

## A Study of Somatic Cell Counts in the Milk of Holstein-Friesian Cows Managed in Mediterranean Climatic Conditions

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**Abstract:** Somatic cell counts (SCC) in the milk of Holstein-Friesian (HF) cows were determined by direct microscopic SCC technique. For a two-year period, milk samples were collected monthly from buckets during the morning and evening milkings at 4 different dairy farms. In total, 1,464 SCC readings from 88 HF cows were analyzed by using repeated measures. Herd, lactation month, parity, milking time, and herd interaction effects on SCC were statistically significant ( $P < 0.05$ ). The average SCC in milk per herd was between 296,483 and 688,811 cells/ml. SCC in milk increased as parity increased. The average SCC in milk from evening milking was about 83,165 cells/ml higher than from morning milking. Improving milking management, reducing stress, providing extra care during the first month of lactation, and milking at a uniform interval will help to decrease SCC in milk and the prevalence of mastitis.

**Key Words:** Cows, Holstein-Friesian, milk, somatic cell counts

### Akdeniz İklim Şartlarında Yetiştirilen Siyah-Alaca Sığırların Sütteki Somatik Hücre Sayıları Üzerine Bir Araştırma

**Özet:** Siyah-Alaca (SA) sığırların sütteki somatik hücre sayıları (SHS) direkt mikroskopik SHS yöntemine göre belirlenmiştir. İşletmeler iki yıl süreyle aylık olarak ziyaret edilerek akşam ve sabah sağımalarında sağım kovanından süt örnekleri alınmıştır. Toplam olarak 88 baş SA ineğe ait 1,464 SHS verisi tekrarlanan ölçümler yöntemine göre analiz edilmiştir. SHS üzerine, sürü, laktasyon ayı, laktasyon sırası, sağım zamanı ve laktasyon ayı x sürü interaksyon etkileri önemli bulunmuştur ( $P < 0.05$ ). SHS sürü ortalamaları 296,483 ile 688,811 hücre/ml arasında bulunmuştur. Akşam sağımından elde edilen sütteki SHS ortalaması sabah sağımından 83,165 hücre daha fazla bulunmuştur. Sonuç olarak, sağım yönetiminin iyileştirilmesi, stres faktörlerinin azaltılması, laktasyonun ilk ayındaki ineklere özen gösterilmesi ve hayvanların eşit sağım aralığında sağılması süt sığırı sürülerinde sütteki SHS'yi ve mastitisin yaygınlık düzeyini azaltıcı etkiye sahip olacaktır.

**Anahtar Sözcükler:** Sığır, Siyah-Alaca, süt, somatik hücre sayısı

### Introduction

Somatic cells are always present in milk and they increase due to mammary gland infections. When udders are healthy the somatic cell count (SCC) in milk is between 50,000 and 100,000 cells/ml (1). If the SCC is greater than 200,000 cells/ml, it is assumed to be a threshold distinguishing a healthy udder from a diseased udder (1,2). High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf life and flavor, as well as cheese and butterfat yield (1). Due to human health and animal welfare concerns, several

countries (EU nations and Switzerland) set 400,000 cells/ml as the upper limit for SCC in milk. SCC in milk is influenced by parity, age, stage of lactation, season, stress, milking interval, and environmental and managerial factors (3-6). The breed of cow also affects SCC in milk (7,8); however, the main factor affecting SCC is mammary gland infection (3,9).

The objectives of this study were to determine SCC and the factors affecting SCC of milk produced by Holstein-Friesian (HF) cows managed in Mediterranean climatic conditions in Turkey.

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**Material and Methods**

This study was conducted with 88 HF cows managed at 4 dairy farms in Aydin Province, Turkey. The characteristics of the farms are given in the Table.

Farms were visited monthly from August 2003 to July 2005. Representative milk samples from each cow were collected from bucket milk from both the morning and evening milkings. SCC in the samples was determined by direct microscopic SCC method. Samples from morning milking were analyzed the same day, but evening samples were stored in a refrigerator during the night and analyzed the next day.

Data from first-, second-, and third-parity cows with lactation periods records between 4 and 12 months were included in the analysis. Data outside these parameters were discarded from the study. Hence, for the statistical analyses, a total of 1464 SCC readings were used.

The data set in this study contained multiple SCC measurements made during the lactation months for each cow. This type of data collection is commonly called univariate repeated measurement, or longitudinal data; however, due to the possibility that differences in the management practices of the 4 dairy farms might affect morning and evening milking SCC data, morning and evening milking times were considered 2 different response variables, data collection known as multivariate repeated measures or doubly multivariate data. Statistical

analysis of the multivariate repeated SCC data was carried out by a linear model with a Kronecker product structured error covariance matrix, after applying 10-base logarithmic transformation (10), in order to provide a normality assumption:

$$\text{Log}_{10}\text{SCC}_{ijklm} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + \gamma_l + (\alpha\gamma)_{il} + \epsilon_{ijklm}$$

where  $\mu$  = overall mean,  $\alpha_i$  =  $i^{\text{th}}$  herd effect (1,2,3,4),  $\beta_j$  =  $j^{\text{th}}$  parity effect (1,2,3),  $\delta_k$  =  $k^{\text{th}}$  lactation month effect (1,2,...,12),  $(\alpha\delta)_{ik}$  = interaction between herd and lactation month,  $(\beta\delta)_{jk}$  = interaction between parity and lactation month,  $\gamma_l$  =  $l^{\text{th}}$  milking time effect (morning and evening milking times) and  $(\alpha\gamma)_{il}$  = interaction between herd and milking time, and  $\epsilon_{ijklm}$  = residual random error.

The analysis of repeated measures data, especially double repeated data, requires special care because measurements of the same individual made on different occasions may, quite likely, be correlated. Covariance measures the degree of association between variables, or in this case, between repeated measures of the same variable. Repeated measures covariance structures more specifically estimate the association between residuals of repeated measurements from the same experimental units (11). The SAS MIXED procedure (12) was used to fit the linear model above to the corresponding matrix, which is assumed to be in the form of:

Table. Characteristics of the dairy farms.

	Farm A	Farm B	Farm C	Farm D
No. of cows	26	26	18	18
Barn type	Open Free stall-natural	Open Free stall-natural	Open Free stall-natural	Open Free stall-natural
Milking Milking machine	At the barn Pipe-line, stable	At the parlor Pipe-line, stable	At the barn Pipe-line, mobile	At the barn Pipe-line, stable
Feeding	During milking	After milking	During milking	After milking
Post milking teat-dipping	No	Yes	No	No
Dry cow therapy	No	Yes	No	No
Periodic use of CMT	No	Yes	No	No
Post-milking udder massage	No	Yes	No	Yes
Milking interval (hours)	9-15	11-13	9-15	11-13

$$\text{Cov}(\epsilon_{ijklm}) = \Omega = V \otimes \Sigma = \begin{bmatrix} 1 & \hat{\rho} & \dots & \hat{\rho}^{11} \\ \hat{\rho} & 1 & \dots & \hat{\rho} \\ \vdots & \vdots & \ddots & \hat{\rho} \\ \hat{\rho}^{11} & \dots & \hat{\rho} & 1 \end{bmatrix} \otimes \begin{bmatrix} \hat{\sigma}_1^2 & \hat{\sigma}_2^2 \\ \hat{\sigma}_2^2 & \hat{\sigma}_1^2 \end{bmatrix}$$

where  $V_{12 \times 12}$  and  $\Sigma_{2 \times 2}$  are symmetric positive definite matrices. The  $V_{12 \times 12}$  matrix represents the correlation between repeated SCC in the milk of a cow during the lactation months for a given milking time. Likewise, the  $\Sigma_{2 \times 2}$  matrix represents the covariance between milking times (morning and evening) of a given cow and for a given time point (lactation month) (11,13). After the structure of  $\Omega$  covariance matrix and the significant effects of fixed factors were identified, differences between LSM of fixed factor levels were considered significant at  $P < 0.05$  (2-tailed), based on the Tukey adjustment type I error rate.

## Results

The matrix of correlation of repeated measures,  $V_{12 \times 12}$ , for SCC data between morning and evening milking time can have compound symmetry or the autoregressive covariance structure of order 1 (AR1). For this data set, AR1, which was determined based on Schwarz's Bayesian criterion, was a realistic structure since data were collected at equispaced time intervals (months) and SCCs close to each other in time duration were likely to be more closely associated. The estimated error covariance matrix was as follows:

$$\hat{\Omega} = \begin{bmatrix} 1 & 0.3703 & \dots & 0.3703^{11} \\ 0.3703 & 1 & \dots & \vdots \\ \vdots & \vdots & \ddots & 0.3703 \\ 0.3703^{11} & \dots & 0.3703 & \mathbf{I} \end{bmatrix} \otimes \begin{bmatrix} 0.1159 & 0.0741 \\ 0.0741 & 0.1003 \end{bmatrix}$$

The covariance structure given above indicates that for milking times, the correlation structure between repeated SCC measures remains the same and that covariance between milking times does not depend on time, but remains constant for all lactation months. In addition, the estimated values of  $\Sigma_{2 \times 2}$  showed that

milking times had different variances ( $\sigma_{\text{morning}}^2 = 0.1159$  and  $\sigma_{\text{evening}}^2 = 0.1003$ ) and a moderate association ( $r = 0.69$ ), which might have been a result of the different management practices used for each herd, such as unequal milking intervals and some stress factors that the animals experienced during the daytime between each milking time. As seen in Figure 1, when SCC measurements within each milking time become further separated in lactation month, the association between them decreases from 0.3703 to zero, which is a meaningful covariance pattern for biological data.

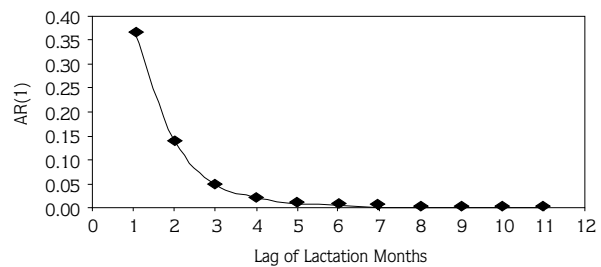


Figure 1. Autoregressive covariance structure of order 1 (AR1), indicating the relationship between lactation months.

Statistical analysis showed that the effects of herd, parity, lactation month, and milking time were statistically significant ( $P < 0.01$ ). The interaction of herd-milking time was also found to be statistically significant ( $P < 0.05$ ).

As seen in Figure 2, the differences between the marginal SCC means among herds were statistically significant ( $P < 0.01$ ). The SCC means for herds were between 296,483 cells/ml in Herd II and 688,811 cells/ml in Herd III. The difference between these 2 herds was very large; amounting to 392,000 cells/ml. Herd II had the lowest mean SCC due to different managerial practices, such as milking cows in the parlor, teat-dipping and dry cow therapy, equal milking interval, etc. On the other hand, Herd I mean SCC was similar to that of Herd IV, but was different from those of the other 2 herds ( $P < 0.01$ ). In Herd I and IV cows were milked in the barn by a pipeline milking machine and had a dryer environment than Herd III; therefore, their SCCs were lower than those of Herd III, but higher than those of Herd II.

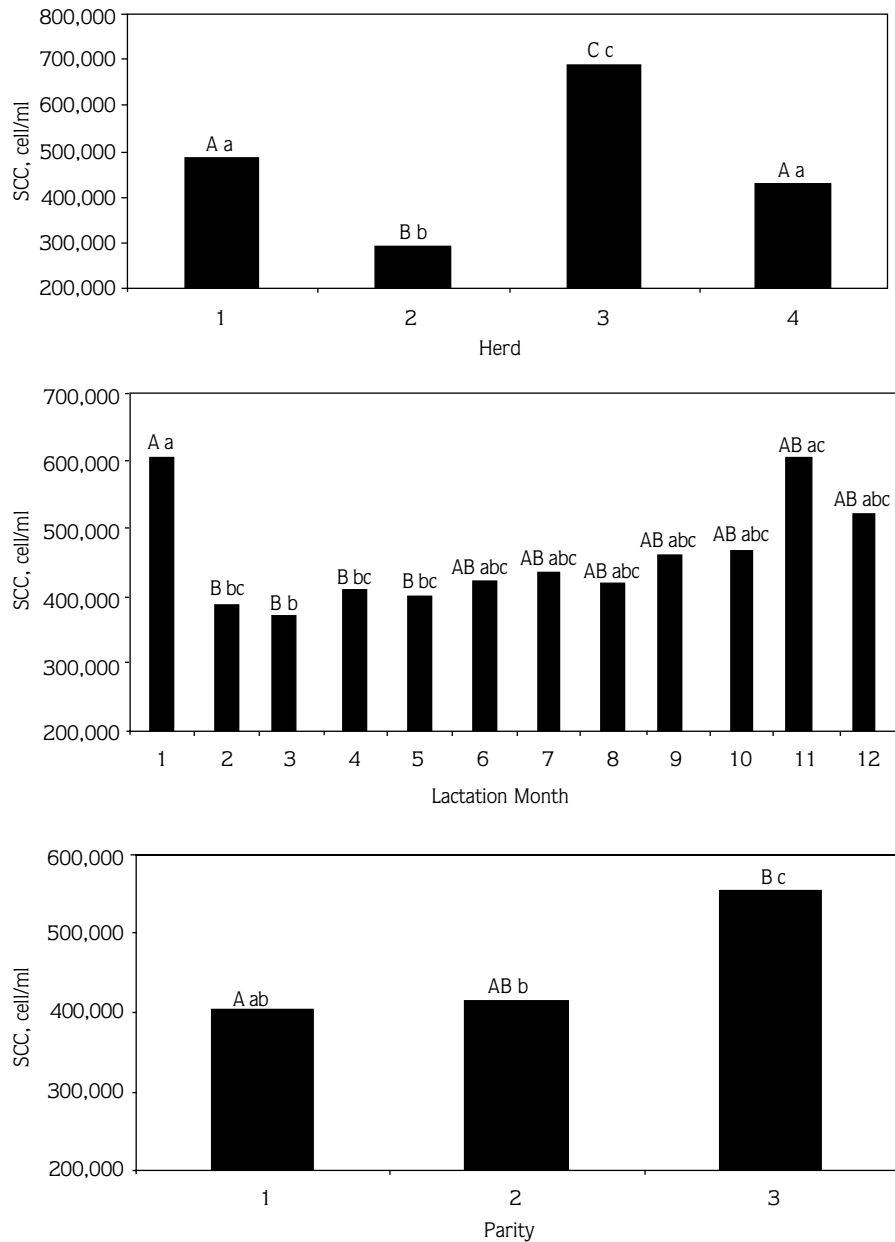


Figure 2. Mean SCCs in milk and differences for herds, parity, and lactation months. A, B, and C show significance at  $P < 0.01$ ; a and b show significance at  $P < 0.05$ .

Lactation month had a significant effect ( $P < 0.01$ ) on SCC in milk (Figure 2). SCC means for lactation months increased gradually from the second month of lactation to the end of lactation. SCC in the first month of lactation was 607,295 cells/ml and dropped to 387,525 in the second month of lactation.

As seen in Figure 2, the lowest SCC was in the second and third lactation months. SCC in these months was below 400,000 cells/ml and started to increase in the fourth month, continuing to increase until the end of lactation. From month 4 to 8, SCC was between 400,000 and 500,000 cells/ml. Then, it increased to over 500,000 cells/ml in the last 2 months of the lactation.

Mean SCC for the first lactation month was different ( $P < 0.01$ ) from the means of months 2-5, but similar to the means of months 6-12. Lactation month 3 had the lowest mean SCC (371,878 cells/ml), which was different from months 1 ( $P < 0.01$ ) and 11 ( $P < 0.05$ ). On the other hand, lactation month 11 had the highest mean SCC (609,116 cells/ml) and the difference between lactation months 3 and 11 was 237,238 cells/ml ( $P < 0.05$ ).

Parity had a significant effect ( $P < 0.01$ ) on SCC (Figure 2). Mean SCC increased as parity increased (Figure 2). The means for parity 1, 2, and 3 were 404,296, 416,582, and 555,393 cells/ml, respectively. Parity 3 was different from parities 1 ( $P < 0.01$ ) and 2 ( $P < 0.05$ ), and the differences between the means were about 151,097 and 138,811 cells/ml, respectively.

Milking time had a significant effect on SCC ( $P < 0.01$ ). The morning and evening milking mean SCCs were 414,572 and 497,737 cells/ml, respectively (Figure 3). For all herds, SCC was lower in morning milking than in evening milking.

The interaction between milking time and herd (Figure 3) was statistically significant ( $P < 0.05$ ). Morning and evening milking mean SCCs were different ( $P < 0.05$ ) in Herd I and Herd III, but the means of Herd II and IV were similar. The difference between morning and evening milking for Herd III was about 200,000 cells/ml. This difference for Herd I was 114,323 cells/ml.

As seen in Figure 3, the morning and evening milking mean SCCs of Herd II were the lowest among all the

herds. The morning mean SCC for Herd II was similar to the morning mean SCC for Herd IV, but the evening mean SCC for Herd II was different from the evening mean SCCs of the other 3 herds ( $P < 0.01$ ). On the other hand, mean SCCs for Herd I and IV were similar for both milkings.

## Discussion

SCC in milk is higher in reports from Turkey (6,9,14) than from some EU countries (1,8,15). In the present study, except for Herd II, mean SCCs were similar to those of previous studies in Turkey (7) and Kenya (16).

SCC during the first month of lactation was higher than in other months. This result agrees with the results of some other studies (3,4,17-19). Sederevicius et al. (3) reported a temporary increase in SCC just after calving due to adaptation of the udder from non-lactating to lactating status. Nikodémusz et al. (18) and De Haas (4) reported a high SCC in the first month of lactation, which dropped in the second month and then increased towards the end of lactation.

In the present study, SCC in milk increased as parity increased. Sederevicius (3), Göncü and Özkütük (9), and De Haas (4) also reported higher SCC for later parities. De Haas (4) indicated that there was a different defense mechanism against mammary infection at younger and older ages.

A significant milking time effect on SCC was found in the present study, which is in agreement with the results

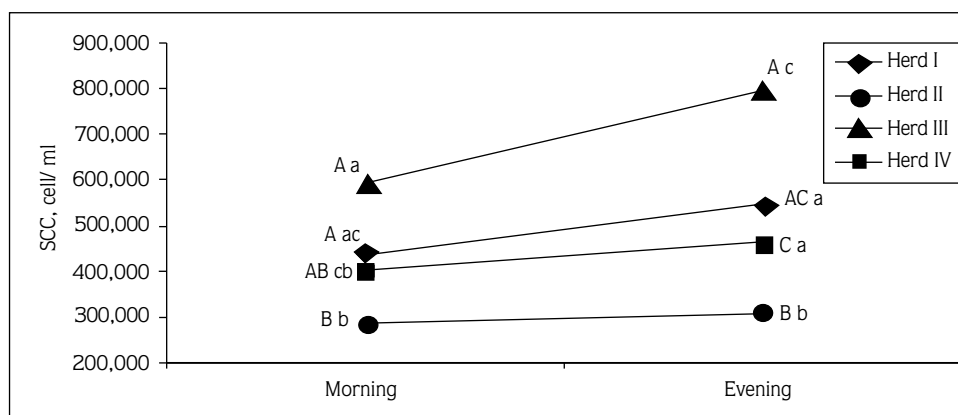


Figure 3. Mean SCCs, and differences between milking time and herds. A, B, and C show significance at  $P < 0.01$ ; a and b show significance at  $P < 0.05$ .

of Koç (7) and Barkema et al. (20). Milking interval is an important factor affecting pressure in the udder and can cause elevated SCC in milk (5). The differences in SCC between the milking times could have been attributed to the dilution effect of longer milking intervals (9,20). Therefore, a shorter milking interval may have caused an increase in the SCC in milk (5), especially in the evening milking of Herd I and Herd III in this study. On the other hand, the difference between mean SCCs for Herd II and III can be explained by the different milking intervals and management practices of these 2 herds.

Results of this study indicate that the first month of lactation is a critical time for new intra-mammary infection, so appropriate care should be given to cows during this time, as well as before calving. Milking cows in the parlor, milking at uniform intervals, feeding cows after milking, using dry cow therapy, periodic use of CMT, post milking udder massage, and teat dipping resulted in decreased SCC in 4 dairy herds. These practices could also reduce the prevalence of mastitis.

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