Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Performance and health

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ABSTRACT

The aim of the study was to evaluate the effects of *Saccharomyces cerevisiae* fermentation products (SCFP) on performance and health of calves during the first 63 d of age. Sixty Holstein calves (30 males and 30 females) at 2 d of age were blocked by sex and date of birth then randomly assigned within blocks to 1 of 3 treatments. A texturized calf starter was fed ad libitum containing 0 (control), 0.5, or 1% SCFP (Original XPC, Diamond V, Cedar Rapids, IA) of DM. In addition, the supplemented calves were fed 1 g/d SCFP (SmartCare, Diamond V) in milk until d 30. All calves were fed 4 L of colostrum within 1 h of birth and were subsequently fed milk twice daily until weaned at 56 d of age. Male calves were harvested on d 56. Performance and health of weaned female calves were monitored until 63 d of age to determine the effect of preweaning treatment of SCFP on weaning stress. Starter intake, fecal scores, and medical treatments were recorded daily. Body weight measures and blood samples were collected on d 2, 28, 56, and 63. Serum was analyzed for blood urea nitrogen, fatty acids, insulin-like growth factor-1, glucose, and total protein. Oxidative biomarkers and total antioxidant capacity were also evaluated in the serum. Body weight, DMI, blood parameters, and oxidative biomarkers did not differ among treatments. Supplementation of SCFP lowered fecal scores in the pre- and postweaning periods. *Saccharomyces cerevisiae* fermentation products can be used to reduce the diarrhea in calves grown under normal commercial conditions.

Key words: calf, *Saccharomyces cerevisiae* fermentation products, health, stress

INTRODUCTION

Raising replacement heifers is dependent on successful management of the calf in the preweaning period. It has been shown that growth of the calf and future performance of the cow is enhanced by a higher plane of nutrition in the preweaning period (Soberon et al., 2012). Moreover, reduction of disease and number of treatments in the preweaning period has a significant effect on production during the first lactation (Heinrichs and Heinrichs, 2011). It is thus imperative that dairy farms implement strategies to optimize health and growth early in the life of a calf. Although preventive strategies such as proper hygiene may be practiced on the farm, colonization of the gut by pathogenic bacteria is still a common phenomenon on many intensive-rearing systems (Fuller, 1989). When the gut is infected, calves will often experience poor digestion and reduced absorption of nutrients (Signorini et al., 2012); these digestive problems often precipitate into diarrhea (Magalhães et al., 2008; NAHMS, 2011). In the report provided by the National Animal Health Monitoring System (NAHMS, 2011), more than 25% of preweaning calves experienced digestive problems, out of which 70% were treated with antibiotics. This does not only lead to poor growth in calves, but has a negative ripple effect on farm profits. Strategies to mitigate the negative effects of diarrhea include good nutrition and health management. Ensuring a calf transitions smoothly from milk to solid feed offers another challenge to producers, as the calf may experience stress leading to impaired resistance to diseases.

Saccharomyces cerevisiae fermentation products (SCFP) have been extensively used in the dairy industry with beneficial effects on cow production parameters (Poppy et al., 2012). In calves, DMI, BW gain, and health were improved when SCFP was included in the calf starter (Cole et al., 1992; Lesmeister et al., 2004). Improvement in rumen fermentation, stability in gut microbiota, as well as stimulation of the gut morphology could explain the observed results (Quigley

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et al., 1992; Brewer et al., 2014). Alternatively, SCFP may independently modulate the immune system in addition to conventionally proposed modes of action, as seen in nonruminants (Shen et al., 2009) and ruminants (Zaworski et al., 2014). As a component of the immune system, the antioxidative system can also be stimulated by SCFP (Jensen et al., 2008).

Several factors may determine the efficacy of SCFP. The nature of the diet, physiological status of the animal, and, possibly, the mode of supplementation of feed additives may influence the effect of SCFP in calves (Chaucheyras-Durand et al., 2008). Previous experiments have shown that health effects of SCFP in dairy calves are observed more apparently in animals under stress, such as weaning or poor health (Lesmeister et al., 2004; Brewer et al., 2014). Magalhães et al. (2008) fed preweaning calves SCFP at 2% of DM in the starter and found the risk of mortality was 6-fold lower past 13 d of age when calves consumed significant amount of SCFP through the starter. *Saccharomyces cerevisiae* fermentation products can be supplemented in milk, milk replacer, or calf starter. Supplementing calves with SCFP in milk or milk replacer can ensure the consumption of the product from a very early age. Brewer et al. (2014) demonstrated a reduction in diarrhea, fever, days of shedding, and intestinal colonization of *Salmonella* following a *Salmonella* challenge when calves were supplemented with SCFP in both starter and liquid feed. Experiments with unchallenged calves managed on a commercial dairy would give us a practical indication into the efficacy of SCFP. We hypothesized that SCFP supplemented in both milk and calf starter would lead to improved performance and health in the first 63 d of life under normal feeding and management conditions. The specific aims of our study were to determine the effects of SCFP on preweaning performance and weaning stress.

MATERIALS AND METHODS

Treatments, Animals, and Feeding

The study was conducted at the Dairy Teaching and Research Farm (Shunyi, Beijing). This facility houses approximately 4,000 dairy cattle, 2,000 of which are milking cows. Sixty (60) Holstein calves (30 male and 30 female) were separated from their dams immediately after birth. The experiment was conducted as a randomized complete block design. Calves were enrolled in the experiment over a period of 1 mo from May to June 2014 and were blocked by date of birth in separate rows for male and female. In total, we used 10 blocks of 6 (3 male or 3 female) calves for the trial. Calves

were housed in individual hutches that contained straw bedding that was renewed weekly. Calves (2 ± 1 d of age) within each block were randomly assigned to 1 of the 3 treatments: no SmartCare or XPC (**CON**; both from Diamond V, Cedar Rapids, IA), 1 g of SmartCare in the milk + 0.5% XPC in the starter (**SCFP1**), and 1 g of SmartCare in the milk + 1% XPC in the starter (**SCFP2**). All calves received 4 L of colostrum within 1 h of birth and were fed pasteurized nonsaleable whole milk twice daily at 0700 and 1500 h. Calves received 6 L/d of milk from d 2 to 10 and 8 L/d from d 11 to 42. Stepwise weaning was applied where calves received 6 L/d from d 43 to 49 and 4 L/d afterward until weaning on d 56. Nonsaleable milk was pasteurized using a milk pasteurizer, where milk temperature was increased and held at 60°C for 30 min and then cooled to 37°C. At weaning (d 56), male calves were harvested for analysis of ruminal parameters (reported in Xiao et al., 2016), whereas female calves remained on the study until d 63. All calves were fed the same calf starter, with the exception of the substitution of XPC for corn germ meal in the treatment groups (Table 1) that met or exceeded nutrient requirements for preweaning and early weaned Holstein calves as suggested by NRC (2001). Starter grain (Honneur Nutritional Technology Co. Ltd., Beijing, China) was offered once every morning after milk feeding during the first 56 d (male calves) or 63 d of life (female calves) for ad libitum intake. Newly fed and refused starter were recorded daily. Water was also offered ad libitum.

Sample Collection

Body Measurements and Feed Samples. Both male and female calves were weighed on a digital scale on the day of study enrollment (d 2) and again on d 28 and 56, and then only female calves were weighed on d 63. Body length, withers height, hip width, and heart girth were also recorded at these time points. Representative samples of starter from the control and 2 SCFP treatments were collected in the first and last week of the experiment. The samples were analyzed for nutritional composition separately at the end of the experiment. Dry matter, CP, NDF and ADF were analyzed following AOAC International (1995).

Fecal Scoring. For consistency purposes, feces were scored daily before the morning milk feeding using a 1 to 4 scale (Magalhães et al., 2008). Fecal consistency was scored as 1 when firm, 2 when soft or of moderate consistency, 3 when runny or as mild diarrhea, and 4 when watery and profuse diarrhea. A diarrheic day was defined when the fecal score (**FS**) was >2 . The scoring was done by 1 trained observer throughout the experi-

mental period, but the calf hutchers had no apparent treatment markings to influence his scoring decision.

Incidence of Health Disorders, Treatments, and Costs Associated with Treatments. Antibiotics and electrolytes administered were recorded daily for each calf. Calves were checked on a daily basis for incidences of health disorders before morning and afternoon milk feeding by the farm veterinary and a member of the research team. Inappetence, breathing difficulties, feces with blood, and attitude scores were recorded for individual calves. If calves had FS >2 or had signs such as fever with temperatures $\geq 39.5^{\circ}\text{C}$, inappetence, droopy ears, nasal discharge, and labored breathing, they were treated according to the recommendations and protocols established by the farm veterinarian. Calves were treated with ofloxacin (Tianjin Pharmaceutical Co. Ltd., Tianjin, China) for a maximum of 5 d through an intramuscular injection (10 mL) at the neck muscles. Antibiotics treatment was stopped for 2 d; if there was no improvement in FS, calves were further treated for 3 d. Calves that showed severe diarrhea were treated using ofloxacin (10 mL) and *Andrographis paniculata*

injection (10 mL; Dazheng Tec-Phar. Co. Ltd., Changchun, China). Kaolin (30 g per feeding; Aether Centre Enterprises Ltd., Beijing, China), a nonantibiotic, was also added in milk until the calf fully recovered from diarrhea. One calf each in CON and SCFP2 developed breathing complications and were treated with 2.5 mg/kg of BW of Draxxin injection (Pfizer Animal Health, Hampstead, NC). Electrolytes were also administered intravenously through the jugular vein to calves that had FS >2 or were visually dehydrated until they had fully recovered, 1 to 2 h after morning or evening milk feeding or both. The electrolyte therapy included 0.5 L of sodium bicarbonate (Kangquan Animal Pharmaceutical Co. Ltd., Hebei, China), 1.0 L of glucose and sodium chloride injection (50 g of glucose and 9 g of sodium chloride, Kexing Pharmaceutical Co. Ltd., Hebei, China), and 0.5 L of compound sodium chloride (Kexing Pharmaceutical Co. Ltd.).

Blood Collection and Analysis. Blood was sampled on d 2, 28, 56, and 63 to determine concentrations of BUN, fatty acids, IGF-1, glucose, total protein (TP), and oxidative biomarkers [glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAOC)]. Briefly, blood was collected via the jugular vein using evacuated tubes containing no anticoagulant for serum separation. Blood was then centrifuged at $3,500 \times g$ for 15 min in a refrigerated centrifuge at 4°C for separation of serum. A subsample of serum was used to determine total serum protein by a hand-held optical refractometer (model WZ-312, Honneur Nutritional Technology Co. Ltd.) and glucose concentration using a hand-held digital glucometer (model Accusmart 720, Yuwell Co. Ltd., Jiangsu, China). Remaining serum was partitioned into aliquots and stored in 1.5-mL microcentrifuge tubes at -20°C for later analysis. Oxidative biomarkers (MDA, SOD, GSH-Px, and TAOC) were analyzed using the thiobarbituric acid, xanthine oxidase reaction, hydroxylamine, and calorimetry methods, respectively, according to kit instructions (Jiancheng Bioengineering Institute, Nanjing, China). Insulin-like growth factor was analyzed using ELISA kits (Shanghai Huole Biotechnological Science and Technology Co. Ltd., Shanghai, China). Blood urea nitrogen was analyzed using enzymatic method adapted from Talke and Schubert (1965) using commercial kits per manufacturer's instructions (Shandong Boke Co. Ltd., Zibo, China). Fatty acids were analyzed by calorimetry (Jiancheng Bioengineering Institute) using commercial kits per the manufacturer's instructions. Concentrations of BUN, fatty acids, IGF-1, GSH-Px, SOD, MDA, and TAOC were determined by an automated biochemistry Analyzer (Model GF-D200, Caihong Co., Shandong,

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

Item	CON	SCFP1 ¹	SCFP2 ¹
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
Dry distillers grain with solubles	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 absorbents	0.1	0.1	0.1
Premix compound for calf ²	0.5	0.5	0.5
XPC	0	0.5	1.0
Nutrient composition			
DM, %	90.9	91.8	90.5
CP, % of DM	19.6	18.9	18.7
NE _L , Mcal/kg	1.73	1.73	1.73
NDF, % of DM	21.3	20.3	20.7
ADF, % of DM	8.2	7.9	8.1
Calcium, % of DM	0.97	0.97	0.97
Phosphorous, % of DM	0.69	0.69	0.69

¹SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

²Premix compound contained (mg/kg) copper 60, iron 60, zinc 80, manganese 80, cobalt 0.4, iodine 1, and selenium 1, and also contained (IU/g) vitamin A 12,000, vitamin D 3,000, and vitamin E 120.

³Calcium and phosphorus were as provided by the feed manufacturer.

China). Their intra- and interassay coefficients of variation were 3.2 and 6.5, 5.0 and 6.4, 4.4 and 8.9, 6.9 and 11.0, 8.3 and 10.0, 7.3 and 10.0, and 8.1 and 9.9 for BUN, fatty acids, IGF-1, GSH-Px, SOD, MDA, and TAOC, respectively.

Statistical Analysis

During the experiment, 1 male calf in the control group died on d 50 from peritonitis. Data from this calf were not included in the analysis. Data were analyzed as a randomized complete block design. Dependent variables (starter intake, BW, ADG, feed efficiency, body measurements, blood metabolites, and oxidative biomarkers) were analyzed using the PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The model included block, treatment, time, and interaction between treatment and time as fixed effects. The sex effect was not included in the model, as it was tested and found not significant. Degrees of freedom were estimated by using the Kenward-Roger method in the model statement. The subject of the repeated statement was calf nested within treatment. The covariance structure was modeled according to Littell et al. (1998). Compound symmetry and anteregressive (1) were the covariance structures tested for the within-calf measurements; the one with the best fit determined by the lowest Akaike information criterion was used. The effect of dietary treatment on the categorical responses

related to FS (number of days calf within each treatment had a specific FS in each period), use of medication (antibiotics and nonantibiotics), and electrolytes parameters were tested using the GLIMMIX procedure of SAS version 9.2, with Gaussian distribution for FS and Poisson distribution for medication and electrolytes usages. The model included the effects of treatment and time. For antibiotics and a combination of antibiotics and nonantibiotics, data were first reduced to the number of times a calf was treated. Each time consisted of 5 or 3 consecutive days for the first and second rounds of treatment, respectively. Conversely, kaolin and electrolytes was analyzed based on the days treated for each calf within a specific period. 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). Significance was declared at $P \leq 0.05$ and a trend was declared at $0.05 < P \leq 0.10$. Data were reported as least squares means $\pm$ standard error of the mean. The calf was the experimental unit.

RESULTS

Feed Intake, ADG, and Feed Efficiency

Calves consumed all the milk offered to them daily. Starter intake was not different between the control and treatment groups in the pre- or postweaning peri-

Table 2. Least squares means for starter intake, calf BW, ADG, and feed efficiency (FE) for calves fed diets with or without *Saccharomyces cerevisiae* fermentation products (SCFP)

Item	Treatment ¹			SEM	P-value	
	CON	SCFP1	SCFP2		CON vs. SCFP	SCFP1 vs. SCFP2
Starter intake, g/d						
d 4–28	91.4	82.4	93.8	9.79	0.79	0.41
d 29–56	484.1	486.3	542.1	50.35	0.64	0.44
Prewaning (d 4–56)	313.5	305.7	341.4	31.25	0.79	0.41
Postweaning (d 57–63)	2,154.8	2,188.1	2,084.1	115.87	0.90	0.53
BW, kg						
Initial (d 2)	40.0	39.6	39.3	0.76	0.56	0.78
d 28	52.7	53.6	53.2	1.00	0.59	0.74
Weaning (d 56)	77.9	78.9	78.9	1.37	0.56	0.99
Final (d 63)	83.8	85.6	85.8	1.87	0.41	0.93
ADG, kg/d						
d 4–28	0.49	0.54	0.53	0.03	0.13	0.84
d 29–56	0.90	0.90	0.92	0.03	0.70	0.59
Prewaning (d 4–56)	0.70	0.72	0.73	0.04	0.21	0.83
Postweaning (d 57–63)	1.09	1.13	1.12	0.06	0.62	0.97
FE ²						
d 4–28	0.20	0.15	0.17	0.02	0.17	0.53
d 29–56	0.54	0.53	0.57	0.05	0.96	0.62
Prewaning (d 4–56)	0.37	0.34	0.37	0.04	0.74	0.63
Postweaning (d 57–63)	2.10	2.11	1.83	0.15	0.46	0.20

¹CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

²FE = feed intake/gain.

ods (Table 2). During the preweaning period, SCFP2 consumed numerically more starter than CON and SCFP1 groups. Starter intake increased as calves grew older, but increased more rapidly from d 42 when milk was reduced ($P = 0.001$; data not shown).

Initial, d 28, weaning, and final mean BW were similar among the treatment groups (Table 2). Average daily gain was not influenced by SCFP during preweaning ($P = 0.21$) or postweaning ($P = 0.62$). Likewise, pre- and postweaning feed efficiency was similar between the control and SCFP groups.

Structural Measurements and Blood Metabolites

Least squares means for initial, d 28, weaning, and postweaning withers height, body length, heart girth, and hip width are shown in Table 3. No treatment effects were observed at any of the times sampled for structural growth measurements ($P > 0.05$).

The least squares means for concentrations of glucose, fatty acids, TP, BUN, and IGF-1 are shown in Table 4. We found no treatment effects on glucose, fatty acids, TP, BUN, or IGF-1 concentrations at the different points of sampling. An effect of time (i.e., age of calves) was observed in all the blood parameters ($P < 0.001$) and IGF-1 ($P = 0.02$). Glucose ($P < 0.001$) concentrations decreased as calves grew older. Fatty acid values were highest on d 2 (Table 4), which could be attributed to fat mobilization, as the calves were fed colostrum only once before blood was collected 24 h

later. Supplementation of SCFP tended to increase TP on d 28 ($P = 0.09$). Urea in serum tended to be lower with SCFP2 compared with SCFP1 on d 28 ($P = 0.08$). No time $\times$ treatment interaction was observed for glucose, fatty acids, TP, BUN, or IGF-1 concentrations.

Oxidative Biomarkers

Table 5 shows the effect of SCFP supplementation on the oxidative status parameters. Supplementation of SCFP had no effect on serum concentrations of GSH-Px, SOD, MDA, and TAOC ($P > 0.05$). We found time-dependent changes in the concentration of oxidative biomarkers ($P < 0.001$), but no differences due to SCFP supplementation were observed at specific time points sampled. Superoxide dismutase activity was highest at weaning, whereas GSH-Px activity increased with age throughout the experimental period ($P < 0.001$). The levels of MDA were highest at weaning but lower on d 28 and 63. The activity of TAOC decreased with time until weaning and then increased postweaning ($P < 0.001$).

Calf Health

Almost all calves experienced diarrhea during the experimental period, based on the fecal scores (Table 6). Diarrhea, defined as calves with FS > 2 was prevalent in the first 28 d. During this period, calves fed SCFP had fewer diarrhea days by having higher number of

Table 3. Least squares means for calf structural growth for calves fed diets with or without *Saccharomyces cerevisiae* fermentation products (SCFP)

Item	Treatment ¹			SEM	P-value	
	CON	SCFP1	SCFP2		CON vs. SCFP	SCFP1 vs. SCFP2
Withers height, cm						
Initial (d 2)	75.9	76.1	75.6	0.49	0.94	0.48
d 28	80.8	80.7	80.7	0.55	0.94	0.97
Weaning (d 56)	89.6	89.7	89.6	0.60	0.97	0.89
Postweaning (d 63)	90.8	90.7	90.5	0.72	0.87	0.83
Heart girth, cm						
Initial (d 2)	78.9	78.4	78.5	0.59	0.56	0.94
d 28	85.3	85.6	86.3	0.66	0.57	0.30
Weaning (d 56)	96.3	96.8	95.6	0.76	0.92	0.24
Postweaning (d 63)	98.1	98.7	98.4	0.81	0.66	0.84
Body length, cm						
Initial (d 2)	69.2	70.1	69.6	0.58	0.38	0.49
d 28	76.0	77.3	76.6	0.60	0.20	0.41
Weaning (d 56)	86.1	88.0	87.2	0.69	0.09	0.39
Postweaning (d 63)	88.7	89.2	89.3	0.95	0.64	0.97
Hip width, cm						
Initial (d 2)	74.2	74.4	74.1	0.70	0.94	0.71
d 28	80.0	80.8	81.2	0.86	0.37	0.73
Weaning (d 56)	92.1	92.5	91.4	0.93	0.86	0.43
Postweaning (d 63)	95.5	97.9	96.4	1.23	0.29	0.39

¹CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

Table 4. Least squares means for blood metabolites for calves fed diets with or without *Saccharomyces cerevisiae* fermentation products (SCFP)

Item	Treatment ¹			SEM	P-value	
	CON	SCFP1	SCFP2		CON vs. SCFP	SCFP1 vs. SCFP2
Glucose, mmol/L						
Initial (d 2)	6.34	6.31	6.45	0.25	0.90	0.70
d 28	5.64	5.49	5.78	0.17	0.97	0.21
Weaning (d 56)	5.14	5.25	5.05	0.10	0.94	0.17
Postweaning (d 63)	5.00	5.13	5.15	0.12	0.36	0.91
Fatty acids, μ mol/L						
Initial (d 2)	114.9	114.5	110.4	3.46	0.58	0.40
d 28	89.4	86.2	87.9	3.19	0.57	0.70
Weaning (d 56)	89.7	90.9	91.5	2.38	0.62	0.86
Postweaning (d 63)	92.3	93.3	93.6	1.65	0.57	0.90
Total protein, g/dL						
Initial (d 2)	5.89	5.96	6.03	0.09	0.36	0.57
d 28	5.05	5.14	5.29	0.08	0.09	0.19
Weaning (d 56)	5.52	5.54	5.61	0.06	0.49	0.42
Postweaning (d 63)	5.61	5.72	5.65	0.08	0.45	0.56
Urea, mmol/L						
Initial (d 2)	3.06	3.09	2.97	0.16	0.89	0.60
d 28	4.21	4.23	3.76	0.19	0.36	0.08
Weaning (d 56)	5.06	4.86	5.27	0.51	0.99	0.57
Postweaning (d 63)	3.57	3.36	3.25	0.23	0.36	0.73
IGF-1, ng/mL						
Initial (d 2)	263.0	263.2	272.9	17.99	0.83	0.69
d 28	241.4	244.1	243.1	7.44	0.81	0.92
Weaning (d 56)	250.3	257.8	254.1	5.86	0.44	0.66
Postweaning (d 63)	236.1	239.9	239.4	6.40	0.64	0.96

¹CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

Table 5. Least squares means for oxidative biomarkers for calves fed diets with or without *Saccharomyces cerevisiae* fermentation products (SCFP)

Item ¹	Treatment ²			SEM	P-value	
	CON	SCFP1	SCFP2		CON vs. SCFP	SCFP1 vs. SCFP2
GSH-Px, μ mol/mL						
Initial (d 2)	99.7	95.9	87.3	10.84	0.56	0.56
d 28	120.5	102.8	114.1	24.29	0.68	0.74
Weaning (d 56)	407.7	409.4	396.4	31.80	0.90	0.77
Postweaning (d 63)	858.7	795.6	835.7	50.82	0.50	0.59
SOD, U/mL						
Initial (d 2)	155.3	149.2	152.7	1.92	0.08	0.19
d 28	152.7	152.8	152.6	2.68	0.98	0.96
Weaning (d 56)	167.9	167.6	167.1	1.83	0.81	0.82
Postweaning (d 63)	163.4	163.1	166.1	2.00	0.65	0.30
MDA, nmol/L						
Initial (d 2)	2.54	2.83	2.66	0.30	0.58	0.68
d 28	2.35	2.22	2.17	0.25	0.62	0.89
Weaning (d 56)	3.60	3.34	3.30	0.16	0.16	0.87
Postweaning (d 63)	2.51	2.52	2.69	0.21	0.71	0.60
TAOC, U/mL						
Initial (d 2)	4.97	4.92	5.01	1.05	0.99	0.95
d 28	3.03	2.40	2.65	0.42	0.33	0.68
Weaning (d 56)	1.61	1.99	1.44	0.37	0.82	0.30
Postweaning (d 63)	1.90	2.57	2.49	0.52	0.32	0.92

¹GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde; TAOC = total antioxidant count.

²CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

Table 6. Effect of *Saccharomyces cerevisiae* fermentation products (SCFP) on fecal score (FS) in calves

Item	Treatment ^{1,2}			SEM	P-value	
	CON	SCFP1	SCFP2		CON vs. SCFP	SCFP1 vs. SCFP2
d 4–28	n = 19	n = 20	n = 20			
FS1	9.1	12.9	13.4	0.78	0.001	0.62
FS2	7.7	7.9	7.2	0.51	0.77	0.34
FS3	6.7	4.9	4.6	0.53	0.001	0.74
FS4	2.7	0.4	0.6	0.26	0.001	0.68
d 29–56	n = 19	n = 20	n = 20			
FS1	13.2	18.1	18.0	0.97	0.001	0.91
FS2	10.7	7.9	8.2	0.93	0.02	0.82
FS3	2.5	0.8	0.3	0.29	0.001	0.23
FS4	0.2	0.1	0.2	0.12	0.83	0.77
d 57–63	n = 10	n = 10	n = 10			
FS1	1.3	2.5	2.8	0.65	0.10	0.77
FS2	3.2	2.8	3.2	0.64	0.80	0.20
FS3	2.3	1.0	0.1	0.46	0.001	0.18
FS4	0.0	0.2	0.0	0.12	0.49	0.23

¹CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

²Least squares means of days calves were in fecal score 1 to 4 (FS1 = fecal score 1, FS2 = fecal score 2, FS3 = fecal score 3, FS4 = fecal score 3).

days with FS of 1 and lower number of days with FS >2 ($P < 0.001$) compared with control calves. Fecal score 2 was similar between SCFP and CON groups in the same period. Calves in CON group had more days with FS of 3 ($P < 0.001$) in d 29 to 56 and d

57 to 63. Calves were treated by both antibiotics and nonantibiotics (Table 7). Number of times calves were treated using antibiotics ofloxacin or a combination of ofloxacin and *Andrographis paniculata* were not different between SCFP and CON ($P > 0.05$) in the first

Table 7. Effect of *Saccharomyces cerevisiae* fermentation products (SCFP) on medical treatments in calves

Item	Treatment ¹			SEM	P-value	
	CON (n = 19)	SCFP1 (n = 20)	SCFP2 (n = 20)		CON vs. SCFP	SCFP1 vs. SCFP2
First round antibiotics ²						
d 4–28	2	2	2	0.2	0.17	0.77
d 29–56	0	0	0	0.1	0.05	0.94
Second round antibiotics ³						
d 4–28	0	0	0	0.1	0.04	0.30
d 29–56	ND ⁴	ND	ND	ND	ND	ND
Combination ⁵						
d 4–28	0	0	0	0.1	0.30	0.76
d 29–56	ND	ND	ND	ND	ND	ND
Kaolin ⁶						
d 4–28	8	7	7	0.8	0.19	0.94
d 29–56	1	0	0	0.3	0.02	0.45
Electrolytes ⁷						
d 4–28	1	1	1	0.2	0.18	0.52
d 29–56	ND	ND	ND	ND	ND	ND

¹CON = control (no SCFP); SCFP1 = XPC (Diamond V, Cedar Rapids, IA) at 0.5% DM in the starter and 1 g/d of SmartCare (Diamond V) in milk; SCFP2 = XPC at 1% DM in the starter and 1 g/d of SmartCare in milk.

²Least squares means for times treated with ofloxacin only in the first round. Each time did not exceed 5 d.

³Least squares means for times treated with ofloxacin only in the second round. Each time lasted for 3 d.

⁴ND = not determined

⁵Least squares means for times treated with a combination of ofloxacin and *Andrographis paniculata* injection. Each time did not exceed 5 d.

⁶Least squares means for days calves were treated with Kaolin (Aether Centre Enterprises Ltd., Beijing, China).

⁷Least squares means for days calves were given electrolytes.

month. Some calves did not respond to the first round of treatment and, thus, were given a second round of treatment. Although we noted differences between the CON and SCFP groups in their second round of antibiotics medication ($P < 0.05$) in the first 28 d, the results were insignificant. Similarly calves that received Kaolin did not show any differences in the average number of days treated. Use of electrolytes was similar between the CON and SCFP-supplemented calves (Table 7).

DISCUSSION

Starter Intake, ADG, and Feed Efficiency

Starter intake of calves in this study was very low in the first 28 d, which was lower than that reported by Magalhães et al. (2008), who fed their calves 3.8 L of milk until 55 d of age. Supplying calves high levels of milk (6 L/d of milk from d 2 to 10 and 8 L/d from d 11 to 42) can reduce calf starter intake, as reported elsewhere (Khan et al., 2008), and may mask the effect of SCFP. Some trials have found SCFP increased starter intake (Lesmeister et al., 2004; Brewer et al., 2014). Lesmeister et al. (2004) reported that calves consumed more starter when SCFP (Original XP, Diamond V) was added at both 1 and 2% on a DM basis in starter grains. Differences in starter intake could be attributed to factors such as amount of milk fed, health status, degree of stress suffered, and exposure to pathogens, as well as feeding and management of the calves (Signorini et al., 2012). Consistent with starter intake, we observed no treatment effects on BW, ADG, and feed efficiency. In previous studies, SCFP increased final BW of calves that were not challenged (Lesmeister et al., 2004) and experimentally infected with *Salmonella* after 2 wk of SCFP supplementation (Brewer et al., 2014). In those 2 studies, rumen development was stimulated with SCFP supplementation, as indicated by longer papillae length with similar or greater papillae width. The numerical differences in ADG could be attributed to better rumen development and, hence, efficient absorption of nutrients, especially butyrate produced by *Butyrivibrio* bacteria in the SCFP-supplemented calves (Xiao et al., 2016).

Structural Measurements and Blood Metabolites

The structural measurements were similar in our trial probably as a result of similar starter intake and volumes of milk fed (Khan et al., 2007). Lesmeister et al. (2004) observed that hip height and hip width were increased in calves supplemented with SCFP (Original XP) at 2% (equivalent to 0.5% of XPC); those authors

attributed the growth to increased intake of energy and nutrients in the SCFP-supplemented calves.

Blood metabolites (glucose, fatty acids, TP, and BUN) were within the normal ranges reported in literature; supplementation of SCFP tended to increase TP on d 28 ($P = 0.08$). Total protein in serum is an important biomarker that can reflect both the health of the animal and protein metabolism. As an indicator of health, the amount of TP in blood is a common and convenient method used to determine whether the calf has achieved passive immunity after consuming colostrum. In research examining the relationship between passive immunity and morbidity and mortality, Donovan et al. (1998) demonstrated that morbidity and mortality rates are higher in calves that have low TP. Moreover, the increased risk of mortality could last for up to 6 mo of age. In young animals, variation in TP is positively correlated with IgG concentration in the blood (Quigley and Wolfe, 2003). The trend seen in TP on d 28 of experiment could be linked to improvements in immunity in the calves that were supplemented with SCFP (Zaworski et al., 2014), which was reflected in reduced FS and related health parameters. The tendency for higher TP in SCFP-supplemented calves could be attributed to a reduced number of pathogens when calves are recovering from high incidences of diarrhea. The SCFP used in this experiment contained mannans, which can bind onto the active sites in the gut epithelium, thus preventing pathogenic bacteria such as *Escherichia coli* and *Salmonella* from attaching on the same binding sites (Heinrichs et al., 2003; Brewer et al., 2014). We found a tendency for lower BUN in SCFP2 compared with SCFP1 at d 28 ($P = 0.08$). Possibly the effect was from a more robust ruminal microbial population, resulting in increased microbial growth and, thus, an increase in ruminal nitrogen requirements and a reduction in BUN. Urea nitrogen in blood may reflect the circulating N in blood. Lower BUN in SCFP2 might be an indication of greater microbial protein synthesis relative to dietary protein ingestion. Also, the SCFP2 calves at this age consumed more calf starter (93.86 vs. 82.47 g/d), whereas the TP was higher in blood (5.29 vs. 5.14 g/dL).

Oxidative Biomarkers

Changes in oxidative biomarkers (GSH-Px, SOD, MDA, and TAOC) can be used as an indicator of the physiological and health status of an animal (Gaál et al., 2006; Ranade et al., 2014). For example, increased levels of lipid peroxides have been reported in calves with diarrhea and respiratory diseases (Chirase et al., 2004). The concentrations of oxidative biomarkers were

similar among the treatments in this trial. However, activity of TAOC decreased with age until weaning, but its activity increased and was numerically higher in the SCFP-supplemented calves postweaning. Calves supplemented with SCFP1 and SCFP2 had 2.41 and 2.49 U/mL, respectively, whereas control calves had 1.90 U/mL. Whether this was due to decreased stress as indicated by the positive effects of SCFP on FS needs more research. Human studies have shown that SCFP improved the capacity of serum antioxidant protection in healthy subjects (Jensen et al., 2007). The differences in digestive systems between dairy and humans may contribute to the lack of differences in observations in our trial. Moreover, the lack of changes in serum oxidative stress biomarkers does not rule out the possibility of SCFP having an effect, as oxidative damage may occur only in a few tissues and at a particular time (Celi et al., 2010). Alterations in serum antioxidants are not always consistent with that of the gut, as the intestinal mucosa and other compartments of the immune system are much different (Wittig and Zeitz, 2003). Because SCFP might act locally on the gut tissues, further research is warranted on effects to the gut mucosa, which when under oxidative stress exacerbates weaning diarrhea (Song et al., 2011).

Calf Health

Maintaining good calf health is essential in ensuring that the calf attains its potential during the productive phase. Poor health may contribute to poor weaning weight and postweaning growth as a result of low growth rates, which cannot be compensated through future nutrition (Jasper and Weary, 2002). Calf diarrhea is the leading cause of mortality and morbidity in calves and is caused by a sum of various factors including the calf, nutrition, infectious agents, and the environment.

Supplementation of SCFP has the capacity to ameliorate scours and, hence, promote gut health and daily growth rates (Brewer et al., 2014). In our calves, we observed improved FS in calves supplemented with SCFP throughout the experimental period, which is consistent with other research where calves were fed SCFP (Magalhães et al., 2008); however, others reported no significant differences in FS when SCFP was supplemented only in starter feed (Lesmeister et al., 2004), which could be as a result of low infection rates observed in those calves. Supplementation of SCFP in milk (SmartCare) may have had a greater effect on decreasing FS in the first 28 d, as the starter consumption in this period was relatively low. The SCFP in milk was delivered to the abomasum through liquid feeding and could have contributed to stabilizing the gut microbiota

and reducing the risk of pathogen colonization in the small intestine where high proliferation of coliform bacteria occurs during infections (Berge et al., 2009). An increase in pathogenic bacteria can lead to reductions in lactobacilli and bifidobacteria populations in the gut (Vlková et al., 2006). Brewer et al. (2014) fed SCFP (SmartCare in milk and Original XPC in starter) to calves that were infected with *Salmonella*; they showed that fecal shedding in control calves continued 4 more days after shedding had ended in SCFP-supplemented calves. Previous research has attributed the positive changes to bioactive components in SCFP, which may inhibit colonization of the gut by pathogenic microorganisms while encouraging the growth of healthy microflora (Jensen et al., 2008a). In a companion to the current paper, *Butyrivibrio* genus richness as well as butyrate concentrations were higher in SCFP-supplemented calves (Xiao et al., 2016). Butyrate has multiple functions in the gut, such as increasing cell proliferation and reduction of diarrhea in calves (Guilloteau et al., 2010); thus, it is possible that the improvement in FS observed in the present study could be a result of higher butyrate production, which also resulted in better intestinal development (Xiao et al., 2016). Furthermore, the immune system is improved when the animal is protected from recurrence of infection, as demonstrated by the improvement in FS. This could be of importance during the weaning period when the animal's immune system tends to be compromised. The scouring in calves increased when the weaning process started; however, SCFP-supplemented calves seemed to be less affected during this period compared with CON. Research in mice and nursery piglets has shown that SCFP can modulate the immune system in the weaning period (Shen et al., 2009; Evans et al., 2012). Further research could help elucidate the mechanisms by which SCFP can reduce diarrhea during weaning.

The use of antibiotics and electrolytes were also investigated. Antibiotics and electrolytes use in calves contribute to form a major economic input for dairy enterprises. Limiting the use of antibiotics in calves would be beneficial to the animals, human beings, environment, and farm economics. One of the major advantages of reduced use of antibiotics is a potential decrease in antibiotic-resistant microflora being transferred into the ecosystem (Quigley et al., 2002). An additive that can decrease the duration and severity of diarrhea would have greater economic value due to less medical costs and labor costs for therapy. Calves were treated with several medications in this trial, as the experiment was designed to mimic the commercial dairy situations. As a result, several antibiotics and nonantibiotics were used to treat diarrhea and pneumonia. Most of the medications and all of the electrolytes in

the current study were offered in the first 28 d of age. Although no differences were observed in this trial, we speculate that a larger sample size would give us a clear picture on the effect of SCFP in preweaned calves. The reduced FS suggested an improved gut health in calves supplemented with SCFP.

CONCLUSIONS

This trial illustrates that SCFP supplementation in milk (SmartCare) and calf starter (Original XPC) had a positive effect on the health of preweaning calves raised under normal commercial conditions when offered high volumes of milk. The health effects of SCFP stem from reduced FS. *Saccharomyces cerevisiae* fermentation products can be used to reduce diarrhea in calves grown under normal commercial conditions.

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