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Effect of supplementing probiotic, prebiotic and synbiotic on blood antioxidant activity and mineral profile of weaned gir calves

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Abstract
Use of probiotic, prebiotics and combination of it may be a viable option to increase proliferation of commensal bacteria in gastrointestinal tract, modulate feeding behavior, and increase immune function to optimize calf health. Twenty-four weaned Gir calves were distributed into four homogenous groups on the basis of their live body weight and sex. The control (T1) group was offered a basal diet consisting of concentrate, green sorghum and dry fodder 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, synbiotic @ 20 gm/calf/day, respectively, for a period of 180 days. Treatment effect was non-significant (P>0.05), on value of serum total antioxidant activity of weaned Gir calves. Analysis of variance did not reveal statistically significant (P>0.05) difference in mean value of serum catalase activity among the groups at 0 days, 90th days as well as 180th day of experiment.

Keywords: Gir calves, Feed conversion ratio, Prebiotic, Probiotic, Synbiotic

Introduction
Man and cow have an essential relationship in India. An important part of Indian culture and economy is the cow. The significance of cows for us is demonstrated by expressions like "Laxmi of home" and "Cow is foster mother of human race." The raising of indigenous cattle uplifts the traditional livelihood by providing employment, supplementary income, wealth, religion, enjoyment and salvation are accomplished with the service of the cow.
According to the National Bureau of Animal Genetic Resources, Gir cow whose population was 23,00,090 is the top indigenous cow in the country (Anonymous, 2022) [2]. The Gir breed of dairy cattle is highly regarded by milk purpose breed due to its exceptional ability to produce milk, good fertility, heat tolerance, ease of maintenance, resistance to diseases and long lifespan. Gir, a pure dairy breed of zebu cattle, gets its name from the Gir forest and hills in the southern part of Gujarat's Saurashtra region, namely from the districts of Junagadh, Gir-Somnath, Amreli, Bhavnagar, Porbandar, Rajkot and Jamnagar.
Dairy calves are widely recognized as the future of the herd. Thus, management approaches have an impact on calf performance. Inadequate nutrition prior to weaning can lead to low weaning weight and weakened immunity, which exacerbates morbidity-related losses. Since long time among the various feed additives, antibiotic is the most frequently and extensively used in livestock diets due to its therapeutic importance (Cho *et al.*, 2005) [3]. Antibiotics help in checking diarrhoea and enhance body weight gain by modifying gut microflora in growing calves (Novak and Katz, 2006) [5]. But the growing concern of the consumers for clean and safe products have restricted the use of antibiotic as feed additive as growth promoters. The worldwide criticism over the use of antibiotics as growth promoters due to their antibiotic resistance, probiotic, prebiotic and synbiotic came up as an alternative to antibiotics.

Materials and Methods

The present experiment was conducted at Cattle Breeding Farm, Kamdhenu University, Junagadh, Gujarat (India), after obtaining permission from institutional animal ethics committee (KU-JVC-IAEC-LA-105-23). A total of twenty four weaned Gir calves of similar body weight and age (4-6 months) were selected and randomly blocked in four treatment groups with six animals each *viz.*, Control (T1), Probiotic group (T2), Prebiotic group (T3) and Synbiotic group (T4). The selected weaned Gir calves were assured for the health and disease.

Probiotic, prebiotic and synbiotic were purchased from Gujarat Enzyme, Ahmedabad, Gujarat, India. Duration of experiment was 180 days. Information on treatment details during the study period are provided in Table 1.

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

Treatment Groups	Treatment Schedule	N	Dose
Control (T1)	Basal diet	6	No supplements
Probiotic group (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.
Prebiotic group (T3)	Basal diet + Prebiotic mannanoligosaccharides	6	10 g/day/calf
Synbiotic group(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

Catalase activity from serum sample was estimated by kinetic method. Buffer solution and substrate solution were incubated at 37°C for 10 minutes. Chromogenic application solution was prepared by dissolved by 1 vial of chromogenic reagent into 24 ml of double distilled water. 1 mmol/ml H_2O_2standard solution was prepared by dilute 1 mmol/ml H_2O_2standard solution with double distilled water diluents. After prepared reagent, two EP tube was taken and added 20 µl buffer solution and 20 µl substrate solution into each tubes, mixed well and added 200 µl chromogenic application solution. 20 µl clarificant and 20 µl sample was added in to sample tube while, in control tube only 20 µl clarificant was added. After 10 minute 200 µl of reaction solution was taken in microplate. Decomposed H_2O_2 by catalase could be quickly stopped by ammonium molybdate produced. Residual H_2O_2reacted with ammonium molybdate and generated yellowish complex then OD value was measured at 405 nm by microplate reader. Constructed a standard curve by plotting absolute OD value of standard as X-axis against concentration on Y-axis. Created a standard curve (y = ax + b) with graph software.

Total antioxidant activity from serum sample was estimated by randox method. Two kinds of antioxidant system, one was enzyme antioxidant system including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). The other was non-enzymatic antioxidant systems, including uric acid, vitamin C, vitamin E, glutathione, bilirubin, a-lipoic acid, carotenoid. Antioxidant capacity was cumulative effect of all antioxidants in blood and body fluids that reflected the total antioxidant capacity in the system. Many antioxidants in the body could be reduced Fe^{3+} to Fe^{2+} and Fe could be form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) could be calculated by measuring the absorbance at 520 nm.

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 $p<0.05$.

Results and Discussion

Table 2: Mean Serum total antioxidant activity and catalase activity values in experimental weaned Gir calves

Parameter	Days	0	90	180	Overall
Total antioxidant activity (ng/dl)	T1	0.78±0.02	0.81±0.03	0.72±0.02	0.77±0.04
	T2	0.81±0.02	1.17±0.03	0.77±0.03	0.75±0.01
	T3	0.96±0.14	1.17±0.16	0.82±0.06	0.99±0.14
	T4	0.83±0.03	0.91±0.05	0.75±0.03	0.84±0.05
	P value	0.407	0.430	0.387	0.118
Catalase activity (ng/dl)	T1	0.67±0.10	0.57±0.04	0.47^{c}±0.05	0.59±0.04
	T2	0.61±0.04	0.60±0.03	0.51^{bc}±0.04	0.56±0.03
	T3	0.58±0.04	0.51±0.04	0.73^{a}±0.04	0.68±0.05
	T4	0.62±0.04	0.56±0.02	0.57^{ab}±0.02	0.61±0.03
	P value	0.237	0.315	0.008	0.611

Means with different superscript (a, b, c) between treatments differ significantly ($p<0.01$)

Total Antioxidant Activity (ng/ml)

Mean values of serum total antioxidant activity (ng/ml) in different groups are presented in table 2. Mean serum total antioxidant activity in T1, T2, T3 and T4 groups at start of experiment was 0.78±0.02, 0.81±0.02, 0.96±0.14 and 0.83±0.03, ng/ml, respectively, Overall mean serum total antioxidant activity for T1, T2, T3 and T4 groups recorded was 0.77±0.04, 0.75±0.01, 0.99±0.14 and 0.84±0.05, ng/ml, respectively. Analysis of variance revealed statistically non-significant (P>0.05) difference in mean value of serum total antioxidant activity (ng/dl) among the groups at 0 days as well as and at end of (180^{th}) of experiment. Overall mean value of serum total antioxidant activity (ng/dl) was also statistically non-significant among the treatment groups. Wang *et al.* (2021) [8] reported that there was non- significant (P>0.05) effect on serum total antioxidant capacity (U/ml) when supplemented with compound probiotic in Holstein calves.

Catalase Activity (ng/ml)

Mean serum catalase activity levels (ng/ml) for different groups are given in table 2. Initially, mean serum catalase activity for T1, T2, T3 and T4 groups was 0.67±0.10,

0.61±0.04, 0.58±0.04 and 0.62±0.04, ng/ml, respectively. whereas the mean serum total antioxidant activity at 180 days of dietary treatments in four different groups averaged 0.56±0.02, ng/ml and 0.47±0.05, 0.51±0.04, 0.73±0.04 and 0.57±0.02 respectively. Overall mean serum catalase activity for T1, T2, T3 and T4 groups recorded was 0.59±0.04, 0.56±0.03, 0.68±0.05 and 0.61±0.03, ng/ml, respectively. Analysis of variance revealed statistically non-significant (P>0.05) difference in mean value of serum catalase activity (ng/dl) among the groups at 0 day, however at end of the experiment (180th), serum catalase activity (ng/dl) was significantly (p<0.05 higher in prebiotic supplemented group as compared to other groups. Sharma *et al.* (2023) [6] reported that serum catalase activity (µmol of H_2O_2) of Murrah buffalo calves was significantly (P>0.05) higher in synbiotic supplemented group than control group.

Mineral Profile

Table 3: Mean Serum calcium, phosphorus and magnesium values in weaned Gir calves during different experimental periods

Parameters	Days	0	90	180	Overall
Calcium (mg/dl)	T1	9.87±0.58	10.29±0.47	10.07±0.20	10.08±0.30
	T2	10.87±0.71	10.53±0.70	10.68±0.35	10.69±0.52
	T3	10.71±0.49	10.90±0.69	11.10±0.51	10.90±0.52
	T4	10.24 ±0.35	10.18±0.41	10.00±0.26	10.14±0.25
	P value	0.555	0.831	0.109	0.427
Phosphorus (mg/dl)	T1	5.28±0.23	5.50±0.23	5.77±0.16	5.52±0.08
	T2	5.39±0.22	5.71±0.21	5.36±0.29	5.48±0.15
	T3	5.59±0.27	5.60±0.34	5.45±0.27	5.55±0.15
	T4	5.59±0.23	5.47±0.20	5.68±0.23	5.58±0.13
	P value	0.738	0.906	0.608	0.962
Magnesium (mg/dl)	T1	2.13±0.12	1.85±0.12	2.00±0.09	1.88±0.08
	T2	1.90±0.12	1.98±0.07	1.98±0.17	1.92±0.10
	T3	1.93±0.08	1.88±0.15	2.02±0.11	2.00±0.13
	T4	1.99±0.09	1.91±0.08	2.00±0.06	1.93±0.05
	P value	0.284	0.947	0.870	0.798

Serum Calcium (mg/dl)

Mean values of serum calcium concentration (mg/dl) in different groups are presented in table 3. Mean serum calcium concentration (mg/dl) in T1, T2, T3 and T4 groups at start of experiment was 9.87±0.58, 10.87±0.71, 10.71±0.49 and 10.24±0.35, mg/dl, respectively, whereas the mean serum calcium concentration (mg/dl) at the end of 180th days of dietary treatments in four different groups averaged 10.07±0.20, 10.68±0.35, 11.10±0.51 and 10.00±0.26, mg/dl, respectively. Overall mean serum calcium concentration (mg/dl) for T1, T2, T3 and T4 groups recorded was 10.08±0.30, 10.69±0.52, 10.90±0.52 and 10.14±0.25, mg/dl, respectively. Analysis of variance revealed statistically non-significant (P>0.05) difference in mean value of serum calcium concentration (mg/dl) among the groups at 0 days as well as last day (180th) of experiment. Overall mean value of serum calcium concentration (mg/dl) was also statistically non-significant (P>0.05) among the treatment groups and serum calcium concentration (mg/dl) varied within the normal physiological range of the calves. Al-Saiady. M. Y. (2010) [1] observed that probiotic supplemented group had not statistically (P>0.05) difference on serum calcium concentration (mg/dl) in Holstein calves. Dimova *et al.* (2013) [4] found no significant (P>0.05) difference on serum calcium concentration (mg/dl) between probiotic and control group.

Serum Phosphorus (mg/dl)

Mean values of serum phosphorus concentration (mg/dl) in different groups are presented in table 3. Mean serum phosphorus concentration (mg/dl) in T1, T2, T3 and T4 groups at start of experiment was 5.28±0.23, 5.39±0.22, 5.59±0.27 and 5.59±0.23, mg/dl, respectively, whereas the mean serum phosphorus concentration (mg/dl) at the end of 180 days of dietary treatments in four different groups averaged 5.77±0.16, 5.36±0.29, 5.45±0.27 and 5.68±0.23, mg/dl, respectively. Overall mean serum phosphorus concentration (mg/dl) for T1, T2, T3 and T4 groups recorded was 5.52±0.08, 5.48±0.15, 5.55±0.15 and 5.58±0.13, mg/dl, respectively. Analysis of variance revealed statistically non-significant (P>0.05) difference in mean value of serum phosphorus concentration (mg/dl) among the groups at 0 days as well as last day (180th) of experiment. Overall mean value of serum phosphorus concentration (mg/dl) was also statistically non-significant (P>0.05) among the treatment groups and serum phosphorus concentration (mg/dl) varied within the normal physiological range of the calves. Dimova *et al.* (2013) [4] reported that the supplementation of probiotic had non- significantly (P>0.05) effect on serum phosphorus concentration (mg/dl) in Bulgarian brown and black and white breeds female calves.

Serum Magnesium (mg/dl)

Mean values of serum magnesium concentration (mg/dl) in different groups are presented in table 3. Mean serum magnesium concentration (mg/dl) in T1, T2, T3 and T4 groups at start of experiment was 2.13±0.12, 1.90±0.12, 1.93±0.08 and 1.99±0.09, mg/dl, respectively, whereas the mean serum magnesium concentration (mg/dl) at the end of 180 days of dietary treatments in four different groups averaged 2.00±0.09, 1.98±0.17, 2.02±0.11 and 2.00±0.06, mg/dl, respectively. Overall mean serum magnesium concentration (mg/dl) for T1, T2, T3 and T4 groups recorded was 1.88±0.08, 1.92±0.10, 2.00±0.13 and 1.93±0.05, mg/dl, respectively. Analysis of variance revealed statistically non-significant (P>0.05) difference in mean value of serum magnesium concentration (mg/dl) among the groups at 0 days as well as last day (180th) of experiment. Overall mean value of serum magnesium concentration (mg/dl) was also statistically non-significant (P>0.05) among the treatment groups and serum magnesium concentration (mg/dl) varied within the normal physiological range of the calves. Al-Saiady. M.Y. (2010) [1] reported non-significant (P>0.05) effect on serum magnesium concentration (mg/dl) when supplemented with probiotic in Kankrej calves.

Conclusions

The findings of the present study revealed that supplementation of probiotic and prebiotic either alone or in combination (synbiotic), Supplementation of probiotic and prebiotic either alone or in combination (synbiotic) have no significant effect on hematological parameters of weaned Gir calves. All the hematological parameters were in normal range during experimental period. Probiotic supplemented group of Gir calves showed significantly higher catalase activity on 180th day as compared to other treatment groups.

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Conflict of Interest: None

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