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THE EFFECTS OF STORAGE TEMPERATURE AND STORAGE TIME ON THE SOMATIC CELL COUNT OF ANATOLIAN BUFFALOES

Aziz Şahin[1,*], Arda Yildirim[2] and Zafer Ulutaş[3]

ABSTRACT

This research examined the effects of storage temperature and storage time on the somatic cell count (SCC) of milk from Anatolian buffaloes, which was measured with the DeLaval cell counter (DCC). Storage temperature and time are among the different factors that potentially affect the SCC of Anatolian buffalo milk. In this context, 20 milk samples were collected from Anatolian Buffaloes and analyzed. The milk samples were divided into two groups according to their measured level of SCC. These two groups were the low score (≤3.16 cell/ml) group and the high score (>3.16 cell/ml) group. The mean logSCC values of the low score and the high score groups were determined as 2.27±0.045 and 4.06±0.019 cells/ml, respectively.

In this research, the effects of storage temperature (4ºC, 21ºC) and storage time (fresh milk, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 h) on logSCC were determined to be statistically significant (P<0.01). Thus, increases in storage temperature and storage time were associated with an increase in the logSCC of the milk samples.

Keywords: *Bubalus bubalis*, buffaloes, SCC, Anatolian buffaloes, milk samples, group, milk sample

INTRODUCTION

Buffalo milk represents one of the most valuable agricultural products of Turkey. Buffalo milk is not only an important protein source for poor rural breeders, but also a significant source of income for the rural economy (Borghese, 2005; Yilmaz *et al*., 2011). In Turkey, buffaloes are especially bred for milk production, and later slaughtered for meat production once they pass their productive ages (Sekerden, 2001). The SCC of milk is, according to national and international regulations, a key parameter of milk quality, as well as an signal of mammary health and of the frequency of clinical and subclinical mastitis in dairy cows (O'brien *et al*., 2009). SCC serves to indicate the level of infection and inflammation in the mammary glands of dairy buffaloes. SCC in milk are mainly formed of leukocytes, whose levels vary according to the intensity of the immune response, while the other somatic cells in milk originate from the mammary tissues. High SCC levels have various undesirable consequences, such as the reduction of milk yield, significant changes

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in milk composition, and the decrease of milk shelf-life (Singh and Ludri, 2001; Koc, 2008). Such production and quality-related changes can result in significant economic losses for dairy businesses. For this reason, it is important for milk SCC levels to be within acceptable limits. SCC levels that are above the acceptable thresholds can result not only in significant quality-related issues for dairy operations but may also lead to health problems among animal and humans (Randolph *et al*., 1971; Manlongat *et al*., 1998).

SCC is considered as one of the most reliable parameters for determining milk quality and subclinical mastitis (Lievaart *et al*., 2007). For this reason, it is necessary to develop simpler and more rapid analysis methods that would allow the determination of SCC levels in farmlands where buffaloes are raised. The number of studies regarding the SCC levels of buffalo milk is very limited (especially concerning the SCC levels of buffalo milk obtained in the Tokat province). Due to concerns relating to human and animal health, many countries (EU nations and Switzerland) have established an SCC upper limit value of 400,000 cells/ml for milk (Hillerton, 2001; Cero´N-Mun˜oz *et al*., 2002; Sederevicius *et al*., 2006; Sharma *et al*., 2011; Kasikci *et al*., 2012). On the other hand, the upper limit specified for milk by the Turkish Food Codex is 500,000 cells/ml (Anonymous, 2000).

Previous studies have determined SCC values for buffalo milk ranging from 50,000 to 375,000 cells/ml (Dhakal *et al*., 1992; Silva and Silva, 1994; Singh and Ludri, 2001; Moroni *et al*., 2006). The factors that affect SCC in cows, sheep and goats have been extensively studied. In dairy animals; the use of milk preservatives (Vermunt *et al*., 1995; Sıerra *et al*., 2006), the analysis temperature (Mıller *et al*., 1986), the storage temperature (Sıerra *et al*., 2006; Erdem *et al*., 2012) and the milk age (Vermunt *et al*., 1995; Erdem *et al*., 2012) have been detected as features that affect the SCC.

A number of researches have investigated the effects of storage circumstances on the SCC of Anatolian buffalo milk. Erdem *et al*., (2012) previously reported that the SCC levels in cow milk samples obtained 15 days post collection at RT (refrigeration) and 5 days post collection at FT (freezing) can safely be used to assess raw milk quality, and also for planning effective herd management programs.

In practice, SCC is measured directly by using a microscopy method, by using advanced measurement devices (Fossomatic, Somacount, Somascope) in laboratory settings, or by using portable measurement instruments such as the C-reader system (Moon *et al*., 2007) and DeLaval cell counter (DCC) (Ruegg *et al*., 2005; Sarıkaya *et al*., 2006), which can be used both in farm and laboratory settings. Van Werven *et al.* (2005) emphasized that DCC provides more accurate results in comparison to indirect cell counting methods.

The DCC is a movable and battery-operated visual device that measures the SCC of a sample in less than one 60 seconds. Prior to cell counting, a cassette filled with propidium iodide (a fluorescent stain) is used for collecting the milk sample. The sample is then mechanically carried towards the screen, where it is exposed to light emitted by a diode. Fluorescent indicators shaped by the propidium iodide- stained nuclei are noted as a picture, and the produced picture is then used to define the SCC of the milk sample. The highest SCC value that can be measured by the DCC is 500×10^3 cells/ml. The DCC is a device that was designed specifically for determining SCC in raw bovine milk (Gonzalo *et al*., 2006).

However, the effect of storage temperature (including temperature ranges encountered in regions of the world where refrigeration may not be possible) and storage time on the SCC levels following milking habitual have not been evaluated or recognized with the DCC method. Therefore, the goal of the current investigation was to investigate the effects of storage temperature and storage period on the SCC level of Anatolian Buffalo milk by using the DCC device. Sanchez-Macias *et al*. (2010) previously reported that the use of milk preservatives, the analysis temperature, the storage temperature, and the milk age are the main factors that affect the SCC of cow milk. In addition, Sánchez *et al.* (2005) described that knowledge regarding the different methods of preservation, the storage temperatures, and the interaction of these two factors with storage time could assist in optimizing current analysis methods and approaches used for cow milk. Although SCC records have previously been used in selecting cows for treatment or culling within the context of dairy operations (Barkema *et al*., 1997), we believe that determining SCC levels in milk samples can also assist in the control of subclinical mastitis. Although a number of studies have been performed regarding the effect of storage temperature on SCC (Martınez *et al*., 2003; Sanchez *et al*., 2005; Zeng *et al*., 2007), these studies were mainly conducted on sheep and goat milk. Studies on cattle (Barkema *et al*., 1997; Hachana *et al*., 2008; Malınowskı *et al*., 2008), on the other hand, have generally focused on the efficiency of electronic cell counters or on the characteristics of milk samples obtained from different udder quarters. The objective of this research was to identify the effects of storage temperature (refrigerator temperature at 4ºC and room temperature at 21ºC) and storage time on the SCC level of milk samples from Anatolian Buffaloes. The aim of this research was to identify the effects of storage temperature and storage period on the SCC level of milk samples from Anatolian buffaloes.

MATERIALS AND METHODS

In this study, samples were collected from Anatolian buffaloes raised under farm conditions in the Tokat province of Turkey. Approval for all of the live animal procedures of this study was obtained from a University's Institutional Animal Care and Use Committee. Milk samples of 500 ml were collected under sterile circumstances from 20 predetermined healthful Anatolian buffaloes, which were determined based on previous SCC records. The samples were collected in healthy quarters before the morning milking. SCC was measured immediately following sample collection by using a DCC device at the farm. The analysis of these milk samples was performed at 560. Depending of their SCC levels, the examples were divided as high score (>3.16 cells/ml, n=20) and low score (≤3.16 cells/ml, n=20) samples. Following this, the milk samples were seperated into twenty aliquots, and stored at four various temperatures (from refrigerator temperature at 4ºC to room temperature at 21ºC).

The initial analysis of the milk samples was performed under farm conditions. The milk samples were first manually agitated, and the SCC values of the samples were recorded with the aid of a DCC device at 1 h intervals for a total period of 12 h and 24 h. The initial analysis of the milk samples was performed under farm conditions. The milk samples were first manually agitated, and the SCC values of the samples were recorded with the aid of a DCC device at one-hour intervals

for a total period of 12 h (fresh milk, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 h) and 24 h. In accordance to a previously described method for bovine milk samples (Gonzalo *et al*., 2006), all of the milk samples from Anatolian buffaloes were placed into the cassettes one minute before the analysis.

Following the measurement of the SCC values, a normal distribution was achieved by completing a $\log_{10}$ transformation. The repeated measures General Linear Model was used for evaluating the effects of different storage temperatures and storage times on the milk SCC. The effect of various groups was defined using a Duncan's post hoc test (1955).

RESULT AND DISCUSSION

In this study, milk examples were catagorized as high score (>3.16 cell/ml) and low score (≤3.16 cell/ml) samples according to their SCC values. Median values were taken into consideration when forming these groups. The mean value of the low SSC group (SHS low; Figure 1) was determined as 2.27±0.045 cells/ml, while the mean value of the high group (SHS high; Figure 2) was determined as 4.06±0.091 cells/ml. The average somatic cell count was determined as 3.17±0.236 cell/ml.

The logSCC values of the Anatolian Buffalo milk samples are shown in the figures (Figure 1 and Figure 2) with respect to storage time (fresh milk, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 h) and storage temperature (4°C and 21°C).

The logSCC values for the high and low SCC score groups that were determined within the 24 h following collection are shown in the figures. It was observed that the storage time had a discernable effect on the SCC. However, Souza *et al.* (2012) previously reported that the SCC of milk samples stored for 1, 3, 5 and 7 days was not significantly affected by storage temperature and time. The SCC values of the fresh samples were lower ($P<0.01$) than the values of the samples stored at all storage temperatures and times (low score). In addition, the SCC values of the fresh samples were lower ($P<0.01$) than that of the samples stored for different times (3, 4 and 7 h) at 4°C (especially among the high SCC examples). Similarly, the SCC values of the fresh samples were lower ($P<0.01$) than that of the samples stored for different times (1, 2, 3 and 4 h) at 21°C (high score). The results of this study were in agreement with the findings of Miller *et al.* (1986), which demonstrated that the logSCC of milk samples stored at 24 h was higher than that of fresh milk samples. Miller *et al.* (1986) also described that the SCC of unpreserved milk samples incubated at 60°C was higher than that of samples incubated at 40°C, probably due to higher dye penetration consequently of heat-induced harm to the cells. This finding was in contrast with the results of Erdem *et al.* (2012), which demonstrated a linear decrease in the SCC values of milk samples stored at different temperatures (+4°C and -20°C) and for different periods of time (1d, 2d, 5d, 8d and 15d). On the other hand, Barkema *et al.* (1997) observed a 10% decrease in the SCC of frozen cow milk (-20°C) that was stored for 28 days.

SCC in milk samples stored at higher temperatures may rise owing to the traumatic effects of heating on the cells. In addition, the study results also indicated higher SCC values in samples stored at 21ºC in comparison to samples stored at 4ºC for the same duration. This was possibly due to greater dye penetration in these cells as a result of heat-induced damage (in both low and high scores samples). Sanchez-Macias *et al.* (2010) previously examined the effect of storage

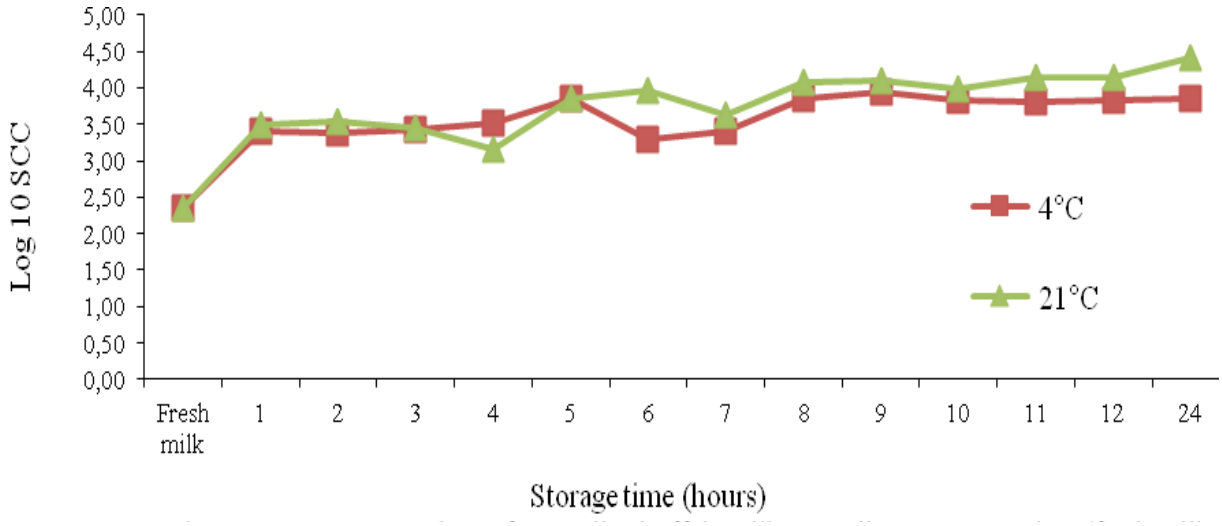

Figure 1. Log_{10} SCC values of Anatolian buffalo milk according to storage time (fresh milk, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 24 h) and storage temperature (4°C and 21°C).

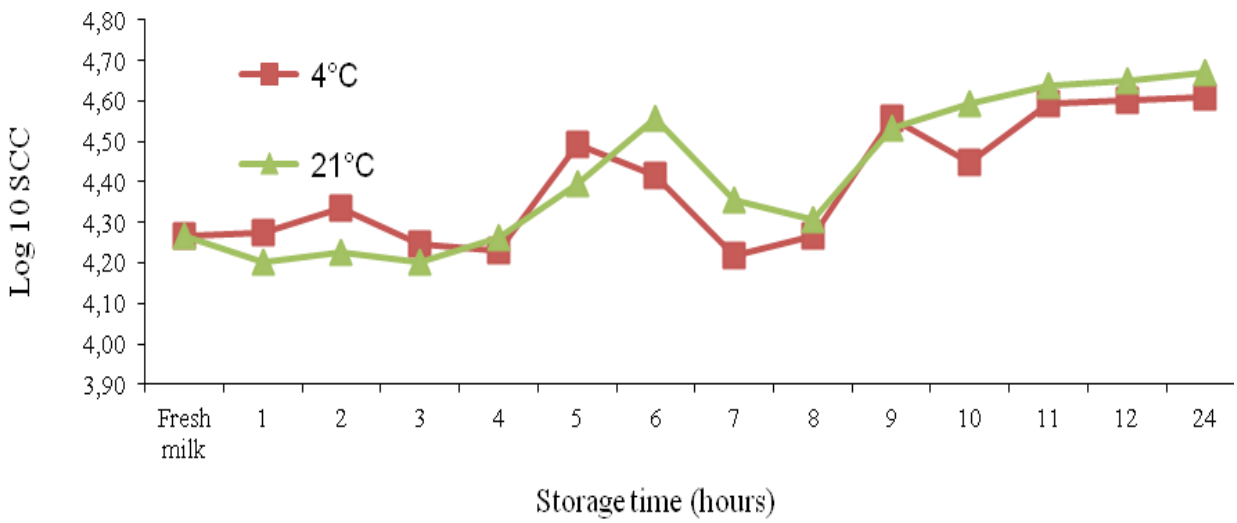

Figure 2. Log_{10} SCC values of Anatolian buffalo milk according to storage time (fresh milk, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 24 h) and storage temperature (4°C and 21°C).

temperature and storage time on goat milk SCC by using the DeLaval cell counter (DCC). SCC were evaluated in 40 Majorera goat milk examples, and the examples were catagorized as high score (>2.750×10^3 cells/mL) and low score (<630×10^3 cell/mL) according to their SCC values. Every milk sample was catagorized into four aliquots, and then stored at four various temperatures (4°C, 21°C, 36°C or 45°C). In their study, Sanchez-Macias *et al.* (2010) observed that the SCC value of the milk samples was lower after 1 h of storage. Sanchez *et al.* (2010) had similarly observed that the SCC of their goat milk samples was lower next 60 minutes of storage at any of the evaluated temperatures.

Storage of high SCC goat milk samples for 3, 4, and 7 h at 4°C, and for 1, 2, 3 and 4 h at 21°C led to a decrease in SCC values in comparison to fresh milk. Similarly, Sanchez *et al.* (2010) had observed that storing high SCC goat milk samples for 1 or 2 h at 21°C led to a decrease in the SCC value in comparison to fresh milk. However, the findings of our current study are not in agreement with previous research (Sanchez *et al*., 2005) that reported that the storage of goat milk samples in a refrigerator (4°C) without any preservatives allows stable counts to be obtained for a period of 10 days. In addition, the consequences found in the current research were in dissimilarity with the findings of an previous study by Sanchez *et al.* (2005), which presented no change in the logSCC values of goat milk samples stored at room temperature for 24 h to 13 days subsequent gathering.

In our study, no difference was observed in the logSCC values of the samples that were storaged at 4°C and 21°C for 5 h (Figure 1). In both the low and high SCC score samples (Figure 1 and Figure 2), the SCC values obtained with the DCC apparatus were considerably influenced by the storage period (h/days). Consequently, the logSCC values of the high and low SCC samples were also significantly affected.

It was observed that the logSCC of the milk samples in the current study varied according to storage temperature and storage time. Storage period had a considerable impect on the logSCC, independently of whether the samples were high or low SCC score samples. The DCC apparatus was an effective instrument for assessing SCC in the fresh milk samples and demonstrated that storage at 4°C and 21°C led to an increase in the logSCC within 1 to 12 h for low SCC samples, and within 24 h for high SCC milk.

However, a similar experiment previously conducted with goat milk (Sierra *et al*., 2006) indicated that the SCC values did not vary between the analytical temperatures of 40°C and 60°C. In other studies performed on bovine milk samples, Gonzalo *et al.* (2004) determined that the analytical temperature did not affect the SCC.

In the current study, the effect of storage temperature on logSCC was significant. In other studies, the SCC was lower in frozen samples compared to refrigerated samples. This effect may be associated with the preservation and analytical temperature of milk (Martinez *et al*., 2003). Souza *et al.* (2012) reported that the storage temperature and milk age did not significantly affect the SCC values of the milk samples (P>0.05). The SCC values of milk samples incubated at 10°C, 20°C and 30°C were slightly lower than those incubated at 5°C.

Storage temperature significantly affected SCC values in both the low and high SCC samples. Among low score samples that were stored 1 to 12 h and 24 h (Figure 1), the samples stored at 21°C had higher logSCC values than the samples stored at 4°C. Similarly, among high-score samples that were stored for 1 to 3 h (Figure 2), the samples

stored at 21°C had higher logSCC values than the samples stored at 4°C.

The effect of storage time on logSCC values were statistically significant. The results in the current study were inconsistent with the findings previously reported by Zeng *et al.* (2007), which described that the SCC values of goat milk (as measured by a Fossomatic machine) did not vary during a 7 day storage period under farm conditions.

Malinowski *et al.* (2008) described that the measurement of SCC values with a DCC can also be performed for milk samples stored at 4°C for 24 or 72 h. In unlike to these consequences, Martinez *et al.* (2003) declared that the analytical temperature of 60°C caused a significant decrease in logSCC in comparison to the analytical temperature of 40°C. For frozen sheep milk samples, the SCC at the analytical temperature 60°C decreased significantly, possibly due to increasing dye penetration consequently of heat-induced harm to the cells. Furthermore, Martinez *et al.* (2003) also reported that the decrease in SCC may be associated with the adverse effects of thawing and storage time on cells. It was similarly reported that freezing temperatures led to lower SCC values in milk compared to refrigeration temperatures. The decrease in SCC was possibly associated with mistakes/errors made during the refrigeration and melting of the examples. The method of thawing (slow or rapid) did not significantly affect the SCC values of the milk samples analyzed at 40°C.

Furthermore, the consequences attained in the current investigation were in opposite with the findings of an earlier study conducted by Sanchez *et al.* (2005), which presented no differences in the logSCC of goat milk samples when they were stored at room temperature (4°C) for 1 days to 13 days subsequent gathering.

Similar somatic cell counts were obtained for milk samples (low SCC score samples, to be exact) that were incubated at -20°C and 4°C for 4 h.

These results were not in agreement with previously finding for milk samples obtained from cows (Barkema *et al*., 1997) and ewes (Martinez *et al*., 2003). In this study, it was observed that the log SCC values varied with to the storage period and storage temperature. The results of the current study were not in aligment with the earlier findings study from Gonzalo *et al.* (1993).

The effect of storage time (24 h) on logSCC was significant (Figure 1 and Figure 2) when the milk samples were stored at 4ºC. This result is in alignment with the results of Zeng *et al.* (1999), who determined that the milk samples could be storage at refrigerator temperatures (3 to 5°C) for a maximum three days. In addition, Dohoo *et al.* (1981) reported that the milk samples stored at ambient temperatures become unacceptable in terms of their SCC values in about 16 hours after collection. On the other hand, Souza *et al.* (2005) reported that milk samples must be retain under cooling till examination, and that the SCC must be evaluated in 7 days of example gathering.

The results of this study were not in compatible with the conclusions of earlier researches in the literature. There is a possibility that the study results might have been affected by the fact that cell nuclei can easily degenerate during storage. Temperature and time were factors that contributed significantly to the increase in somatic cell count. Similar observations were previously made by Barkema *et al.* (1997); Sierra *et al.* (2006). Due to the limited number of studies on this area, there is currently insufficient data and information to develop a clear picture regarding the effects of storage temperatures and storage times on SCC. Thus, additional and more comprehensive

studies need to be conducted to further clarify and elucidate this subject.

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