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Effect of storage temperature on somatic cell count of buffalo milk using direct microscopic method

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ABSTRACT

This study was made to determine the effect of storage temperature and storage time on somatic cell counts (SCCs) in buffalo milk samples from quarters with no signs of clinical mastitis. The critical limit of SCC in raw buffalo milk is around 400,000 cells/mL. SCCs were measured in 32 Murrah buffalo milk samples by direct microscopic method. Milk samples were grouped from high score (> 3.50×10^5 cells/mL) to low score (< 1.50×10^5 cells/mL) according to the SCC. Each milk sample was divided into 3 aliquots and stored at 3 different temperatures (4°C, 21°C and 37°C). The SCC was recorded every hour up to 4 h and every 2 h up to 12 h. Storage of buffalo milk with a high SCC for 4 h at 4°C and 2 h at both 21°C and 37°C, respectively, decreased the $\log_{10}$ value of SCC compared to fresh milk. The $\log_{10}$ value of buffalo milk SCC was lower after 1 h of storage than that determined for fresh milk at any tested temperature in low SCC samples. The results sugges that irrespective of storage temperature, buffalo milk samples should not be stored for more than 2 h before measurement of SCC by direct microscopic method.

Key words: Buffalo milk, Microscopic method, Somatic cell count, Storage temperature

India, the leader in buffalo production across the world, produces approximately 96 million tonnes of milk annually from buffalo, which is superior to cow milk due to higher total solids. Besides presence of immunoglobulin, lactoferrin, lactoperoxidase and lysozymes make it a good dietary and health food (Iqbal *et al*. 2012). Measurement of SCC has become one of the most reliable indicators for determining milk quality and the price of raw milk within the dairy industry (Köster *et al*. 2006, Moon *et al*. 2009, Pantoja *et al*. 2009). Automated counted methods such as electronic particle counting method and fluoro-optic electronic cell counting method by disk cytometry or flow cytometry have gained popularity in dairy industry for rapidly determining the SCC of milk samples. Details of the procedures were published by various workers (Heeschen 1975, Gonzalo *et al*. 2003). Although, these devices are very accurate and reliable instrumental method, however, upfront cost of the instrument, time-consuming instrument maintenance and setup and engagement of trained manpower are major constraints for the farms and laboratories of inadequate funding (Persson and Olofsson 2011). These devices are also not always affordable for those laboratories where the grant for research is insufficient. Therefore, direct microscopic method is an option being inexpensive and easy to perform. The direct microscopic method with methylene blue staining is the reference method for making a direct estimation of the cells, and this method is commonly used in India (Sharma *et al*. 2011). A good correlation between direct microscopic somatic cell counting and flow cytometry, Fossomatic cell counting methods and optically cell counting method (DeLaval cell counter) have been reported in raw milk samples of buffalo and other mammals (Schmidt-Madsen 1975, Miller *et al*. 1986, Gunasekera *et al*. 2003, Bansal *et al*. 2007).

Moreover, milk SCC, a useful indicator of the status of bovine intramammary infection (IMI), is monitored to assess udder health (Koop *et al*. 2012). Apart from IMI, there are several factors that influence milk SCC at individual and herd level. Among these, temperature and duration of storage, counting methods immensely affect SCC of milk samples. Researchers have reported temperature and storage effect on milk SCC using automated devices in cattle, goat and ewes (Barkema *et al*. 1997, Sanchez-Macias *et al*. 2010, Sanchez *et al*. 2005, Martinez *et al*. 2003). Reports on the effect of storage temperature (a wide range of temperatures across India where the refrigeration facilities may not be available) and time between collecting and analyzing the sample following the milking have not been established for SCC determination by the direct microscopic method of buffalo milk. Hence, the present study was undertaken to investigate the influence of storage time and temperature for determination of buffalo milk SCC using direct microscopic method.

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MATERIALS AND METHODS

In the present study, milk samples were collected from Murrah buffaloes of the local dairy herds of Bareilly, Uttar Pradesh, India. The milk samples (50 mL) were randomly collected from 47 quarters of healthy buffaloes in sterile vials after cleaning the teat orifice with 70% ethyl alcohol and after discarding few streams of milk. The SCC was measured immediately as per Schalm *et al*. (1971). Total number of cells per mL was then calculated from microscopic factor (work factor 14,700). Based on SCC, milk samples were grouped into 2 groups: milk with high SCC score (> 3.5×10^5 cells/mL; n=16) and low SCC score (< 1.5×10^5 cells/mL; n=16). Subsequently, each milk sample was divided into three aliquots and stored at three different temperatures (4°C, 21°C and 37°C). Each milk sample was mixed properly and then SCC of each sample was determined at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. Each SCC value was transformed to $\log_{10}$/mL to achieve a normal distribution. The data were analyzed using statistical software package SPSS (Version 17.0). A repeated measure model was used to evaluate the effect of different storage temperatures and times after milking on buffalo milk SCC. The effect of different groups was determined using a Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The changes of SCC values ($\log_{10}$/mL) following storage at different temperatures for the high and low SCC score groups during the 12 h following collection are depicted in Fig. 1a, b. There was a noticeable effect of storage duration on SCC as determined by direct microscopic method for the high SCC samples. Fresh sample SCC values (0 hr) were significantly (P<0.05) higher than for samples stored for 4 h at 4°C. The SCC was significantly lower (P<0.05) at 2 h of storage at both 21°C and 36°C as compared to fresh samples.

Storage temperature significantly affected SCC in high-SCC samples. SCC $\log_{10}$ transformation of samples stored at 4°C was higher (P<0.05) than that obtained at 21°C when evaluating samples stored for 4 and 10 h. The SCC $\log_{10}$ transformation of samples stored at 4°C was higher (P<0.05) than that obtained at 37°C when evaluating samples stored from 4 to 10 h. There was no difference in SCC $\log_{10}$ transformation values up to 3 h of experimental period for samples stored at 4°, 21° and 37°C.

SCC values determined by direct microscopic method were markedly affected by storage time in low SCC score samples (Fig. 1b). The SCC in samples stored for 2 h at 4°C and 37°C was significantly (P<0.05) lower than that seen in fresh samples, whereas, it was 3 h for 21°C.

Storage temperature did not have a considerable impact on $\log_{10}$ values of SCC in low SCC samples between storage temperatures during the first 8 hours of the experiment. However, SCC values of samples storage at 37°C were significantly higher than samples storage at 4°C and 21°C during 10 to 12 h.

In the present study, storage time had a significant effect

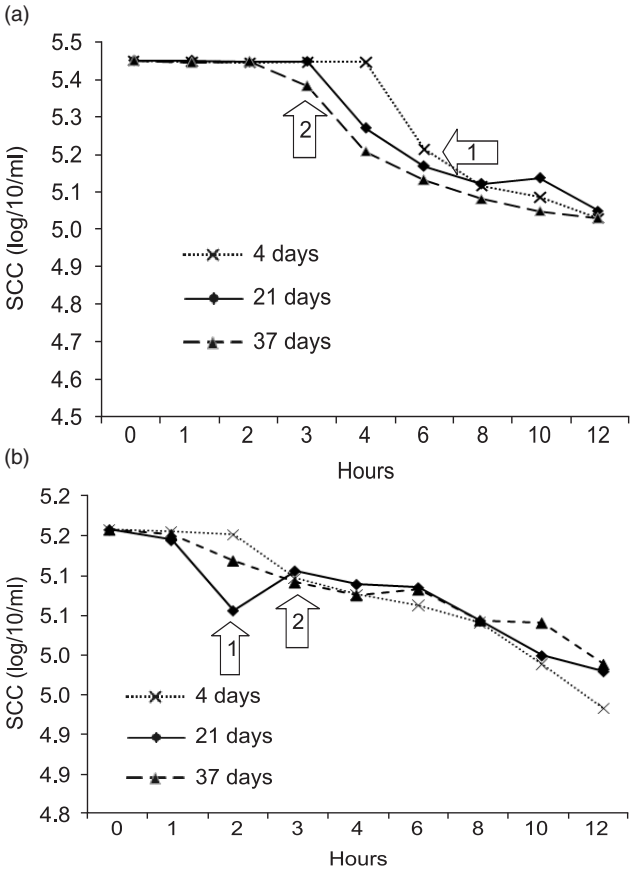

Fig. 1a-b. **a.** Buffalo milk SCC $\log_{10}$ transformation in samples possessing high SCCs during 12 h of storage at 4°C, 21°C and 37°C. Arrow 1, statistical differences between fresh samples and those stored for 4 h at 4°C arrow 2, statistical differences between fresh samples and those stored for 2 h at 21°C and 37°C. **b.** Buffalo milk SCC $\log_{10}$ transformation in samples possessing low SCCs during 12 h of storage at 4°C, 21°C and 37°C. Arrow 1, statistical differences between fresh samples and those stored for 4 h at 4°C and 37°C, arrow 2, statistical differences between fresh samples and those stored for 2 h at 37°C.

on $\log_{10}$ values of SCC, independent of high or low SCC category. Hence, the direct microscopic method is a feasible tool for evaluating the SCC in fresh milk samples because refrigeration at 4°C reduced $\log_{10}$ values of SCC at 4 h and 2 h in high-SCC and low-SCC milk, respectively. This time could be shortened where the environmental temperature will be higher like tropical countries. Although no such reports are available on effect of temperature and duration of storage on SCC determination of buffalo milk by microscopic method, however, Erdem *et al*. (2012) reported that SCC measured by direct microscopic cell counting method were not affected for 15 days and 8 days storage of cow milk at 4°C and –20°C. Additionally, the results obtained in the present study were in contrast to an earlier report by Dohoo *et al*. (1981), which showed that fresh cow milk samples stored at 21°C became acceptable for somatic cell count using a Coulter Electronic milk cell counter up to 8 h while those stored in the refrigerator (3–5°C) were acceptable for up to 3 days. These SCC reductions as a result of storage time could be reduced using milk

preservatives. The SCC from cow milk samples preserved with potassium dichromate, azidiol, and bronopol measured using a Fossomatic was more precise than the SCC measured using unpreserved samples (Martinez *et al*. 2003). While several temperature–storage time combinations were suggested as optimal, the use of potassium dichromate as a preservative and glutaraldehyde as a fixative provided the best results (Dohoo *et al*. 1981). This indicated that storage conditions (such as temperature, duration) produce greater effects when using the direct microscopic method as opposed to automatic devices. This may be due to specificity and sensitivity of the counting device and compositional variation of cow and buffalo milk. Most of the automatic devices evaluate nuclear material based on fluoro-optoelectronic identification rather than intact cells, whereas, by direct microscopic method, cells are counted on the basis of intact cellular morphology (Vermunt *et al*. 1995). Therefore, disintegration of cellular details over an incubation period might have some effect on the determination of SCC by the microscopic method than that by automatic device. As regards to composition, buffalo milk contains much more fat percentage as compared to cow milk. Milk fat globules possess a number of attributes which severely hinder accurate somatic cell counting by microscopic method. In buffalo milk, fat globules are significantly more abundant than cells and often form aggregate with cells upon sticking following milk age. Sometimes, fat globules overlaps with somatic cells as because size of fat globules (diameter range: 0.1–20 µm) is more than somatic cell diameter (8.5–10 µm).

Our results suggested that the time from milk sampling to milk SCC analysis by direct microscopic method should be reduced to less than 2 h in buffalo milk samples in Indian climatic condition. However, the analysis could be carried out during the milking process if milking were to take longer than two hours. The storage at different freezing temperatures and effect of preservatives on buffalo milk SCC using direct microscopic method warrants further investigation. In conclusion, regardless of the storage temperature, buffalo milk samples should not be stored for more than 2 h before SCC analysis using direct microscopic method.

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