazad et al. (2016) were more severe than that induced in our study. Hence, the impact of the SARA challenges, including their effects on the microbiota and epithelia in the rumen, may have been lower, and, therefore, the recovery from SARA more rapid in our study. The fecal pH also did not differ between the first and second SARA challenges, suggesting that the recovery of the hindgut pH from SARA challenges is also rapid.

Reasons why SARA can reduce DMI include reduced rumen motility, increased rumen concentrations of VFA and endotoxins, inflammation, and increased rumen osmolality (Kleen et al. 2003; Plaizier et al. 2008, 2012). Rumen motility and osmolality were not measured in our study, but the effects of the SARA challenges on the pH, VFA concentrations, and acute phase proteins in blood plasma (Guo et al. 2018) were limited. Whereas a severe grain-based SARA reduces feed intake, a mild SARA can increase DMI (Plaizier et al. 2008; Khafipour et al. 2009; Pourazad et al. 2016). The latter effect may

**Table 6.** Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) treatment (control, SCFPa, SCFPb-1X, and SCFPb-2X) and stage of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on rumen volatile fatty acid (VFA), lactate, and ammonia-nitrogen.

	Treatment				Stage					Effects, <i>P</i>			
	Control	SCFPa	SCFPb-1X	SCFPb-2X	Pre-	Post-		Post-		SEM	Treatment	Stage	Treatment × Stage
					SARA1	SARA1	SARA1	SARA2	SARA2				
Acetate (mmol·L <sup>-1</sup> )	81.9	81.0	77.6	78.6	83.6a	73.2b	83.4a	76.9b	82.9a	2.01	0.23	<0.001	0.97
Propionate (mmol·L <sup>-1</sup> )	30.5ab	30.2ab	31.1a	26.8b	20.9b	44.3a	21.2b	44.3a	20.9b	1.78	<0.01	<0.001	0.76
Butyrate (mmol·L <sup>-1</sup> )	12.6	13.9	12.9	12.5	11.2c	14.5a	12.2b	14.2a	12.5b	0.96	0.24	<0.001	0.96
Other VFA (mmol·L <sup>-1</sup> )	5.3	5.2	5.4	5.6	6.0a	4.0c	6.7a	4.5c	5.7b	0.38	0.38	<0.001	0.87
Total VFA (mmol·L <sup>-1</sup> )	130.2x	130.5x	126.9xy	122.1y	121.4c	131.7a	122.4c	140.4a	122.1c	4.4	0.09	<0.001	0.94
Ac/Pr	3.15ab	3.13ab	3.07b	3.48a	4.13a	1.97b	3.98a	1.91b	4.06a	0.22	<0.01	<0.01	0.70
Lactate (mmol·L <sup>-1</sup> )	6.65	6.61	6.12	6.72	5.22b	7.44a	5.09b	8.26a	5.16b	1.05	0.12	<0.01	0.21
Ammonia-nitrogen (mg·dL <sup>-1</sup> )	11.9a	9.8b	11.7ab	12.9a	14.3a	8.4b	12.5a	8.9b	13.7a	1.31	0.04	<0.01	0.99

**Note:** Means with different lowercase letters (a, b, and c) within SCFP treatment or SARA stage differ ( $P < 0.05$ ). Means with different lowercase letters (x and y) within SCFP treatment or SARA stage tend to differ ( $P < 0.10$ ). SEM, standard error of mean; Ac/Pr, acetate-to-propionate ratio.

**Table 7.** Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) treatment (control, SCFPa, SCFPb-1X, and SCFPb-1X) and stage of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on fecal volatile fatty acid (VFA), lactate, and ammonia-nitrogen.

	Treatment				Stage					Effects, <i>P</i>			
	Control	SCFPa	SCFPb-1X	SCFPb-2X	Pre-	Post-		Post-		SEM	Treatment	Stage	Treatment × Stage
					SARA1	SARA1	SARA1	SARA2	SARA2				
Acetate (mmol·L <sup>-1</sup> )	24.0b	22.1b	21.8b	26.7a	23.8	24.0	22.8	24.4	23.0	1.23	<0.01	0.64	0.11
Propionate (mmol·L <sup>-1</sup> )	4.5b	4.1b	3.8b	5.1a	4.2ab	4.8a	3.9b	4.8a	4.2ab	0.26	<0.01	<0.01	0.22
Butyrate (mmol·L <sup>-1</sup> )	2.2ab	2.1ab	2.0b	2.4a	2.3a	2.2ab	2.1ab	2.2ab	2.0b	0.14	0.03	0.41	0.28
Other VFA (mmol·L <sup>-1</sup> )	1.0	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.65	0.41	0.36	0.10
Total VFA (mmol·L <sup>-1</sup> )	33.5ab	30.5b	29.8b	36.5a	32.4	33.7	31.4	33.8	31.5	1.68	<0.001	0.41	0.11
Ac/Pr	5.5b	5.5b	5.9a	5.4b	5.9a	5.2b	5.9a	5.2b	5.7ab	0.19	0.01	<0.001	0.94
Lactate (mmol·L <sup>-1</sup> )	1.5	1.1	1.2	1.5	1.3	1.4	1.2	1.4	1.5	0.18	0.15	0.70	0.26
Ammonia-nitrogen (mg·dL <sup>-1</sup> )	3.4a	2.7b	2.7b	3.0ab	3.0	2.9	2.8	3.1	3.0	0.25	<0.01	0.70	0.48

**Note:** Means with different lowercase letters (a and b) within SCFP treatment or SARA stage differ ( $P < 0.05$ ). SEM, standard error of mean; Ac/Pr, acetate-to-propionate ratio.

**Table 8.** Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) treatments (control, SCFPa, SCFPb-1X, and SCFPb-2X) and stages of subacute ruminal acidosis (SARA) induction (pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on dry matter intake (DMI), dry matter digestibility (DMD), neutral detergent fiber digestibility (NDFd), and apparent digestibility coefficients (ADC) of phosphorus (P) and starch.

	Treatments										Stages				Effect, P					
	Control					SCFP					Pre-		Post-		Treatment		Stages		Treatment × Stage	
	Control	SCFPa	SCFPb-1X	SCFPb-2X	SCFPb-IX	SCFPa	SCFPb-1X	SCFPb-2X	SARA1	SARA1	SARA1	SARA1	SARA2	SARA2	SARA2	SARA2	SEM	Treatment	Stages	Treatment × Stage
DMI (kg·d <sup>-1</sup> )	21.9	20.4	20.7	20.3	20.3	17.2c	17.2c	19.3c	21.7b	21.7b	23.9a	22.0ab	22.0ab	22.0ab	22.0ab	1.03	0.20	<0.01	0.85	
DMD (%)	67.2	70.0	71.65	66.6	66.6	71.8a	71.8a	72.9a	71.1a	71.1a	59.6b	69.0a	69.0a	69.0a	69.0a	3.40	0.63	0.05	0.29	
NDFd (%)	52.7a	52.2a	55.8ab	61.8b	61.8b	61.1a	61.1a	49.0b	59.9a	59.9a	48.9b	59.1a	59.1a	59.1a	59.1a	3.29	0.01	<0.001	0.82	
ADC P (%)	50.8	52.6	52.91	53.9	53.9	57.7a	57.7a	54.5ab	51.1ab	51.1ab	51.4ab	48.0b	48.0b	48.0b	3.48	0.79	0.02	0.40		
ADC starch (%)	91.5	89.8	91.2	90.8	90.8	89.6y	89.6y	92.9x	89.5y	89.5y	92.4x	89.7y	89.7y	89.7y	1.34	0.81	0.06	0.34		

**Note:** Means with different lowercase letters (a, b, and c) within treatments and stages differ ( $P < 0.05$ ). Means with different lowercase letters (x and y) within SCFP treatment or SARA stage tend to differ ( $P < 0.10$ ). SEM, standard error of mean.

be explained by a reduction in physical rumen fill due to the higher inclusion of grain and the lower inclusion of forages in the diet (Allen 2000; Plaizier et al. 2008, 2018). The SARA challenge diet in our study contained substantially more grain (36% vs. 20% DM) and less forage (44% vs. 55% DM) than the lactating diet, and, therefore, contained less physical fill than the control diet (Allen 2000). Hence, the lower physical fill of the SARA diet may be the reason why the DMI was higher during the SARA2 stage than during the pre-SARA1 stage. A reason for the absence of a difference in DMI between the pre-SARA1 and SARA1 stages may be that it took the conditions in the rumen that affected the DMI more than 1 wk to adapt to the increase in grain feeding during the SARA1 stage. The lower DMI during the pre-SARA1 stage compared with the post-SARA1 and post-SARA2 stages may indicate that it took these conditions more than 1 wk to adapt from a reduction in grain feeding. A parallel study showed that the daily milk yield of the cows did not change between the SARA1 and post-SARA2 stages (Senaratne 2019). Hence, variation in milk yields did not contribute to the variation in DMI among the stages of SARA.

Apparent total tract digestibility coefficients were determined using an internal marker, i.e., AIA (Van Keulen and Young 1977). Chapuis-Lardy et al. (2004) concluded that this method produces satisfactory results and that it has the advantage over the total feces collection technique in that the animal does not have to be restrained in special metabolism stalls for extended periods. Another advantage is that bladder catheters are not needed to collect the urine. Bladder catheters can be problematic as the use of these catheter can lead to inflammation and the subsequent need to use antibiotics (J.C. Plaizier, personal communication, University of Manitoba, Winnipeg, MB). McGeough et al. (2010) compared this method with the total feces collection method, and reported an acceptable agreement between these methods.

The lower NDFd during the SARA challenges compared with the pre-SARA1, post-SARA1, and post-SARA2 stages, and the absence of differences in NDFd among pre-SARA1, post-SARA1, and post-SARA2 stage evidences SARA reduces the NDFd, and that this digestibility recovers quickly from a SARA challenge. The reductions in NDFd in our study were similar to the reductions in rumen NDFd due to grain-based SARA challenges conducted by Plaizier et al. (2001) and Krajcarski-Hunt et al. (2002). Shi et al. (2019a) observed that increasing the dietary starch from 22% to 28% DM reduced the NDFd at day 7 but not at day 21 of lactation. This discrepancy may be explained by the absence of an effect of this increase in the dietary starch content on the rumen pH in the earlier study (Shi et al. 2019b), whereas the increase in the dietary starch content due to the SARA challenge in our study increased the duration of the rumen pH below pH 5.6 from 13.6 to 179 min·d<sup>-1</sup> across

SCFP treatments. However, next to the reduction in the rumen pH, in our study, the SARA challenges resulted in other changes that may have affected the NDFd, including the increase in DMI. In addition, the reduction in the feces pH could be an indication that the challenge reduced the hindgut pH. In addition, the increase in the dietary content of pellets during the SARA challenge, as well as the increase in DMI could have reduced the retention time of digesta in the rumen, and as a result, the digestion in the rumen (Shaver et al. 1986; NRC 2001). However, despite an increase in DMI, the first SARA challenge did not affect the DMD and the increase in DMI due to the SARA challenges was limited. Therefore, we do not believe that an increased digesta passage rate was the main reason for the reduced fiber digestion during these challenges. As the diet fed during the SARA challenge stages contained more grain and pellets of ground grain and less coarse forage than that fed during the other stages, differences between the NDFd of grain and forages could also have contributed to the effects of the SARA challenges on the NDFd of the diet. The differences in the NDFd of the forages and the grains used in our study are, however, not large enough to explain why increasing the dietary grain content reduced the NDFd of the diet (Hoffman et al. 2006). The multifactorial impact of the SARA challenges does show that the effects of an excessive intake of grain are likely not solely due to a reduced rumen pH.

The reduction of the NDFd resulting from SARA may be attributed to the sensitivity of the fibrolytic bacteria to low pH. The optimum pH for fibrolytic bacteria ranges from 6.5 to 7, and conditions below pH 6 is less tolerable (Shi and Weimer 1992). In agreement, Calsamiglia et al. (2002) followed dual-flow continuous culture system and observed that SARA induction in a dual-flow fermentation system reduces simulated NDFd in the rumen from 53.8% at a constant pH of 6.4 to 34.3% at a constant pH of 5.7. However, by alternating cycles of low (5.7) and high pH (6.7), the reduction of the NDFd was intermediate, suggesting that the populations of fibrolytic microorganisms in the rumen recovered the pH depression quickly.

It has been suggested that low pH values cause reductions in the populations of fibrolytic bacteria due to difficulty in attaching to feed particles (Cheng and Costerton 1980) and reductions in rate of their replication (Russell and Dombrowski 1980). In agreement, several studies have shown that SARA challenges reduce the abundances of many fibrolytic bacteria in the rumen and hindgut (Khafipour et al. 2009; Li et al. 2012; Mao et al. 2013), which may explain why these challenges reduce total tract NDFd. However, as many genes are shared by various microbial taxa (Weimer 2015), reduction of the abundances of a few fibrolytic bacteria in the rumen and hindgut does not have to result in a reduction of the fibrolytic activity of the rumen and hindgut

microbiomes unless the impacted species are among the keystone or foundation members of the community. The absence of differences in the NDFd among the pre-SARA1, post-SARA1, and post-SARA2 stages suggests that the fibrolytic functionality of the rumen and hindgut microbiomes recovers rapidly from the SARA challenges. This recovery could be due to increases in the abundances of fibrolytic microorganisms (Russell and Dombrowski 1980).

The lower total tract P digestibility in the post-SARA2 stage compared with the pre-SARA1 stage suggests that, in contrast to NDFd, only prolonged SARA challenges and prolonged feeding of excessively high grain diets reduce this P digestibility. During the SARA challenges, forages were replaced by grain. Despite this, this dietary challenge only increased the dietary P content from 0.45% to 0.47% of DM. However, as grains contain more phytate P than forages (NRC 2001), the grain challenge would have increased the proportion of dietary P consisting of phytate P. This may have reduced the total tract digestibility of total P, but if that were so, then this reduction would have occurred after the first SARA challenge, and the gradual decline in this digestibility over the SARA challenge stages observed in our study would not have occurred. Hence, we believe that an effect of the SARA challenge on phytase activity is a more likely explanation of this gradual decline in P digestibility.

Godoy and Meschy (2001) showed that ruminal phytase may not hydrolyze all phytate P. In support of this, Jarrett et al. (2014) observed that supplementation with exogenous phytase reduced the excretion of total P and phytate P in the feces, suggesting that the microbial breakdown of phytate P is incomplete. Konietzny and Greiner (2004) concluded that phytase is produced by several anaerobic rumen bacteria, including *Selenomonas ruminantium*, *Megasphaera elsdenii*, and *Prevotella* sp. Short grain-based SARA challenges lasting only several days do not greatly reduce the abundances of these rumen bacteria (Khafipour et al. 2009; Petri et al. 2013; Plaizier et al. 2017), although Mao et al. (2013) observed that such a challenge reduced the relative abundance of *Prevotella* sp. Hence, phytase-producing bacteria may not respond rapidly to increased grain feeding and a reduction in rumen pH, as the acid sensitive fibrolytic bacteria. Therefore, the response of phytase production in the rumen to grain-based SARA challenges could be slower than the response of fiber digestion to these challenges. However, to prove this, the direct monitoring of phytase activity in the rumen following these challenges will be needed.

Both our study and that of Shi et al. (2019a) determined the effect of increasing the dietary starch content on the total tract starch digestibility in multiparous cows. Whereas our study found that increasing this starch content increased the starch digestibility, the earlier study observed the opposite at day 21 of lactation. In the study of Shi et al. (2019a), the digestibility of starch

was increased from 22.1% to 28.3% of DM, whereas in our study, it was increased from 18.6% to 27.9% DM. Hence, the difference between the studies may also have been due to the larger increase in dietary starch, which may have resulted in a larger increase in rumen bypass starch in our study, thereby compensating for a reduction in the ruminal digestion of starch (Rémond et al. 2004).

#### Effects of SCFP treatments

Results on the effects of SCFP supplementation on DMI vary among studies, but the meta-analysis by Poppy et al. (2012) on 61 research publications concluded that, across these studies, SCFP increased DMI by  $0.62 \text{ kg}\cdot\text{d}^{-1}$ . Due to the high variation among cows, many individual studies may not have had sufficient statistical power to find significant effects of SCFP supplementation on feed intake. Several individual studies did indeed not observe an effect of SCFP on DMI (Allen and Ying 2012; Li et al. 2016; Olagaray et al. 2019). However, whereas Shi et al. (2019a, 2019b) reported that DMI during the close-up and fresh periods was not affected by supplementation with  $19 \text{ g}\cdot\text{d}^{-1}$  of NutriTek<sup>®</sup>, but they found that SCFP supplementation during post-fresh period tended to reduce DMI. In contrast, Dann et al. (2000) observed that supplementation with  $60 \text{ g}\cdot\text{d}^{-1}$  of Diamond V XP increased DMI of dairy cows during the immediate pre- and post-partum periods, but not later in lactation. These authors attributed this increase in DMI to an improved fiber digestion and reduced fill in the rumen. The reason that this supplementation did not increase DMI later in lactation could be that at that stage of lactation, physical fill is no longer rate limiting for DMI (Allen 2000). Ramsing et al. (2009) also reported an improved DMI before calving as well as an increase in milk production of 10% in dairy cows supplemented with 57 and  $227 \text{ g}\cdot\text{d}^{-1}$  of SCFP (XP, fully fermented yeast culture of *S. cerevisiae*; Diamond V). Despite this, these authors did not find that supplementation with SCFP increased DMI after calving.

An explanation for the effect of SCFP on the rumen pH of cattle on high grain diets may be that the fermentation of starch in the rumen is reduced, whereas that in the hindgut is increased (Allen and Ying 2012; Shen et al. 2018). This is confirmed by our study, as SCFPb-2X treatment increased the rumen pH during the SARA challenges and decreased the concentration of total VFA in the rumen and the fecal pH, and increased the concentration of propionate in feces. Across stages of SARA, cows on the SCFPb-2X treatment had lower rumen concentrations of propionate and a higher acetate-to-propionate ratio than cows on the SCFPb-1X treatment. In addition, the concentration of total VFA in the rumen was lower during the SCFPb-2X treatment than during the control and SCFPa treatments. In contrast, the concentrations of acetate and propionate was highest in SCFPb-2X cows among all treatments, and the concentration of total VFA was higher than SCFPb-1X and SCFPa,

and the acetate-to-propionate ratio in the feces were lower during the SCFPb-1X treatment than during the other treatments. Together with the effects of the SCFPb-2X treatment on the rumen and feces pH, these changes in VFA show that the SCFPb-2X treatment shifted fermentation from the rumen to the hindgut, thereby creating a more favourable rumen environment during SARA. Such a shift in fermentation may not be entirely beneficial when it induces hindgut acidosis (Gressley et al. 2011; Plaizier et al. 2018). However, the fecal pH values during the SCFPb-2X treatment do not suggest that hindgut acidosis occurred. Our results also show that an increased digestion of starch in the small intestine must not have compensated for the reduced fermentation of starch in the rumen, as the increased fermentation in the hindgut during the SCFPb-2X treatments was likely the result of an increased starch content of hindgut digesta during this treatment (Li et al. 2012, 2016).

It has also been proposed that the positive effects of yeast products are not through direct action on pH, but rather through a modulatory effect on the fermentation process and ruminal microbiome, such as by stimulation of lactate utilizers and an increase in certain cellulolytic bacteria and fungi (Calsamiglia et al. 2012). The soluble growth factors in SCFP have been shown to stimulate growth of pure cultures of ruminal bacteria that digest cellulose and utilize lactate in vitro (Callaway and Martin 1997). In addition, Calsamiglia et al. (2012) proposed that positive effects of yeast products are modulatory effects on the fermentation process and ruminal microbiome, such as by stimulation of lactate utilizers and fibrolytic microorganisms. This may explain why Mao et al. (2013) observed that XP (Diamond V) SCFP increased the populations of fibrolytic microorganisms including protozoa, fungi, *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* during in vitro incubations. In agreement, Harrison et al. (1988) found that supplementation with  $114 \text{ g}\cdot\text{d}^{-1}$  of a SCFP tended to increase the abundance of anaerobic bacteria and increased that of cellulolytic bacteria in the rumen of dairy cows. Desnoyers et al. (2009) conducted a meta-analysis on the effects of supplementations with live yeast and yeast cultures and concluded that, on average, these supplementations increased total-tract organic matter digestibility. However, the majority of the studies included in their analysis used live yeasts. Hence, it is not clear how representative their results are for our study. Miller-Webster et al. (2002) observed that Diamond-V XP SCFP (Diamond V) increased the digestibility of DM, but not that of organic matter, NDF, acid detergent fiber, and nonstructural carbohydrates. Yoon and Stern (1996) reported that supplementation with Diamond V XP SCFP (Diamond V) increased the ruminal organic matter and crude protein digestions, without affecting the ruminal NDF digestion. Hristov et al. (2010) observed that supplementation with  $56 \text{ g}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$  of XP (Diamond V) did not affect the

total-tract apparent digestibility of DM, organic matter, nitrogen, NDF, and starch in lactating dairy cows. Similarly, Allen and Ying (2012) also reported that 56 g·head<sup>-1</sup>·d<sup>-1</sup> of XP (Diamond V) did not affect the NDFd, and that this SCFP increased the starch digestibility of lactating cows with a DMI of less than 26 kg·d<sup>-1</sup> prior to the study, whereas it decreased starch digestibility of cows that had higher feed intakes immediately before the study. Also, Shi et al. (2019a) observed that, across low- and high-starch diets, a supplementation of 19 g·d<sup>-1</sup> of NutriTek® did not affect the NDFd of dairy cows at days 7 and 21 of lactation. The above studies show that the effects of SCFP on the digestion of DMD and NDFd vary. Reasons for this variation may include differences in basal diet, stages of lactation and milk production, feed intake, and the technology used to produce the SCFP. However, the improvement of the NDFd by the SCFPb-2X treatment suggests that at a dose of 38 g·d<sup>-1</sup> of NutriTek® increases the NDFd of lactating dairy cows that are beyond the transition period.

A main finding from our study is that the SCFPb-2X treatment is able to attenuate the impact of SARA on the rumen pH depression and increase the NDFd, whereas the effects of other SCFP treatments on these impacts were not significant. Despite this, a parallel study (H. Khalouei, unpublished data) found that SCFPb-2X did not reduce the effects of the SARA challenges on milk yield, milk fat yield, and milk protein yield, although more experimental cows may have been needed to detect these effects.

The SCFP NutriTek® is different from XPC in that it functions differently by providing enhanced bioactive compounds which include those found in XPC, new fermentation metabolites, and additional propriety antioxidants, including polyphenols (Diamond V). Several of the symptoms of grain-induced SARA, including inflammation, might be due to the accompanying oxidative stress (Gabel et al. 2002; Karmin et al. 2011; Plaizier et al. 2018). Polyphenols have antioxidative and anti-inflammatory effects in mammals (Gessner et al. 2017). In agreement, De Nardi et al. (2014) observed that administration of 100 g·d<sup>-1</sup> of a polyphenol mixture increased the rumen pH and reduced the concentrations of neutrophils and acute phase proteins in peripheral blood in dairy heifers on a high grain diet. In addition, polyphenols may increase the activity of lactating-consuming bacteria and the growth of propionate-producing bacteria (Balcells et al. 2012). Hence, the higher content of polyphenols and other antioxidants in NutriTek® compared with XPC may explain why NutriTek® at a dose of 38 g·d<sup>-1</sup> had the larger effect on attenuating the drop in rumen pH and NDFd caused by the SARA challenges.

## Conclusions

The changes in rumen pH, feces pH, feed intake, fiber digestibility, and the concentrations of fermentation

acids in the rumen and feces show that the SARA challenges induced a mild form of SARA in cows under the control, SCFPa, and SCFPb-1X treatment. Based on the rumen pH data, the SARA challenges did not induce SARA in cows on the SCFPb-2X treatment. The latter may have been due to a shift of the digestion of starch from the rumen to the intestines, creating a better rumen environment. The rumen pH of the cows recovered quickly from the SARA challenges, and the second SARA challenge did not have more severe effects than the first SARA challenge. The SCFP treatments did not affect the DMD and the total tract digestibilities of crude protein and P, but the SCFPb-2X treatment increased the NDFd across the stages of the SARA induction. The combination of both SARA challenges reduced the total tract digestibility of P. Our data suggest that the NutriTek® supplementation at a dose of 38 g·d<sup>-1</sup> limits the depression of the rumen pH and fiber digestion that is commonly associated with high grain feeding.

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