Influence of yeast culture and feed antibiotics on ruminal fermentation and site and extent of digestion in beef heifers fed high grain rations[1]

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ABSTRACT: The study objective was to investigate the effects of site of delivering *Saccharomyces cerevisiae* fermentation product (**SCFP**) on ruminal pH and fermentation characteristics, and the site and extent of feed digestion in the digestive tract of beef heifers fed high-grain diets. Examining the ruminal and postruminal effects of SCFP is important for understanding the potential use of SCFP as an alternative for current industry-standard antibiotics used in beef cattle rations. Five beef heifers (initial BW = 561 ± 11.7 kg) equipped with ruminal and duodenal cannulas were used in a 5 × 5 Latin square design with 28-d periods, including 21 d for adaption and 7 d for data collection. Five treatments were as follows: 1) control diet that contained 10% barley silage and 90% barley concentrate mix (DM basis); 2) control diet supplemented with antibiotics (ANT; 330-mg monensin/d and 110-mg tylosin/d per head); 3) ruminal (top dress) delivery of SCFP (**rSCFP**; NaturSafe, Diamond V, 18-g SCFP/d); 4) duodenal delivery of SCFP (**dSCFP**; 18-g SCFP/d, via duodenal cannula); and 5) a combination of rSCFP and dSCFP (**rdSCFP**; 18-g rSCFP and 18-g dSCFP). Intake of DM tended ($P < 0.10$) to be greater by heifers fed rdSCFP than those fed control, ANT and rSCFP diets. Minimum ruminal pH was greater ($P < 0.05$) with rSCFP than control and rdSCFP treatments. The duration of ruminal pH < 5.6 tended ($P < 0.10$) to be less with rSCFP than control and ANT. Heifers fed the rSCFP diet had greater ($P < 0.03$) protozoa counts and proportion of acetate than the other treatments. Nutrient flows to the duodenum did not differ ($P > 0.19$), whereas the amount of truly fermented OM was greater ($P < 0.03$) with rdSCFP than the other treatments. Ruminal OM digestibility was highest with rSCFP and rdSCFP, intermediate with dSCFP and ANT, and lowest with control ($P < 0.03$). Intestinal digestibility was similar among treatments. As a result, total tract digestibility of OM ($P < 0.07$) and NDF ($P < 0.01$) was greater with rSCFP and rdSCFP than control and ANT. Fecal IgA concentration was highest with ANT, intermediate with dSCFP and rdSCFP, and lowest with control and rSCFP ($P < 0.03$). These results demonstrate that feeding SCFP improved stability of ruminal pH and digestibility of OM and NDF. Delivery of SCFP to the duodenum appeared to have little effect on nutrient digestibility but improved intestinal immune response. Feeding SCFP performed better or at least equal to antibiotics currently used in beef cattle rations and could be a natural alternative for beef cattle production.

Key words: antibiotics, beef heifers, rumen pH and fermentation, *Saccharomyces cerevisiae* fermentation product, site and extent of digestion

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INTRODUCTION

Saccharomyces cerevisiae fermentation product (**SCFP**) is a product of yeast fermentation, as opposed to live yeast, which has not gone through the fermentation process. SCFP is produced via an anaerobic fermentation process by fermenting selected liquid (e.g., cane molasses) and cereal grain raw ingredients (e.g., roughage products and processed grain by-products) with *S. cerevisiae*. SCFP is used extensively in dairy cattle as a dietary supplement to support milk production and feed efficiency (Poppy et al., 2012). A meta-analysis evaluating the effects of SCFP on feedlot performance of beef cattle reported increases in ADG, DMI, and G:F (Wagner et al., 2016). It has been proposed that SCFP contains fermentation metabolites as stimulatory nutrients to specific fiber-digesting (Wiedmeier et al., 1987) and lactate-utilizing (Callaway and Martin, 1997) bacteria. In calves, an increased papilla length in the rumen and increased villus height-to-crypt depth ratio in all segments of the small intestine were seen when SCFP was added to milk replacer and starter grains (Xiao et al., 2016). However, little data are available in the area of lower gut health and function in adult ruminants. It is not known whether SCFP are resistant to ruminal digestion and whether they are metabolically active in the intestine. In addition, feeding monensin and tylosin is a common practice in North American feedlot operations to improve feed efficiency and prevent liver abscesses (Meyer et al., 2009). However, antimicrobial use in animals has been blamed as a contributing factor for reducing the effectiveness of antimicrobial drugs for treating human disease. In response to public concerns, the animal industry has been diligently seeking natural alternatives that provide similar performance without compromising animal health. The present study tested the hypothesis that adding SCFP in the ration of finishing beef cattle would be metabolically active in the rumen and intestine, and SCFP would exhibit similar or better activity than monensin and tylosin. The study objective was to investigate the effects of site of delivering SCFP on ruminal pH and fermentation characteristics, and the site and extent of feed digestion in the digestive tract of beef heifers fed high-grain diets. Examining the ruminal and postruminal effects of SCFP is important for understanding the potential use of SCFP as an alternative for current industry-standard antibiotics used in beef cattle rations.

MATERIALS AND METHODS

Experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the Lethbridge Research and Development Centre (Lethbridge, Canada). Animals were cared for and managed according to the guidelines of the Canadian Council on Animal Care (2009).

Animal, Treatment, and Design

Five ruminally and duodenally cannulated beef heifers (initial BW = 561 ± 11.7 kg) were used in a 5 × 5 Latin square design with 28-d periods, including 21 d for adaption to new treatments and 7 d for data and sample collection in each period. The treatments were as follows: 1) control (no antibiotics, no SCFP); 2) control diet supplemented with antibiotics (**ANT**; 330-mg monensin/d and 110-mg tylosin/d per head; top dressed); 3) ruminal (top dressed) delivery of SCFP (**rSCFP**; 18-g SCFP/d); 4) duodenal delivery of SCFP (**dSCFP**; 18-g SCFP encapsulated in gelatin capsules, and delivered via duodenal cannula); and 5) a combination of rSCFP and dSCFP (**rdSCFP**; 18-g rSCFP and 18-g dSCFP). Monensin and tylosin are commonly used to improve feed efficiency and to prevent liver abscess, respectively, in feedlot cattle (Tedeschi et al., 2003; Meyer et al., 2009). SCFP used in this trial was NaturSafe from Diamond V (Cedar Rapids, Iowa). NaturSafe is a newly developed SCFP specifically formulated to optimize beef cattle health, performance, and balanced immunity. Each treatment was mixed with 72-g ground barley and 18-g molasses, split into 2 portions and top dressed twice daily at 0900 and 1700 h. The control and dSCFP animals were top-dressed only with the same amount of ground barley and molasses with no antibiotics or SCFP.

Heifers were housed in individual tie stalls on rubber mats and bedded with wood shavings. Beef heifers were allowed free-choice access to fresh water throughout the experiment. Heifers were exercised in an outdoor pen for 1 h daily, as the measurement and sampling schedule permitted. Diets consisted of 10% barley silage, 87% dry rolled barley grain, and 3% vitamin and mineral pellet (Table 1; DM basis) and were formulated to

meet or exceed nutrient requirements of beef cattle (NASEM, 2016). The diet was prepared daily using a feed mixer (Data Ranger, American Calan, Inc., Northwood, NH), and heifers were fed once daily (0900 h) ad libitum to ensure at least 5% refusals. Animals were weighed at the same time each day at the first day of the first period and at the last day of each subsequent period.

Intake, Duodenal Flows, Digestibility, and Microbial Protein Synthesis

Feed offered and feed refusals were recorded daily for each heifer during the experiment. A sample of the diet and each feed ingredient were collected weekly to determine DM content. Feed refusals were collected during the last 7 d of each period and pooled together by heifer and by period. Both feed and feed refusal samples were oven dried at 55 °C for 48 h and then ground through a 1-mm screen (standard model 4 Wiley Mill; Arthur H. Thomas, Philadelphia, PA) prior to chemical analyses. Daily feed intake was calculated as the difference between feed offered and feed refusal.

Nutrient digestibility was determined using Yb ($YbCl_3 \cdot 6H_2O$) as an external digestive marker. Microbial protein synthesis in the rumen was measured using ammonia ^{15}N ($[^{15}NH_4]_2SO_4$) as a label. Starting at day 18 of each period, the digesta marker (2.7 g of $YbCl_3 \cdot 6H_2O$, 1.2 g of Yb), microbial marker (3 g of $[^{15}NH_4]_2SO_4$), and treatments (SCFP and antibiotics) were mixed together with ground barley and molasses and top dressed twice daily as described above. The duodenal delivery of SCFP followed the same schedule as top dressing.

Table 1. Ingredients and chemical composition of the experimental diet

Ingredient, % of DM	
Barley silage[1]	10.0
Barley grain,[2] dry-rolled	87.0
Barley, ground	1.64
Canola meal	0.29
Calcium carbonate	0.73
Molasses	0.07
Salt	0.15
Feedlot premix[3]	0.03
Urea	0.06
Vitamin E (500,000 IU/kg)	0.002
Canola oil	0.03
Chemical composition, % of DM	
DM	79.8
OM	96.6
NDF	29.7
ADF	8.0
Starch	52.8
CP	13.2

[1]Composition (% of DM): 39.4 DM, 92.7 OM, 47.5 NDF, 24.7 ADF, 22.1 starch, and 13.7 CP.

[2]Composition (% of DM): 90.2 DM, 97.0 OM, 27.8 NDF, 6.4 ADF, 56.2 starch, and 14.0 CP.

[3]Supplied per kilogram of dietary DM: 15 mg Cu, 65 mg Zn, 28 mg Mn, 0.7 mg I, 0.2 mg Co, 0.3 mg Se, 6,000 IU vitamin A, 600 IU vitamin D, and 47 IU vitamin E.

Duodenal samples (~250 mL per sample) were collected through the duodenal cannula 3 times daily, moving ahead 2 h each day from days 25 to 28. Samples were immediately split into 3 fractions and pooled by heifer and by period to determine DM content, NH_3-N, and nutrient analyses, respectively. Duodenal DM flows were calculated as the ratio of daily Yb consumed to Yb concentration in duodenal content. Fecal samples (~150 g wet weight per sample) were collected from the rectum of each heifer following the same schedule as for duodenal sampling. Feces (50 g wet weight) were immediately subsampled after mixing and composited across sampling times for each heifer within period. Samples were dried at 55 °C for 48 h, ground through a 1-mm screen (standard model 4 Wiley Mill; Arthur H. Thomas, Philadelphia, PA), and stored until analyzed.

Ruminal bacterial pellets were prepared as described by Yang et al. (2014). Briefly, whole ruminal contents (~750 g/sample) were collected twice at 0930 h on day 26 and 1430 h on day 28 in each period from 4 different locations in the rumen and immediately squeezed through 4 layers of cheesecloth. The particles retained on the cheesecloth were blended with an equal amount of 0.9% sodium chloride in a waring blender for 1 min and then squeezed again. Both filtrates from squeezed and strained homogenate were mixed to obtain a mixed bacterial pellet using differentiation centrifuge techniques (Yang et al., 2014). Bacterial pellets were accumulated by heifer within period, freeze-dried, ground using a ball mill, and analyzed for OM, ^{15}N, and total N. These samples were used as a reference to calculate ruminal microbial protein synthesis as described by Yang et al. (2014).

Ruminal pH and Fermentation Characteristics

Ruminal pH was monitored continuously for 4 d from days 22 to 26 of each period using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA). Ruminal pH readings were taken every 30 s and stored by the data logger (model M1b-pH-1KRTD, Dascor, Escondido, CA). Ruminal pH

data were summarized daily and included mean, minimum and maximum, and duration of pH < 5.8, < 5.6, and < 5.2. The determination of ruminal fermentation characteristics was carried out on days 22 and 23 at 1, 3, 5 and 7 h daily after the morning feeding. Ruminal fluid was collected from 4 locations within the rumen (i.e., 2 locations across the top and 2 locations at the bottom of the rumen) and immediately squeezed using a nylon mesh (pore size 355 µm; PeCAP, B & SH Thompson, Ville Mont-Royal, QC, Canada) to obtain filtrate. Two subsamples of 5 mL of filtrate were preserved with 1 mL of 25% (wt/vol) HPO_3 and 1 mL of 1% (wt/vol) H_2SO_4 for VFA and NH_3-N determination, respectively. The samples were subsequently stored at −20 °C until analyzed. Two and a half milliliters of filtrate were mixed with an equal volume of methyl green-formalin-saline for protozoa count. Protozoa were enumerated by light microscopy using a Levy-Hausser counting chamber (Hausser Scientific, Horsham, PA).

Blood Sampling and Analyses

At day 28 of each experimental period, blood samples were collected from the jugular vein at 2 h after the morning feeding. Vacuum tubes with Na heparin were used to collect plasma. Vacuum tubes without any additive were used to collect serum. Blood metabolites including glucose and NEFA were determined as described by Yang et al. (2010). Concentration of serum amyloid A (**SAA**) and LPS-binding protein (**LBP**) was determined using commercial ELISA kits (SEA885Bo and SEB406Bo, respectively; Cloud-Clone Corp, Katy) as detailed by Ametaj et al. (2005).

Chemical, Fermentation, and LPS Analyses

All chemical analyses were conducted in duplicate. Samples were oven dried at 135 °C for 2 h to determine the analytical DM content (AOAC, 2005; method 930.15). Ash content was determined by combustion at 550 °C overnight. Organic matter content was calculated as 100 minus ash content (AOAC, 2005; method 942.05). Neutral detergent fiber was measured by following the method of Van Soest et al. (1991) using heat stable α-amylase and sodium sulfite. Acid detergent fiber was determined according to AOAC (2005), method 973.18. Starch was determined as described by Rode et al. (1999). Concentrations of Yb in diet, feed refusal, and duodenal and fecal samples were determined using inductively coupled plasma optical emission spectroscopy (AOAC, 2005; method 968.08). The flash combustion and thermal conductivity detection technique (model 1500, Carlo Erba Instruments, Milan, Italy) were used to analyze the concentration of total N in feed offered, feed refusals, duodenal samples, fecal samples, and bacterial pellets. A combustion analyzer interfaced with a stable isotope ratio mass spectrometer (VG Isotech, Middlewich, UK) was used for the measurement of ^{15}N in the bacterial pellets and duodenal samples. A gas chromatograph (model 5890, Hewlett-Packard Lab, Palo Alto, CA) equipped with a capillary column (30 m × 0.32 mm i.d., 1-µm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame ionization detection was used to determine the concentration of VFA and lactic acid in rumen fluid. The oven temperature was held at 170 °C for 4 min, which was then increased by 5 °C/min to 185 °C, and then by 3 °C/min to 220 °C, and held at this temperature for 1 min. The injector and detector temperatures were 225 °C and 250 °C, respectively. The carrier gas was helium. Concentrations of NH_3-N in the ruminal contents were determined as described by Rhine et al. (1998). Ruminal, duodenal, fecal, and plasma LPS were determined using a chromogenic Limulus amoebocyte lysate end-point assay (QCL-1000, Lonza Group Ltd., Basel, Switzerland) as described by Khafipour et al. (2009). For IgA determination, 1 g of feces was weighed and placed immediately in ultra-purified water at a concentration of 10% (wt/vol) by adding 9 mL of water in a 15-mL centrifuge tube. The tubes were vortexed and incubated overnight prior to centrifuging at 2,000 × *g* for 15 min at 4 °C. The supernatants were collected and analyzed for total IgA (Bovine IgA ELISA Quantitation Set, Bethyl Laboratories, Montgomery, TX).

Calculations and Statistical Analyses

Flow of duodenal DM and fecal DM was calculated by dividing consumed Yb (mg/d) by Yb concentration (mg/kg DM) in duodenal digesta or feces, respectively. Flows of other nutrients in duodenal or fecal samples were calculated by multiplying the DM flow with the concentration of nutrients in either duodenal digesta or feces. Ruminal microbial protein synthesis was calculated as the ratio of microbial ^{15}N flow (total ^{15}N – ammonia ^{15}N) at the duodenum to ^{15}N concentration of the bacterial pellets isolated from rumen contents. The protozoa count data were normalized by $\log_{10}$ transformation prior to statistical analysis.

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a 5 × 5 Latin square design. Treatment was the fixed effect, whereas the heifer and period were random effects in the MIXED model. Sampling day and sampling time points were considered as a repeated measurement. For repeated measures, various covariance structures were tested and AR(1) was selected based on the lowest value for Akaike's information criteria. The PDIFF option adjusted by the Tukey method was included in the LSMEANS statement to account for multiple comparisons among treatments. Differences between treatments were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS

Ruminal pH and Fermentation Characteristics

Mean and maximum ruminal pH were similar ($P > 0.58$) among treatments, whereas minimum pH was greater ($P < 0.05$) for heifers fed rSCFP and dSCFP compared with heifers fed control and rdSCFP (Table 2). No treatment effects on the duration of ruminal pH < 5.8 and < 5.2 were observed, but the duration of ruminal pH < 5.6 tended ($P < 0.10$) to be shorter for heifers fed rSCFP than for heifers fed control and ANT. Ruminal total VFA concentration was similar ($P > 0.56$) among treatments, whereas the molar proportion of acetate tended ($P < 0.10$) to be greater for heifers fed rSCFP than heifers fed control, ANT, and rdSCFP. In contrast, the molar proportion of butyrate was greatest ($P < 0.05$) for heifers fed rdSCFP, least ($P < 0.05$) for heifers fed rSCFP, and intermediate for heifers fed control, ANT, or dSCFP. The molar proportion of propionate and lactic acid concentrations did not differ ($P > 0.38$), and no treatment effect ($P > 0.43$) on ruminal NH_3–N concentration was observed. Total protozoa counts were greater ($P < 0.05$) in the rumen fluid for heifers fed rSCFP compared with heifers that received the other treatments.

Intake, Duodenal Flows, and Digestibility

Intakes of DM, OM, NDF, and starch tended ($P < 0.10$) to be greater for heifers fed rdSCFP compared with heifers fed control, ANT, and rSCFP (Table 3). Duodenal flow of total OM, microbial OM, NDF, and starch was similar ($P > 0.20$) among treatments, whereas the amount of OM truly fermented in the rumen was greater ($P < 0.05$) for heifers fed rdSCFP compared with heifers that received the other treatments. Digestibility of OM in the rumen was greatest ($P < 0.05$) for heifers fed rdSCFP, least ($P < 0.05$) for heifers fed control, and intermediate for heifers fed ANT, rSCFP, and dSCFP. Ruminal NDF digestibility was greater ($P < 0.05$) for heifers

Table 2. Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on ruminal pH and fermentation in finishing heifers

	Treatments[1]						
Item	Control	ANT	rSCFP	dSCFP	rdSCFP	SEM	*P*-value
Ruminal pH							
Mean	5.77	5.81	5.96	5.87	5.87	0.085	0.58
Minimum	5.02^{c}	5.13^{bc}	5.30^{a}	5.21^{ab}	5.05^{c}	0.070	0.03
Maximum	6.74	6.69	6.70	6.60	6.77	0.083	0.65
pH < 5.8, h/d	12.7	12.2	9.0	11.7	11.6	1.81	0.48
pH < 5.6, h/d	11.6	10.4	5.6	8.6	8.7	1.80	0.09
pH < 5.2, h/d	4.3	2.2	0.3	2.1	2.4	1.35	0.30
Volatile fatty acids (VFA)							
Total, m*M*	141.9	136.6	132.6	141.6	134.5	6.54	0.56
Acetate (A), mol/100 mol	47.2	47.2	51.8	47.3	45.1	2.32	0.06
Propionate (P), mol/100 mol	28.5	31.4	29.4	30.0	26.8	5.54	0.68
Butyrate, mol/100 mol	18.6^{ab}	15.4^{bc}	13.4^{c}	16.8^{bc}	22.3^{a}	3.40	0.01
A:P ratio	1.86	1.74	2.10	1.76	1.88	0.367	0.58
Lactic acid, m*M*	0.19	0.11	0.12	0.08	0.08	0.060	0.38
NH_3-N, m*M*	9.35	9.40	11.80	7.46	9.19	2.591	0.43
Protozoa, $\times 10^5$/mL	3.53^{b}	2.83^{b}	9.93^{a}	2.78^{b}	4.35^{b}	1.55	0.03

[a–c]Different superscripts within a row indicate significant difference ($P < 0.05$).

[1]Treatments: 1) control diet (no antibiotics, no SCFP addition), control plus 2) antibiotics (ANT; 330 mg monensin + 110 mg tylosin/d per heifer; top-dressed), 3) ruminal delivery of SCFP (rSCFP; 18 g/d per heifer; top dressed), 4) duodenal delivery of SCFP (dSCFP; 18 g/d per heifer; via duodenal cannula), or 5) combination of treatments #3 and #4 (rdSCFP; 18 g rSCFP + 18 g dSCFP/d per heifer).

fed rSCFP and rdSCFP compared with heifers fed control and ANT. No difference ($P > 0.14$) in ruminal starch digestibility was observed among treatments. Intestinal digestibility of OM, NDF, and starch, expressed as percentage of intake, did not differ ($P > 0.19$) among treatments. Total tract OM digestibility tended ($P < 0.10$) to be greater for heifers fed rdSCFP than for heifers fed control and ANT. Moreover, total tract NDF digestibility was greater ($P < 0.05$) for heifers fed rSCFP or rdSCFP compared with heifers fed control and ANT. However, there was no difference ($P > 0.35$) in total tract starch digestibility among treatments. Average BW did not differ ($P > 0.21$) among treatments.

Nitrogen Metabolism and Ruminal Microbial Protein Synthesis

Intake of N tended ($P < 0.10$) to be greater for heifers fed rdSCFP than for heifers fed control, ANT, or rSCFP diets (Table 4). However, duodenal flows of total N, microbial N, and feed N were similar ($P > 0.71$) among treatments. No difference ($P > 0.32$) in microbial protein efficiency was observed among treatments as well. Although ruminal and intestinal N digestibility was similar ($P > 0.92$) among treatments, total tract N digestibility tended ($P < 0.10$) to be greater for heifers fed rdSCFP compared with heifers fed control or ANT.

LPS Concentrations and Immune Responses

Ruminal, duodenal, and blood concentrations of LPS were similar ($P > 0.49$) among treatments, whereas fecal LPS concentration tended ($P < 0.06$) to be reduced for heifers fed ANT than for heifers that received the other treatments (Table 5). Fecal IgA concentration was greater ($P < 0.03$) for heifers

Table 3. Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on feed intake, duodenal flow, site, and extent of digestion in finishing beef heifers

	Treatments[1]						
Item	Control	ANT	rSCFP	dSCFP	rdSCFP	SEM	*P*-value
Intake, kg/d							
DM	12.2	11.8	11.8	12.6	13.0	0.66	0.09
OM	11.2	10.8	10.8	11.6	11.9	0.58	0.10
NDF	3.9	3.8	3.8	4.0	4.2	0.23	0.07
Starch	6.6	6.4	6.3	6.8	7.1	0.54	0.08
Average BW, kg	685	677	675	681	689	32.6	0.21
Duodenal Flow, kg/d							
OM	6.42	5.81	5.04	6.07	5.33	0.585	0.20
Microbial OM	1.48	1.52	1.22	1.25	1.43	0.167	0.20
NDF	1.68	1.63	1.47	1.60	1.54	0.158	0.65
Starch	1.50	1.37	1.08	1.42	1.08	0.181	0.24
RFOM[2]	5.54^{b}	5.73^{b}	6.20^{b}	6.18^{b}	7.19^{a}	0.366	0.03
Digestibility coefficient							
Rumen							
OM (truly)[3]	53.1^{c}	57.3^{bc}	62.7^{ab}	56.3^{bc}	64.8^{a}	2.84	0.03
NDF	41.3^{b}	39.3^{b}	52.9^{a}	46.6^{ab}	52.5^{a}	3.29	0.01
Starch	75.4	77.5	81.4	77.8	83.3	2.68	0.14
Intestinal							
OM	38.1	35.2	30.2	34.8	29.8	3.23	0.19
NDF	14.9	15.8	14.9	15.8	13.7	3.49	0.99
Starch	20.8	18.7	16.1	18.8	13.6	2.59	0.21
Total							
OM	77.2	77.4	80.7	79.8	81.7	1.23	0.07
NDF	56.2^{bc}	55.1^{c}	67.7^{a}	62.4^{ab}	66.1^{a}	2.55	0.01
Starch	96.3	96.2	97.5	96.6	96.9	0.55	0.35

[a–c]Different superscripts within a row indicate significant difference ($P < 0.05$).

[1]Treatments: 1) control diet (no antibiotics, no SCFP addition), control plus 2) antibiotics (ANT; 330 mg monensin + 110 mg tylosin/d per heifer; top-dressed), 3) ruminal delivery of SCFP (rSCFP; 18 g/d per heifer; top dressed), 4) duodenal delivery of SCFP (dSCFP; 18 g/d per heifer; via duodenal cannula), or 5) combination of treatments #3 and #4 (rdSCFP; 18 g rSCFP + 18 g dSCFP/d per heifer).

[2]RFOM: OM truly fermented in the rumen calculated by correcting for microbial OM.

[3]Corrected for microbial portion.

Table 4. Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on N metabolism in finishing beef heifers

	Treatments[1]						
Item	Control	ANT	rSCFP	dSCFP	rdSCFP	SEM	*P*-value
Intake, kg/d	275	268	266	285	296	18.9	0.07
Flow to duodenum							
Total N, g/d	272	261	256	279	285	32.0	0.85
Dietary N[2], g/d	113	107	115	132	130	17.8	0.71
Microbial N, g/d	151	147	135	139	149	19.5	0.81
Microbial efficiency[3]	27.5	26.2	21.3	23.1	20.9	3.24	0.32
Digestibility, % of intake							
Ruminal (truly)	56.3	57.4	54.9	52.1	52.5	5.68	0.92
Intestinal	69.4	71.5	70.9	69.7	76.3	7.03	0.93
Total tract	68.1	70.1	72.6	71.3	75.3	1.75	0.09

[1]Treatments: 1) control diet (no antibiotics, no SCFP addition), control plus 2) antibiotics (ANT; 330 mg monensin + 110 mg tylosin/d per heifer; top-dressed), 3) ruminal delivery of SCFP (rSCFP; 18 g/d per heifer; top dressed), 4) duodenal delivery of SCFP (dSCFP; 18 g/d per heifer; via duodenal cannula), or 5) combination of treatments #3 and #4 (rdSCFP; 18 g rSCFP + 18 g dSCFP/d per heifer).

[2]Feed N + endogenous N.

[3]Gram of microbial N/kg of OM truly fermented.

fed ANT compared with heifers fed control and rSCFP. However, fecal IgA was similar ($P > 0.15$) between heifers fed dSCFP and rdSCFP. No treatment effects ($P > 0.11$) on blood concentrations of glucose, urea N, NEFA, SAA, and LBP were observed.

DISCUSSION

Effects of SCFP Supplementation

The subacute ruminal acidosis (**SARA**) is characterized as the duration of ruminal pH < 5.6 exceeding 180 min (Plaizier et al., 2008). Supplementation of rSCFP improved ruminal pH status and potentially reduced the risks of SARA. Minimum pH was increased by 0.28 units, and pH < 5.6 was reduced by 6 h daily (11.6 vs. 5.6 h/d). However, the reduced effect on ruminal pH with rdSCFP (8.7 h/d of pH < 5.6) appeared to be attributed to a trend of greater DMI and more rumen fermented OM, which could offset the positive effect of SCFP on ruminal pH. The improved ruminal pH with SCFP confirmed the claim that SCFP helps prevent the decline in ruminal pH following feed ingestion (Williams et al., 1991). Several mechanisms by which the supplementation of SCFP maintained greater ruminal pH have been proposed. Williams et al. (1991) believed that the greater ruminal pH of steers supplemented with SCFP was a result of the decreased concentration of lactic acid. Callaway and Martin (1997) found that the supplementation of SCFP stimulated the growth of the lactic acid utilizing bacteria and the subsequent uptake of lactic acid. It has been suggested that organic acids and other growth factors (B vitamins, AA) provided by SCFP stimulate the growth of lactic acid utilizing bacteria in the rumen (Callaway and Martin, 1997). In the present study, SCFP supplementation numerically decreased ruminal concentration of lactic acid by 37% to 58%. Furthermore, rumen protozoa have been shown to stabilize ruminal pH by engulfing starch granules, thereby restricting starch access to amylolytic bacteria and reducing the rate of starch degradation in the rumen (Williams and Coleman, 1997). The greater ruminal protozoa counts with rSCFP confirmed the evidence that SCFP can modulate the concentration and generic composition of ruminal protozoa (Arakaki et al., 2000). The improved ruminal pH with rSCFP may partially be attributed to the increased protozoa counts. The reason for lack of effect of rdSCFP on protozoa count is not clear, but may be associated with lower ruminal minimum pH. Clarke (1977) reported that rumen protozoa are sensitive to changes in ruminal pH as they cannot survive at pH > 7.8 or pH < 5.0. Owens et al. (1998) suggested that with high-concentrate diets, the prevalence of protozoa in the rumen typically declines. Overall, no differences in ruminal pH profiles between control and dSCFP were expected since no SCFP was added into the rumen.

The effects of supplementation of SCFP on total VFA concentrations are inconsistent in the literature. In the present study, there was a lack of effect of treatment on total ruminal VFA, which is similar to results found by Moya et al. (2009)

Table 5. Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on concentrations of lipopolysaccharide (LPS), fecal IgA, blood metabolites and acute phase protein in finishing beef heifers

	Treatments[1]						
Item	Control	ANT	rSCFP	dSCFP	rdSCFP	SEM	*P*-value
LPS							
Ruminal, ×10^5 EU[2]/mL	14.45	14.13	9.33	16.22	12.88	3.18	0.49
Duodenal, ×10^5 EU/mL	0.71	0.59	0.42	0.50	0.42	0.15	0.83
Fecal, ×10^5 EU/g	10.72	5.75	8.13	10.96	9.77	2.08	0.06
Blood, EU/mL	0.11	0.10	0.10	0.11	0.11	0.006	0.52
Fecal IgA, µg/g	59.7^{b}	85.5^{a}	45.1^{bc}	72.6^{ab}	79.4^{ab}	10.7	0.03
Blood metabolites							
Glucose, mg/dL	76.3	74.5	69.7	71.9	74.8	3.05	0.37
Urea N, mg/dL	15.2	16.0	16.4	15.6	13.9	1.84	0.50
NEFA, µ*M*	47.1	50.4	58.0	44.4	47.2	6.81	0.70
Acute phase protein, µg/mL							
Serum amyloid A	38.0	21.3	34.4	24.8	47.3	7.19	0.11
LPS-binding protein	201.2	211.9	195.4	173.6	200.6	31.56	0.87

$^{a-c}$Different superscripts within a row indicate significant difference ($P < 0.05$).

[1]Treatments: 1) control diet (no antibiotics, no SCFP addition), control plus 2) antibiotics (ANT; 330 mg monensin + 110 mg tylosin/d per heifer; top-dressed), 3) ruminal delivery of SCFP (rSCFP; 18 g/d per heifer; top dressed), 4) duodenal delivery of SCFP (dSCFP; 18 g/d per heifer; via duodenal cannula), or 5) combination of treatments #3 and #4 (rdSCFP; 18 g rSCFP + 18 g dSCFP/d per heifer).

[2]EU = endotoxin unit.

and Li et al. (2016). Although variation of the total VFA concentration is in agreement with the variability of ruminal mean pH, it is not consistent with the amount of ruminal fermented OM, which was greater with heifers fed rdSCFP. In fact, ruminal total VFA concentration often does not reflect the amount of VFA produced in the rumen, because there is a dynamic balance between production and disappearance (absorption and passage) of VFA. The trend for a greater proportion of acetate with rSCFP or greater proportion of the sum of acetate and butyrate with rdSCFP is in accordance with the improved ruminal NDF digestibility.

The lack of the SCFP effect on ruminal NH_3-N concentration is in agreement with a previous study (Lehloenya et al., 2008). Acharya et al. (2017) found either no difference or decrease of ruminal NH_3-N concentration, depending on the source of SCFP supplemented in lactating dairy cows. No treatment effects on protein metabolism and microbial protein synthesis may explain the similar ruminal NH_3-N concentration among treatments.

Response of DMI to SCFP supplementation has been inconsistent with more studies showing no impact of SCFP than negative or positive effects on DMI (Beauchemin et al., 2006). Supplementation with rSCFP or dSCFP did not alter DMI compared with control, which agreed with studies evaluating SCFP by Swyers et al. (2014) in beef steers fed high-concentrate diet or by Acharya et al. (2017) in lactating dairy cows in mid-lactation. Altering ruminal digestibility or passage rate of feeds out of the rumen can influence feed intake. The trend of greater DMI with rdSCFP is consistent with higher ruminal OM digestibility. In addition, DMI with dSCFP was numerically greater ($P < 0.12$) than rSCFP treatment, suggesting an additive effect of dSCFP in combination with rSCFP which may have explained the improved effect of rdSCFP on DMI and ruminal digestion. The lack of an effect of SCFP on the flows of OM, NDF, and starch to the duodenum agrees with a previous study using beef steers supplemented SCFP (Lehloenya et al., 2008). Yoon and Stern (1996) suggested that a decrease in ruminal liquid dilution rate was attributed to an increase in ruminal OM digestibility and reduction of flows to the duodenum for cows fed SCFP. The trend for greater DMI was offset by the improved ruminal OM digestibility, thus resulting in the absence of differences in the duodenal OM flows.

The improved ruminal OM digestibility with rSCFP or rdSCFP compared with control was primarily due to the increased NDF digestibility and the numerically improved ruminal starch digestibility. In agreement with this study, Yoon and Stern (1996) reported that dairy cows fed SCFP improved ruminal true OM digestion by 12% compared with cows fed the control diet. In beef steers supplemented with SCFP, Lehloenya et al. (2008) found improved ruminal digestibility of OM, NDF, and

ADF in steers fed SCFP. The increased fiber digestion in the rumen has been one of the most consistently reported effects of SCFP supplementation (Tang et al., 2008). The increased ruminal NDF digestibility with SCFP supplementation could be explained by an increased number of cellulolytic bacteria and fungi (Yoon and Stern, 1996; Mao et al., 2013) and improved ruminal pH status.

The absence of significant effects of SCFP on intestinal digestibility appears to be in agreement with a study where lactating dairy cows were fed SCFP (Yoon and Stern, 1996). In that study, the authors reported that although ruminal OM digestibility improved by adding SCFP, total tract OM digestibility did not differ. This indicates that there were no effects of SCFP supplementation on the intestinal digestibility. However, Lehloenya et al. (2008) found a trend of increased total tract digestibility of OM and NDF even though there were no effects of SCFP supplementation on ruminal digestibility of OM and NDF in beef steers. This suggests a possible improvement in intestinal digestibility of OM and NDF by feeding SCFP. The discrepancy between the present and previous studies (Lehloenya et al., 2008) in the effects of SCFP on intestinal digestibility could be attributed to the difference in SCFP used, dosage of SCFP (18 vs. 56 g/d, respectively), or the diet (high concentrate vs. high forage diet, respectively). In the present study, direct input of SCFP into the small intestine via the duodenal cannula ensured that the SCFP was not digested in the rumen or abomasum and maintained its activity in the intestine. Therefore, the lack of SCFP supplementing effect on intestinal digestibility of OM and NDF might be due to insufficient dosage of SCFP compared with the study by Lehloenya et al. (2008) or feeding a high concentrate diet that had limited extent for fiber digestion to be improved in the intestine by SCFP.

Supplementation of SCFP appeared to have a lesser impact on ruminal degradation and intestinal digestion of CP than on NDF digestion. The tendency of greater N intake in heifers fed rdSCFP mirrored the greater DMI for heifers fed rdSCFP. Similarly, Lehloenya et al. (2008) reported a trend for a difference in N intake, but no impact on microbial protein synthesis and protein metabolism in the digestive tract of steers supplemented with SCFP. Increases in the amount of ruminal OM digestion with rdSCFP did not support greater microbial protein synthesis, which is in agreement with Yoon and Stern (1996).

An increased concentration of ruminal LPS is usually associated with a decreased ruminal pH (Khafipour et al., 2009). Li et al. (2016) reported that supplementation of SCFP tended to reduce the LPS concentration in rumen fluid (about 37%) when dairy cows were experiencing grain-induced SARA. Blood LPS, mainly translocated from the rumen and hindgut, is a strong inflammatory factor and can stimulate the release of many inflammatory cytokines (Plaizier et al., 2012). The LBP and SAA are two important acute phase proteins in cattle and can be stimulated by blood LPS. The LBP is reported to transfer and magnify the signal of LPS and stimulate the inflammatory response (Plaizier et al., 2012), whereas SAA is reported to modulate the innate immune reaction or reduce oxidative damage (Ceciliani et al., 2012). Therefore, the lack of difference in blood LBP and SAA between heifers fed control and heifers fed SCFP can be explained by their similar ruminal and fecal LPS concentrations.

The IgA secreted by the gut plays crucial roles in the mucosal defense by entrapping microorganisms, preventing the adherence of pathogens to the mucosal surface and maintaining a stable gut microbiota (Neutra and Kozlowski, 2006). Hence, fecal IgA concentration has been used as an indicator of mucosal immunity (Suzuki et al., 2004). The trend for greater fecal IgA concentration with duodenal delivery of SCFP (dSCFP and rdSCFP) compared with control may suggest an improvement of mucosal immunity by SCFP. Feye et al. (2016) reported that feedlot heifers fed SCFP reduced fecal shedding of *Salmonella* and *Escherichia coli*, which may be associated with the improved mucosal immunity.

Similar blood glucose concentrations between SCFP supplemented heifers and control heifers are consistent with the lack of difference among treatments for ruminal propionate concentrations and intestinal starch digestibility. Propionate is an important precursor of glucose, and more net glucose can be transferred from the small intestine to the liver if more starch is digested in the small intestine. No treatment effects of adding SCFP on glucose concentration were reported in a previous study using beef cattle (Lehloenya et al., 2008). Both glucose and NEFA are important indicators of energy status. Although the short-term periods of the Latin square design are not conducive to measuring changes in weight gain in cattle, the lack of treatment effect on average BW and ADG (data not shown) in the present study is consistent with the similar blood concentrations of glucose and NEFA. These results indicated that the energy status of beef heifers was not influenced

by the treatments under the current experimental conditions.

Effects of Antibiotics vs. SCFP

Monensin is widely used in commercial beef cattle production for altering ruminal fermentation patterns and to improve feed efficiency (Meyer et al., 2009). Tylosin is commonly used in beef cattle production for preventing liver abscesses. The missing effects of monensin on DMI, rumen fermentation pattern, and protein degradation seemed to be inconsistent with the general known mode of action of monensin in the rumen (Tedeschi et al., 2003). DiLorenzo and Galyean (2010) indicated that high energy diets containing high grain or high fat may need a greater amount of monensin than recommended (CFIA, 2018) because of a lesser response to the effects of monensin with a high energy diet than a high-forage diet (Duffield et al., 2012). In a recent study, we found a decrease in DMI and ruminal ratio of acetate to propionate using a high (48 mg/kg diet DM) dose of monensin, but found no difference with a low (28 mg/kg diet DM) dose of monensin (Xu et al., 2013; Yang et al., 2014). The recommended monensin dose by Canadian Food Inspection Agent was used in the present study (330 mg/d), which may partly explain the lack of monensin effects.

Compared with the ANT diet, rSCFP supplementation improved ruminal pH status, had greater protozoa counts, and improved digestibility of OM and NDF in the rumen and total digestive tract. In general, SCFP improves fiber digestion (Swyers et al., 2014) by stimulating ruminal cellulolytic bacteria, particularly in ruminants fed high-roughage diets (Newbold et al., 1993). However, feeding monensin inhibits fiber digestibility in beef cattle (Nagaraja et al., 1997). It has been reported that adding monensin reduced ruminal gene copies of fiber digesting bacteria *Ruminococcus flavefaciens* and *Ruminococcus albus* (Narvaez et al., 2013; Jiao et al., 2017), which may explain the reduction of NDF digestion with monensin compared with SCFP supplementation. Furthermore, fewer protozoa counts with monensin may partly explain the lesser ruminal NDF digestibility for ANT compared with SCFP. Monensin is known to inhibit ruminal protein degradation and decrease flow of microbial protein to the intestine (Ruiz et al., 2001). It appears that both monensin and SCFP had little effects on ruminal protein degradation and microbial protein synthesis under current experimental conditions. Recently, several studies (Swyers et al., 2014; Scott et al., 2016) reported that replacing monensin and tylosin in conventional feedlot diets with SCFP resulted in similar growth performance, carcass characteristics, and liver abscess prevalence in finishing beef cattle. These results suggest that feeding SCFP demonstrates a beneficial impact on improved ruminal pH status and fiber digestibility over monensin and tylosin without compromising feed efficiency and increasing the risk of liver abscess. The lack of difference in blood LBP and SAA between heifers fed ANT and heifers fed SCFP may be explained by their similar ruminal and blood LPS concentrations even though the fecal LPS concentration of heifers fed ANT vs. SCFP diets tended to be lower. In fact, the trend of lower fecal LPS concentration of heifers fed ANT did not change the blood LPS concentration although it was reported that translocation of endotoxins into bloodstream appeared to be greater from hindgut than rumen (Khafipour et al., 2009). Moreover, the fact that fecal IgA concentration of heifers fed ANT was greater than heifers fed control or rSCFP but it was similar to heifers fed either dSCFP or rdSCFP suggests comparable activity between ANT and SCFP in the improvement of mucosal immunity. The results also emphasize that ruminal passage of SCFP may reduce their activity in the intestine.

In conclusion, supplementation of a high grain diet with rSCFP elevated the ruminal minimum pH and reduced the duration of pH < 5.6 by 6 h compared with control heifers. A trend ($P < 0.10$) of increasing molar proportion of acetate and increased rumen protozoa counts along with improved ruminal and total NDF digestibility with rSCFP compared with control indicate that adding SCFP to high concentrate diets may alleviate ruminal acidosis and increase fibrolytic microbial activity. Supplementing rdSCFP increased ruminally fermented OM and ruminal and total tract digestibility of OM and NDF. The duodenal delivery of SCFP resulted in a trend for greater fecal IgA concentration suggesting potential increase immune response. The present study also demonstrated an advantage of feeding SCFP over ANT (monensin and tylosin) in reducing the risk of rumen acidosis and improving nutrient digestibility. These results suggest that SCFP could be a natural alternative to monensin and tylosin in feedlot cattle.

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