Research Article

Effect of feeding emulsifier and probiotic supplement with sodium butyrate on blood biochemical profile and haircoat condition in pre-ruminant crossbred calves

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Abstract

The present study evaluated the effect of supplementation of emulsifier and probiotic supplement with sodium butyrate in milk replacer on haircoat condition and blood biochemical profile in pre-ruminant crossbred calves. Twelve healthy calves, seven days old, were randomly assigned to two treatment groups (n = 6) for a 90-day feeding trial following step-down feeding regimen. The T1 group received milk replacer containing lysolecithin at 4g/calf/day and Lactobacillus rhamnosus GG (10^{10} CFU) at 1g/calf /day, while the T2 group was provided the same diet with sodium butyrate at 2 g/calf/day. Calf starter and green fodder were offered ad libitum to all animals. Alopecia was assessed weekly using a 0–3 point scoring scale and blood biochemical indices including haemoglobin, serum glucose, total protein, albumin, globulin, A:G ratio, blood urea nitrogen, calcium, and phosphorus were measured at the end of the trial. Results showed that alopecia was evident in both groups between the 2nd and 6th weeks, but scores were significantly (P < 0.05) higher in T1 calves during the 3rd, 4th, and 5th weeks, indicating more severe hair loss. Supplementation of emulsifier and probiotic feed additive with sodium butyrate reduced the severity of alopecia, suggesting its role in improving nutrient absorption and haircoat. All measured blood biochemical parameters remained within the normal physiological range, with no significant differences between groups. These findings indicated that sodium butyrate supplementation in milk replacer in addition to probiotic supplement and emulsifier supported normal metabolic function while improving hair coat condition in pre-ruminant crossbred calves.

Keywords: *Milk replacer, alopecia, lysolecithin, probiotic, sodium butyrate*

Milk replacers are widely employed in calf rearing as cost-effective substitutes for whole milk. However, calves reared solely on milk replacers are often predisposed to dermatological problems such as alopecia, possibly due to altered nutrient utilization or reduced digestibility (Abhijith et al., 2023).

Haircoat quality serves as an important visual indicator of health and nutritional status in calves. Alopecia, though not always associated with pruritus or infectious disease, is a frequently observed dermatological condition in young calves. Nutritional and management-related factors are increasingly recognized as important contributors to this disorder. Wieland et al., (2019) reported a case series of Belgian Blue crossbred calves that developed moderate to severe, non-pruritic alopecia despite adequate vitamin and mineral supplementation and in the absence of infectious or parasitic disease. The underlying cause was traced to malabsorption of dietary fat, associated with errors in milk replacer preparation. This observation highlighted that nutritional imbalances or husbandry practices alone can trigger alopecia, reinforcing the need for optimized feeding strategies in pre-ruminant calves.

Apart from external clinical signs such as haircoat condition, evaluation of blood biochemical parameters provides valuable insights into the nutritional and metabolic status of calves. Parameters including haemoglobin, blood glucose, serum protein, albumin, globulin, A:G ratio, blood urea nitrogen, calcium, and phosphorus reflect energy balance, protein metabolism, and mineral utilization in calves (Kamalahasan, 2018). Any dietary intervention aimed at improving growth and health should ideally maintain these indices within normal physiological ranges. Functional fortification with emulsifiers, probiotics and amino acids has been shown to improve the nutritional adequacy of milk replacers (Reis et al., 2021; Zhang et al., 2025). Still, alopecia and metabolic disturbances remain areas of concern in calves reared on such diets.

Sodium butyrate, a sodium salt of butyric acid (short chain fatty acid), has been extensively studied for its beneficial effects on gut health. It provides energy to intestinal epithelial cells, promotes villi development, and enhances nutrient absorption (Gorka et al., 2009). Improved nutrient assimilation, particularly of proteins and fats, may indirectly contribute to healthier skin and hair coat condition. Additionally, sodium butyrate may help to stabilize blood metabolites by reducing systemic inflammation and supporting better nutrient utilization, thereby reflecting positively in haematobiochemical profiles.

Considering these aspects, the present study was designed to evaluate the effect of sodium butyrate supplementation in milk replacer in addition to probiotic supplement and emulsifier on haematobiochemical parameters and haircoat condition in pre-ruminant crossbred calves.

Materials and methods

Experimental animals and design

The study was conducted for 90 days at the University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Mannuthy, using 12 healthy crossbred calves (7 days old) of either sex. Calves were randomly assigned to two treatment groups (n=6) in a completely randomized design. The average initial body weights were 34.20 ± 1.85 kg for T1 and 34.25 ± 1.44 kg for T2, (P>0.05). All calves were dewormed before the start of the experimental trial and were housed individually in well-ventilated pens under uniform management conditions of the farm.

Feeding regimen

During the first week of life, all calves received colostrum and transition milk. Thereafter, three dietary treatments were followed:

- **T1:** Milk replacer with lysolecithin (4 g/calf/day, (Reis et al., 2021) and *Lactobacillus rhamnosus* GG (10^{10} CFU, Han et al., 2025) at 1g/ calf/day.

- **T2:** Same as T1, with additional sodium butyrate at 2 g/ calf/day (Wu et al., 2024: Zhang et al., 2025)

All Calves received milk replacer at a rate of $1/10^{th}$ of body weight during first six weeks, $1/15^{th}$ body weight during next four weeks and $1/20^{th}$ body weight during the last two weeks. All calves were fed as per ICAR (2013) feeding standards and were also fed calf starter and green fodder *ad libitum*. Clean drinking water was always made available.

Dermatological examination

Alopecia was evaluated at weekly intervals by visual inspection. Data on the severity of hair fall and number of locations having hair fall were recorded. The severity of hair loss was scored using a 0-3 point scale (0 = nil, 1 = mild, 2 = moderate, 3 = severe)

Blood biochemical parameters

Towards the end of the feeding trial, blood samples were collected from all calves. Blood was analysed for haemoglobin (cyanmethaemoglobin method). While serum was analysed for glucose (GOD-PAP methodology), serum total protein (Jong and Vegeter, 1950), serum albumin (Bromocresol green method), serum calcium (AOAC, 2016), serum phosphorous (Bernhart and Wreath, 1955) and blood urea nitrogen (modified Berthelot method) using standard kits.

Statistical analysis

Data were subjected to independent t-test as per Snedecor and Cochran (1994). Dermatological scores were analysed using the Kruskal–Wallis non-parametric test.

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Results and discussion

Chemical composition

The per cent chemical composition of milk replacer, calf starter and green fodder provided to all experimental calves are presented in Table 1. It was found that the chemical composition of milk replacer was in accordance with the nutrient requirements for dairy calves outlined by NRC (2001). The nutrient composition of calf starter and green fodder were comparable to the results of Karade (2024) in pre-ruminant crossbred calves.

Alopecia score

The weekly alopecia scores of crossbred calves from both experimental groups are presented in Table 2. No calves displayed itching, scaling or any indications of infectious causes of alopecia. Alopecia was observed in all calves between the second and sixth weeks of the trial. Throughout this period, higher alopecia scores were recorded for calves in the T1 group consistently compared to those in T2. Notably, during the 3rd, 4th, and 5th weeks, the alopecia scores of T1 calves were significantly (P<0.05) higher, with mean values indicative of severe hair loss.

These results align with earlier reports suggesting that alopecia in calves is influenced by nutritional and metabolic factors, particularly when milk replacers are fed. Wieland et al. (2019) documented a series of Belgian Blue crossbred calves in which alopecia was attributed to dietary fat malabsorption and disturbances in lipid metabolism, largely due to errors in milk replacer formulation and feeding practices. In the present study, calves receiving a plant-protein and plant oil-based milk replacer supplemented with probiotic supplement and emulsifier without sodium butyrate supplementation (T1) showed the most severe alopecia. This may be linked to poor fat absorption and the presence of unphysiological fatty acids that could disrupt lipid metabolism and sebaceous gland activity.

Conversely, supplementation of the same milk replacer with sodium butyrate (T2) appeared to mitigate severity of alopecia, as reflected by intermediate scores. This suggested a protective role of sodium butyrate in promoting intestinal and rumen development, stabilizing gut function, enhancing absorptive capacity, and ultimately improving nutrient utilisation in addition to the effect of probiotic feed additive and emulsifier in the diet.

Blood biochemical parameters

The blood biochemical parameters of the experimental crossbred calves, including haemoglobin, serum glucose, serum calcium, serum phosphorus, serum total protein, serum albumin, serum globulin, albumin-to-

Table 2. Weekly alopecia score[1] of experimental crossbred calves

Week	T1	T2	P value
Week 1	0(0)	0(0)	1.000[ns]
Week 2	1(1)	0(1)	0.213 [ns]
Week 3	3(2.75) [b]	1.5(2.25) [a]	0.029 [*]
Week 4	3(0.25) [b]	2(2.25) [a]	0.007 [**]
Week 5	3(1.5) [b]	2(3) [a]	0.035 [*]
Week 6	1(2)	0(0.5)	0.448 [ns]
Week 7	0(0)	0(0)	1.000[ns]
Week 8	0(0)	0(0)	1.000[ns]
Week 9	0(0)	0(0)	1.000[ns]
Week 10	0(0)	0(0)	1.000[ns]
Week 11	0(0)	0(0)	1.000[ns]
Week 12	0(0)	0(0)	1.000[ns]

[1]Median values of six replicates with Inter Quartile Range (IQR)

ns-non significant (P>0.05)

[*]Means with different superscripts within a row differ significantly(P<0.05)

[**]Means with different superscripts within a row differ significantly(P<0.01)

Table 1. Chemical composition[1] of milk replacer, calf starter, and green fodder

Nutrients (%)	Milk replacer	Calf starter	Green fodder
Moisture	7.93±0.02	9.55±0.34	81.44±0.04
Crude protein[*]	23.12±0.24	23.48±0.18	10.89±0.20
Crude fibre[*]	1.54±0.01	6.04±0.03	31.13±0.47
Ether extract[*]	18.50±0.11	4.41±0.07	2.14±0.12
Total ash[*]	6.10±0.02	8.28±0.07	11.47±0.06
NFE[*]	50.74±0.29	57.80±0.28	44.37±0.49
NDF[*]	9.92±0.04	24.97±0.08	62.17±0.20
ADF[*]	3.93±0.01	12.91±0.08	38.83±0.18
Calcium[*]	1.37±0.11	1.80±0.08	0.68±0.05
Phosphorus[*]	0.66±0.01	0.82±0.01	0.33±0.02

[1]Mean±SE values of six replicates   [*]Items on DM basis

globulin ratio, and blood urea nitrogen were recorded at the end of the 12th week and are summarised in Table 3. All the values obtained in this study were within the normal physiological range for pre-ruminant crossbred calves as reported by Bouda and Jagos (1983).

The mean haemoglobin concentrations were 13.17 ± 0.94 g/dL in T1 and 13.46 ± 0.84 g/dL in T2, with no significant differences (P>0.05) between the treatments. These findings agree with Suresh (2022), who observed haemoglobin levels of 12.34 ± 0.19 g/dL in calves fed whole milk and 12.20 ± 0.19 g/dL in those fed milk replacer after 16 weeks. Similarly, Abhijith (2022) reported comparable haemoglobin values among calves fed whole milk (12.21 ± 0.13 g/dL), soy-based replacer (12.25 ± 0.15 g/dL), and whey-based replacer (12.26 ± 0.08 g/dL), indicating that the type of liquid feed does not markedly affect haemoglobin synthesis.

The average serum glucose concentrations were 80.63 ± 1.71 mg/dL in T1 and 82.07 ± 3.48 mg/dL in T2, with no statistical difference (P>0.05). These values are consistent with the range reported by Jith (2004), who observed serum glucose levels of 71.35 mg/dL and 71.15 mg/dL in calves fed a 23% crude protein calf starter, confirming stable energy metabolism across different diets.

Serum calcium concentrations were 11.17 ± 0.86 mg/dL in T1 and 11.11 ± 0.72 mg/dL in T2, showing no significant differences (P>0.05). These results agreed with the findings of Suresh (2022), who recorded serum calcium levels of 11.26 ± 0.02 mg/dL in calves fed whole milk and 11.06 ± 0.20 mg/dL in calves fed milk replacer, demonstrating that replacers effectively support calcium homeostasis.

The mean serum phosphorus concentrations were 5.77 ± 0.58 mg/dL in T1 and 5.90 ± 0.38 mg/dL in T2, with no significant differences (P>0.05). These findings agreed with Suresh (2022), who reported phosphorus levels of 6.72 ± 0.00 mg/dL in whole milk-fed calves and 6.71 ± 0.01 mg/dL in replacer-fed calves, suggesting minimal influence of liquid feed on phosphorus status.

The average serum total protein values were 6.48 ± 0.14 g/dL in T1 and 6.48 ± 0.29 g/dL in T2, with no significant variation between treatments (P>0.05). Comparable results were reported by Thamothiran et al. (2020), who observed that substituting whole milk with skim milk had no adverse effect on serum protein metabolism in crossbred calves.

Serum albumin concentrations were 3.53 ± 0.08 g/dL in T1 and 3.41 ± 0.17 g/dL in T2, with no significant differences between groups (P>0.05). Thamothiran et al. (2020) also reported no significant changes in serum albumin levels when whole milk was replaced by skimmed milk in calf diets. Globulin concentrations were 2.96 g/dL in T1 and 3.06 g/dL in T2, with no significant differences between groups (P>0.05). The A:G ratio was 1.21 in T1 and 1.24 in T2, showing non-significant variation (P>0.05). Blood urea nitrogen (BUN) values were 12.87 mg/dL in T1 and 12.69 mg/dL in T2, with no significant differences observed (P>0.05). Thamothiran et al. (2020) likewise reported no significant alterations in these parameters when whole milk was substituted with skimmed milk in calf diets.

Overall, the blood biochemical parameters studied remained within the normal physiological range, and the absence of significant treatment effects indicates that sodium butyrate supplementation in milk replacer in addition to probiotic feed additive and emulsifier supported normal metabolic functioning in crossbred calves.

Conclusion

The present study demonstrated that sodium butyrate supplementation in addition to probiotic feed additive and emulsifier in milk replacer improved haircoat condition by reducing the severity of alopecia in pre-ruminant crossbred calves. Calves fed milk replacer without sodium butyrate exhibited higher alopecia scores, whereas supplementation with sodium butyrate effectively mitigated hair loss, highlighting its beneficial role in maintaining hair growth and haircoat. Notably, the blood biochemical parameters such as haemoglobin, serum glucose, calcium, phosphorus, total protein, albumin,

Table 3. Blood biochemical parameters of experimental calves

Parameters	T1	T2	P value
Haemoglobin (g/dL)	13.17±0.94	13.46±0.84	0.820 [ns]
Glucose (mg/dL)	80.63±1.71	82.07±3.48	0.717 [ns]
Calcium (mg/dL)	11.17±0.86	11.11±0.72	0.957 [ns]
Phosphorous (mg/dL)	5.77±0.58	5.90±0.38	0.854 [ns]
Total protein (g/dL)	6.48±0.14	6.48±0.29	0.990 [ns]
Albumin (g/dL)	3.53±0.08	3.41±0.17	0.552 [ns]
Globulin (g/dL)	2.96±0.13	3.06±0.38	0.790 [ns]
A: G ratio	1.21±0.07	1.24±0.24	0.884 [ns]
BUN (mg/dL)	12.87±0.75	12.69±0.90	0.878 [ns]

globulin, AG ratio and BUN remained within the normal physiological range across treatments, with no significant differences between groups. These findings indicated that supplementation of emulsifier and probiotic with or without sodium butyrate supplementation supported normal metabolic functions without adverse effects on nutrient utilization. Overall, the dietary inclusion of sodium butyrate at 2 g/calf/day in milk replacer emerged as a promising nutritional strategy to enhance dermatological health and sustain metabolic balance in pre-ruminant calves.

Acknowledgement

Conflicts of interest

The authors declare that they have no conflict of interest.

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