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ORIGINAL RESEARCH ARTICLE

Determinants of milk composition and somatic cell count in buffaloes from the Terai belt of Nepal

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ABSTRACT

This study aimed to investigate the relationship between somatic cell count (SCC) and various milk quality parameters of buffaloes in the Chitwan district of Nepal during the year 2024. A total of 122 buffalo-owned households were selected using a cross-sectional survey design. Results indicated that average daily milk yield was highest during the first lactation (4.74 kg/day), followed by the second (4.54 kg/day), and third lactation (3.21 kg/day). Conversely, milk fat content showed a decreasing trend across successive lactations. Lactation stage significantly ($p<0.05$) influenced the milk's fat, solid-not-fat (SNF), protein, and lactose content. While parity exhibited a marginal correlation with lactose concentration, it did not significantly affect milk yield, fat, SNF, or protein content. A notable finding was that high-yielding buffaloes had significantly ($p<0.01$) lower fat content than low-yielding counterparts. In contrast, SNF, protein, and lactose percentages were significantly ($p<0.01$) higher in high-yielding groups compared to low and medium-yielding ones, indicating a substantial impact of production level on milk composition. No statistically significant ($p>0.05$) differences were observed in SCC and electrical conductivity (EC) across varying levels of milk production, parity, or lactation stages. These findings suggested that SCC and EC are not strongly influenced by these factors under current management conditions. Key challenges identified in the study area included poor hygiene of feeding and milking equipment, unbalanced feeding practices, inadequate waste management, limited infrastructure for milk marketing, and slow adoption of modern dairy technologies.

INTRODUCTION

One of the nation's fastest-growing agricultural sub-sectors is the cattle industry. Buffalo milk plays a vital role in Nepal's agrarian economy, serving as both a key nutritional source and a major contributor to household income. About 24.01% of the entire agricultural GDP came from livestock, which is an essential component of agriculture (MoALD, 2021/22). Buffalo are one of the primary livestock species cultivated in Nepal. In the nation's agricultural economy, buffaloes are used for their draft, meat, milk, and manure, which helps rural farmers make a living

(Paneru *et al.*, 2021). According to the Nepalese Agriculture 2078/2079 statistics report, there is an estimated 51,32,931 buffalo and 16,66,827 milking buffalo. In fiscal year 2078/2079, buffalo produced around 1,464,802 metric tons of milk. The number of buffalo in the Chitwan region is 77,896, and there are 28,992 buffalo that provide milk. In fiscal year 2078/2079, buffalo produced 27,944 metric tons of milk (MoALD, 2023). Despite being an issue with animal welfare, mastitis is the largest economic concern and a problem with food safety. Mastitis is characterized by physical, chemical, and bacteriological abnormalities in the milk as well as pathological changes in

the glandular tissue of the udder. In the dairy sector, mastitis is the most expensive illness, mostly due to the higher rate of cow replacement, treatment costs, lost output, and more labor. Mammary gland inflammation may be detected with high sensitivity by measuring the SCC (Kaskous *et al.*, 2022), or concentration of milk cells. The primary factor influencing variations in SCC is the migration of leukocytes from blood to tissue and ultimately to milk, which often occurs in response to an inflammatory response in the mammary tissue caused by bacteria invading the mammary gland (Sumon *et al.*, 2020). Sub-clinical mastitis (SCM) is characterized by a high somatic cell count (SCC) in milk, but no obvious systemic symptoms or abnormalities of the breast or milk are present. When compared to clinical mastitis, which causes significant financial losses, SCM is far more frequent in dairy cows.

Somatic cell count (SCC) is a frequently used indicator of udder health and milk quality in dairy animals. Due to the general disdain for farm sanitation, several infections, including mastitis, have been contracted by farm animals, especially cattle and buffalo. Mastitis is a frequent udder disease that can change the milk's composition, reduce milk production, and compromise food safety. Stress, milking techniques and frequency, breed, age and parity, shed management, lactation stage, body condition score, and body weight are some of the factors that influence the variation in somatic cell count(Viana *et al.*, 2025). Therefore, the primary problems facing the dairy sector are inadequate milk distribution marketing, uncontrolled waste, a lack of balanced diet, poor farm and feeding utensil hygiene, and a lack of use of current technology. For human survival, nourishment is necessary. One of the foods that is most frequently used and consumed is milk. Water, proteins, amino acids, minerals, lipids, vitamins, and fatty acids make up its composition. There are several functions and unique physiological roles for each of these components. Because buffalo milk has higher levels of fat, lactose, protein, and ash than cow milk, it is more nutritious overall and costs more (Chandrakar *et al.*, 2018). Buffalo milk has more protein than cow's milk. Caseins make up over 80% of the proteins in buffalo milk, followed by whey proteins (20%) and trace quantities of other proteins. Buffalo milk has nearly twice as much fat as cow's milk. Buffalo milk has more lactose than milk from cows, goats, sheep, and camels.

A typical indicator of milk quality and udder health in dairy cows is the somatic cell count (SCC). Farm animals, especially cattle and buffalo, have been infected with a variety of illnesses (including mastitis) because of the general disdain for farm sanitation. A prevalent udder ailment, mastitis can reduce milk production, change the milk's composition, and compromise food safety. Stress, milking methods and frequency, breed, age and parity, shed management, lactation stage, body condition score, and body weight are some of the factors that influence the variation in somatic cell count. Thus, the dairy industry's primary problems include inadequate milk distribution marketing, uncontrolled waste, a lack of balanced rations, poor farm and feeding utensil hygiene, and a lack of use of contemporary technology. The udder becomes infected with germs, which results in

mastitis. Milk's typical somatic cell population, which is minimal, tries to address this IMI right away. Leukocytes and bacteria in the diseased area both emit compounds, many of which function as chemo-attractants (Abdi *et al.*, 2021). A rise in somatic cells in milk is the result of neutrophils moving quickly from the circulation into the secretions of affected quarters. Restoring the gland's health will only require a small inflammatory episode if the bacteria are eliminated since neutrophils recruitment into the gland will stop (Rienesl *et al.*, 2022). Numerous epidemiological investigations have attempted to address the relationship between resistance to mastitis and SCC levels. Furthermore, bacteriological studies have seldom been used to identify causative microorganisms. While sub-clinical mastitis is frequently approximated from cow SCC levels during the lactation period, clinical mastitis is typically observed. The association between the rate of clinical mastitis and the herd-level somatic cell count (as determined by bulk milk SCC) appears to be influenced by the kind of infection that is frequently observed in herds. Since somatic cell counts are a measure of a cow's resistance or susceptibility to the illness, they may also be used to track the prevalence or severity of sub-clinical mastitis in herds or individual cows. Since SCC is a good indicator of intra-mammary infection (IMI), it plays a significant role in evaluating the quality, cleanliness, and mastitis management of milk (Pegolo *et al.*, 2021). Therefore, this study was conducted to assess the relationship between somatic cell count (SCC) in buffalo milk and several aspects of milk characteristics.

MATERIALS AND METHODS

The study was carried out on buffalo ranches in the south-central Nepalese region of Bharatpur Metropolitan City, Chitwan District, Bagmati Province of Nepal. The area is mostly agricultural, with a sizeable section of the populace working in the production of rice, wheat, and vegetables as well as raising animals, including buffalo, cattle, and goats. In the region, stall-feeding and grazing methods use locally accessible feed and fodder are frequently used in commercial dairy production. Even though agriculture and livestock are becoming more and more important, little is known about commercial agricultural methods and farm management. A cross-sectional investigation was conducted, and 122 buffalo milk samples were aseptically taken from the Bharatpur Metropolitan Area. From May to June 2024, this study was carried out at the National Cattle Research Program's Research Laboratory in Rampur, which is situated in the Bharatpur Metropolitan City of Chitwan. Primary data were collected through structured face-to-face interviews and direct milk sampling, while secondary data were sourced from published literature, journals, research reports, and official government documents from the Ministry of Agriculture and Livestock Development (MoALD) and the Ministry of Agricultural Development (MoAD). Null hypothesis: (Ho: μ1=μ2): The somatic cell count in buffalo milk does not significantly correlate with various milk characteristics. Alternative hypothesis: (H1: μ1≠μ2): The somatic cell count in buffalo milk is significantly correlated

with several milk characteristics.

To gather quantitative data from the various animal farms, the owner was questioned using a pre-made closed-type questionnaire. After the animals were done milking, we went to farms and collected milk samples. Additionally, research papers on the somatic cell count and milk attributes in livestock milk were also collected. The purpose of this action was to augment the original data. To preserve the integrity of the samples, stringent hygiene protocols were adhered to prior to collection. A milk-sampling dipper was used to extract the milk sample from the whole milk, minimizing the chance of sampling mistakes. Each animal's 40 ml milk sample was then taken and placed in a sterile plastic tube. The labels on the plastic tube bore the name of the owner and the animal identification of the farm. The LACTOS-CAN SCC Somatic Cell Counter was used to analyze the somatic cell count (SCC). Raw milk samples were homogenized using a small vortex mixer before examination. A microtube containing lyophilized SOFIA GREEN dye was then filled with 100 μL of homogenized raw milk and carefully mixed. To prevent bubble formation and splashing during pipetting, an aliquot of 8 μL of the produced mixture was carefully placed into the LACTOCHIP x4's microfluidic chamber. After 30 to 60 seconds of equilibration, the loaded LACTOCHIP x4 was placed into the device cartridge. The LACTOSCAN SCC software interface was used to start the analysis. Following sample loading, the pipette tip was removed using the appropriate release button and disposed of appropriately by leaving it within the sample-containing microtube. The Milko-Tester-Master Eco (Ultrasonic Milk Analyzer) was also used to assess the percentages of fat, solids-not-fat (SNF), protein, lactose, electrical conductivity, and density. To guarantee cleanliness, the equipment was cleaned with distilled water before analysis. Afte, milk sample to guarantee homogeneity, 10–15 mL of the sample was added to the analyzer. The findings for every measured parameter were shown on the device screen when the analysis, which took about a minute, was finished. After every measurement, distilled water was used to cleanse the analyzer once again. Every sample was examined using the same methodology. Before every sample analysis, the analyzer was cleaned using a daily cleaning solution to preserve measurement accuracy. For analytical purposes, buffaloes were categorized based on parity number, daily milk yield, and lactation stage. Statistical analysis was conducted using Microsoft Excel, SPSS version 26, and the General Linear Model (GLM), with a significance threshold set at p < 0.05. To ensure normalization, SCC data were log-transformed (log_{10}).

RESULTS AND DISCUSSION

Effect of lactation stage on physio-chemical properties and somatic cell count of buffalo milk

The variations in milk yield and milk composition, such as fat, SNF, protein, and lactose, across different stages of lactation in dairy animals, with statistically significant differences across stages of lactation; however, the EC and SCC values across lactation stages are relatively close, with no statistically significant difference (P > 0.05).Milk yield is highest during the 1st of lactation (4.74 ± 0.21) and decreases in the 2nd (4.54 ± 0.23) and 3rd stages (3.21 ± 0.18) (Table 1). The F-value of 11.27 and (P < 0.01) indicate a statistically significant decline in milk yield as lactation progresses, which is similarly finding with Baloch *et al.* (2023) reported the milk yield is highest during early lactation and then decreases subsequently with the progress of lactation. Some research on Murrah buffalo reported a significant effect of lactation stage on milk yield as the milk yield declined significantly during the late stage of lactation with a significant concomitant increase in milk fat. Similarly, Chisowa (2023) was also noted on the milk of Holstein cows, as the highest milk yield was recorded in the mid stage of lactation, followed by the early and late stages of lactation. Fat content is lowest during the 1st stage (7.54 ± 0.24) and gradually increases, reaching its peak in the 3rd stage (9.35 ± 35). The F-value of 8.72 and significant P-value show that fat content increases significantly as lactation progresses. Also, Shazinosh *et al.* (2024) reported that fat content increased as the lactation progressed. The overall average fat percent observed in buffalo milk 8.10±0.18 which was in close agreement with that reported in Purnathadi buffaloes (Kuralkar & Kuralkar, 2022) and Surti buffaloes and higher than present in other buffalo like Murrah, Nili Ravi (Zhao *et al.*, 2023). Numerous factors, including animal health, breed, lactation stage, season, feeding system, milking equipment and procedures, nutrition, and genetics, can affect the relative percentage of milk components. SNF content is highest in the 1st stage (9.25 ± 0.166) and decreases through the 2nd (8.49 ± 0.22) and 3rd stages (7.98 ± 0.23) but other reported data on SNF are 8.34% in the non- descriptive buffaloes and 10.09% in Pakistani buffaloes (Lutsenko *et al.*, 2021). The F-value of 10.24 and P-value of 0.000 indicated significant differences across stages, with SNF decreasing overtime. Protein content starts highest in the 1st stage (3.40 ± 0.06) and decreases through the 2nd (3.13 ± 0.08) and 3rd stages (2.94 ± 0.08). The F-value of 10.15, with a P-value of 0.0001, highlights significant variations in protein content across lactation stages, indicating metabolic changes that influence milk protein levels over time. The overall average protein percent in present study was observed as 3.19±0.05 which was in close agreement with finding of research on Mehsana, Surti and Bhadwari buffalo and lower that reported in Murrah buffalo and nearly similar with the research in water and swamp buffaloes (Emakpor *et al.*, 2024). Also, Bhattarai (2020) reported that lactation number was also found to have a highly significant effect (P < 0.01) on milk fat %, protein %, and a significant effect (P< 0.05) on 305-day milk yield. Lactose content is highest in the 1st stage (5.11 ± 0.90) and gradually declines through the 2nd (4.70 ± 0.12) and 3rd stages (4.43 ± 0.12). With an F-value of 10.00 and a P-value of 0.0001, the data show that lactose content significantly decreases across lactation stages. The overall average lactose percentage observed was 4.84±0.67, which was lower than present in Murrah (5.18), Nili-Ravi (5.28), and the triple crossbreed (5.13) as reported by Zhao *et al.* (2023). Likewise, Tanmaie *et al.* (2022) reported that the mean SCC values in the milk of first lactation were higher during the early lactation

stage, which dropped during the mid-lactation stage and increased marginally during the late lactation stage in buffaloes. This observation aligns with a previous study by Balabanova & Ivanova (2021), revealing that the non-significant effects of lactation stage on milk SCC. However, there was a significant relationship (P< 0.01) between SCC and stage of lactation due to the infection status of the quarter changing somatic cell concentration as lactation progressed (Costa *et al.*, 2020).

Effect of parity on physio-chemical properties and somatic cell count of buffalo milk

The effect of parity on milk yield and milk composition, somatic cell count, broken down by three groups: early, mid, and late lactation periods (Table 2). The milk yield increases from early (3.73 ± 0.29 kg) to mid (4.41 ± 0.19 kg) parity and is comparatively high in late parity (4.47 ± 0.25 kg). There is no statistically significant difference in milk yield across the different parity groups, as indicated by the F-value for milk yield of 1.51 and the p-value of 0.225. Our findings align with studies by Bhattarai (2020) which reported there is an increase in milk yield up to 5th parity and a decline within each group, the fat content changes slightly, with early parity showing 8.25 ± 0.43%, mid-parity at 7.85 ± 0.25%, and late parity at 8.39 ± 0.30%. There is no significant impact of parity on fat content, as indicated by the F-value

of 0.95 and the p-value of 0.38. Early parity has the highest SNF content: 9.29 ± 0.25% drops in late parity: 8.91 ± 0.19%. The F-value for SNF is 2.99 with a p-value of 0.05. There is no significant effect of parity on SNF content. The protein content marginally increases in late parity (3.28 ± 0.07) but declines from early (3.42 ± 0.34%) to mid parity (3.12 ± 0.06%). Early parity has the highest lactose level (5.14 ± 0.13%), mid-parity has the lowest (4.69 ± 0.10%), and late-parity has the slightest rise (4.93 ± 0.10%). Bhattarai (2020) reported a significant effect of parity on milk composition, and milk lactose increased in the second parity compared to late, i.e., fifth parity. Overall, the milk yield, fat%, SNF%, and protein contents are not significantly affected by parity, but lactose content has a marginal effect on parity. The SCC and EC values across the parity (Early, Mid, Late) are fairly close. F-value and P-value according to the p-value (P< 0.05), this suggested that parity had no statistically significant impact on SCC and electric conductivity (EC). Similarly, Tanmaie *et al.* (2022) revealing that milk SCC is affected by parity with first calves having low SCC. Similar results were reported by Balabanova & Ivanova (2021) who suggested that the non-significant effects of parity on milk SCC. As a result, it increased as lactation advanced, with progressively higher conductivities for quarters infected with minor or severe pathogens.

Table 1. Effect of lactation stage on physio-chemical properties and somatic cell count of buffalo milk.

Stage of lactation	Milk Yield (L)	Milk yield, milk composition and somatic cell count					
		Fat	SNF	Protein	Lactose	EC	SCC x 10^5
Stage 1st	4.74±0.21[a]	7.54±0.24[b]	9.25±0.166[a]	3.40±0.06[a]	5.11±0.90[a]	3.47± 0.05	6.21±0.11
Stage 2nd	4.54±0.23[a]	8.05±0.33[b]	8.49±0.22[b]	3.13±0.08[b]	4.70±0.12[b]	3.41± 0.70	6.32±0.14
Stage 3rd	3.21±0.18[b]	9.35±35[a]	7.98±0.23[b]	2.94±0.08[b]	4.43±0.12[b]	3.38±0.10	6.44±0.78
Total	4.33±0.14	8.10±0.18	8.75±0.12	3.22±0.04	4.84±0.67	3.43± 0.04	6.29 ± 0.78
F-valve	11.27	8.72	10.24	10.15	10	0.386	0.683
P-valve	0	0	0	0	0	0.68	0.507
Sig. level	**	**	**	**	**	NS	NS

** P<0.01 Mean Superscripted by different letters differed significantly from each other.

Table 2. Effect of parity on physio-chemical properties and somatic cell count of buffalo milk.

Parity	Milk yield (L)	Milk yield, milk constituents & somatic cell count (SCC)					
		Fat	SNF	Protein	Lactose	EC	SCC x 10^5
Early	3.73± 0.29	8.25 ± 0.43	9.29 ± 0.25[a]	3.42 ± 0.34[a]	5.14± 0.13[a]	3.41± 0.10	6.07±0.16
Mid	4.41± 0.19	7.85 ± 0.25	8.46 ± 0.18[b]	3.12 ± 0.06[b]	4.69± 0.10[b]	3.44±0.06	6.39±0.11
Late	4.47± 0.25	8.39 ± 0.30	8.91 ± 0.19[ab]	3.28 ± 0.07[ab]	4.93±0.10[ab]	3.43±3.31	6.25±0.12
Total	4.33± 0.14	8.10 ± 0.18	8.75 ± 0.12	3.22 ± 0.50	4.84 ± 0.06	3.43±0.04	6.29± 0.17
F-value	1.51	0.95	2.99	3.05	3.12	0.039	1.013
P-value	0.225	0.38	0.05	0.051	0.04	0.961	0.366
Sig-value	NS	NS	NS	NS	*	NS	NS

* P<0.05, [NS] P>0.05, DMRT was used to analyzed Mean Superscripted by different letters differed significantly from each other.

Table 3. Effect of different production levels on physio-chemical properties & somatic cell count of buffalo milk.

Production level	Milk yield, milk constituents & somatic cell count (SCC)					
	Fat %	SNF%	Protein%	Lactose %	EC	SCC
Low	8.95 ± 0.35[a]	8.49 ± 0.24[b]	3.13 ± 0.08[b]	4.71 ± 0.13[b]	3.47 ± 0.77	6.27± 0.13
Medium	7.94 ± 0.24[b]	8.58 ± 0.16[b]	3.16 ± 0.06[b]	4.75 ± 0.09[b]	3.37 ±0.06	6.32± 0.11
High	7.23 ± 0.26 [b]	9.47 ±0.23[a]	3.48 ± 0.85[a]	5.23 ± 0.12[a]	3.52 ± 0.07	6.27± 0.16
Total	8.10 ± 0.18	8.75 ± 0.12	3.22 ± 0.45	4.84 ± 0.06	3.43 ± 0.04	6.29± 0.86
F-value	6.694	5.101	5.064	4.964	0.039	1.227
P-value	0.002	0.007	0.008	0.008	0.963	0.297
Sig-value	**	**	**	**	NS	NS

** $P < 0.01$, DMRT was used to analyzed Mean Superscripted by different letters differed significantly from each other.

Effect of different production levels on physio-chemical properties and somatic cell count of buffalo milk

Table 3 gives a thorough summary of how production level affects the composition of milk and somatic cell level. Low production level showing the highest fat content (8.95 ± 0.35) and high production level showing low fat content (7.23 ± 0.26), so this difference is statistically significant (P< 0.01). SNF, protein, and lactose % are highest at high production levels and much lower at low and medium production levels. For all milk components-fat, SNF, protein, and lactose-these changes are statistically significant across the production level, according to the F-value and P-values. Overall, the production level has a highly significant effect on the composition of milk. Araújo de Melo *et al.* (2020) have reported the fat concentration increases and decreases in lactose concentration with the decrease in milk production. These results agree with the results of my study. The SCC value across the production levels (Low, Mid, High) is close, with values of 6.27±0.13, 6.32±0.11, and 6.27±0.16, respectively. The mean EC value across the production levels (Low, Mid, High) is nearly close, with values of 3.47±0.77, 3.37±0.06, and 3.52±0.07, respectively. F-value and P-value indicate that there is no significant difference in SCC and EC across the different production levels.

Conclusion

In the present study, 122 buffalo milk samples were randomly collected from the Bharatpur Metropolitan area of Chitwan District, Nepal, to investigate the association between SCC and milk compositional traits. Milk composition, particularly fat, solid-not-fat (SNF), protein, and lactose content, exhibited a significant association with production levels. While parity did not have a statistically significant effect on milk yield, fat, SNF, or protein, a minor correlation was observed with lactose concentration. No significant differences in SCC or electrical conductivity (EC) were detected across varying lactation stages or milk production levels. These findings suggest that SCC and EC remain relatively stable across physiological and production variables under the prevailing farm conditions. The results highlight the importance of routine monitoring of SCC and milk quality traits, as they are integral to optimizing buffalo milk productivity and ensuring udder health in dairy systems. Extremely low somatic cell counts (SCC) in dairy animals may suggest an increased susceptibility to

mastitis, potentially reflecting a phenotypic trait associated with altered immune modulation. This observation underscores the importance of conducting targeted studies to better understand the immunological basis of low SCC, rather than relying solely on phenotypic selection for breeding decisions. Evidence from divergent selection experiments indicates that intentional selection for reduced SCC in dairy cows does not necessarily compromise resistance to mammary gland (MG) infections. Hence, low SCC should not be universally interpreted as a marker of high mastitis susceptibility. Previous research has demonstrated that higher SCC levels are often associated with elevated MG immune reactivity, potentially due to basal inflammation. This heightened reactivity may serve as a primed state of the innate immune system, which, while associated with inflammatory drawbacks, could confer a protective advantage under certain pathogenic challenges. Nonetheless, elevated baseline inflammation may also render herds more susceptible to mastitis in the presence of ongoing bacterial colonization. Importantly, selection based on somatic cell score (SCS) appears not to alter SCC levels in healthy, uninfected mammary glands, nor does it increase susceptibility to infection.

DECLARATIONS

Author's contribution: Conceptualization, N.S. and P.H.; methodology, N.S., R.K., and P.H.; software, N.S. and R.K.; validation, R.K. and P.H.; formal analysis, N.S. and R.K.; investigation, N.S. and R.K.; resources, P.H. and R.K.; data curation, P.H. and R.K.; writing—original draft preparation, P.H., N.S. and R.K.; writing—review and editing, P.H. and R.K.; visualization, R.K.; supervision, P.H.; project administration, P.H.; funding acquisition, P.H. All authors have read and agreed to the published version of the manuscript.

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