

## QUALITY ASSESSMENT AND COMPARATIVE STUDY OF SOMATIC CELL FLUCTUATION AT DIFFERENT LACTATION STAGES OF BUFFALO'S MILK

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### ABSTRACT

The aim of current study is to check the health status and the quality of milk by counting somatic cells. Total 50 samples of fresh raw buffalo milk were gathered and divided into five groups on the basis of lactation stages i.e early, mid, late, buffalo at parturition and post parturition (colostrum). Fresh samples were collected and assessed with quality assessment test i.e. pH analysis, lactometer reading test, clots on boiling (COB), alcohol precipitate test (APT) and acidity % age test and the somatic cell count (SCC) through Neubauer chamber under light microscope. Data were evaluated by the stage of lactation with the SPSS programme. The total mean values of pH, Lr and acidity % at each lactation stages were ( $\pm 6.96$ ,  $\pm 31$  and  $\pm 0.19$ ). Significant effect of SCC on different stages were observed ( $P < 0.05$ ). The average SCC was determined to be  $10.1000 \pm 5.8121$  cells/ml. The effects lactation stages on the SCC value between group were statistically significant ( $P < 0.05$ ). The mean SCC values for early, mid, late, parturition and post parturition stages were ( $\pm 6.4750$ ,  $\pm 5.7500$ ,  $\pm 10.8750$ ,  $\pm 16.2750$  and  $\pm 11.1250$ ). The significant differences recognized

between the groups indicates that there is a fluctuation of somatic cells at different lactation stages. The number of somatic cells increase from early stage, then decrease gradually in mid stage and starts increasing till the end of lactation and have no significant difference ( $P < 0.05$ ), whereas the mean comparison is highly significant in parturition and colostrum stage.

**Keywords:** *Bubalus bubalis*, buffaloes, Raw buffalo milk, Somatic cell count (SCC), Hemocytometer (Neubauer chamber), lactation stages

### INTRODUCTION

Pakistan ranks among the world's top ten milk producers with 35.5 billion liters of milk annually (FAOSTAT, 2010). After India and Bangladesh, Pakistan is the world's second largest producer of buffalo and goat milk with production of 22.3 and 0.7 billion litres, respectively. Buffaloes are widely reared in Pakistan on a limited scale for family feed, due to their higher milk fat content than cattle. Milk is a nutritive fluid secreted by female

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mammary gland and has a significant role for the growth and development of neonates (Yarabbi *et al.*, 2014). Besides, there is a noteworthy effect on all parts of dairy chain according to request of high caliber of milk and dairy items, by customer (Javaid *et al.*, 2009). One of the critical illnesses that affects the quality of milk is a sickness of the mammary organs, called Mastitis (Vlieghe *et al.*, 2012) caused by microorganisms, including species of *Pseudomonas*, *Klebsiella* and *Staphylococcus* (Saini *et al.*, 2012).

SCC of milk is a main component of international and national regulations for quality of milk, and is an alarming signal of teats health and the occurrence of mastitis in dairy farming (Sharma *et al.*, 2011). Sc's are mostly leukocytes. The quantity of somatic cells in milk is increase when an infection occurs. High SCC impacts many factors, such as reduction of milk yield, changes in cell composition, and durability of milk, and can be the reason of substantial losses for dairy industry. Apart from intramammary contamination, there are numerous elements that impact the milk physical cell check of milk at the individual and herd. The ability to correctly interpret SCC depends on considering the factors that can affect somatic cell counts (Lakshmi *et al.*, 2009). The variables influencing are mammary organ infection level (IMI), period of individual, seasonal variation, diurnal variation, parity and stages of lactation.

In most created dairy industry, there are a wide range of administrative limits that applied to milk for human utilization. The European Union Directives (92/46CEE and 94/71 CEE) set a tolerant scope of 4,00,000 ( $4.0 \times 10^5$ ) cells/ml for SCC in raw milk of buffalo and when the raw milk is used for creating dairy item. In US, the breaking point is 7,50,000 ( $7.5 \times 10^5$ ) cells/ml, New Zealand,

Australia and a few parts of Europe has an impediment of 4,00,000 ( $4.0 \times 10^5$ ) cells/ml though Canada has 5,00,000 ( $5.0 \times 10^5$ ) cells/ml of crude milk. However, in developing countries, milk is still sold based on its fat percentage (Alhussien, and Dang, 2018). There is no tolerate range set for developing countries like Pakistan, so in this study the threshold limit is set  $>500,000$  cells/ ml.

The aim and objective of this research is to access the quality of milk and to compare the counted cell of different lactation stages to check the variations of somatic cells in individuals.

This research is also an attempt to create a sort of awareness about the buffalo's health status and milk quantity based on somatic cell count.

## MATERIALS AND METHODS

### Materials

The materials that were used for current studies include, Beakers, Pipettes, Burette, Suction pump, Dropper, Conical flask, Eppendorf bottles, Neubauer chamber (Tiefe. Depth profondeur 0.100 mm, Marienfeld Germany), Cover slips, Micropipette (capable of dispensing volumes in the range of 50 to 100  $\mu$ l), Lactometer, Thermometer, Water bath, Microscope, Test tubes, Test tube holder, pH meter, Bunsen burner, Petri dish and Cylinder.

### Chemicals

The chemicals used for the purpose of this research work include, Methylene blue (stain), KCl, NaCl,  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ , 0.1% NaOH, Phenolphthalein and Ethanol 68%. These chemicals were used. All the apparatus and chemicals were taken and used from Food and Biotechnology lab of PCSIR, Lahore.

### Collection, types and grouping of samples

Fresh buffalo milk samples at different stages were collected from different areas of Lahore i.e. Jaman and heir pind and divided into 5 groups and each group contains 10 samples. The stages of lactations and grouping were: early stage (8<sup>th</sup> day to 3<sup>rd</sup> months), mid stag (4<sup>th</sup> to 6<sup>th</sup> months), late stage (7<sup>th</sup> to 9<sup>th</sup> months), buffalo at parturition (1<sup>st</sup> to 10<sup>th</sup> months) and post parturition/Colostrum (0 to 7<sup>th</sup> days).

### Quality test of milk

To assess the quality of milk following tests were applied on raw milk sample.

#### pH test

Freshly drawn milk shows an amphoteric reaction, i.e. turning red litmus into blue and blue litmus into red. Normal fresh milk has an approximate pH of 6.6 to 6.9, indicating a slightly acidic milk. pH higher or lower than this ranges will show high acidity i.e. in colostrum.

#### Lactometer test

The lactometer is a tool used to measure milk density. Pure milk has a density of 30 to 35 g/100 ml (specific gravity). Adding water or other substances will change the density. Adding water decreases the density, while adding solids significantly increases the density. When concentration exceeds the normal range, this means that the milk has been adulterated.

#### Clots on boiling test

2 ml of fresh milk sample was taken in the test tube for boiling. If there is any coagulation or precipitation then the milk has failed the test and can be rejected for further process.

### Alcohol precipitation test

This experiment was also conducted by Marshall's 1992 method; the analysis is created on the proteins (volatility) and the acid or rennet concentration is increased. The alcohol test was performed using an ethanol solution of 68%. In a sterile test tube, 2 ml of the raw milk sample was taken and assorted with 68% ethanol solution.

### Acidity test

Acidity was measured in various samples of milk using the method described by Marshall in 1992. Milk's total acidity is 0.16 - 0.26%. 9 ml of fresh milk sample took in a beaker and then added few drops of phenolphthalein in it as an indicator and then solution was titrated against 0.1% NaOH, drop by drop by using pipette or biuret. Stop it, until the milk color changes to persistent pink color.

$$\text{Acidity \%} = \frac{\text{ml} \times \text{N} \times 90 \times 100}{\text{V} \times 1000}$$

### Sample preparation for SCC

For the preparation of sample 20 ml of milk and 10 ml of Pbs was taken in a Centrifuge tubes which then subjected to Centrifuge machine at 4000 rpm for 15 minutes at 25°C<sup>[11]</sup>. After centrifugation, supernatant was removed by pasture pipette and in remaining pallet 10 ml PBS was added again to remove fats and centrifuge at 8000 rpm for 5 minutes <sup>[12]</sup>. After the process of centrifugation, the supernatant was removed and 2 ml PBS was added again in fatless pallet and left it undisturbed for 5 minutes. 50 µl of methylene blue dye was added to 50 µl of fatless mixture in Eppendorf serum tubes and left it undisturbed for 10 to 15 minutes. With the help of micropipette inject 50 µl of sample in Neubauer chamber and observe the sample under microscope.

### Statistical analysis

All facts were stated as Mean  $\pm$  S.D. ANOVA and Standard deviation was determined by using SPSS. Means of different lactation stages were determined by using one-way ANOVA ( $P < 0.05$ ) and comparison of different stages means were evaluated by Tukey HSD test.

## RESULTS AND DISCUSSIONS

### Effect of pH, Lr and acidity on milk quality

The total mean values of pH, Lr and acidity % ( $\pm 6.96$ ,  $\pm 31$  and  $\pm 0.19$ ) at each lactation stages was shown in Table 1. At each lactation stage such as early, mid, late, parturition and post parturition (colostrum), the mean value of pH ( $\pm 6.8$ ,  $\pm 6.76$ ,  $\pm 6.88$ ,  $\pm 6.93$  and  $\pm 7.46$ ), Lr ( $\pm 31$ ,  $\pm 31$ ,  $\pm 30.8$ ,  $\pm 30.6$  and  $\pm 34$ ) and acidity % ( $\pm 0.19$ ,  $\pm 0.16$ ,  $\pm 0.23$ ,  $\pm 0.16$ ,  $\pm 0.22$ ) were shown in Table 1.

Normal raw milk has a pH of about 6.6 to 6.9, indicating that the milk is little acidic. By using phenolphthalein as an indicator if ordinary fresh milk is titrated with an alkali sol, acidity varies from 0.10 to 0.26%. The acidity is believed to be due to lactic acid, but genuine fresh milk does not contain lactic acid. It is known that the acidity of raw milk is due to the phosphates in milk, protein and the existence of  $\text{CO}_2$  and citrates (Bilal and Ahmad, 2004). The findings agree with Tripaldi *et al.* (2003), who stated that, the milk pH amplified expressively, from 6.74 to 6.80 at when the somatic cells inclined from  $0.30 \times 10^5$  to  $0.39 \times 10^5$  to  $0.50 \times 10^5$  to  $1.0 \times 10^5$  cells/ml. The findings of this study are also in streak with Haggag's work in 1991 and Horvath's work in 1980, which showed that the specimens increased with the severity of mastitis pH. Similarly, it was reported that milk pH in dairy animals increased by 0.13 per unit increase in CMT

score and pH could serve as the best indicator for assessing udder health status (Charjan-Ku *et al.*, 2000). According to Ishwari and Hajime, it is commonly accepted that mastitis grounds an increase in milk pH. This is mainly due to outflow of blood bicarbonate into milk after mammalian epithelium has been damaged (Ishwari and Hajime, 2018). The higher pH reduces the action of enzymes used to coagulate milk significantly, thus having allegations for dairy products in the production potential of milk (Subedi and Dhakal, 2002). The average pH of standard buffalo milk was 6.96 as shown in Table 1, similar to the reported by Silva *et al.* (1995), of 6.5 (6.1 to 7.0) in Sri Lanka. The possible explanation for the rise in pH due to mastitis severity can be attributed to the decreased acidity as observed in mastitic milk. The decreased acidity in mastic milk is due to decreased lactose content as the production of lactic acid in this case is limited (Horvath *et al.*, 1980; Haggag *et al.*, 1991). The pH can serve as the best indicator for assessing the animal and food quality of the milk's udder health status. As mastitis frequency increased, the pH value also increased increasing the milk food value.

### SCC at early, mid and late lactation stages

The SCC means, standard errors (S.E) and mean differences for each lactation stages is shown in Table 2. In the current study, the average SCC was determined as  $10.100 \pm 5.8121$  cells/ml. The mean SCC values in the early stage of lactation were high ( $P < 0.05$ ) ( $6.4750 \pm 1.9594$  cells/ml), that start decreasing during the mid-lactation stage ( $5.7500 \pm 2.0581$  cells/ml), and then inclined once more during the late lactation stage ( $10.8750 \pm 3.0075$  cells/ml).

As illustrated in Table 2, the mean SCC values was determined as ( $10.100 \pm 5.8121$  cells/

ml). The results of this study were in agreement, to the finding of Sahin *et al.* (2016), which also showed that the lactation stages had a significant effect ( $P < 0.05$ ) on SCC, the mean values of SCC were high ( $P < 0.05$ ) in the early lactation stage ( $\pm 11.456$  cells/ml), that decline in the mid-stage of lactation ( $\pm 13.542$  cells/ml) and then rise again during the late stage of lactation ( $\pm 45.211$  cells/ml). The recent findings were also reliable with those of Singh and Ludri (2001), who expressed that the mean values of SCC were high ( $P < 0.05$ ) in initial 90 - day lactation (range:  $1.10 \times 10^5$  to  $1.27 \times 10^5$  cells/ml), decreased to a low lactation value of 90 to 120 days ( $0.90 \times 10^5$  to  $0.99 \times 10^5$  cells/ml) and inclined a little bit in late stage of lactation ( $0.97 \times 10^5$  to  $1.07 \times 10^5$  cells/ml). The milk SCC increased towards the termination of lactation due to the higher prevalence of mastitis, normal involution of the udder and reduced milk production.

On the other hand, Thomas (2004) reported that it has not been possible to establish a consistent threshold value for a healthy buffalo milk SCC; however, according to the studies (Singh and Ludri, 2001; Dhakal, 2006), a SCC  $> 2.0 \times 10^5$  cells/ml appears likely to be indicative of mastitis. SCC is a measure broadly used for mammalian health assessment. It has been documented that a  $2.0 \times 10^5$  cells/ml or higher raw buffalo milk SCC can be utilized as the threshold for early exposure of subclinical mastitis (Tripaldi *et al.*, 2010). Mastitis can be indicated by an SCC of over  $2.0 \times 10^5$  cells/ml, while values under  $1.0 \times 10^5$  cells/ml can define healthy quarters (Fagiolo and Lai, 2007). It claimed that milk SCC could be specified to assess the quality of milk / teat health in buffaloes, and that SCC can best be calculated directly by a (DCC) DeLaval cell counter (Hamann *et al.*, 2010).

### SCC during parturition

With progressing lactation, the SCC values tend to increase; and particularly in later stages of lactation. As shown in Table 2, the SCC in parturition stage showed maximum SCC ( $16.2750 \pm 6.4285$  cells/ml) and can be observed in Figure 1. The results were reliable with the finding of Aysar *et al.* (2013), who reported that in the late pregnant animals or the drying stage, the mean number of somatic cells was between  $18.0 \times 10^5$  to  $55.0 \times 10^5$  cells/ml and the mean value was  $\pm 22.50$ . In 2001, Meglia found that neutrophilia, eosinopenia, lymphopenia, and monocytosis characterized the weeks just before and after parturition, but there is no influence of time on neutrophil phagocytosis and oxidative bursts (Meglia *et al.*, 2001). Cortisol increases dramatically during calving and might have a harmful effect on near-calving mammary immunity (Hill *et al.*, 1979). At parturition, SCC is frequently advanced than 1 million per ml and falls to  $1.0 \times 10^5$  cells/ml in the 7 to 10 days of post-partum (Jensen and Eberhart, 1981).

### SCC at Post-parturition (colostrum)

A study conducted by Dang and other co-workers reported the amount of SCC was  $4.7 \times 10^5$  cells/ml on the first day of calving, in buffalo milk, and after first week it decreases (Dang *et al.*, 2007). In present study Table 2 shown the higher level of SCC in initial days after calving which started decreasing gradually. The current finding, reported similar to the work by Barkema *et al.* (1999), that lactation stage effects the SCC as the amount of SC is high at parturition that start decreases to the normal count within 4 to 5 days of calving. It was also reported by Schalm and co-workers that in colostrum, the occurrence of large number of cells seems due to exfoliation of epithelial cells abundantly, in a small volume of milk that causes

Table 1. Mean value of pH, Lr and acidity % of all stages.

Test	Early	Mid	Late	Parturition	Post parturition	Total mean
pH	±6.8	±6.76	±6.88	±6.93	±7.46	±6.96
Lr	±31	±31	±30.8	±30.6	±34	±31.48
Acidity %	±0.19	±0.16	±0.23	±0.163	±0.22	±0.19

Table 2. Means and standard errors of the Somatic cell count for stage of lactation and significance levels of the factors and differences between the means.

Descriptives								
SCC								
Stage of lactation	N	Mean (X)	SD	Std. Error (SE)	95% Confidence interval for mean		Min.	Max.
					Lower bound	Upper bound		
Early	10	6.475 <sup>b</sup>	1.9594	0.6196	5.0733	7.8767	3.75	10.00
Mid	10	5.750 <sup>b</sup>	2.0581	0.6508	4.2777	7.2223	2.50	8.75
Late	10	10.875 <sup>a,b</sup>	3.0075	0.9510	8.7236	13.0264	7.50	16.25
Parturition	10	16.275 <sup>a</sup>	6.4285	2.0328	11.6763	20.8737	8.75	26.25
Colostrum	10	11.125 <sup>a,b</sup>	6.7559	2.1364	6.2921	15.9579	5.00	26.25
Total	50	10.100	5.8121	0.8219	8.4482	11.7518	2.50	26.25

a-b: differences between groups with same letter in the same column are insignificant, but differences with different letter are significant  $P < 0.05$ .

Table 3. One-way ANOVA at all the stages of lactation.

ANOVA					
SCC					
Analysis	Sum of Squares	d.f	Mean Square (X) <sup>2</sup>	F	Sig.
Between Groups	718.450	4	179.613	8.628	0.000*
Within Groups	936.800	45	20.818		
Total	1655.250	49			

\*Mean value is significant when  $P < 0.05$

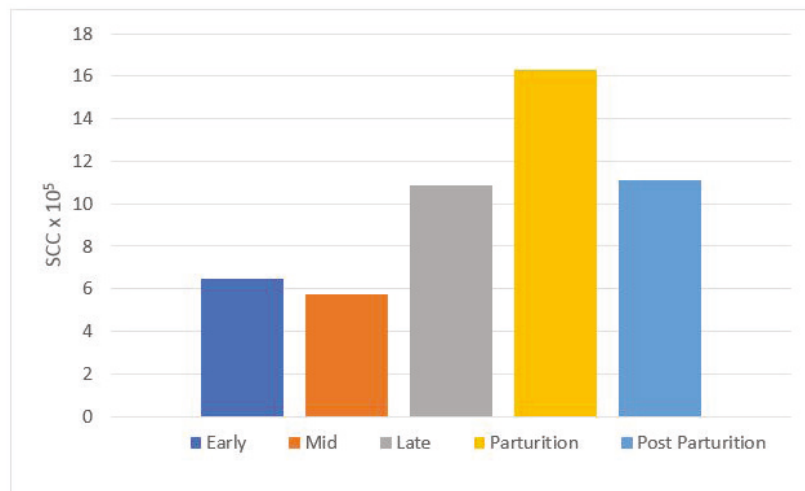


Figure 1. Graphical representation of SCC at each level of stage.

functional activity to be resumed after a resting duration (Schalm *et al.*, 1971).

On the other hand, in 2010, De and others expressed that buffaloes have an incredible resistance system against mastitis because of their constricted nipple sphincter and long, limited nipple channel, which can be relied upon to keep bacteria from attacking the udder successfully. It may also be due to the lower cisternal part of buffalo contrasted and dairy cows (De *et al.*, 2010).

## CONCLUSION

In conclusion, the present research work clearly indicates about somatic cells fluctuation. By the research work it is concluded that there is fluctuation in somatic cell at different lactation stages. There is no significant difference within group but mean difference is highly significant between groups. The change in high concentration of somatic cells was observed and highly significant when compared to parturition and post parturition stage because of high acidic level of milk.

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