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Influence of Probiotic, Prebiotic and Synbiotic Additives on Feed intake and Conversion ratio in Jaffarabadi Buffalo Calves during Early and Late Post-natal Phases

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ABSTRACT

A study was conducted to evaluate the impact of probiotic, prebiotic and synbiotic supplementation on the feed intake and feed conversion ratio of newborn Jaffarabadi buffalo calves (early post-natal stage: 2nd to 13th week and late stage: 14th to 26th week of age). Twenty-four 8-day-old calves were chosen and divided into four groups of six at random: probiotic (T1), prebiotic (T2), synbiotic (T3) and control (C). 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). Average dry matter intake (DMI) as % of body weight varied slightly among groups, with pooled values of 3.20%, 3.28%, 3.13% and 3.36% for C, T1, T2 and T3, respectively. DMI increased by 2.40% (T1) and 5.08% (T3) and decreased by 2.21% (T2) compared to control, though differences were not significant (p>0.05). Overall feed conversion ratio (FCR) remained unaffected statistically but showed numerically lower values in supplemented groups, particularly in T2 (5.78) compared to control (6.34). Results suggest potential benefits of supplementation on feed efficiency without significant changes in FCR. Overall fecal coliform count was also reduced in the feces of supplemented buffalo calves than control.

KEYWORDS: FCR. Fecal coliform count, Feed intake, Jaffarabadi calves, Probiotic, Prebiotic, Synbiotic.

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INTRODUCTION

The Jaffarabadi buffalo, indigenous to the Saurashtra region of Gujarat, stands out as the heaviest and one of the most productive Indian breeds. It is renowned for its high milk yield and rich fat content, making it a valuable genetic resource for dairy improvement programs (Jayebhaye et al., 2020). However, the long-term productivity and profitability of such breeds are closely linked to the health and management of their calves. Effective calf management is essential to ensure the survival, growth and future productivity of buffalo herds. Neonatal calf mortality in India ranges from 12.5% to 30%, largely due to inadequate care and nutrition during the early stages of life (Singh et al., 2009). Therefore, implementing sound management practices from birth is critical for reducing mortality and enhancing the lifetime performance of dairy animals. During the neonatal phase, calves are highly susceptible to infections and growth setbacks. Optimal nutrition, particularly the establishment of a healthy gut microbiota, plays a crucial role in improving immunity, digestion and overall development. Scientific evidence highlights that appropriate dietary interventions, such as the use of probiotics, prebiotics and synbiotics, can significantly influence gut health and promote better growth outcomes in young calves (Lucas et al., 2007). Hence, early nutritional strategies that support gastrointestinal health are integral to sustainable and efficient buffalo farming.

The supplementation of probiotics, prebiotics and synbiotics in calf diets has been shown to significantly improve health by promoting a balanced gut microbiota, enhancing immune response and lowering the risk of gastrointestinal infections (Heyman and Menard, 2002; Markowiak and Œli¿ewska, 2017). Probiotics such as *Saccharomyces cerevisiae* and *Lactobacillus* species aid in digestion and inhibit pathogenic microbes (Dawson et al., 1990; Nocek and Kautz, 2006), while prebiotics like fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) support the proliferation of beneficial bacteria and suppress harmful strains like *E. coli* (Cangiano et al., 2020). Synbiotics, which combine probiotics and prebiotics, offer synergistic effects, further enhancing gut health and growth performance (Alloui et al., 2013). Effective early-life nutrition during this period directly influences growth rate, immune development and long-term productivity. Research in exotic and crossbred calves has demonstrated that dietary supplementation with probiotics, prebiotics and synbiotics significantly improves feed conversion efficiency and overall performance (Ratre et al., 2019).

Keeping in view the importance of topic, a study was conducted to assess the feed intake and feed conversion ratio of Jaffarabadi buffalo calves receiving diets enriched with probiotics, prebiotics and their combination as synbiotics.

MATERIALS AND METHODS

A study was undertaken to evaluate the impact of dietary supplementation with probiotic, prebiotic and their combination (synbiotic) on the feed intake and feed conversion ratio of Jaffarabadi buffalo calves. The trial involved 24 calves (Average body weight and age in days), divided into four equal groups (n=6 per group) and was conducted at the Cattle Breeding Farm, Kamdhenu University, Junagadh, following approval from the Institutional Animal Ethics Committee (Protocol No. KU-JVC-IAEC-LA-99-22). The experimental period spanned from 8 to 182 days of age. Calves were allocated to groups based on birth weight, dam parity, previous and current average milk yield of the dam and calf sex, ensuring equal distribution (3 males and 3 females per group).

Pelleted concentrate was offered to meet protein requirements as per ICAR (2013) feeding standards and mineral mixture @10-15 g/h/ (Table 1). Representative of feed and fodders samples were tested for proximate principles (AOAC, 2023). Dry matter intake (kg/day, % body weight, g/kg Wp ·w u) and feed conversion ratio (kg DMI/kg body weight gain) data of experimental Jaffarabadi buffalo calves were recorded and analyzed across two post-natal phases: early (2nd –13th week) and late (14th –26th week) stages.

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 (*Lactobacillus sporogenes* 5x10^7 c.f.u./g, *Saccharomyces cerevisiae* 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 (*Lactobacillus sporogenes* 5x10^7 c.f.u./g, *Saccharomyces cerevisiae* 1.5x10^8 c.f.u./g (in 1:1)@ 2.5g/day/calf + mannan-oligosaccharides @ 2.5g/day/calf)

c.f.u: Colony Forming Units

Statistical analysis

Statistical analysis was performed using ANOVA, following the method of Snedecor and Cochran (1994). Group differences were assessed using Duncan's Multiple Range Test (Duncan, 1955) with SPSS software version 16.0 (SPSS Inc., Chicago, USA). Results are expressed as mean ± standard error, with statistical significance considered at $p \leq 0.05$ and $p \leq 0.01$ levels.

RESULTS AND DISCUSSION

Proximate Composition of Feed Stuff

Among all the feedstuffs, the compound concentrate mixture exhibited the highest crude protein (CP) content, making it the most protein-rich component of the diet. This was followed by groundnut haulms, while green fodders such as green sorghum and Napier grass had relatively lower protein levels. Crude fiber exhibited an inverse pattern, being lowest in the compound concentrate mixture. Additionally, the compound concentrate mixture contained lower levels of ether extract and total ash (Table 2).

Table 2. Proximate composition of seasonal green fodder, dry fodder and compound concentrate mixture (% DM basis)

Nutrients	Green Sorghum	Napier grass	Groundnut Haulms	Compound concentrate mixure
DM	30.02	25.30	91.20	90.00
OM	90.45	91.78	89.70	92.29
CP	06.80	05.58	10.67	20.42
CF	32.02	28.15	32.30	10.14
EE	02.56	02.27	03.25	02.85
NFE	49.07	55.78	43.48	58.88
Total Ash	09.55	08.22	10.30	07.71

Dry Matter Intake (Kg/d)

Dry matter intake (DMI) was measured to assess the nutritional intake of Jaffarabadi buffalo calves. As experimental groups were similar in key traits, milk suckling was not considered in feed intake and efficiency calculations. DMI was monitored weekly during two post-natal phases: early (2nd – 13th week) and late (14th –26th week).

At the initial phase (week 2), DMI was 0.71±0.023, 0.77±0.024, 0.83±0.076 and 0.73±0.031 kg/day in control, T1, T2 and T3 groups, respectively, which increased to 2.14±0.09, 2.17±0.05, 2.13±0.14 and 2.23±0.077 kg/day by week 13. Treatment groups showed higher DMI than control, with T1 showing the highest increase (0.08 kg/day, 5.37%), followed by T3 (0.06 kg/day, 4.28%) and T2 (0.05 kg/day, 3.57%). However, differences were statistically non-significant ($p>0.05$). Overall average DMI during this phase was 1.40±0.06, 1.48±0.04, 1.45±0.08 and 1.46±0.04 kg/day in control, T1, T2 and T3, respectively (Figure 1). The overall mean DMI for the late phase were 3.18±0.09, 3.32±0.05, 3.09±0.20 and 3.50±0.14 kg/day in control, T1, T2 and T3 groups, respectively, difference being insignificant. The group T3 exhibited the highest overall increase in DMI (10.06%), followed by T1 (4.40%) and T2 (-2.83%) when compared with the control (Figure 1).

The pooled mean DMI for both the phases combined were 2.32±0.07, 2.43±0.04, 2.30±0.14 and 2.52±0.08 for control, T1, T2 and T3 groups, respectively. T3 group of calves showed the highest increase (0.20 kg/day, 8.62%) over the control, while T2 showed a slight decrease (-0.02 kg/day, -0.86%). the overall trend indicated a non-significantly ($p>0.05$) increase in intake for the treatment groups.

From weeks 14th to 26th of age, buffalo calves fed with prebiotics showed a reduction in dry matter intake (DMI) compared to those fed with control, probiotic and synbiotic. While all groups generally showed increasing feed intake with age, the prebiotic group had consistently lower DMI values, indicating that prebiotics may slightly suppress appetite or enhance feed efficiency, leading to reduced intake. In contrast, probiotic and synbiotic groups maintained higher DMI, suggesting better palatability or digestive stimulation.

The findings of the current study are consistent with those of Riddell et al. (2010), who found that the inclusion of probiotics in the diet did not significantly affect feed intake (p>0.05). Similarly, Uzmay et al. (2011) reported no significant (p>0.001) differences in feed intake across different age periods, although calves receiving MOS feeding consumed 19.9% more calf starter as compared to those on the control diet. Dimova et al. (2013) also concluded that there were no significant (p>0.05) differences in daily feed intake between the treatment and control groups. Hossain et al. (2012) conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U./g) and found that DM intake (kg/day) did not differ statistically (p>0.05), but were numerically higher in treatment groups than control. Singh et al. (2014) in their experiment revealed that no any significant difference (p>0.05) found in dry matter intake between groups.

In week 2, the DMI (% of b.wt.) for T1, T2 and T3 were 2.04±0.08, 2.23±0.24 and 1.98±0.09, respectively, slightly higher as compared to 1.93±0.07 for the control. Over 13 weeks, DMI increased to 3.31 in T1, 3.26 in T2 and 3.41 in T3 (p>0.05). Overall DMI observations in percent body weight were 2.72±0.10, 2.83±0.03, 2.80±0.05 and 2.81±0.08 in the control, T1, T2 and T3 groups, respectively. During the early post-natal phase, average DMI (% of body weight) increased from 1.93% in week 2 (control) to 3.41% in week 13 (Figure 2).

Initial observations (14th week) of the late post-natal phases were 3.35±0.33, 3.29±0.14, 3.22±0.24 and 3.48±0.19 % in the control, T1, T2 and T3 group of calves, respectively. Final observations (26th week) were 3.91±0.42, 4.04±0.19, 3.71±0.24 and 4.25±0.21 % in the control, T1 T2 and T3 group of calves, respectively. Similar to the early phase, the treatment groups showed some variations in their DMI, with T1 and T2 generally having slightly lower DMI values in comparison to the control group. Overall DMI observations in percent body weight during 14th to 26th weeks of age were estimated to be 3.64±0.10, 3.69±0.03, 3.43±0.22 and 3.87±0.17 in the control, T1, T2 and T3 groups, respectively (p>0.05) (Figure 2).

Pooled DMI percent of body weight were 3.20±0.09, 3.28± 0.03, 3.13 ± 0.18 and 3.36± 0.11 of the control, T1, T2 and T3 group of calves respectively (p>0.05). The treatment groups had changes of 2.40% (increase) for T1, 2.21% (decrease) for T2 and 5.08% (increase) for T3 in DMI relative to the control.

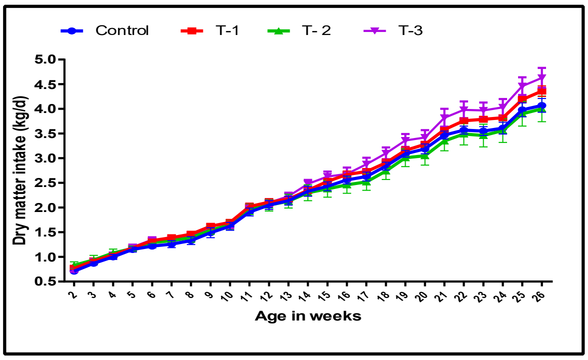

Figure 1. Change in DMI (kg/d) in early and late post-natal phases of experimental Jaffarabadi buffalo calves

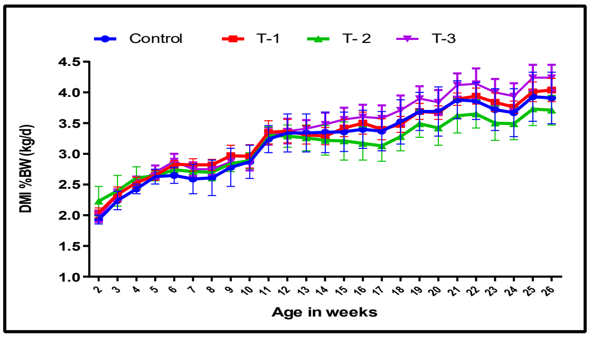

Figure 2. Change in DMI expressed as % of BW in early and late phase of experimental Jaffarabadi buffalo calves

The findings of the current study are consistent with those of Riddell et al. (2010), who found that the inclusion of probiotics in the diet did not significantly affect feed intake (p>0.05). Similarly, Uzmay et al. (2011) reported no significant (p>0.05) differences in feed intake across different age periods, although calves receiving MOS feeding consumed 19.9% more calf starter as compared to those on the control diet. Dimova et al. (2013) also concluded that there were no significant (p>0.05) differences in daily feed intake between the treatment and control groups. Hossain et al. (2012) conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U./g) and found that DM intake (kg/100 kg B.wt.) did not differ statistically (p>0.05), but were numerically higher in treatment groups than control. Singh et al. (2014) in their experiment revealed that no any significant difference (p>0.05) found in dry matter intake between groups.

The average DMI (g/kg $W^{0.75}$) of experimental Jaffarabadi buffalo calves during the first observation period was 125.43±1.30, 130.71±1.12, 143.21±5.10 and 127.11±2.93 in control, T1, T2 and T3 group, respectively. End day of experiment observations were 124.79±4.75, 122.42±3.24, 113.41±8.05 and 127.06±5.54 in the control, T1, T2 and T3, respectively. Overall mean showed no statistically significant (p>0.05) differences between treatments, with the overall mean values being 137.53±2.84 for the control group, 136.30±1.09 for T1, 132.07±5.35 for T2 and 138.38±3.27 for T3 group. There was an increasing trend till 5th/6th weeks of age no definite trend from 7th to 12th week (Figure 3) and, thereafter, from 13th week of age it tended to decline till 26th week of age (Figure 3).

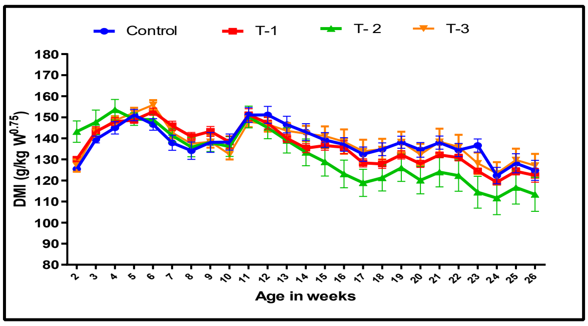

Figure 3. Change in DMI in terms of g/kg $w^{0.75}$ in early and late post-natal phase of Jaffarabadi buffalo calves

These results aligned with the finding of Hossain et al. (2012), who conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U. /g) and found that DM intake (g/kg $W^{0.75}$) did not differ statistically (p>0.05), but numerically higher in treatment groups than control. Kara et al. (2015) observed the effects of mannan-oligosaccharides (MOS) feeding on Holstein cattle calves and revealed that average daily feed intake (ADFI) was statistically similar (p>0.05) in supplemented group but ADFI was numerically higher by 10.97%, in MOS than in control calves.

Feed Conversion Ratio

Initial observations were 2.22±0.05, 2.37±0.04, 2.54±0.20 and 2.17±0.13 in the control, T1, T2 and T3 group of calves, respectively. The weekly values were relatively close to the control group, with slight increases in the treatment groups (Figure 4).

The average feed conversion ratio for the control group was 4.09±0.09 kg, while the treatment groups T1, T2 and T3 had mean of 3.96±0.04, 3.96±0.15 and 3.83±0.08, respectively. These represented differences of 3.17%, 3.17% and 6.35% as compared to the control group (p>0.05).

First observations of experimental Jaffarabadi buffalo calves in late post-natal phase were 6.36±0.13, 6.06±0.06, 5.72±0.21 and 5.99±0.13 in the control, T1, T2 and T3 groups, respectively. Overall means of the late Post-natal phase were 8.60±0.10 in the control and the treatment groups T1, T2 and T3, 8.04±0.03, 7.61±0.22 and 8.19±0.16, respectively (Figure 4). The differences between control and treatment groups for the overall period were -6.51% for T1, -11.51% for T2 and -4.77% for T3 group of Jaffarabadi buffalo calves, indicating favourable effect of prebiotic feeding on FCR of the calves.

The treatment groups did not show statistically significant differences when compared with the control group (p>0.05). Pooled means were 6.34±0.09, 6.00±0.03, 5.78±0.18 and 6.01±0.11 of the control, T1, T2 and T3 group respectively. Treatment groups T1 and T3 had relatively positive-favourable impact by decrease of 5.36% and 5.20%, whereas treatment T2 (prebiotic feeding) exerted a greater favourable results by lowering the FCR by 8.83%, these differences were not statically significant (p>0.05).

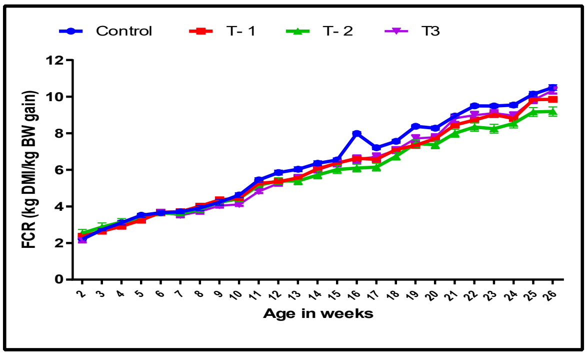

Figure 4. Change in FCR (kg DMI/kg body weight gain) in early and late post-natal of Jaffarabadi buffalo calves

In this study, buffalo calves supplemented with prebiotics, probiotics, or synbiotics showed a reduction in feed conversion ratio (FCR) compared to the control group, indicating enhanced feed efficiency. Prebiotics, in particular, led to the greatest improvement, which may be attributed to better nutrient absorption and more favorable microbial activity. These results differ from the findings of Sri Lekha et al. (2021), who reported a significantly lower FCR in Murrah buffalo calves receiving synbiotic supplementation. Similarly, Liu et al. (2020) found that Holstein calves fed a combination of essential oils and prebiotics had a significantly improved FCR compared to the non-supplemented group, reinforcing the beneficial role of prebiotics in improving feed utilization.

Fecal Sampling and measurements

Fecal samples were collected on days 8th day (Initiation of experiment, 84th day (midpoint of the experiment) and at the end of the experiment (175th day of the experiment). (Samples were collected directly from the rectum using sterile rubber gloves and placed into clean, sterile containers.). All samples were kept at 4°C and processed within a maximum of two hours. For microbial analysis, one gram of each fecal sample was homogenized in 9 ml of 0.1% sterile peptone water. (A series of tenfold dilutions was prepared). Dilutions a 10{ u , 10{ v , and 10{ w were plated using the pour plate method to facilitate colony enumeration. The plates were incubated at 37°C for 48 hours. Colony growth was assessed on Eosin Methylene Blue (EMB) agar (Lab Supply Company, Heliopolis, Cairo, Egypt), with observations made after 24 and 48 hours of incubation. Colonies exhibiting a characteristic green metallic sheen were identified and counted. (Only plates showing 30–300 colonies were considered for enumeration). (The mean colony count from three replicate plates was calculated and expressed as log € colony-forming units (CFU) per gram of feces) to estimate the *E. coli* population.

Table 3. Effect of supplementation on mean coliform count (CFU/g ± SE) in feces of Jaffrabadi buffalo calves

Days	Control	T1 (Probiotic)	T2 (Prebiotic)	T3 (Synbiotic)	P value
8th day	6.88±0.12	6.37±0.07	6.41±0.17	6.60±0.17	0.06
84th day	6.90±0.09a	5.17±0.09b	6.02±0.17c	6.15±0.17c	0.01
175th day	6.93±0.10a	4.62±0.10b	5.52±0.21c	5.68±0.16c	0.01
Overall	6.90±0.04a	5.38±0.17b	5.98±0.08c	6.14±0.08c	0.01

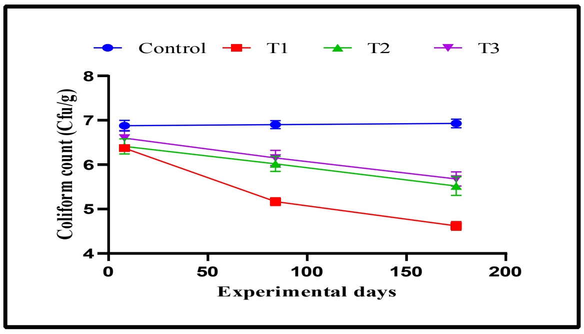

Figure 5. Change in faecal coliform count (Cfu/g) in Jaffarabadi buffalo calves

Coliform counts were measured in four groups, control, probiotic (T1), prebiotic (T2) and synbiotic (T3) across three-time period. On the 8th day (At the beginning), there were no significant differences among groups. By the 84th and 175th days, probiotic supplementation (T1) significantly reduced coliform counts compared to control and other treatments ($P \leq 0.01$). Overall, the probiotic group showed the lowest mean coliform levels, followed by prebiotic and synbiotic groups, all significantly lower than the control ($P \leq 0.01$).

The findings of this study are consistent with earlier reports. Agazzi et al. (2014) observed a higher lactic acid bacteria to *E. coli* ratio in Holstein calves supplemented with probiotics and a corresponding decrease in diarrheal incidence. Similarly, Omran et al. (2020) reported significantly lower faecal *E. coli* counts in probiotic-fed buffalo calves compared to non-supplemented controls. Ayala-Monter et al. (2019) also demonstrated the beneficial impact of inulin and *Lactobacillus casei* on reducing coliform loads and improving gut health in lambs.

All in all, the results clearly show that adding beneficial microbes to the diet can play an important role in shaping the gut bacteria and improving overall gut health in young ruminants. Among the different approaches, probiotics stood out as the most effective and reliable in reducing harmful coliform bacteria in the calves' faeces. This suggests that probiotic supplementation could be a simple and practical way to help protect buffalo calves from gut-related infections and health problems early in life.

CONCLUSION

DMI increased by 2.40% with supplementation of probiotic and 5.08% with synbiotic supplementation, where as slight reduction of 2.21% was observed with prebiotic feeding compared to the control group. In terms of FCR, prebiotic-fed calves showed the most efficient feed utilization (5.78), followed by equal values in probiotic and synbiotic groups (6.0), all of which performed better than the control group (6.34). Total coliform count was decreased in the treated groups than control. These findings suggest potential improvements in nutrient use efficiency and health with additive supplementation to Jaffrabadi calves for long term enhanced productivity and performance.

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