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Effect of Various Environmental Factors and Management Practices on Somatic Cell Count in the Raw Milk of Anatolian Buffaloes

Aziz Sahin[1]* Arda Yıldırım [2] and Zafer Ulutas[3]
[1]*Department of Animal Science, Faculty of Agriculture, Ahi Evran University, 40100 Kırşehir, Turkey*
[2]*Department of Animal Science, Faculty of Agriculture, Gaziosmanpasa University, 60250, Tokat, Turkey.*
[3]*Department of Animal Production and Technologies, Nigde University, 51240 Nigde, Turkey*

ABSTRACT

The aim of this study was to determine the effects of various environmental factors on the somatic cell count (SCC) of Anatolian Buffaloes raised under different herd conditions in Turkey. Data were evaluated according to the stage of lactation (early, mid, and late), herd, lactation month, milking time, and parity. Analysis of the data was performed using the SAS package program. For a one-year period, farms were visited on a monthly basis to collect milk samples from each buffalo, in milkings performed both in the morning and evening. A total of 1200 SCC readings from 100 Anatolian Buffaloes were analyzed using repeated measures. The average SCC was determined to be 134,731±18,500 cells/ml. The effects of herd, parity, lactation month, milking time and stage of lactation on the SCC value were statistically significant ($P<0.05$). The mean SCC for morning milking (173,118 cells/ml) was higher than evening milking (148,562 cells/ml). The fourth month of lactation had the highest mean SCC value (186,418 cells/ml), which was statistically different from the values observed during the first, second and fifth months of lactation ($P<0.05$), as well as the sixth month of lactation ($P<0.05$). The SCC level was the highest in the first parity (177,844 cells/ml) and the lowest in buffaloes in their third and fourth parity ($P<0.05$). Mean SCC values were high ($P<0.05$) for late lactation (203,498 cells/ml), low for mid-lactation (81,975 cells/ml). The SCC was low in herd 6 (37,481 cells/ml), and high in herd 1 (223,000 cells/ml). The significant differences identified between the herds indicated differences in management methods, milking hygiene, and barn conditions. To reduce the SCC levels of milk, while also improving udder health, it is necessary to take certain precautions and measures such as improving milking management; improving hygiene and barn conditions; carrying out milking at uniform intervals; feeding the buffaloes after milking; and implementing a mastitis control program. In this context, further studies are necessary to investigate and identify the threshold SCC values that are applicable for Anatolian buffaloes and their associated conditions.

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Key words
Anatolian buffaloes, somatic cell count, parity, stage of lactation.

INTRODUCTION

Buffaloes in Turkey, which are generally referred to as Anatolian buffaloes, are species of the Mediterranean Buffalo, which in turn is a subgroup of River Buffaloes (Soysal *et al*., 2005). In Turkey, Anatolian buffaloes are the second most important species for dairy production. Anatolian buffaloes are the preferred breed in certain parts of Turkey, owing to their resistance to diseases and lower feed consumption. They are mostly bred in North, Middle, West, East, and Southeast Anatolia in Turkey (Atasever and Erdem, 2008). In Turkey, Anatolian buffaloes are mainly bred for the production of meat and milk, being generally slaughtered for meat at the end of their productive years (Sekerden, 2001). For the dairy industry, milk quality is as important as the quantity of milk produced (Dogru, 2015). Somatic cell count (SCC) is a key component of national and international regulations concerning milk quality, as well as an important indicator of udder health and the prevalence of clinical and subclinical mastitis in dairy farming (Lievaart *et al*., 2007; O'Brien *et al*., 2009). SCC reflects the level of infection and resulting inflammation in the mammary gland of dairy animals. High SCC affects a number of factors, leading to a decrease in milk yield, notable changes in milk composition, and reduced shelf life for milk; it can consequently result in considerable economic losses for dairy breeders. On the other hand, it is known that factors such as breed, parity, calving age, stage of lactation, season, stress, milking interval, and environmental and managerial factors can all affect SCC levels in buffalo milk (Muggli, 1995; Singh and Ludri, 2001; Koc, 2008). While somatic cells are always present in milk, their levels increase significantly during mammary gland infections. In milk from cows with healthy udders, the SCC level is normally between 50,000 and 100,000

*Corresponding author: azizsahin78@mynet.com
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cells/ml (Skrzypek *et al*., 2004), while an SCC value of 200,000 cells/ml is considered as the threshold value distinguishing healthy udders from a diseased udders (Harmon, 2001; Skrzypek *et al*., 2004). High SCC values in milk have the effect of reducing the quality of both milk and dairy products. They also affect milk shelf-life and flavor, as well as cheese and butterfat yield (Skrzypek *et al*., 2004). It is important to maintain milk SCC within acceptable limits, since high SCC values can lead to significant risks for animal and human health (Manlongat *et al*., 1998), and contribute to various quality-related problems in the processing of dairy products (Randolph *et al*., 1971). Due to human health and animal welfare concerns, several countries (EU nations and Switzerland) have determined a threshold value of 400,000 cells/ml for SCC in milk (Hillerton, 2001; Cero´n-Mun˜oz *et al*., 2002; Sederevicius *et al*., 2006; Sharma *et al*., 2011, Kasıkcı *et al*., 2012). In Turkey, the threshold value specified by the Turkish Food Codex is >500,000 cell/ml (Anonymous, 2000).

The number of previous studies on SCC levels in buffalo milk are somewhat limited, especially for the buffalo milk produced in Tokat province. In this context, the current study aimed to determine the normal values of SCC - as well as the variations observed in these values - according to different stage of lactation, herd, parity, milking time, lactation month etc. in Anatolian buffaloes.

MATERIALS AND METHODS

Location of the study

This study was carried out at the Tokat province in the Mid-Black Sea Region of Turkey. Tokat is located between 35° 27’ and 37° 39’ East longitudes, and 39° 52’ and 40° 55’ North latitudes. The province has a transitional climate, with features similar to both the Black Sea maritime climate and the Anatolian continental climate. Long-term average annual temperature varies between 8.1°C and 14.2°C. Average relative humidity ranges between 56 and 73% (MARA, 2014).

Buffalo management

The lactating buffaloes grazed outside between the months of April to December, while being kept and fed indoors through the winter. During the grazing period, the buffaloes were allowed to graze between eight to seventeen hours (without any concentrates being fed), and then kept indoors at night. The buffaloes were fed a total mixed ration all year round. The buffaloes were mated naturally, and hand milked twice a day. Buffalo calves were fed on milk in the morning and evening, and weaned at approximately 120 days of age.

Data collection

This study was conducted with buffaloes from 12 herds in Tokat Province, Turkey. Samples from the healthy quarters of 100 healthy buffaloes were taken under different farm conditions during the morning and evening milking. SCC was measured immediately following sample collection by using a DeLaval cell counter (DCC) in the farm. Hamann *et al*. (2010) describes the DCC as the best method to directly determine somatic cell count. Lactating buffaloes were divided three groups based on lactation stages (1^{st}, 2^{nd} month (1: early); 3^{rd}, 4^{th} month (2: mid); and 5^{th}, 6^{th} month (3: late), and into a total of seven groups based on parity (1 to $\geq$7 parities). Season for calving were spring (March, April, May), summer (June, July, August) and winter (December, January, February). Anatolian buffaloes raised in different herds of Tokat were examined starting from February. Data were collected in year 2012 to 2014. The data were obtained from the Anatolian buffaloes in their 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} lactation months, while the age at calving for the buffaloes ranged from 30 to 48, 36 to 60 and 48 to 72 months. Milk samples were collected in the morning and evening on every fifteenth day of each month. To determine the SCC values, milk samples were obtained for the first six months. Data outside these parameters mentioned above were excluded from the study. Hence, for the statistical analyses, a total of 1200 SCC readings were used.

Statistical analysis

The data obtained in this study included multiple SCC readings collected during the lactation months of the buffaloes. Such type of data collection is generally referred to as longitudinal data, or univariate repeated measurement. However, due to the possibility of differences in the management practices of the 12 buffalo farms that might affect the morning and evening milking SCC values/data; morning and evening milking times were considered as two different response variables. This particular type of data collection, on the other hand, is known as multivariate repeated measures, or doubly multivariate data. Statistical analysis of the multivariate repeated SCC data was performed using a linear model with a Kronecker product structured error covariance matrix, after applying 10 base logarithmic transformation (SAS Inst. 2003) in order to provide a normality assumption.

Buffaloes with at least four months and at most five months of lactation data were included into the analysis. The total number of observations used in the analyses were 1200 test day SCC values. Base 10 logarithmic transformation was applied to the SCC data

to create a normal distribution (SHOOK, 1982), and the linear mixed model was applied. In this context, the following statistical model was used:

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + (af)_{im} + (ad)_{il} + (bf)_{jm} + e_{ijklmn}$$

where is μ the overall mean; a_i the i^{th} herd effect (i =1,2,3,…….12); b_j the j^{th} lactation number effect (j =1, 2 ,3, 4, 5, 6, 7); c_k the k^{th} stage of lactation (k = 1: early lactation, 2: mid lactation, 3: late lactation); d_l the d^{th} milking time effect (m =morning, evening); f_m: f^{th} lactation month effect (l = 1, 2, 3, 4, 5, 6); $(af)_{im}$ the interaction between herd and lactation month; $(ad)_{il}$ the interaction between herd and milking time; $(bf)_{jm}$ the interaction between lactation number and lactation month; and e_{ijklmn} the residual random error.

The SAS mixed procedure (SAS Inst. 2003) was used to fit the linear mixed model shown in equation 1 with corresponding R matrix, which is a block diagonal with blocks corresponding to the individuals, and with each block having the compound-symmetry (CS) structure. The form of the R matrix was as follows:

$$R = \begin{bmatrix} R_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & R_{60} \end{bmatrix}$$

where

$$R_i = \begin{bmatrix} \sigma^2 + \sigma_1 & \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma^2 + \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma_1 & \sigma^2 + \sigma_1 \end{bmatrix}$$

and *i*:1,2, 3,…….100 buffaloes

At each time interval, or lactation month, individual observations were considered as repeated measurements of the relevant experimental unit (buffalo within herd). The compound-symmetry covariance structure, which was optimal for the $\log_{10}$SCC data set, was determined with the Schwarz's Bayesian Criterion (Littell *et al*., 1997). Two unknown parameters, one modeling a common covariance (σ), and the other a residual variance (σ^2) of R matrix and the common correlation $\sigma^1/(\sigma^1 + \sigma^2)$, were estimated with SAS. After the significant effects of the fixed factors were identified, differences between least square means of fixed factor levels were found to be significant at P<0.05 (2-tailed) based on the Tukey adjustment type I error rate.

RESULTS

The SCC least squares means, standard errors and differences between the means for parity, herds, stage of lactation, month of lactation and milking time are shown in Table I. In this research, the average SCC was determined as 134,731±18,500 cells/ml. The SCC in different parity, lactation month, milking time, herd and stage of lactations have been presented in Table I. The statistical analysis showed that the effects of herd, parity, stage of lactation, lactation month and milking time were statistically significant (P<0.05) for SCC. None of the interactions were found to be significant. Changes in SCC levels based on parity, herd, stage of lactation, lactation month, and milking time are shown in Figure 1.

The SCC value for morning milking (173,118 cells/ml) was significantly higher compared to the evening milking (148,562 cells/ml). The fourth month of lactation had the highest SCC mean (186,418 cells/ml), and was statistically different from the SCC values for the first, second and fifth months of lactation (P<0.05), as well as the sixth month of lactation (P<0.05). The SCC level was the highest in the first parity (177,844 cells/ml). The SCC level decreased in the later parities, and was statistically different in buffaloes with three, four and five parity (P<0.05), as well as those with two parity (P<0.05). The SCC level was the highest in the first (223,000 cells/ml) and fifth (213,119 cells/ml) herds with the levels observed in these two herds being statistically different from the others. As shown in Table I, herd 6 had the lowest mean SCC value (37,481 cells/ml).

The stage of lactation also had a significant effect on SCC (P<0.05). As shown in Figure 1C, while the mean SCC values were high (P<0.05) in the early stage of lactation (109,415±11,456 cells/ml), they decreased during the mid-stage of lactation (81,975±13,542 cells/ml), and then increased once again during the later stage of lactation (203,498±45,211 cells/ml).

DISCUSSION

The average SCC was determined as 134,731±18,500 cells/ml. This result was in parallel with the findings of many previous studies (Dhakal *et al.*, 1992; Silva and Silva, 1994; Singh and Ludri, 2001; Moroni *et al.*, 2006), which observed that the SCC values for buffaloes varied between 50,000 and 375,000 cell/ml. The average SCC of Mediterranean buffaloes was reported as 169,000 cells/ml by Esposito *et al.* (1997), while in another study, the mean SCC value was determined as 309,000 cells/ml for water buffaloes (Tantillo *et al.*, 1997). Tripaldi *et al.* (2003) reported that the SCC value ranged between 50,000 and 300,000 cells/ml, and that the mean value was 221,280 cells/ml. The mean SCC value was determined as 137,000 cells/ml for Murrah and Mediterranean buffaloes (Coelho *et al.*, 2004), while Lopes (2009) reported the mean SCC value

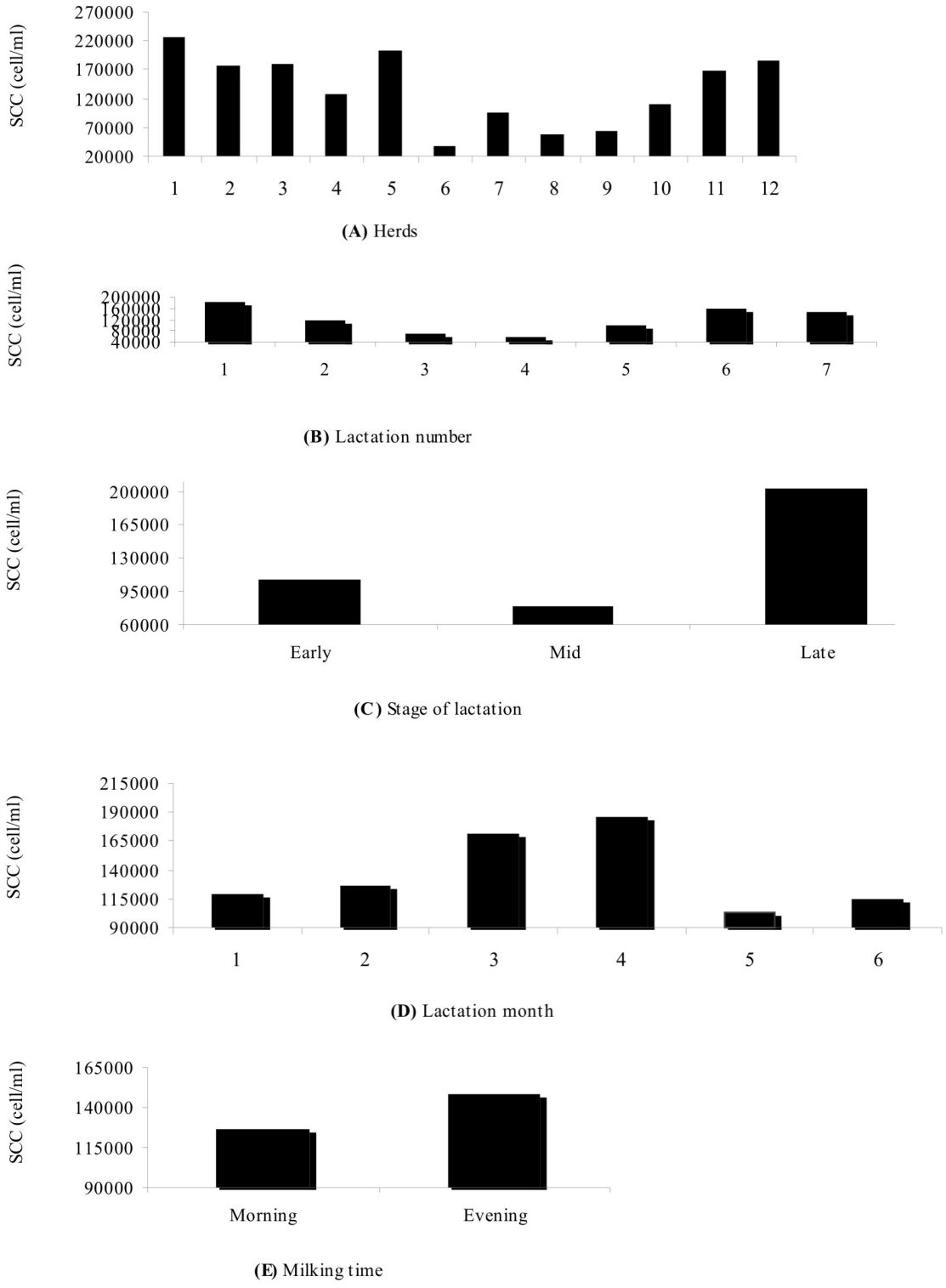

Fig. 1. Effect of herd (A), lactation number (B), stage of lactation (C), lactation month (D) and milking time (E) on mean SCCS in milk.

Table I.- Least square means and standart errors of the Somatic cell count for lactation number, herd, stage of lactation, lactation month, milking time, and significance levels of the factors and differences between the means.

Factors	N	SCC	S_X	$Log_{10}SCC$	S_X
Parity					
1	282	177844[c]	45112	5.25004	4.65429
2	226	114524[b]	43225	5.0589	4.63574
3	196	69656[a]	11280	4.84296	4.05231
4	92	58495[a]	9875	4.76712	3.99454
5	152	97365[ab]	21375	4.9884	4.32991
6	112	156640[bc]	28656	5.1949	4.45722
7	136	145524[bc]	33255	5.16293	4.52186
Herd					
1	72	223000[d]	11875	5.3483	4.07463
2	86	173500[c]	58445	5.2393	4.76675
3	78	176814[c]	66988	5.24752	4.826
4	126	125341[bc]	33145	5.09809	4.52042
5	142	213119[d]	61256	5.32862	4.78715
6	134	37481[a]	9987	4.57381	3.99944
7	72	94865[b]	8275	4.97711	3.91777
8	110	53412[ab]	12524	4.72764	4.09774
9	90	58497[ab]	9318	4.76713	3.96932
10	156	111978[bc]	41578	5.04913	4.61886
11	78	177133[c]	18145	5.2483	4.25876
12	52	183612[cd]	16251	5.2639	4.21088
Stage of lactation					
Early	400	109415[b]	11456	5.03908	4.05903
Mid	400	81975[a]	13542	4.91368	4.13168
Late	400	203498[c]	45211	5.30856	4.65524
Lactation months					
1	200	116561[ab]	18151	5.06655	4.2589
2	200	129987[b]	22355	5.1139	4.34937
3	200	183870[c]	58233	5.26451	4.76517
4	200	186418[c]	27354	5.27049	4.43702
5	200	102671[a]	18645	5.01145	4.27056
6	200	115879[ab]	15263	5.064	4.18364
Milking time					
Morning	600	173118[a]	17489	5.23834	4.24276
Evening	600	148562[b]	9578	5.17191	3.98127
Total	1200	134731	18500	5.12947	4.26717

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

in buffalo milk as 269,590 cells/ml. The mean SCC value was 112,765±75,269 and 50,222±24,952 cells/ml for the Murrah and Mediterranean breeds, respectively (Damé *et al.*, 2010). Based on data obtained for the 400 mammary quarters of 60 buffaloes under conditions in Nepal and India, Dhakal (2006) reported a mean SCC value of 151,000 cells/ml for clinically normal Murrah buffaloes.

In the current study, the mean SCC value for the evening milking (148,562 cells/ml) was statistically lower than that of the morning milking (173,118 cells/ml). A statistically significant effect of milking time on SCC was agreement with the findings of Baltay (2002), Koç (2004), Nielsen *et al.* (2005), and Koç and Kızılkaya (2009). Erskine (2001) as well as Koç and Kızılkaya (2009) described that the SCC level in morning milking was lower compared to the evening milking. The statistically significant difference in mean SCC values observed at different milking times may be due to the differences in milking intervals and milk yield.

The fourth lactation month had the highest mean SCC (186,418 cells/ml), and was statistically different from the mean SCC observed in the first, second and fifth lactation months (P<0.05), as well as the sixth lactation month (P<0.05). The higher SCC level observed in the fourth lactation month during this study is not agreement with the findings of Haas (2003) and Hinrichs *et al.* (2006). In contrast to the current study; Erskine (2001), Santos *et al.* (2004) and Hinrichs *et al.* (2006) described a gradual increase in mean SCC levels towards the end of lactation, before drying off for buffaloes.

The SCC level was the highest in the first parity (177,844±45,112 cells/ml); this level decreased linearly with third and fourth parity, and increased in other parities. For all parities, the SCC was between the range of 58,495±9,875 and 177,844±45,112 cells/ml. The variations in SCC values for different parities were significant (P<0.05; Fig. 2). However, with regards to variations associated with parity, the findings of the current study do not appear to be in line with previous studies (Singh and Ludri, 2001). In the current study, parity had a significant effect on SCC, which indicated that the secretion of somatic cells in milk changes with increasing and decreasing parity. In this respect, while the findings of the current study were supported by certain studies in the literature (Muggli, 1995; Singh and Ludri, 2001), they were in disagreement with various other previous studies (De *et al*. 2010) which observed that milk SCC levels do not increase significantly from the first to the fourth parity.

Imbayarwo-Chikosi *et al.* (2001), Goncu and Ozkutuk (2002), Amin (2001), Haas (2003), Bielfeldt *et al*. (2004) and Hinrichs *et al*. (2006) previously reported that SCC levels increase gradually with increasing parity. A higher SCC level in the first parity might have stemmed from a different defense mechanisms exhibited against mammary infection at a younger stage in life (Haas, 2003).

The stage of lactation also had a significant effect on SCC (P<0.05). As shown in Figure 1C; while the mean SCC values were high (P<0.05) in the early stage of lactation (109,415 cells/ml), they decreased during the

mid-stage of lactation (81,975 cells/ml), and then increased once again during the later stage of lactation (203,498 cells/ml). In this respect, the findings of the current study were in agreement with the results of Singh and Ludri's (2001) study, which also indicated higher SCC levels ($P<0.05$) in the early stage of lactation for 90 days decrease in SCC levels during the mid-lactation stage between days 90 and 120 (90,000 to 99,000 cells/ml), and then an increase in SCC during the late stage of lactation (97,000 to 107,000 cells/ml). Physiologically, dairy buffaloes tend to exhibit increasing SCC levels as their productive period progresses (Dohoo *et al*., 1984; Sharma *et al*., 2011). This trend is inversely related with milk production (Dohoo *et al*., 1984; Sharma *et al*., 2011). Consequently, the SCC levels in buffalo milk were at such high levels towards the end of lactation that, as described by McDonald and Anderson (1981), distinguishing between healthy and unhealthy glands by using SCC values was not possible. Various researchers have described that SCC tends to increase during lactation due to a dilution effect (Miller *et al*., 1991; Bergonier *et al*., 1993; Sing and Ludri, 2001; Zeng *et al*., 1996). The SCC level and milk production appear to have a strong negative correlation (Sing and Ludri, 2001; Zeng and Escobar, 1995).

SCC values tend to increase with progressing lactation (and especially in later stages of lactation) indepedently of whether the cow has an infection (Dohoo and Meek, 1982). Elevated SCC levels have been associated with the buffaloe's immune response, in relation to its preparation for calving, and for enhancing immune/defense mechanism in mammary gland tissues (Reichmuth, 1975). The ratio of neutrophils increases during early and late lactation, while the ratio of lymphocytes decreases (McDonald and Anderson, 1981). SCC levels tend to reach values higher than 1,000,000 cells/ml immediately after paturation, but then decrease to 100,000 cells/ml by the 7^{th} to 10^{th} days post-partum (Jensen and Eberhart, 1981).

The increase we observed in SCC levels is in agreement with other studies, since it has been reported that later lactation stages and higher parity are associated with increased and repeated exposure to pathogens and bacterial diseases, which lead to permanent grandular damage in this area (Cero´n-Mun˜oz *et al*., 2002).

As shown in Table I, the differences in the mean SCC levels between the herds were statistically significant ($P<0.05$). The mean SCC for the herds varied from 37,481 cells/ml in herd 6 to 223,000 cells/ml in herd 1. The differences in SCC levels between the herds was determined to be significant (Fig. 1A, $P<0.05$). The mean SCC levels obtained for herds 2, 3, 4, 6, 7, 8, 9, 10, 11, and 12 reflected the values for healthy buffaloes (*i.e.* without mastitis). The mean SCC levels for herds 1 and 5, on the other hand, reflected the values for unhealthy buffaloes (*i.e.* with mastitis), with the mean count in these herds exceeding 200,000 cells/ml. The significant differences observed between the 12 herds indicated differences in management methods, milking hygiene, and barn conditions. To reduce the SCC levels of milk, while also improving udder health, it is necessary to take certain precautions and measures such as improving milking management; improving hygiene and barn conditions; carrying out milking at uniform intervals; feeding the buffaloes after milking; and implementing a mastitis control program. The results we observed in this study can be explained by the large differences that existed between the herds with respect to in milking management methods and hygiene. However, elevated mean SCC levels in certain herds indicated that additional precautions and measures are necessary to reduce these values to more acceptable levels. Possible precautions and measures that could be applied for reducing SCC include improving managerial factors, barn conditions and hygiene; feeding the buffalo after milking; milking the buffalo in a parlor; applying udder massage; using dry buffalo therapy; using teat dipping before and after milking; practicing CMT periodically; maintaining uniform milking intervals; and giving extra care to the buffalo just before and after calving.

In conclusion, the lower mean SCC levels observed in this study compared to previous studies in Turkey could have been due to increasing efforts for producing milk of higher quality, through approaches aiming to improve managerial factors, barn conditions and hygiene. In this context, further studies are necessary to investigate and identify the threshold SCC values that are applicable for Anatolian buffaloes and their associated conditions.

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