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Effects of supplementation of lactic acid bacteria on growth performance, blood metabolites and fecal coliform and lactobacilli of young dairy calves

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ABSTRACT

To evaluate the effects of supplementation of lactic acid bacteria (LAB) on growth of calves, twenty four female Holstein calves, immediately after birth, were used. Claves were randomly assigned into 3 treatments as follow: control (CON; milk without any probiotic), laboratory produced probiotic (LPP; milk containing 2 g/d/calf) and commercial produced probiotic (CPP; milk containing 2 g/d/calf). Calves were weaned abruptly if they consumed 900 g dry matter of starter per day for three consecutive days. Starter intake was measured every day and fecal scoring conducted daily. Calves were weight weekly and blood samples were obtained on days 7, 21, 42 and 90 after birth. To assess the effect of probiotics on weaning stress, blood samples were obtained at −168, 24 and 168 h after weaning day. To assess the effect on the gut flora, fecal samples were collected on days 14, 21, 28 and 45 after birth. Compared with control, incorporation of the probiotics in the diet had significantly effect on final body weight. There was no significant effect on starter intake and daily body weight gain, although there were trend to increase by supplementation of probiotics in diets. Including probiotic into diets resulted to decrease weaning time compare to control group. Feeding probiotics to calves had not remarkable effects on blood metabolites during abrupt weaning. On days 14 and 28 the fecal population of lactic acid bacteria was no different ($P > 0.05$) between treatments; however the average fecal population of LAB was greater ($P < 0.05$) with LPP than other treatments. The results of this study showed that incorporation of probiotics in the diet can affect the calves' growth performance, although observed benefits from treatments in several area were likely minimized.

1. Introduction

In the newborn calves, the digestive tracts are sterile in the womb of their mothers. Upon birth, this tract is naturally colonized by a variety of microorganisms from the environment (Savage, 1987). Under normal conditions, useful microorganisms colonize in the rumen and lower part of gut in a symbiotic relationship with the host. The activity of gastrointestinal

Abbreviations: ADG, average daily gain; BW, body weight; CBC, complete blood count; CPP, commercial produced probiotic; CSPB, calf specific multistrain probiotic; DM, dry matter; LAB, lactic acid bacteria; LPP, laboratory produced probiotic; MSPB, multispecies probiotic; PTCC, persian type collection culture.

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microbes and subsequently their metabolites affects the performance of farm animals in many ways, especially young ones subjected to environmental stress. Gut microbes supply nutrients, aid in digestion and compete with potential pathogenic microbes.

Due to the intensive rearing and management methods of today, calves are very susceptible to enteric bacterial imbalance and usually suffer from diarrhea and respiratory diseases, leading to inefficient digestion and absorption of nutrients and consequently retarded growth.

In order to solve these difficulties, diets have been supplemented with antibiotics, which have been widely used as feed additives. Many researchers reported that these additives are very effective to improve body weight gains (Felsman et al., 1973; Quigley et al., 1997; Berge et al., 2005) feed efficiency and reducing diarrhea (Parker and Armstrong, 1987; Quigley et al., 1997). However, the use of antibiotics has been associated with the development of antibiotic-resistant strains may interfere with the use of veterinary antibiotics (Hedges and Linton, 1988) and decrease the efficiency of antibiotics. Possible residues in the animal products and cross-resistance with human pathogens may cause do not respond to commonly prescribed antibiotics for humans, this renders the treatment of human diseases more challenging (Cheeke, 1999). To avoid these problems, probiotics are used as an alternative agent and has been investigated in replacing antibiotics.

According FAO/WHO (2001), probiotics are "live microorganisms which when administered in adequate amounts conferring a health benefit on the host". Probiotics have been used for many years to improve the health of humans and the health and productivity of production animals, both ruminants and monogastrics. The microorganisms normally used as probiotics include the *Lactic acid bacteria* (LAB), *Lactobacilli Bifidobacteria* and *Enterococcus*.

Some researchers have reported that these probiotics decrease the incidence of diarrhea (Abe et al., 1995; Galvao et al., 2005; Timmerman et al., 2005), improved body weight gain and feed conversion (Abe et al., 1995) and decreased mortality (Gorgulu et al., 2003).

Since the beneficial effects of probiotics are strain dependent, it has been suggested that combinations of different probiotic strains may be more effective than single strain probiotics (Timmerman et al., 2004). The rationale for multiple organisms comes from potential synergistic actions. However, probiotics contain one or more of these bacteria which can be of human origin or animal origin. It seems that probiotic strains of animal origin due to "host specific effect" (Fuller, 1997) are more effective, and there are some studies that show animals generally benefit from probiotic microorganisms isolated from their own digestive tracts (Walter, 2005). However, Timmerman et al. (2005) used a multispecies probiotic (MSPB) and a calf specific multistrain probiotic (CSPB) and found no clear difference in the efficiency of the MSPB and CSPB preparations.

Due to the limited research in calves fed prebiotics containing strains of human origin, the purpose of this study was to investigate and compare the effects of feeding probiotics (a laboratory produced probiotic (LPP) by using of four strains of human origin and a commercial probiotic (CPP) on growth performance, blood metabolites, incidence of scours and numbers of fecal coliforms and lactobacilli of milk feeding dairy calves.

2. Materials and methods

2.1. Probiotic strains

In this study, a commercially produced probiotic (CPP) and a laboratory produced probiotic (LPP) that was produced in laboratory condition, were used. The CPP contained *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidium* and *Enterococcus faecium* as fermentation product dehydrated.

To produce LPP some freeze-dried probiotic strains of human origin including: *Lactobacillus acidophilus* PTCC (Persian Type Collection Culture) 1643, *Lactobacillus rhamnosus* PTCC 1637, *Lactobacillus casei* PTCC 1608 and *Lactobacillus delbrueckii* PTCC 1333 were used. These strains were purchased from Iranian Scientific and Industrial Organization. Stock cultures of freeze-dried strains were individually inoculated into 5 mL MRS (de Man Rogosa Sharpe) broth (Merck KGaA Germany) and incubated at 37 °C for 24 h under aerobic conditions. Samples were taken for viable cell count and analysis of each strain. Pour plate counts in MRS agar were used to numeration of each culture.

After incubation, 1 mL of cultures were transferred into 100 mL skim milk and allowed to grow for 48 h. Every 12 h, fresh skim milk was injected into bottles to assure growth of all strains. The cultures were moved into a freeze drier for 72 h. Products of freeze dried cultures were mixed (in equal amounts) and added to whey as a carrier. Samples were taken to determine the total viable cell count of the finished product. Surviving bacteria were numerated by pour plate counts in MRS agar after incubation at 37 °C and the counts were expressed as mean log CFU/g. The product was prepares every 2 weeks and was kept at 4 °C. During storage, the total cell count was checked weekly to control stability of the product.

2.2. Animal and housing and diet

Twenty-four Holstein female calves were separated from their dams immediately after birth. They were randomly assigned in groups of 8 within three treatments, balanced by BW. Calves were subjected to experiment when they have 3 days old. Calves were housed in a naturally ventilated barn and kept in individual pens bedded with wood shavings. All calves were weaned suddenly, to determine effects of probiotics on stress during weaning stress, if they consumed 900 g DM of starter per day for three consecutive days. Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf). The LPP or CPP containing a total of 2.0×10^8 CFU, were dissolved into

Table 1
Ingredient and chemical composition of calf starter (g/kg DM).

Ingredient	
Barley grain ground	400
Corn grain ground	228
Soybean meal	342
$CaCo_3$	50
Mineral/vitamin premix	10
Salt	5
Shell powder	10
Chemical composition	
DM	902.7
CP	226.2
NDFom	384
ADFom	85
Ash	66

DM: dry matter; CP: crude protein; NDFom: neutral detergent fiber; ADFom: acid detergent fiber.

milk and administered with morning milk feeding. Calves were maintained on the study from 4 through 90 d of age. All calves received 4.5 kg whole milk which divided into two equal portions and fed at 04:00 and 16:00 h. Calves had access to clean fresh, water and dry pelleted starter feed at all times (Table 1).

Standard methods as described in AOAC (1984) were used for determination of dry matter (DM method 930.15) ash (method 924.05) and N (method 984.13). Ash-free neutral detergent fiber (NDFom) was determined using sodium sulfite according to the method of Van Soest et al. (1991) and ash-free acid detergent fiber (ADFom method 973.18) was determined based on AOAC (1984).

2.3. Experimental measurements

Weekly measurements of BW were made and feed intake and orts were measured daily. Refusals plus wastage were used to calculate DM intake of starter. Calves were observed daily to check health status and calves that were suffering from diarrhea were treated with electrolyte solution and when it was necessary, antibiotic treatment was used. Feces were scored for consistency daily, using a three point scale (1 = normal, 2 = moderate and 3 = watery) according to Abu-Tarboush et al. (1996). Structural growth measurements of body length, withers height, hip height, heart girth and hip width were recorded weekly using the procedure of Lesmeister et al. (2004).

2.4. Rumen fluid and blood metabolite sampling

Rumen fluid was collected by an esophageal tube from calves at 21, 42, 60 and 90 d, 4 h after the morning milk feeding without restriction of feeding to determine pH and NH_3-N concentrations. Samples were filtered through four layers of cheesecloth and pH was measured immediately by glass electrode (Metrohm 691 models). For determination of NH_3-N, 10 mL of filtered rumen fluid were added to 10 mL of 0.2 N HCl (vol/vol) and were frozen immediately at −20 °C until they were assayed. Blood samples were collected from the jugular vein at 7, 21, 45, 60 and 90 d of study 4 h after the a.m. meal. After collection, plasma was harvested by centrifugation (3000 × *g* for 15 min), placed in storage tubes and frozen (−20 °C) until further analysis. To monitor complete blood count (CBC), 1.5 mL from the blood samples poured in vacutainer tubes with EDTA at −168, 24 and 168 h after weaning. These samples were kept in room temperature until analyzing for CBC. Total serum proteins were estimated for all calves using refractometer method.

2.5. Fecal collection and enumeration of lactobacilli and coliforms

The procedure of Ellinger et al. (1980) was applied to collect fecal samples on 14, 21, 28 and 45 d to enumerate fecal pH, coliforms and lactobacilli. The samples were collected at approximately 07:00 h into sterile 50 mL falcon tubes and then transported to the laboratory. A subsample (1 g) of the feces was placed in a 50 mL falcon tube and mixed with 9 mL of distilled water. The mixture was vortexed until homogenous, the pH was determined with pH meter (Metrohm, 691 models).

Bacterial enumeration was carried out using selective growth media and growth conditions. Each fecal subsample (1 g) was serially diluted 10-fold with 9 mL of sterilized saline water dilution from 10^{-1} to 10^{-8}. From each dilution, 100 μl of suspension was plated out, in triplicate on the MRS agar (Merck, Darmstadt, Germany) and eosin methylene Blue agar (oxoid) for the determination of the total cell count of *Lactobacillus* spp. and coliforms, respectively. The MRS broth agar plates were incubated anaerobically at 37 °C for 48 h. Eosin methylene blue agar plates were incubated aerobically at 37 °C for 48 h. After incubation, the agar plates were assessed for growth and colonies counted. The total cell counts of lactobacilli and coliforms per gram of fecal material were calculated.

2.6. Statistical analysis

Performance data from this experiment including daily body weight gain, structural growth, starter intake and average days to weaning were analyzed as a randomized complete design using the General linear model of the SAS (Version 9.1, SAS Institute) based on the statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} = dependent variable of the jth calf on the ith treatment
μ = overall mean
T_i = the fixed effect of ith treatment effect (i = 1, 2, 3)
e_{ij} = random residual (error) associated with the dependent variable from the jth calf on The ith treatment. Means were tested using Duncan's multiple range tests.

Fecal bacteria counts were transformed by $\log_2(x+1)$ before statistical analysis. Continuous data collected over time (*i.e.*, weakly BW, weekly average fecal score, ruminal pH, ruminal ammonia N, blood metabolites and fecal pH) were analyzed as repeated measures by the 'MIXED' procedure of SAS (Version 9.1, SAS Institute). Variables were analyzed using the linear model:

$$Y_{ijk} = \mu + T_i + P_j + (TP)_{ij} + e_{ijk}$$

where μ = general mean, T_i = effect of ith treatment P_j = effect of jth period $(TP)_{ij}$ = interaction effect of ith treatment with jth period e_{ijk} = random error. Treatment difference with ($P < 0.05$) were considered as a significant statistic.

3. Results

3.1. Performance and body structural growth of calves

Daily mean BW and average daily DM intake during the experiment are shown in Table 2. Analysis of variance revealed that there was no difference between treatments on daily mean BW. Initial BW and weaning weight were similar between treatments; however, groups treated with probiotics had higher final BW than control groups ($P < 0.05$). Least squares means for weekly body weight for calves fed CON, LPP and CPP treatments are presented in Fig 1. The results indicated that calves in control group lost 2.7% of their initial body weight on second week of ages ($P > 0.05$).

Statistical analysis exposed that there were no differences between treatments on starter intake. However, calves in control group had lowest starter intake compared to other treatments. Calves in LPP and CPP tended to consume more starter compared to control group.

In this study, calves were weaned when they consumed 900 g of starter for 3 consequent days. Obtain results shows that supplementation of probiotic in diet resulted to decrease milk feeding periods in calves compare to CON treatment.

Least squares means for initial and final of body structural are presented in Table 3. There were no differences between treatments for initial structural growth and final body length, heart girth and hip height ($P > 0.05$). The final wither height and hip height of calves in group CON were lower than other treatments ($P < 0.05$).

Table 2
Least square means of average daily gain and dry matter consumption of calves fed probiotics.

Item	Treatment*			SEM
	CON	LPP	CPP	
Calves, *n*	8	8	8	
Average days to weaning	48.90	42.90	43.1	6.79
Initial BW (kg)	37.00	38.25	38.7	1.68
Weaning BW (kg)	57.54	58.25	57.62	2.30
Final BW (kg)	82.10[a]	86.71[b]	87.50[b]	1.65
Daily BW gain (kg/day)				
Pre-Weaning	0.353	0.381	0.370	0.071
Post-Weaning	0.807	0.794	0.802	0.131
Overall	0.498	0.549	0.542	0.082
Starter intake (kg/day)				
Pre-Weaning	0.503	0.535	0.555	0.105
Post-Weaning	1.880	2.013	2.076	0.270
Overall	0.973	1.136	1.197	0.310

[a,b]Differences in superscript indicate significance at $P < 0.05$.
* Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf).

Table 3
Least squares means for structural growth measurements (cm) of calves fed probiotics.

	Treatment*			SEM
	CON	LPP	CPP	
Body length				
Initial	37.06	37.87	37.25	1.45
Final	47.00	49.00	48.50	1.4
Wither height				
Initial	70.62	72.62	72.00	2.76
Final	82.00[a]	86.50[b]	85.75[b]	1.69
Hip height				
Initial	70.87	75.12	74.62	2.05
Final	84.12[a]	90.37[b]	89.87[b]	2.5
Heart girth				
Initial	74.12	75.00	76.25	2.88
Final	96.12	99.37	102.25	3.46
Hip width				
Initial	15.50	16.00	15.62	0.68
Final	23.25	24.50	24.00	0.75

[a,b]Differences in superscript indicate significance at $P < 0.05$.
* Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf).

3.2. Fecal pH and fecal bacterial counts

There were no differences between treatments on fecal pH (Table 4). Fecal pH in the control group was high in comparison with other treatments all time ($P > 0.05$).

Fecal samples were taken on days 14 and 28 after birth to enumerate the number of fecal lactobacilli and coliforms (Table 4). In this study, the number of fecal lactobacilli was higher in treatment LPP compared to other treatments. The average numbers of LAB was highest in treatment LPP ($P < 0.05$). However, there were no differences between treatments on days 14 and 28 treatments. Coliform concentrations in the feces tended to increase by supplementation of probiotics from d 14 to 28. The number of coliform in treatment CPP was high on d 28 compared with other treatments ($P < 0.05$). There were no differences between treatments on d 14 and average on coliform population.

Weekly average fecal consistency scores are shown across treatments in Fig. 1. There were differences between treatments at first and seventh weeks ($P < 0.05$). High values recorded of fecal score were related to the first two weeks during the 8 weeks recording. In first week, treatment LPP had lowest value of fecal pH in comparison with other treatments ($P < 0.05$). In present study, number of calves that suffered from scour was 3 in control group and 1 in treatments LPP and CPP (Table 4).

Table 4
Fecal pH coliform and Lactobacillus counts* and morbidity of calves fed probiotics.

	Treatment**			SEM
	CON	LPP	CPP	
Fecal pH				
14 d	7.47	6.94	7.52	0.472
21 d	8.02	7.47	7.81	0.315
28 d	8.10	7.73	7.89	0.210
45 d	7.81	7.61	7.71	0.250
Lactobacilli				
14 d	2.59	2.94	2.72	0.262
28 d	2.87	3.10	2.68	0.220
average	2.73[a]	3.04[b]	2.76[a]	0.115
Coliforms				
14 d	2.302	2.260	2.160	0.395
28 d	2.677[a]	2.590[a]	2.977[b]	0.159
Average	2.490	2.420	2.570	0.178
Number of scouring	3	1	1	
Number of pneumonia	1	0	0	
Number of off- feed	2	0	0	

[a,b]Differences in superscript indicate significance at $P < 0.05$.
* Observed data were cfu/mL; transformed data are presented: $\log_2(x+1)$.
** Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf).

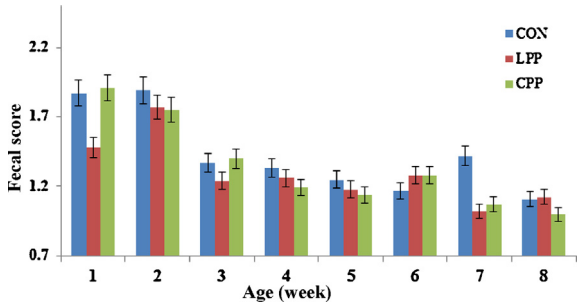

Fig. 1. Least squares means for average body of calves fed diets containing no additives (CON), laboratory produced probiotic (LP) (2 g/d/calf) and commercial probiotic (CP) (2 g/d/calf).

Table 5
Least squares means of rumen pH and NH_3 concentration.

	Treatment*			SEM
	CON	LPP	CPP	
pH				
21	5.39[a]	5.71[b]	5.45[a]	0.123
45	5.47	5.63	5.63	0.170
60	5.37	5.57	5.38	0.112
90	5.78	5.84	5.63	0.215
NH_3 (mmol/l)				
21	16.76	13.09	12.91	3.06
45	14.06	13.70	11.74	2.51
60	9.35	6.91	6.31	2.63
90	7.67[b]	4.92[ab]	3.43[a]	1.40

[a,b]Differences in superscript indicate significance at $P < 0.05$.
* Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf).

3.3. Rumen fermentation

Least squares means of treatments effect on ruminal pH and N-NH_3 concentration are presented in Table 5. Ruminal pH in Calves fed treatment LPP was higher than others ($P < 0.05$). The results were constant and had low fluctuation throughout the study. All calves showed lowest pH on d 60 compared with other sampling days.

There were no differences between treatments at d 21, 42 and 60 after birth for the rumen N-NH_3 concentration ($P > 0.05$), but on d 90 rumen NH_3 concentration in treatment CON was higher than other treatments ($P < 0.05$).

3.4. Blood metabolites

Least squares means of plasma β-Hydroxybutyrate (BHBA) concentrations are presented in Fig. 2. As calves aged, BHBA levels of calves for all treatments increased. There were no differences between treatments except on d 60 after birth that BHBA concentration in treatment LPP higher than others ($P < 0.05$) (Fig. 3).

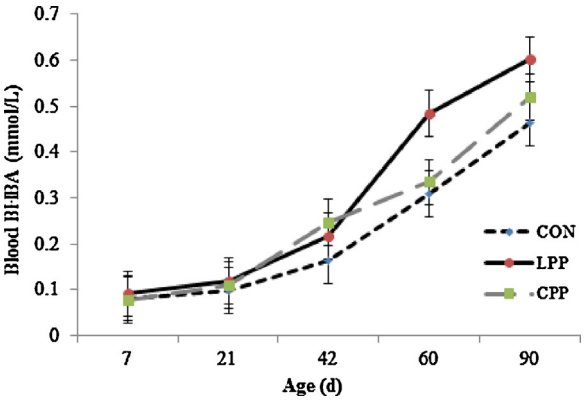

Fig. 2. Least squares means for faecal consistency scores of calves fed diets containing no additives (CON) laboratory produced probiotic (LPP) (2 g/d/calf) and commercial produced probiotic (CPP) (2 g/d/calf).

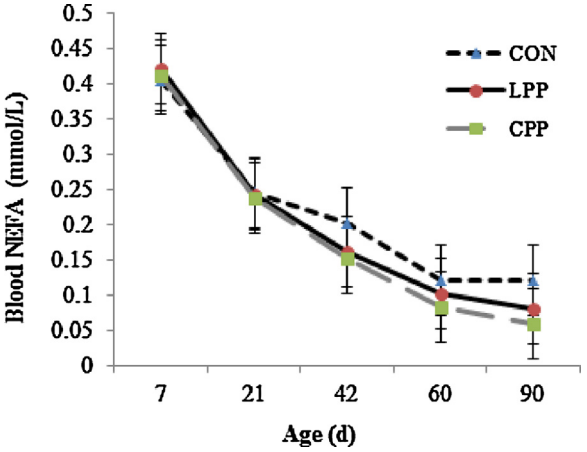

Fig. 3. Concentration of Beta-hydroxybutyrate in plasma of calves fed diets containing no additives (CON) laboratory produced probiotic (LPP) (2 g/dcalf) and commercial produced probiotic (CPP) (2 g/d/calf).

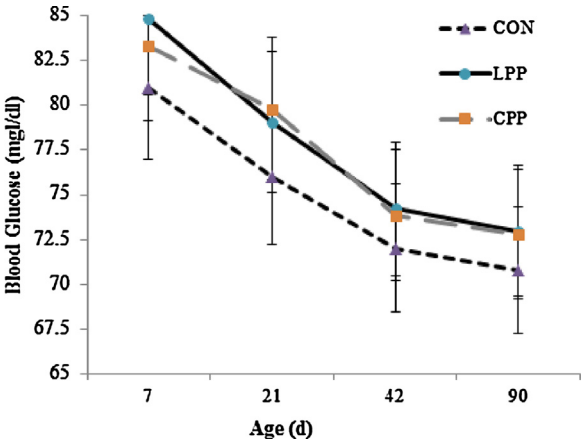

Fig. 4. Concentration of nonesterified fatty acids of calves fed diets containing no additives (CON) laboratory produced probiotic (LPP) (2 g/d/calf) and commercial produced probiotic (CPP) (2 g/d/calf).

The nonesterified fatty acids (NEFA) concentrations in blood decreased throughout the study. However, the reduction rate of NEFA concentrations till d 21 was similar for all treatments, but after that calves fed treatment CON had high concentration of NEFA compared to other treatments (Fig. 4).

No differences between treatments were observed for glucose concentrations in plasma (Fig. 5). However, calves in CON group had lowest glucose concentrations compared with calves fed probiotics ($P > 0.05$).

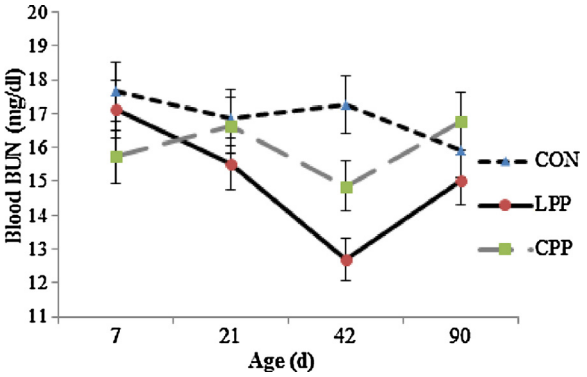

Fig. 5. Concentration of blood glucose of calves fed diets containing no additives (CON) laboratory produced probiotic (LPP) (2 g/d/calf) and commercial produced probiotic (CPP) (2 g/d/calf).

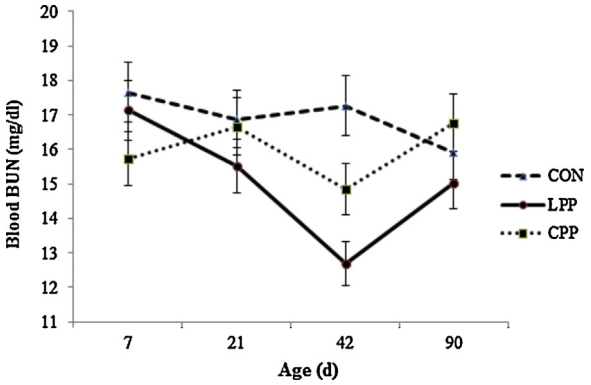

Fig. 6. Concentration of blood urea nitrogen of calves fed diets containing no additives (CON) laboratory produced probiotic (LPP) (2 g/d/calf) and commercial produced probiotic (CPP) (2 g/d/calf).

Table 6
Least squares means of total protein Hematocrit, White blood cell and cortisol at −168, 24 and 168 h after weaning.

	Treatment[a]			SEM
	CON	LPP	CPP	
Total protein (g/dl)				
−168	6.01	6.08	6.23	0.577
24	5.88	5.85	5.98	0.406
168	6.12	6.65	6.52	0.798
Hematocrit (%)				
−168	34.70	36.50	36.50	2.70
24	35.42	31.62	36.00	5.01
168	31.63	33.89	35.92	2.60
WBC ($\times 10^3/\mu l$)				
−168	5.74	5.89	7.97	1.87
24	7.78	7.12	7.30	1.08
168	5.77	8.60	7.95	1.47
Cortisol (mmol/l)				
−168	8.13	7.23	7.66	0.658
24	14.33	13.30	13.26	0.809
168	10.83	9.90	10.00	0.536

[a] Treatments were: control (CON), laboratory produced probiotic (LPP; 2 g/d/calf) and commercial produced probiotic (CPP; 2 g/d/calf).

Calves fed diet L had lower blood urea nitrogen (BUN) concentrations compared to CON at d 45 ($P < 0.05$), although calves fed CON tended to have greater BUN concentrations than another treatments (Fig. 6).

3.5. Abrupt weaning and blood metabolites

No differences were obtained between treatments in total serum protein, hematocrit, white blood cell and cortisol during the weaning period (Table 6). Although, there was no difference between treatments on total serum protein, but after weaning total serum protein decreased ($P > 0.05$).

4. Discussion

The supplementation of probiotics improved daily BW gain in pre-weaning and overall period of experiment ($P > 0.05$). These results are in agreement with other studies (Abe et al., 1995; Timmerman et al., 2005; Cruywagen et al., 1995) who found that average daily gain (ADG) improved when applying probiotics ($P < 0.05$). Cruywagen et al. (1995) reported that ADG increased in calves receiving *Lactobacillus* probiotic. In this study, calves in control group lost 2.7% of their initial body weight on second week consistent with Cruywagen et al. (1995) who found calves lost 4% of initial body weight during the first 2 weeks of experiment. Improvement in ADG attributed to improvement of DM intake and agreed with the result of the present study.

During the first days of life, calves accessed to starter but consumption of DM was practically very low, close to zero, for all calves. In the present study, the calves receiving probiotics consumed slightly more DM than control group. The results of previous studies to calves fed probiotics containing LAB are quite inconsistent. Abe et al. (1995) administered *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus* to neonatal calves and reported improvement in feed intake although, there were no differences between two probiotic groups ($P > 0.05$). Timmerman et al. (2005) and Cruywagen et al. (1995) reported that

calves benefited from Lactobacillus probiotics during the first two weeks. A possible explanation is that treatments LPP and CPP received probiotics and LAB colonized the intestinal tract before pathogen could be colonized (Higginbotham and Bath, 1993; Schwab et al., 1980).

The results of weaning age suggested that feeding probiotic can affect the age of weaning. The age at weaning or the number of days to weaning is a useful measurement for assessing diet effects on digestive tract development in calves. Some researchers used a fixed time for weaning in their studies (Terre et al., 2007). Consumption of 0.9 kg of calf starter was used as index for development of rumen and readiness for weaning. The average of weaning can show the effect of probiotics on accelerating to start DM intake and calf development. Normally, calves are weaned at 2 months on dairy farms but some studies have shown that calves can be weaned by 4 week of age (Anderson et al., 1987).

Final wither height and hip height on probiotic supplemented treatments were higher than treatment CON ($P < 0.05$). These results can be attributed to DM intake that was more in treatment LPP and CPP than control group. Calves receiving probiotic also had higher daily BW gain, hence structural growth measurements could be affected. Jenny et al. (1991) found no differences in these measurements with probiotic inclusion, but Lesmeister et al. (2004) reported that hip height increased when calves were fed a starter supplemented with yeast culture.

Previous studies have used of some characteristics of feces including score, to assess consistency of the feces as an indicator of the severity and the presence of diarrhea (Cruywagen et al., 1995; Galvao et al., 2005), population of key bacteria in the sample to determine the health condition (Jenny et al., 1991; Timmerman et al., 2005; Rada et al., 2006). Fecal pH can be used to assess gut health because it is linked to the activity of enteric pathogenic bacteria such as *E. coli* (Buchko et al., 2000; Berg et al., 2004).

Fecal pH in the treatment LPP was lower compared with other treatments all time may have been due to production of high amounts of lactic acid during fermentation of carbohydrate by LAB. The population of LAB in treatment LPP was higher than other treatments ($P > 0.05$) and hence, produced high levels of lactate in feces. Many researchers have reported that there is a relationship between diet and age of calves on fecal pH (Sato and Koiwa, 2008). Sato and Koiwa (2008) reported higher fecal concentrations of lactate and lower fecal pH during the first two weeks of age; but when the calves were 4–6 weeks, fecal pH increased. LAB as beneficial bacteria normally associated with a balanced normal in the gut flora. Increases in numbers of lactobacilli can show a normal occurrence in the development of intestinal flora of calves (Gilliland and Speck, 1977). In this study, results showed that probiotics had no effect on LAB population on days 14 and 28 although, there was a tendency to increase in treatment LPP consistent with results of other studies (Ellinger et al., 1980; Jenny et al., 1991).

It is predictable that fecal counts of lactobacilli are normally higher than counts of coliforms in healthy calves and are lower in calves suffering from scours (Sandine, 1979). Results of this study confirm this hypothesis but fecal counts of coliforms at d 14 was lower than d 28 as well calves suffering from scours more after 14 d.

The results of fecal consistency scores showed that calves had high fecal score during the first two weeks. These results are in agreement with the number of calves that suffered from diarrhea. The number of calves that suffering from diarrhea was high in control group compared with those received probiotics. Similarly, Timmerman et al. (2005) and Abe et al. (1995), both using LAB based probiotics, reported a decrease in occurrences of diarrhea in calves. Magalhaes et al. (2008) used yeast culture and reported improvement in fecal scores in calves ($P > 0.05$). In contrast, Cruywagen et al. (1995) reported no positive effects on general health by feeding probiotics.

There were no differences between treatments for the ruminal pH except at d 21 where treatment LPP had higher pH than other treatments ($P < 0.05$). Beharka et al. (1998) similar to this study founded a quadratic change in the relationship between pH and age of the calf. The low rumen pH is expected as calves were fed only concentrates and no forages (Lesmeister et al., 2004). In the present study, calves consumed starter and they did not receive forage. On the other hand, starter had high level of easily digestible carbohydrates and caused microbial growth and increased production of VFA. It has shown that increasing in production of VFA especially lactate can decrease the rumen pH (Lesmeister et al., 2004).

As calves aged, rumen N-NH_3 concentrations decreased because the rumen in the newborn calves is not functional and stabilization of microbial population is formed gradually with age as the animal matures (Karney et al., 1986). Decreasing rumen N-NH_3 concentrations is attributed to ruminal microbial proliferation and increased incorporation of N-NH_3 into microbial protein (Crocker et al., 1998). Anderson et al. (1987) observed higher NH_3 concentrations in unweaned calves than in weaned calves. However, starter intake with progress of age increased and supply more energy and substrate for ruminal microbial and N-NH_3 was used.

The results of BHBA levels in this study agreed with other studies e.g. Coverdale et al. (2004) and Quigley et al. (1991). Concentrations of BHBA in plasma reflects starter intake and can be considered as an indicator of rumen development (Quigley et al. (1991)). Most of BHBA is formed from conversion of butyrate in the rumen wall before release into portal circulation, but as mentioned rumen in the newborn calves is metabolically nonfunctional. Hence, BHBA concentrations were low at early age of calves. After initiation of solid feed intake by calves and the subsequent establishment of microbial population and ruminal fermentation, the development of physically and metabolically rumen occurs and the ruminal epithelium becomes the primary source of production of BHBA. BHBA concentrations were high in probiotic supplemented treatments all time. These results may indicate that probiotic supplemented treatments had greater development of rumen.

The values obtained for glucose concentrations in this study are similar to that obtained by Quigley et al. (1991). Calves receiving LPP and CPP had higher glucose concentration than control group. Calves fed probiotics had higher starter intake than control group and it may be explained the differences between treatments on glucose concentrations.

The BUN concentrations can be used to measure the efficiency of utilization of dietary protein. In this study, treatments LPP and CPP showed a decreasing on d 45, but after that BUN concentration increased. This may indicate fermentation of dietary protein and absorption of ammonia to the blood stream.

Abrupt weaning is known to cause stress in calves, although there is a lack of research especially addressing the effects of weaning stress alone. In this study, feeding probiotics to calves had no remarkable effects on blood metabolites during abrupt weaning. Probiotics can stimulate the immune system, through acceleration of the maturation of the gut immune system resulting in lower morbidity and mortality and alleviate the effects of stress in newborn calves. Total serum protein is an indirect method of measuring serum Ig concentrations and has high correlation with the Ig concentration (Nocek et al., 1984; Perino et al., 1993). No differences were observed between treatments in hematocrit throughout the study; this result agree with the finding of Adams et al. (2008) who found no variation between probiotic treated calves and their control counterparts in overall hematocrit.

It has been observed that weaning alone does not increase cortisol levels in valves (Lefcourt and Elsasser, 1995). In this study, one of the hypotheses was that the abrupt weaning of calves will cause stress and increase the cortisol concentration due to attenuation of immune system and addition of LAB based probiotics in the diet of calves would stimulate immune function. Inclusion of probiotics in diets of calves had no effect on plasma cortisol concentration in comparison with calves in the control group.

5. Conclusions

The results of the present experiment indicated that feeding of probiotics improved final body weight daily BW gain and starter intake. It seems that to achieve a maximum effect of probiotics the calves had to contain an unbalanced gut microflora. The results indicated that the calves were in a healthy condition and it is probable that significant effects of probiotics were not observed in this study due to the fact that probiotics are generally only significantly effective in the animals that are under stress conditions.

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