



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(6): 165-170

© 2026 VET

www.veterinarypaper.com

Received: 15-03-2026

Accepted: 21-04-2026

Gyan Deepak Kumar

Ph.D., Department of Livestock
Production Management, College of
Veterinary and Animal Sciences, G.B.
Pant University of Agriculture and
Technology Pantnagar, Udham Singh
Nagar, Uttarakhand, India

Brijesh Singh

Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Jyoti Palod

Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

RK Sharma

Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

SK Singh

Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Shive Kumar

Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Neeraj Singh

Department of Animal Husbandry
and Dairying, Chandra Shekhar Azad
University of Agriculture and
Technology, Kanpur, Uttar Pradesh,
India

Corresponding Author:

Gyan Deepak Kumar

Ph.D., Department of Livestock
Production Management, College of
Veterinary and Animal Sciences, G.B.
Pant University of Agriculture and
Technology Pantnagar, Udham Singh
Nagar, Uttarakhand, India

Association between Haematological traits and milk mineral composition in lactating Sahiwal cattle supplemented with *Leptadenia reticulata* and *Moringa oleifera*

**Gyan Deepak Kumar, Brijesh Singh, Jyoti Palod, RK Sharma, SK Singh,
Shive Kumar and Neeraj Singh**

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i6c.3514>

Abstract

The present study was undertaken to investigate the association between selected haematological traits and milk mineral composition in lactating Sahiwal cattle receiving herbal feed supplementation with *Leptadenia reticulata* and *Moringa oleifera* under farm conditions. Twenty-eight healthy lactating Sahiwal cows were randomly allocated into four treatment groups comprising a control group (basal diet), a *Leptadenia reticulata*-supplemented group, a *Moringa oleifera*-supplemented group and a combined herbal supplementation group, with seven animals in each treatment. The feeding trial was conducted for 120 days, and blood and milk samples were collected at 30-day intervals (Days 0, 30, 60, 90 and 120), generating a total of 140 longitudinal observations. Haematological parameters, including haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC), were determined using standard veterinary procedures, whereas milk samples were analysed for calcium (Ca), phosphorus (P), magnesium (Mg) and zinc (Zn) concentrations following established analytical methods. Linear mixed-effects models were employed to evaluate the associations between haematological traits and milk minerals while accounting for repeated measurements within animals. Animal identification number was included as a random effect, whereas herbal treatment group and sampling day were fitted as fixed effects.

The mixed-effects analysis revealed that packed cell volume was positively associated with milk phosphorus concentration, with each unit increase in PCV corresponding to an estimated increase of 0.981 mg/dL in milk phosphorus levels ($\beta = 0.981$; $P = 0.011$). Similarly, total leukocyte count emerged as a significant positive predictor of milk zinc concentration, whereby each unit increase in TLC was associated with an increase of 0.073 mg/dL in milk zinc concentration ($\beta = 0.073$; $P < 0.001$). Herbal feed supplementation per se did not exert independent effects on milk phosphorus or zinc concentrations in the adjusted mixed-effects models ($P > 0.05$), whereas milk zinc concentration exhibited a significant decline at Day 120 compared with baseline observations ($P = 0.019$). Importantly, the observed associations remained significant after adjustment for herbal treatment effects and within-animal correlations, indicating that these relationships were independent of the phyto-genic supplementation regimes evaluated in the present study.

The findings suggest that routine haematological traits reflecting erythrocytic and immune status may serve as supportive indicators of selected milk minerals in indigenous dairy cattle receiving herbal feed additives. The persistence of the PCV-phosphorus and TLC-zinc associations under mixed-effects modelling provides novel insights into the physiological interplay among blood characteristics, herbal nutrition and mammary nutrient partitioning during lactation. These findings may contribute to the development of integrated strategies for monitoring animal health and milk nutritional quality in indigenous dairy production systems. Further studies involving larger populations and diverse physiological stages are warranted to validate these associations and elucidate the biological mechanisms underlying mineral transfer into milk.

Keywords: Sahiwal cattle, herbal feed additives, *Leptadenia reticulata*, *Moringa oleifera*, haematological traits, milk mineral composition, phosphorus, zinc, linear mixed-effects model

1. Introduction

Milk is universally recognized as one of the most complete natural foods owing to its balanced composition of proteins, lipids, lactose, vitamins and minerals that collectively contribute to growth, development and maintenance of physiological functions. Beyond its nutritional importance, the quality of milk determines its technological suitability for the manufacture of

dairy products and influences its commercial value. Among the various constituents of milk, mineral components have attracted increasing scientific attention because of their direct implications for human nutrition, neonatal development and dairy processing characteristics. Calcium and phosphorus are indispensable for skeletal development and maintenance, magnesium acts as a cofactor in numerous enzymatic reactions, while zinc participates in immune regulation, antioxidant defence and cellular metabolism (Walstra *et al.*, 2006; Fox and McSweeney, 2015) ^[18, 5]. Consequently, understanding the biological factors that influence milk mineral composition is of considerable importance for both animal production and public health.

India occupies the leading position in global milk production and contributes substantially to the world's dairy economy. According to the Department of Animal Husbandry and Dairying (DAHD, 2025) ^[3], the dairy sector not only serves as a major source of nutrition but also provides livelihood security to millions of rural households. Indigenous cattle breeds constitute an important component of this production system because of their adaptability to tropical climates, resistance to endemic diseases and ability to perform under relatively low-input management conditions. Among these breeds, the Sahiwal cattle hold a distinctive place owing to their superior heat tolerance, reproductive efficiency, longevity and comparatively high milk production among zebu breeds. The breed is particularly valued for its ability to maintain productivity under stressful environmental conditions, making it a suitable genetic resource for sustainable dairy farming in tropical regions. Despite these advantages, efforts aimed at improving milk quality in indigenous cattle remain limited compared with those directed toward enhancing milk yield.

Milk mineral composition is influenced by a multitude of factors including breed, stage of lactation, parity, nutritional status, environmental conditions and mammary gland physiology. Previous investigations have largely focused on evaluating the direct effects of dietary interventions on milk yield and major milk constituents such as fat, protein and lactose. However, the physiological determinants governing the secretion of minerals into milk have received relatively less attention. The mammary gland functions as a highly specialized organ capable of selectively transporting nutrients from systemic circulation into milk through tightly regulated homeostatic mechanisms. Therefore, identifying physiological indicators associated with variations in milk mineral composition may contribute to a deeper understanding of nutrient partitioning during lactation and facilitate the development of practical approaches for monitoring milk quality.

Haematological parameters are among the most commonly employed indicators of health and physiological status in domestic animals. Routine blood examinations provide valuable information regarding oxygen-carrying capacity, immune competence, metabolic adaptation and responses to nutritional or environmental challenges. Haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) have long been used to assess the health status of dairy cattle and to detect subclinical alterations before the appearance of overt clinical signs (Jain, 1993) ^[8]. Packed cell volume reflects erythrocytic mass and is considered an indicator of circulatory efficiency and hydration status, whereas total leukocyte count provides insights into immune responsiveness and inflammatory activity. Weiss and Wardrop (2010) ^[19]

emphasized that changes in these parameters may occur in response to nutritional deficiencies, oxidative stress, infectious conditions and physiological adjustments associated with lactation.

Emerging evidence suggests that the physiological state of an animal may influence the transfer of nutrients into milk. Phosphorus, for instance, constitutes an essential component of erythrocyte metabolism and participates in adenosine triphosphate (ATP) generation and 2,3-diphosphoglycerate synthesis, thereby contributing to cellular energy homeostasis and red blood cell function (Kaneko *et al.*, 2008) ^[9]. Disturbances in phosphorus metabolism have been associated with impaired erythrocyte integrity, reduced productivity and altered physiological performance in livestock (Underwood and Suttle, 1999; Radostits *et al.*, 2007) ^[17, 15]. Similarly, zinc is intimately linked with immune competence and serves as a structural and catalytic component of numerous enzymes involved in lymphocyte proliferation, neutrophil activity and antioxidant defence mechanisms. Zinc deficiency has been shown to impair both innate and adaptive immune responses, resulting in compromised disease resistance (Prasad, 2008) ^[14]. Given these biological roles, it is plausible that indicators of erythrocytic and leukocytic status may be associated with the secretion of specific minerals into milk.

In recent years, there has been growing interest in the use of phytogetic feed additives as natural alternatives to synthetic growth promoters and health modulators in livestock production. Medicinal plants possess a diverse array of bioactive compounds capable of influencing metabolism, immunity and oxidative balance. Among these, *Leptadenia reticulata* (Jivanti) has been extensively used in Ayurvedic medicine because of its galactagogue, immunomodulatory, adaptogenic and antioxidant properties. The beneficial effects of *Leptadenia reticulata* have been attributed to the presence of flavonoids, glycosides, sterols and alkaloids that may improve physiological resilience and productive performance (Dash *et al.*, 1972; Kumar *et al.*, 2012) ^[4, 1]. Experimental studies have indicated that supplementation with *Leptadenia reticulata* may positively influence haematological traits and reproductive performance without exerting adverse effects on animal health (Pathak, 2017) ^[13].

Similarly, *Moringa oleifera* has emerged as a promising fodder tree owing to its exceptional nutritional profile characterized by high-quality protein, essential amino acids, vitamins, minerals and antioxidant compounds. The inclusion of *Moringa oleifera* in livestock diets has been associated with improved nutrient digestibility, enhanced immune responsiveness and favourable effects on productive traits in ruminants (Babeker and Abdalbagi, 2015; Gopalakrishnan *et al.*, 2016) ^[2, 6]. Owing to these properties, phytogetic supplementation has attracted considerable attention as a strategy to improve animal performance while addressing consumer concerns regarding the excessive use of synthetic additives.

Although several investigations have independently examined the effects of *Leptadenia reticulata* and *Moringa oleifera* on haematological responses, milk production and compositional attributes, the biological relationship between blood profile and milk mineral secretion remains poorly understood. Most published studies have concentrated on treatment-induced changes in individual traits rather than exploring whether routine haematological parameters themselves are associated with the mineral composition of milk. Understanding such associations may provide valuable insights into the physiological processes governing nutrient allocation between

systemic circulation and mammary secretion and may identify practical biomarkers useful for herd-level monitoring.

Despite increasing interest in herbal feed additives and milk quality enhancement in indigenous dairy cattle, evidence linking routine haematological traits with milk mineral composition under field conditions remains limited. Moreover, information pertaining specifically to lactating Sahiwal cattle supplemented with *Leptadenia reticulata* and *Moringa oleifera* is virtually unavailable. Therefore, the present investigation was undertaken to evaluate the association between selected haematological parameters, namely haemoglobin concentration, packed cell volume, total erythrocyte count and total leukocyte count, and milk mineral constituents including calcium, phosphorus, magnesium and zinc in lactating Sahiwal cattle receiving herbal feed supplementation. It was hypothesized that specific haematological traits reflecting erythrocytic and immunological status may be associated with the secretion of selected minerals into milk. The findings of this study may contribute to a better understanding of the interplay between physiological health and milk nutritional quality and identify supportive indicators of milk mineral dynamics in indigenous dairy cattle.

2. Materials and Methods

Experimental Location and Ethical Considerations

The present investigation was conducted at the Instructional Dairy Farm, Nagla, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India. All experimental procedures involving animals were carried out in accordance with the institutional guidelines for the care and use of animals in research. Animal handling, sampling and management practices were performed under the supervision of qualified veterinarians to minimize animal discomfort and ensure welfare throughout the experimental period.

Experimental Animals and Management

A total of twenty-eight apparently healthy lactating Sahiwal cows maintained under uniform management conditions were selected for the study. Animals were chosen based on similar physiological status, stage of lactation, parity and health condition to minimize biological variation unrelated to the dietary treatments. Prior to the commencement of the experiment, all animals underwent routine clinical examination to confirm the absence of metabolic disorders, infectious diseases or other health abnormalities. The selected animals were housed in well-ventilated sheds under standard farm conditions and were provided with adequate space, clean drinking water and routine health care throughout the study period. Milking was performed twice daily following the standard management practices of the farm. The basal feeding regimen was formulated according to the nutrient requirements recommended by the Indian Council of Agricultural Research (ICAR, 2013) [7], ensuring that all animals received nutritionally adequate diets throughout the trial.

Experimental Design and Dietary Treatments

The feeding trial was conducted for a period of 120 days following a randomized block design. Twenty-eight lactating Sahiwal cows were randomly allocated into four treatment groups comprising seven animals each. Animals in the control group (T₀) received the basal diet without herbal

supplementation. Cows in T₁ received the basal diet supplemented with 90 g/day of *Leptadenia reticulata* powder, whereas those in T₂ were supplemented with 150 g/day of *Moringa oleifera* leaf powder. Animals assigned to T₃ received a combination of *Leptadenia reticulata* and *Moringa oleifera* according to the experimental protocol. The herbal supplements were incorporated into the concentrate mixture at levels standardized for the primary feeding trial, while the control group received only the basal ration without phyto-genic additives. The rationale for the selected supplementation doses and their effects on productive and haematological traits in the same experimental cohort has been reported previously (Kumar *et al.*, 2025; Kumar *et al.*, 2026) [10, 11].

Collection of Blood Samples and Haematological Analysis

Blood samples were collected from each experimental animal at five sampling intervals corresponding to Day 0 (baseline), Day 30, Day 60, Day 90 and Day 120 of the feeding trial. Approximately 5 mL of blood was aseptically collected from the jugular vein using sterile disposable needles and vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were transported immediately to the laboratory under refrigerated conditions and processed for haematological analysis. The use of anticoagulated blood minimized clot formation and ensured the reliability of the measured parameters.

Haematological analyses were performed according to the standard procedures described by Jain (1993) [8] and Weiss and Wardrop (2010) [19]. Haemoglobin concentration was estimated using the cyanmethemoglobin method and expressed as g/dL. Packed cell volume was determined using the microhaematocrit technique with capillary tubes and centrifugation and expressed as percentage (%). Total erythrocyte count and total leukocyte count were estimated using standard haemocytometric procedures and expressed as $\times 10^6/\mu\text{L}$ and $\times 10^3/\mu\text{L}$, respectively. These haematological indices were selected because they reflect oxygen-carrying capacity, erythrocytic status and immune competence, which may influence nutrient metabolism and partitioning during lactation.

Collection and Analysis of Milk Samples

Milk samples were collected from individual cows during the morning milking at the same sampling intervals used for blood collection (Days 0, 30, 60, 90 and 120). Representative milk samples were obtained after thorough mixing to ensure homogeneity and were transported immediately to the laboratory for mineral analysis. Milk mineral estimation was carried out according to the standard analytical procedures recommended by AOAC (2016) [1]. Calcium, phosphorus and magnesium concentrations were determined using established colorimetric and spectrophotometric methods and expressed as mg/dL. Zinc concentration was determined following acid digestion using an Atomic Absorption Spectrophotometer (AAS) according to standard mineral analytical procedures and expressed as mg/dL. The selected minerals were evaluated because of their nutritional importance and physiological relevance in lactating dairy cattle. Although the absolute concentrations and temporal trends of these milk minerals under herbal supplementation have been reported previously (Kumar *et al.*, 2026) [11], the present study specifically investigates their association with selected haematological traits using linear mixed-effects modelling.

Statistical Analysis

The study generated repeated observations from the same animals over the 120-day experimental period, resulting in a total of 140 observations (28 cows $\times$ 5 sampling intervals). Therefore, linear mixed-effects models (LMM) were employed to appropriately account for within-animal correlations arising from repeated measurements. All statistical analyses were performed using IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA). The covariance structure for repeated observations was specified as compound symmetry (CS), which provided an adequate fit for the longitudinal data. Animal identification number was included as a random effect, whereas treatment group and sampling day were fitted as fixed effects. The respective haematological trait (packed cell volume or total leukocyte count) was included as a continuous covariate in the mixed-effects models to evaluate its association with milk phosphorus and milk zinc concentrations while accounting for within-animal dependency arising from repeated measurements.

The linear mixed-effects model used in the present study can be represented as follows

$$[Y_{ijk} = \mu + T_i + D_j + \beta X_{ijk} + A_k + \epsilon_{ijk}]$$

where (Y_{ijk}) is the observed milk mineral concentration, (μ) is the overall mean, (T_i) is the fixed effect of the (i^{th}) treatment group, (D_j) is the fixed effect of the (j^{th}) sampling day, (β) is the regression coefficient associated with the haematological predictor, (X_{ijk}) is the value of the corresponding haematological trait, (A_k) is the random effect of the (k^{th}) animal to account for repeated observations within individuals, and (ϵ_{ijk}) is the residual error term.

Statistical significance was declared at $P < 0.05$, whereas $P < 0.001$ was considered highly significant. Results are presented as parameter estimates, standard errors, t-values and corresponding probability values.

3. Results and Discussion

3.1 Association Between Erythrocytic Status (PCV) and Milk Phosphorus Concentration

To appropriately account for the repeated-measures design and within-animal dependency generated during the 120-day feeding trial, linear mixed-effects models (LMM) were employed. In these models, Animal ID was treated as a random effect, whereas treatment groups and sampling days were included as fixed effects along with the selected haematological predictor.

Table 1: Linear mixed-effects model evaluating factors associated with milk phosphorus concentration in lactating Sahiwal cattle (n = 28; 140 observations)

Variable	Estimate (β)	SE	t-value	P-value
Intercept	58.296	12.891	4.522	<0.001
Treatment (T_1)	1.379	2.392	0.577	0.564
Treatment (T_2)	3.811	2.409	1.582	0.114
Treatment (T_3)	1.462	2.368	0.617	0.537
Day 30	1.458	2.652	0.550	0.583
Day 60	0.576	2.708	0.213	0.831
Day 90	0.168	2.679	0.063	0.950
Day 120	-1.142	2.659	-0.430	0.667
Packed Cell Volume (PCV)	0.981	0.387	2.535	0.011

$P < 0.05$ indicates statistical significance. Reference categories: T_0 (Control) and Day 0.

The mixed-effects analysis demonstrated that packed cell volume (PCV) maintained an independent and statistically significant positive association with milk phosphorus concentration after accounting for treatment effects, sampling day and repeated observations within individual animals. The estimated regression coefficient indicated that each one-unit increase in PCV was associated with an average increase of 0.981 mg/dL in milk phosphorus concentration ($P = 0.011$). None of the treatment groups or sampling intervals exerted significant effects on milk phosphorus levels ($P > 0.05$).

Phosphorus is a vital constituent of erythrocyte metabolism and contributes to ATP generation and the synthesis of 2,3-diphosphoglycerate (2,3-DPG), which regulates oxygen delivery through alterations in haemoglobin affinity (Kaneko *et al.*, 2008) [9]. Adequate phosphorus availability is therefore essential for maintaining cellular energy balance and normal erythrocyte function. Disturbances in phosphorus metabolism have been reported to adversely affect physiological performance and metabolic efficiency in livestock species (Underwood and Suttle, 1999) [17]. Furthermore, phosphorus deficiency has been associated with impaired erythrocytic function, reduced feed utilization efficiency and decreased productive performance in ruminants (Radostits *et al.*, 2007) [15].

The persistence of the association between PCV and milk phosphorus concentration following adjustment for repeated measurements suggests that erythrocytic status may be linked with phosphorus partitioning into milk. Since phosphorus is simultaneously required for maintaining red blood cell metabolism and supporting mammary secretory activity, this observation may indicate a potential physiological relationship between erythrocytic status and phosphorus dynamics during lactation. Although the precise physiological pathways underlying this association were not investigated in the present study, the findings suggest that routinely measured haematological parameters may provide valuable insights into mineral dynamics in lactating dairy cattle. However, further mechanistic investigations are required to elucidate the biological pathways underlying these associations.

3.2 Association Between Leukocytic Status (TLC) and Milk Zinc Concentration

A separate linear mixed-effects model was developed to evaluate the relationship between total leukocyte count (TLC) and milk zinc concentration while accounting for treatment allocation, sampling day and repeated measurements within animals.

Table 2: Linear mixed-effects model evaluating factors associated with milk zinc concentration (mg/dL) in lactating Sahiwal cattle (n = 28; 140 observations)

Variable	Estimate (β)	SE	t-value	P-value
Intercept	-0.067	0.136	-0.494	0.621
Treatment (T_1)	0.005	0.018	0.255	0.799
Treatment (T_2)	-0.027	0.019	-1.433	0.152
Treatment (T_3)	-0.012	0.018	-0.669	0.504
Day 30	-0.004	0.020	-0.211	0.833
Day 60	-0.017	0.020	-0.873	0.383
Day 90	-0.023	0.020	-1.155	0.248
Day 120	-0.047	0.020	-2.345	0.019
Total Leukocyte Count (TLC)	0.073	0.016	4.579	<0.001

$P < 0.05$ indicates statistical significance. Reference categories: T_0 (Control) and Day 0.

The mixed-effects model identified total leukocyte count (TLC) as a significant positive predictor of milk zinc

concentration. After controlling for treatment group, sampling interval and within-animal correlations, each unit increase in TLC was associated with an average increase of 0.073 mg/dL in milk zinc concentration ($P < 0.001$). Treatment groups did not significantly influence zinc concentration ($P > 0.05$). However, milk zinc concentration at Day 120 was significantly lower than baseline observations ($P = 0.019$), suggesting temporal variation during the later phase of lactation. The decline in milk zinc concentration observed at Day 120 might be attributed to the advancing stage of lactation and physiological shifts in mammary nutrient partitioning, which may influence trace mineral concentrations in milk.

Zinc is an indispensable trace mineral involved in numerous immunological processes, including lymphocyte proliferation, neutrophil activity and antioxidant defence. Prasad (2008) [14] reported that zinc deficiency compromises both innate and adaptive immunity by impairing leukocyte development and function. Spears and Weiss (2008) [16] further emphasized the importance of zinc in maintaining disease resistance and immune competence in dairy cattle. Underwood and Suttle (1999) [17] also documented the close relationship between zinc status and immunological responsiveness in livestock.

The significant association observed between TLC and milk zinc concentration suggests that immune status may be linked with zinc secretion into milk. Although zinc transporter proteins such as the ZnT and ZIP families were not evaluated in the present investigation, previous studies have demonstrated their importance in regulating zinc transport across mammary epithelial cells. Higher TLC values within the physiological range may reflect enhanced immune responsiveness, which could influence zinc mobilization and subsequent secretion into milk. Nevertheless, further mechanistic investigations are required to elucidate the biological pathways underlying these associations.

3.3 General Discussion and Practical Implications

The present study provides evidence supporting the existence of associations between selected haematological traits and specific milk minerals in lactating Sahiwal cattle receiving herbal supplementation. Importantly, these relationships remained statistically significant after accounting for treatment effects, sampling day and repeated measurements through mixed-effects modelling. This analytical approach minimized the potential bias associated with pseudoreplication and strengthened the reliability of the observed findings.

The persistence of the PCV-phosphorus and TLC-zinc associations indicates that routine haematological parameters may reflect aspects of nutrient partitioning occurring during lactation. Notably, the absence of significant treatment effects in both mixed models suggests that these relationships were not substantially influenced by supplementation with *Leptadenia reticulata* or *Moringa oleifera*. Instead, the findings may reflect physiological interactions between circulatory function, immune competence and mammary secretory processes.

Although the present study involved a relatively small experimental population maintained under a single management system, the utilization of 140 longitudinal observations analysed through mixed-effects models enhanced statistical efficiency by accounting for within-animal variability over time. Nevertheless, caution should be exercised while extrapolating these findings to other breeds and production environments, and validation studies

involving larger populations and diverse production systems are warranted. From a practical perspective, routine blood profiling may provide supportive information regarding milk mineral dynamics under field conditions and could potentially complement existing approaches used to monitor animal health and milk quality.

Further investigations involving larger populations, different breeds and diverse physiological stages are warranted to validate these findings and elucidate the molecular mechanisms governing the transfer of minerals between blood and milk in dairy cattle.

4. Conclusion

The present investigation demonstrated that selected haematological traits were significantly associated with specific milk minerals in lactating Sahiwal cattle supplemented with *Leptadenia reticulata* and *Moringa oleifera*. Using linear mixed-effects modelling to account for repeated measurements within animals, packed cell volume (PCV) was identified as an independent positive predictor of milk phosphorus concentration, whereas total leukocyte count (TLC) emerged as a significant positive predictor of milk zinc concentration. Importantly, these associations remained significant even after adjustment for treatment group and sampling day, thereby strengthening the validity and reliability of the observed relationships.

The absence of significant treatment effects in the mixed-effects models suggests that the associations between PCV and phosphorus and between TLC and zinc were not substantially influenced by phytogenic supplementation under the conditions of the present study. Rather, these findings indicate that physiological characteristics related to erythrocytic status and immune competence may contribute to the regulation of mineral partitioning between blood circulation and mammary secretion during lactation.

From a practical perspective, routinely measured haematological parameters may serve as supportive indicators of selected milk mineral dynamics in indigenous dairy cattle maintained under field conditions. Such indicators could complement conventional approaches used for monitoring animal health and evaluating aspects of milk nutritional quality. Although the exact biological mechanisms underlying these associations remain to be elucidated, the present findings provide a basis for future investigations exploring the interactions among blood physiology, mammary function and nutrient transport.

Further studies involving larger animal populations, diverse physiological stages and different production systems are warranted to validate these findings and elucidate the mechanisms governing mineral transfer into milk. Such investigations may facilitate the development of evidence-based approaches integrating physiological biomarkers with milk quality assessment and may contribute to improved nutritional monitoring and sustainable management strategies in indigenous dairy production systems.

Acknowledgements

The authors are grateful to the Department of Livestock Production and Management, College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, for providing the necessary facilities and support required to conduct this investigation. The authors also acknowledge the staff of the Instructional Dairy Farm, Nagla, for their assistance in animal management, sample collection and

execution of the feeding trial.

Conflict of Interest

The authors declare that they have no conflict of interest related to this study.

Author Contributions

Gyan Deepak: Conceptualization, investigation, data collection, statistical analysis, interpretation of results and manuscript preparation.

Dr. Brijesh Singh: Supervision, methodology development, critical review of the manuscript and overall guidance throughout the study.

Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

References

1. AOAC. Official methods of analysis of AOAC International. 20th ed. Gaithersburg (MD): Association of Official Analytical Chemists; 2016.
2. Babeker EA, Abdalbagi YMA. Effect of feeding different levels of *Moringa oleifera* leaves on performance, haematological, biochemical and some physiological parameters of Sudan Nubian goats. *Online Journal of Animal and Feed Research*. 2015;5:50-61.
3. Department of Animal Husbandry and Dairying (DAHD). Basic animal husbandry statistics 2025. New Delhi: Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; 2025.
4. Dash B, Sharma BK, Chopra IC. Studies on the pharmacological properties of *Leptadenia reticulata*. *Bulletin of the Indian Institute of History of Medicine*. 1972;2:1-10.
5. Fox PF, McSweeney PLH. Dairy chemistry and biochemistry. New York: Springer; 2015.
6. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*. 2016;5:49-56.
7. Indian Council of Agricultural Research (ICAR). Nutrient requirements of cattle and buffalo. New Delhi: ICAR; 2013.
8. Jain NC. Essentials of veterinary hematology. Philadelphia (PA): Lea and Febiger; 1993.
9. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th ed. San Diego (CA): Academic Press; 2008.
10. Kumar GD, Singh B, Sharma RK, Singh SK, Kumar S, Palod J, et al. Effects of *Leptadenia reticulata* and *Moringa oleifera* feeding on hematological profile of lactating Sahiwal cattle under farm conditions. *International Journal of Advanced Biochemistry Research*. 2025;9(8):1012-1015. <https://doi.org/10.33545/26174693.2025.v9.i8m.5405>
11. Kumar GD, Singh B, Palod J, Sharma RK, Kumar S, Singh SK, et al. Effect of *Leptadenia reticulata* and *Moringa oleifera* supplementation on physico-chemical properties and mineral composition of milk in lactating Sahiwal cattle. *International Journal of Advanced Biochemistry Research*. 2026;10(6):139-142. <https://doi.org/10.33545/26174693.2026.v10.i6b.8573>

12. Kumar D, Kumar A, Prakash O. Pharmacogenetic and phytochemical evaluation of *Leptadenia reticulata* (Retz.) Wight & Arn. *Journal of Medicinal Plants Research*. 2017;11(7):131-136.
13. Pathak S. Studies on therapeutic efficacy of herbal galactogogues in hypogalactic buffaloes. Jabalpur (MP): Nanaji Deshmukh Veterinary Science University; 2017.
14. Prasad AS. Zinc in human health: Effect of zinc on immune cells. *Molecular Medicine*. 2008;14(5-6):353-357.
15. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. Edinburgh: Saunders Elsevier; 2007.
16. Spears JW, Weiss WP. Role of antioxidants and trace elements in health and immunity of transition dairy cows. *The Veterinary Journal*. 2008;176:70-76.
17. Underwood EJ, Suttle NF. The mineral nutrition of livestock. 3rd ed. Wallingford (UK): CABI Publishing; 1999.
18. Walstra P, Wouters JTM, Geurts TJ. Dairy science and technology. 2nd ed. Boca Raton (FL): CRC Press; 2006.
19. Weiss DJ, Wardrop KJ. Schalm's veterinary hematology. 6th ed. Ames (IA): Wiley-Blackwell; 2010.

How to Cite This Article

Kumar GD, Singh B, Palod J, Sharma RK, Singh SK, Kumar S, et al. Association between Haematological traits and milk mineral composition in lactating Sahiwal cattle supplemented with *Leptadenia reticulata* and *Moringa oleifera*. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(6):165-170.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.