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The effects of a prebiotic supplement (Prebio Support) on fecal and salivary IgA in neonatal dairy calves

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ABSTRACT

The newborn calf's gastrointestinal tract is sterile at birth, but by 3 days of age coliforms, *Lactobacilli*, and *Bifidobacteria* are the predominant flora in the feces. During the preweaning period, calves are susceptible to diarrhea that can lead to high levels of morbidity and mortality. Diarrhea has been related with a decrease of beneficial microbiota and an increase of coliform counts in feces. Prebiotic supplements are believed to decrease diarrhea and positively affect some parameters of the immune system. In calves, these supplements have shown some promising effects on intestinal microbial populations but there is limited information about effects on immunity. The main objectives of this study were to evaluate effects of a prebiotic supplement containing fermentation products of lactic acid bacteria on the mucosal immune system by measuring fecal and salivary IgA and to evaluate calf health and growth performance. In this trial 40 Holstein calves were randomly assigned to receive milk replacer with a prebiotic supplement (20 g/day Prebio Support™; Meiji Feed Co., Ltd. Tokyo, Japan) or the same milk replacer with no prebiotic (control). Fecal and salivary IgA, calf health, plasma IgG, and lymphocyte counts were not affected by treatment. *Lactobacilli* count in feces was higher ($P=0.05$) and *Bifidobacteria* tended to be higher ($P=0.07$) in calves fed prebiotic. Prebiotic supplement increased beneficial bacteria in calves, but did not decrease overall incidence of diarrhea in this trial. Calves in this study were all affected by cryptosporidiosis and some were treated with antibiotics, so it is possible that this limited some of the effects of the prebiotic product. Fecal IgA seemed to be a good measure of mucosal immunity, and more studies are needed to develop methods to measure this type of immunity in calves.

1. Introduction

The gastrointestinal tract of newborn calves is sterile; microbes are introduced from the environment and from the dam's birth canal and colonize the gastrointestinal tract (Ewaschuk et al., 2004; Ouwehand et al., 2002). By 3 days after birth, coliforms, *Lactobacilli*, and *Bifidobacteria* are the predominant flora in the feces (Ouwehand et al., 2002; Vlková et al., 2006). However, in the neonatal calf the microbial population is in transition and extremely sensitive. Sudden changes in diet or environment, disease, or other stress can cause alterations (Krehbiel et al., 2003; Ouwehand et al., 2002) in this microbial system. Newborn calves are often exposed to high levels of stress during the first days of life because they experience changes in environment, diet, feeding conditions, handling, and immunity. It is during this period that calves develop diarrhea, the most common health concern and cause of death during the preweaning period. Calves with diarrhea require prompt attention and care; failure to treat these calves can lead to high levels of morbidity and mortality (Kertz, 2003; Lundborg, 2004; Ribeiro et al., 2009). Diarrhea has been related to an increase of coliform bacteria counts in the intestines and a decrease in *Lactobacilli* and *Bifidobacteria* counts (Krehbiel et al., 2003; Ouwehand et al., 2002). The increase of coliform bacteria in the intestines may produce putrefactive substances and harm the host (Fujisawa et al., 2010). As a result, gut

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microbiota are important to the health and development of the host (Ng et al., 2009; Rowland et al., 2010).

Lactic acid bacteria, especially *Lactobacillus* and *Bifidobacterium* spp., have been used as feed supplements to influence the gut microbiota to stimulate immune responses in the host (He et al., 2000). The bacteria in these supplements, like *Lactobacillus rhamnosus strain GG* (Ewaschuk et al., 2004), *Lactobacillus acidophilus* (Higginbotham and Bath, 1993), and *Lactobacillus gasseri* K7 (Bogovič et al., 2006), had been proven to survive the gastrointestinal tract to then colonize the intestinal mucosa. Once in the intestines the bacteria in the supplements are believed to improve intestinal microbial balance by decreasing the adherence of pathogens in the lumen of the intestinal mucosa and affecting the mucosal immune system (Isolauri et al., 2001; Ng et al., 2009). There are many studies with lactic acid bacteria supplements in humans and rodents, and they have shown beneficial effects at the intestinal level, such as decreasing diarrhea in children (Rowland et al., 2010), decreasing or increasing the numbers of IgA and CD4+ T cells in the lamina propria (Perdigon et al., 1999), reducing gastric mucosal inflammation in humans (Sakamoto et al., 2001), and preventing gastric ulcers in rats (Uchida and Kurakazu, 2004). Also other aspects of the immune system are influenced, such as increasing plasma IgA in humans and increasing the amount of IgA in response to *Salmonella typhimurium* inoculation in rodents (Erickson and Hubbard, 2000).

Some reports using these supplements in calves have found promising results on the intestinal microbial population (Fujisawa et al., 2010; Heinrichs et al., 2009). However, more information about effects of lactic acid bacteria supplements on immunity during the preweaning period is needed. The main objectives of this study were to evaluate effects of a prebiotic supplement on the mucosal immune system by measuring fecal and salivary IgA and to evaluate effects on body weight, feed intake, lymphocyte counts, fecal bacteria populations and general health. Additionally we sought to better understand the role of fecal and salivary IgA in the health of dairy calves.

2. Materials and methods

2.1. Animals and treatments

All study procedures were approved by The Pennsylvania State University Institutional Animal Care and Use Committee. Twenty-eight Holstein heifer and 12 Holstein bull calves from the university herd were randomly assigned to 2 groups (20/treatment; equal numbers of heifers and bulls on each treatment) at 1 day of age. Calves were removed from their dams within 1 h of birth, fed pooled frozen colostrum for 2 feedings (4 L/day) and then fed transition milk (second and third milking) from their respective dams for 2 days before being changed to milk replacer. Samples of colostrum (20 mL) were collected and analyzed for IgG and IgA using ELISA (Bethyl Laboratories, Inc. Montgomery, TX, USA) to be used as a baseline in further calf IgG and IgA analyses. Calves were vaccinated after birth for infectious bovine rhinotracheitis and parainfluenza$_3$ (1 intranasal dose TSV-2; Pfizer Animal Health, Exton, PA, USA) and for bovine rota-coronavirus (1 oral dose Calf-Guard; Pfizer Animal Health, Exton, PA, USA). A blood sample was taken between 24 and 48 h for measurement of IgG status. Calves were housed in 1.2×2.4-m, open-sided, individual pens bedded with wood shavings in a naturally ventilated barn. The control group was fed commercial milk replacer (20% crude protein, 20% crude fat; Renaissance Nutrition, Inc., Roaring Spring, PA, USA) containing a coccidiostat (Deccox; 0.05 g/kg; Alpharma, Inc. Bridgewater, NJ, USA) but no other additives. The second group was fed the same milk replacer with Prebio Support (PB; Meiji Feed Co., Ltd. Tokyo, Japan), according to company recommendations (20 g/day) which contained fermentation products of *L. gasseri* OLL2716 and *Propionibacterium freudenreichii* ET-3. Addition of PB began on day 2 and continued through week 5; 10 g of product was added to transition milk or milk replacer at each feeding. All milk replacer was fed twice daily at 6% of birth body weight per feeding. During week 6, calves were fed once a day at 6% of birth weight, and weaned at the end of week 6. Fresh calf starter grain and water were offered ad libitum and fed daily from day 1 of age with refusals weighed weekly to monitor feed intake. Nutrient composition of milk replacer and calf starter is listed in Table 1.

2.2. Health and growth measurements

Calf health was monitored daily by assigning scour (diarrhea), respiratory, and general appearance scores (Lesmeister and Heinrichs, 2005). Body weight, hip height, withers height, and heart girth were measured at day 1 and weekly. Blood samples were collected into evacuated glass tubes containing heparin at weeks 1, 2, 3, 4, and 5 for analysis of lymphocyte populations via flow cytometry; CD3, CD4, CD8, CD21 and γδ T cell markers were determined (Ohtsuka et al., 2006) at the Pennsylvania State University Huck Institute for the Life Sciences. In addition plasma samples were obtained from blood collected at 48 h and weeks 1, 2, 3, 4, and 5 for IgG analysis using ELISA (Bethyl Laboratories, Inc. Montgomery, TX, USA). Fecal grab samples were collected from the rectum at days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of

Table 1
Nutrient composition of milk replacer and calf starter fed to 40 Holstein calves.

	Milk replacer		Calf starter	
	Mean	SD	Mean	SD
Dry matter (DM) (%)	94.7	0.6	84.5	0.7
Crude protein (CP) (% DM)	20.9	0.5	23.7	3.5
Soluble protein (% CP)			15.5	1.1
Fat acid hydrolysis (% DM)	19.9	0.3		
Acid detergent fiber (% DM)			5.6	1.2
Neutral detergent fiber (% DM)			14.2	1.9
Ash (% DM)			7.2	0.6
Calcium (% DM)	0.7	0.1	1.17	0.12
Phosphorus (% DM)	0.7	0.0	0.51	0.01
Magnesium (% DM)			0.35	0.03
Potassium (% DM)			1.34	0.15
Sodium (% DM)	0.9	0.1	0.51	0.04
Iron (ppm)			295	21
Manganese (ppm)			72	5.7
Zinc (ppm)			105	7.2
Copper (ppm)			14	2.1
Total digestible nutrients (% DM)			78.7	0.6
Net energy, maintenance (Mcal/kg)			1.90	0.02
Net energy, gain (Mcal/kg)			1.26	0.02

calf age. Fecal samples from days 2, 6, 10, 14, and 18 were collected and stored under anaerobic conditions until enumeration of *Bifidobacteria*, *Lactobacilli*, lecithinase-positive *Clostridia*, and *Enterobacteriaceae* species on fresh samples (Gilliland et al., 1975; Hadadji et al., 2005; Rada and Petr, 2002). Saliva samples were collected at days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 using a small cotton ball placed inside the calf's mouth until it was reasonably wet (1 to 2 min; Toyoguchi et al., 2001). The cotton with absorbed saliva was placed in a 10-mL syringe and compressed to recover liquid. The recovered liquid was stored at −20 °C for later analysis. All fecal and salivary samples were analyzed for IgA using ELISA (Bethyl Laboratories, Montgomery, TX, USA).

2.3. Statistical analysis

All statistical analyses were conducted in SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA) with the MIXED procedure using a model with fixed effects of treatment and time (day or week) and their interaction and a random effect of calf within treatment. Repeated measurements of time were analyzed using a first-order autoregressive covariance structure (Littell et al., 1998).

To more closely approximate a normal distribution, fecal bacteria counts were transformed by $\log_{10}(x+1)$ before being analyzed with the model described above. Treatment effects were considered significant when $P<0.05$ and trends were identified at $P<0.10$. For least squares means separation tests, Tukey–Kramer adjustment was applied to account for multiple comparisons.

3. Results

3.1. Health performance

All calves completed the study, with the exception of 2 calves that died during the trial. One of the calves died from dehydration because it was not treated correctly with electrolytes and another calf died of injuries incurred at birth. These calves that died during the trial were removed as it was determined that their cause of death was not a result of the treatment. Their samples and information collected during the time they participated in the trial were not included in the results presented.

In general calves were healthy except from 8 to 14 days of age. During this period all calves in the trial developed scours due to *Cryptosporidium parvum*, which is endemic at low levels in our facility, and all of them were given electrolyte treatment (Bluelite C, Tech Mix, Inc., Stewart, MN, USA) for 3 days as a preventative measure. Additionally, 8 calves from the control group and 7 from group PB required antibiotic treatment (Naxcel, Pfizer Animal Health, Exton, PA, USA) for 3 days as a result of severe scours accompanied by cryptosporidiosis. No other major pathogens were isolated from samples. Scour scores were similar for both treatments; however, there was a week effect ($P<0.01$) for scour scores as expected. Scour scores exhibited a normal pattern of increasing at week 2, starting to decrease at week 3, and becoming normal by week 4 and 5 for both treatments (Table 2). Although a week by treatment interaction was observed overall ($P=0.05$), when individual means were compared, no differences were detected. When treated calves were analyzed separately from healthy calves there was no difference between treatments for body weight (P values 0.93 and 0.73 for healthy and treated calves). Other parameters were analyzed with treated calves eliminated and no parameters were different than from the entire data set analysis. Therefore treated calves remained in the data set for all analysis.

Table 2
Least squares means of scours, respiratory, general appearance, and daily health scores[1] of 40 Holstein calves fed milk replacer with or without (control) a prebiotic additive.

	Treatment		SEM
	Control	Prebiotic	
Scour score			
Week 1	1.09[c]	1.30[c]	0.09
Week 2	2.73[a]	2.91[a]	0.09
Week 3	2.19[b]	1.94[b]	0.09
Week 4	1.17[c]	1.06[c]	0.09
Week 5	1.00[c]	1.03[c]	0.09
Respiratory score			
Week 1	1.01	1.01	0.02
Week 2	1.00	1.00	0.02
Week 3	1.00	1.00	0.02
Week 4	1.00	1.05	0.02
Week 5	1.00	1.00	0.02
General appearance score			
Week 1	1.00[b]	1.08	0.03
Week 2	1.14[a]	1.04	0.03
Week 3	1.04[ab]	1.01	0.03
Week 4	1.00[b]	1.00	0.03
Week 5	1.00[b]	1.01	0.03
Total daily score			
Week 1	1.02[c]	1.18[b]	0.07
Week 2	2.04[a]	2.12[a]	0.07
Week 3	1.48[b]	1.29[b]	0.07
Week 4	1.00[c]	1.00[b]	0.07
Week 5	1.00[c]	1.00[b]	0.07

[1]Scored on a 5-point scale, 1 = normal (Lesmeister and Heinrichs, 2005).
[a,b,c]Values within a column with no letters in common indicate significant effects of time (week) at $P<0.05$; no treatment effects were detected. Treatment by week interaction was significant overall for scour and general appearance scores, but no differences were detected between individual means when multiple comparisons were made (Tukey adjustment applied).

There were no cases of pneumonia during the trial and respiratory scores were the same during the 5 weeks for control calves or calves fed PB; as a result there was no treatment effect and no week effect on respiratory scores (Table 2).

General appearance scores were not affected by treatment, but there was a week effect ($P=0.01$) and a treatment by week interaction ($P=0.01$) due to the scours (Table 2). Total daily scores followed the scour scores and showed no treatment effect, but a week effect was present ($P<0.01$; Table 2).

3.2. Immunoglobulins in colostrum and plasma

Intake of IgG and IgA from colostrum is presented in Table 3. There was no difference in colostrum IgG and IgA intakes between control and PB groups and there was no treatment effect on plasma IgG throughout the study (Table 3); however, there was a week effect ($P<0.01$) as IgG decreased over time.

Table 3
IgG and IgA contents of colostrum and weekly plasma IgG of 40 Holstein calves fed milk replacer with or without (control) a prebiotic additive.

	Treatment		SEM	*P* value[1]
	Control	Prebiotic		
Colostrum IgG (g)	227.8	217.8	19.44	0.72
Colostrum IgA (g)	15.7	14.7	1.19	0.58
Plasma IgG (g/L)				
Week 0	22.4[a]	20.7[a]	1.28	1.00
Week 1	18.1[b]	19.2[ab]	1.28	1.00
Week 2	18.2[b]	16.1[b]	1.28	0.99
Week 3	14.4[c]	16.0[bc]	1.28	1.00
Week 4	14.1[c]	14.4[c]	1.28	1.00
Week 5	13.4[c]	14.4[c]	1.28	1.00

[1]Treatment effect.
[a,b,c]Values within a column with no letters in common indicate significant effects of time (week) at $P<0.05$. Treatment by week interaction was significant overall for plasma IgG, but no differences were detected between individual means when multiple comparisons were made (Tukey adjustment applied).

3.3. Lymphocyte populations

Lymphocyte populations over time are presented in Table 4. All lymphocyte populations demonstrated a week effect ($P \leq 0.01$) but they were not affected by treatment and no treatment by week interactions were detected. There was a tendency toward interaction of treatment and week overall ($P = 0.07$), but no differences between individual means were detected.

3.4. Fecal bacteria

The population of beneficial bacteria in feces was affected by treatment under the conditions of this experiment; however the pathogenic bacteria in feces were not affected. When compared over all weeks, calves on PB treatment had more *Lactobacilli* in their feces (9.06 versus 8.86 ± 0.07 $\log_{10}$ cfu/g of wet feces; $P = 0.05$) and tended to have more *Bifidobacteria* (9.14 versus 8.81 ± 0.12 $\log_{10}$ cfu/g of wet feces; $P = 0.07$) than control calves. Populations of *Clostridia* (3.32 and 3.58 ± 0.53 $\log_{10}$ cfu/g of wet feces for PB and control respectively) and *Enterobacteriaceae* (6.28 and 5.99 ± 0.45 $\log_{10}$ cfu/g of wet feces for PB and Control respectively) were similar for both groups. All bacteria populations in this experiment demonstrated significant changes over time ($P<0.01$; Table 5). No interaction of time and treatment was observed.

3.5. Fecal and salivary IgA

There was no treatment effect on overall fecal and salivary IgA, but there was a time effect. Fecal IgA over time is presented in Fig. 1 and salivary IgA in Fig. 2. There was no correlation between salivary and fecal IgA ($r = 0.01$).

3.6. Growth performance and dry matter intake

There was no significant difference in average daily gains between control (338 ± 22 g/day) and PB calves (324 ± 22 g/day). Grain intake was not affected by treatment (1.598 ± 0.195 kg/day for control calves and 1.325 ± 0.195 kg/day for PB calves). The addition of Prebio Support did not affect hip height, withers height or hearth girth. The total dry matter intake was not affected between control (2.265 ± 0.169 kg/day) and PB calves (2.007 ± 0.169 kg/day). However there was a tendency ($P<.06$) for overall feed efficiency (kg ADG/kg feed intake) to be improved with the prebiotic additive ($0.157 \pm .011$ for control and $0.171 \pm .011$ for PB).

Table 4
Least square means of lymphocyte populations in 40 Holstein calves fed milk replacer with or without (control) a prebiotic additive.

Cell type	Control	Prebiotic	SEM
CD3 (%)			
Week 1	9.6[a]	8.7[b]	3.12
Week 2	14.5[ab]	17.3[ab]	3.04
Week 3	18.4[bc]	17.2[ab]	3.07
Week 4	21.1[c]	22.8[a]	3.04
Week 5	20.7[c]	26.3[a]	3.04
CD4 (%)			
Week 1	3.4[a]	3.0[b]	0.72
Week 2	3.3[a]	4.6[a]	0.70
Week 3	4.5[ab]	5.8[a]	0.71
Week 4	5.4[b]	4.6[a]	0.70
Week 5	6.2[b]	5.9[a]	0.70
CD8 (%)			
Week 1	6.0[a]	4.9[b]	1.25
Week 2	6.4[a]	5.4[ab]	1.22
Week 3	5.7[a]	7.3[ab]	1.23
Week 4	6.2[a]	7.7[ab]	1.22
Week 5	8.2[a]	10.3[a]	1.22
CD21 (%)			
Week 1	4.1[c]	4.2[d]	2.49
Week 2	9.3[bc]	10.2[cd]	2.40
Week 3	16.2[b]	16.4[c]	2.48
Week 4	25.3[a]	26.2[b]	2.45
Week 5	30.8[a]	35.3[a]	2.38
γδ T-cell (%)			
Week 1	8.3[b]	7.4[b]	2.20
Week 2	10.7[ab]	13.0[ab]	2.16
Week 3	15.2[ab]	12.6[ab]	2.16
Week 4	18.8[a]	16.1[a]	2.16
Week 5	18.4[a]	19.9[a]	2.16

[a,b,c,d]Values within a column with no letters in common indicate significant effects of time (week) at $P<0.05$. No significant effects of treatment or interactions of treatment and week were detected.

4. Discussion

In this study, *Lactobacilli* and *Bifidobacteria* counts in feces increased from day 2 to day 6, while *Clostridia* and *Enterobacteria* counts decreased. The increase of beneficial bacteria during this time is related with the beginning of the bacteria population in the intestine of the newborn calf as reported by Vlková et al. (2006). However, from day 6 to day 10 *Lactobacilli* and *Bifidobacteria* counts decreased and *Clostridia* counts increased. This change of bacteria is probably related to the diarrhea that calves developed. Previously, diarrhea has been related with an increase of coliforms and a decrease in *Lactobacilli* and *Bifidobacteria* counts (Abu-Tarboush et al., 1996). Higher counts of *Bifidobacteria* in calves supplemented with this prebiotic product have been found at 21 days of age (Fujisawa et al., 2010). Since there was some degree of

Table 5
Least squares means of fecal bacteria counts (log_{10} cfu/g wet feces) by day for 40 Holstein calves fed milk replacer with or without (control) a prebiotic additive.

Bacteria species	Treatment		SEM
	Control	Prebiotic	
Lactobacilli			
Day 2	8.75[ab]	9.02[ab]	0.13
Day 6	9.18[a]	9.48[a]	0.13
Day 10	8.62[b]	8.89[b]	0.13
Day 14	8.82[ab]	8.95[ab]	0.13
Day 18	8.92[ab]	9.03[ab]	0.13
Bifidobacteria			
Day 2	8.24[b]	9.00[ab]	0.25
Day 6	9.40[a]	9.48[b]	0.25
Day 10	8.66[ab]	8.89[a]	0.25
Day 14	9.05[ab]	9.04[ab]	0.25
Day 18	8.69[ab]	9.32[ab]	0.25
Clostridia			
Day 2	6.40[a]	6.43[a]	0.91
Day 6	0.89[b]	2.06[b]	0.91
Day 10	4.37[ab]	2.82[ab]	0.91
Day 14	5.35[a]	4.34[ab]	0.91
Day 18	0.87[b]	0.93[b]	0.91
Enterobacteriaceae			
Day 2	7.45[a]	8.49[a]	0.72
Day 6	5.63[ab]	6.01[ab]	0.72
Day 10	7.62[a]	5.94[ab]	0.72
Day 14	6.16[a]	6.74[ab]	0.72
Day 18	3.11[b]	4.24[b]	0.72

[a,b]Values within a column with no letters in common indicate significant effects of time (day) at $P<0.05$. No significant interactions of treatment and day were detected.

variability in *Clostridia* levels in the calves and this seems to correspond to when calves had higher scour scores, this finding may merit further investigation to determine if prebiotic treatment could help decrease clostridial colonization in the intestine during this critical time.

In general, PB increased *Lactobacilli* counts and tended to increase *Bifidobacteria* counts in the feces of dairy calves, but did not significantly decrease *Clostridia* and *Enterobacteria*. The

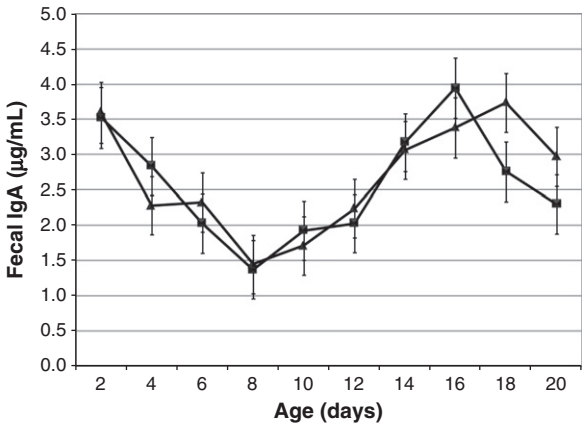

Fig. 1. Concentration of IgA in feces of 40 Holstein calves fed milk replacer containing 20 g/day of a prebiotic supplement (▲) or no prebiotic (■).

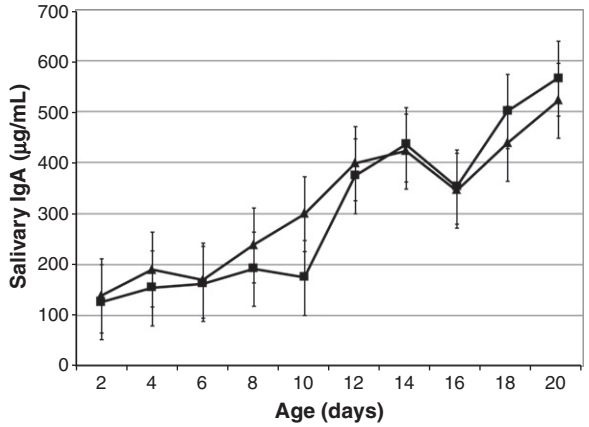

Fig. 2. Concentration of IgA in saliva of 40 Holstein calves fed milk replacer containing 20 g/day of a prebiotic supplement (▲) or no prebiotic (■).

increase of *Lactobacilli* has been found before in calves supplemented with mixed *Lactobacilli* during 9 weeks (Abu-Tarboush et al., 1996) and with *L. acidophilus* isolated from humans and calves (Bruce et al., 1979). However, in a previous study with this product (PB) in calves, only an increase in *Bifidobacteria*, not *Lactobacilli*, was observed (Fujisawa et al., 2010). The effects of *Lactobacilli* supplementation on coliforms have been positive in some trials but not in others and may be related to the specific bacteria used. Bruce et al. (1979) found a decrease of coliforms in calves when an increase of *Lactobacilli* was found, but Fujisawa et al. (2010) and Abu-Tarboush et al. (1996) did not find this decrease in coliform counts. Lactic acid bacteria supplements are believed to increase beneficial bacteria in the intestine and to decrease various pathogens. However, bacteria in the intestines could be modified by many factors, such as diet, disease, stress, or various drugs (Mitsuoka, 2000). In this trial *C. parvum* was a problem for all calves and the use of antibiotics was necessary in some calves to help their recovery. Previous results using a supplement of *L. acidophilus* in waste milk did not find any effect (Higginbotham and Bath, 1993), but when they used this supplement in milk replacer without medication they found positive results (Higginbotham and Bath, 1993). Penicillin, chlortetracycline, and tylosin tend to inhibit the growth of mixed cultures of bacteria. It is possible that we could not realize the potential benefits of PB because of antibiotic sensitivity to cephalosporin (Higginbotham and Bath, 1993). It is possible that because in this trial pathogens did not decrease with the increase of *Lactobacilli* and *Bifidobacteria*, we did not find any effects of PB on the health of calves as reported before in calves fed lactic acid bacteria supplements (Abe et al., 1995; Higginbotham and Bath, 1993). However, some have reported that lactic acid bacteria supplements decrease diarrhea scores at weeks 5, 7, and 8 in calves (Abu-Tarboush et al., 1996); these later weeks were not measured in the present study.

Supplementation with PB did not affect body weight, growth performance, or grain intake of calves in this trial. A lack of effects on weights has been found before using different types of lactic acid bacteria supplements (Görgülü et al., 2003; Higginbotham and Bath, 1993). However, some positive results have been found

in older calves. Calves supplemented with a mixture of lactic acid bacteria for 90 days had higher body weight gains at 3 months of age (Mokhber-Dezfouli et al., 2007), and calves supplemented with *Lactobacilli* or *Bifidobacteria* from 7 days of age had higher weights after 56 days of supplementation (Abe et al., 1995). The use of PB and the increase in beneficial intestinal flora populations did not have any effects on circulating blood lymphocytes as reported before by Heinrichs et al. (2009). CD4 and CD8 lymphocytes increased slightly from week 1 to week 5 as reported previously by Kampen et al. (2006); values reported here were lower compared with means reported by Kampen et al. (2006) but fit within the range reported in that study.

CD21 lymphocytes increased through time from weeks 1 to 5 in both groups and the values reported here were similar to the ones reported before (Kampen et al., 2006). This rapid increase is the result of the young animal being exposed for the first time to large numbers of environmental antigens. The proliferation and maturation of B cells are expected during this period of time (Kampen et al., 2006).

γδ T lymphocytes increased from week 1 to week 5 in both groups and the values reported here are between the range reported by Burton and Kehrli (1996) of 5 to 35% in calves of around 1 year of age and lower than the 25% reported by Wilson et al. (1996) at day 30. The reason for this difference in values is likely because γδ T lymphocytes values vary greatly among individual animals of the same age (Burton and Kehrli, 1996).

IgG values from both groups decreased from week 0 to week 5 as reported by Franklin et al. (1998) and as expected, as colostral IgG declines and calves develop their own immune system. Supplementation with PB did not affect the levels of plasma IgG in calves. Similar results have been found before in chickens supplemented with a mix of *Lactobacilli*, *Bifidobacteria*, and *Streptococci* (Haghighi et al., 2006). However, calves supplemented with *L. acidophilus* and *L. plantarum* or *L. acidophilus* 27SC for 9 weeks had higher serum IgG levels (Al-Saiady, 2010). One of the possible reasons why we did not find any effect of PB on IgG is because plasma IgG levels in both groups at week 0 were higher than minimum recommended levels (>10 mg/mL of IgG) to avoid compromising survival, indicating successful passive immunity transfer. These values were still high at week 5, so it is likely that the calves' own immune systems were functional by 5 weeks of age.

The increase of beneficial bacteria did not have any effects on the production of IgA in feces or saliva. Fecal IgA decreased from day 2 to day 8, then increased from day 8 to 16, and decreased by day 20. High levels of IgA in feces at day 2 likely reflect IgA obtained from maternal colostrum. Any re-secretion of IgA back into the intestines that originated from colostrum could not be determined in this study. The decline of IgA levels reflects the decrease of IgA from colostrum ingestion. However at day 8, when calves started to develop diarrhea, IgA levels increased. This increase of IgA was likely a cellular response to the onset of diarrhea. At day 16 the levels decreased again, likely due to less intestinal stress as enteric damage healed and infections were cleared.

Salivary IgA values increased with age in both treatments. This increase over time has been reported before in humans (Jafarzadeh et al., 2008). Although not statistically significant, at day 10, PB calves had higher levels of saliva IgA and an increase from day 8, compared to a decrease between days 8 and 10 for controls. A decrease in saliva IgA levels is an indicator of stress and disease as reported before in dogs and humans (Kikkawa et al., 2003).

5. Conclusion

Supplementing PB in milk replacer increased the beneficial bacteria in feces during the first days of life; however this increase did not decrease coliform bacteria or affect the observed health of calves. The presence of *C. parvum* in all calves at the time of the trial could have affected the observed health and growth of calves. Growth was not different however feed efficiency tended to be improved for the PB calves. IgA in saliva was not a good measure of immunity in calves, but IgA in feces appeared to be a good measure of mucosal immunity. More studies are needed on influencing IgA in feces to develop a new method to measure immunity on calves during the first days of life when they are more susceptible to disease and death.

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