

Effect of *Tinospora cordifolia* Supplementation on Murrah Buffalo Milk Yield and Composition

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Abstract

The present study evaluated the effect of nutritional treatments and other factors on milk production parameters in Murrah buffaloes maintained across ten farms in Mansa district, Punjab. Thirty lactating buffaloes were randomly assigned to three treatment groups: T1 (extra concentrate ration), T2 (mineral mixture supplementation at 50 g/day for 2 months), and T3 (Giloy powder @100 g/day for 15 days with mineral mixture @50 g/day for 2 months). Milk samples were collected during morning and evening milking once in a week over eight consecutive weeks after calving. The statistical model included treatment group, week of observation, time of day, season of calving, and lactation number as fixed effects, with farm and animal nested within farm as random effects. Type III ANOVA (Satterthwaite's method) revealed that treatment significantly influenced both milk fat percentage and milk yield, with Group 3 showing the highest values, followed by Group 2 and Group 1 (all significantly different; $p < 0.05$). Week of observation had a strong effect on all traits, while time of day and lactation number showed limited or no effect. Solid-not-fat (SNF) percentage was not significantly affected by nutritional treatments, though week and treatment $\times$ week interactions were significant. These results highlight that targeted nutritional interventions, especially the combination of Giloy and mineral mixture (T3), can enhance milk fat and yield but have limited impact on SNF. This provides practical implications for feeding strategies aimed at improving dairy productivity under smallholder conditions in Punjab.

Keywords: Murrah buffalo, Giloy, Milk fat, Milk yield, SNF, Nutritional treatments, Punjab

Introduction

In India, the dairy industry is crucial to increasing rural income since it produces fuel for rural households, nutrient-dense milk for human consumption, and draft power for transportation and farming (Patbandha *et al.*, 2015). The best buffalo germplasm in the world is thought to be found in India. With a population of 109.85 million, buffaloes make up around 20.45% of India's overall livestock population (Livestock Census, 2019). Buffaloes are an important contributor to India's milk production, accounting for approximately 44.8% of total milk production in 2022–23 (DAHD, 2023). Murrah, the predominant buffalo breed in India accounts for 42.8% of the buffalo population according to the breed-wise report of the 20th Livestock Census (DAHD, 2022).

A balanced diet is essential to helping an animal reach its full genetic potential. An animal can retain optimal vital functions and reproduce and produce in a physiologically appropriate manner by being fed the right amount of each required nutrient. Micronutrients, particularly minerals, are essential for sustaining the body's regular physiological and metabolic processes. According to Bhanderi *et al.*, (2016), the amount of minerals in widely accessible feed products varies and is typically insufficient to meet the needs of animals at various phases of production. Farmers can increase the total productivity of dairy animals and generate extra revenue from the same animals by supplementing their diet with a mineral mixture (Islam *et al.*, 2023).

The list of synthetic galactagogues includes dopamine antagonists, anti-psychotics (Sulpiride and Chlorpromazine), anti-emetics (Metoclopramide and Domperidone), and hormone synthetic analogs (Oxytocin, Recombinant bovine somatotropin, Thyrotropin-releasing hormone, and Medroxyprogesterone) that are used to promote lactation (Tabares *et al.*, 2014). Synthetic medications have a negative impact on the nursing animal's neuroendocrine system. Long-term usage of them may result in toxicity, which creates a risk to both humans' and animals' normal health. Traditional herbs as galactagogue are therefore of great interest to researchers due to their accessibility, affordability, and potential to leave no harmful residues in milk (Mohanty *et al.*, 2014 Kachhawaha *et al.*, 2025 Patel *et al.*, 2016).

Many herbal plants can be used as herbal medicine to help milch cows let down their milk since they contain a lot of chemical active ingredients with galactogenic qualities. One of the ayurvedic medicinal plants, *T. cordifolia*, has the potential to be a highly valued plant with a wide range of therapeutic, ethno-pharmacological, phytochemical, and numerous properties. Numerous pharmacological effects, such as anti-diabetic, anti-inflammatory, anti-arthritic, antioxidant, hepatoprotective, cardioprotective, anti-allergic, anti-stress, and many more, have been demonstrated by prior studies on *T. cordifolia* (Bala *et al.*, 2015). Through these bioactivities—which include reducing oxidative stress, improving nutrient utilization, and fostering systemic health—giloy may have an impact on lactational physiology (Rajneesh *et al.*, 2022).

Despite the known medicinal and health-promoting benefits of giloy, its role in influencing milk yield and milk quality parameters in buffaloes remains inadequately studied. A few experiments in buffaloes and cows have reported increases in milk yield and improvements in certain compositional traits when giloy was fed, either alone or in combination with bypass nutrients (Mallick and Prakash 2011, Rajneesh *et al.*, 2021). However systematic scientific data on the combined supplementation of giloy and mineral mixture in buffaloes under field conditions are scarce. This represents a significant research gap, especially considering the importance of buffalo milk to India's dairy sector and the growing demand for natural and sustainable feeding interventions.

Therefore, the present study was designed to investigate the effect of feeding giloy powder in combination with mineral mixture on milk yield, fat percentage, and SNF percentage in Murrah buffaloes under field conditions in Mansa district of Punjab. This research aims to provide scientific evidence for the potential use of giloy as a natural feed additive in improving productivity and milk quality, thereby contributing to the sustainable development of buffalo dairy farming.

Materials and Methods

Experimental Site and Duration

The present field trial was conducted in Mansa district (29°58'44.83" N latitude and 75°23'48.02" E longitude) Punjab, India, during the year 2021–22. The region falls under a semi-arid climate, with hot summers and relatively cold winters, and the trial was carried out under practical field conditions on organized buffalo-owning households. The total duration of the experiment was two months, starting immediately after calving of the selected animals.

Experimental Animals and Management

A total of 30 lactating Murrah buffaloes of similar body condition, age (4–7 years), and parity (2nd to 4th lactation) were selected immediately after calving. All animals were apparently healthy and maintained under similar feeding and management conditions throughout the study. Standard housing and hygienic practices were followed, and routine deworming and vaccination schedules were ensured before the trial commenced.

Experimental Design

The animals were randomly divided into three groups (n=10 per group) following a completely randomized design (CRD). The experimental groups were as follows:

T1 (Control): Basal diet without any additional supplementation. T2: Feeding Basal diet + mineral mixture @ 50 g/day (South-zone specific mineral mixture formulated by GADVASU). T3: Feeding Basal diet + mineral mixture @ 50 g per animal per day + Giloy (*Tinospora cordifolia*) powder @ 100 g per animal per day

The Giloy (*Tinospora cordifolia*) powder was procured from a local market pansari shop at Rs. 200 per kg, while the mineral mixture cost was Rs. 90 per kg. Thus, at the supplementation rates of 100 g and 50 g per animal per day, respectively, the supplementation costs were Rs. 20 and Rs. 4.50 per animal per day. The dose of Giloy powder (100 g per animal per day) was selected based on previously reported use of *Tinospora cordifolia* in lactating Murrah buffaloes. Mir *et al.* (2014) reported beneficial effects at 120 g Giloy stem powder per buffalo per day; therefore, 100 g per animal per day was selected as a practical field-level dose while remaining within the range previously investigated in Murrah buffaloes. The basal diet consisted of seasonal green fodder, dry roughages (wheat straw), and concentrate mixture provided as per NRC 2001 feeding standards. Clean and fresh drinking water was provided ad libitum.

Data Recording

Milk samples were collected during morning and evening milking once a week over a period of 8 consecutive weeks. Milk yield (kg/milking) fat percentage and solids-not-fat (SNF) percentage of milk samples were determined for each buffalo using a Lactoscan milk analyser. Thus, a total of 8 weekly observations were recorded during the two-month experimental period.

Classification of season of calving

Calving seasons were classified according to Punjab's climatic conditions: spring (March–mid-April), summer (mid-April–June), rainy (July–September), pre-winter (October–November), and winter (December–February).

Traits Recorded- Milk fat percentage (%), Milk yield (L/milking), Solid-not-fat (SNF, %).

Statistical Model

To evaluate the effects of nutritional treatment and other factors on milk production traits (milk fat percentage, milk yield, and solid-not-fat content), a linear mixed-effects model was fitted. The general form of the model was:

$$Y_{ijklmno} = \mu + T_i + W_j + D_k + C_l + L_m + (T \times W)_{ij} + (T \times D)_{ik} + (W \times D)_{jk} + (T \times W \times D)_{ijk} + F_n + A_{o(n)} + \varepsilon_{ijklmno}$$

Where

$Y_{ijklmno}$ = observed response variable (milk fat %, milk yield, or SNF).

μ = overall mean.

T_i = fixed effect of the i^{th} treatment group ($i = 1, 2, 3$).

W_j = fixed effect of the j^{th} week of observation ($j = 1, \dots, 8$).

D_k = fixed effect of time of day of sampling ($k = \text{morning, evening}$).

C_l = fixed effect of the l^{th} season of calving ($l = 1, \dots, 5$).

L_m = fixed effect of lactation number

$(T \times W)_{ij}, (T \times D)_{ik}, (W \times D)_{jk}, (T \times W \times D)_{ijk}$ = interaction effects among treatment, week, and time of day.

F_n = random effect of the n^{th} farm $\sim N(0, \sigma^2_F)$.

$A_{o(n)}$ = random effect of the o^{th} animal nested within farm $\sim N(0, \sigma^2_A)$.

$\varepsilon_{ijklmno}$ = residual error term $\sim N(0, \sigma^2)$.

Model Fitting

Models were fitted using the lmer() function in the lme4 package and the lme() function in the nlme package (R software). Degrees of freedom and p-values for fixed effects were estimated using the Satterthwaite approximation (via lmerTest). Pairwise comparisons of estimated marginal means (EMMeans) were conducted with Tukey's adjustment for multiple testing.

Results

Milk Yield

A highly significant effect ($p < 0.001$) of treatment group on milk yield, and of week of observation, was observed. The interaction between the treatment group and week was highly significant ($p < 0.001$), demonstrating that the effect of dietary treatments on milk yield varies over different weeks of lactation (Figure 1). The time of day when the samples were taken had a small but significant effect on yield ($p = 0.049$). Its interactions with week ($p < 0.001$) and treatment $\times$ week ($p < 0.001$) were also significant, which means that the changes in yield between day and night depended on both the stage of lactation and the therapy. Season of calving ($p = 0.323$) and lactation number ($p = 0.107$) did not significantly affect yield.

Significant differences between all groups for mean milk yield were confirmed by Tukey-adjusted pairwise contrasts (Table 1). Group 3 had a significantly higher mean milk yield (3.53 L/milking) than groups 2 and 1, whereas group 2 (3.37 L/milking) had a significantly higher mean milk yield than group 1 (3.19 L/milking).

In comparison to the control, the milk yield was greatly increased when Giloy was supplemented with a mineral mixture. These outcomes are consistent with those of Mir *et al.* (2014), who gave Murrah buffaloes Giloy stem powder per day at a dose rate of 120 g/day/buffalo and observed a significant ($P < 0.05$) increase in milk yield and improved milk quality. Mir *et al.* (2015) observed similar results, showing that supplementing Murrah buffalo with *T. cordifolia* boosted milk output by 10.10 percent. Mallick and Prakash (2011) found that supplementing Karan Fries cattle with *T. cordifolia* increased their milk output. Similar results were found in Elmann *et al.* (2013) in which milk yield increased significantly with the increased levels of fenugreek seed supplementation in Nubian does.

Milk Fat Percentage

The week of observation and the treatment group had a highly significant ($p < 0.001$) effect on the percentage of milk fat. The interaction between treatment group and week was also statistically significant ($p = 0.023$), suggesting that the effect of treatment on milk fat varied across weeks (Figure 2). Conversely, time of day of sampling ($p = 0.207$), Season of calving ($p = 0.526$), and lactation number ($p = 0.551$) did not show significant effects on milk fat percentage. Other two- and three-way interactions were not statistically significant.

For milk fat percentage in Murrah buffaloes, all three groups differed significantly (Table 1). Group 3 had the highest mean fat percentage (7.82%), followed by Group 2 (7.68%), while Group 1 recorded the lowest (7.50%). These findings contradict those reported by Mir *et al.* (2014), Mallick and Prakash (2011), and Mir *et al.* (2015). These researchers discovered that supplementing with *T. cordifolia* did not significantly alter the percentage of milk fat.

Solid-Not-Fat (SNF) Content

Statistical analysis of the experimental data found that the SNF content of milk was not significantly influenced by treatment group ($p = 0.299$). In contrast, week of the study emerged as a highly significant ($p < 0.0001$) factor, indicating notable temporal variation in SNF levels over the course of the investigation. A significant interaction was also observed between treatment group and week (0.0383), suggesting that changes in SNF content across weeks differed modestly among the treatment groups. The interaction between week and time-of-day was also significant, reflecting that SNF content fluctuated differently by time-of-day over the study period. However, neither season of calving, lactation number, the interaction between treatment group and time-of-day, nor the three-way interaction among treatment group, week, and time-of-day had a significant effect on SNF content (all $p > 0.20$).

The results of this investigation are consistent with those of Mallick and Prakash (2011), who found that supplementing Karan Fries cattle with *T. cordifolia* did not significantly alter the levels of SNF, protein, or lactose in their milk. In a study conducted by Srivastav *et al.* (2024), Milk protein quality was improved by supplementation of fenugreek, shatavari and giloy to Sahiwal cattle. Milk protein was recorded highest with fenugreek seed powder supplementation while improved total solids content was found in 100 g giloy supplementation group. In contrast to this, Elmann *et al.* (2013) found no discernible pattern in the SNF, protein, and lactose levels in the milk of Nubian goats given fenugreek.

The estimated marginal means for solid-not-fat (SNF) across treatment groups were 8.79% in Group 1, 8.84% in Group 2, and 8.85% in Group 3 (Table 1). Statistical evaluation using Tukey's post hoc pairwise comparisons indicated no significant differences among the treatment groups ($p > 0.05$), as shown in Table 1. Consequently, all groups were assigned the same superscript letter (a), confirming that SNF values remained statistically consistent across treatments throughout the study.

Conclusion

The study demonstrated that supplementation of Giloy powder (100 g/animal/day) with mineral mixture (50 g/animal/day) significantly improved milk yield and fat percentage in Murrah buffaloes under field conditions, while SNF content remained unaffected. These findings highlight the potential of combining conventional mineral supplementation with herbal additives for improving dairy productivity. Further studies including a Giloy-only treatment are required to determine the independent effect of Giloy on milk production and composition. Future research with larger herds, longer durations, and mechanistic insights is warranted to develop farmer-friendly and sustainable feeding recommendations.

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