Original Article

The effects of probiotic *Lactobacillus acidophilus* and/or prebiotic mannan oligosaccharides on growth performance, nutrient utilization, blood metabolites, faecal bacteria, and economics of crossbred calves

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Abstract

Background: The ban on antibiotics as growth promoters paved the way for probiotics and prebiotics as growth promoters in animal production. **Aims:** The present study was conducted to evaluate the effect of probiotic *Lactobacillus acidophilus* and/or prebiotic Mannan oligosaccharides on growth performance, blood biochemical variables, and faecal bacterial count in crossbred calves. **Methods:** Fifteen-day-old crossbred calves (n=24) were divided into four groups, each consisting of six calves, and subjected to different experimental diets. The control group (T_0) received a basal diet without any additives. The T_1 and T_2 groups received the basal diet and the probiotic (*L. acidophilus*, 2×10^{10} cfu/g) @ 1 g/calf per day and prebiotic (Mannan oligosaccharide) @ 4 g/calf per day, respectively. Calves of the T_3 group were offered a basal diet and synbiotic (*L. acidophilus*, 0.5 g + Mannan oligosaccharide, 2 g/calf per day). The feed additives were mixed in milk. **Results**: The results of 90 days feeding trial showed that calves of the T_3 and T_1 groups had higher (P<0.05) body weight (BW) gain and dry matter digestibility than the control. Feeding the probiotic showed a positive effect (P<0.05) on body length at the first, second, and third months, compared to the control. The blood serum total protein and globulin concentrations in the T_1 group, on days 30 and 90, and T_3 group, on day 90, were higher (P<0.05) than those of the control. All the treatment groups (T_1, T_2, and T_3) showed a reduction (P<0.05) in faecal coliform and *E. coli* count, compared to the control, on the 15th and 30th days of the study. Additionally, the T_2 group showed a significant coliform count reduction on days 45 and 60 of the study. **Conclusion:** The dietary addition of *L. acidophilus*, 2×10^{10} cfu/g @ 1 g/calf per day and the combination of *L. acidophilus*, 0.5 g + Mannan oligosaccharide, 2 g/calf per day improved growth performance, serum biochemical values, and favourable gut microbiota.

Key words: Body weight gain, *Lactobacillus acidophilus*, Prebiotic, Probiotic, Synbiotic

Introduction

Calves play a significant role in dairy economics and are future replacement herds. Therefore, a healthy calf promises a better return in the future in terms of the production of good-quality milk. The twentieth century witnessed a rise in the use of antibiotics as growth promoters in young calves. However, the development of antibiotic resistance and transference of antibiotic-resistant genes from animal to human microbiota (Mathur and Singh, 2005) led to worldwide concern and a ban on the use of antibiotics as growth promoters. The antibiotic ban was followed by tremendous pressure on the livestock and poultry industry, which made scientists look for alternatives that can replace antibiotics as growth promoters. To overcome these problems, the use of probiotics (Kim *et al*., 2011), prebiotics (Konstantinov *et al*., 2004), and a combination of probiotics and prebiotics (synbiotics) came up as a better alternative to antibiotics (Heinrichs *et al*., 2003). The intensively managed animals are subjected to alterations in immunity due to stress that leads to unhealthy animals (Nonnecke *et al*., 2003). The alteration in immunity makes calves susceptible to diarrhea and other diseases. Probiotics and prebiotics help maintain intestinal microbial balance and a healthy gut (Timmerman *et al*., 2005; Morrison *et al*., 2010). Probiotics have a broad range of beneficial effects such as regulating intestinal microbial homeostasis, an enzymatic activity that enhances nutrient absorption (Timmerman *et al*., 2005), and immunomodulatory effects (Timmerman *et al*., 2005; Kim *et al*., 2011), intrusion with pathogenic colonization and many more. Additionally, probiotics protect young animals against gastrointestinal disorders like diarrhea (Timmerman *et al*., 2005), improve faecal score (Satık, 2017), body weight gain, feed efficiency (Lesmeister *et al*., 2004), and health status (Frizzo *et al*., 2010). Prebiotics have been found to be the most effective additives at times of stress or increased pathogen exposure in calves (Morrison *et al*., 2010). Mannan oligosaccharides (MOS) addition in calves' diets has been reported to increase average daily weight gain (ADG) (Ghosh and Mehla, 2012; Roodposhti and Dabiri, 2012; Heinrichs *et al*., 2013), improve faecal score (Morrison *et al*., 2010), enhance the level of gamma-globulin, as well as create better passive immunity transfer (Król, 2011). *Lactobacillus acidophilus* is one of the lactobacilli found in animal intestines (Sandine *et al*., 1972) known to have probiotic effects (Shah, 2007). *Lactobacillus acidophilus* in calves improves body weight gain and intestinal microbial balance (Lesmeister *et al*., 2004; Ghosh and Mehla, 2012). Variable results of feeding probiotics and prebiotics need further investigation to validate their efficacy on calves' growth and health. Earlier, most of the studies focused on either probiotic or prebiotic addition. The present study was conducted to evaluate the influence of feeding a commercial probiotic (*L. acidophilus*), prebiotic (Mannan oligosaccharide), and synbiotic (*L. acidophilus* + MOS) on growth performance, nutrient utilization, blood serum metabolites, faecal coliform and *E. coli* count, and the economics of intensively managed crossbred calves.

Materials and Methods

Experimental animals and diet

The study was approved by Institutional Animal Ethics Committee (IAEC) under sanction letter-number IAEC/LPM/CVASC-227 as per article 13 of CPSCEA rules laid down by the Government of India. Throughout the study, animal welfare was given full consideration. The present experiment was conducted using 24 crossbred (Sahiwal × Holstein Friesian) calves (average 15 days old) consisting of sixteen female and eight male calves, reared at the Instructional Dairy Farm (IDF) Nagla, College of Veterinary Sciences & Animal Sciences, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand. The calves were randomly divided into four groups of six animals each (four female and two male calves) in a completely randomized design. The experiment lasted 90 days along with a digestion trial of 7 days. The calves were housed in well-ventilated calf pens (2×1 m^2) and the animal shed was cleaned and disinfected before the entry of the calves. The calves were assigned to different dietary treatments viz T_0, a basal diet without any additive, T_1, basal diet + *L. acidophilus* @ 1 g/calf per day (2×10^{10} cfu/g), T_2, basal diet + MOS @ 4 g/calf per day, and T_3, basal diet + 0.5 g *L. acidophilus* @ and 2 g MOS/calf per day. One g of probiotic contained *L. acidophilus* concentration of 2×10^{10} cfu. The commercial probiotic *L acidophilus* with brand name *L. acidophilus* and prebiotic Mannan oligosaccharide under brand name MOS were purchased from Hexon Laboratories Pvt. Ltd, Maharastra, India. The probiotic, prebiotic, and synbiotic were mixed in milk and fed early in the morning (6.00 am). The routine of milk feeding was twice a day (06:00 and 16:00). Milk was offered according to the Indian Council of Agriculture Research (ICAR) guidelines. Whole milk was fed to the calves at 1/10th of actual BW up to 2 weeks, 1/15th of actual BW in the third and fourth week, 1/20th of actual BW in the fifth and sixth week, and 1/25th from the seventh week till 90 days (weaning age) of the study. The basal diet consisted of calf starter and mixed greens (berseem + oats + mustard). The mixed green fodder contained green berseem, mustard, and green oats in the ratio of 1:1:1. The roughages (green fodder) and calf starter were fed separately to all groups of crossbred calves as *ad libitum* with equal opportunity to treatment and control groups in the morning and evening hours. Calf starter was offered from the second week onwards @ 100 g/animal per day and was increased fortnightly by 100 g till weaning (Table 1). The concentrate mixture consisted of maize, groundnut cake, soybean meal, mustard oil cake, wheat bran, rice polish, mineral mixture, and salt at the ratio of 30:15:12:13:17:10:2:1. The chemical composition of the commercial calf starter and mixed green fodder fed to experimental calves during the digestibility trial is given in Table 1. All the calves had 24 h access to *ad libitum* clean water.

Experimental measurements

Body weight (BW) and body measurements (body length, chest girth, and height) of calves were taken in the morning before feeding and watering at fortnightly and monthly intervals, respectively. Body weight was measured with a digital electronic weighing scale, whereas body measurements were taken with a measuring tape.

Blood samples were taken aseptically from the

Table 1: Chemical composition of feeds and fodders (% DM basis) fed during the digestion trial

Feeding stuff	DM (g/kg as-fed)	CP	EE	CF	NFE	TA	OM	AIA
Calf starter	89.75	22	3	7	61.1	6.9	91	2.5
Mixed green fodder	25	14.5	4	25	45.5	11	89	3

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NFE: Nitrogen free extract, TA: Total ash, OM: Organic matter, and AIA: Acid insoluble ash jugular vein using a sterilized syringe with the 18-gauge needle at 15 days intervals. The blood serum was separated and collected for determining total protein, albumin, globulin, and A/G ratio. Serum analysis of total protein (Biuret method), albumin (BCG Dye method), and globulin was calculated from the difference between total protein and albumin. The calves were kept in individual calf pens during the digestion trial to ensure proper recording. The daily record of feed offered and residues were kept and the representative samples were taken for nutritional analysis. Whole faeces and urine were collected separately manually on a daily basis. The faeces collected were mixed thoroughly and representative samples were taken for nutritional analysis. All the representative samples from daily collections of feed offered, feed residues, and feces voided were pre-dried in a hot air oven at 65°C for 48 h, ground through 1 mm mill screen openings, and stored in zip lock airtight polythene bags for further analysis. Representative samples (feed, residue, and faeces) taken during the digestion trial were analysed for dry matter, crude protein, crude fibre, ether extract, and total ash as per the procedure of AOAC 1995, whereas nitrogen-free extract was calculated by difference.

For bacterial examination, faecal samples were collected by sterilized plastic containers and bacterial populations were calculated by serial 10-fold dilutions (mixing 1 g homogenized faeces and 9 ml normal saline). The procedure was standardized and the dilutions of 10^{-5}, 10^{-6}, and 10^{-7} were used on Mac Conkey agar (Hi-media) and EMB agar (Hi-media) for coliform and *E. coli*, respectively, using the pour plate technique. The agar plates were incubated at 37°C for 24 and 48 h and colonies with colony count 30 to 300 were selected for calculations and expressed as log colony-forming units per g of faeces.

Statistical analysis

Statistical analysis was conducted using SPSS 16.0 software. Statistical significance of mean comparisons was calculated using a one-way ANOVA test. Data for variables involving periodic collections were analysed adopting repeated measures procedure using GLM of SPSS; the analysis included between-subjects main effect of treatment, within-subjects main effect of period of sampling, and the periods of sampling × treatment. A p-value of <0.05 was considered statistically significant.

Results

Body weight gain

The variation in body weight gain and ADG of crossbred calves at different intervals under different treatment groups is presented in Tables 2 and 3, respectively. Synbiotic and probiotic received groups showed a significant (P<0.05) increase in body weight gain than the control from the second fortnight to the 90th day of the experimental study, whereas no significant difference was observed between the prebiotic and control group. The mean value for the overall body weight gain in 90 days experimental trial for T_0, T_1, T_2, and T_3 were 29.38 ± 0.88, 38.03 ± 1.23, 32.10 ± 1.36, and 40.10 ± 1.32 kg, respectively. The ADG throughout the experimental period showed an increase of 29.44%, 9.26%, and 36.49% in the T_1, T_2, and T_3 groups, respectively, compared to the T_0 group. Thus, the overall body weight gain obtained in probiotic and synbiotic groups was significantly (P<0.05) higher than that of the control and prebiotic groups.

Table 2: Effect of probiotic, prebiotic, and synbiotic addition on body weight gain (kg) of crossbred calves

Fortnight	T_0	T_1	T_2	T_3
1	4.00 ± 2.27	5.14 ± 1.20	4.35 ± 1.57	5.09 ± 2.27
2	4.58 ± 1.20^b	6.00 ± 0.90^a	5.10 ± 1.00^b	6.10 ± 1.25^a
3	4.88 ± 0.87^b	6.76 ± 1.27^a	5.5 ± 0.90^b	6.00 ± 1.30^a
4	5.10 ± 1.50^b	7.00 ± 0.80^a	5.83 ± 1.2^b	6.96 ± 0.97^a
5	5.42 ± 0.50^b	7.13 ± 1.80^a	5.90 ± 1.30^b	7.93 ± 2.27^a
6	5.40 ± 0.27^b	7.00 ± 1.20^a	6.06 ± 1.50^b	8.00 ± 1.80^a

Means bearing different superscripts in a row differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3; Symbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

Table 3: Effect of probiotic, prebiotic, and synbiotic on growth performance (mean±SE) of crossbred calves

Group	T_0	T_1	T_2	T_3
Initial body weight (kg)	32.00 ± 0.96	32.08 ± 0.74	32.08 ± 1.08	32.08 ± 0.83
Final body weight (kg)	61.38 ± 1.13^b	70.11 ± 1.27^a	64.18 ± 1.04^b	72.18 ± 1.21^a
Total body weight gain 90 days (kg)	29.38 ± 0.88^b	38.03 ± 1.23^a	32.10 ± 1.36^b	40.10 ± 1.32^a
Average daily gain (g/day)	322.89 ± 9.72^b	417.91 ± 13.54^a	352.75 ± 14.95^a	440.66 ± 14.53^a

Means in row with different superscripts differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Symbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

Morphometrical parameters

The effects of feeding probiotic, prebiotic, and synbiotic on biometrical parameters (body length, chest girth, and height) are shown in Table 4. The probiotic fed groups showed significantly higher body length at the first, second, and third month of the experimental period than the control and prebiotic group. The synbiotic group also showed a significantly higher body length on the second and third month of the experimental trial compared to the control and prebiotic groups. Furthermore, significantly higher chest girth and height were observed in the probiotic and synbiotic groups at the end of the third month compared to the control group.

Nutrient intake and digestibility

According to Table 5, no significant differences were observed in the intake of crude protein, ether extract, crude fiber, and organic matter among different experimental groups. However, the significantly higher (P<0.05) values of dry matter digestibility were observed in the probiotic and synbiotic groups than in the control group.

Blood biochemical variables

The results of feeding probiotic, prebiotic, and synbiotic on the serum concentration of total protein, albumin, globulin, and A/G ratio are shown in Table 6. It was observed that all the treatment groups showed a significant increase (P<0.05) in the serum total protein and globulin concentration at the 90th day of the study compared to the control group. Moreover, the T_1 group showed a significant increase (P<0.05) for the same item on the 30th day of the study.

Faecal microbial count

The results of the faecal microbial count, i.e., faecal coliform and *E. coli* count, are given in Figs. 1 and 2, respectively. All the treatment groups (T_1, T_2, and T_3) showed significant differences (P<0.05) in faecal coliform and *E. coli* count from the control group on the 15th day and 30th day. Prebiotic supplemented calves also showed a significant reduction of coliform count on the 45th and 60th day of the study.

Table 4: Effect of probiotic, prebiotic, and synbiotic on biometrical parameters (cm) of crossbred calves

Parameters	Month	T_0	T_1	T_2	T_3
Body length	0	64.09 ± 0.62	64.17 ± 0.47	63.66 ± 0.58	64.45 ± 0.38
	1	67.89 ± 0.62^{b}	69.68 ± 0.48^{a}	69.14 ± 0.22^{ab}	68.36 ± 0.28^{ab}
	2	71.28 ± 0.37^{b}	73.24 ± 0.36^{a}	71.83 ± 0.34^{b}	73.88 ± 0.57^{a}
	3	75.73 ± 0.78^{b}	80.48 ± 1.09^{a}	77.03 ± 0.78^{b}	81.63 ± 0.54^{a}
Chest girth	0	69.80 ± 0.70	70.61 ± 0.42	69.77 ± 0.91	70.38 ± 0.66
	1	75.12 ± 0.70	76.02 ± 0.30	75.42 ± 0.53	76.22 ± 0.42
	2	79.37 ± 0.76	79.88 ± 0.31	79.40 ± 0.64	79.80 ± 0.29
	3	83.73 ± 0.51^{b}	86.77 ± 0.84^{a}	84.45 ± 0.20^{b}	87.71 ± 0.95^{a}
Height	0	68.89 ± 0.54	69.26 ± 0.41	69.19 ± 0.63	68.98 ± 0.39
	1	72.45 ± 0.52	73.07 ± 0.28	72.79 ± 0.48	73.56 ± 0.24
	2	76.36 ± 0.28	77.10 ± 0.80	76.49 ± 0.33	77.75 ± 0.26
	3	81.59 ± 0.43^{b}	84.20 ± 0.40^{a}	82.09 ± 0.57^{b}	84.43 ± 0.69^{a}

Means bearing different superscripts in a row differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2×10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Symbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

Table 5: Effect of probiotic, prebiotic, and synbiotic on nutrients intake (kg/d) and digestibility coefficients (%) in crossbred calves during digestion trial

Particulars	T_0	T_1	T_2	T_3
DMI	2.12 ± 0.24	2.30 ± 0.05	2.19 ± 0.07	2.37 ± 0.12
Digestibility of DM	67.86 ± 0.46^{b}	72.32 ± 1.26^{a}	70.06 ± 1.19^{ab}	72.76 ± 1.13^{a}
CP intake	0.35 ± 0.04	0.38 ± 0.01	0.39 ± 0.02	0.39 ± 0.02
Digestibility of CP	73.74 ± 0.31	77.34 ± 1.01	77.24 ± 2.72	77.78 ± 0.89
EE intake	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	0.08 ± 0.00
Digestibility of EE	75.10 ± 0.29	78.52 ± 0.96	78.41 ± 2.57	78.94 ± 0.84
CF intake	0.39 ± 0.05	0.43 ± 0.01	0.43 ± 0.01	0.44 ± 0.02
Digestibility of CF	47.05 ± 1.44	54.59 ± 2.18	52.99 ± 3.99	54.89 ± 2.12
OM intake	1.91 ± 0.21	2.08 ± 0.05	2.18 ± 0.14	2.15 ± 0.105
Digestibility of OM	68.70 ± 0.44	73.04 ± 1.22	73.01 ± 3.33	73.47 ± 1.10

Means bearing different superscripts in a row differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2×10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Symbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day. DMI: Dry matter intake, DM: Dry matter, CPI: Crude protein, EE: Ether extract, and OM: Organic matter

Table 6: Effect of probiotic, prebiotic, and synbiotic on the blood serum biochemical metabolites in crossbred calves

Parameters	Month	T_0	T_1	T_2	T_3
Total protein (g/dl)	0	5.41 ± 0.44	5.47 ± 0.27	5.78 ± 0.33	5.23 ± 0.19
	1	5.34 ± 0.27^{b}	6.84 ± 0.05^{a}	5.58 ± 0.29^{b}	5.09 ± 0.40^{b}
	2	5.30 ± 0.09	6.25 ± 0.36	6.47 ± 0.80	6.07 ± 0.23
	3	5.44 ± 0.22^{b}	6.79 ± 0.11^{a}	6.48 ± 0.12^{a}	6.71 ± 0.13^{a}
Average value of total protein (g/dl)		5.37 ± 0.03	6.33 ± 0.32	6.07 ± 0.23	5.77 ± 0.38
Albumin (g/dl)	0	2.91 ± 0.29	3.10 ± 0.34	3.13 ± 0.04	3.08 ± 0.45
	1	3.00 ± 0.09	3.36 ± 0.20	3.40 ± 0.27	3.49 ± 0.35
	2	3.00 ± 0.05	3.61 ± 0.13	3.56 ± 0.49	3.52 ± 0.17
	3	3.16 ± 0.07	3.49 ± 0.20	3.59 ± 0.32	3.51 ± 0.24
Average value of albumin (g/dl)		3.02 ± 0.05	3.39 ± 0.11	3.42 ± 0.11	3.40 ± 0.11
Globulin (g/dl)	0	2.50 ± 0.72	2.37 ± 0.09	2.65 ± 0.36	2.14 ± 0.41
	1	2.34 ± 0.30^{ab}	3.48 ± 0.15^{a}	2.18 ± 0.56^{ab}	1.60 ± 0.63^{b}
	2	2.30 ± 0.07	2.64 ± 0.48	2.90 ± 1.09	2.54 ± 0.32
	3	2.28 ± 0.23^{b}	3.29 ± 0.31^{a}	2.89 ± 0.33^{ab}	3.20 ± 0.11^{a}
Average value of globulin (g/dl)		2.36 ± 0.05	2.95 ± 0.26	2.66 ± 0.17	2.37 ± 0.34
A/G ratio	0	1.16 ± 0.57	1.31 ± 0.47	1.18 ± 0.19	1.43 ± 0.44
	1	1.52 ± 0.57	1.07 ± 0.13	1.23 ± 0.19	1.60 ± 0.44
	2	1.34 ± 0.60	1.48 ± 0.23	1.92 ± 1.04	1.57 ± 0.46
	3	1.21 ± 0.15	1.23 ± 0.29	1.07 ± 0.23	1.02 ± 0.24
Average value of A/G ratio		1.31 ± 0.08	1.27 ± 0.09	1.35 ± 0.19	1.41 ± 0.13

Means bearing different superscripts in a row differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Symbiotic group: basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

Table 7: Body weight gain and average expenditure (USD) per kg additional weight gain above the control group in probiotic, prebiotic, and synbiotic group

Parameters	Control	Probiotic	Prebiotic	Synbiotic
Average total body weight gain (kg)	29.38 ± 0.88^{b}	38.03 ± 1.23^{a}	32.10 ± 1.36^{b}	40.10 ± 1.32^{a}
Weight gain (kg) over and above the control group		8.65	2.72	10.72
Cost		0.64	1.40	1.02
Cost/kg weight		0.074	0.52	0.095

Means bearing different superscripts in a row differ significantly (P<0.05). T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Synbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

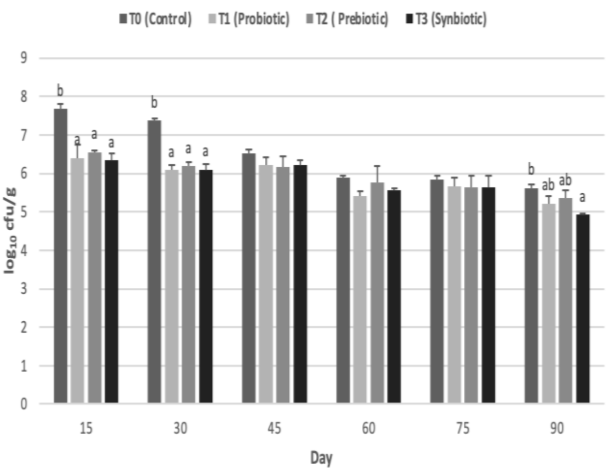

Fig. 1: Average faecal coliform count of crossbred calves under different treatments. T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Synbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

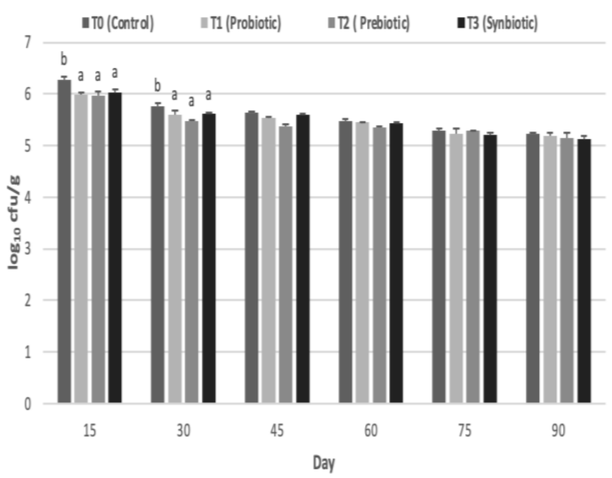

Fig. 2: Average faecal *E. coli* count of crossbred calves under different treatments. T_0: Control group, basal diet without any additive; T_1: Probiotic group, basal diet + *Lactobacillus acidophilus* @ 1 g/calf per day (2 × 10^{10} cfu/g); T_2: Prebiotic group, basal diet + Mannan oligosaccharide @ 4 g/calf per day; and T_3: Symbiotic group, basal diet + *Lactobacillus acidophilus* @ 0.5 g and 2 g MOS/calf per day

Economics

The production cost of 1 kg additional BW in probiotic, prebiotic, and synbiotic supplemented groups has been presented in Table 7. An additional gain of 8.65 kg was observed in the T_1 group compared with the control group and the same cost about 0.074 USD/kg additional body weight gain. The T_2 group gained an additional 2.72 kg than the control group and the same cost about 0.52 USD/kg additional body weight gain. Likewise, the T_3 group resulted in an additional 10.72 kg more weight gain than the control group, costing about 0.095 USD/kg additional body weight gain. The present study showed that probiotics are more economical followed by synbiotics and prebiotics in raising dairy calves.

Discussion

Body weight gain

In the present study, probiotic and synbiotic groups performed better and showed significant improvement in body weight gain compared to prebiotic and control groups and this might be due to better gut microbial balance. Synbiotics have been found to show the synergistic effect as prebiotics support the growth of the probiotic and beneficial organism in the gut, which has an encouraging effect on the overall performance of calves, thus showing an enhanced effect in the present study. The present study results are consistent with the results of Adams *et al*. (2008), who reported significantly higher body weight gain in probiotic supplemented calves. Similarly, in line with the present results, higher weight gain was observed in probiotic supplemented calves (Lesmeister *et al*., 2004; Roodposhti and Dabiri, 2012). However, growth performance was not affected by *L. acidophilus* 27SC (Hossain *et al*., 2007) and bacillus-based probiotic (Nonnecke *et al*., 2003) supplementation in young dairy calves. Kamra *et al*. (2002) also did not find any difference in the body weight gain of cross-bred calves with probiotic supplementation. Mannan oligosaccharide (prebiotic) supplementation could not evoke any effect on the growth performance of crossbred calves and previous researchers have reported similar findings in Holstein and in other dairy calves (Konstantinov *et al*., 2004; Terre *et al*., 2007; Hill *et al*., 2009; Uzmay *et al*., 2011; Roodposhti and Dabiri, 2012; Kara *et al*., 2015). However, many studies show that MOS supplementation in calves has a significant effect on the growth of Holstein cross calves (Ghosh and Mehla, 2012; Heinrichs *et al*., 2013). Such inconsistent results of MOS supplementation on growth performance in different studies may be attributed to level, source, duration of MOS supplementation, health status, intestinal bacterial population, and environmental conditions (Zhao *et al*., 2012).

Morphometrical parameters

Calves of probiotic and synbiotic supplemented groups performed better in terms of morphometry (body length, chest girth, height) than those of the control and prebiotic groups due to better nutrient absorption and/or favorable gut conditions. Better skeletal growths and improved gut conditions in the calves of the probiotic and synbiotic groups were responsible for significant body weight gain. These results agree with Noori *et al*. (2016), who reported a significant increase in the body length and wither height in Holstein calves supplemented with probiotic yoghurt. Contrary to the present results, no significant change in biometrical parameters of probiotic supplemented calves was observed (Lesmeister *et al*., 2004; Gupta *et al*., 2015). Also, changes in heart girth, hip-width, and wither height were unaffected by bacillus-based probiotic supplementation in young calves (Riddell *et al*., 2010). MOS supplementation did not affect morphometric parameters (heart girth, hip-width, wither height) of calves (Heinrichs *et al*., 2003; Uzmay *et al*., 2011; Da Silva *et al*., 2012).

Nutrient intake and digestibility

In spite of the similar feed intake of the experimental calves, the better dry matter digestibility observed in probiotic and synbiotic supplemented groups is attributable to the thinning of intestinal surface, improvement in gut health, and increase in beneficial microbial populations which led to better absorption of nutrients. However, these findings are in agreement with the earlier reports (Pandey and Agrawal, 2002; Di Francia *et al*., 2008; Riddell *et al*., 2010). Inconsistent with these results, higher dry matter intake was reported in yeast supplemented calves (Di Francia *et al*., 2008). Abu-Tarboush *et al*. (1996) also observed no effect of *L. acidophilus* 27SC supplementation on apparent digestibility of nutrients in dairy calves. Quigley *et al*. (2002) also reported that intakes of dry matter, protein, and fat in calves were unaffected by prebiotic, fructooligosaccharide supplementation. There are reports that MOS tends to increase starter intakes in calves (Heinrichs *et al*., 2003; Terre *et al*., 2007). Contrary to the present study, Hossain *et al*. (2012) observed higher digestibility of organic matter, crude protein, ether extract, and crude fibre in Kankrej calves with yeast supplementation.

Blood biochemical variables

Blood serum total protein, albumin, globulin, and A/G ratio were in normal range in all groups, and this represents the good health status of the calves. The increase in serum total protein in the T_1, T_2, and T_3 groups might be due to improved hepatic functions and increased beneficial rumen microbiome which favours protein metabolism. Serum protein level (albumin and globulin) is an essential indicator of hormonal, nutritional, and water balance in the animal body, thus affecting health status. The better globulin levels in the calves of the probiotic and synbiotic group indicate good immune status as serum globulins provide immunity to animals (Jain, 1993). Similar results of an increase in total serum protein with probiotic supplementation were reported by El-Ashry *et al*. (2003) in sheep and by Tang *et al*. (2005) in pigs. In another study, MOS supplementation in calves' diets did not affect blood protein levels (Heinrichs *et al*., 2003), and this contradicts the present study. There are also other reports that serum proteins are unaffected by prebiotic supplementation (Quigley *et al*., 2002). The A/G ratio in calves was unaffected by probiotic, which is also reported in other studies (Bakhshi *et al*., 2006; Hossain *et al*., 2012). Furthermore, Al-Saiady (2010) reported comparable serum albumin and globulin values between probiotic supplemented and control groups in *L. acidophilus* 27SC supplementation in calves. Contrary to the present study, Lesmesister *et al*. (2004) and Chaudhary *et al*. (2008) reported no significant effect of probiotics on serum albumin, globulin, and total protein concentration of neonatal dairy calves and crossbred calves, respectively. Abdel Fattah and Farrah (2009) reported no effect of prebiotic and probiotic on serum protein, albumin, and globulin in chicken. However, they reported a significant increase in serum total protein, albumin, and globulin concentration in the synbiotic supplemented group.

Faecal microbial count

In the present study, the addition of prebiotics in the feed produced no significant effect on the faecal composition except coliform count on the 45th and 60th days. The treatments offered to calves in the present study reduced the faecal coliform and *E. coli* counts, and this might be due to the contribution of probiotic and prebiotic in maintaining intestinal microbial balance and competition for attachment site (Pandey and Agrawal, 2002). The balance of microbiota is essential for maintaining health status and preventing infections when the immune system is immature and not fully developed in calves. Supplementing probiotics to calves helps in maintaining the microbial balance by increasing the number of beneficial bacteria and decreasing the number of pathogenic bacteria, as confirmed by the present study and other studies (Gaggìa *et al*., 2010; Al-Saiady, 2010). This could also explain the present study's better growth performance in the probiotic and synbiotic groups. Ghosh and Mehla (2012) who reported that MOS supplementation in calves increased ADG and reduced Coliforms and *E. coli* count further supported this. Conversely, MOS supplementation did not change the faecal bacterial populations of calves (Hill *et al*., 2009; Kara *et al*., 2015). Similar results have been reported by Shim (2005) and Roodposhti and Dabiri (2012) for probiotic, prebiotic, and synbiotic supplementation of suckling pigs and calves, respectively. However, the effect of feed supplementation with prebiotics, probiotics, and synbiotics is population-specific and it is dependent on various genetic and non-genetic factors.

Feeding probiotic and synbiotic improved body weight gain, morphometric and blood biochemical variables in crossbred calves, but prebiotic alone had no significant effect. Thus, *L. acidophilus* (2×10^{10} cfu/g) at the dosage of 1 g/calf per day as a probiotic and a combination of *L. acidophilus*, 0.5 g + Mannan oligosaccharide, 2 g/calf per day as a synbiotic may be used as effective growth promoters in the rearing of cross bred dairy calves and are cost effective for body weight gain.

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Conflict of interest

The authors declare there is no conflict of interest regarding the current study and its publication.

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