RESEARCH ARTICLE

Effect of Supplementing Probiotic, Prebiotic and Synbiotic on Haematological Parameters in Weaned Gir Calves

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Abstract

Good management practices to optimize nutrition, growth, immune status and decrease the risk of disease are vital in livestock. The use of probiotic, prebiotics and synbiotic may be a viable option to increase the proliferation of commensal bacteria in the gastrointestinal tract, modulate feeding behaviour, and optimize calf health. Twenty-four weaned Gir calves (4-6 month old) were distributed into four equal homogenous groups on the basis of their live body weight and sex in completely randomized design (CRD). The control (T1) group was offered a basal diet consisting of concentrate, green sorghum and dry fodder (Groundnut haulms) without any additional supplementation, while T2, T3 and T4 groups were supplemented basal diet with probiotic @ 10 gm/calf/day, prebiotic @ 10 gm/calf/day, symbiotic @ 20 gm/calf/day, respectively, for a period of 180 days. The haematological parameters and blood glucose levels did not differ significantly among the groups and were within the normal physiological range throughout the experimental period. However, overall mean value of serum growth hormone (GH) was significantly higher in T4 followed by T1, T2 and T3 groups. Thus, it can be inferred that, probiotic, prebiotic and symbiotic should be added in the diets of weaned Gir calves to maximize overall performance and overall profit for well-being of farmers.

Key words: Gir calves, Growth hormone, Prebiotic, Probiotic, Synbiotic.

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Introduction

Inadequate nutrition prior to weaning can lead to low weaning weight and weakened immunity, which exacerbates morbidity-related losses in livestock species. Weaned calves experience discomfort and upset stomach because of the change in microbiota in their digestive tract and the transition from a liquid to a solid diet. According to Bach *et al.* (2011), this diet change can occasionally cause the creation of undesirable gastro-intestinal microbiota, which impairs performance. Additionally, the use of different antibiotics to treat the gastrointestinal tract infection has led to additional disruptions in the rumen microbiota. These illnesses and circumstances can sometimes be too much for a calf to handle, which can result in death or stunted growth in later life. Prebiotic supplements, however, can reverse dysbiosis brought on by gastrointestinal disorders (Raza *et al.,* 2022).

Prebiotics are described as "non-digestible food ingredients that selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health and having a beneficial effect on the host". Prebiotics are carbohydrates that must be easily available and naturally occurring since they are indigestible to animal enzymes, degraded by gut acids, and not absorbed in the upper gastrointestinal system (Gibson and Roberfroid, 1995). Fructooligosaccharide (FOS), mannan oligosaccharides (MOS) and polysaccharide-protein complexes made from the yeast *S. cerevisiae* are a few examples of common prebiotics (Xu and Gorden, 2003). Prebiotic supplements increase feed intake (Terre *et al.,* 2007), average daily gain, feed efficiency (Xu and Gorden, 2003), improve growth, lower the count of faecal coliform in the intestines (Ghosh and Mehla, 2012), boost immunity

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(Fleige *et al.,* 2009), and are most beneficial during stressful times or during periods when the calf is exposed to more pathogens (Heinrichs *et al.,* 2009).

Probiotics are defined by FAO/WHO (2001) as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". The microbe that supports the equilibrium of microbes in the gut is known as a probiotic. Enterococcus, Lactobacilli, Bifidobacteria, and Lactic acid bacteria (LAB) are among the microorganisms commonly employed as probiotics. Additionally, yeasts can be utilized as probiotics, and studies suggest that using these items to reduce diarrhea in calves may be beneficial (Timmerman *et al.,* 2005). The positive effects of synbiotics on feed consumption, growth performance, and gastrointestinal health are widely recognized (Sharma *et al.,* 2023). In light of the fact that antibiotics are prohibited in many nations (Abd El-Tavab *et al.,* 2016) and public health concerns regarding their use, the hunt for appropriate substitutes has escalated. Probiotics, prebiotics, and synbiotics are three such alternatives that are thought to be safe, effective, and emerging for improving the performance of farm animals. Hence, this study was planned to evaluate the effect of supplementing probiotic, prebiotic and synbiotic on haematological parameters in weaned Gir calves

Materials and Methods

The present experiment was conducted on 24 weaned Gir calves, 4 to 6 months of age weighing 56 to 100 kg, at Cattle Breeding Farm, Kamdhenu University, Junagadh, Gujarat (India). The research protocol was approved by the Animal Ethics Committee of the College of Veterinary Science, Junagadh, vide protocol No. KU-JVC-IAEC-LA-105-23.

Probiotic, prebiotic and synbiotic were purchased from Gujarat Enzyme, Ahmedabad, Gujarat, India. Probiotic contained *Lactobacillus sporogenes* @ $5x10^7$ c.f.u./g and yeast, *Saccharomyces cerevisiae* @ $1.5x10^8$ c.f.u./g; Prebiotic (M-MOS Powder) contained, a mannan-oligosaccharides, and Synbiotic contained *Saccharomyces cerevisiae* @ $1.5x10^8$ c.f.u./g, *Lactobacillus sporogens* @ $5x10^7$ c.f.u./g + mannan oligosaccharides.

Experimental Animals and Duration of study

The selected weaned Gir calves (n=24) were assured for the health and disease. They were randomly allotted to four equal groups with six calves in each, *viz.,* Control (T1), Probiotic group (T2), Prebiotic group (T3) and Synbiotic group (T4). Difference in mean initial body weight of experimental groups was non-significant. Duration of experiment was 180 days. Information on treatment details during the study period are provided in Table 1.

Standard Managerial Practices

All the experimental animals were housed in a well-ventilated shed having tying arrangement for individual feeding and watering without having access to the other animal's diet. They were kept tied all the time and were let loose for two hours (7-9 am) in the morning for exercise in an open covered area. Each animal was given individual care. The experimental shed area was thoroughly cleaned daily in the morning. Hygienic conditions were maintained during entire experimental period to prevent any incidence of infectious and contagious diseases. Deworming of all experimental animals was carried out before start of experiment with broad spectrum anthelmintic.

Blood Collection and Haematology

Approximately 5 mL whole blood was collected from jugular vein with all aseptic precautions from each calf on day 0, 90^{th} and 180^{th} of experiment in Na-EDTA vacutainer for haematological parameters. For separation of serum, 10 mL blood was collected in a tube without anticoagulant and kept in slanting position. These tubes were incubated for 1 h at 37^oC. Blood clots were broken and tubes were centrifuged at 1765 x g for 20 min.

Haematological studies were performed on fresh blood samples. Haemoglobin, PCV, RBC, WBC, MCV, MCH, MCHC, and Platelets counts were estimated by automated blood analyser (Mindray BC - 2800 Vet). Blood glucose concentrations were determined by Glucometer. Growth hormone from serum sample was estimated by Enzyme-Linked Immunosorbent Assay (ELISA) technique and Bovine Growth hormone ELISA kit procured from PUREGENE.

Statistical Analysis

The data were analyzed for descriptive statistics (mean and standard error). Treatment and period effects on different parameters were analyzed by two-way analysis of variance (ANOVA) according to Snedecor and Cochran (1994). Pair-wise mean differences between groups were compared by DMRT test, and the mean differences were considered significant at $p<0.05$.

Table 1: Schedule of supplementation of probiotic, prebiotic and symbiotic

Treatment Groups	Treatment Schedule	N	Dose
Control (T1)	Basal diet	6	No supplements
Treatment-2 (T2)	Basal diet + Probiotic *Lactobacillus sporogenes* @ $5x10^7$c.f.u./g, *Saccharomyces cerevisiae* @ $1.5x10^8$c.f.u./g	6	10 g/day/calf.
Treatment-3 (T3)	Basal diet + Prebiotic mannanoligosaccharides	6	10 g/day/calf
Treatment-4 (T4)	Basal diet + Synbiotic *Lactobacillus sporogenes* @ $5x10^7$c.f.u./g, *Saccharomyces cerevisiae* @ $1.5x10^8$c.f.u./g + mannanoligosaccharides	6	10 g Probiotic + 10 g Prebiotic/ day/calf

Results and Discussion

The mean values of haematological parameters, *viz.*, Hb, PCV, RBC, WBC and platelet counts on days 0, 90th day, 180th and overall of calves supplemented with probiotic, prebiotic and synbiotic for 180 days are presented in Table 2. The statistical analyses revealed that none of these parameters differed significantly between days within treatment or between groups overall or at any of the treatment period. However, numerically higher overall values of Hb concentrations were observed in T2 as compared to T1, T4 and T3 group; PCV in T3 than other groups; RBCs in T2 followed by T3, T1 and T4; WBCs in T2 followed by T4, T3 and T1; and platelets counts in T1 followed by T3 and others.

The results of present study on Hb concentration were in agreement with Adams *et al*. (2008) that inclusion of direct fed microbial had no effect on Hb in treatment groups. Riddell *et al.* (2010) reported non-significant difference in PCV (%) among probiotic and control groups. Sharma *et al.* (2023) observed that haemoglobin content of buffalo calves was not affected ($p>0.05$) by the supplementation of synbiotics formulation. The present findings were in accordance with Shehta *et al.* (2019), who did not find any effect of probiotic supplementation on PCV, RBC and WBC counts in buffalo calves. Sri Lekha *et al.* (2021) also did not find significant effect of probiotic, prebiotic and synbiotic supplementation on Hb, PCV or RBC counts. Roodposhti and Dabiri (2012) reported that dietary supplementation of prebiotic and probiotic had no significant effect on WBC count of Holstein female calves.

The current results are however in contrast to Dimova *et al.* (2013), who recorded significantly ($p<0.05$) increase in Hb concentration with addition of probiotic in the diet of Bulgarian female calves. Dar *et al.* (2017) also reported significantly ($p<0.05$) increased PCV (%) in crossbred calves supplemented with probiotic, prebiotic and synbiotic as compared to control group. Yashmin *et al.* (2021) reported significantly ($p<0.05$) increased Hb, PCV, RBC and WBC counts in probiotic supplemented group of growing male calves than control group.

The mean values of haematological indices, blood glucose and serum growth hormone concentrations on days 0, 90th day, 180th and overall of calves supplemented with

Table 2: Mean haematological values of weaned Gir calves over the experimental period (Mean ± SE)

Parameter	Days	0	90	180	Overall
Haemoglobin (g/dL)	T1	8.92 ± 0.20	8.03 ± 0.24	8.93 ± 0.65	8.63 ± 0.26
	T2	8.98 ± 0.62	8.35 ± 0.56	8.75 ± 0.49	8.69 ± 0.52
	T3	9.03 ± 0.26	8.03 ± 0.35	8.30 ± 0.32	8.46 ± 0.27
	T4	8.78 ± 0.60	8.65 ± 0.60	8.37 ± 0.41	8.60 ± 0.48
	P value	0.983	0.749	0.756	0.979
PCV (%)	T1	38.20 ± 0.14	38.20 ± 0.14	38.48 ± 0.06	38.28 ± 0.06
	T2	38.60 ± 0.32	38.00 ± 0.27	38.23 ± 0.17	38.26 ± 0.20
	T3	38.30± 0.37	38.58 ± 0.30	38.63 ± 0.19	38.51 ± 0.19
	T4	38.60 ± 0.16	37.97 ± 0.23	38.17 ± 0.30	38.25 ± 0.16
	P value	0.598	0.300	0.346	0.681
RBC ($\times10^6/\mu L$)	T1	9.21 ± 0.35	9.26 ± 0.24	9.50 ± 0.19	9.32 ± 0.23
	T2	9.53 ± 0.68	9.62 ± 0.42	9.89 ± 0.48	9.68 ± 0.52
	T3	9.45 ± 0.39	9.67 ± 0.40	9.67 ± 0.40	9.60 ± 0.38
	T4	9.05 ± 0.65	9.24 ± 0.62	9.23 ± 0.62	9.16 ± 0.65
	P value	0.813	0.483	0.473	0.927
WBC ($\times10^3/\mu L$)	T1	8.00 ± 0.32	7.45 ± 0.93	8.23 ± 0.87	7.89 ± 0.62
	T2	9.56 ± 1.14	7.90 ± 0.40	9.47 ± 0.41	8.98 ± 0.60
	T3	8.55 ± 0.82	7.10 ± 0.81	9.17 ± 1.04	8.27 ± 0.74
	T4	9.08 ± 0.86	7.93 ± 0.44	9.25 ± 0.51	8.76 ± 0.34
	P value	0.599	0.802	0.676	0.584
Platelets ($x\ 10^5/\mu L$)	T1	3.04 ± 0.36	3.07 ± 0.41	3.04 ± 0.47	3.05 ± 0.35
	T2	2.88 ± 0.14	2.97 ± 0.35	2.58 ± 0.07	2.81 ± 0.14
	T3	3.08 ± 0.31	2.9 ± 0.07	2.95 ± 0.20	2.98 ± 0.09
	T4	2.64 ± 0.41	2.85 ± 0.34	2.98 ± 0.33	2.82 ± 0.28
	P value	0.756	0.966	0.714	0.864

T1 control - basal diet consisting of concentrate, green sorghum and Groundnut haulms. T2, T3 and T4 groups - supplemented basal diet with probiotic @ 10 gm/calf/day, prebiotic @ 10 gm/calf/day, and symbiotic @ 20 gm/calf/day, respectively probiotic, prebiotic and synbiotic for 180 days are presented in Table 3. Analysis of variance revealed statistically non-significant differences in mean values of MCV, MCH, MCHC between days within treatment or between groups overall or at any of the treatment period. Numerically higher MCHC values were observed in T2 group than in T3, T1 and T4. Dar *et al.* (2017) reported non-significant effect of probiotic, prebiotic and synbiotic on MCV, MCH in crossbred calves, although MCHC was increased significantly in treated groups, whereas, Yashmin *et al.* (2021) found significantly ($p<0.05$) higher MCV in probiotic supplemented group than control group.

The mean values of blood glucose and serum growth hormone concentrations on days 0, 90th day, 180th and overall of calves supplemented with probiotic, prebiotic and synbiotic for 180 days did not show statistical variations between days within treatment or between groups overall or at any of the treatment period, however, numerically higher values of blood glucose were observed in T4 group than in T2, T1 and T3 groups, yet the values were within the normal physiological range. Similar non-significant effect on blood glucose of crossbred calves supplemented with direct fed microbial (Chaudhary *et al.,* 2008) or probiotic, prebiotic and synbiotic (Dar *et al.,* 2017) has been reported earlier. Wang *et al.* (2021) also reported non-significant effect on blood glucose concentration with compound probiotic in Holstein calves. Hossain *et al.* (2012) however found significantly ($p<0.05$) higher blood glucose concentration in probiotic supplemented Kankrej calves when compared with control group, while Yashmin *et al.* (2021) observed significantly ($p<0.05$) lowered serum glucose in probiotic treated groups than in control.

Analysis of variance for serum growth hormone concentration revealed statistically non-significant difference among the groups at 0 day, 90th day & 180th day of experiment, however, overall mean value of serum GH concentration was significantly ($p<0.05$) higher in T4 than in T1, T2 and T3 groups, which were at par with each other. Wang *et al.* (2021) reported non-significant ($p>0.05$) effect on serum IGF-I concentration in Holstein calves when supplemented with probiotic.

Table 3: Mean haematological indices, blood glucose and growth hormone levels in weaned Gir calves during different days of experimental period (Mean ± SE)

Parameter	Days	0	90	180	Overall
MCV (fl)	T1	57.14 ± 0.51	53.13 ± 4.73	58.60 ± 0.31	56.30 ± 1.46
	T2	57.64 ± 0.76	58.10 ± 0.34	57.50 ± 0.61	57.75 ± 0.24
	T3	57.38 ± 0.56	58.25± 0.28	58.10 ± 0.18	57.91 ± 0.12
	T4	57.62 ± 0.32	58.05 ± 0.50	57.80 ± 0.25	57.83± 0.25
	P value	0.916	0.372	0.231	0.395
MCH (pg)	T1	9.65 ± 0.28	18.40 ± 0.59	20.00 ± 0.48	16.00 ± 0.33
	T2	9.42 ± 0.25	18.10 ± 0.29	20.70 ± 0.31	16.10 ± 0.18
	T3	10.10 ± 0.35	18.50 ± 0.26	20.70 ± 0.39	16.40 ± 0.25
	T4	8.68 ± 1.14	17.90 ± 0.37	20.70 ± 0.62	15.80 ± 0.47
	P value	0.479	0.692	0.604	0.579
MCHC (g/dL)	T1	28.54 ± 0.24	28.66 ± 0.36	27.93 ± 0.75	28.39 ± 0.29
	T2	28.20 ± 0.32	28.55 ± 0.22	28.58 ± 0.32	28.45 ± 0.12
	T3	28.23 ± 0.51	28.51 ± 0.51	28.51 ± 0.31	28.41± 0.29
	T4	28.34 ± 0.30	28.42 ± 0.24	27.98 ± 0.31	28.25 ± 0.20
	P value	0.911	0.971	0.724	0.938
Blood glucose (mg/dL)	T1	74.33 ± 3.30	74.83 ± 3.35	77.00 ± 2.84	75.39 ± 3.04
	T2	76.83 ± 3.19	77.50 ± 3.34	76.33 ± 2.86	76.89 ± 2.93
	T3	70.00 ± 4.34	72.00 ± 4.68	72.00 ± 3.95	71.33 ± 4.22
	T4	76.33 ± 3.28	79.00 ± 2.67	77.00 ± 2.07	77.44 ± 2.55
	P value	0.528	0.542	0.596	0.550
GH (ng/mL)	T1	5.16 ± 0.37	6.51 ± 1.02	5.93 ± 0.37	5.86^{b} ± 0.34
	T2	5.07 ± 0.24	5.89 ± 0.59	5.67 ± 0.28	5.55^{b} ± 0.08
	T3	5.16 ± 0.23	5.40 ± 0.33	5.24 ± 0.22	5.27^{b} ± 0.10
	T4	6.94 ± 0.99	6.07 ± 0.54	5.68 ± 0.16	6.23^{a} ± 0.26
	P value	0.070	0.700	0.370	0.040

Means with different superscript (a, b) between treatment differ significantly ($p<0.05$)

Conclusions

The findings of the present study revealed that the diet supplemented with probiotic, prebiotic and synbiotic have no significant effect on haematological parameters in weaned Gir calves, and values varied within normal physiological range. However, the concentration of serum growth hormone showed significantly ($p<0.05$) increased values. It is possible that the duration of the study or the specific dosages of probiotic, prebiotic and synbiotic supplementation might not have been sufficient to elicit significant changes in haematological parameters and blood glucose in weaned calves. Additionally, individual variability in response to supplementation and the specific mechanisms through which interact with haematological processes may also play a role that needs to be understood.

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