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Supplementing a yeast probiotic to pre-weaning Holstein calves: Feed intake, growth and fecal biomarkers of gut health

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ABSTRACT

Diarrhea, resulting from gastrointestinal infection by pathogens, is a common cause of the high mortality and morbidity of neonatal calves. The objective of this study was to evaluate the effects of supplementing a yeast product in milk replacer (MR) on growth and health of calves, and on fecal populations of some targeted microorganisms related to calf health and growth (*i.e.*, total bacteria, *Escherichia coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Bifidobacterium* spp.). We hypothesized that feeding a *Saccharomyces cerevisiae* var *boulardii* (SCB) product would improve gastrointestinal health and growth performance of calves. Forty-two Holstein bull calves (42.6 ± 0.77 kg at birth) were randomly assigned on day 2 of age to either a control or SCB treatment. The SCB was supplemented in MR and fed at 5 g/d per head to supply 10 billion colony-forming units per day. All calves received high quality colostrum (> 50 mg/mL of immunoglobulin G) during the first 24 h of life, and were fed with 8 L MR (150 g/L mixed with 40 °C water) daily from day 2–35, and 4 L daily from day 35–42. Calves were also fed calf starter *ad libitum* from day 7–56. Daily MR and starter offered and refused, daily fecal scores, nasal scores, ear scores, and weekly body weight of calves were recorded. Fecal samples were collected on day 7, 35 and 56 after the first feeding of that day for microbial targets analysis. Overall, there is no serious disease challenge for all the calves during the entire experimental period. No differences were observed in MR intake, starter intake, metabolizable energy (ME) intake, average daily gain, ME intake to gain ratio, fecal score, nasal score, eye score or any targeted microorganisms between treatments throughout the experiment. These results suggest that supplementing SCB in MR has no additive effects on animal growth or fecal biomarkers of gut health when calves do not show deteriorated health status.

1. Introduction

The high rate of neonatal calf mortality – approximately 7.8% of pre-weaned heifers across North America (USDA, 2010) – remains a long standing challenge for the dairy industry. Gastrointestinal infections and subsequent diarrhea and dehydration account for the majority of mortality and morbidity of neonatal calves (USDA, 2010). Common practices employed by producers to treat diarrhea include electrolyte therapy to replace lost fluids, or the administration of antibiotics to combat invading pathogens

Abbreviations: ADG, average daily gain; BW, body weight; DM, dry matter; *E. coli*, *Escherichia coli*; ME, metabolizable energy; MR, milk replacer; PCR, polymerase chain reaction; SC, *Saccharomyces cerevisiae*; SCB, *Saccharomyces cerevisiae* var *boulardii*; STP, serum total protein; xfp, xylulose-5-phosphate/fructose-6-phosphate phosphoketolase

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(Lorenz et al., 2011). However, the extensive use of antibiotics may result in antibiotic resistant pathogens, posing a potential risk of exposure for calves and humans (Friedman et al., 2007). As such, alternatives to antibiotics for preventing and treating diarrhea are desirable.

Probiotics, including live bacteria and yeasts, are microbial food supplements that may beneficially affect the host by improving its intestinal microbial balance and have been widely studied as production enhancers (Hume, 2011). Recent studies in monogastrics have demonstrated that feeding *Saccharomyces cerevisiae* var *boulardii* (SCB), a subtype of *Saccharomyces cerevisiae* (SC) species, could reduce the risk of enteric diseases by blocking pathogenic toxin receptors sites, destructing pathogenic toxin receptors, inhibiting the growth of some pathogens within the intestinal lumen, and/or regulating immune responses (McFarland, 2010; Kelesidis and Pothoulakis, 2012). At birth, the digestive system of a calf functions similarly to that of a monogastric animal (Drackley, 2008). Because of this similarity, we hypothesized that feeding SCB would have similar effects in calves, thereby improving their gastrointestinal health and growth. The objective of this study was to evaluate the effects of supplementing SCB in milk replacer (MR) during the pre-weaning period on intake, growth and health of calves.

Moreover, because of the growing concept that establishing a proper microflora population in the gut can defend against pathogenic infections (Williams, 2010) and early bacterial colonization may impact the growth and development of animals later in life (Malmuthuge et al., 2015), our second objective was to evaluate if the gut microflora was influenced by SCB supplementation. Five general microbial markers associated with animal growth and health, including *Escherichia coli* (*E. coli*; Muktar et al., 2015), *Clostridium* cluster XIVa (Lopetuso et al., 2013), *Faecalibacterium prausnitzii* (Uyeno et al., 2010) and *Bifidobacterium* spp. (Picard et al., 2005) were evaluated by quantifying their populations in feces.

2. Materials and methods

2.1. Animals and diets

This study was approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta, Edmonton, Canada, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009) at Eckerlea Acres Dairy Farm in Seaforth, Ontario, Canada. Forty-two male Holstein calves, averaging 42.6 ± 0.77 kg at birth, were used. The calves were removed from the dam within 2 h of birth, ear tagged, weighed, and housed in individual pens with straw bedding. The calves were given 4 L of quality colostrum (> 50 mg/mL of immunoglobulin G) within 3 h of birth and another 3 L of fresh colostrum at the next set feeding time. After the second colostrum feeding, calves were randomly assigned to 1 of 2 dietary treatments: 1) Control: a treatment fed commercial MR; or 2) Treat: a treatment fed commercial MR additionally supplemented with 5 g of live SCB product per day which was expected to supply 10 billion colony-forming units of SCB to each calf daily. The SCB product (ProTernative® Milk; Levucell SB20 containing the Pasteur Institute CNCM I-1079 strain of SCB; Lallemand Animal Nutrition, Montreal, Quebec, Canada) was mixed with MR in a bucket for each calf daily before morning feeding and given to them at the first feeding of the day. All calves were fed the MR solution by a nipple bottle (Super Calf Nipple; Merrick's, Middleton, Wisconsin, USA) during the first week of life, and then transitioned at 7 ± 3 d to a gate-mounted artificial teat (Peach Teats; Skellerup Industries Ltd., Woolston, New Zealand). The artificial teat feeding setup consisted of the teat mounted at the front of the pen, attached to a tube fitted with a one-way valve, running into a 8-L bucket placed outside the pen. The MR used in this study contained 260 g/kg crude protein, 160 g/kg crude fat, and 4.58 Mcal/kg metabolizable energy (ME) on a dry matter (DM) basis (MapleviewAgri, Palmerston, Ontario, Canada), and were mixed to 150 g/L with 40 °C water. For the first 2 days, MR was fed 3 times per day (0700, 1600 and 2100 h) with 3 L given at each of the first 2 feedings and 2 L at the third feeding. From day 3 until weaning at day 42, MR was fed in 2 equal volumes daily at 0700 and 1600 h, with 8 L offered daily from day 3 to 34, and 4 L daily from day 35 to 42. Calves also received *ad libitum* calf starter containing 220 g/kg crude protein, 37 g/kg crude fat, 57 g/kg crude fibre and 2.63 Mcal/kg ME on a DM basis (Nieuwland Feeds Elora Ltd., Elora, Ontario, Canada) from day 7 of age. They also had free access to water throughout the experiment.

Calves were given First Defense bolus (Immucell, Portland, Maine, USA) and Dystosel vitamin E/selenium (Zoetis, Kirkland, Quebec, Canada) to protect against *E. coli* K99+ and coronavirus and prevent white muscle disease after birth, respectively. Calves were vaccinated against Bovine Respiratory Syncytial Virus on day 1 of age using Inforce 3 (Zoetis, Kirkland, Quebec, Canada). Draxxin (tulathromycin; Zoetis, Kirkland, Quebec, Canada) was administered as a preventative for bovine respiratory disease on day 5 of age. Calves with diarrhea that required treatment received Metacam (Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) subcutaneously and 2–4 L of oral electrolytes fluids in the evening after 9 pm. Any calves with digestive and respiratory problems were also treated and remained in the study for its duration, which was 56 days.

2.2. Feed intake, animal growth, health measurements, and sample collection

Calf starter offered and orts were weighed and recorded daily until 56 days of age to determine daily feed intakes. The calves were weighed at birth, and weekly thereafter until week 8, to determine weekly average daily gain (ADG). Fecal scores, nasal scores, and ear scores were recorded daily before the morning feeding using a 0–3 (fecal score and nasal score) or 0–4 (ear score) scale developed by University of Wisconsin-Madison (McGuirk, 2008). Fecal scores were 0: normal, 1: semi-formed, 2: loose, and 3: watery, with fecal score ≥ 2 considered as diarrhea. Nasal scores were categorized as 0: no discharge, 1: small amount of cloudy discharge from one nostril, 2: cloudy discharge from both nostrils, and 3: excessive thick cloudy discharge from both nostrils. Ear scores were categorized as 0: normal, 1: one ear droopy, 2: both ears slightly droopy, 3: both ears straight downward, and 4: head tilt.

Blood samples were collected via jugular venipuncture from the calves into vacutainer tubes 24 h after colostrum administration.

Table 1
Primers used in the present study for real-time polymerase chain reaction analysis.

Targets	Primers	Annealing temperature	Reference
Total bacteria	F: actcctacgggaggcag R: gactaccagggtatctaatcc	62 °C	Stevenson and Weimer (2007)
Escherichia coli	F:ggaagaagcttgcttctttgctgac R:agcccggggatttcacatctgactta	62 °C	Sabat et al. (2000)
Clostridium cluster XIVa	F: cggtacctgactaagaagc R: agtttyattcttgcgaacg	60 °C	Ramirez-Farias et al. (2009)
Faecalibacterium prausnitzii	F: ggaggaagaaggtcttcgg R: aattccgcctacctctgcact	60 °C	Ramirez-Farias et al. (2009)
Bifidobacterium phosphoketolase	F: atcttcggaccbgaygagac R: cgatvacgtgvacgaaggac	60 °C	Cleusix et al. (2010)

After clotting, the samples were centrifuged at 3000 × *g* for 10 min, and then serum samples were collected and stored at −20 °C until analysis. Fecal samples were collected on day 7, 35 and 56 after the first feeding of that day using sterile gloves. A finger was inserted into the anus of the calf to stimulate defecation. A cup was used to catch the feces, and the sample was stored at −20 °C.

2.3. Serum total protein determination

Serum total protein (STP) concentrations were determined using a temperature compensating refractometer (TS Meter; American Optical, Buffalo, NY, USA).

2.4. Real-time polymerase chain reaction analysis of fecal bacterial targets

Total DNA from fecal samples was isolated with the repeated bead beating plus column (RBB + C) method (Yu and Morrison, 2004). DNA quality and quantity were measured using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Total DNA was then diluted to a final concentration of 20 ng/μL, and stored at −20 °C until analysis. The populations of total bacteria, *E. coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Bifidobacterium* spp. were estimated using specific primers (Table 1) and SYBR green chemistry (fast SYBR® green master mix; Applied Biosystems, Foster City, CA, USA) with a StepOne Plus real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA). The real-time PCR conditions were 95 °C for 20 s (5 min for total bacteria), followed by 40 cycles at 95 °C for 3 s (20 s for total bacteria) and annealing temperatures (as in Table 1) for 30 s. Each PCR (20 μL) contained 10 μL SYBR Green, 1 μL (0.5 μL for total bacteria) of each primer (20 mM), 1 μL of template DNA, and 7 μL (8 μL for total bacteria) of sterile H_2O. Three replicates of each DNA template were used, and negative controls (sterile distilled water) were also loaded on each plate to exclude possible contamination.

The bacterial populations were determined by calculating the copy numbers of 16S rRNA or specific genes according to the method of Zhou et al. (2009). Standard curves were constructed by using the bacterial-specific primers based on a serial dilution of plasmid DNA from the domain bacteria, *E. coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii*, and the xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (xfp) amplicon, respectively. Xylulose-5-phosphate/fructose-6-phosphate phosphoketolase is the key enzyme of the F6P-phosphoketolase pathway in *Bifidobacteria* and has been widely used to characterize *Bifidobacteria* (Cleusix et al., 2010). The copy numbers of 16S rRNA genes targeting the specific bacteria and xfp gene copy number were calculated through the equation ($N_s = (Q_c \times C \times V)/(S \times W)$), where N_s is the copy number in every gram of fresh fecal sample; Q_c is the quantitative copy number from the standard curve; C (ng/μL) is the DNA concentration of sample; V (μL) is the dilution volume of isolated DNA; S (ng) is the amount of DNA analyzed; and W (g) is the weight of sample used for DNA isolation.

2.5. Statistical analysis

Prior to analysis, daily measurements of intake, growth performance and healthy scores were reduced to weekly means. Microbial population data were transformed using the logarithmic transformation (base 10) to achieve normal distribution. All data were analyzed using the PROC MIXED procedure of SAS (2002). Calf was the experimental unit. Treatment, time (week or day) and their interaction were fixed effects with week/day being considered as a repeated effect. If the interaction was not significant, it was subsequently excluded from the final model (Wallenstein et al., 1980). The probability difference (PDIFF) option and the Kenward-Roger test were applied to evaluate multiple comparisons among the treat group means and the weekly group means. Mean values were considered to be significantly different when $P < 0.05$. Results are reported as least squares means with standard error of the mean.

3. Results

All calves were healthy at the start of the study and had adequate transfer of passive immunity; STP was 5.91 ± 0.13 and 5.94 ± 0.14 g/dL for control and treated calves, respectively. There were no interactions between treatment and time for any

Table 2
Intake, growth performance and fecal score of claves treated with or without yeast probiotic (*Saccharomyces cerevisiae* var *boulardii*) during the first 8 weeks.

	Treatment		SEM	Week								SEM	P value		
	CON	Treat		1	2	3	4	5	6	7	8		Treat	Week	Treat × week
MR intake, L/d	6.7	6.7	0.11	5.7^{c}	6.9^{b}	7.8^{a}	7.9^{a}	7.7^{a}	4.0^{d}	–	–	0.13	0.96	< 0.01	0.99
Starter intake, g/d	944	871	47.1	–	31^{f}	103^{ef}	179^{e}	381^{d}	892^{c}	2061^{b}	2706^{a}	46.4	0.26	< 0.01	0.87
ME intake, Mcal/d	5.6	5.4	0.13	3.9^{g}	4.8^{f}	5.6^{d}	5.9^{c}	6.3^{b}	5.1^{e}	5.4^{d}	7.1^{a}	0.14	0.36	< 0.01	0.91
ADG, g/d	789	838	33.3	908^{ab}	422^{d}	841^{b}	859^{b}	649^{c}	748^{bc}	1040^{a}	1044^{a}	67.5	0.29	< 0.01	0.09
ME intake: gain, Mcal/kg	8.6	7.7	0.43	4.9^{e}	11.2^{a}	8.5^{bc}	7.3^{cd}	11.0^{a}	10.1^{ab}	5.4^{de}	7.0^{cde}	0.97	0.15	< 0.01	0.86
Fecal Score	0.48	0.47	0.028	1.04^{b}	1.56^{a}	0.82^{c}	0.25^{d}	0.05^{e}	0.02^{e}	0.04^{e}	0.00^{e}	0.049	0.80	< 0.01	0.56

MR = milk replacer; ADG = average daily gain; ME = metabolizable energy; the ME of milk replacer and starter were estimated based on dairy cattle NRC (2001); $^{a–f}$ Weekly means with different superscript letters are significantly different ($P < 0.05$).

measurements in this study. Providing SCB treatment in the MR did not alter MR intake, which averaged 7.2 L/d during the first 5 weeks and 4.0 L/d during week 6. Starter intake also was not affected by treatment; however, it did significantly increase with age, averaging 173 g/d during the first 5 weeks, 892 g/d at week 6 and 2383 g/d during week 7 and 8 (Table 2).

The MR intake (Fig. 1A) and starter intake (Fig. 1B) of calves were similar between treatments. The most rapid increase of starter intake occurred during the week of weaning. There was no difference between treatments for ADG (Fig. 1C) and final BW, which was 88.02 ± 1.92 kg for Control and 87.42 ± 1.52 kg for Treat at week 8 of life (data not shown). Ratio of ME intake:gain was unaffected by treatment (Table 2), and averaged 8.64 ± 0.54 Mcal/kg and 7.68 ± 0.42 Mcal/kg for Control and Treat, respectively. In addition, fecal score (Fig. 1D) was also not affected by treatment. However, there were significant age effects ($P < 0.01$) for ME intake, ADG, ME intake:gain and fecal score (Table 2). The highest values for ME intake and ADG were both at week 8 (7.11 Mcal/g and 1044 g/d, respectively), whereas the lowest values were at week 6 (5.09 Mcal/g) and week 2 (422 g/d), respectively. The highest ME intake:gain was at week 2 (11.22 Mcal/kg) and the lowest was at week 1 (4.89 Mcal/kg). The highest fecal scores were at week 2 (averaged 1.56), and the values dropped close to 0 from week 5 of age to the end of the study. The number of days in which calves had diarrhea (fecal score ≥ 2) averaged 5.14, and were not different between treatments. Moreover, the nasal and ear scores of calves were not altered by treatment. Almost all calves scored 0 (*i.e.*, normal) during the entire study (data not shown).

There was no treatment effect on fecal populations of total bacteria, *E. coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* or *Bifidobacterium* (Table 3). However, there were significant time effects ($P < 0.01$) for populations of total bacteria, *E. coli*, *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii*. The fecal *E. coli* population was reduced ($P < 0.01$) with age, with the highest value being at day 7. In contrast, populations of total bacteria and *Clostridium* cluster XIVa from day 35 and 56 were higher ($P < 0.01$) than those from day 7. In addition, the *Faecalibacterium prausnitzii* population was increased ($P < 0.01$) from day 7 to

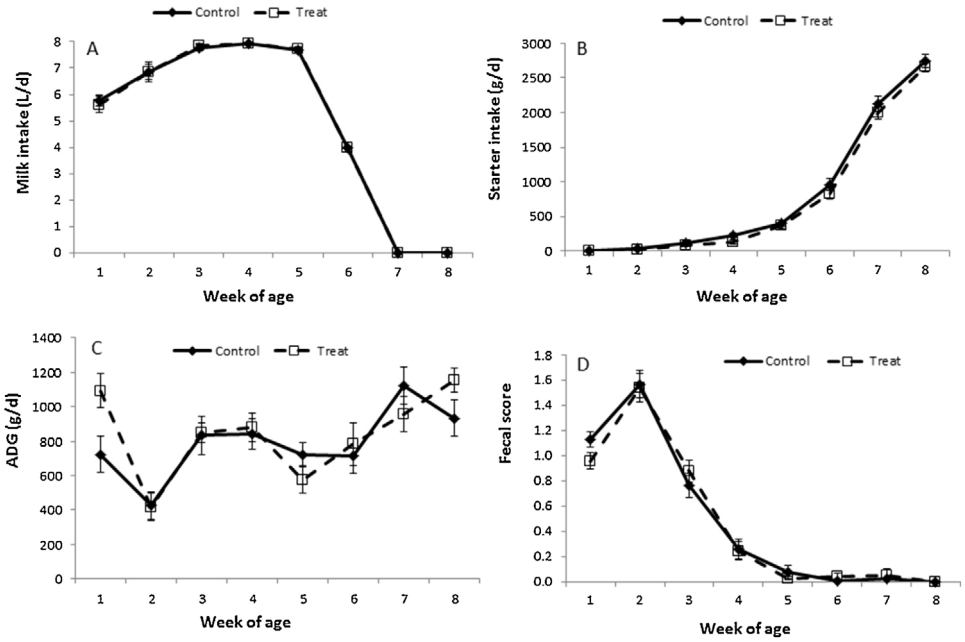

Fig. 1. Milk replacer intake (A), starter intake (B), average daily gain (ADG; C), and fecal score (D) of calves fed with or without yeast probiotic (*Saccharomyces cerevisiae* var *boulardii*) during the first 8 weeks.

Table 3
Targeted bacterial populations (copy numbers (log10)/g) in feces from calves treated with or without yeast probiotic (*Saccharomyces cerevisiae* var *boulardii*) during the first 8 weeks.

	Treatment		SEM	Day			SEM	P value		
	Control	Treat		7	35	56		Treat	Day	Treat × Day
Total bacteria	11.99	11.85	0.101	11.48^{b}	12.14^{a}	12.14^{a}	0.118	0.36	< 0.01	0.30
Escherichia coli	8.45	8.48	0.120	8.99^{a}	8.40^{b}	8.02^{c}	0.140	0.84	< 0.01	0.19
Clostridium cluster XIVa	11.40	11.28	0.163	10.81^{b}	11.59^{a}	11.61^{a}	0.220	0.6	< 0.01	0.33
Faecalibacterium prausnitzii	9.78	9.80	0.120	9.09^{c}	10.33^{a}	9.93^{b}	0.139	0.93	< 0.01	0.99
Bifidobacterium phosphoketolase	8.75	8.69	0.145	8.77	8.62	8.78	0.177	0.77	0.78	0.59

Data were transformed using the logarithmic transformation (base 10) to achieve normal distribution. $^{a–c}$ Weekly means with different superscript letters are significantly different ($P < 0.05$).

day 35, but decreased ($P < 0.01$) from day 35 to day 56.

4. Discussion

Globally, the use of probiotics as an alternative to antibiotics in agriculture practice has increased because of the increasing concerns around antibiotic resistance (Hume, 2011). A variety of microbial species, such as *Bacillus* spp., *Enterococcus* spp. and *Saccharomyces* yeast (Simon et al., 2001), have been used in livestock as probiotics products. The present study investigated the effects of supplementing a *Saccharomyces* yeast product on dairy calf growth performance and its potential to improve animal health. The lack of treatment effect on intake and performance in this study was in agreement with Quigley et al. (1992), who observed no difference in feed intake or BW gain of calves fed a yeast culture product supplemented in a starter feed. In contrast, Lesmeister et al. (2004) reported that including 20 g/kg of SC yeast culture in a starter feed improved DM intake and BW gain of young calves, but there was no effect when the supplementing dose of yeast culture was reduced to 1 g/kg. Galvão et al. (2005) indicated that feeding a live SC supplemented into starter increased grain intake and BW gain of calves, but there was no effect when a live SCB yeast was incorporated into MR. Magalhães et al. (2008) observed no difference in overall starter intake throughout the experimental period (week 1–week 10) between control and SC group but a marginal increase from week 1 to 4 for calves fed a SC yeast culture product. Differing responses to feeding yeast products on intake and BW gain may relate to the type of yeast product (live yeast *vs.* yeast culture), strain of yeast, amount fed to the animal, delivery method of yeast (in milk *vs.* in starter), and disease challenge present.

The lack of treatment effect on fecal scores of calves was unexpected. In young calves, although fecal scores were similar between treatments, Galvão et al. (2005) reported that the calves fed SCB in MR had fewer days with diarrhea during pre-weaning (control *vs.* SCB: 5.83 *vs.* 4.00 days), which was inconsistent with our results. In that study, there was no difference on days with diarrhea post-weaning (control *vs.* SCB: 2.08 *vs.* 2.83 days) between control and SCB treatment; however, the high incidences of diarrhea observed after weaning, which averaged 2.45 days, suggested that the calves were still under stress post-weaning. In the current study, the fecal scores of all calves were almost 0 after weaning, and even during the most stressful week (*i.e.*, week 2) the averaged fecal score of calves were below 2 (*i.e.*, 1.56), which suggested that the severity level of diarrhea was very low although the days with diarrhea lasted approximately 5 days. The relatively low disease challenge of calves during this study may be partially resulted from the adequate colostrum feeding strategy. It has been commonly accepted that the consumption of an adequate quantity of high quality colostrum within the first 24 h of birth is vital for calves to successfully acquire passive immunity (Godden, 2008), which is inversely correlated with morbidity and mortality rates of calves (Meganck et al., 2014). In the current study, all calves received adequate colostrum and had STP concentrations in excess of 5.2 g/dL, which is considered to be indicative of adequate transfer of passive immunity (Weaver et al., 2000). Moreover, the good management of pre-weaned calves with a conventional medication protocol may also be a factor contributing to the relatively low disease challenge. In support, the ADG of calves during pre-weaning (over 700 g/d) even if during the most stressful period (week 2; 422 g/d) were relatively high as compared to that reported in the study of Galvão et al. (2005); 438 g/d for calves fed control MR. The healthy condition of calves was also reinforced by the fact that all the calves had low nasal and ear scores (mostly scored 0) in this study. Therefore, the results of this study suggest the SCB effect on gastrointestinal health of calves is minimal when calves are healthy and well-managed.

Benefits of feeding probiotics in ruminants to improve animal health by balancing the gastrointestinal microbial ecosystem are of growing concern (Uyeno et al., 2015) and studies on the effect of probiotics of the calf's gut microbiome are limited. Since it has been reported that the bacterial community in fecal samples from dairy calves could represent the composition of the large-intestinal bacterial community (Uyeno et al., 2010), the current study compared 5 microbial markers associated with animal growth and health in feces from control and treated calves to evaluate the probiotics effect on gut microbiota. Since *E. coli* is one of the most common and important causes of neonatal diarrhea in dairy calves (Muktar et al., 2015), the lack of an SCB treatment effect on the fecal *E. coli* population was consistent with the fecal score results and suggests that feeding SCB may not alter the gastrointestinal *E. coli* population in healthy calves. Our results also provided quantified data that the calves with an averaged $10^{8} \sim 10^{9}$ 16S rRNA gene copy/g (up to 0.3% of total bacteria) fecal *E. coli* population at day 7 after birth did not show significant symptoms of diarrhea. Moreover, the lack of beneficial bacterial population changes (*i.e.*, *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii* and *Bifidobacterium*) in fecal samples from treated calves suggested that feeding SCB may have no effect on gut microflora.

To our knowledge, this was the first time that the specific microbial targets *Clostridium*, *Faecalibacterium* and *Bifidobacterium* influenced by week of age in dairy calves were quantified. In the current study, *Clostridium* were present in the feces of dairy calves in great abundance, and the population of *Clostridium* cluster XIVa increased with week of age, from 7.8×10^{10} (21.40% of total bacteria) at day 7 after birth to 3.9×10^{11} gene copy/g (27.86% of total bacteria) at day 35. The population of *Faecalibacterium prausnitzii* was also affected by week of age, which increased from 1.2×10^{9} (0.41% of total bacteria) at day 7 to 2.2×10^{10} gene copy/g (1.57% of total bacteria) at day 35. The dynamic changes on fecal bacterial populations of dairy calves during the first 8 weeks corresponded to a previous study by Uyeno et al. (2010) who found that the Chao1 index gradually increased from the first to the seventh week of life. In fact, during the first 8 weeks after birth, calves not only experienced an age change, but were also challenged by a diet transition from milk to solid feed (Khan et al., 2016). It has been reported that solid feed consumption altered gut microbiome during weaning transition (Malmuthuge et al., 2013), and the shift of gut microbiomes affected by solid feed could be toward to the mature ruminant state (Meale et al., 2016). By targeting specific bacteria, the current results suggest that the fecal microbial population in dairy calves can be influenced by week of age and weaning, and the natural microbial changes occurring over time in the life of a health calf appears to be more important than the expected SCB effect. Nevertheless, since there were only fecal samples tested in the present study, it should be noted that the gut bacterial community can be further investigated to confirm the SCB and age effects on gut microbiota.

5. Conclusion

In the present study, supplementing SCB in MR did not affect intake, ADG or feed efficiency. Fecal, nasal and ear scores were low throughout the study and were not influenced by dietary treatment, which suggests minimal potential benefit from feeding SCB to improve calf's performance and health when they are in good health. Moreover, the lack of changes in fecal populations of *E. coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Bifidobacterium* by dietary treatment further implies that feeding yeast probiotics has no additive effect when there is no disease challenge for calves.

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References

Canadian Council on Animal Care (CCAC), 2009. Guide to the Care and Use of Farm Animals in Research, Teaching and Testing. CCAC, Ottawa, ON.
Cleusix, V., Lacroix, C., Dasen, G., Leo, M., Le Blay, G., 2010. Comparative study of a new quantitative real-time PCR targeting the xylulose-5-phosphate/fructose-6-phosphate phosphoketolase bifidobacterial gene (xfp) in faecal samples with two fluorescence in situ hybridization methods. J. Appl. Microbiol. 108, 181–193.
Drackley, J.K., 2008. Calf nutrition from birth to breeding. Vet. Clin. Food Anim. 24, 55–86.
Friedman, D.B., Kanwat, C.P., Headrick, M.L., Patterson, N.J., Neely, J.C., Smith, L.U., 2007. Importance of prudent antibiotic use on dairy farms in south Carolina: a pilot project on farmers' knowledge, attitudes, and practices. Zoonoses Public Health 54, 366–375.
Galvão, K.N., Santos, J.E., Coscioni, A., Villaseñor, M., Sischo, W.M., Berge, A.C., 2005. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal Escherichia coli. Reprod. Nutr. Dev. 45, 427–440.
Godden, S., 2008. Colostrum management for dairy calves. Vet. Clin. North Am. Food Anim. Pract. 24, 19–39.
Hume, M.E., 2011. Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. Poult. Sci. 90, 2663–2669.
Kelesidis, T., Pothoulakis, C., 2012. Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. Therap. Adv. Gastroenterol. 5, 111–125.
Khan, M.A., Bach, A., Weary, D.M., von Keyserlingk, M.A., 2016. Invited review: transitioning from milk to solid feed in dairy heifers. J. Dairy Sci. 99, 885–902.
Lesmeister, K.E., Heinrichs, A.J., Gabler, M.T., 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci. 87, 1832–1839.
Lopetuso, L.R., Scaldaferri, F., Petito, V., Gasbarrini, A., 2013. Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog. 5, 23.
Lorenz, I., Fagan, J., More, S.J., 2011. Calf health from birth to weaning: II. Management of diarrhoea in pre-weaned calves. Irish. Vet. J. 64, 9–14.
Magalhães, V.J.A., Susca, F., Lima, F.S., Branco, A.F., Yoon, I., Santos, J.E.P., 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci. 91, 1497–1509.
Malmuthuge, N., Li, M., Goonewardene, L.A., Oba, M., Guan, L.L., 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. J. Dairy Sci. 96, 3189–3200.
Malmuthuge, N., Griebel, P.J., Guan, L.L., 2015. The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. Front Vet. Sci. 2, 36.
McFarland, L.V., 2010. Systemic review and meta-analysis of Saccharomyces boulardii in adult patients. World J. Gastroenterol. 16, 2202–2222.
McGuirk, S.M., 2008. Disease management of dairy calves and heifers. Vet. Clin. North Am. Food Anim. Pract. 24, 139–153.
Meale, S.J., Li, S.C., Azevedo, P., Derakhshani, H., Plaizier, J.C., Khafipour, E., Steele, M.A., 2016. Development of ruminal and fecal microbiomes are affected by weaning but not weaning strategy in dairy calves. Front. Microbiol. 7, 582.
Meganck, V., Hoflack, G., Opsomer, G., 2014. Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. Acta Vet. Scand. 56, 75–83.
Muktar, Y., Mamo, G., Tesfaye, B., Belina, D., 2015. A review on major bacterial causes of calf diarrhea and its diagnostic method. J. Vet. Med. Anim. Health 7, 173–185.
National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle (7th Rev). Natl. Acad. Press, Washington, DC.
Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., Matuchansky, C., 2005. Review article: bifidobacteria as probiotic agents – physiological effects and clinical benefits. Aliment. Pharmacol. Ther. 22, 495–512.
Quigley, J.D., Wallis, L.B., Dowlen, H.H., Heitmann, R.N., 1992. Sodium bicarbonate and yeast culture effects on ruminal fermentation growth, and intake in dairy calves. J. Dairy Sci. 75, 3531–3538.
Ramirez-Farias, C., Slezak, K., Fuller, Z., Duncan, A., Holtrop, G., Louis, P., 2009. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium

adolescentis and Faecalibacterium prausnitzii. Br. J. Nutr. 101, 533–542.
SAS Institute, 2002. SAS User's Guide: Statistics Ver 9.0. SAS Institute Cary, NC, USA.
Sabat, G., Rose, P., Hickey, W.J., Harkin, J.M., 2000. Selective and sensitive method for PCR amplification of Escherichia coli 16S rRNA genes in soil. Appl. Environ. Microb. 66, 844–849.
Simon, O., Jadamus, A., Vahjen, W., 2001. Probiotic feed additives—effectiveness and expected modes of action. J. Anim. Feed Sci. 10, 51–67.
Stevenson, D.M., Weimer, P.J., 2007. Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl. Microbiol. Biotechnol. 75, 165–174.
USDA, 2007. Dairy 2007, Heifer Calf Health and Management Practices on U.S. Dairy Operations. USDA :APHIS:VS, CEAH, Fort Collins, CO.
Uyeno, Y., Sekiguchi, Y., Kamagata, Y., 2010. rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves. Appl. Microbiol. 51, 570–577.
Uyeno, Y., Shigemori, S., Shimosato, T., 2015. Effect of probiotics/prebiotics on cattle health and productivity. Microbes Environ. 30, 126–132.
Wallenstein, S., Zucker, C.L., Fleiss, J.L., 1980. Some statistical methods useful in circulation research. Circ. Res. 47, 1–9.
Weaver, D.M., Tyler, J.W., VanMetre, D.C., Hostetler, D.E., Barrington, G.M., 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14, 569–577.
Williams, N.T., 2010. Probiotics. Am. J. Health-Syst. Pharm. 67, 449–458.
Yu, Z.T., Morrison, M., 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36, 808–812.
Zhou, M., Hernandez-Sanabria, E., Guan, L.L., 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microb. 75, 6524–6533.