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Immunomodulatory effects of *Leptadenia reticulata* and *Moringa oleifera* Supplementation on neutrophil-to-lymphocyte ratio in lactating Sahiwal cattle

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Abstract

The present study was conducted to evaluate the immunomodulatory effects of *Leptadenia reticulata* and *Moringa oleifera* supplementation on differential leukocyte response and neutrophil-to-lymphocyte (N: L) ratio in lactating Sahiwal cattle. Twenty-eight lactating Sahiwal cows were randomly allocated into four treatment groups comprising seven animals each. The control group (T₀) received the basal diet, while T₁ received *Leptadenia reticulata* root powder @ 90 g/day/animal, T₂ received *Moringa oleifera* leaf powder @ 150 g/day/animal and T₃ received a combination of *Leptadenia reticulata* (45 g) and *Moringa oleifera* (75 g) per day. The feeding trial was conducted for 120 days.

Differential leukocyte count (DLC) and neutrophil-to-lymphocyte ratio were evaluated at 0, 30, 60, 90 and 120 days of the experimental period. Statistical analysis revealed significant ($P < 0.05$) improvements in neutrophil percentage in the *Moringa* supplemented group during the later stages of the experiment. The overall neutrophil percentage was significantly higher in T₂ (28.37%) compared to the control group (26.74%). The neutrophil-to-lymphocyte ratio was significantly influenced by dietary treatments, with the highest overall value observed in T₂ (0.437) followed by T₃ (0.422), T₁ (0.413) and T₀ (0.402). Significant differences in N:L ratio were observed at 90 and 120 days of supplementation, and the overall treatment effect was also significant ($P < 0.05$).

The findings indicate that *Moringa oleifera* supplementation exerts a positive immunomodulatory effect by enhancing neutrophil response and improving N:L ratio in lactating Sahiwal cattle. The increase in N:L ratio suggests improved innate immune competence and disease resistance without adversely affecting physiological homeostasis.

Keywords: *Moringa oleifera*, *Leptadenia reticulata*, Sahiwal cattle, Immunomodulation, Neutrophils, Lymphocytes, Neutrophil-to-Lymphocyte Ratio

1. Introduction

India possesses one of the largest cattle populations in the world, and indigenous breeds contribute significantly to the country's dairy sector. Among these breeds, Sahiwal cattle are highly valued for their superior milk production, adaptability to tropical climates and resistance to various environmental stresses. Maintenance of optimum health and immune competence in dairy animals is essential for sustaining productivity, reproductive efficiency and overall farm profitability. In recent years, increasing emphasis has been placed on the utilization of natural feed additives to enhance animal health and reduce dependence on synthetic growth promoters and chemotherapeutic agents.

The growing concern regarding antimicrobial resistance and drug residues in animal-derived food products has encouraged researchers to explore alternative strategies for improving livestock health. Medicinal plants have emerged as promising natural supplements owing to their rich content of bioactive compounds possessing antioxidant, antimicrobial and immunomodulatory properties. Herbal feed additives can improve physiological functions, support

immune responses and promote sustainable livestock production without adversely affecting animal health.

Leptadenia reticulata (Jivanti) is an important medicinal plant widely used in traditional Ayurvedic medicine. The plant contains numerous biologically active compounds such as flavonoids, alkaloids, glycosides, terpenoids and phenolic substances. These phytochemicals have been reported to exhibit antioxidant, adaptogenic and immunostimulatory properties. Previous studies have demonstrated beneficial effects of *Leptadenia reticulata* on hematological parameters, metabolic functions and immune responses in livestock and laboratory animals (Singh, 2011; Pathak, 2017) [12, 9]. The herb is therefore considered a potential natural immunomodulator capable of enhancing disease resistance and physiological well-being.

Moringa oleifera, commonly known as drumstick tree, has gained considerable attention as a multipurpose fodder and medicinal plant. Moringa leaves are rich in high-quality protein, essential amino acids, vitamins, minerals and antioxidant compounds including flavonoids and phenolics. Several studies have reported that supplementation of

Moringa oleifera improves nutrient utilization, growth performance, antioxidant status and immune function in livestock species (Babeker and Abdalbagi, 2015; Jiwuba *et al.*, 2017; Mahmood *et al.*, 2018; Sonkar *et al.*, 2020) [1, 4, 6, 13]. The abundance of natural antioxidants and bioactive compounds makes *Moringa* an attractive feed supplement for enhancing animal health and productivity.

Evaluation of immune status in animals often involves assessment of leukocyte populations, particularly neutrophils and lymphocytes. Neutrophils constitute the first line of defense against invading pathogens and play a critical role in innate immunity through phagocytosis and microbial killing. Lymphocytes are essential components of adaptive immunity and are involved in antibody production and cell-mediated immune responses. Alterations in the proportion of these leukocyte populations may reflect changes in immune responsiveness, physiological adaptation and health status.

The neutrophil-to-lymphocyte (N: L) ratio has recently emerged as a simple, reliable and cost-effective indicator of immune competence, inflammatory status and physiological stress in animals. Unlike individual leukocyte counts, the N:L ratio integrates information from both innate and adaptive immune systems, thereby providing a more comprehensive assessment of immune modulation. An increase in N:L ratio within the normal physiological range is often associated with enhanced innate immune responsiveness and improved ability to withstand pathogenic challenges. Consequently, N:L ratio has gained considerable importance as a biomarker for evaluating the immunomodulatory effects of dietary interventions.

Although the beneficial effects of *Leptadenia reticulata* and *Moringa oleifera* on animal performance and hematological parameters have been documented, information regarding their influence on neutrophil-to-lymphocyte ratio in indigenous dairy cattle remains limited. Furthermore, comparative evaluation of these herbal supplements under practical farm conditions in lactating Sahiwal cattle has not been extensively investigated. Therefore, the present study was undertaken to assess the immunomodulatory effects of *Leptadenia reticulata* and *Moringa oleifera* supplementation on neutrophil and lymphocyte responses and the neutrophil-to-lymphocyte ratio in lactating Sahiwal cattle.

Materials and Methods

Experimental Location and Animals

The experiment was conducted at the Instructional Dairy Farm, Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Twenty-eight healthy lactating Sahiwal cows of comparable age, parity and stage of lactation were selected for the study. The animals were maintained under uniform housing, feeding and management conditions throughout the experimental period. Clean drinking water was made available ad libitum.

Experimental Design and Dietary Treatments

The feeding trial was conducted for a period of 120 days. Twenty-eight lactating Sahiwal cows were randomly allocated into four treatment groups comprising seven animals each.

The treatment groups were as follows:

To: Basal diet (Control)

T₁: Basal diet + *Leptadenia reticulata* root powder @ 90 g/day/animal

T₂: Basal diet + *Moringa oleifera* leaf powder @ 150 g/day/animal

T₃: Basal diet + *Leptadenia reticulata* root powder @ 45 g/day + *Moringa oleifera* leaf powder @ 75 g/day/animal

The herbal supplements were administered orally along with the concentrate mixture throughout the experimental period.

Feeding and Management

All experimental animals were maintained under identical feeding and management conditions. The basal ration consisted of green fodder, dry fodder and concentrate mixture formulated according to the nutrient requirements of lactating Sahiwal cattle. Feeding was carried out twice daily and animals were monitored regularly for their health status.

Blood Collection and Differential Leukocyte Analysis

Blood samples were collected aseptically from the jugular vein of each animal at 0, 30, 60, 90 and 120 days of the feeding trial. Approximately 5 mL of blood was collected into sterile tubes containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant.

The collected samples were subjected to differential leukocyte count (DLC) analysis, including neutrophils, lymphocytes, monocytes, eosinophils and basophils, using standard hematological procedures. Particular emphasis was given to neutrophil and lymphocyte percentages due to their role in evaluating immune responsiveness.

Determination of Neutrophil-to-Lymphocyte Ratio

The neutrophil-to-lymphocyte (N: L) ratio was calculated for each animal at each sampling interval using the following equation:

$$N:L \text{ Ratio} = \text{Neutrophil (\%)} / \text{Lymphocyte (\%)}$$

The N:L ratio was used as an indicator of immune status and immunomodulatory response to dietary herbal supplementation.

Statistical Analysis

The experimental data were analyzed using Statistical Package for Social Sciences (SPSS) software. One-way Analysis of Variance (ANOVA) was employed to determine the effect of dietary treatments on neutrophil percentage, lymphocyte percentage and neutrophil-to-lymphocyte ratio. Duncan's Multiple Range Test (DMRT) was used for post-hoc comparison of treatment means whenever significant differences were detected. Differences among treatment means were considered statistically significant at $P < 0.05$.

Results and Discussion

The differential leukocyte profile serves as an important indicator of immune status and physiological adaptation in dairy animals. The effect of dietary supplementation of *Leptadenia reticulata* and *Moringa oleifera* on neutrophil and lymphocyte percentages in lactating Sahiwal cattle is presented in Table 1.

Table 1: Effect of *Leptadenia reticulata* and *Moringa oleifera* supplementation on lymphocyte and neutrophil percentages in lactating Sahiwal cattle

Lymphocyte (%)				
Days	T ₀	T ₁	T ₂	T ₃
0	68.05 ± 0.81	69.24 ± 1.21	66.33 ± 0.75	65.85 ± 1.12
30	66.53 ± 1.13	66.57 ± 1.04	65.25 ± 1.41	67.25 ± 1.22
60	66.16 ± 0.64	65.74 ± 1.06	64.53 ± 1.32	66.75 ± 0.94
90	65.61 ± 1.75	65.42 ± 1.39	63.52 ± 0.93	63.13 ± 0.78
120	66.81 ± 0.38	63.60 ± 0.62	65.07 ± 0.69	65.30 ± 1.32
Overall Mean	66.63 ± 0.53	66.11 ± 0.49	64.94 ± 0.64	65.66 ± 0.72
Neutrophil (%)				
Days	T ₀	T ₁	T ₂	T ₃
0	25.09 ± 1.14	26.45 ± 0.88	25.80 ± 1.15	26.86 ± 0.75
30	26.10 ± 0.85	26.67 ± 0.92	27.05 ± 0.69	28.55 ± 0.92
60	27.47 ± 0.81	27.70 ± 0.70	27.22 ± 1.08	27.77 ± 0.83
90	27.36 ± 0.51 ^a	27.32 ± 0.34 ^a	30.75 ± 1.04 ^b	27.79 ± 0.29 ^a
120	27.67 ± 0.58 ^a	28.33 ± 0.35 ^a	31.00 ± 1.16 ^b	27.27 ± 0.51 ^a
Overall Mean	26.74 ± 0.42 ^a	27.29 ± 0.36 ^{ab}	28.37 ± 0.39 ^b	27.65 ± 0.35 ^{ab}

Means bearing different superscripts within a row differ significantly ($P < 0.05$).

Lymphocytes and neutrophils are important components of the immune system and serve as indicators of adaptive and innate immune responses, respectively.

The overall mean lymphocyte percentages recorded in T₀, T₁, T₂ and T₃ groups were 66.63 ± 0.53, 66.11 ± 0.49, 64.94 ± 0.64 and 65.66 ± 0.72 per cent, respectively. Statistical analysis revealed that dietary supplementation of *Leptadenia reticulata* and *Moringa oleifera* had no significant ($P > 0.05$) effect on lymphocyte percentage throughout the experimental period. The lymphocyte values remained within the normal physiological range reported for healthy cattle, indicating that herbal supplementation did not adversely affect adaptive immune functions. Similar findings were reported by Dash *et al.* (1972), who observed non-significant changes in lymphocyte percentage following supplementation of *Leptadenia reticulata*. Mishra (2008) [7] and Kumar *et al.* (2020) [5] also reported non-significant effects of *Moringa* supplementation on lymphocyte counts in dairy animals and goats, respectively.

In contrast, neutrophil percentage was significantly influenced by dietary treatments. The overall mean neutrophil percentages in T₀, T₁, T₂ and T₃ groups were 26.74 ± 0.42, 27.29 ± 0.36, 28.37 ± 0.39 and 27.65 ± 0.35 per cent, respectively. The highest neutrophil percentage was observed in the *Moringa oleifera* supplemented group (T₂). Furthermore, neutrophil percentages recorded at 90 and 120 days were significantly ($P < 0.05$) higher in T₂ compared to the control and other treatment groups.

The increase in neutrophil percentage observed in the

Moringa supplemented group may be attributed to the presence of bioactive compounds such as flavonoids, phenolic compounds, vitamins and antioxidants that enhance immune responsiveness and stimulate leukocyte activity. Neutrophils represent the first line of defense against invading pathogens and play a major role in phagocytosis and destruction of microorganisms. Therefore, the increased neutrophil percentage observed in the present study may indicate enhanced innate immune competence and improved disease resistance.

The present findings are in agreement with Babeker and Abdalbagi (2015) [1], who reported significant improvements in leukocyte populations in goats supplemented with *Moringa oleifera*. Fadiyimu *et al.* (2010) [3] also reported beneficial effects of *Moringa* supplementation on differential leukocyte profile. However, Kumar *et al.* (2020) [5] reported non-significant changes in neutrophil percentage in goats supplemented with *Moringa* leaf powder. With regard to *Leptadenia reticulata*, Dash *et al.* (1972) reported non-significant effects on neutrophil percentage in dairy cattle, whereas Pravansha *et al.* (2012) [10] demonstrated enhanced neutrophil activity and phagocytic response following administration of *Leptadenia* extracts.

Overall, the results indicate that *Moringa oleifera* supplementation positively modulated neutrophil response while maintaining normal lymphocyte levels, thereby improving immune competence in lactating Sahiwal cattle without disturbing physiological homeostasis.

Table 2: Effect of *Leptadenia reticulata* and *Moringa oleifera* supplementation on Neutrophil-to-Lymphocyte (N: L) Ratio in lactating Sahiwal cattle

Days	T ₀	T ₁	T ₂	T ₃
0	0.370 ± 0.019	0.383 ± 0.015	0.389 ± 0.018	0.409 ± 0.015
30	0.394 ± 0.018	0.401 ± 0.012	0.417 ± 0.017	0.425 ± 0.012
60	0.416 ± 0.014	0.421 ± 0.009	0.422 ± 0.016	0.416 ± 0.013
90	0.420 ± 0.017 ^a	0.419 ± 0.008 ^a	0.484 ± 0.013 ^b	0.441 ± 0.009 ^a
120	0.414 ± 0.010 ^a	0.446 ± 0.009 ^{ab}	0.477 ± 0.021 ^b	0.418 ± 0.006 ^a
Overall Mean	0.402 ± 0.009 ^a	0.413 ± 0.007 ^a	0.437 ± 0.007 ^b	0.422 ± 0.007 ^{ab}

Means bearing different superscripts (a,b) within a row differ significantly ($P < 0.05$).

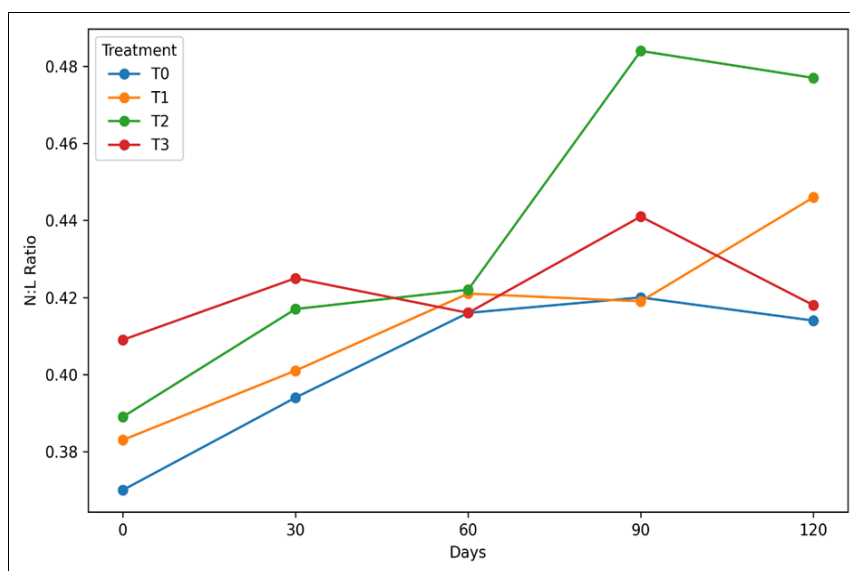


Fig 1: Effect of *Leptadenia reticulata* and *Moringa oleifera* supplementation on neutrophil-to-lymphocyte (N: L) ratio in lactating Sahiwal cattle.

The neutrophil-to-lymphocyte (N: L) ratio is considered a reliable indicator of immune competence, inflammatory status and physiological adaptation in animals. The effect of dietary supplementation of *Leptadenia reticulata* and *Moringa oleifera* on N:L ratio in lactating Sahiwal cattle is presented in Table 2.

The N:L ratio did not differ significantly ($P>0.05$) among treatment groups at 0, 30 and 60 days of the experiment. However, significant differences ($P<0.05$) were observed at 90 and 120 days of supplementation. At 90 days, the N:L ratio was significantly higher in the *Moringa* supplemented group (T_2 ; 0.484 ± 0.013) compared to T_0 (0.420 ± 0.017), T_1 (0.419 ± 0.008) and T_3 (0.441 ± 0.009). Similarly, at 120 days, T_2 recorded the highest N:L ratio (0.477 ± 0.021), which was significantly greater than the control and combination groups.

The overall mean N:L ratio was significantly ($P<0.05$) affected by dietary treatments. The highest overall value was observed in the *Moringa* supplemented group (0.437 ± 0.007), followed by T_3 (0.422 ± 0.007), T_1 (0.413 ± 0.007) and T_0 (0.402 ± 0.009). The significant increase in N:L ratio observed in T_2 may be attributed to the higher neutrophil percentage recorded in this group, while lymphocyte counts remained within the normal physiological range.

Moringa oleifera leaves are rich sources of vitamins, minerals, flavonoids, phenolic compounds and natural antioxidants that have been reported to enhance immune function and leukocyte activity. The improvement in N:L ratio observed in the present study suggests enhanced innate immune responsiveness and better preparedness of animals to combat infectious challenges. Neutrophils are the primary cellular components involved in phagocytosis and pathogen destruction, whereas lymphocytes are responsible for adaptive immune responses. Therefore, the balance between these two leukocyte populations provides valuable information regarding the immune status of animals.

The maintenance of normal lymphocyte percentages along with increased neutrophil values indicates that the observed increase in N:L ratio was associated with improved immune competence rather than physiological stress. Similar

immunomodulatory effects of *Moringa oleifera* have been reported by Babeker and Abdalbagi (2015) ^[1], who observed improved leukocyte responses in goats supplemented with *Moringa* leaves. Fadiyimu *et al.* (2010) ^[3] also reported favorable changes in differential leukocyte profile following *Moringa* supplementation. Furthermore, Pravansha *et al.* (2012) ^[10] demonstrated enhanced neutrophil activity and phagocytic responses following administration of *Leptadenia reticulata* extracts.

The results of the present investigation indicate that prolonged supplementation of *Moringa oleifera* positively modulates the neutrophil-to-lymphocyte ratio and enhances immune responsiveness in lactating Sahiwal cattle. The significant increase observed at 90 and 120 days suggests that the immunomodulatory effects of herbal supplementation become more pronounced with continued feeding.

Effect on Other Differential Leukocyte Parameters

Monocyte, eosinophil and basophil percentages were not significantly ($P>0.05$) influenced by supplementation of *Leptadenia reticulata* and *Moringa oleifera* throughout the experimental period. The values remained within normal physiological limits, indicating that herbal supplementation had no adverse effect on leukocyte distribution and immune homeostasis. Similar observations were reported by Dash *et al.* (1972), Mishra (2008) ^[7] and Kumar *et al.* (2020) ^[5].

Conclusion

The present study demonstrated that dietary supplementation of *Leptadenia reticulata* and *Moringa oleifera* influenced differential leukocyte responses and neutrophil-to-lymphocyte ratio in lactating Sahiwal cattle. Lymphocyte percentage remained unaffected by dietary treatments, whereas neutrophil percentage increased significantly in animals supplemented with *Moringa oleifera*. Consequently, the neutrophil-to-lymphocyte ratio was significantly enhanced at 90 and 120 days of supplementation as well as in the overall treatment means. The observed improvement in N:L ratio indicates enhanced

innate immune competence and immunomodulatory activity without disturbing physiological homeostasis. The findings suggest that *Moringa oleifera* may serve as a promising natural feed additive for improving immune responsiveness and health status in lactating Sahiwal cattle under farm conditions.

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