Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community

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ABSTRACT

The aim of this study was to evaluate the effects of *Saccharomyces cerevisiae* fermentation products (SCFP) in the calf starter and milk on ruminal fermentation, gastrointestinal morphology, and microbial community in the first 56 d of life. Thirty Holstein bull calves were randomly assigned to 1 of 3 groups: a texturized calf starter containing 0 (CON), 0.5, or 1% SCFP (XPC, Diamond V, Cedar Rapids, IA) of dry matter from d 4 to 56. In addition, the XPC-supplemented calves were fed with 1 g/d SCFP (SmartCare, Diamond V, Cedar Rapids, IA) in milk from d 2 to 30. All calves were fed 4 L of colostrum within 1 h of birth and were subsequently fed milk twice daily until weaned on d 56. Rumen fluid was collected by an esophageal tube 4 h after the morning feeding on d 28 and 56 to determine ruminal pH, ammonia-N, and volatile fatty acids concentrations. On d 56, 15 (5 per treatment) calves were harvested and slaughter weight, gastrointestinal morphology parameters, and bacteria community were recorded. Papilla length, width, and surface area were measured from 5 locations within the rumen. Villus height, width, surface area, crypt depth, and villus height-to-crypt depth ratio were measured in the duodenum, jejunum, and ileum. Next-generation sequencing technology was used to test the microbial community of the rumen and duodenum samples on d 28 and 56. Data were analyzed by MIXED procedure in SAS (SAS Institute Inc., Cary, NC) with contrast statements to declare CON versus all SCFP and 0.5 versus 1% SCFP in starter grains. Ruminal pH, ammonia-N, and total volatile fatty acids were not altered by SCFP. However, the supplemented groups exhibited higher ruminal butyrate concentrations coinciding with higher *Butyrivibrio* and lower *Prevotella* richness than CON group. Supplementation of SCFP increased papilla length in the rumen. In the small intestine, SCFP

reduced crypt depth of jejunum, and increased villus height-to-crypt depth ratio in all segments of the small intestine, especially when supplemented at a higher dosage in the starter. In conclusion, *Saccharomyces cerevisiae* fermentation products improved gastrointestinal morphology, possibly due to increased *Butyrivibrio* and decreased *Prevotella* richness of the rumen fluid, which resulted in an increase in butyrate production, and the effect was slightly greater with the higher dosage of SCFP in the starter.

Key words: calf, *Saccharomyces cerevisiae* fermentation products, gastrointestinal morphology, microbial community

INTRODUCTION

Saccharomyces cerevisiae fermentation products (SCFP) are commonly used as feed additives, which contain compounds including oligosaccharides, organic acids, AA, and peptides, which can benefit the various types of bacteria (Callaway and Martin, 1997), protozoa (Arakaki et al., 2000), and fungi (Mao et al., 2013) in the gastrointestinal tract. In mature ruminants, SCFP has been shown to influence milk production (Poppy et al., 2012), VFA concentrations, ruminal pH (Yoon and Stern, 1996), and DMI (Dann et al., 2000). In preruminant calves, supplementation of SCFP has been shown to improve survival of calves, which resulted in greater profit margin (Magalhães et al., 2008), better ADG, body structure (Lesmeister et al., 2004a), and slightly promoted VFA production (Quigley et al., 1992) and ruminal morphology development (Brewer et al., 2014). However, the effect of supplementing SCFP in diets of calves on gastrointestinal epithelium development and microbial community has not been fully evaluated.

The rumen is typically the main digestive and absorptive organ for VFA and excess ammonia-N in ruminants, whereas protein occurs further down the tract. As important parameters of gastrointestinal health and growth, rumen microbes, ruminal pH, ammonia-N, and VFA are of interest. Volatile fatty acids, as the main energy substrate produced in the rumen by microbial fermentation (Bergman, 1990), are rapidly absorbed

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across the rumen epithelium and can contribute up to 80% of the energy requirements of ruminants (Gäbel and Sehested, 1997). The development of rumen morphology is closely associated with VFA production and absorption and is especially affected by butyrate and propionate concentrations (Saner et al., 1959; Weigand et al., 1975; Sakata and Tamate, 1978). *Saccharomyces cerevisiae* fermentation products have the ability to accelerate the production of VFA (Quigley et al., 1992); thus, improvement in rumen morphology may be found when SCFP is supplemented in the starter. As for the intestines, previous studies have illustrated that piglets (Shen et al., 2009) and broilers (Gao et al., 2008) fed SCFP had a greater improvement in morphology; however, this kind of study is lacking in calves. Therefore, we hypothesized that the inclusion of SCFP both in milk and starter in calf feeding might accelerate the rumen and intestinal epithelium development, with the higher inclusion in the starter exerting a greater response. Conversely, gastrointestinal microbiota plays an important role in digestive tract, which is also unstable in preweaning calves (Li et al., 2012). Another objective of our work was to investigate the effect of SCFP on the microbial colonization of the rumen and intestine of preweaning calves.

MATERIALS AND METHODS

Treatments, Animals, and Feeding

This study was conducted at the Dairy Teaching and Research Farm (Shunyi, Beijing, China), which has approximately 2,000 milking cows. The experiment was conducted as a randomized complete block design with 30 male calves. Initial BW was measured on d 2 and calves selected ranged from 35 to 45 kg to reduce the difference among individuals. Only calves with successful passive transfer of immunity, determined by clinical refractometer 24 h after birth, were used [≥ 5.5 g/dL of serum total protein (TP); Trotz-Williams et al., 2008]. Calves were enrolled in the experiment over a period of 1 mo, from May to June 2014, and 3 calves were grouped as a block by an order of birth date, within each block. Selected calves (initial BW = 40 ± 5 kg; TP ≥ 5.5 g/dL) were randomly assigned to 1 of 3 treatments: control [CON; no SmartCare or XPC (Diamond V, Cedar Rapids, IA)], 1 g/d SmartCare in the milk + 0.5% XPC in the starter (SCFP1), and 1 g/d SmartCare in the milk + 1% XPC in the starter (SCFP2). Calves were subsequently housed in individual hutches and placed on straw that was replaced weekly. The XPC (Original XPC, Diamond V) was incorporated in the starter at rates of 0, 0.5, and 1% on an as-fed basis from d 4 to 56, mixed with other ingredients, and pelleted. SmartCare

(Diamond V) was added into milk daily at 0 or 1 g/head per day in the morning milk feeding from d 2 to 30.

All calves received 4 L of colostrum immediately after birth and thereafter were fed pasteurized nonsaleable milk twice per day at 0800 and 1500 h [3 L/feeding (6 L/d) from d 2 to 10, 4 L/feeding (8 L/d) from d 11 to 42, 3 L/feeding (6 L/d) from d 43 to 49, and 2 L/feeding (4 L/d) from d 50 to 56]. Nonsaleable milk was pasteurized using a milk pasteurizer where milk temperature was elevated and held at 60°C for 30 min and then cooled to 37°C.

Calf starter ingredients and nutrient composition are presented in Table 1. Ingredient composition was identical between treatments with the exception of SCFP content, which replaced corn germ meal. Nutrient composition of starter met or exceeded the requirements for pre-weaned Holstein calves as suggested by NRC (2001). Starter grain (Honneur Nutritional Technology Co. Ltd., Beijing, China) was offered once every day in the morning, immediately after milk feeding, for ad libitum intake during the first 56 d of age. Water was provided ad libitum after 1 h of milk feeding.

Sample Collection

Blood Collection and Analysis. Blood was sampled from all calves before the morning feeding on d 28 and 56 for BHB analysis as an indicator of energy status of calves. Using evacuated tubes containing no anticoagulant, blood was collected via the jugular vein and then centrifuged at $3,500 \times g$ for 15 min at 4°C. Serum was collected in 1.5-mL microtubes and stored at -20°C for later analysis. Blood BHB concentrations were determined based on the enzymatic oxidation of BHB to acetoacetate and concomitant reduction of NAD^{+} to NADH (Williamson et al., 1962) by using an automated biochemistry Analyzer (model GF-D200, Caihong Co., Shandong, China) and a spectrophotometer (model 722, Caihong Co.).

Harvest. On d 56 of age, 15 calves (5 per treatment) were harvested and samples of gastrointestinal tissues and contents were collected. After harvest, the abdominal cavity was immediately opened and each region of digestive tract (rumen, duodenum, jejunum, and ileum) was isolated and tied off. Samples were collected as described below.

Rumen Fluid and Content. Rumen contents from the harvested, tied-off rumens were collected and separated into the solid and liquid portions by squeezing through 4 layers of cheesecloth. Solid contents were placed into sterile tubes, quick-frozen in liquid nitrogen, and then stored at -80°C for later analysis of microbial composition and populations (Prates et al., 2010;

Fliegerova et al., 2014). Rumen fluid was also collected on d 28 and 56 of age by a flexible esophageal tube (2 mm of wall thickness and 6 mm of internal diameter; Anscitech Co. Ltd., Wuhan, Hubei, China) from all calves 4 h after the morning milk feeding (Morales et al., 2014). Rumen pH was measured immediately with a glass electrode pH meter. Five milliliters of rumen fluid was stored at -80°C for microbial analysis and another 5 mL was preserved at -20°C for later analysis of VFA (Erwin et al., 1961) and ammonia-N concentrations (Broderick and Kang, 1980).

Intestinal Contents. Intestinal contents from duodenum were collected into sterile tubes and stored at -80°C for later microbial analysis. All samples were separately stored and no samples were pooled (Vlková et al., 2006; Suchodolski et al., 2008).

Rumen and Intestinal Tissues. After removing the digesta, tissues from the rumen, duodenum, jejunum, and ileum were rinsed in saline. Two 1-cm² rumen tissue samples were removed from the center of each area of cranial ventral sac (**RA**), ventral portion of caudal ventral sac (**RB**), caudal portion of caudal ventral sac (**RC**), cranial dorsal sac (**RD**), and caudal dorsal sac (**RE**). The tissue samples were then fixed in 4% phosphate-buffered paraformaldehyde solution (4% PBPS) for later analysis. From each rumen tissue sample, 5 papillae ($n = 10$ in each area) were randomly selected for papillae length (**PL**) and papillae

width (**PW**) measurement (Lesmeister et al., 2004b). The cross-sectional papillae surface area (**PSA**) was determined by multiplying PL by PW. For small intestines, a 4-cm-long looped section from mid-duodenum, mid-jejunum, and mid-ileum was placed into 4% PBPS (Bühler et al., 1998; Roffler et al., 2003) and sent for morphological analysis (Beijing Raise Dragon Co. Ltd., Beijing, China). The samples were separately cut into two 1-mm-thick cross-sections, washed, and then embedded in paraffin blocks. For each block, 5 cuts of 3- to 4- μm thick sections were isolated, resulting in 10 regions of each intestinal site for morphological quantitative and qualitative analysis (Zitnan et al., 2003). Section pictures (Olympus CX 31, Olympus Corp., Tokyo, Japan) were taken and quantitative morphometric analyses (Mirs Image Pro. Zip, Beijing Research Technology Develop Co. Ltd., Beijing, China) were conducted to measure villus height (**VH**), villus width (**VW**), and crypt depth (**CD**). Cross-sectional villus surface area (**VSA**) was calculated by multiplying VH by VW, whereas villus height-to-crypt depth ratio (**VCR**) was determined by dividing VH by CD. Finally, the rinsed rumen, reticulum, omasum, and abomasum were weighed and values were recorded after sampling.

Bacteria (Genus) Richness. Next-generation sequencing technology was used to test the microbial community in samples of rumen liquid collected on d 28 (**RL28**) and 56 (**RL56**), rumen solid content col-

Table 1. Ingredient and nutrient composition of the starter without (CON) or with *Saccharomyces cerevisiae* fermentation products (XPC)

Item	CON	0.5% XPC	1% XPC
Ingredient (%)			
Steam-flaked corn	33.1	33.1	33.1
Wheat bran	7.6	7.6	7.6
Soybean meal	14.3	14.3	14.3
Extruded soybean	4.2	4.2	4.2
Canola meal	8.0	8.0	8.0
Corn germ meal	8.5	8.0	7.5
Rice powder	4.2	4.2	4.2
Milk powder	0.4	0.4	0.4
DDGS	16.0	16.0	16.0
CaCO ₃	1.4	1.4	1.4
CaHPO ₄	1.0	1.0	1.0
NaCl	0.7	0.7	0.7
Mycotoxin binder	0.1	0.1	0.1
Premix compound for calf ¹	0.5	0.5	0.5
XPC ²	0	0.5	1.0
Nutrient composition, DM basis (% , unless noted)			
DM	90.9	91.8	90.5
CP	19.6	18.9	18.7
NE _L (Mcal/kg)	1.73	1.73	1.73
NDF	21.3	20.3	20.7
ADF	8.2	7.9	8.1
Ca	0.97	0.97	0.97
P	0.69	0.69	0.69

¹Premix compound contained (mg/kg) copper 60, iron 60, zinc 80, manganese 80, cobalt 0.4, iodine 1, and selenium 1. The premix also contained (IU/g) vitamin A 12,000, vitamin D 3,000, and vitamin E 120.

²Diamond V (Cedar Rapids, IA).

lected on d 56 (**RS56**), and duodenal content collected on d 56 (**DC56**). A total of 60 samples (5 calves $\times$ 3 treatments $\times$ 4 samples) were collected (not pooled) for DNA extraction, amplification, and sequencing. Based on the V3 region of 16S rDNA, trimmed DNA samples were sequenced using the pair-end method (Bartram et al., 2011) by Illumina HiSeq platform (Beijing Computing Center, Beijing, China). After sequencing, the high-quality sequences were clustered (UCLUST v1.2.22, <http://www.drive5.com/usearch/index.html>; Edgar, 2010) into operational taxonomic units (**OTU**), defined as comprising sequences with less than a 3% difference as detected in DOTUR (Schloss and Handelsman, 2005) by the furthest-neighbor method. For OTU comparison among each sample, we standardized the OTU for later diversity and abundance analyses through equating the sequence number to 70,000 reads. Total sequences from OTU were used to compare the databases (Greengene and RDP, http://greengenes.secondgenome.com/downloads/database/13_5; Malmuthuge and Griebel, 2014) to assign in different taxa (QIIME). Microbial composition graphs were then generated at a genus level.

Statistical Analyses

Data were analyzed as a randomized complete block design. Continuous variables with repeated measurements including ruminal VFA, ammonia-N and pH, blood BHB, and ruminal and intestinal bacteria richness within the same calf were analyzed using MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the fixed effects of treatment, day of measurement, interaction between treatment and day of measurement, and the random effect of calf nested within treatment. Variables of gastrointestinal morphological parameters with repeated measures of different area were also analyzed using MIXED with the fixed effects of treatment, area of measurement (rumen: RA, RB, RC, RD, RE; small intestines: duodenum, jejunum, ileum), and the random effect of calf nested within treatment. Variables

with a single measurement during the study, such as harvested weight, were analyzed with the fixed effects of treatment of calf and the random effect of calf nested within treatment. Orthogonal contrasts were completed using contrast statements to declare CON versus SCFP (SCFP1 and SCFP2) and SCFP1 (0.5% SCFP in starter) versus SCFP2 (1% SCFP in starter). All treatment results are reported as least squares means. Significant differences were indicated at $P \leq 0.05$, with trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Rumen Fermentation, Blood BHB, and Slaughter Weight

Least squares means for ruminal pH, ammonia-N, and blood BHB are in Table 2. Throughout the study, ruminal pH was similar ($P > 0.05$) between treatments and averaged 5.71 and 5.29 on d 28 and 56, respectively. Likewise, ammonia-N was not different between CON and treatment groups on d 28 ($P = 0.28$) or 56 ($P = 0.31$). There tended to be a treatment by day interaction ($P < 0.10$) for concentration of ammonia-N. It was interesting to find that ammonia-N increased by 2.2 mg/dL from d 28 to 56 in the CON calves and decreased by 1.27 and 0.76 mg/dL when calves were supplemented with 0.5 and 1% SCFP in starter, respectively. We observed no effects of treatment on BHB concentrations.

No differences between treatment and CON group for VFA concentrations were found, except for butyrate concentrations (Table 3). Calves supplemented with SCFP exhibited higher ($P < 0.05$) ruminal butyrate concentrations than CON calves (9.38 vs. 4.07 and 13.27 vs. 8.46 mmol/L for d 28 and 56, respectively). Some numerical differences were noted in acetate, propionate, isovalerate, valerate, and total VFA concentrations among treatments, but these parameters did not significantly differ. As expected, we found a significant time effect ($P < 0.001$) on VFA concentrations and ru-

Table 2. The effect of *Saccharomyces cerevisiae* fermentation products (SCFP)¹ on ruminal pH, ammonia-N, and blood BHB in dairy calves

Item	N ²	Day	Mean			SEM	P-value	
			CON	SCFP1	SCFP2		CON vs. SCFP	SCFP 1 vs. 2
pH	30	28	5.76	5.78	5.59	0.19	0.75	0.45
	30	56	5.23	5.32	5.33	0.26	0.70	0.97
Ammonia N (mg/dL)	30	28	10.06	12.03	11.25	1.20	0.28	0.61
	30	56	12.26	10.81	10.48	1.29	0.31	0.85
BHB ² (mmol/L)	30	28	0.34	0.31	0.36	0.04	0.90	0.15
	30	56	0.30	0.32	0.30	0.04	0.78	0.62

¹CON = no SmartCare or XPC; SCFP1 = 1 g/head/per day SmartCare in milk + 0.5% XPC in the starter grains; SCFP2 = 1 g/head per day SmartCare in milk + 1% XPC in the starter grains (Diamond V, Cedar Rapids, IA).

²N = number of calves used (CON = 10, SCFP1 = 10, SCFP 2 = 10).

Table 3. The effect of *Saccharomyces cerevisiae* fermentation products (SCFP)¹ on ruminal VFA² in dairy calves

Item	N ³	Day	Mean			SEM	P-value	
			CON	SCFP1	SCFP2		CON vs. SCFP	SCFP 1 vs. 2
Acetate (mmol/L)	30	28	24.71	25.38	26.50	2.54	0.69	0.74
	30	56	69.79	74.92	73.57	5.22	0.49	0.85
Propionate (mmol/L)	30	28	19.41	20.59	22.34	2.23	0.45	0.55
	30	56	67.16	75.23	71.37	5.75	0.38	0.62
Acetate-to-propionate ratio	30	28	1.27	1.27	1.21	0.07	0.78	0.51
	30	56	1.05	1.00	1.04	0.03	0.43	0.30
Butyrate (mmol/L)	30	28	4.07	7.69	11.08	1.57	0.01	0.11
	30	56	8.46	12.28	14.27	2.21	0.08	0.51
Isovalerate (mmol/L)	30	28	0.33	0.61	0.42	0.14	0.27	0.29
	30	56	1.63	2.05	2.24	0.48	0.38	0.76
Valerate (mmol/L)	30	28	0.59	0.64	0.83	0.17	0.49	0.42
	30	56	4.31	4.76	4.00	0.55	0.92	0.32
Total (mmol/L)	30	28	50.29	56.04	62.25	5.33	0.17	0.38
	30	56	152.40	170.20	166.56	12.62	0.30	0.83

¹CON = no SmartCare and XPC; SCFP1 = 1 g/head per day SmartCare in milk + 0.5% XPC in the starter grains; SCFP2 = 1 g/head per day SmartCare in milk + 1% XPC in the starter grains (Diamond V, Cedar Rapids, IA).

²Total and individual VFA values were from ruminal fluid obtained via esophageal tubing.

³N = number of calves used (CON = 10, SCFP1 = 10, SCFP 2 = 10).

minal pH as the calves grew older and DMI increased. Rumen (average 948.5 g), reticulum (157.1 g), omasum (220.5 g), abomasum (427.9 g), and harvest BW (69.2 kg) recorded on d 56 showed no significant treatment effect (Table 4).

Rumen Morphology

Effects of SCFP on PL and PW in the rumen are presented in Table 5. Calves fed SCFP exhibited greater PL of RA ($P = 0.04$), RB ($P = 0.01$), RE ($P = 0.03$), and ER ($P = 0.002$), showed significantly higher PW of ER ($P = 0.002$), and tended to have greater PW of RB ($P = 0.08$) and RC ($P = 0.06$) than CON calves. The PSA of RA ($P = 0.03$), RB ($P = 0.02$), RC ($P = 0.09$), RD ($P = 0.06$), RE ($P = 0.02$), and ER ($P < 0.001$) were found to be greater in SCFP treated calves compared with CON.

Evaluation of all rumen areas (RA, RB, RC, RD, RE, and ER) indicated no significant difference between SCFP1 and SCFP2 among PL, PW, and PSA, with the exception of PL of RD and ER. Calves supplemented with SCFP2 tended to have greater PL of RD ($P = 0.07$) and ER ($P = 0.08$) compared with SCFP1. In short, calves supplemented with SCFP increased PL, PW and PSA growth compared with those fed CON diet, with the higher dosage rate showing potentially better function in promoting PL growth, but not PW and PSA.

Intestine Morphology

Least squares means for VH, VW, VSA, CD, and VCR were measured to determine the effect of SCFP on intestine morphology (Table 6). With the exception of higher VH of ESI ($P = 0.002$) in SCFP, numerical

Table 4. The effect of *Saccharomyces cerevisiae* fermentation products (SCFP)¹ on harvested weight in dairy calves

Item ²	N ³	Mean			SEM	P-value	
		CON	SCFP1	SCFP2		CON vs. SCFP	SCFP 1 vs. 2
Rumen (g)	15	928.2	975.8	941.6	55.31	0.66	0.67
Reticulum (g)	15	145.2	163.0	163.2	11.48	0.23	0.99
Reti-rumen (g)	15	1,073.4	1,138.8	1,104.8	63.27	0.54	0.71
Omasum (g)	15	209.6	223.8	228.2	21.2	0.54	0.89
Abomasum (g)	15	436.4	443.0	404.2	26.58	0.70	0.32
Total (g)	15	1,719.4	1,805.6	1,737.2	94.93	0.66	0.62
Harvest (kg)	15	68.59	69.07	69.92	1.26	0.57	0.64

¹CON = no SmartCare and XPC; SCFP1 = 1 g/head per day SmartCare in milk + 0.5% XPC in the starter grains; SCFP2 = 1 g/head per day SmartCare in milk + 1% XPC in the starter grains (Diamond V, Cedar Rapids, IA).

²Reti-rumen (g) = reticulum (g) + rumen (g); Total (g) = whole stomach weight; Harvest weight (g) = BW excluding blood and gastrointestinal chyme.

³N = number of calves used (CON = 5, SCFP1 = 5, SCFP 2 = 5).

differences ($P > 0.05$) were detected between SCFP and CON for VH, VW, or VSA of any segments in the duodenum, jejunum, and ileum. And calves fed SCFP showed lower CD ($P < 0.05$) of jejunum and ESI ($P = 0.07$) than those fed the CON diet. Calves supplemented with SCFP also had significantly improved VCR in the jejunum ($P < 0.01$), ileum ($P = 0.02$), and ESI ($P = 0.002$). No differences were noted between SCFP1 and SCFP2 in VH, VW, VSA, and CD; however, calves supplemented with the higher dosage of SCFP (SCFP2) exhibited a greater VCR in the ESI ($P = 0.03$) and a tendency for greater VCR in the duodenum ($P = 0.09$).

Although it had limited effects on VW and VSA, SCFP had an ability to increase VH and decrease the CD, which resulted in improved VCR. A greater effect was observed when supplemented at the higher dosage of SCFP (SCFP2) in the starter compared with the lower dosage (SCFP1).

Bacteria (Genus) Richness

After sequencing all 60 collected samples, observed bacterial species of RL28, RL56, RS56, and DC56 were between 634 and 1,366, 703 and 1,056, 809 and 1,248, and 643 and 2,280 OTU, respectively. The relative richness of the top 10 bacteria genus (percent of total reads) is presented in Figure 1. These bacteria were identified

mainly as *Prevotella* (21.8, 36.3, 40.3, and 43.5% average occurrence for RL28, RL56, RS56, and DC56, respectively), unknown genus (40.4, 36.5, 30.9, and 31.6%), and *Butyrivibrio* (22.1, 15.8, 15.5, and 5.7%). No treatment effects were observed in the RS56 and DC56 for any bacteria; however, SCFP supplementation was found to decrease relative occurrence of *Prevotella* at RL28 (4.2 fold compared with the CON, $P = 0.01$) and a tendency at RL56 (1.6 fold, $P = 0.08$). An increase of relative occurrence of *Butyrivibrio* of RL on d 28 was noted. On average, 30.2% of OTU were dominated by members of genus *Butyrivibrio* when supplemented SCFP in the diet, which was much higher ($P = 0.02$) compared with CON (5.6%). On d 56, the percentage of *Butyrivibrio* for CON, SCFP1, and SCFP2 was 11.7, 18.9, and 16.6 of total reads, respectively. Supplementation of SCFP also decreased ($P < 0.05$) the richness of *Shuttleworthia* of RL28 and *Desulfovibrio* of RL56. No significant effects were observed between SCFP1 and SCFP2 for bacteria richness in all 4 samples.

DISCUSSION

Rumen Fermentation and Slaughter Weight

Very little research exists evaluating the effects of SCFP on ruminal VFA concentrations in calves. The

Table 5. The effect of *Saccharomyces cerevisiae* fermentation products (SCFP)¹ on rumen morphology measurement in dairy calves

Item ²	N ³	Mean			SEM	P-value	
		CON	SCFP1	SCFP2		CON vs. SCFP	SCFP 1 vs. 2
PL (μm)							
RA	15	1,900.3	2,006.3	1,963.7	30.9	0.04	0.32
RB	15	755.9	869.0	911.4	37.7	0.01	0.44
RC	15	872.7	898.4	969.9	35.7	0.18	0.18
RD	15	713.4	739.3	838.2	35.8	0.11	0.07
RE	15	756.7	816.4	886.7	32.4	0.03	0.15
ER	15	1,002.3	1,065.2	1,114.7	26.45	0.002	0.08
PW (μm)							
RA	15	218.3	239.8	226.6	7.2	0.11	0.21
RB	15	168.1	180.9	187.6	7.1	0.08	0.52
RC	15	181.2	201.6	187.4	5.1	0.06	0.07
RD	15	193.8	233.9	224.2	19.7	0.17	0.73
RE	15	193.0	220.6	211.1	12.2	0.15	0.59
ER	15	190.7	216.1	207.6	6.8	0.002	0.23
PSA (μm ²)							
RA	15	418,121	483,114	447,813	15,687	0.11	0.21
RB	15	129,227	158,549	171,679	11,451	0.08	0.52
RC	15	159,175	182,453	182,979	10,602	0.06	0.07
RD	15	137,759	170,476	190,037	16,421	0.17	0.73
RE	15	147,019	179,114	187,860	10,973	0.15	0.59
ER	15	198,619	235,013	236,697	8,075	<0.0001	0.83

¹CON = no SmartCare and XPC; SCFP1 = 1 g/head per day SmartCare in milk + 0.5% XPC in the starter grains; SCFP2 = 1 g/head per day SmartCare in milk + 1% XPC in the starter grains (Diamond V, Cedar Rapids, IA).

²PL = papillae length; PW = papillae width; PSA = papillae surface area; RA = cranial ventral sac; RB = ventral portion of the caudal ventral sac; RC = caudal portion of the caudal ventral sac; RD = cranial dorsal sac; RE = caudal dorsal sac; ER = entire rumen (RA + RB + RC + RD + RE).

³N = number of calves used (CON = 5, SCFP1 = 5, SCFP 2 = 5).

SCFP tested in this experiment increased ruminal butyrate (statistically) and other individual VFA (numerically) compared with CON on d 28 and 56. The changes in bacterial richness, particularly the increase in *Butyrivibrio* with SCFP supplementation, could contribute to an increased butyrate concentration. Butyrate and, to a lesser extent, propionate are used as energy sources by the rumen epithelium and subsequently have great influence on rumen epithelial development (Tamate et al., 1962). Therefore, the increased concentration of butyrate and numerically higher concentration of propionate might result in the greater ruminal papillae length observed in calves supplemented with SCFP.

Previous studies concluded that VFA production increased and pH decreased in preweaning calves over time (McCarthy and Kesler, 1956; Anderson et al., 1987; Quigley et al., 1991), which is similar to what was shown in this experiment. These changes could be attributed to the increase in calf starter intake, which was exhibited in another study (G. Alugongo, J. Xiao, Y. Chung, S. Z. Dong, S. Li, I. Yoon, Z. Wu, and Z. Cao, unpublished data). No treatment or day effect was observed for ammonia-N; however, a tendency was

found for an interaction between treatment and day ($P < 0.10$) for ammonia-N. This indicates that with an increase in age and feed intake, SCFP may improve the utilization of ruminal ammonia-N, which may be related to SCFP increasing fiber-degrading bacteria that have a high preference for ammonia as their N source (Hristov et al., 2010). Other researchers, in agreement with our study, suggested a decrease in ruminal ammonia-N when calves were fed SCFP supplemented diets (Kumar et al., 1994).

Supplementation with SCFP has been shown to improve BW and ADG of animals (Lesmeister et al., 2004a; Gao et al., 2008); however, we did not find statistical differences between SCFP and CON in slaughter weight. Only numerical improvements were observed in rumen, reticulum, omasum, and harvest weights when SCFP was added in the diet in the present study.

Blood BHB

Calves rely upon glucose as the primary energy substrate due to the limited utilization of VFA in undeveloped epithelium (Heinrichs and Lesmeister, 2005). However, as starter intake increases and rumen

Table 6. The effect of *Saccharomyces cerevisiae* fermentation products (SCFP)¹ on small intestinal morphology measurement in dairy calves

Item ²	N ³	Mean			SEM	P-value	
		CON	SCFP1	SCFP2		CON vs. SCFP	SCFP 1 vs. 2
VH (μm)							
Duodenum	15	394.5	429.2	415.1	13.3	0.11	0.46
Jejunum	15	640.5	669.5	709.4	35.0	0.27	0.43
Ileum	15	411.9	443.5	431.7	18.4	0.27	0.66
ESI	15	482.3	513.0	518.6	11.5	0.002	0.63
VW (μm)							
Duodenum	15	123.6	122.2	125.8	6.8	0.97	0.71
Jejunum	15	121.6	115.4	116.0	3.4	0.15	0.91
Ileum	15	145.8	141.4	149.0	9.6	0.96	0.58
ESI	15	129.9	125.6	130.3	7.4	0.76	0.54
VSA (μm ²)							
Duodenum	15	48,597	52,802	52,345	3,153	0.32	0.92
Jejunum	15	77,353	77,023	81,698	4,209	0.69	0.44
Ileum	15	60,024	63,026	65,568	6,139	0.58	0.77
ESI	15	61,879	63,867	66,604	4,011	0.35	0.35
CD (μm)							
Duodenum	15	235.3	243.9	193.9	18.6	0.49	0.08
Jejunum	15	266.0	230.6	234.7	9.0	0.01	0.75
Ileum	15	228.5	222.8	198.7	12.7	0.27	0.20
ESI	15	244.1	232.6	209.1	13.6	0.07	0.11
VCR							
Duodenum	15	1.78	1.89	2.29	0.15	0.13	0.09
Jejunum	15	2.47	2.92	3.13	0.12	0.002	0.26
Ileum	15	1.87	2.07	2.28	0.09	0.02	0.11
ESI	15	2.05	2.29	2.56	0.11	0.002	0.03

¹CON = no SmartCare and XPC; SCFP1 = 1 g/head per day SmartCare in milk + 0.5% XPC in the starter grains; SCFP2 = 1 g/head per day SmartCare in milk + 1% XPC in the starter grains (Diamond V, Cedar Rapids, IA).

²VH = villus height; VW = villus width; VSA = villus surface area; CD = crypt depth; VCR = villus-to-crypt ratio; ESI = entire small intestines (duodenum + jejunum + ileum).

³N = number of calves used (CON = 5, SCFP1 = 5, SCFP 2 = 5).

Figure 1. Top 10 bacteria percentage in genus level of total operational taxonomic unit number of control [CON; no SmartCare and XPC (Diamond V, Cedar Rapids, IA), *Saccharomyces cerevisiae* fermentation products 1 (SCFP1; 1 g/head per day of SmartCare in milk + 0.5% XPC in starter grains), and SCFP2 (1 g/head per day of SmartCare in milk + 1% XPC in the starter grains) in rumen liquid (RL) sampled on d 28 and 56, and rumen solid content (RS) and duodenal content (DC) on d 56. Fifteen calves were used (CON = 5, SCFP1 = 5, SCFP2 = 5). * $P < 0.1$, ** $P < 0.05$ between CON and SCFP (SCFP1 and SCFP2). The error bars represent the standard deviation.

development progresses, the contribution of VFA to the energy needs of calves also increases. Most metabolic BHB is converted from butyrate uptake by ruminal papillae (Weigand et al., 1975); as with other ketone bodies, it is a major energy source for the body and an indicator of rumen development.

No treatment and time effects on blood BHB were observed in this and most previous studies (Lesmeister et al., 2004a; Laborde, 2008; Magalhães et al., 2008). In contrast, Coverdale et al. (2004) reported that BHB levels of calves increased with age. Also, Quigley et al. (1992) observed that SCFP increased blood BHB coinciding with an increase in butyrate concentrations. These shifts were only exhibited 4 h after feeding. In our study, we collected blood samples only once before morning feeding, and the difference in sampling time

may explain the lack of treatment effect noted in our study.

Rumen Morphology

Rumen epithelium plays an important role in short-chain fatty acid absorption (Kramer et al., 1996; Penner et al., 2009), regulation of the protons to prevent severe cytosolic acidosis (Gäbel et al., 2002), and nitrogen transportation and urea recycling (Abdoun et al., 2006; Penner et al., 2009). The normal growth of stratified rumen epithelium that consists of countless papillae as major absorptive structures is critical for nutrient utilization (Graham and Simmons, 2005). Rumen PL and PW have been used to assess the relative development of the rumen (Lesmeister et al., 2004b).

Most of the findings have shown treatment differences for gastrointestinal morphological measurements when milk feeding (Bühler et al., 1998; Khan et al., 2007) or diet forms were altered (Lesmeister and Heinrichs, 2004). The results from these studies indicated that gastrointestinal morphology was sensitive to physical or chemical changes in the diet.

The effect of SCFP on ruminal morphology has been reported previously. *Saccharomyces cerevisiae* fermentation products enhanced PL and PW (Lesmeister et al., 2004a; Brewer et al., 2014), and similar positive stimulation of ruminal papillae was obtained in the present study. Besides, papillae surface area was also improved, which means a greater rumen absorption area of VFA and other nutrients substrates. All dorsal and ventral sacs showed statistically or numerically greater PL, PW, and PSA when calves were fed SCFP. As stated above, butyrate serves as a preferred energy source for rumen epithelial cells and plays an important role in the normal development of ruminal papillae. The improvement of papillae might be induced by higher butyrate production by ruminal microbes such as *Butyrivibrio*.

Intestine Morphology

Previous studies illustrate that short villi resulted in a reduction of villi surface area and digestive enzymes (Graham et al., 1984). Villus shortening occurs via an increase in rate of cell loss, leading to increased crypt cell production and, finally, increased crypt depth (Pluske et al., 1997). Deeper crypts coinciding with lower VCR value may lead to nutrient malabsorption, which may lead to osmotic diarrhea (Pearson et al., 1978). Thus, SCFP could improve fecal score of calves by improving intestinal absorption of nutrients due to an increased VCR. These proposed benefits were verified in another experiment, which showed an improvement in fecal score and a decrease in diarrhea rate when SCFP was supplemented in the diet (G. Alugongo, J. Xiao, Y. Chung, S. Z. Dong, S. Li, I. Yoon, Z. Wu, and Z. Cao, unpublished data).

Ours is the first study to determine the effect of SCFP on intestinal morphology in calves. We found a significant increase in VH of ESI and a tended decrease in CD of ESI, which resulted in an increase in VCR of the jejunum and ileum and ESI. Furthermore, when calves were supplemented with 1% of SCFP in the starter, the duodenum and ESI exhibited greater VCR compared with lower dosage of SCFP at 0.5%. Similar results were observed by Shen et al. (2009), who claimed that dietary supplementation of SCFP (Original XP, Diamond V) resulted in greater jejunal VH and VCR compared with CON in piglets. Gao et

al. (2008) also illustrated that broilers fed SCFP (Original XP, Diamond V) reported improved morphological performance. The mechanism of the effect of SCFP on intestinal structural development of broiler, piglets, and calves needs further elucidation.

Saccharomyces cerevisiae fermentation products contain glucans and oligosaccharides, which provide a protective function to the mucosa by preventing pathogens from binding to the villi in the gut (Bode, 2012). Brewer et al. (2014) reported enhanced ruminal papillae development and reduced *Salmonella* intestinal colonization in SCFP-fed calves when orally challenged with *Salmonella*; this effect may enhance proliferation of epithelial cells to build the villus (Mourão et al., 2006). More studies should focus on absorptive and metabolic mechanism of action of SCFP in gastrointestinal development and the difference between the supplementation of SCFP in milk and grain.

Bacteria (Genus) Richness

With the advance of the next-generation sequencing technologies, ours is first study to conduct the 16s RNA gene sequencing of microbes to analyze the effect of SCFP on the microbial community and richness in digestive tract of calves. Compared with culture-dependent and denaturing gradient gel electrophoresis methods, using next-generation sequencing technology to achieve high coverage can more accurately reflect the community of the gastrointestinal microbiome.

Our sequencing data, spanning the V3 region, was not able to identify all the bacterial groups at species level, but allowed identification of numerous genera (Chakravorty et al., 2007). Over the totality of samples, 244 genera were detected. Among these genera, most sequences belonged to *Prevotella* followed by *Butyrivibrio*, which is in accordance with a previous study (Li et al., 2012).

The primary motile butyrate-producing anaerobic bacteria in the rumen are mainly of *Butyrivibrio fibrisolvens* strains, and the major characteristic of bacteria belonging to genus *Butyrivibrio* is the production of butyric acid (Bryant and Small, 1956; Mrázek et al., 2006). Improvements in cellulolytic bacteria (e.g., *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*) when supplemented with SCFP were reported in previous studies (Harrison et al., 1988; Mao et al., 2013); however, studies that reported the effect of SCFP on *Butyrivibrio* are limited. In vitro studies have shown that SCFP reduce the lag time for growth of *Butyrivibrio fibrisolvens* D1 (Gumus and Sehu, 2012). In our study, *Butyrivibrio* richness was increased with SCFP, whereas no significant differences were observed between dosages (SCFP1 vs. SCFP2). Bacteria richness

was 5.4 fold higher in SCFP than CON at RL28, with no significant effects at RL56 (1.5 fold increase) and RS56 (1.5 fold decrease). The SCFP was supplemented in milk from d 2 to 30 and in starter from d 4 to 56. This may imply that SCFP had a stronger effect on *Butyrivibrio* on d 28 when SCFP was supplemented in both milk and starter than d 56 when SCFP was only supplemented via the starter. Additionally, as rumen bacteria are not stable in the first month (Jami et al., 2013), it may be reasonable that SCFP in the starter fast-tracked the establishment of *Butyrivibrio* by d 28. Ours is the first study to indicate the effect of SCFP on *Butyrivibrio* richness in calves. Higher *Butyrivibrio* genus richness in SCFP potentially contributed to higher butyrate concentration, which resulted in greater ruminal morphology.

Prevotella has been reported to account for a numerically dominant microbial population in the rumen of cattle (Meyer et al., 2008). In agreement with our results, Li et al. (2012) indicated that *Prevotella* was the most dominant genus at 33.1% in young calves. *Prevotella* are highly active hemicellulolytic and proteolytic bacteria that mainly produce acetate and propionate but not butyrate (Matsui et al., 2000). Compared with CON, a lower richness of *Prevotella* of SCFP treatment at RL28 (4.2 fold decrease) and RL56 (1.6 fold decrease) might also contribute to a higher butyrate concentration.

Furthermore, 2 genus bacteria abundance (*Prevotella* and *Desulfovibrio*) were altered in RL56 when calves were supplemented with SCFP compared with CON, but no significant differences were present in RS56. We hypothesized that the changes in richness of genus in RL56, in contrast with those of RS56 (unchanged), may imply that SCFP is more likely to influence liquid-associated rumen bacteria rather than solid-associated ones. The solid fraction is regarded as the main ruminal fraction with highest bacteria population in dairy cows. That most bacteria are attached to solid rather than liquid fractions was demonstrated by Yang et al. (2001), who also indicated that differences in diet composition can influence the proportion of bacteria in 2 fractions. Increasing the forage-to-concentrate ratio is usually beneficial for the pool of bacteria in solid fraction, as more fibrolytic bacteria attach to the forage particles. Faichney (1980) reported that the percentage of solid fraction bacteria reached 90% in sheep fed only forage, whereas it declined to 50% for steers fed equal proportions of forage and concentrate (Merry and McAllan, 1983). In the current experiment, we did not feed any forage to calves; as a result, we think the liquid-associated bacteria would represent the general rumen population. However, limited research has been

done with calves in this area and further research could help explain why SCFP is more likely to influence liquid-associated rumen bacteria and whether the liquid fraction plays a more important role than the solid fraction in manipulating rumen bacteria or vice versa.

As for genus of *Shuttleworthia* and *Desulfovibrio*, limited studies have been reported in calves. *Shuttleworthia* are saccharolytic bacteria that produce acetate, butyrate, and lactate as end-products of glucose fermentation (Downes et al., 2002). *Desulfovibrio* are known as the group of sulfate-reducing bacteria (Lobo et al., 2007). No research has been reported on the relationship between SCFP and *Desulfovibrio* or *Shuttleworthia*. Although differences were noted between CON and SCFP in these 2 genera, average relative abundances were just 1.3 and 0.7%, respectively, which may lack significance in their regulatory roles.

In general, even though SCFP had positive effects on ruminal microbial establishment and butyrate production, which might connect to the improvement of rumen morphology, some limitations existed in our study. The effects of SCFP on butyrate and *Butyrivibrio* were significant only on d 28 but not on d 56, and the effect on rumen bacteria was only in the liquid fraction but not in the solid fraction. Based on these results, we suggest further research with more calves focused on the mechanism of SCFP in regulating the rumen microbial community to explain the changes found in the present study.

CONCLUSIONS

Saccharomyces cerevisiae fermentation products fed to preweaning calves via milk and starter grains improved ruminal morphology. This was possibly due to manipulating microbial community, which resulted in an increase in ruminal butyrate production, and supplying SCFP, which might provide a protective function to the mucosa by preventing pathogens from binding to the villi in the gut. Furthermore, compared with SCFP1 (0.5% of XPC in starter), SCFP2 (1% of XPC) tended to exhibit slightly greater effect on gastrointestinal morphology.

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