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Effects of feeding probiotic on daily gain, fecal characteristics and blood metabolites in calf

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ABSTRACT

Feed substrate containing probiotic culture of *Lactobacillus acidophilus, Bacillus subtilis* and *Sacharomyces cerevicae* were prepared for feeding calves. Twelve 2 weeks old calves were distributed equally in two groups (6 calves in each) and supplemented with (treated) or without probiotic feed (Control). Calves were reared in an individual pan and provided with *ad libitum* suckling, calf starter, and soft green grass. In 90 days trial period data were collected on growth performance, blood metabolic profile, immune status, fecal microbial load, morbidity etc. It was observed that milk intake, dry matter intake (DMI), daily gain and feed conversion ratio (FCR) were not differed (P>0.05) between treatments. Claves under the probiotic fed group voided feces of better physical properties (color, odor and consistency) compared to the control. Weekly *E. coli* count (log_{10}CFU/g) in feces was found lower (P<0.01) in the probiotic group compared to the control. The plasma IgG (ng/ml) concentration was found higher (P<0.05) in probiotic group than in the control, and total cholesterol level tended to be high (P=0.071) in the same group. It is concluded that, probiotic feed improved fecal characteristics, lesser *E. coli* load in feces, lowered diarrheal incidence and improved immunoglobulin status of calves from 15th to 105th days of age.

Introduction

Malnutrition caused by inadequate colostrum feeding and suckling is very much common in the calf-rearing system in Bangladesh. As a result, calf morbidity and mortality up to 12 months of age are much higher in the country. Hossain *et al.* (2014) reported an average of 5.6% calf mortality based on 16 years data in Central Cattle Breeding and Dairy Farm (CCBDF) with a range of 1.05 to 11.58% and about 70% of total mortality was reported up to 12 months of age. On the other hand, unjustified use of antibiotics for treating calves is common at farm level. Extensive and prolonged use of antibiotics may impair the intestinal microflora ecosystem by gaining resistance to the antibiotics and increasing susceptibility of calves to some pathogenic organisms, consequently, increase the risk for diarrhea and malabsorption in intestines. Feeding probiotics, which is defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002) are potential alternative to antibiotic for increasing feed intake and weight gain, earlier weaning, increased immunity, decreased scours and fecal coliform count in calves.

The work on probiotic for cattle has increased in recent years and positive effects have been found for feed intake, weight gain, milk yield and quality, early weaning, decrease of scouring and fecal coliform count and reduced demand for antibiotic treatment (Retta, 2016; Roodposhti and Dabiri, 2012; Frizzo *et al*., 2011; Seo *et al*., 2010). Frizzo *et al*. (2010) reported that calves fed probiotic had higher daily gain, total feed

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intake, and starter diet intake as well as lower incidence of diarrhea. Malik and Bandla (2010) reported higher average daily gain and feed efficiency in calves fed probiotic (*L. acidophilus* and *S. cerevisiae)* plus enzyme supplements. Reduced diarrhea incidence in calves by feeding probiotics were reported by many scientists earlier (Roodposhti and Dabiri, 2012; Frizzo *et al*., 2011; Seo *et al*., 2010). Probiotics in ruminant feeds mainly help to stabilize intestinal flora, enhance the development of adult rumen microflora, improve digestion and nitrogen flow towards the lower digestive tract, and improve meat and milk production (Retta, 2016). Probiotics administration improves the health status of animals by competing nutrient utilization of pathogenic microbes by having a positive influence on gut microflora. Furthermore, their anti-pathogenic activity may reduce the stress on animals (Seo *et al.* 2010).

The most commonly used microbial additive is *Lactobacillus spp.* These microbes have specific roles in the host's body, primarily responsible for the exclusion of enterotoxigenic bacteria (Fuller 1989). Nevertheless, probiotic microorganisms for ruminants include species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus* and *Propionibacterium* (Seo *et al*., 2010). Although bacterial probiotics are emphasized, fungal probiotics are also common feed additives to ruminant diets (Kung Jr, 2001). Yeast products based on *Saccharomyces cerevisiae* have been used as feed additives in dairy production systems for more than two decades (Jiang *et al*., 2017). It is typically fed in dairy cattle rations to alter rumen fermentation in an attempt to improve nutrient digestion, N utilization, reduce the risk of rumen acidosis and improve animal performance (Seo *et al*., 2010). Recent multi-study analyses performed both in dairy and beef cattle have shown significant benefits with live yeast (*Saccharomyces cerevisiae*) on milk yield and feed efficiency (Jiang *et al*., 2017). Tolerance of microorganisms to heat is also important for probiotic since they have to survive processing during feed production. Spore-forming bacteria have advantages as probiotics (Ripamonti *et al*., 2009) as they provide higher resistance to stresses during production and storage processes (Hyronimus *et al*., 2000) as well as higher resistance to gastric and intestinal environmental conditions (Hong *et al*., 2005). Bacillus species have specific mechanisms that inhibit gastrointestinal infection by pathogens or by producing antimicrobials (Song *et al*., 2014).

The probiotic culture for feeding ruminants may be single or mixed strains of more microorganisms. The combination of probiotics strains could improve the beneficial health effects compared with individual strains, because of their synergetic adhesion effects (Collado *et al*., 2007). The mechanisms by which multi-strain probiotics exert their effects include cell–cell communications, interactions with the host tissues, and modulation of the immune systems (Kwoji *et al*., 2021). Cangiano *et al*. (2020) suggested that the mode of action of bacterial probiotics is species and strain specific and therefore, supplementation of multispecies and multistrain probiotics typically result in improved beneficial effects on dairy calves due to a combination of their different effects. Therefore, this study was conducted to evaluate effects of mixed culture of *L. acidophilus, B. subtilis* and *S. cerevicae* on daily gain, fecal characteristics and blood metabolites in local Red Chittagong Cattle (RCC) calves.

Materials and Methods

Location of the experiment

This research was conducted in Biotechnology Division and animal experiment was conducted at the cattle farm of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341 (23.8887° N, 90.2739° E). The duration of the study was 91 days.

Table 1. Formulation of probiotic mix

Ingredients	Amount
Wheat bran	500 g
Molasses	100 g
Water	370 ml
L. acidophilus (Broth)	10 ml
S. cerevicae (Broth)	10 ml
B. subtilis (Broth)	10 ml

Preparation of mixed probiotic culture

Commercial sources of *Lactobacillus acidophilus, Bacillus subtilis* and *Sacharomyces cerevicae* were collected and cultured in 'De Man, Rogosa and Sharpe (MRS) agar (BD-Oxoid, USA), Mannitol Yolk Polymyxin (MYP) agar (BD-Oxoid, USA) and Potato Dextrose (PD) agar (BD-Oxoid, USA), respectively. Firstly, 1g of commercial source containing specific strain was diluted into 9 ml of 0.85% of saline solution and then serial dilution was made up to ten-fold. A 100 ml aliquot of three consecutive dilutions (10^{-3} to 10^{-5}) were plated in triplicate onto the respective selective agar medium. Plates were placed in an incubator at 37 ◦ C for 24 hours. After 24 hours colonies were counted, selected colonies were picked and multiplied in mass through culturing in MRS, MYP and PD broth, respectively, at 37◦C for 48 hours.

Autoclaved wheat bran (as substrate) was used to produce mixed probiotic culture for feeding animal. Formulation to produce roughly 1 kg of such probiotic mixture is illustrated in Table 1. Water content is variable depending on concentration of molasses used.

Molasses, water and microbial culture were first mixed thoroughly in a solution and then sprayed on wheat bran to mix thoroughly. All ingredients were autoclaved (except microbial culture) before mixing. Then they all together were mixed homogenously, packed and incubated at 37◦C for 6 days. The substrate was stirred 2 times daily for preventing clumps formation and facilitating vigorous fermentation. Stirring was done by hands wearing sterile hand gloves and the process was performed inside the vertical laminar flow cabinet (ESCO, USA) for avoiding contamination. After 6 days, it was taken out and evaluated for chemical and microbial characteristics.

Table 2. Feeding chart (Basal feed) for the experimental calves

Age (week)	Milk (liter)	Calf starter (gram)*	Soft green German grass (gram)
1	Ad libitum suckling (roughly around 10% of calf's live weight)	0	0
2		0	0
3		50	300
4		300	500
5		400	550
6		600	600
7		700	700
8		800	800
9		1000	1000
10		1200	1100
11		1300	1200
12		1400	1400
13		1700	1900

**According to manufacturer's instruction*

An aqueous extract of probiotic feed was prepared by macerating 20 g of feed with 180 ml of sterile ultra-pure water in a laboratory blender for 30 s, and then, filtered through 2 layers cheesecloth. The fresh extract was used to determine pH and the counts of microorganisms at the day of opening. The pH was determined from the extract using a pH meter (SENSION^{TM}+ PH^3, Spain). The microbial enumeration was done as stated above. The dry matter (DM), organic matter (OM), crude protein (CP) and ammonia-N content was determined following the method of AOAC (2005). It was also evaluated for the same parameters at fresh conditions before incubation.

Experimental calves, dietary treatments and management

Twelve Red Chittagong Cattle (RCC) calves of two weeks of age (Average live weight: 20.94±0.37 Kg) were selected and distributed into two treatments having 6 calves (3 male, 3 female) in each treatment. The calves irrespective of treatments were supplied with *ad libitum* suckling, commercial calf starter (Bovino Calf Starter, ACI-Godrej Bangladesh Ltd.) and soft green grass (German grass; *Echinochloa polystachya*) according to the feeding chart given in Table 2.

Table 3. Chemical compositions of milk, calf starter, German grass and mineral block

Compositions	Milk	Calf starter	Grass	Mineral block
Total solids, %	14.0	-	-	-
Fat	4.7	-	-	-
SNF	9.3	-	-	-
Protein	3.4	-	-	-
Lactose	5.0	-	-	-
DM, %	-	90.3	13.9	-
CP, % of DM	-	22.0	13.2	-
EE	-	2.7	3.5	-
CF	-	8.0	26.0	-
Ash	-	13.9	7.4	-
TDN	-	65.0	-	-
Ca	-	1.0	-	-
P	-	0.5	-	-
Mg, mg/kg	-	-	-	300.0
Mn, mg/kg	-	-	-	100.0
I, mg/kg	-	-	-	10.0
Zn, mg/kg	-	-	-	150.0
Fe mg/kg	-	-	-	500.0
Cu, mg/kg	-	-	-	40.0
Co, mg/kg	-	-	-	6.0
Se, mg/kg	-	-	-	2.0
Enzymes, %	-	-	-	0.05
Molasses, %	-	-	-	0.5

They were also provided with mineral blocks for licking. For each 100 kg of live weight 25 grams of probiotic feed were supplied to the animals under probiotic fed groups. However, the probiotic feed contained some ingredients as substrate for microbes, which may incur added effect from their nutrient contents along with effects from probiotic microbes. Therefore, to minimize this effect, animals under the control

group were fed all ingredients in similar amount and proportion as in the probiotic feed substrate, but un-inoculated with probiotic bacteria. It was made sure the 100% intake of probiotic and control feed to animals. The chemical compositions of milk, calf starter, German grass and mineral blocks are given below in Table 3. Claves were housed in individual calf pan provided with a feeder and waterer. Stalls were washed with clean water and disinfected (with iodine-based commercial disinfectant) once daily throughout the trial period. A weighed amount of calf starter and green grass were supplied in two halves; once in the morning (around 08:00 am) and another half at the evening (around 03:00 pm). The daily orts from calf starter and green grass were weighed to calculate the intake. The trial was continued for 91 days.

Data collection, sample collection and laboratory analysis

Data were collected on growth performance, blood metabolic profile, immune status, fecal microbial load and morbidity during the trial period. Live weight was measured weekly on digital calf weighing balance to calculate daily gain. Milk intake was measured fortnightly at 3 consecutive days by taking weight of animal before and after feeding. Calf starter, green grass, probiotic feed or placebo feed intake was recorded daily. Feces sample was collected fortnightly from each animal for *E. coli* and *Salmonella* enumeration and at 90 day for *L. acidophilus, B. subtilis* and *S. cerevicae* enumeration. Feces were observed daily for physical property evaluation, which included color, odor and consistency. The consistency properties of feces, scores and cases of diarrhea were recorded following the method of Amanullah *et al*. (2018) with some modifications. Consistency was recorded as hard (constipation), normal, tended to be liquid and watery and a score value of 1, 2, 3 and 4 was given, respectively for each consistency class to each calf in each day. The average value was used to derive consistency score. Persistency of score 3 and 4 for consecutive 3 days was considered as the 'case of diarrhea'. At the end of the trial (88^{th} to 90^{th} day) blood sample were collected from jugular vein at 3 consecutive days in vacutainer tubes containing sodium heparin (BD Vacutainer). Plasma were separated by centrifuging it at 3000 rpm for 15 minutes at 4 ◦ C in a centrifugation machine (Hanil, South Korea) and stored at -20 ◦ C until analysis. The metabolic profile and other biochemical properties including blood glucose, blood urea nitrogen (BUN), insulin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, Immunoglobulin G (IgG), insulin-like growth factor-1 (IgF-1) and cortisol were determined followed by the methods as described by Das *et al*. (2019). Representative samples of calf starter, green grass and probiotic/placebo feed were used in duplicate to determine dry matter, organic matter, ash, crude protein and crude fiber following the method of AOAC (2005). The percentage of SNF (solid not fat), fat, protein, and lactose of milk samples were determined by using a Lactostar (Funke-Gurber, Germany). Microbial enumeration of feed and feces were done according to Amanullah *et al*. (2018).

Data were analyzed using paired sample *t*-test in computer package program SPSS-20. Significance were declared at $P<0.05$, while tendency was declared at $P<0.10$.

Results and Discussion

Nutritional and microbial characteristics of mixed probiotic feed substrate

The chemical compositions (DM, OM and CP), microbial load (*L. acidophilus, B. subtilis* and *S. cerevicae*) and fermentation characteristics (pH and NH_3-N) of fresh and probiotic feed substrate are presented in Table 4. It was observed that the DM concentration in feed substrate at fresh remained almost similar in probiotic feed after 6 days of incubation and so in the case of OM concentration.

The DM concentration was found good enough to avoid dustiness as well as to prevent clump formation in feed. Similarly, CP content of feed at fresh was found 14.3%, which remained after 6 days of incubation. Inoculated microbes might utilize sugar and protein for their multiplication and, thereby slight reduction in DM, OM and CP occurred.

The pH and NH_3-N (mg/100g) contents in feed at fresh were observed 6.17 and 28.04, respectively. After 6 days of incubation pH was dropped down to 4.34, which indicated vigorous microbial growth during incubation that helped to produce sufficient lactic acid to reduce pH at such a low level. On the other hand, the NH_3-N contents were increased to 42.55 mg/100g after 6 days of incubation. This result indicated an increased amination in feed substrate. The range of pH, CP and NH_3-N contents in feeds found good to maintain probiotic feed characteristics. It was observed that the concentration *L. acidophilus* in fresh feed was 4.9 $\log_{10}$ CFU/g, but after incubation that was increased to almost double in probiotic feed substrate (8.8 $\log_{10}$ CFU/g feed).

Similar results were obtained in the case of *B. subtilis* and *S. cerevicae*. Initial concentrations of *B. subtilis* and *S. cerevicae* in fresh feed were 5.5 and 4.5 log_{10} CFU/g, respectively, which increased to 9.1 and 7.5 log_{10} CFU/g, respectively in probiotic feed substrate after 6 days of incubation. High concentrations of all three microbes at a level of >7.0 log_{10} CFU/g was considered sufficient to be a probiotic feed as most of the commercial sources contained probiotic microbes.

Effects of mixed probiotic on growth performance of calves

Effects of feeding probiotic feed supplement on intake and growth performances of calves were presented in Table 5. There were no significant differences (P>0.05) between the control and probiotic treatment in final live weight, milk intake, total dry matter intake (DMI), total crude protein intake, daily gain and feed conversion ratio (FCR). In agreement with the present study, Frizzo *et al*. (2011) reported no effect of probiotic supplement in feed intake, live weight gain and FCR in calves.

Table 4. Chemical, microbial and fermentation characteristics of fresh and probiotic feed substrate

	Fresh substrate	**Probiotic feed after 6 days incubation**
Dry matter, %	50.46 ± 1.84	49.69 ± 0.05
Organic matter, %	96.94 ± 0.02	96.40 ± 0.05
Crude protein, %	14.30 ± 0.11	14.10 ± 0.25
NH_3-N, mg/100 g	28.04 ± 0.18	42.55 ± 1.88
pH	6.17 ± 0.01	4.34 ± 0.01
Microbes, log_{10} CFU/g		
L. acidophilus	4.9± 0.27	8.8 ± 0.19
B. subtilis	5.5 ± 0.30	9.1 ± 0.23
S. cerevicae	4.5 ± 0.26	7.5 ± 0.22

Table 5. Effect of feeding probiotic feed supplement on intake and growth performance of calves

	Control	**Probiotic**	**SEM**	**Sig.**
Initial LW, kg	20.68	21.20	0.718	NS
Milk intake, kg/d	1.00	1.03	0.033	NS
Total DMI, g/d	406.32	423.36	14.08	NS
Total CPI, g/d	94.70	96.97	3.36	NS
Final LW, kg	34.60	36.14	1.925	NS
Daily gain, g/d	152.97	164.19	14.32	NS
FCR	2.66	2.59	0.231	NS

LW, Live weight; DMI, Dry matter intake; CPI, Crude protein intake; FCR, Feed conversion ratio; NS, Not significant

Table 6. Effect of feeding probiotic feed supplement on physical properties of feces of calves

	Control (n=540)	**Probiotic (n=540)**
Color		
Normal	470 (87.03%)	504 (93.33%)
Yellowish-Yellow	28 (5.2%)	11 (2.03%)
Yellow green	42 (7.77%)	25 (4.63%)
Odor		
Normal	470 (87.03%)	504 (93.33%)
Bad	70 (12.96%)	36 (6.66%)
Consistency		
Hard (+)	6 (1.11%)	19 (3.52%)
Normal (++)	468 (86.66%)	486 (90.0%)
Tended to be liquid (+++)	43 (7.96%)	26 (4.81%)
Watery (++++)	23 (4.26%)	9 (1.66%)
Consistency Score (1-4)	2.12	2.04
Cases of Diarrhoea	14 (2.60%)	7 (1.30%)

n, number of observations (no. of replications × days = 6 × 90 = 540)

Similar results were also observed by Saleem *et al*. (2017) on milk intake, average daily gain (ADG) and total gain in pre-weaning lamb and by Ataşoğlu *et al*. (2010) in the case of pre-weaning goat kids, where kefir was supplied as the source of probiotic. Better management and feeding systems irrespective of treatments might dim the effect of probiotic on intake of calves in this

study. Earlier, Ruppert *et al*. (1994) stated that probiotic supplementation in feed may affect calf's feed intake only when they were kept under stressful condition. Further, no differences in feed intake might be the underlying reason for unaffected daily gain of calves in this study.

Effects on physical properties of feces

Effects of feeding probiotic feed supplement on physical properties of feces and frequencies of diarrhea in calves were presented in Table 6. The physical properties of feces consisting color, odor and consistency was observed better in probiotic group compared to the control diet-fed group. Results (Table 6) revealed that in probiotic feed supplemented calves 93.3% cases were found normal feces in terms of color, while it was 87.03% in the control. Yellowish to yellow and yellow green color of feces were considered as abnormal color, which was found 5.2 and 7.77% *vs* 2.03 and 4.63% in the control *vs* probiotic-feed supplemented calves, respectively. Similarly, normal and bad odor, which was practically relevant to color properties were found at 87.03 and 12.96% *vs* 93.33 and 6.66% in the control *vs* probiotic-fed calves, respectively. It was found that the percentage of hard, normal, tended to be liquid and watery feces in the control group was 1.11, 86.66, 7.97 and 4.26%, respectively and all together they derive a consistency score of 2.12 in the range of 1 to 4 scale. While in the probiotic-fed group, they were 3.52, 90.0, 4.81 and 1.66%, respectively and they gave a consistency score of 2.04.

Table 7. Effect of feeding probiotic feed supplement on fecal microbial load

	Control (log_{10} CFU/g)	Probiotic (log_{10} CFU/g)	SEM	*p*-value
Eschericia coli				
0 d	7.87	8.16	0.259	0.315
15 d	8.22	8.24	0.159	0.874
30 d	8.34	7.44	0.086	<0.001
45 d	8.45	7.36	0.052	<0.001
60 d	8.33	7.46	0.066	<0.001
75 d	8.27	6.71	0.306	<0.01
90 d	7.28	6.14	0.128	<0.001
Salmonella spp.	nd	nd	-	-
Lactic acid bacteria	6.96	9.11	0.428	<0.01
Bacillus subtilis	nd	4.11	-	-
Sacharomyces cerevicae	nd	3.63	-	-

Table 8. Effect of feeding probiotic feed supplement on blood metabolic profile of milk-fed calves

	Control	Probiotic	SEM	*p*-value
Blood glucose (mmol/l)	3.7	3.9	0.118	0.152
BUN (mg/dl)	31.78	31.68	1.566	0.952
Total Cholesterol (mg/dl)	157.2	171.52	23.857	0.575
HDL	25.8	37.55	5.136	0.071
LDL	76.45	82.26	7.562	0.477
Triglyceride (mg/dl)	8.02	10.82	3.733	0.489
Cortisol (µg/dl)	0.52	0.66	0.147	0.401
IgG (ng/ml)	8.38	12.62	1.353	0.026
IgF-1 (g/l)	0.62	0.76	0.419	0.738
Insulin (mIU/ml)	0.76	0.52	0.212	0.319

BUN, Blood urea nitrogen; HDL, High density lipoprotein; LDL, Low density lipoprotein; IgG, Immunoglobulin G; IgF-1, Insulin-like growth factor-1/Somatotropin-c

There were 14 cases (2.60%) of diarrhea observed in the control group compared to 7 cases (1.30%) in probiotic group. The decreased frequency of diarrhea in probiotic fed calves as observed in this study was in agreement with previous findings (Isyk *et al*., 2004; Abe *et al*., 1995). A trend for reduced diarrhea in this study may be explained by an antagonistic action of probiotic microbes towards diarrhoeagenic *E. coli* and implantation of probiotic microorganisms in the intestinal tract (Amanullah *et al*., 2009; Yamazaki *et al*., 1991; Namioka *et al*. 1991; Ozawa *et al*., 1983). Significant decrease in fecal *E. coli* shedding in probiotic fed calves (Table 7) is

supporting the said reason for reducing diarrhea in this study. In addition, significantly increased serum IgG concentration in probiotic fed calves (Table 8) might also contributed to prevent diarrhea in this study.

Effects on microbial load in feces

The fecal count of *E. coli, Salmonella spp., Lactic acid bacteria (LAB), Bacillus subtilis and Sacharomyces cerevicae* at different days during the experimental period were illustrated in Table 7. It was found that the *E. coli* concentration (log_{10} CFU/g) in the feces from calves under the probiotic-fed group was significantly (P<0.01) reduced from day 30 onwards (30, 45, 60, 75 and 90 days). No *Salmonella* was detected in feces of calves irrespective of dietary groups at any days. The feces of calves under both the control and probiotic-fed group contained LAB at 90 day, however, the probiotic-fed group had significantly higher (P<0.01) concentration compared to the control. On the other hand, *B. subtilis* and *S. cerevicae* were not detected in the feces of calves under the control group, but in the probiotic-fed group.

In agreement with the present study, decreased fecal count of *E. coli* in female calves resulted from probiotic feeding was also reported by Mohamadi Roodposhti and Dabiri (2012). Earlier, few mechanisms were suggested by which probiotics may reduce harmful bacteria like *E. coli* in intestinal tract and feces in calves (FAO, 2016). Probiotic microorganisms produce inhibitory substances such as organic acids, hydrogen peroxide and bacteriocins, which acts as antimicrobial-like compounds. Secondly, probiotic bacteria may inhibit competitively by expelling harmful bacteria like *E. coli* on intestinal epithelial surfaces. Elam *et al*. (2003) also reported decreased fecal *E. coli* shedding in beef steers fed *Lactobacillus acidophilus.* The LAB as beneficial bacteria normally associated with a balanced normal in the gut flora (Bayatkoushar *et al*., 2013). Increases in numbers of *Lactobacilli* can show a normal occurrence in the development of intestinal flora of calves (Gilliland and Speck, 1977). Amanullah *et al*. (2009) found significantly increased number of LAB, while significantly reduced *E. coli* on intestinal surfaces of calves fed LAB probiotic compared to that in the control.

Effects on blood metabolites and immunity

Effects of feeding probiotic feed supplement on serum metabolic profiles in calves were presented in Table 8. In blood profiling of calves under control and probiotic feed-supplemented group glucose and blood urea nitrogen (BUN) were not differ significantly (P>0.05). These results are in agreement with other previous findings (Noori *et al*., 2016 and Frizzo *et al*., 2010). Similarly, Antunovic *et al*. (2006) reported no change in blood glucose concentration in probiotic-supplemented lambs.

The total cholesterol and low density lipoprotein (LDL) concentrations in control and probiotic fed calves were not significant (P>0.05). Usually, it is believed to decrease serum cholesterol by probiotic feeding and there are two proposed mechanisms for the reduction of serum cholesterol level in animals fed on probiotics (Noori *et al*., 2016). Zarate *et al*. (2002) suggested an increase in degradation of cholesterol across the gastrointestinal tract, while Farnades *et al*. (1987) suggested simultaneous sediment of cholesterol and deconjugation of bile acids in animals fed probiotic. However, in the present study, no such results were observed. Moreover, the HDL concentration in calves was tended (P<0.10) to be higher in probiotic fed group than that in the control. This result was in agreement with Deroos and Katan (2000), who showed that dietary inclusion of probiotic, resulted in an increased serum HDL concentration. In contrast, some others reported no effects of probiotic on HDL concentration in animals (Noori *et al*., 2016 and Panda *et al*., 2000).

The triglyceride concentrations in control *vs* probiotic-fed calves were also did not differ significantly (P>0.05) in this study. The effects of probiotic feeding on serum triglyceride contents in animals were found variable. In agreement with our finding, Panda *et al*. (2000) reported no change in serum triglyceride in pig. Unlike this study, Noori *et al*. (2016) reported a significant increase in serum triglycerides of calves fed yogurt probiotic (pH 3.8). They suggested, probiotic feeding might reduce conversion of primary bile acids to secondary one, and in turn, fat metabolism was increased. However, effects also might come from probiotic career yogurt as it contained added fat. On the other hand, Chiofalo *et al*. (2004) observed significantly decreased serum triglycerides in kids as a result of feeding dietary probiotics.

The immunoglobulin G (IgG) content was significantly (P<0.05) increased in probiotic fed calves than that in the control. Riddell *et al*. (2010) reported an increasing tendency of serum IgG1 in pre-ruminant calves at day 42 fed *Bacillus* based probiotic. It was suggested that addition of a *Bacillus* based probiotic to the diet would stimulate an increase in IgG1 levels mediated through an anti-spore immune response (Hong *et al*., 2005). Duc *et al*. (2004) indicated

an increase in IgG1 levels in mice dosed with *B. subtilis.* In contrast, some researchers reported no effect of probiotic on serum immunoglobulin (Mohamadi Roodposhti and Dabiri, 2012; Morill *et al*., 1995).

Conclusion

Results showed that probiotic feed prepared based on wheat bran and molasses containing *L. acidophilus*, *B. subtilis* and *S. cerevicae* improved fecal physical properties, reduced *coliform* and increased probiotic bacteria in the feces of calves. This probiotic feed supplement also reduced diarrheal frequency and increased immunoglobulin status as indicated by serum IgG concentration in probiotic-fed calves compared to the control. However, effects of probiotic feed supplement were not reflected in feed intake, daily gain or feed conversion ratio (FCR). The beneficial effects from improved fecal health, reduced diarrheal frequency and improved immunoglobulin status on production performances might be achieved later in advanced age

Author's contribution

SM Amanullah: Conceptualizing the research, project panning, supervision, data analysis, manuscript writing and editing; R Jahan: Execute the research trial and lab analysis; MM Rahman: Helped in experimentation and data generation; MA Samad: Helped in project planning, supervision and editing; SMJ Hossain: Overall supervision of the research.

Conflict of interest

The authors declare no conflict of interest.

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Data Availability

All the necessary data used in this research will be made available as per the authorization of the authors.

Ethical approval

This research was approved by the Ethical Committee of BLRI

Consent to participate

The authors provide full consent to participate as per need.

Consent for publication

All the author has fully agreed to publish this research in Bangladesh Journal of Animal Science.

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