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Effect of dilution ratio on determination of somatic cell count in buffalo milk by direct microscopy

Manda sütünde doğrudan mikroskobik somatik hücre sayısının belirlenmesinde sulandırma oranının etkisi

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ABSTRACT

The objective of this study was to investigate the accurate dilution ratio for determination of somatic cell count (SCC) in buffalo milk by direct microscopic method. Milk samples taken from four Anatolian water buffalo raising farms of two towns of Turkey were diluted with distilled water at 1:1, 1:2, 1:4 and 1:6 dilution ratio (RD) and tested by SCC using direct microscopic method. While 1:1 and 1:2 RD were different from 1:4 and 1:6 RD ($P< 0.05$), SCC groups were not different by towns and farms. Correlation coefficient ($r= -0.481$) between SCC and RD was estimated to be significant ($P< 0.05$). The results reveal that water buffalo milk samples should not be diluted higher than 1: 2 RD for obtaining true SCC data.

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Somatik hücre sayısı
Manda
Süt
Sulandırma
Doğrudan mikroskobik yöntem

ÖZ

Bu çalışma, manda sütlerinde doğrudan mikroskobik somatik hücre sayım yöntemindeki en uygun sulandırma oranı (SO)'nın belirlenmesini amaçlamaktadır. İki ilçedeki dört manda çiftliğinden toplanan süt örnekleri saf suyla 1:1, 1:2, 1:4 ve 1:6 oranında sulandırılarak doğrudan mikroskobik yöntemle somatik hücre sayısı (SHS) bakımından test edilmiştir. 1:1 ve 1:2 SO değerleri 1:4 ve 1:6 SO değerlerine göre farklılık gösterirken ($P< 0.05$), ilçeler ve işletmelere ait SHS grupları arasında fark bulunmamıştır. SHS ve SO arasındaki korelasyon katsayısı ($r= -0.481$) istatistiksel olarak önemlidir ($P<0.05$). Varılan sonuçlar, manda sütlerinde doğru SHS verileri elde edebilmek için sulandırma oranının 1:2'den yüksek tutulmaması gerektiğini ortaya koymaktadır.

1. Introduction

Buffaloes, recognized to have economic significance among livestock animals in terms of milk and meat yields as well as work purposes, are bred in many countries of the world (Ozenci et al. 2008). The advantages of buffalo breeding are: ability to subsist on a low quality and high roughage diet; converting low quality roughages to high quality protein; high adaptability; and use of buffalo skin in leather industry (Khosroshahi et al. 2011). However, number of the water buffalo was decreased in Turkey due to entrance to the intensive animal production and elevated popularity of dairy cattle breeding in last years (Atasever and Erdem 2008). In this view, boosting the number of buffalo population and quality of their yields are seen an indispensability. Currently, different techniques are used for measuring milk quality. Of these, somatic cell count (SCC) is usually used as a reliable reflector to diagnose infection status of udder gland of animals and determination of raw milk quality. Briefly, somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining og gland and leukocytes that have entered the mammary gland in response to infection (Sharma et al. 2011) and The European Union Directives (92/46CEE and 94/71 CEE) set a limit of $400x10^3$ cells/ml for SCC in of bovine and buffalo raw milk (Cerón-Muñoz et al. 2002). In SCC analysis, direct microscopy is known as the standard method by IDF (International Dairy Federation). However, high dry matter and fat levels of buffalo milk can cause false evaluation in reading values in SCC by microscopy. Moreover, little is known about the relationship between the dilution of buffalo milk and accurate SCC readings. The fact that, accurately recording SCC data will help to farm owners for regularly assessing raw milk quality and udder

health of lactating buffaloes. Therefore, the present study was proposed to investigate the accurate dilution ratio for SCC tests using direct microscopy.

2. Materials and Methods

Milk samples were taken from four Anatolian water buffalo raising farms of two counties (Bafra and Carsamba) of Samsun province, Turkey. For each county, two farms, which had similar feeding and management conditions, were chosen for collecting milk samples. Thus, about 50 ml bucket milk samples were taken from each farm and immediately reached to the laboratory via a closed bag including ice-boxes and SCC analysis was performed within the same day. Before SCC test, raw milk samples were applied to dilution process. For this aim, raw milks were diluted with distilled water to 1:1, 1:2, 1:4 and 1:6 (milk:water) dilution ratio (RD). SCC determination was performed by direct microscopy (Packard et al. 1992). For each farm, ten slides were prepared for evaluating SCC. In the analysis, dye solution was composed of 0.6 g certified methylene blue, 52 ml ethyl alcohol (95%), 44 ml tetrachlorethane and 4 ml glacial acetic acid. Total number of fields counted per slide was 40 and the working factor (WF) was 13225. Recorded SCC values, obtained from dilution process, were converted to real SCC values as obtained from raw milks by direct proportion.

In the statistical work, SCC values were transformed to $\log_{10}$ for normality and homogeneity of variances. The data were evaluated by analysis of variance (One-Way ANOVA) and group means were compared by Tukey test. The linear model was as follows:

$$y_{ijkl} = \mu + a_i + b_j + e_{ijk}$$

where;
y_{ijkl} is observation value,
μ is population mean,
a_i is effect of dilution ratio (i= 1,2,3,4),
b_j is effect of farm (j= 1,2,3,4),
e_{ijk} is the random residual effect.

Besides, comparison of logSCC means of the towns were evaluated by Paired Simple *t*-test. To estimate correlations between SCC and RD values, Pearson's correlation coefficient analysis was applied. All statistical analyses were performed using SPSS 17.0 for Windows at the 0.05 significance level.

3. Results

Descriptions for logSCC values by different RD is presented in Table 1. As seen that no significant difference was determined in logSCC by 1:1 and 1:2 RD groups, and also in 1:4 and 1:6 RD groups. However, first two RD means were different from the later RD values (P<0.05).

Table 1. Descriptives of logSCC of buffalo milk in different dilution rates.

Çizelge 1. Farklı düzeyde sulandırılan manda sütlerinde logSHS ile ilgili tanımlayıcılar.

RD	n	Mean (±SD)	Minimum	Maximum
1	40	5.72±0.12[a]	5.48	5.87
2	40	5.65±0.18[a]	5.30	5.96
3	40	5.52±0.13[b]	5.27	5.77
4	40	5.51±0.17[b]	5.31	6.28
Overall	160	5.60±0.18	5.27	6.28

RD: dilution rate (milk/ distilled water; 1= 1/1; 2=1/2; 3= 1/4 and 4= 1/6)
Within the columns the numbers with different superscripts differ significantly (P< 0.05)

Change of logSCC values by towns were given in Table 2. As seen that no statistically significant difference was found between two counties.

Table 2. Means (±SD) logSCC of buffalo milk by towns.

Çizelge 2. İlçelere göre manda sütü logSHS ortalamaları (±SD).

Town	n	Mean (±SD)
1	80	5.61±0.17
2	80	5.59±0.18

(Towns: 1=Carsamba, 2= Bafra)

Distribution of logSCC means of four farms investigated in the investigation is shown in Figure 1. In spite of logSCC mean of Farm 3 (5.55±0.14) was calculated as lower than Farm 1, 2 and 4 (5.60±0.14; 5.62±0.19 and 5.61±0.20, respectively), no significant difference was determined, statistically.

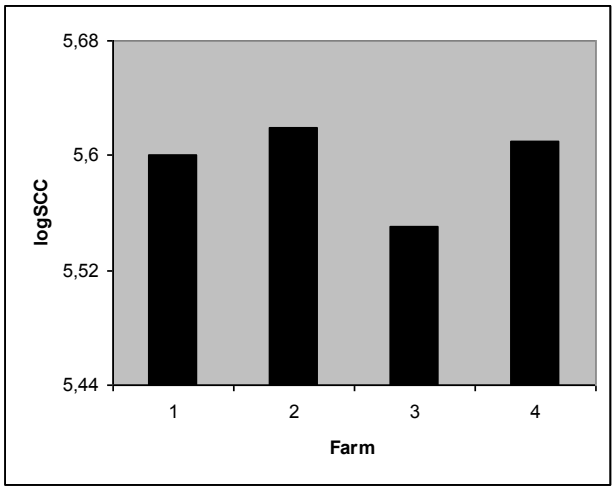

Figure. 1. Change of logSCC means by buffalo farms.

Şekil 1. LogSHS ortalamalarının manda işletmelerine göre değişimi.

Relationship between logSCC and RD values are presented in Figure 2. It could be understood that logSCC numbers declined with elevated RD.

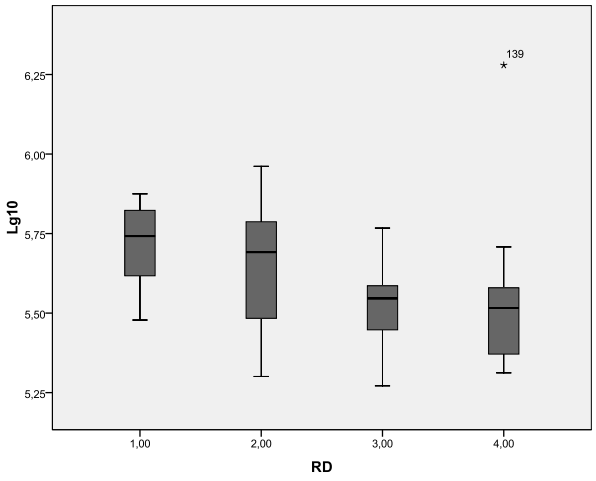

Figure 2. Box plots of RD values by logSCC.

Şekil 2. LogSHS değerlerine bağlı olarak sulandırma oranlarının box-plot dağılımları.

4. Discussion and Conclusion

In this investigation, untransformed SCC data was calculated to be 436978±203893 cells/ml and this value was found as lower than the study results (Atasever et al. 2011) that conducted in the same region, but higher than the results of Syed et al. (2009). Also, obtained mean was found as higher than threshold of EU Directives. As known that elevated milk SCC is associated with altered protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity and higher pH of raw milk (Ogola et al. 2007). In this point, buffalo herd owners of the region should be advised to check their husbandrial practices in the farms.

As seen that logSCC values were declined with advanced RD (Table 1). This case could be assumed as an expected result that occurred by dilution effect. In other words, higher RD might be caused to significant errors in SCC readings. This result could clearly be understood from min.(5.27) and max. (6.28) logSCC values. Actually, the range was very high among the logSCC data and this case clearly indicates to significant variation by SCC readings in different RD levels.

Similar logSCC means of the present investigation (Table 2) could be assumed as an expected case. Because of the farms and towns selected as the study material were located in the similar geoagraphic and climatic region could be caused to this result. Such that, result of Atasever (2012) in the same region on bovine milk supported this finding.

In farm evaluation (Figure 1), logSCC means were found as nonsignificant. Actually, in addition to similar managemental conditions of the farms, uniform animal breed and localization used in the present work might be referred as the main reasons of this finding. Besides, notifications of Atasever et al. (2011) and Sekerden (2011) were found to be parallel with these results.

When the Figure 2 was evaluated by the change of the parameters, it can be pointed out that logSCC numbers declined with elevated RD. Besides, estimated correlation coefficient ($r = -0.481$; $P < 0.05$) was found harmoniously with this finding.

In conclusion, some basic adjustments in microscopic analysis are still needed for ensuring true data for water buffalo milks.. In this context, the current investigation revealed that buffalo milk samples should not be diluted higher than 1: 2 (milk: distilled water) to prevent false readings on SCC by direct microscopy.

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