

Effect of dietary probiotics, prebiotics and synbiotics on Jaffarabadi buffalo calves

B.A. Pata^{1*}, M.R. Gadariya², H.H. Savsani³, T. Patbandha⁴, K.C. Gamit², V.V. Gamit², M.D. Odedra⁵, G.P. Sabapara⁶, K.B. Vala⁷

¹Gir Cow Sanctuary, Kamdhenu University, Dharampur, Porbandar-360550, Gujarat, India. ²Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India. ³Bull Mother Farm, Kamdhenu University, Paratpara, Amreli-365601, Gujarat, India. ⁴Polytechnic in Animal Husbandry, ⁵Cattle Breeding Farm, ⁶Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, ⁷Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India

*Corresponding Author's Email: bapata@kamdhenuuni.edu.in

Journal of Livestock Science (ISSN online 2277-6214) 17: 206-210

Received on 26/5/25; Accepted on 4/4/26; Published on 10/4/26

doi. 10.33259/JLivestSci.2026.206-210

Abstract

An experiment was conducted to evaluate the effects of probiotic, prebiotic and synbiotic administration on blood enzyme profile, antioxidant activity and health status in neonatal Jaffarabadi buffalo calves. Twenty-four calves, randomized into four groups of six each—control (C), probiotic (T1), prebiotic (T2) and synbiotic (T3)—were selected at 8 days of age. All calves received restricted suckling plus a basal diet and pelleted concentrate as per ICAR (2013) standards. T1 calves were fed probiotics (*L. sporogenes* and *S. cerevisiae*, 5 g/day), T2 received prebiotics (mannan-oligosaccharides, 5 g/day) and T3 were given a synbiotic mix (2.5 g each of probiotic and prebiotic per day). Blood enzyme parameters and antioxidant activity were measured on days 0, 84 and 175 of the experiment. Calves were monitored daily for signs of illness. Enzyme levels- including Lactate Dehydrogenase, Alkaline Phosphatase, Aspartate Aminotransferase and antioxidant Superoxide Dismutase-were not significantly affected by feed additives. However, blood Catalase activity was significantly higher ($p \leq 0.05$) in the treatment groups (T1, T2, T3: 54.4–57.9 ng/ml) compared to the control (51.46 ng/ml). Supplementation with probiotic (0%), synbiotic (33.33%) and prebiotic (50%) reduced the incidence of colibacillosis, pneumonia and gastroenteritis compared to control (66.67%). Pyrexia, illness rates were lowest in the probiotic (16.67%) and synbiotic (66.67%) groups, while higher in the prebiotic and control groups (83.33%). In conclusion, feed additives enhanced antioxidant activity and probiotic and synbiotic supplements helped reduce common illnesses in calves.

Keywords: Jaffarabadi buffalo calves, Probiotic, Prebiotic, Synbiotic, Antioxidant

Introduction

Livestock is vital to the Indian economy, supporting two-thirds of the rural population and contributing 5.5% to the national GVA (BAHS, 2024). Jaffarabadi buffalo, native to Gujarat's Saurashtra region, is the heaviest Indian breed, known for high milk yield and fat content (Sharma et al., 2016; Jayebhaye et al., 2020). Effective calf management is crucial, as early care reduces mortality (12.5–30%) and enhances future productivity (Shinde et al., 2019). Optimal growth and health in calves, especially during the vulnerable neonatal stage, depend on proper nutrition and gut health management (Ghosh and Mehla, 2012).

Probiotics have been used in cows (Litonia et al., 2022); monogastric animals (Ovcharova et al., 2022) and poultry (Tsogoeva et al., 2021). Feeding probiotics, prebiotics and synbiotics improves calf health by enhancing gut microbial balance, boosting immunity and reducing disease risk (Markowiak and Slizewska, 2017). Synbiotics combine both, showing synergistic benefits in improving growth and gut health (Roodposhti and Dabiri, 2012; Alloui et al., 2013).

With a focus on this fact, an experiment was carried out to evaluate the blood enzyme, antioxidant activity and health status of Jaffarabadi buffalo calves fed diets treated with probiotics, prebiotics, both individually and in combination as synbiotics, in order to assess the impact of these dietary additives on calf health.

Materials and methods

Experimental animals

An investigation was carried out to study the inclusion of probiotic and prebiotic alone and in combination in the diet of Jaffarabadi buffalo calves (N=24) on their blood enzyme profile, antioxidant activity and health status after receiving approval from the IAEC (Protocol No. KU-JVC-IAEC-LA-99-22) at Cattle Breeding Farm, Kamdhenu University, Junagadh. The Cattle Breeding Farm, in Gujarat is located at 70.5° west longitude, 21.4° north latitude of 60 meters above mean sea level. Experimental period was from 8 to 182 days of age of the calves by allocated into 4 groups based on birth weight, parity of dam of calves, previous average milk yield and current average milk yield of dam and sex of the calves (3 male and 3 female in each group). Calves selected for the experiment had birth weights ranging from 28 to 38 kg, with group averages between 34.01 and 34.61 kg. Their dams were mostly in their 2nd to 4th parity and had an average daily milk yield of 6.24 to 7.03 liters in the previous lactation and 5.10 to 5.42 liters during the first 7 days of the current lactation. Restricted suckling was practiced: calves were allowed to suckle for 50–60 seconds (maximum one minute) before and after milking up to 12 weeks of age. After 12 weeks, suckling was limited to 20–30 seconds (maximum half a minute) only for milk let-down.

Treatment details

The calves of all the groups were subjected to restricted suckling milk of their dam + basal diet, the calves of C group did not receive any additive, while calves of T1 group were additionally fed probiotic (*L. sporogenes* 5x10⁷ c.f.u./g, *S. cerevisiae* 1.5x10⁸ c.f.u./g, in 1:1 @ 5 g/d/h; T2 group were additionally fed prebiotic (mannan-oligosaccharides) @ 5 g/d/h and T3 group was additionally fed with synbiotic (probiotic @ 2.5g/d/h + prebiotic @ 2.5g/d/h) orally. Each animal was individually provided with a basal diet consisting of up to 3 kg of seasonal green fodder and dry fodder offered *ad lib*. Pelleted concentrate was offered to meet protein requirements as per ICAR (2013) feeding standards and mineral mixture @10-15 g/h/d (Table 1). The probiotic and prebiotic were prepared at Gujarat enzymes company, Ahmedabad, Gujarat, India.

Table 1. Schedule for probiotic, prebiotic and synbiotic inclusion in feed

Treatment Groups	No. of Animals	Dietary treatment details
Control (C)	6	Restricted suckling milk of their dam + basal diet
Probiotic (T-1)	6	Restricted suckling milk of their dam + basal diet +supplementation of probiotic (<i>Lactobacillus sporogenes</i> 5x10 ⁷ c.f.u./g, <i>Saccharomyces cerevisiae</i> 1.5x10 ⁸ c.f.u./g (in 1:1) @ 5 g/day/calf.
Prebiotic (T-2)	6	Restricted suckling milk of their dam + basal diet +supplementation of prebiotic (mannan-oligosaccharides) @ 5 g/day/calf
Synbiotic (T-3)	6	Restricted suckling milk of their dam + basal diet+ supplementation of synbiotic (<i>Lactobacillus sporogenes</i> 5x10 ⁷ c.f.u./g, <i>Saccharomyces cerevisiae</i> 1.5x10 ⁸ c.f.u./g (in 1:1) @ 2.5g/day/calf + mannan-oligosaccharides @ 2.5g/day/calf)

Estimation of blood enzyme parameters, antioxidant activity and health status

Total of three blood samples were collected from each calf: at the start of the experiment (day 0), mid-experiment (day 84) and at the end of the experiment (day 175). Blood enzyme parameters and antioxidant activity viz., Lactate Dehydrogenase, Alkaline Phosphatase, Aspartate Aminotransferase, antioxidant Superoxide

Dismutase and catalase activity were determined using commercially available standard analytical kits. Each calf was observed daily for illness and occurrence of different calf ailments *viz.*, Colibacillosis, gastroenteritis, pneumonia and other sickness if any, was recorded. Morbidity pattern as no. of calves affected with disease per group and incidence (%) of various health disorders in different experimental group were studied and compared.

Statistical analysis

The data were subjected to analysis of variance (Snedecor and Cochran, 1994). The significance of the differences among the groups were assessed by Duncan's Multiple Range Test (Duncan, 1955). SPSS 16.0 (SPSS for windows, Version 16.0 Chicago, USA, SPSS Inc) package program was used for statistical evaluation. Results were given mean \pm standard error of means. Means were considered significantly different at $p \leq 0.05$ and $p \leq 0.01$, *i.e.*, respectively, at 5 and 1 % levels of significance.

Result and discussion

Enzymes and antioxidant activity of experimental Jaffarabadi buffalo calves

Enzymes and antioxidant activity of Jaffarabadi buffalo calves in control (C), probiotic (T1), prebiotic (T2) and synbiotic (T3) supplemented groups are presented in Table 2

Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Superoxide Dismutase (SOD) levels were measured on days 0, 84 and 175 of the study. Across all treatment groups (T1, T2, T3) and the control, no significant differences ($p > 0.05$) were observed in LDH, ALP, AST and SOD levels throughout the experimental period. However, Catalase activity showed a significant increase ($p \leq 0.05$) in the treatment groups by the end of the experiment. The highest activity was recorded in T3 (57.89 ± 1.18 ng/ml), followed by T1 (56.28 ± 2.08 ng/ml) and T2 (54.44 ± 1.26 ng/ml), while the control group had the lowest value (51.46 ± 1.41 ng/ml). Feeding probiotics, prebiotics and synbiotics to calves enhances catalase activity primarily by reducing oxidative stress, improving gut health and supporting nutrient absorption and immune balance. This reflects a stronger antioxidant defense, contributing to better health and resilience in calves.

The findings of this study align with previous research. While Wang *et al.* (2022) reported a significant ($p \leq 0.05$) reduction in serum LDH levels in probiotic-supplemented Holstein calves, the current study observed no significant changes in LDH across treatment groups. Similar to current results, Hossain *et al.* (2012) and Wang *et al.* (2022) found no significant effect ($p > 0.05$) of probiotic supplementation on serum ALP levels in Kankrej calves and Holstein calves, respectively. Catalase activity was significantly higher in the treatment groups in our study, which is consistent with the findings of Varada *et al.* (2022) and Sharma *et al.* (2023), who reported increased ($p \leq 0.05$) catalase activity in calves supplemented with probiotics and synbiotics.

Assessment of health status based on morbidity of experimental Jaffarabadi buffalo calves

The study aimed to evaluate the effect of probiotic, prebiotic and synbiotic supplementation on the health status of Jaffarabadi buffalo calves by monitoring disease occurrence across treatment groups. Table 3. Shows the Summary information on observed number (%) of Jaffarabadi buffalo calves affected with various disorders.

Daily monitoring of all calves revealed that the incidence of colibacillosis, pneumonia and gastrointestinal (G.I.) disorders was significantly reduced in the supplemented groups. No cases were observed in the probiotic-fed group (0.0%), while the incidence was lower in the synbiotic (33.33%) and prebiotic-fed groups (50%) compared to the control group (66.67%). Including pyrexia in the analysis, overall health disorders were still substantially lower in treated calves: 16.67% in the probiotic group, 50.00% in the synbiotic group and 83.33% in the prebiotic and control group. These findings highlight the beneficial role of probiotic and synbiotic supplementation in improving calf health and reducing the prevalence of major diseases in Jaffarabadi buffalo calves.

Probiotic and synbiotic supplementation support early development of stable gut flora, helping prevent diarrhea, pneumonia and G.I. disorders in calves (Malmuthuge and Guan, 2017). Synbiotics, combining probiotics and prebiotics like FOS or inulin, enhance probiotic survival and colonization, leading to better pathogen control and gut health (Markowiak and Śliżewska, 2018). While prebiotics alone can help, their effect depends on existing gut microbes, which are still developing in young calves. The results were supported the findings of Dar *et al.* (2018) who evaluated the effect of synbiotic on health of crossbred calves. In the experiment synbiotic group was fed a basal diet with *Lactobacillus acidophilus* @ 0.5 g (2×10^{10} C.F.U./g) + 2 g MOS per calf/day and a significant reduction was found in faecal shedding of coliform and *E. coli* in synbiotic group indicating reduced severity of scours in calves. Similarly, the findings were also supported by Sharma *et al.* (2023) observed effect of synbiotic on buffalo calves and they observed that Incidence of diarrhoea and faecal scores were reduced in treated groups.

Tables 2: Blood enzymes and antioxidant activity of Jaffarabadi buffalo calves supplemented with probiotic, prebiotic and synbiotic

Days of Experiment	Control (C)	Probiotic (T1)	Prebiotic (T2)	Synbiotic (T3)	'p' value
Lactate Dehydrogenase (LDH, IU/L)					
0 day	424.83±4.12	422.00±4.52	419.50±6.29	416.17±3.43	0.61
84 th day	414.67±14.6	413.30±4.20	410.50±7.11	410.33±2.18	0.94
175 th day	412.0±6.41	403.50±3.74	402.83±6.46	398.28±4.42	0.36
Overall	417.16±4.06	413.22±2.65	409.23±6.50	408.26±2.91	0.46
Alkaline phosphatase (ALP, IU/L)					
0 day	137.97±4.83	140.93±3.37	140.27±4.16	137.87±1.10	0.09
84 th day	143.43±3.33	137.27±2.55	135.30±1.94	133.63±3.28	0.32
175 th day	140.55±3.25	135.57±2.73	132.57±1.78	131.08±2.61	0.81
Overall	140.65±3.21	137.92±2.07	136.04±1.94	134.19±1.37	0.24
Aspartate aminotransferase (AST, IU/L)					
0 day	69.00 ± 1.13	71.75± 1.04	70.50 ±0.76	71.33±1.08	0.35
84 th day	66.50 ±1.14	69.38± 1.35	68.60± 1.47	68.83±1.19	0.43
175 th day	64.50 ± 1.40	67.43± 1.20	67.67 ±1.56	67.71±1.14	0.28
Overall	66.67± 1.20	69.52± 1.20	68.94 ±1.20	69.27±1.04	0.31
Superoxide dismutase (SOD, IU/L)					
0 day	21.70 ±0.22	21.50±0.18	22.60±1.21	22.00±0.80	0.73
84 th day	21.00 ±0.38	20.40±0.27	20.30±0.31	20.00±0.30	0.19
175 th day	20.98 ±0.45	20.00±0.42	19.60±0.38	19.50±0.40	0.07
Overall	21.23 ±0.28	20.60±0.28	20.80±0.51	20.50±0.30	0.49
Catalase activity (ng/mL)					
0 day	50.25±1.37	51.50±1.57	51.17±0.84	51.25±0.48	0.88
84 th day	51.83±2.39	56.17±2.28	54.17±1.27	57.50±1.32	0.19
175 th day	52.28±2.01 ^a	58.33±2.22 ^{ab}	56.00±1.41 ^{ab}	60.54±0.83 ^b	0.02
Overall	51.46±1.41 ^a	56.28±2.08 ^{ab}	54.44±1.26 ^{ab}	57.89±1.18 ^b	0.04

Means with different superscripts (a, b) differ significantly (p<0.05)

Table 3. Summary information on observed number (%) of Jaffarabadi buffalo calves affected with various disorders

Health disorder	Control	T1(Probiotic)	T2 (Prebiotic)	T3 (Synbiotic)
Colibacillosis	01	00	01	00
Gastroenteritis	03	00	02	02
Pneumonia	00	00	00	00
Total (i)	04 (66.67%)	00 (0.00%)	03 (50.00%)	02 (33.33%)
Pyrexia /Fever	01	01	02	02
Total (ii)	05 (83.33%)	01(16.67%)	05 (83.33%)	04 (66.67%)
Other sickness	02 (33.33%)	02 (33.33%)	01(16.67%)	03 (50.00%)

Conclusions

The study demonstrates that supplementation with probiotics and synbiotics in neonatal calves positively influences health status by reducing the incidence of common ailments such as colibacillosis, pneumonia, gastroenteritis and pyrexia. While most blood enzyme parameters remained unaffected, a significant increase in blood Catalase antioxidant activity in treated groups indicates enhanced oxidative defense. Overall, probiotic and synbiotic supplementation proved effective in improving calf immunity and reducing disease occurrence, supporting their beneficial role in early calf nutrition and health management.

Acknowledgement

Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, and the Cattle Breeding Farm, Kamdhenu University, Junagadh, are gratefully acknowledged for providing the necessary facilities that enabled the timely and successful completion of this work.

Conflict of interest

The authors declare no conflict of interest.

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