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Impact of dietary rumen-protected protein and fat supplementation on milk yield and composition in Murrah buffaloes

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Abstract

The present study was undertaken to assess the effects of dietary supplementation with rumen protected protein and fat on milk production and milk composition in transition Murrah buffaloes. Ten Murrah buffaloes were selected based on their Most Probable Producing Ability (MPPA) and randomly assigned to two dietary treatment groups, each comprising five animals. The experimental feeding was carried out from 30 days before calving until 90 days after calving. The control group (T_0) was offered a conventional concentrate mixture, whereas the treatment group (T_1) received formaldehyde-treated groundnut cake as a source of rumen protected protein along with rumen protected fat supplemented at the rate of 15 g/L of milk produced. Milk yield and milk composition parameters were evaluated at fortnightly intervals throughout the study period. The average daily milk yield was significantly ($P < 0.05$) greater in T_1 (7.62 kg/day) than in T_0 (6.67 kg/day). Likewise, the average 6% fat-corrected milk (FCM) yield was significantly ($P < 0.05$) higher in the supplemented group (8.79 kg/day) compared with the control group (7.32 kg/day). Milk fat percentage also increased significantly ($P < 0.01$) in T_1 (7.31%) relative to T_0 (6.85%). In contrast, no significant differences were observed between the treatment groups with respect to milk protein, lactose, solids-not-fat (SNF), and total solids. The observed improvement in milk yield and milk fat percentage may be associated with the greater post-ruminal availability of amino acids and the enhanced energy supply provided by the combined supplementation of rumen protected protein and fat. These findings suggest that strategic supplementation during the transition period can enhance lactational performance without adversely affecting milk composition. Therefore, the combined inclusion of rumen protected protein and fat may serve as an effective nutritional approach for improving the productivity of Murrah buffaloes during early lactation.

Keywords: Murrah buffaloes, rumen-protected protein, rumen-protected fat, transition period, milk yield, milk composition, fat-corrected milk, lactational performance

1. Introduction

The transition period, extending from approximately three weeks before calving to three weeks after calving, is recognized as one of the most critical phases in the productive life of dairy animals due to the profound physiological, metabolic, and endocrine changes that occur during this stage. Rapid fetal growth during late gestation, coupled with the onset of lactation, substantially increases nutrient requirements, which often exceed voluntary feed intake, resulting in a state of negative energy balance (NRC, 2001) [14]. This nutritional imbalance may adversely affect milk yield, milk composition, health status, reproductive performance, and overall productive efficiency.

Buffaloes play a vital role in the dairy sector of many Asian countries, particularly India, where Murrah buffaloes are highly valued for their superior milk-producing ability, adaptability, and economic importance. Despite their high genetic potential, the productive performance of buffaloes is often limited by inadequate nutritional management during the transition period. Conventional feeding practices frequently fail to satisfy the increased energy and protein requirements associated with early lactation, leading to inefficient nutrient utilization and suboptimal productive performance.

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In ruminants, a considerable proportion of dietary protein is degraded in the rumen, producing ammonia and resulting in the inefficient utilization of dietary nitrogen and associated energy losses. Similarly, supplementation with unprotected fats may impair rumen fermentation by reducing fiber digestion and altering microbial activity. Consequently, nutritional strategies that enhance the post-ruminal availability of nutrients without adversely affecting rumen function have received considerable attention. Among these, rumen protected protein and rumen protected fat have been extensively investigated for their potential to improve nutrient utilization and lactational performance.

Rumen protected protein, commonly produced by formaldehyde treatment of oilseed cakes, minimizes ruminal protein degradation and increases the flow of undegraded dietary protein to the small intestine, thereby improving the availability of absorbable amino acids for productive functions (Chatterjee and Walli, 2003; Walli, 2005) [5, 28]. Likewise, rumen protected fat provides a concentrated source of energy without interfering with normal rumen fermentation, thereby improving energy balance and supporting milk synthesis (Palmquist and Jenkins, 1980; Palmquist, 1991) [15, 16].

Several studies have reported beneficial effects of supplementing rumen protected fat or rumen protected protein individually on milk yield and milk composition in dairy animals (Garg *et al.*, 2003a; Ranjan *et al.*, 2012; Sirohi *et al.*, 2013) [9, 18, 22]. Furthermore, the combined supplementation of rumen protected protein and rumen protected fat has been shown to enhance lactational performance by improving nutrient availability and optimizing the synchronization of energy and protein utilization (Shelke *et al.*, 2012b; Vahora *et al.*, 2013; Katiyar *et al.*, 2019; Mane *et al.*, 2016) [20, 26, 11, 12]. However, information regarding the combined effects of these nutritional interventions in transition Murrah buffaloes under Indian feeding conditions remains limited.

Therefore, the present study was undertaken to evaluate the effects of dietary supplementation with rumen protected protein and rumen protected fat on milk yield and milk composition in Murrah buffaloes during the transition period.

2. Materials and Methods

2.1 Experimental site and duration

The experiment was conducted at the Dairy Farm of the Department of Animal Husbandry and Dairy Science, College of Agriculture, Pune, Maharashtra, India, from August to December 2016. The experimental period extended from 30 days before calving to 90 days after calving, during which all animals were maintained under standard farm management practices.

2.2 Experimental animals and design

Ten pregnant Murrah buffaloes were selected from the institute herd based on their Most Probable Producing Ability (MPPA) of approximately 1800 L milk yield. The animals were randomly allocated into two experimental groups, each comprising five buffaloes, and were maintained under identical housing and management conditions throughout the study. The control group (T₀) was fed a conventional basal ration, whereas the treatment group (T₁) received the basal ration supplemented with rumen protected protein and rumen protected fat.

2.3 Feeding treatments

The composition of the experimental diets offered to the

animals is presented in Table 1. The concentrate mixture was provided twice daily according to the individual milk production of each buffalo throughout the experimental period.

Table 1: Composition of concentrate mixture and supplementation strategy for experimental groups

Ingredient (%)	T ₀ (Control)	T ₁ (Supplemented)
Maize	35	35
Tur chuni	22	22
Wheat bran	20	20
Groundnut cake	20 (untreated)	20 (formaldehyde treated)
Mineral mixture	2	2
Common salt	1	1
Rumen protected fat	-	15 g/L of milk production

2.4 Preparation of rumen protected protein

Groundnut cake was treated with 40% formaldehyde at the rate of 1.0 g formaldehyde per 100 g crude protein following the procedure described by Chatterjee and Walli (2003) [5]. Briefly, the required quantity of formaldehyde was diluted with water, uniformly sprayed onto the groundnut cake, and thoroughly mixed to ensure even distribution. The treated material was subsequently stored in airtight plastic bags for seven days before feeding to facilitate protein protection and stabilization.

2.5 Rumen protected fat supplementation

Commercial rumen protected fat (calcium salts of palm fatty acids) was procured from Berg and Schmidt Ingredients Pvt. Ltd., Pune, India. Buffaloes in the treatment group received rumen protected fat at the rate of 15 g per litre of milk produced throughout the experimental period. The supplement was thoroughly mixed with the concentrate mixture and offered during feeding.

2.6 Animal management

All experimental buffaloes were housed in a well-ventilated dairy shed with individual feeding facilities to prevent cross-feeding among animals. The animal shed was cleaned twice daily to maintain proper hygiene, and fresh drinking water was made available *ad libitum* throughout the experimental period.

2.7 Milk yield and milk composition

Individual milk yield was recorded daily by combining the morning and evening milkings throughout the study. Milk samples were collected at fortnightly intervals and analyzed using a pre-calibrated milk analyzer to determine milk fat, protein, lactose, solids-not-fat (SNF), and total solids (TS).

2.8 Chemical analysis

Representative samples of feed ingredients and concentrate mixtures were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), total ash (TA), and nitrogen-free extract (NFE) following the standard analytical procedures of the Association of Official Analytical Chemists (AOAC, 1995) [2].

2.9 Statistical analysis

The experimental data were subjected to statistical analysis using an independent samples Student's *t*-test as described by Snedecor and Cochran (1995) [24]. Mean values of the two treatment groups (T₀ and T₁) were compared for all measured parameters. The results are expressed as mean $\pm$ standard

error (SE), and differences between treatment means were considered statistically significant at $P < 0.05$. Individual animals were considered the experimental units for all statistical analyses.

3. Results and Discussion

3.1 Milk yield

Milk yield exhibited the typical lactation pattern in both experimental groups, with production increasing during early lactation and gradually declining towards the end of the experimental period. Throughout the study, however, buffaloes supplemented with rumen protected protein and rumen protected fat (T_1) consistently produced higher milk yield than those in the control group (T_0) (Table 2).

The average daily milk yield was significantly ($P < 0.05$) greater in T_1 (7.62 ± 0.15 kg/day) than in T_0 (6.67 ± 0.21 kg/day), representing an increase of approximately 14.2%. The maximum milk yield was recorded during the third fortnight in T_1 (8.08 ± 0.68 kg/day), whereas the minimum yield was observed during the first fortnight in T_0 (6.36 ± 0.07 kg/day).

The improvement in milk yield may be attributed to the enhanced post-ruminal availability of nutrients. Formaldehyde treatment reduced the ruminal degradation of dietary protein and increased the flow of undegraded dietary protein to the small intestine, thereby improving the supply of absorbable amino acids for mammary gland function (Chatterjee and Walli, 2003; Walli, 2005) [5, 28]. In addition, supplementation with rumen protected fat increased the energy density of the diet without adversely affecting rumen microbial fermentation, thereby providing additional energy to support milk synthesis (Palmquist and Jenkins, 1980; Palmquist, 1991) [15, 16].

Table 2: Fortnightly average milk yield (kg/day) in Murrah buffaloes fed diets with or without rumen protected protein and fat

Fortnight	T_0 (Mean $\pm$ SE)	T_1 (Mean $\pm$ SE)	t-value	P-value
1	6.36 ± 0.07^a	6.82 ± 0.01^b	6.47	<0.001
2	7.01 ± 0.29^c	8.01 ± 0.23^d	2.69	0.028
3	6.61 ± 0.38	8.08 ± 0.68	1.83	0.105
4	6.69 ± 0.10	7.54 ± 0.47	1.77	0.114
5	6.80 ± 0.45	7.85 ± 0.17	2.18	0.061
6	6.54 ± 0.35	7.42 ± 0.22	2.14	0.065
Average	6.67 ± 0.21^c	7.62 ± 0.15^d	3.68	0.006

Table 3: Fortnightly average 6% fat-corrected milk (FCM) yield (kg/day) in Murrah buffaloes fed diets with or without rumen protected protein and fat

Fortnight	T_0 (Mean $\pm$ SE)	T_1 (Mean $\pm$ SE)	t-value	P-value
1	6.68 ± 0.06^a	7.34 ± 0.01^b	10.86	< 0.001
2	7.40 ± 0.26^c	8.78 ± 0.53^d	2.34	0.047
3	7.13 ± 0.31^c	8.57 ± 0.37^d	2.97	0.018
4	7.51 ± 0.11	8.77 ± 0.57	2.15	0.064
5	7.77 ± 0.42^c	9.29 ± 0.19^d	3.35	0.010
6	7.42 ± 1.02	9.98 ± 0.67	2.12	0.067
Average	7.32 ± 0.21^a	8.79 ± 0.17^b	5.46	< 0.001

The combined supplementation of rumen protected protein and rumen protected fat likely improved the energy–protein balance during the transition period. Early lactation is characterized by increased nutrient demands accompanied by reduced voluntary feed intake, often leading to a negative energy balance. Enhancing the availability of absorbable amino acids together with additional energy substrates may have promoted more efficient nutrient partitioning towards

milk synthesis, thereby improving overall lactational performance.

The findings of the present study are consistent with those of Bhosale *et al.* (2010) [3], Sirohi *et al.* (2010) [23], Ranjan *et al.* (2012) [18], Ramteke *et al.* (2014) [17], Yadav *et al.* (2015) [29], Sharma *et al.* (2016) [19], and Desai *et al.* (2016) [6], who reported significant improvements in milk yield following bypass fat supplementation. Likewise, Dosky *et al.* (2012) [8], Sherasia *et al.* (2012) [21], Vahora *et al.* (2012) [27], Sirohi *et al.* (2013) [22], and Amrutkar *et al.* (2014) [1] observed enhanced milk production in dairy animals supplemented with rumen protected protein. Similar positive responses to the combined supplementation of rumen protected protein and rumen protected fat have also been documented by Shelke *et al.* (2012b) [20], Dhulipalla *et al.* (2013) [7], Vahora *et al.* (2013) [26], Grewal *et al.* (2014) [10], Mane *et al.* (2016) [12] and Katiyar *et al.* (2019) [11].

3.2 Six percent fat-corrected milk (FCM)

Six percent fat-corrected milk (FCM) yield is considered a more reliable indicator of lactational performance because it accounts for variations in milk fat concentration. In the present study, the average 6% FCM yield was significantly ($P < 0.05$) higher in the supplemented group (T_1 ; 8.79 ± 0.17 kg/day) than in the control group (T_0 ; 7.32 ± 0.21 kg/day), representing an improvement of approximately 20.1%.

The maximum 6% FCM yield was recorded during the sixth fortnight in T_1 (9.98 ± 0.67 kg/day), whereas the minimum value was observed during the first fortnight in T_0 (6.68 ± 0.06 kg/day). The higher FCM yield in the supplemented group can be attributed to the concurrent improvement in both milk yield and milk fat percentage.

The increased energy supply provided by rumen protected fat may have enhanced the availability of long-chain fatty acids for milk fat synthesis, whereas the greater availability of absorbable amino acids from rumen protected protein likely supported mammary gland metabolism and milk production. Furthermore, the synchronized supply of energy and protein is considered an important factor contributing to improved nutrient utilization and enhanced lactational efficiency in dairy animals.

The findings of the present study are in agreement with those of Ranjan *et al.* (2012) [18], Ramteke *et al.* (2014) [17], and Yadav *et al.* (2015) [29], who reported increased FCM yield following bypass fat supplementation. Likewise, Vahora *et al.* (2012) [27], Sirohi *et al.* (2013) [22], and Amrutkar *et al.* (2014) [1] observed similar improvements with rumen protected protein supplementation. Positive effects of the combined supplementation of rumen protected protein and rumen protected fat on FCM yield have also been reported by Shelke *et al.* (2012b) [20], Dhulipalla *et al.* (2013) [7], Vahora *et al.* (2013) [26] and Mane *et al.* (2016) [12].

3.3 Effect of dietary supplementation of rumen protected protein and fat on milk composition

3.3.1 Milk fat

Milk fat percentage was significantly ($P < 0.01$) higher in the supplemented group (T_1 ; 7.31%) than in the control group (T_0 ; 6.85%). The increase in milk fat content may be attributed to the greater intestinal availability of long-chain fatty acids supplied by rumen protected fat, which can be directly utilized for milk fat synthesis. Moreover, the enhanced post-ruminal availability of amino acids resulting from rumen protected protein supplementation may have improved nutrient utilization efficiency and supported

mammary gland metabolism.

Approximately half of the fatty acids present in milk fat are synthesized from acetate and butyrate produced during ruminal fermentation, while a considerable proportion is derived from absorbed dietary fatty acids (Palmquist and Jenkins, 1980) [15]. Consequently, increasing the dietary supply of rumen protected fat can enhance milk fat concentration without adversely affecting normal rumen fermentation.

The results of the present study are consistent with those reported by Barley and Baghel (2009), Thakur and Shelke (2010) [20], Sirohi *et al.* (2010) [23], Sharma *et al.* (2016) [19], and Mane *et al.* (2016) [12], who observed significant improvements in milk fat percentage following bypass fat supplementation. Similar findings have also been reported with rumen protected protein supplementation by Dosky *et al.* (2012) [8], Sherasia *et al.* (2012) [21], and Amrutkar *et al.* (2014) [1].

Table 4: Effect of dietary supplementation of rumen protected protein and fat on milk composition in Murrah buffaloes

Parameter (%)	T ₀	T ₁	t-value	P-value
Milk fat	6.85 ± 0.11	7.31 ± 0.00	4.85	0.001**
Milk protein	3.39 ± 0.12	3.42 ± 0.36	0.84	0.423NS
Lactose	5.22 ± 0.25	5.29 ± 0.23	1.12	0.291NS
SNF	10.36 ± 0.03	10.34 ± 0.13	-0.36	0.728NS
Total solids	17.25 ± 0.09	17.59 ± 0.04	1.94	0.089NS

3.3.2 Milk protein

Milk protein concentration was numerically higher in the supplemented group (T₁; 3.42%) than in the control group (T₀; 3.39%); however, the difference was not statistically significant. The lack of a significant treatment effect suggests that the additional supply of absorbable amino acids may have been preferentially utilized to support the increased milk yield rather than increasing the protein concentration of milk.

The present findings are in agreement with those of Sirohi *et al.* (2013) [22], Amrutkar *et al.* (2014) [1], Grewal *et al.* (2014) [10], and Nam *et al.* (2014), who also reported no significant changes in milk protein percentage following supplementation with protected nutrients. Nevertheless, maintaining milk protein concentration while simultaneously increasing milk yield is considered advantageous because it results in a greater overall protein output.

Recent studies have further indicated that supplementation with rumen protected amino acids enhances nitrogen utilization efficiency and supports milk protein synthesis, particularly during early lactation (Sun *et al.*, 2021; Muruz *et al.*, 2024) [25, 13].

3.3.3 Lactose

Milk lactose concentration was not significantly affected by dietary supplementation, with mean values of 5.22% and 5.29% recorded in T₀ and T₁, respectively. Lactose is generally regarded as one of the most stable constituents of milk because its synthesis is largely regulated by mammary osmotic balance and is relatively insensitive to dietary manipulation.

These findings are consistent with those reported by Sirohi *et al.* (2010) [23], Ranjan *et al.* (2012) [18], Sirohi *et al.* (2013) [22], Singh *et al.* (2014), and Yadav *et al.* (2015) [29], who likewise observed minimal effects of bypass nutrient supplementation on milk lactose concentration.

3.3.4 Solids-not-fat (SNF)

No significant differences were observed in solids-not-fat

(SNF) content between the treatment groups. The mean SNF values remained within the normal physiological range for Murrah buffalo milk, indicating that supplementation with rumen protected protein and rumen protected fat enhanced milk production without adversely affecting milk quality.

Similar observations have been reported by Sirohi *et al.* (2013) [22], Amrutkar *et al.* (2014) [1], Shelke *et al.* (2011a) [20], and Mane *et al.* (2016) [12].

3.3.5 Total solids

Total solids content was numerically higher in the supplemented group (T₁; 17.59%) than in the control group (T₀; 17.25%), although the difference was not statistically significant. The slightly higher total solids content may be attributed to the increase in milk fat percentage observed in the supplemented animals.

Murrah buffalo milk is naturally characterized by a high total solids content, which contributes to its excellent processing characteristics and suitability for the manufacture of value-added dairy products. Recent studies have highlighted the importance of nutritional interventions that improve milk production while maintaining the compositional stability and processing quality of buffalo milk.

4. Conclusion

The present study demonstrated that dietary supplementation with rumen protected protein and rumen protected fat during the transition period positively influenced the lactational performance of Murrah buffaloes. Buffaloes receiving the supplemented diet produced significantly higher milk yield, 6% fat-corrected milk (FCM) yield, and milk fat percentage than those fed the conventional diet. In contrast, milk protein, lactose, solids-not-fat (SNF), and total solids were not significantly affected, indicating that the supplementation enhanced milk production without compromising the overall compositional quality of milk.

The improvement in productive performance may be attributed to the greater post-ruminal availability of absorbable amino acids from rumen protected protein and the increased energy supply provided by rumen protected fat. The synchronized availability of protein and energy during early lactation likely improved nutrient utilization efficiency, alleviated the negative energy balance commonly experienced during the transition period, and promoted greater nutrient partitioning towards milk synthesis. These nutritional advantages contributed to enhanced lactational performance while maintaining normal milk composition.

Overall, the findings indicate that strategic supplementation with rumen protected protein and rumen protected fat is an effective nutritional approach for improving milk production in transition Murrah buffaloes. The combined supplementation not only enhanced milk yield and milk fat content but also maintained desirable milk quality characteristics, highlighting its practical value under tropical production conditions. Adoption of this feeding strategy may therefore contribute to improved productivity and profitability of buffalo dairy farming, particularly during the nutritionally demanding transition period. Further studies involving larger animal populations and different stages of lactation are recommended to validate these findings and to optimize supplementation strategies under diverse management and feeding systems.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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