Short Communication

Evaluation of Proximate Composition and Mineral Profile of Raw Milk from Three Livestock Species

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Abstract: This study investigated the variation in raw milk composition as influenced by species and parity among milking animals. Milk samples from buffalo, cow, and goat were randomly collected from livestock farms across three districts in southern Punjab, Pakistan. Alongside milk sampling, detailed animal-related data, including species and parity, were recorded. The milk samples underwent chemical analysis to determine proximate composition and mineral content. The results revealed significant differences ($p < 0.05$) in all proximate variables, including total solids, fat, solids-not-fat, and crude protein, among the three species, while parity had no significant effect on these variables. Mineral parameters also varied significantly among species. Buffalo milk demonstrated the highest total solids (15.6%), and fat content (5.4%) compared to cattle (12.3% and 4.0%) and goat milk (12.1% and 3.88%), respectively. However, buffalo milk exhibited the lowest potassium content (37.7 ± 11.76) compared to cattle (60.7 ± 8.68) and goat milk (50.6 ± 8.05 mg/25 mL). Sodium levels were statistically similar across species. Calcium content was significantly higher in goat milk (647.5 ± 32.75 mg/25 mL) compared to buffalo (329.9 ± 44.38 mg/25 mL) and cattle milk (447.8 ± 30.41 mg/25 mL). While buffalo milk contained higher concentrations of major nutrients, it exhibited lower macro-mineral levels. These findings highlight the significant compositional variation in milk among species, providing a basis for selecting milk sources based on nutritional and mineral requirements.

Keywords: milk composition; buffalo; cattle; goat

1. Introduction

Milk production worldwide is primarily contributed by five animal species: dairy cattle, buffalo, goats, sheep, and camels [1]. In Pakistan, milk production is dominated by buffalo, accounting for 61% of the total, followed by cattle (35.5%), goats (1.6%), camels (1.6%), and sheep (0.07%) [2–3]. The composition of milk varies significantly across species and is influenced by multiple factors, including species type, parity, season, and milking intervals [4–6]. For instance, Walstra et al. [7] observed that buffalo milk differs significantly from cow and goat milk in terms of nutrient composition and its bioavailability. Furthermore, Stokes et al. [6] reported that age and parity influence milk composition; for example, while the fat content of milk remains stable, the protein content gradually decreases with advancing age.

Understanding the variability in milk composition is critical for optimizing its use in human diets and dairy product development. This study aims to evaluate the effects of species and parity on the proximate composition and mineral profile of raw milk from

buffalo, cattle, and goat, providing valuable insights into its nutritional and industrial implications.

2. Materials and Methods

2.1 Study area and sampling

Raw milk samples were collected from three districts in southern Punjab, Pakistan—Rahim Yar Khan (Liaquatpur), Muzaffargarh, and Bahawalpur—between May and August 2016. During this period, the climatic conditions across these districts were relatively uniform, allowing for the exclusion of seasonal effects on milk composition. A total of 41 buffaloes, 46 cows, and 42 goats were randomly selected from animal farms within these districts for sampling. Information regarding breed, age, parity, offspring sex, and feeding practices was recorded alongside the milk samples. The majority of the animals were in the early to mid-lactation stages, and none were in late lactation. Milk samples (150 mL each) were kept at low temperatures during collection and transportation and subsequently frozen at -20 °C until analysis. Formalin, a legally permitted preservative for milk samples intended for compositional analysis, was added at a rate of 0.5 mL/L to ensure long-term preservation. All chemical analyses were conducted in the laboratories of Livestock Management and Animal Nutrition at the Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan.

2.2. Chemical analysis of milk

Total solids (TS) and total ash (TA) were determined following AOAC [8] methods, with TS assessed at 95 °C for 1.5 hours in a hot air oven and TA at 550 °C for 4 hours using a muffle furnace. Fat content was measured using Gerber's method, while crude protein was determined using the Kjeldahl digestion method, as per AOAC [8]. Lactose content was calculated by subtraction using the formula:

Lactose (%) = TS% - (Fat% + Crude Protein% + TA%).

Solids-not-fat (SNF) content was determined using the formula:

SNF (%) = TS% - Fat%

2.3. Determination of minerals of milk

The concentrations of sodium (Na) and potassium (K) in milk samples were determined using a Flame Photometer (Model 410, Sherwood Scientific Ltd, UK). The preparation of milk samples followed these steps: One mL of raw milk was mixed with 10 mL of nitric acid (HNO_3) in a volumetric flask and heated at 60-70 °C for 15 minutes until the solution became clear. The transparent solution was then further heated at 100 °C for 30 minutes. In the second step, 5 mL of perchloric acid ($HClO_4$) was added to the digested material, and the mixture was heated at 100 °C until the volume reduced to 1-2 mL. The digest was then diluted with distilled water to a final volume of 25 mL. The concentrations of Na and K were calculated using the following formula:

C2 = X / C1 × Y

Where X = reading of the standard solution, Y = reading of the sample solution, C1 = concentration of standard solution and C2 = concentration of sample solution

The concentrations of calcium (Ca) and iron (Fe) in the milk samples were measured using an Atomic Absorption Spectrometer (AAS). The samples were digested following the method described earlier, after which they were analyzed using the Atomic Absorption Spectrometer. The metal concentrations were then calculated using the following formula:

Concentration (mg/L or ppm) = Concentration of the element through AAS (ppm) x sample volume/ sample weight.

2.4 Statistical analysis

Statistical analyses were performed using the GLM procedure of Minitab® 16.1.1.0. The effects of species (fixed) and parity (random) parameters were evaluated according to the model:

$$Y_{ijk} = \mu + S_i(P_j) + P_j + \varepsilon_{ij}$$

Where Y_{ijkl} is the dependent variable, μ is the overall mean, $S_i(P_j)$ represents the effect of ith species which was nested under parity, P_j is the effect of jth parity and ε_{ij} is the residual error. Results were presented as least square means with standard error of the means (SEM) and were considered statistically significant when the P-value was ≤ 0.05.

3. Results and Discussion

3.1. Proximate Composition of Milk

The proximate composition of milk from buffalo, cattle, and goats is summarized in Table 1. Species significantly influenced the composition of milk; however, parity did not show any association with milk composition ($p > 0.05$).

Total solids (%) were significantly higher in buffalo milk (15.6 ± 0.7) compared to cattle (12.1 ± 0.5) and goat milk (12.3 ± 0.5; $p < 0.001$). Solids not fat (%) was also higher in buffalo milk (10.2 ± 0.6) than in cattle (8.3 ± 0.4) and goat milk (8.2 ± 0.5; $p = 0.027$). Fat content (%) was highest in buffalo milk (5.4 ± 0.5), followed by goat milk (4.0 ± 0.4) and cattle milk (3.9 ± 0.3; $p = 0.028$). Crude protein (%) was highest in buffalo milk (4.2 ± 0.2), followed by goat milk (3.7 ± 0.1), and cattle milk (3.1 ± 0.2), with significant variation between species ($p < 0.001$). Lactose (%) was highest in buffalo milk (5.29 ± 0.601), followed by cattle (4.18 ± 0.41) and goat milk (4.19 ± 0.44; $p < 0.001$). Total ash (%) was lowest in buffalo milk (0.75 ± 0.03) compared to goat milk (0.93 ± 0.02) and cattle milk (0.69 ± 0.02; $p < 0.001$).

In the present study, the concentration of TS in buffalo milk was the highest than the milk of other two species. Imran et al. [9] observed the similar values and similar trends of TS in their study to our values. However, Mahmood et al. [10] found higher TS values in buffalo milk but the similar values in goat and cow milks than our values. The concentration of TS is a valuable measurement that can give an indirect measurement of water adulteration in milk. However, this measure can be misleading to some extent as adulteration of sand or other solid particles may also increase this value. Milk adulteration leads to economic losses, deterioration of the quality of the end products and a risk to the consumer's safety [11].

Table 1. Influence of species and parity on the proximate composition of raw milk collected from three districts in southern Punjab.

Parameters	Livestock Species, means ± SEM [1]			*p* Value	
	Buffalo	Cow	Goat	Species	Parity
Samples size, n	41	46	42		
Total solids, %	15.6 ± 0.7 [a]	12.1 ± 0.5 [b]	12.3 ± 0.5 [b]	<0.001	0.238
Solids not fat, %	10.2 ± 0.6 [a]	8.3 ± 0.4 [b]	8.2 ± 0.5 [b]	0.027	0.228
Fat, %	5.4 ± 0.5 [a]	3.9 ± 0.3 [b]	4.0 ± 0.4 [b]	0.028	0.155
Crude protein, %	4.2 ± 0.2 [a]	3.7 ± 0.1 [b]	3.1 ± 0.2 [c]	<0.001	0.175
Lactose, %	5.29 ± 0.60 [a]	4.18 ± 0.41 [b]	4.19 ± 0.44 [b]	<0.001	0.217
Total ash, %	0.75 ± 0.03 [a]	0.69 ± 0.02 [b]	0.93 ± 0.02 [c]	<0.001	0.425

[1] SEM: Standard error of mean. [a–c] Values with different superscripts in a row are significantly different ($p ≤ 0.05$).

In the present study, buffalo milk exhibited the highest fat content. Similar trends were observed by Mahmood et al. [10], who reported comparable fat levels in goat and cow milk but found buffalo milk to contain higher fat content (7.97%) than in our study. Mansour et al. [12] also reported the highest fat content in buffalo milk, followed by goat and cow milk. These findings align with the literature, which indicates that milk fat concentration is influenced by both species and breed of the milking animal [6].

The protein concentration in the milk samples ranged from 3.11% to 4.16%, with buffalo milk being a richer source of both protein and fat compared to cow and goat milk. Similar protein values were reported in previous literature [9, 13] who studied milk

samples from cows and buffaloes. Mahmood et al. [10] also reported protein values fairly close to those observed in the present study.

Lactose, commonly known as milk sugar, ranged from 3.84% to 5.30% in the tested milk samples, with buffalo milk containing the highest amount. These results align with previous studies [10], both in terms of the range and trends. Lactose concentration was not significantly affected by the parity of the milking animals. In contrast, Enb et al. [14] reported higher lactose content in cow milk (5%) compared to buffalo milk (4.8%). Králíčková et al. [15] found that lactose content in goat milk remained consistent across different lactation stages. Diseases, such as subclinical mastitis may reduce lactose levels in milk [6].

In the present study, goat milk had the highest concentration of total ash (TA), suggesting that it is a rich source of essential minerals required by the human body. Similar trends in TA were observed by others [9], whose findings align with those of our study. Enb et al. [14] and Mahmood et al. [10] also reported similar TA values for buffalo and cow milk to our results; however, Mahmood et al. [10] found a lower TA (0.75%) in goat milk.

In the present study, the effect of parity in milking animals was found to be non-significant for all examined parameters, including total solids (TS), solids-not-fat (SNF), fat, crude protein, lactose, and total ash (TA). However, Králíčková et al. [15] found that the number of parities significantly influenced milk yield and composition in goats. While milk fat remained consistent, milk protein gradually diminished with increasing age. An overview of Holstein Dairy Herd Improvement Association lactation records revealed that milk protein typically decreases by 0.10 to 0.15 units over five or more lactations, or approximately 0.02 to 0.05 units per lactation [6]. Similarly, Şahin et al. [16] reported that the number of parities in water buffalo significantly impacted the levels of most milk constituents.

3.2. Mineral Profile of Milk

The mineral profile of raw milk samples from buffalo, cow, and goat are shown in Table 2. The results showed significant differences across species for potassium, calcium, and iron, while sodium concentrations did not exhibit a significant variation.

Potassium concentrations (mg/25 mL) were significantly higher in goat milk (60.7 mg/25 mL) compared to buffalo (37.7 mg/25 mL) and cow milk (50.6 mg/25 mL), with a *p*-value of 0.044. For calcium (mg/25 mL), goat milk contained the highest concentration (647.5 mg/25 mL), while buffalo milk had the lowest (329.9 mg/25 mL). Iron concentration (mg/25 mL) was also significantly different across species, with goat milk showing the highest iron content (0.54 mg/25 mL), followed by buffalo milk (0.42 mg/25 mL) and cow milk (0.41 mg/25 mL). Sodium content (mg/25 mL) ranged from the lowest in buffalo milk (15.5 mg/25 mL) to the highest in cow milk (23.8 mg/25 mL), with goat milk having intermediate levels (19.6 mg/25 mL).

Table 2. Influence of species and parity on the mineral composition of raw milk collected from three districts in southern Punjab.

Parameters	Livestock Species, means ± SEM [1]			*p* Value	
	Buffalo	**Cow**	**Goat**	**Species**	**Parity**
Samples size, n	41	46	42		
Potassium, mg/25 mL	37.7 ± 11.8 [a]	50.6 ± 8.1 [b]	60.7 ± 8.7 [b]	0.044	0.965
Sodium, mg/25 mL	15.5 ± 4.4	19.6 ± 3.0	23.8 ± 3.2	0.293	0.869
Calcium, mg/25 mL	329.9 ± 44.4 [a]	447.8 ± 30.4 [b]	647.5 ± 32.8 [c]	<0.001	<0.001
Iron, mg/25 mL	0.42 ± 0.04 [a]	0.41 ± 0.03 [a]	0.54 ± 0.03 [b]	0.003	0.437

[1] SEM: Standard error of mean. [a–c] Values with different superscripts in a row are significantly different ($p \leq 0.05$).

Milk is a rich source of sodium (Na) and a fair source of potassium (K), making milk and dairy products important dietary contributors of these two essential macro-minerals [17]. Sodium plays a critical role in maintaining extracellular fluid balance, osmotic pressure, and activating various enzymes in the body, such as amylase [17]. In the present study, goat milk exhibited the highest concentration of potassium. However, parity did not affect the concentrations of sodium or potassium in the milk samples.

The sodium concentrations observed in all species studied were lower than those reported in previous studies [18–19], and much lower than the values recommended by the WHO [20]. Our findings, however, align more closely with those reported by Imran et al. [9], while Kravić et al. [21] reported even lower sodium concentrations than ours. The variation in sodium concentrations could be attributed to differences in the animal's diet, breed, geographical location, and farming practices, as sodium intake from feed and water sources can significantly impact the mineral content in milk.

Overall, the potassium levels observed in the present study were lower than those reported by others [9, 19], and much lower than the values suggested by other researchers [20]. The variation in potassium concentrations might be due to differences in feeding practices, lactation stage, breed, or the overall health status of the animals. Potassium content can also be influenced by environmental factors such as soil composition and water quality, which affect the mineral uptake of livestock. Additionally, methodological differences in sample collection, handling, and analysis between studies could contribute to these discrepancies.

In the present study, goat milk exhibited the highest concentrations of calcium (Ca) and iron (Fe) compared to buffalo and cow milk. The observed levels of Ca in buffalo and cow milk were surprisingly lower than those reported in the literature [9, 18–19]. Additionally, calcium concentrations showed significant variation among the different parities of milking animals in the present study. Published literature supports the finding that Ca concentrations can vary with parity, breed, or species of milking animals [22]. Some studies report that calcium levels decrease with increasing parity, likely due to higher calcium demands during late lactation and increased maternal mineral mobilization to support offspring growth [23].

When comparing iron concentrations to the available literature, the values obtained in this study were similar to those reported by Hussain et al. [24], though higher values were reported by others [14]. The variation in minerals concentrations between studies may stem from differences in the analytical methods, differences in feeding practices, lactation stage, breed, or the overall health status of the animals.

4. CONCLUSIONS

This study demonstrates that milk composition, particularly proximate and mineral content, varies significantly across species, with buffalo milk showing the highest concentrations of total solids, fat, and crude protein, while goat milk contained the highest levels of calcium. Parity did not have a significant effect on these variables. These findings emphasize the importance of species selection in dairy production, especially in meeting specific nutritional needs. Further research is needed to explore the factors influencing these variations for enhancing milk quality based on the desired nutrient profile.

Institutional Review Board Statement: Not Applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Barlowska, J.; Szwajkowska, M.; Litwinczuk, Z.; Krol, J. Nutritional Value and Technological Suitability of Milk from Various Animal Species Used for Dairy Production. *Compr. Rev. Food Sci. Food Saf.* **2011**, *10*, 291–302. [Google Scholar] [CrossRef]

2. Tahir, M. N.; Riaz, R.; Bilal, M.; Nouman, H. M. Current Standing and Future Challenges of Dairying in Pakistan: A Status Update. In *Milk Production, Processing and Marketing*; Javed, K., Ed.; InTech Open, **2019**; ISBN 978-1-78985-730-6, DOI: 10.5772/intechopen.73442. [Google Scholar] [CrossRef]

3. Economic Survey of Pakistan 2023–24; Ministry of Finance, Government of Pakistan: Islamabad, 2024. [Online Link] (Accessed November 10, 2024)

4. Bansal, B. K.; Hamann, J.; Grabowski, N. T.; Singh, K. B. Variation in the Composition of Selected Milk Fraction Samples from Healthy and Mastitis Quarters and Its Significance for Mastitis Diagnosis. *J. Dairy Res.* **2005**, *75*, 144–152. [Google Scholar] [CrossRef]

5. Ahmad, S.; Anjum, F. M.; Huma, N.; Sameen, A.; Zahoor, T. Composition and Physicochemical Characteristics of Buffalo Milk with Particular Emphasis on Lipids, Proteins, Minerals, Enzymes, and Vitamins. *J. Anim. Plant Sci.* **2013**, *23*, 62–74. [Google Scholar]

6. Stokes, S. R.; Waldner, D. N.; Jordan, E. R.; Looper, M. L. Managing Milk Composition: Normal Sources of Variation; AgriLife Extension, Texas A&M University System, 2014. [Online Link] Accessed November 10, 2024.

7. Walstra, P.; Wouters, J. T. M.; Geurts, T. J. Milk Main Characteristics; Milk Components. In *Dairy Science and Technology*, 2nd ed.; CRC, Taylor and Francis Group: London, New York, 2006; pp 12–103.

8. AOAC International. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, 2000. [Google Scholar]

9. Imran, M.; Khan, H.; Hassan, S. S.; Khan, R. Physicochemical Characteristics of Various Milk Samples Available in Pakistan. *J. Zhejiang Univ. Sci. B* **2008**, *9*, 546–551. [Google Scholar]

10. Mahmood, A.; Usman, S. A Comparative Study on the Physicochemical Parameters of Milk Samples Collected from Buffalo, Cow, Goat, and Sheep of Gujrat, Pakistan. *Pak. J. Nutr.* **2010**, *9*, 1192–1197. [Google Scholar]

11. Mabrook, M. F.; Petty, M. C. A Novel Technique for the Detection of Added Water to Full-Fat Milk Using Single-Frequency Admittance Measurements. *Sens. Actuators, B* **2003**, *96*, 215–218. [Google Scholar] [CrossRef]

12. Mansour, A. I. A.; El-Loly, M. M.; Ahmed, R. O. A Preliminary Detection of Physical and Chemical Properties, Inhibitory Substances, and Preservatives in Raw Milk. *Int. J. Food Saf.* **2012**, *14*, 93–103. [Google Scholar]

13. Elvingson, L. Calcium in Water Buffalo (*Bubalus bubalis*) Milk: Implications for Mozzarella Production. Swed. Univ. Agric. Sci. **2013**, *377*. [Google Scholar]

14. Enb, A.; Abou-Donia, M. A.; Abd-Rabou, N. S.; Abou-Arab, A. A. K.; El-Senaity, M. H. Chemical Composition of Raw Milk and Heavy Metals Behavior during Processing of Milk Products. *Glob. Vet.* **2009**, *3*, 268–275. [Google Scholar]

15. Králíčková, Š.; Kuchtík, J.; Filipčík, R.; Lužová, T.; Šustová, K. Effect of Chosen Factors on Milk Yield, Basic Composition, and Somatic Cell Count of Organic Milk of Brown Short-Haired Goats. *Acta Univ. Agric. Silvic. Mendelianae Brun.* **2013**, *61*, 99–105. [Google Scholar] [CrossRef]

16. Şahin, A.; Ulutaş, Z.; Yıldırım, A.; Kul, E.; Aksoy, Y.; Sözen, E. U. Ö.; Kaplan, Y. The Effect of Some Environmental Factors on Milk Composition of Anatolian Buffaloes. *Sci. Pap., Ser. D Anim. Sci.* **2016**, *59*, 57–64. [Google Scholar]

1. Belitz, H. D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 3rd ed.; Springer-Verlag: Heidelberg, Berlin, 2009; pp 421–423.

2. Ikem, A.; Nwankwoala, A.; Odueyungbo, S.; Nyavor, K.; Egiebor, N. Levels of 26 Elements in Infant Formula from USA, UK, and Nigeria by Microwave Digestion and ICP-OES. *Food Chem.* **2002**, *77*, 439–447. [Google Scholar] [CrossRef]

3. Jaffar, M.; Shah, M. H.; Shaheen, N.; Khaliq, A.; Tariq, S. R.; Manzoor, S.; Saqib, M. Pre- and Post-Expiry Metal Levels in Canned Dry Milk. *Nutr. Food Sci.* **2004**, *34*, 65–71. [Google Scholar] [CrossRef]

4. WHO. Minor and Trace Elements in Breast Milk. In *Report of Joint WHO/IAEA Collaborative Study*; World Health Organization: Geneva, 1989; pp 157–159. [Online Link]

5. Kravić, S. Z.; Suturović, Z. J.; Đurović, A. D.; Brezo, T. Z.; Milanović, S. D.; Malbaša, R. V.; Vukić, V. R. Direct Determination of Calcium, Sodium, and Potassium in Fermented Milk Products. *Acta Period. Technol.* **2012**, *43*, 43–49. [Google Scholar] [CrossRef]

6. Patino, E. M.; Pochon, D. O.; Faisal, E. L.; Cedres, J. F.; Mendez, F. I.; Stefani, C. G.; Pochon, D. O. Influence of Breed, Year, Season, and Lactation Stage on Buffalo Milk Minerals Content. *Ital. J. Anim. Sci.* **2007**, *19–22*. [Google Scholar] [CrossRef]

7. Kume, S.; Yamamoto, E.; Kudo, T.; Toharmat, T.; Nonaka, I. Effect of Parity on Mineral Composition in Milk and Plasma of Holstein Cows during Early Lactation. *Asian-Australas. J. Anim. Sci.* **1998**, *11*, 133–138. [Google Scholar] [CrossRef]

8. Hussain, Z. A.; Nazir, U.; Shafique, S.; Salm, M. Comparative Study for the Determination of Metals in Milk Samples Using Flame-AAS and EDTA Complex Metric Titration. *J. Sci. Res.* **2010**, *40*, 31–36. [Google Scholar]