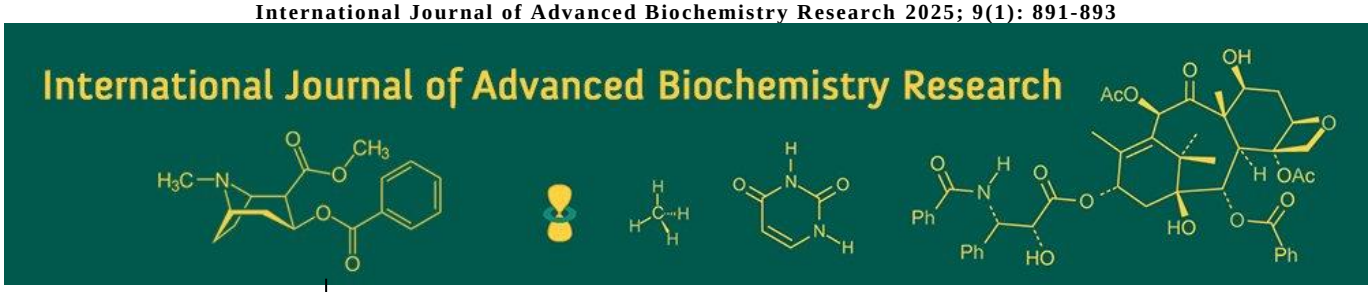

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Effect of different stages of lactation on somatic cell count in local buffalo of Jammu region

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Abstract
The present study was planned to study the variations in the somatic cell count at different physiological stages of lactation in local buffalo of the Jammu region. The milk somatic cells (SCC) are one of the most illustrative of mammary gland tissues and can be used to study the expression kinetics of mammary gland specific genes. The aim of the study was to analyze the effect of different physiological stages of lactation on the amount of milk somatic cells of local buffaloes of Jammu region. Milk was collected from 300 multiparous healthy local buffaloes, which were divided into various groups according to their stage of lactation. i.e. early (60±30 days), mid (120±30 days), late lactation (180±30 days) buffalo of Jammu. The selection was based on health status, parity and on average lactation length of about 290 days. Early morning milk sample were collected in a sterile container by discarding the first few milk streams of milk. We soaked the teats in 0.5% iodine or 4% hypochlorite for at least 20 to 30 seconds while the udder received lukewarm water preparation prior to milking. The preparation of milk sample smears for SCC, analysis were done immediately following collection during the first hour to avoid somatic cell degradation. A version of the SCC determination method used for milk samples followed Schalm *et al.* (1971) [1] after slight modification. The results obtained revealed that the mean values of somatic cell count were 5.85 ± 2.05,6.47 ± 1.95 and 10.85 ± 3.07 x 10^5cells / ml during mid, early and late lactation respectively. The values of somatic cell count followed no trend. The results suggested an increase in the early lactation, then it decreased in mid lactation with a further increase in late lactation stage.

Keywords: Buffaloes, milk, SCC, physiological stages, lactation

Introduction
Livestock holds a significant position in the Indian economy and is a vital component of the agricultural sector. India boasts a rich genetic diversity of bovines, with an estimated population of approximately 302.79 million according to the 2019 livestock Census. Ownership of livestock is primarily among landless laborers and marginal farmers, with a smaller fraction belonging to private and organized sectors. These animals act as a crucial safety net against the unpredictability of nature, including droughts, famines and other natural calamities. The dairy industry plays an essential role in boosting rural income by providing nutritious milk, organic fertilizer from manure, fuel for rural households, and draft power for farming and transportation (Pathbanda *et al*., 2015) [3]. As of the 2019 Livestock Census, buffaloes in India number 109.85 million, contributing approximately 20.45 percent to the country's total livestock population and accounting for around 49.2 percent of total milk production (DAHD, 2017–18). Milk output in India rose from 155.5 million tonnes during 2015-16 fiscal year to 165.4 million tonnes in the 206-17 fiscal year, reflecting an annual growth rate of 6.37 percent. In 2016–17, the per capita milk availability stood at about 355 grams per day (DAHD, 2017–18). Indigenous buffaloes, with a production average of 5.92 kg of milk per day per animal, significantly outperform indigenous cattle, which average 3.54 kg per day per animal. This superior feed conversion efficiency and adaptability highlight the buffalo's significance as a dairy animal in India (DAHD, 2017–18). The buffalo genetic resources of the country are represented by 16 recognized breeds of buffalo (NBAGR, 2019). There exists some lesser known buffalo populations in our country, which needs to be known. Local buffalo of Jammu region is one such un characterized non-descript type of buffalo population.

Thus, making it imperative to identify and characterize this population of buffaloes. The dispersion of buffaloes in Jammu & Kashmir is 7.975 lakh buffaloes in Jammu region and Kashmir region shares 0.214 lakhs buffaloes with 293.70 Th MTs (18.19%) of buffalo milk out of 164.57 thousand MTs (Ministry of Agriculture, Department of Animal Husbandry, Dairying& Fisheries, GOI The developing countries have so far contributed 39 to 40 percent in milk production worldwide. The milk production volume remains high yet insufficient milk quality focus presents barriers to establish exports of milk and dairy products at scale. The continued importance of clean milk creation alongside healthy herd care but particularly udder health stands as major production challenges. National milk quality assessment relies heavily on somatic cell count data as an indicator of mammary gland functional health (Dang *et al*., 2007) [4]. The research examined local buffaloes' somatic cell counts throughout lactation periods because of global milk SCC significance.

Materials and Methods

Research was conducted at the Division of Veterinary Physiology and Biochemistry within the Faculty of Veterinary Sciences and Animal Husbandry at Sher-e-Kashmir University of Agricultural Sciences and Technology, located in R. S. Pura, Jammu, 181102. For the study, 300 healthy multiparous local buffaloes, managed by local Gujjars at various sites in the Jammu region, were selected. The animals were randomly divided into three groups of 100 each based on their stage of lactation: early (60 ± 30 days), mid (120 ± 30 days), and late (180 ± 30 days). The selection criteria included health status, parity, and an average lactation length of approximately 290 days. These buffaloes were raised under semi-migratory conditions.

Sample collection and processing of samples

Milk samples were obtained during in the early morning hours. Three separate milk samples were taken from each animal at different lactation stages. Each sample, 50 ml in volume, was collected in clean, sterile tubes and maintained at a temperature between 2ºC-8ºC from collection until analysis. Morning milk samples were collected in sterile vials after discarding the first few streams of milk using the manual full hand milking method. Prior to the milking process, the udder was cleaned with lukewarm water, and the teats were dipped in a 0.5% iodine or 4% hypochlorite solution for 20-30 seconds. For SCC determination, slides were prepared within one hour of sample collection. The SCC was measured following a modified method of Schalm *et al*. (1971) [1]. The milk samples were thoroughly mixed, and 10 µl (0.01ml) of milk was placed on a pre-drawn one cm square marked area on a clean, grease-free glass slide using a sterilized bacteriological platinum loop and smears were air-dried and fixed by applying a few drops of alcohol before being dried again.

For staining, the prepared smears were immersed in Newman's Lampert stain for 1 to 2 minutes. After staining technicians applied a few alcohol drops onto smears before allowing them to rest for 1-2 minutes before rinsing with tap water and letting them dry in open air. Microscopic evaluation of dried and stained smears occurred at 100x magnification under oil immersion.

Counting of Cells: Cells were counted in 30 different fields under an oil immersion objective lens (100x). Thirty fields per smear were counted to estimate the somatic cell count. The mean cell count was multiplied by microscope's magnification factor to determine the number of cells per milliliter of milk.

Microscopic Factor: View field determination under a 10x eyepiece alongside the oil immersion lens utilized micrometer stage measurement according to Shukla (1980) [5]. Each slide contained a 0.01 milliliter sample of the test solution.

SCC/ml of milk = Average no. of cell per field x MF x 100
= Average no. of cell x 3931.744 x 100

The calibrated microscope remained consistent for the entire research duration.

Statistical Analysis The collected data was formulated in MS Excel sheets for descriptive statistical analysis (Mean, Standard error). The collected results were presented as mean ± standard error. The difference between the means were assessed using Turkey's least significant difference test, with a p-value of less than 0.05 considered statistically significant.

Results and Discussion

Somatic cell count measurements from experimental data highlight mean cell values of 5.85 ± 2.05 x10^5 cells/ml measured in mid lactation and early values of 6.47± 1.95 x 10^5 cells / ml also in the early period and late values of 10.85 ± 3.07 x 10^5 cells / ml in late lactation. The study results parallel Sahin *et al*. (2017) [6] research showing SCC dependence upon lactation period. Analysis confirmed that the early lactation stage showed higher mean somatic cell counts of 96,134 cells/ml which reduced to 68,966 cells/ml in mid lactation and returned to 103,776 cells/ml during late lactation. Analytics from Tripaldi *et al*. (2003) [7] revealed that most tested milk samples contained SCC within the range of 50,000 to 300,000 cells/ml with an average measurement of 221,280 cells/ml in agreement with this present investigation. Brazilian buffalo milk revealed a mean SCC of 269,590 cells/ml according to Lopes (2009) study. The researcher Dame *et al*. (2010) [8] detected that Mediterranean breed displayed SCC levels of 50222 ± 24,952 cells/ml yet Murrah buffaloes revealed SCC levels at 112,765 ± 75,269 cells/ml.

Managuli *et al*. (2014) [9] categorized buffaloes into three equal groups based on lactation stages: early (0-100 days), mid (101-200 days), and late (beyond 200). The lowest somatic cell counts were detected in the mid-lactation period showing statistically significant variations (p<0.05) between the various lactation stages. Rana *et al*. (2023) [10] performed a comparative investigation of somatic cell counts between buffalo groups at different lactation periods which yielded significant statistical results (p<0.05), with highly significant differences (p<0.05) among the early, mid and late lactation stages. Researchers measured somatic cell counts in five different lactation periods: early, mid, late, parturition and post parturition with statistics reporting (±6.4750, ±5.7500, ±10.8750, ±16.2750 and ±11.1250) respectively. These different groups demonstrate pronounced changes in somatic cell counts across multiple

periods of lactation. There is a positive association between somatic cell count and the stages of lactation, with higher scores observed during the early phase, a gradual decrease in the mid-lactation stage, and a subsequent increase in the late stage (Pyorala. 2003., Alhussien & Dang, 2018) [11, 12]. High-yielding cows often experience a negative energy balance during the early lactation phase, leading to elevate levels of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHB). This condition can impair the phagocytic function of the mammary gland, increasing the risk of mastitis (Ottalwar *et al*., 2018) [13]. Regardless of infection status, late lactation phases typically exhibit higher somatic cell counts, which is associated with the preparation for calving and enhancement of mammary defense mechanisms (Sharma *et al*., 2011). However Dang *et al*. (2007) [4] reported no significant (p<0.05) changes in milk somatic cell counts in early, mid and late lactation. Same observations were made by De *et al*. (2011) [14] found no significant changes in milk somatic cell counts across the early, mid and late lactation in Murrah buffalo and also, they found that milk somatic cell counts were significantly higher (p<0.01) in day-1 colostrum samples and then decreased when colostrums transformed into milk. Węglarz *et al*. (2008) [15] reported a contrasting observation in their analysis of the cytological quality of milk from Holstein Friesian cattle. They found that the mean somatic cell count (SCC) was highest in milk collected at an early stage of lactation (1,097,740 cells/ml), while the mid and late stages exhibited lower and similar SCC values (893,910 and 914,150 cells/ml, respectively).

Table 1: Effect of different stages of lactation on Somatic cell count (Mean±SE;10^5×cells/ml) in local buffalo of Jammu region

Stage of lactation	Mean ± SE; ×10^5cells/ml
Early stage	6.47^a±1.95
Mid stage	5.85^a±2.05
Late stage	10.85^b±3.07

Conclusion

The analysis shows significant p<0.05 differences between somatic cell counts across different lactation periods. During the initial lactation period somatic cell numbers show statistically significant increases (p<0.05) followed by a sustained decrease through mid-lactation yet they progressively rise during late lactation. The end lactation increase in somatic cells counts likely results from natural udder involution and increased mastitis incidence along with reduced milk production creating less diluted leukocytes.

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