Influence of *Saccharomyces cerevisiae* fermentation products, SmartCare in milk replacer and Original XPC in calf starter, on the performance and health of preweaned Holstein calves challenged with *Salmonella enterica* serotype Typhimurium¹

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ABSTRACT

This study was designed to investigate the effects of supplementing SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and Original XPC (XPC; Diamond V) in calf starter on performance and health of preweaned calves following an oral challenge with *Salmonella enterica*. The study was performed in two 35-d periods with 30 Holstein bull calves (2 ± 1 d of age) per period. In each period, calves were blocked by location in the barn and randomly assigned to treatments that included control, base milk replacer and calf starter with no added *Saccharomyces cerevisiae* fermentation products; SC, milk replacer with 1 g of SC/calf per day and base calf starter; and SC+XPC, milk replacer with 1 g of SC/calf per day and calf starter with 0.5% XPC on a dry matter basis. Calves were fed 350 g of milk replacer solids at 14% dry matter twice daily at 0700 and 1700 h. Calf starter and water were offered ad libitum and intakes were recorded daily. Calves were challenged with 108 cfu of sulfamethazine-resistant *Salmonella enterica* serotype Typhimurium orally on d 14 of the study. Fecal *Salmonella* shedding was determined on d 14 to 21 (daily), 24, 28, and 35 using selective media. Blood samples were collected on d 0, 7, 14, 16, 18, 21, 24, 28, and 35 and analyzed for hematology; plasma were analyzed for haptoglobin concentrations. All data were reported as CON, SC, and SC+XPC, respectively. Calf starter intake was increased from d 22 to 35 among SC+XPC calves and from d 29 to 35 among SC calves. The SC+XPC calves had a lower neutrophil-to-lymphocyte ratio (0.81, 0.83, and 0.69 ± 0.051) throughout the study. The SC+XPC calves also

had lower hematocrits (35.1, 35.3, and $33.4 \pm 0.54\%$) and hemoglobin concentrations (10.8, 10.6, and 10.1 ± 0.16 mg/dL) throughout the study. We found a tendency for calves fed SC and SC+XPC to have more solid fecal scores during the week after the challenge. We observed no treatment or treatment $\times$ time differences on plasma haptoglobin concentrations (63, 48, and 60 ± 0.5 μ g/mL). No treatment differences were observed in the fecal shedding of the *Salmonella*; however, we noted a tendency for a treatment difference in the percentage of calves positive for *Salmonella* present in the ileal tissue at d 21 after the challenge (25, 50, and 60%). Supplementing preweaned Holstein calves with both SC in milk replacer and XPC in calf starter improved starter intake and improved fecal consistency immediately after a mild *Salmonella enterica* challenge, but more data are needed to further understand how these yeast fermentation products influence the immune responses to *Salmonella enterica*.

Key words: calf, health, yeast fermentation product

INTRODUCTION

Enteric disease continues to account for a large proportion of morbidity and mortality among dairy calves in the first few weeks of life. According to the USDA National Animal Health and Monitoring System (NAHMS, 2002, 2007), from 48 h of life until weaning, the mortality rate of dairy calves is between 7.8 and 10.8%. *Salmonella* is one of the major enteric pathogens that can cause severe local and systemic disease with a high risk of mortality, especially among young calves. Further, *Salmonella* is a zoonotic disease that poses both food safety and labor safety risks. Therefore, reducing colonization and shedding of enteric pathogens, such as *Salmonella*, would improve calf health and reduce the risk of disease in humans.

Major concern exists with the use of metaphylactic antibiotics in livestock, which is driving an interest in

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finding alternatives to antibiotic use. Nutrition is an attractive approach to reduce the risk of enteric disease, because nutrients or biologically active compounds can be delivered directly to the sites of infection and disease. Fermentation products, specifically derived from *Saccharomyces cerevisiae* were reported to improve enteric health and increase leukocyte function (Jensen et al., 2008; Magalhães et al., 2008; Brewer et al., 2014). SmartCare (SC; Diamond V, Cedar Rapids, IA) and XPC (XPC; Diamond V) are *Saccharomyces cerevisiae* fermentation products that can be supplemented to calf diets via milk (or milk replacer) and starter grains, respectively.

The objectives of the current study were to determine the effects of SC added in milk replacer and XPC added in the calf starter on performance characteristics, clinical signs of disease (diarrhea, fever, hematology), and gastrointestinal development of preweaned Holstein calves before and after they were experimentally challenged with *Salmonella enterica* serovar Typhimurium. It was hypothesized that SC and XPC would minimize performance losses, attenuate the severity of clinical signs, and improve rumen development in Holstein calves during salmonellosis.

MATERIALS AND METHODS

Study Design

The study was reviewed and approved the Animal Care and Use Committee of the Livestock Issues Research Unit of the USDA-Agricultural Research Service (ARS) in Lubbock, Texas. The study was conducted as a complete randomized block design study. The study consisted of 2 periods of 35 d each with 30 Holstein bull calves in each period. Calves were blocked by location in the barn and randomly assigned to 1 of 3 dietary treatments. Treatments included (1) control, base milk replacer and calf starter alone; (2) SmartCare (SC), base milk replacer + 1 g/calf per day of SmartCare added to the milk replacer and base calf starter; and (3) SmartCare + XPC (SC+XPC), base milk replacer + 1 g/calf per day of SmartCare added to the milk replacer and XPC included in the base calf starter at 0.5%.

All calves were housed at the USDA-ARS Livestock Issues Research Unit enclosed calf barn and enrolled in the study at 2 ± 1 d of age. At enrollment, BW of calves were recorded. In addition, peripheral blood samples were collected from each calf and total serum protein was immediately measured using a refractometer to estimate passive immunity status. To ensure adequate randomization, no differences among treatments

in initial BW and total serum protein were confirmed immediately after enrollment in each period.

Calves were challenged orally with 108 stationary-growth colony forming units of *Salmonella enterica* serotype Typhimurium strain SL1344. The *Salmonella* challenge was administered in the morning feeding on d 14 of the study and was administered in the calves' milk replacer. Ten milliliters of a 20% sodium bicarbonate and 20% calcium carbonate buffer was also added in the milk replacer to aid in buffering the pH of the abomasum to increase the survival of the *Salmonella* to the lower gastrointestinal tract. This strain of *Salmonella* is sulfamethazine-resistant, which was used to determine fecal shedding and tissue colonization of the bacteria after challenge. The observation period continued until 35 d after enrollment.

Calf Selection and Housing

All calves were received from 1 large commercial dairy located approximately 8 km east of Plainview, Texas. Only healthy calves that were provided 4 L of pooled colostrum via an esophageal tube within 6 h of birth were picked up from the dairy. The calves were transported from the dairy to the research facility each morning within 24 h of birth.

Calves were housed in the USDA-ARS livestock issues research unit calf research facility located approximately 25 km northwest of Lubbock, Texas. The facility is a Biosafety Level II Calf Research Facility that is an indoor, temperature-controlled environment. Calves were housed individually in pens (1.2 × 1.1 m, width × length; Agri-Plastics, Tonawanda, NY). No bedding was provided in the pens, and the floor of the pens was calf mesh Tenderfoot and was raised above a manure pit.

Animal Feeding and Care

Calves were offered a milk replacer with no feed additives twice daily throughout the study at 0700 and 1700 h. The milk replacer consisted of 22% CP and 20% fat (Land O Lakes, Minneapolis, MN; Table 1) and was fed at a rate of 350 g of milk solids per feeding and 14% solids. The milk replacer was offered at 38 to 41°C. All milk replacer was fed using nipple bottles and any milk refusals were measured 15 min after the milk was offered.

Calf starter and water were also offered to calves beginning on d 1 of the study in separate removable plastic buckets. Calf starter was mixed weekly and stored at the calf facility. The ingredient and formulated nutrient composition of the calf starter are reported

in Table 1. Calf starter refusals were measured daily at 0730 h and the amount offered was adjusted daily for an approximate 15% refusal. Water measurements were taken at the same time as calf starter intake and were determined by measuring the quantity of water remaining in the bucket using a ruler measuring to the nearest 0.5 cm. Fresh water was then added to fill each bucket. Data were converted to milliliters before statistical analysis.

Visual exams of each calf were recorded at each feeding, which included a fecal score (1 = well-formed, not hard; 2 = soft, pudding-like; 3 = loose, pancake batter-like; or 4 = watery, pulpy, orange juice-like). Rectal temperatures were collected each morning from 0600 to 0700 h from each calf on 14 to 21 (daily), 24, 28, and 35 d after enrollment using a hand-held thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). Accuracy of the thermometer was confirmed before each period by placing the thermometer in a water bath at various known temperatures between 38 and 41°C.

Sample Collection and Analysis

Performance Data. Calf starter intake was recorded daily. Individual BW were recorded at enrollment and at 14, 21, and 35 d after enrollment (Calf Cart Scale, Digi-Star, Fort Atkinson, WI). Calves were weighed at 0600 h. Total DMI was calculated as milk replacer intake plus calf starter intake. Feed efficiency was calculated as the feed-to-gain ratio for each time interval between BW measurements.

Blood Samples. A peripheral blood sample (12 mL; 9 mL in heparinized and 3 mL in EDTA Vacutainers, Becton Dickinson, Franklin Lakes, NJ) was collected from the jugular vein of each calf at enrollment and at 7, 14, 16, 18, 21, 24, 28, and 35 d after enrollment. Blood samples were obtained before the morning milk replacer feeding. Whole blood was analyzed for hema-

tology, including hematocrit, total leukocyte counts, total neutrophil counts, total monocyte counts, total lymphocyte counts, ratio of neutrophils to lymphocytes, and hemoglobin using an Idexx Procyte Analyzer (Idexx Laboratories, Westbrook, ME) with bovine-specific algorithms. Plasma was collected and stored at -80°C from the heparinized Vacutainer following centrifugation at 1,500 × g for 15 min at 4°C. Plasma haptoglobin was quantified as described by Makimura and Suzuki (1982). A pooled plasma sample was used to calculate intra- and interassay coefficients of variation of 1.8 and 4.2%, respectively, for the haptoglobin assay. The colorimetric data were quantified using a SpectraMax 340PC (Molecular Devices, Sunnyvale, CA). Plasma samples with known concentrations of haptoglobin were used to create a standard curve.

Fecal Samples. Fresh fecal samples were collected via digital stimulation from each calf on 7, 15 to 21 (daily), 24, 28, and 35 d after enrollment. At each time point, 1 g of feces from each sample was weighed and serially diluted in sterile PBS (7.4 pH) before spread plating 100 µL of diluted fecal sample in PBS on selective media [XLD agar (Himedia, Mumbai, India), sulfamethazine included at 512 mg/L of agar (Acros Organics, Thermo Fisher Scientific, Waltham, MA)] contained in petri dishes. The plated petri dishes were then incubated at 37°C for 24 h. After incubation, the dishes were then removed from the incubator and set on the laboratory bench for an additional 24 h. This step aided in differentiation of selected *Salmonella* colonies from other bacterial populations that were sulfamethazine-resistant. After the 2-h laboratory bench incubation, the *Salmonella* colonies were then counted as the colonies with a black center and recorded to determine shedding of *Salmonella* in colony forming units per gram of wet fecal material.

Tissue Samples. After the 35-d observation period was completed, calves were euthanized and multiple samples were taken immediately postmortem. Major organs were removed and weighed, including the reticulorumen, omasum, abomasum, total intestines, liver, spleen, and kidneys. The reticulorumen, omasum, and abomasum were rinsed and cleaned of any residual ingesta before being weighed, whereas the intestines were weighed with the ingesta. The liver, spleen, and kidneys were trimmed of excess non-organ-specific tissue (fat and omentum) before being weighed. Tissue samples of the rumen, duodenum, and ileum were removed, rinsed gently with sterile 1× PBS, and preserved in formalin. These tissues were then sent to the Texas Tech Health Sciences Center pathology laboratory for slide preparation and hematoxylin and eosin staining; thus, tissue morphology could be analyzed under light microscopy.

Table 1. The formulated nutrient contents of the milk replacer and calf starter

Nutrient	Milk replacer	Calf starter
DM, %	97	93
CP, %	22	20
Ether extract, %	20	4.8
ADF (maximum), %	0.15	9
ME, Mcal/kg ²	4.65	3.1
Calcium, %	0.75	0.86
Phosphorus, %	0.7	0.42
Vitamin A (minimum), IU/kg	44,000	44,000
Vitamin D ₃ (minimum), IU/kg	11,000	11,000
Vitamin E (minimum), IU/kg	220	330

Ileal fluid, as well as tissue samples from the ileum and a mesenteric lymph node (MLN) were analyzed for the presence of the challenge *Salmonella enterica*. Ileal fluid was collected by making an incision in the ileum, approximately 10 cm anterior to the ileo-cecal junction, and aseptically transferring approximately 7 mL of fluid from the lumen of the ileum into a sterile 15-mL conical tube. After the ileal fluid was transferred, the ileal tissue was gently rinsed with sterile 1× PBS, cut into 2 pieces, and stored either in sterile PBS or 10% formalin. The third MLN from the ileo-cecal junction was collected and stored in a sterile 15-mL conical tube. One milliliters of ileal fluid was diluted in 10 mL of sterile PBS and 1 g of ileum tissue and MLN were then homogenized with a tissue homogenizer in 10 mL of sterile PBS for 30 s. Subsequently, each respective sample was plated on XLD agar with 512 mg/L of sulfamethazine and incubated for 24 h at 37°C. After 24 h of incubation at 37°C, the petri dishes were removed from the incubator and allowed to incubate at room temperature on a laboratory bench. Once the dishes incubated for an additional 24 h at room temperature, the presence of *Salmonella* colonies were identified as a colony with a black center on each plate.

Statistical Analysis

Prior to any statistical interpretation, the residuals of all continuously distributed data were analyzed using the Univariate procedure of SAS to evaluate suitability of the data for parametric data analysis (SAS ver. 9.2, SAS Institute Inc., Cary, NC). All continuous, repeatedly measured data were analyzed as a repeated measure using the Mixed Procedure of SAS (ver. 9.2). The model included the fixed effects of treatment, time, and the interaction of treatment × time. Period and block were included as random effects. The subject of the repeated statement was calf nested within treatment, and the appropriate covariance structure for the

within-calf measurement was chosen based on the lowest Bayesian information criterion. Initial BW was used as a covariate. A survival analysis was performed on the proportion of calves shedding the challenge *Salmonella enterica* strain in feces using the LifeTest procedure of SAS (ver. 9.2). The percentages of calves positive for the challenge *Salmonella enterica* strain in ileal tissue or MLN and average fecal score were analyzed using a Chi-squared test in the Freq procedure of SAS (ver. 9.2). Pairwise differences were performed for each statistical analysis to further evaluate treatment differences. Differences of $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Calf Losses

Two calves died during the second period. One SC+XPC calf died immediately after consuming milk replacer in the afternoon on d 10 of the experiment. The cause of death is unknown, but was not suspected to be directly associated with the dietary treatment. The second calf was fed the SC treatment and developed a hernia on d 11 of the experiment. Severe discomfort was observed and the calf was euthanized on d 12.

Performance

Although we found no differences in BW either at the beginning or end of the study among treatments, we did note a treatment × time interaction in calf starter intake (Table 2; Figures 1). The SC+XPC calves consumed more calf starter during wk 4 and 5 of the study, whereas calves fed SC also had increased calf starter intake during wk 5. Average daily gain was not different among treatments ($P \geq 0.161$). Further, we found no treatment difference in the feed-to-gain ratio ($P \geq 0.221$) or water intake ($P \geq 0.591$).

Table 2. The effect of treatments on performance measurements in Holstein dairy calves from 1 to 35 d in age

Item	Treatment ¹			Largest SEM	Fixed effects, <i>P</i> -value		
	Control	SC	SC+XPC		Treatment	Time	Treatment × time
Initial BW, kg	40.2	39.9	39.3	1.36	≤0.897	—	—
Final BW, kg	57.8	58.3	60.1	2.97	≤0.612	—	—
Starter intake, kg/d	0.162 ^a	0.207 ^{ab}	0.237 ^b	0.0257	≤0.034	≤0.0001	≤0.021
ADG, kg/d	0.503	0.517	0.576	0.0812	≤0.161	≤0.0001	≤0.851
Feed-to-gain ratio, kg/kg	1.89	1.85	1.66	0.251	≤0.221	≤0.0001	≤0.301
Water intake, L/d	1.65	1.77	1.93	0.313	≤0.591	≤0.002	≤0.994

^{a,b}Differing superscripts within a row indicate a difference between means ($P < 0.05$).

¹Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

Figure 1. The effects of SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and XPC (Diamond V) in a calf starter on calf starter intake in Holstein calves from 1 to 35 d in age. All calves were challenged with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14. Treatments included a control group (CON) of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% XPC. Bars represent SEM. Differing letters (a,b) within a time period indicate a difference ($P < 0.05$). We noted a treatment $\times$ time interaction ($P = 0.021$).

Figure 2. The effects of SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and XPC (Diamond V) in a calf starter on rectal temperatures in Holstein calves after they were challenged with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14. Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% XPC. Bars represent SEM. We found no treatment $\times$ time interaction ($P = 0.396$) or overall treatment differences ($P \geq 0.450$).

Hematology and Health

The oral challenge with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14 after enrollment caused a mild systemic acute phase response, as evident by elevated rectal temperatures (Figure 2) and an increase in the ratio of neutrophils to lymphocytes in peripheral circulation. However, plasma haptoglobin concentrations did not increase following the *Salmonella enterica* challenge (Table 3).

We noted no treatment $\times$ time or treatment differences in either rectal temperature or plasma haptoglobin concentrations following the *Salmonella enterica* challenge (Figure 2; Table 3). No differences in the distribution of calves with different fecal scores were observed among treatments during the first 2 wk, but there were tendencies ($P \leq 0.085$) for treatment differences during wk 3 and 5 of the study (Figure 3). During the third week, a greater proportion of control calves tended ($P = 0.083$) to have scours (fecal score 4)

Table 3. Effects of treatment on hematology and haptoglobin measurements in Holstein dairy calves from 1 to 35 d in age

Item	Treatment ¹			Largest SEM	Fixed effects, <i>P</i> -value		
	Control	SC	SC+XPC		Treatment	Time	Treatment $\times$ time
Total leukocytes, $\times 10^6/\text{mL}$	10.1	10.3	9.7	0.53	≤ 0.636	≤ 0.017	≤ 0.508
Total neutrophils, $\times 10^6/\text{mL}$	4.2	4.4	3.6	0.38	≤ 0.171	≤ 0.0001	≤ 0.595
Total monocytes, $\times 10^6/\text{mL}$	0.58	0.54	0.51	0.043	≤ 0.523	≤ 0.0001	≤ 0.599
Total lymphocytes, $\times 10^6/\text{mL}$	5.3	5.2	5.2	0.13	≤ 0.892	≤ 0.001	≤ 0.407
Neutrophils, %	39.1 ^a	38.9 ^a	35.1 ^b	1.78	≤ 0.008	≤ 0.0001	≤ 0.418
Monocytes, %	5.8	5.6	6	0.44	≤ 0.834	≤ 0.0001	≤ 0.678
Lymphocytes, %	53.3 ^a	53.8 ^a	57.5 ^b	2.07	≤ 0.014	≤ 0.0001	≤ 0.489
Neutrophil:lymphocyte ratio	0.81 ^a	0.83 ^a	0.69 ^b	0.051	≤ 0.016	≤ 0.0001	≤ 0.097
Hematocrit, %	35.1 ^a	35.3 ^a	33.4 ^b	0.54	≤ 0.032	≤ 0.0001	≤ 0.829
Hemoglobin, g/dL	10.8 ^a	10.6 ^a	10.1 ^b	0.16	≤ 0.006	≤ 0.0001	≤ 0.637
Haptoglobin, $\mu\text{g/mL}$	63.0	48.8	60.3	31.68	≤ 0.578	≤ 0.012	≤ 0.510

^{a,b}Differing superscripts within a row indicate a difference between means ($P < 0.05$).
¹Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (SC; Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

Figure 3. The effects of SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and XPC (Diamond V) in a calf starter on the distributions of calves with a given fecal score. Calves were challenged with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14. Fecal scores include 2 = soft, pudding-like; 3 = runny, pancake batter like; 4 = watery, pulpy orange juice. Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% XPC. Analysis was performed using a Chi-squared test in the Freq procedure in SAS (ver. 9.2, SAS Institute Inc., Cary, NC). Statistical significance is reported above each time period.

than either the SC or SC+XPC calves. During the fifth week, the calves fed the SC+XPC treatment tended ($P = 0.085$) to have a greater proportion of calves that had a fecal score of 3.

We observed differences among treatments in the total peripheral blood counts of leukocytes, neutrophils, monocytes, or lymphocytes (Table 3); however, the SC+XPC calves had a lower percentage of neutrophils and greater percentage of lymphocytes in circulation. There was a tendency for a treatment $\times$ time interaction ($P = 0.097$) on the neutrophil-to-lymphocyte ratio in peripheral circulation (Figure 4). Hematocrit and hemoglobin concentrations were both lower among the SC+XPC calves compared with control and SC calves ($P \leq 0.032$; Table 3).

Salmonella Fecal Shedding and Colonization

The *Salmonella enterica* strain was shed in feces from all calves at 1 d after the challenge and persisted among some calves up until 6 d after the challenge, when no more calves were shedding *Salmonella* in feces (Figure 5). We noted no treatment differences ($P \geq 0.115$) in either the median number of days shedding the *Salmonella* or the area under the curve in log₁₀ colony-forming units per gram of fecal material. No calves had *Salmonella* present in ileal fluid when calves were euthanized 21 d after the challenge. A tendency for a treatment difference was observed in the proportion of

Figure 4. The effects of SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and XPC (Diamond V) in a calf starter on neutrophil to lymphocyte ratios in peripheral circulation in Holstein calves. All calves were challenged with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14. Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% XPC. Bars represent SEM. Differing letters (a,b) within a time period indicate a difference ($P < 0.05$). We observed a tendency ($P = 0.097$) for a treatment $\times$ time interaction.

Table 4. Effect of treatment on *Salmonella* Typhimurium shedding in Holstein dairy calves from 1 to 35 d in age

Item	Treatment ¹			Largest SEM	Treatment, <i>P</i> -value
	Control	SC	SC+XPC		
Fecal <i>Salmonella</i> shedding, ² d	3.21	2.84	2.42	0.279	≤0.115
Fecal <i>Salmonella</i> shedding AUC, log ₁₀ (cfu/g)	16.9	16.2	14.4	1.8	≤0.229
Ileal tissue <i>Salmonella</i> colonization, % calves positive	25	50	60	—	≤0.073
MLN ³ <i>Salmonella</i> colonization, % calves positive	40	61	70	—	≤0.145
Ileal tissue <i>Salmonella</i> colonization, cfu/g of tissue ⁴	200	200	200	—	≤0.947
MLN <i>Salmonella</i> colonization, cfu/g of tissue	100	100	100	—	≤0.836

¹Treatments included a control group of calves that were fed a base diet of milk replacer and calf starter; SC were fed a base diet of milk replacer and calf starter plus SmartCare (Diamond V, Cedar Rapids, IA) included in the milk replacer at a rate of 1 g/head per day; SC+XPC were fed a base diet of milk replacer and calf starter with SmartCare included in the milk replacer at a rate of 1 g/head per day and XPC (Diamond V) included at 0.5% of the calf starter.

²Significance reported from the Proc LifeTest (SAS ver. 9.2, SAS Institute Inc., Cary, NC): Wilcoxon-rank sum test.

³MLN = mesenteric lymph node.

⁴Data are reported as the median cfu/g of tissue from *Salmonella*-positive calves only. Data were analyzed as the log base 10 (cfu/g).

calves that were positive for *Salmonella enterica* in ileal tissue at the end of the study (Table 4), but no difference ($P = 0.145$) was found in the proportion of calves that had *Salmonella* present in the MLN. Additionally, there were no differences ($P \geq 0.836$) among treatments in the number of colony-forming units per gram of tissue from *Salmonella*-positive calves only (Table 4).

Figure 5. The effects of SmartCare (SC; Diamond V, Cedar Rapids, IA) in milk replacer and XPC in a calf starter on the proportion of calves shedding *Salmonella* in feces in Holstein calves. All calves were challenged with *Salmonella enterica* serotype Typhimurium strain SL1344 at a dose of 108 stationary-growth colony-forming units on d 14. Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% XPC. Bars represent SEM. Data were analyzed using the Proc LifeTest (SAS ver. 9.2, SAS Institute Inc., Cary, NC). We found no treatment difference ($P = 0.115$) in the median number of days calves shed the *Salmonella* in feces.

Organ Weights and Histology

We found no differences among treatments ($P \geq 0.119$) in organ weights at the time of harvest (Table 5). Additionally, no treatment differences ($P \geq 0.115$) were noted in rumen or duodenum histology (Table 6). There was a tendency ($P = 0.087$) for a treatment difference in villi length in the ileum and a difference ($P = 0.018$) in crypt depth in the ileum, whereas SC+XPC had greater depth (Table 7). Last, we observed no differences ($P \geq 0.536$) in cellular infiltrate scoring in the ileum (Table 8).

DISCUSSION

Both SC and XPC are *Saccharomyces cerevisiae* fermentation products used in the livestock industry. The XPC is a product containing yeast-derived metabolites that is supplemented to calves in calf starter and to heifers and cows in TMR. The SC product reportedly contains similar metabolites found in XPC as well as other metabolites, including those with enhanced antioxidant capacity. Unlike XPC, SC is water-dispersible and can be more easily supplemented to calves in their milk or milk replacer.

Similar to the current study, Brewer et al. (2014) conducted a study that provided SC in milk replacer and 3.5 g of XPC in an oral bolus, each day, to pre-weaned Holstein calves before and after they were challenged with the same *Salmonella enterica* serotype Typhimurium strain SL1344 used in our study. Despite the similarities of using the same challenge microorganism, differences exist between the 2 studies. Brewer et al. (2014) had only 2 treatments (control and SC+XPC), provided the XPC treatment to calves via an oral bolus, had a mixture of heifers and bull calves, treated multiple calves with sulfamethazine before the bacterial

Table 5. Effect of treatment on organ weights of Holstein dairy calves from 1 to 35 d in age

Item	Treatment ¹			Largest SEM	Fixed effect, <i>P</i> -value
	Control	SC	SC+XPC		Treatment
Reticulorumen, kg	0.56	0.64	0.61	0.059	≤0.381
Percent reticulorumen ³	51.8	56.8	54.4	0.367	≤0.119
Omasum, kg	0.14	0.13	0.16	0.024	≤0.651
Percent omasum ³	13.5	11.9	14.3	0.020	≤0.493
Abomasum, kg	0.36	0.35	0.32	0.017	≤0.211
Percent abomasum ²	34.7	31.3	31.2	0.210	≤0.134
Total intestines, kg	3.50	3.56	3.36	0.277	≤0.772
Liver, kg	1.07	1.12	1.06	0.089	≤0.402
Spleen, kg	0.16	0.17	0.17	0.007	≤0.776
Kidney, kg	0.43	0.46	0.37	0.035	≤0.205

¹Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

²Percent weight of each measured compartment of the foregut.

challenge, and inoculated the calves with *Salmonella enterica* using an oral gel capsule. In the present study, SC was fed to calves separately in addition to having a SC+XPC treatment, XPC was fed to calves in the calf starter at a fixed percentage of the DM, and calves in the current study would have to consume 700 g of calf starter DM/d to consume the 3.5 g of XPC given in the bolus in Brewer et al. (2014). Additionally, the present study only used bull calves, did not treat any calves with antibiotics, and were inoculated by adding the *Salmonella enterica* directly to their morning milk replacer along with the addition of sodium bicarbonate and calcium carbonate to act as a buffer. Calves in the SC+XPC at the time of the *Salmonella enterica* chal-

lenge were consuming approximately 0.55 g of XPC/d compared with the 3.5 g in the study by Brewer et al. (2014).

Saccharomyces cerevisiae fermentation products may improve the performance of livestock. Both nonruminants and ruminants reported increased DMI, nutrient digestibility, ADG, gain-to-feed ratio, and overall weight gain (Haddad and Goussous, 2005; Gao et al., 2008). In the present study, dairy calves supplemented with XPC in the calf starter had increased starter intake during the fourth and fifth week of the study when compared with the control calves. Brewer et al. (2014) did not record calf starter intake, ADG, or efficiency, but their data indicated that calves fed SC+XPC had

Table 6. Effects of treatment on rumen and duodenum histology in 35-d-old Holstein dairy calves

Item	Treatment ¹			Largest SEM	Fixed effect, <i>P</i> -value
	Control	SC	SC+XPC		Treatment
Rumen					
Papillae length, μm	1,001	1,181	1,128	92.6	≤0.367
Papillae width, μm	367	354	392	20.1	≤0.295
Papillae density, ² μm	1,682	1,694	1,859	127.8	≤0.544
Rumen wall thickness, ³ μm	2,649	2,540	2,687	213.6	≤0.875
Duodenum					
Villi length, μm	251	266	273	15.6	≤0.551
Villi width, μm	109	120	122	6.5	≤0.326
Villi density, ² μm	753	762	765	36.5	≤0.938
Crypt depth, μm	475	425	453	23.1	≤0.294
Crypt width, μm	57	55	66	4	≤0.115

¹Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

²Density is the distance between 5 papillae.

³Rumen wall thickness is the distance from the epithelium through muscle.

Table 7. Effects of treatment on ileum histology in 35-d-old Holstein dairy calves

Item	Treatment ¹			Largest SEM	Fixed effect, <i>P</i> -value
	Control	SC	SC+XPC		Treatment
Villi length, μm	328	296	343	28.7	≤ 0.087
Villi width, μm	124	124	127	5.7	≤ 0.903
Villi density, ² μm	762	785	797	28.3	≤ 0.685
Crypt depth, μm	367 ^a	401 ^{ab}	478 ^b	27.1	≤ 0.018
Crypt width, μm	53	58	57	1.9	≤ 0.107
Villi length:Crypt depth	0.94	0.82	0.75	0.1	≤ 0.252

^{a,b}Differing superscripts within a row indicate a difference between means ($P < 0.05$).

¹Treatments included a control group of calves that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

²Density is the distance between 5 villi.

a greater BW gain on a percentage basis over the study period when compared with the control calves. Supporting the increase in calf starter intake observed in the present study, Lesmeister et al. (2004) found that a less concentrated form of *Saccharomyces cerevisiae* fermentation product, known as Original XP (**XP**), increased final BW, ADG, and starter consumption. Magalhães et al. (2008) fed XP to 255 preweaned dairy

calves at 2% of calf starter DM. Although they did not observe an improvement in either calf starter intake or BW gain, there was a significant reduction in mortality rate of calves fed XP (7.5 vs. 12.1%).

Hematocrit levels were greater in the control and SC calves compared to the SC+XPC calves throughout the entire experiment. Lesmeister et al. (2004) reported that calves fed XP had lower hematocrit percentages

Table 8. Effects of treatment on ileum cells in 35-d-old Holstein dairy calves

Item	Treatment ¹			Fixed effect, <i>P</i> -value
	Control	SC	SC+XPC	Treatment
Lymphocytes, ² no.	20	19	19	≤ 0.536
Score 1, %	0.15	0.21	0.37	—
Score 2, %	0.75	0.63	0.53	—
Score 3, %	0.10	0.16	0.11	—
Eosinophil, ³ no.	20	19	19	≤ 0.723
Score 1, %	0.45	0.58	0.58	—
Score 2, %	0.40	0.37	0.26	—
Score 3, %	0.15	0.05	0.16	—
Crypt goblets, ⁴ no.	20	19	19	≤ 0.818
Score 1, %	0.45	0.42	0.42	—
Score 2, %	0.45	0.42	0.53	—
Score 3, %	0.10	0.16	0.05	—
Villus goblets, ⁵ no.	20	19	19	≤ 0.777
Score 1, %	0.80	0.68	0.79	—
Score 2, %	0.20	0.26	0.16	—
Score 3, %	0.00	0.05	0.05	—

¹Treatments included a control group that were fed a base milk replacer and calf starter; SC were fed the base milk replacer with 1 g/d of SmartCare (SC; Diamond V, Cedar Rapids, IA) and the base calf starter; and SC+XPC were fed the base milk replacer with 1 g/d of SmartCare and the base calf starter + 0.5% Original XPC (Diamond V).

²Lymphocytes receive a 1 scoring if they were rare or not present, a 2 for intraepithelial or frequent occurrence, and a 3 for exudative occurrence.

³Eosinophils receive a 1 scoring if they were rare or not present, a 2 for intraepithelial or frequent occurrence, and a 3 for exudative occurrence.

⁴Crypt goblets hyperplasia were scored a 1 for normal, a 2 for mild or moderate increase in size and mitoses, and a 3 for marked increase in size and mitoses.

⁵Villus goblets cell mass were scored a 1 for normal mass, a 2 for mild to moderate increases in mass, and a 3 for marked increase in mass.

than calves on the other treatments. The SC+XPC calves tended to have less loose fecal scores than the control calves immediately after the challenge. Brewer et al. (2014) also reported that diarrhea scores were reduced in calves fed SC+XPC than controls after the *Salmonella enterica* challenge. In agreement, although Magalhães et al. (2008) did not experimentally challenge their calves, that group noted that fecal scores and diarrhea incidences were reduced in calves fed XP. However, the difference in hematocrit in the present study likely cannot be completely explained by differences in fecal scores or incidences of diarrhea, because the differences in hematocrit were observed over the course of the entire study.

The *Salmonella enterica* challenge caused similar increases in rectal temperature among all treatments, which returned to baseline temperatures within 6 d post-challenge. These data are in contrast to those of Brewer et al. (2014), who found that SC+XPC calves had reduced rectal temperature compared with control calves from 1 to 6 d postchallenge. Although the present study did not report treatment differences in rectal temperatures after the *Salmonella enterica* challenge, other variables may indicate that the calves fed SC+XPC had attenuated or delayed responses. Calves supplemented with SC+XPC had reduced neutrophil-to-lymphocyte ratios immediately after the *Salmonella enterica* challenge, and did not peak as high as the other 2 treatments. The peak in the neutrophil-to-lymphocyte ratio among SC+XPC calves occurred on d 18, whereas the peak among the control and SC calves occurred more rapidly on d 16. Additionally, if the data are evaluated as the relative increase from immediately before the challenge to peak all treatments had similar increases after the challenge. The biological significance of the delayed increase in the neutrophil-to-lymphocyte ratio needs further investigation because a delay in the activation of neutrophils after an infection can be viewed as a negative response. Alternatively, a delayed or attenuated response may also indicate that response was not needed to the same degree as calves on other treatments because the infection was better controlled locally or by some other component of the immune system. Lastly, it should be noted that none of the treatment groups had elevated plasma haptoglobin concentrations following the *Salmonella enterica* challenge. This suggests that the *Salmonella enterica* challenge in these calves was mild. In fact, none of the 78 calves that were challenged died or needed any antimicrobial or anti-inflammatory interventions following the *Salmonella enterica* challenge.

Neither the median days that calves shed the challenge *Salmonella enterica* nor the area under the curve

of colony-forming units per gram of fecal material were different in the present study. In contrast, Brewer et al. (2014) reported that SC+XPC treatment had a reduced percentage of calves shedding the *Salmonella enterica* than the control calves. However, in the present study, the percentage of SC and SC+XPC calves positive for ileal colonization of the *Salmonella enterica* was greater than that of the control calves. In contrast, Brewer et al. (2014) reported less *Salmonella enterica* colonization in the ileum of SC+XPC-supplemented calves than control calves. There was a difference between the present study and the study conducted by Brewer et al. (2014) in the total counts of *Salmonella enterica*; Brewer et al. (2014) reported approximately 10^3 to 10^4 cfu/g of ileal tissue whereas the present study recorded 10^1 to 10^2 cfu/g of ileal tissue and MLN upon direct plating.

In the present study, no treatment differences were observed in ruminal or duodenal weights or histology. In contrast, Brewer et al. (2014) reported that rumen papillae width increased with SC+XPC. The SC+XPC calves had increased crypt depth in the ileum in the current study. van Beers-Schreurs et al. (1998) reported that crypt depth and width increased in pigs fed weanling diets when compared with pigs only consuming milk. van Beers-Schreurs et al. (1998) concluded that increased nutrients in the gastrointestinal tract promoted greater crypt hyperplasia, which could be explained, at least partially, by the greater calf starter intake among those calves in the last 2 wk of the study.

CONCLUSIONS

Preweaned dairy calves that were fed 1 g/d of SmartCare in milk replacer and 0.5% XPC in calf starter had improved calf starter intake compared with a negative control. Some of the improvements in calf starter intake could be attributed to the SmartCare in milk replacer alone, but the data indicate that greater benefits were observed when the SmartCare in the milk replacer and XPC in the calf starter were fed together.

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