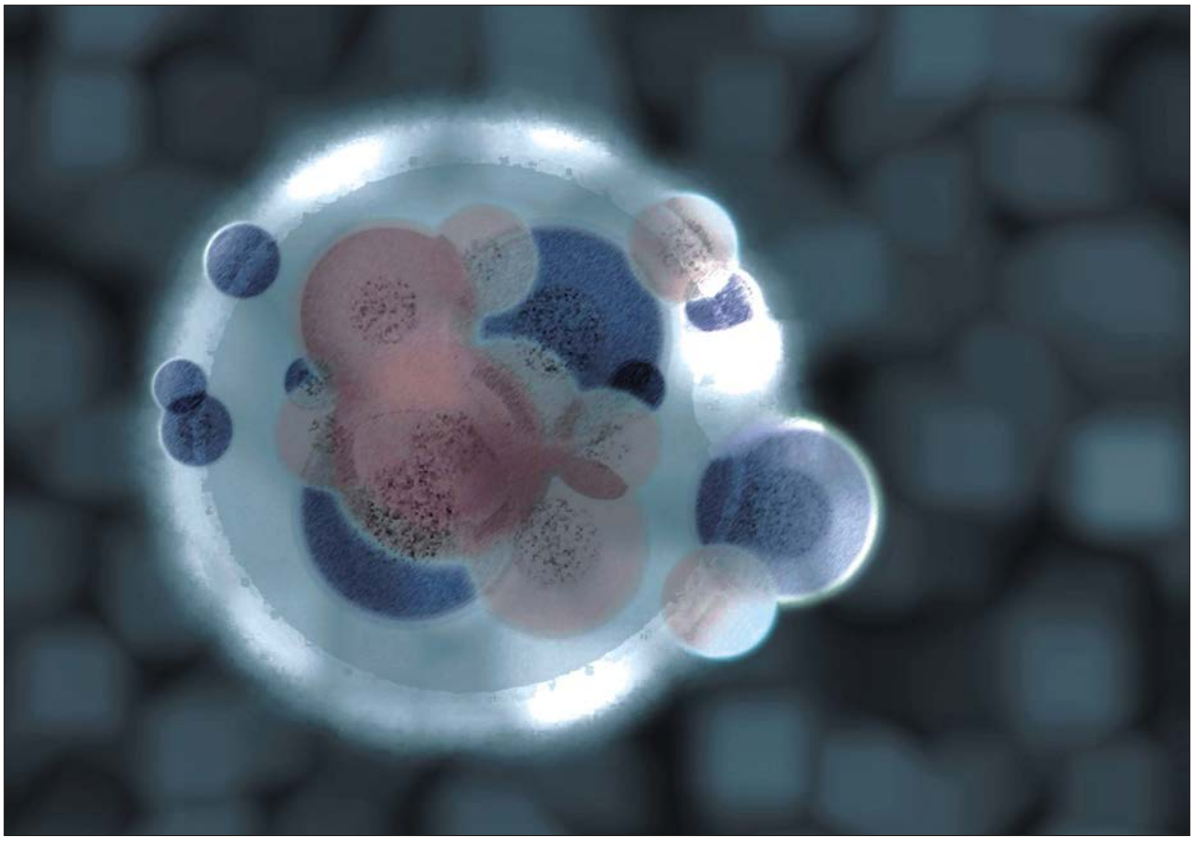

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Analysis of Repeated Milk Somatic Cell Count of Holstein-Friesian Cows Raised in Mediterranean Climatic Conditions

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Abstract: Somatic Cell Counts (SCC) in milk of Holstein-Friesian (HF) cows were monthly determined by direct microscopic SCC technique for two years period. In total, 1,682 SCC data from 95 HF cows were analyzed by using repeated measures. Herd, lactation month, parity, milking time and daily milk yield effects on SCC were found to be statistically significant ($p<0.01$). The averages of SCC in milk for herds were between 319, 448 and 497,279 cells mL^{-1}. SCC in milk increased as parity increased. The average SCC in milk from the evening milking had about 28,768 cells mL^{-1} higher than that of morning milking. Improving barn conditions, managerial practices, milking management, giving an extra care at the first month of lactation and also milking at uniform interval will help to decrease SCC in milk.

Key words: Somatic cell count, repeated measures, milking interval, Holstein-Friesian

INTRODUCTION

Somatic Cells (SC) are always present in milk and the level of SC has been used as an indirect measure of mammary gland inflammation. The SC level of 200,000 cells mL^{-1} is accepted as a threshold distinguishing a healthy and a diseased udder (Dohoo and Leslie, 1991; Skrzypek *et al*., 2004). High SCC in milk reduces the quality of milk and dairy products such as cheese and butterfat and shelf life and flavour of drinking milk (Skrzypek *et al*., 2004; Barbano *et al*., 2006). Due to human health and animal welfare concerns, several countries (EU nations, Australia, Switzerland, etc.) accepted 400,000 mL^{-1} as an upper limit (Skrzypek *et al*., 2004). At some researches in Turkey, on the other hand, higher SCC levels were determined (Uzmay *et al*., 2002; Eyduran, 2002; Göncü and Özkütük, 2002; Koç, 2004).

SCC in milk is increased by infection of udder, as a consequence of lack of management practices (Dohoo and Leslie, 1991; Omore *et al*., 1999; Fernandes *et al*., 2004) and also influenced by cow level factors like breed of cow, parity, age of cow, stage of lactation, etc. (Rice and Bodman, 1997; Barkema *et al*., 1999; Busato *et al*., 2000; Haas, 2003).

The objectives of this study were to analyse the repeated SCC data collected from monthly cow level milk samples over a two-year period and describe the pattern of SCC on randomly selected four different dairy farms in Aydin province of Turkey under the Mediterranean climatic conditions.

MATERIALS AND METHODS

Data: This study was conducted on four different dairy farms rearing Holstein-Friesian (HF) in Aydin Province, Turkey. Farms were visited monthly from August, 2003 to July, 2005. Milk samples were taken from 95 heads of cows for both the morning and evening milkings. Samples used for the analysis taken from the cow having not any visible abnormalities in milk or the udder. The Direct Microscopic Somatic Cell Count (DMSCC) procedure as outlined in Form FDA-2400d was applied to determine SCC in milk samples. Samples from the morning milking were analyzed within the same day, but evening samples were stored in a refrigerator during the night and analyzed on the next day. Cows having at least four and at most eleven lactation months within three parities were evaluated in the analysis.

Statistical analysis: A total of 1,682 SCC data were used for the statistical analyses. Since each cow had multiple SCC over lactation months, this type of data was defined univariate repeated measurements, or longitudinal data. Due to the different SCC variability between morning and evening milking times, the SCC data within milking times was considered as two different response variables and so was defined in the form of multivariate repeated measures or doubly multivariate data over lactation months for each cow.

The statistical analysis of multivariate repeated SCC data was carried out by mixed linear model based on an

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error covariance matrix with a Kronecker product structured. SCC were analysed after applying based-10-logarithmic transformation (Shook, 1982) in order to provide normally distributed data. The statistical model was as follow:

$$Log_{10}SCC_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \omega_l + b(X_{ijklm} - \bar{X}) + \varepsilon_{ijklm}$$

where, μ = overall mean, α_i = ith herd effect (i = 1, 2, 3, 4), β_j = jth parity effect (j = 1, 2, 3), γ_k = kth lactation month effect (k = 1, 2, …, 11), ω_l = lth milking time effect (l = morning and evening milking times), b = regression coefficient of milk yield per milking on Log_{10} SCC, $\bar{X}$: average daily milk yield, X_{ijklm}: daily milk yield and εijklm = residual random error.

The analysis of repeated measures data, especially double repeated data, requires special care because the measurements made on different occasions on the same individual may quite likely be correlated. Covariance measures the degree of association among variables or, in this case, among repeated measures of the same variable. Repeated measures covariance structures more specifically estimate the association among residuals of repeated measurements from the same experimental units (Naik and Rao, 2001). The SAS mixed procedure (SAS, 1999) was used to fit the linear model above with the corresponding Ω matrix, which is assumed to be in the form of:

$$Cov(\epsilon_{ijklm}) = \Omega = V \otimes \Sigma$$

where V_{11x11} and Σ_{2x2} are symmetric positive definite matrices. The matrix V_{11x11} represents the correlation between repeated SCC in the milk of a cow through lactation months for a given milking time. Likewise, Σ_{2x2} represents the covariance between milking times (morning and evening) of a given cow and for a given time point (lactation month) (Naik and Rao, 2001). After the structure of Ω covariance matrix and the significant effects of fixed factors were identified, differences between LSMEANS of fixed factor levels were considered significant at $p<0.05$ (2-tailed) based on the Tukey adjustment type I error rate.

RESULTS

The correlation matrix (V_{11x11}) of repeated SCC data within each morning and evening milking time had the autoregressive covariance structure of order 1 (AR(1)), which was determined based on Schwarz's Bayesian Criterion (Littell *et al.*, 1997). AR(1) for this data set was the realistic structure since data were collected at equispaced time intervals (months) and SCC close to each other in time duration were likely to be more closely associated. The estimated error covariance matrix Ω was as follows:

$$\hat{\Omega} = \begin{bmatrix} 1 & \hat{\rho} & \cdots & \hat{\rho}^{10} \\ \hat{\rho} & 1 & \cdots & \vdots \\ \vdots & \vdots & \ddots & \hat{\rho} \\ \hat{\rho}^{10} & \cdots & \hat{\rho} & 1 \end{bmatrix} \otimes \begin{bmatrix} \hat{\sigma}^2_m & \hat{\sigma}_{me} \\ \hat{\sigma}_{me} & \hat{\sigma}^2_e \end{bmatrix}$$

$$= \begin{bmatrix} 1 & 0.323 & \cdots & 0.323^{10} \\ 0.323 & 1 & \cdots & \vdots \\ \vdots & \vdots & \ddots & 0.323 \\ 0.323^{10} & \cdots & 0.323 & 1 \end{bmatrix} \otimes \begin{bmatrix} 0.1011 & 0.0654 \\ 0.0654 & 0.0847 \end{bmatrix}$$

where ρ^i: relationship between lactation months, $\hat{\sigma}^2_m$: variance of morning milking SCC, $\hat{\sigma}^2_e$: variance of evening milking SCC, $\hat{\sigma}_{me}$: covariance between morning and evening milking SCC.

The results indicated that, when SCC measurements within each milking time become further separated in lactation month, the association between them decreases from $\rho^1 = 0.3230$ to zero ($\rho^{10} = 0.3230^{10}$), that is meaningful covariance pattern for biological data. The estimated values of Σ_{2x2} showed had different variances $\sigma^2_m = 0.1011$ and $\sigma^2_e = 0.0847$ for each milking time and a moderate association (r = 0.71) between SCC from morning and evening milking times for each cow. These results might be a convergence of the unequal milking intervals and some stress factors that the animals revealed during the daytime within each milking time. As shown in Fig. 1 the Daily Milk Yield (DMY) and SCC means estimated from raw data for morning and evening milkings across lactation months were different.

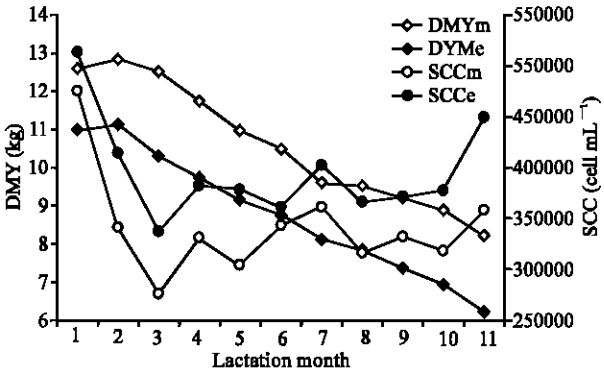

Fig. 1: DMY and SCC means in morning and evening milkings for lactation months

DMY means in morning milking for all lactation months was higher than those of evening milking. However, SCC means in evening milking for all lactation months were higher than those of morning milking.

In the statistical analysis, as seen in Table 1, the effects of herd, lactation month, parity, milking time and covariable DMY were found to be statistically significant ($p<0.01$).

The SCC means were between 319,448 and 497,279 cells mL^{-1} for herds. Herd 2 had statistically significant less SCC (77,378, 104,195 and 177,831 cells mL^{-1}) than Herd 4, Herd 1 and Herd 3, respectively.

Lactation months had a significant effect on SCC in milk ($p<0.01$). The SCC level was sharply dropped from 605,899 cells mL^{-1} in the first month of lactation to the second (454,046 cells mL^{-1}) and third (374,800 cells mL^{-1}) month of lactations. Then, the SCC level fluctuated between 350,000-400,000 cells mL^{-1} from the third lactation month to the month ten (Fig. 2). However, the level increased slightly in the last lactation month. The first lactation month was not different from the last lactation month; however, it was found to be statistically higher (about 200,000-250,000 cells mL^{-1}) than all other lactation months.

SCC means increased as parity increased (Table 1). SCC levels of Parity 1 and Parity 2 were found to be similar but, their SCC levels (345,303 cells mL^{-1}, 390,122 cells mL^{-1}) were significantly different from that of Parity3 (490,456 cells mL^{-1}).

Table 1: Somatic cell count LSMEANS

	Log_{10} somatic cel count (cells mL^{-1})	
	$\bar{X} \pm S_{\bar{X}}$	Back- transformed values
Herd (No. of cows)	p<0.01	
Herd1 (29)	5.6270±0.02494[a]	423,643
Herd2 (30)	5.5044±0.02460[b]	319,448
Herd3 (17)	5.6966±0.03440[a]	497,279
Herd4 (19)	5.5986±0.02900[a]	396,826
Lactation month	p<0.01	
1	5.7824±0.03357[a]	605,899
2	5.6571±0.03215[b]	454,046
3	5.5738±0.03189[b]	374,800
4	5.6048±0.03141[b]	402,532
5	5.5815±0.03145[b]	381,505
6	5.5742±0.03186[b]	375,146
7	5.6047±0.03227[b]	402,439
8	5.5472±0.03236[b]	352,533
9	5.5684±0.03412[b]	370,169
10	5.5615±0.03730[b]	364,334
11	5.6178±0.04192[ab]	414,763
Parity	p<0.01	
Parity1	5.5382±0.01704[a]	345,303
Parity2	5.5912±0.03522[a]	390,122
Parity3	5.6906±0.02636[b]	490,456
Milking time	p<0.01	
Morning	5.5912±0.01727[a]	390,122
Evening	5.6221±0.01638[b]	418,890
Daily milk yield	p<0.01	
	-0.01943±0.002725	

[a,b]: Different letters show the significance between the LSMEANS with each factor

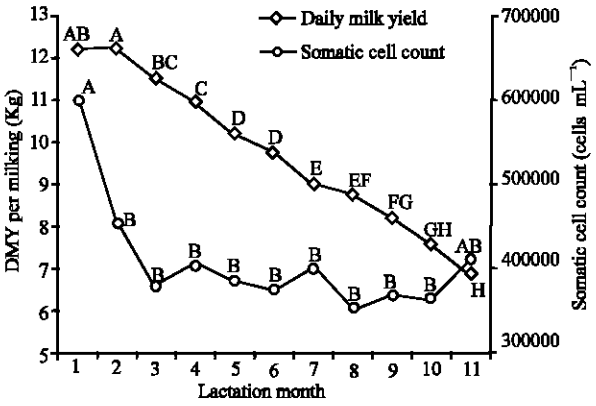

Fig. 2: Changes of lactation month' SCC means and DMY

Milking time had a significant effect on SCC ($p<0.01$). The morning and evening milking SCC means were 390,122 and 418,890 cells mL^{-1}, respectively. The morning milking had statistically significant lower SCC (28,768 cells mL^{-1}) than that of evening milking.

Daily milk yield per milking had also a significant effect on SCC ($p<0.01$). A negative association (-0.01943±0.002725) between SCC and DMY was found (Table 1).

DISCUSSION

The SCC levels for herds in this study were generally lower than some reported works (Uzmay *et al.*, 2002; Eyduran, 2002; Göncü and Özkütük, 2002) but, similar to Koç's (2004) study in Turkey. The results were agreed with Omore *et al.* (1999)'s work in Kenya and Fernandes's *et al.* (2004) work in Brazil.

The differences between the herds SCC means were resulted from different managerial practices, barn conditions, milking management and milking hygiene. The application of having milking parlour, feeding the cow after milking, practicing udder massage, teat-dipping and periodically CMT, dry cow treatment and having an equal milking interval provided Herd 2 with lowest SCC than other. However, the mean for this herd was still higher than the studies conducted in EU countries (Busato *et al.*, 2000; Toledo *et al.*, 2002; Skrzypek *et al.*, 2004) and some extra measures need to be taken like providing dryer environment, using bedding material, culling chronic mastitis cows, milking the mastitis cow at last and more hygienic milking. All these precautions would reduce the SCC level and also prevalence of mastitis and increase the quality of milk for all herds.

A higher SCC level at the first month of lactation found in this study agreed with the results of Deluyker *et al.* (1993), Nikodemusz *et al.* (1994), Rice and Bodman (1997) and Haas (2003). The SCC level was elevated after calving due to several factors like adaptation of the udder from non-lactating to lactating status, higher infection risk, decreased general immune function, humoral defence mechanisms shortly after calving and increased serum cortisol level (Rice and Bodman, 1997; Erskine, 2001). The first month of lactation is critical, so care should be given to the cows at this month (and probably for the second month) and also before calving.

Rice and Bodman (1997), Göncü and Özkütük (2002) and Haas (2003) were also reported higher SCC levels for later parities. Increasing parity was reported a risk factor for increasing prevalence of subclinical mastitis and clinical mastitis incidences (Erskine, 2001). A different defence mechanism against mammary infection in early and late life was also indicated by Haas (2003).

A statistically significant milking time effect on SCC was found in this study agreed with the studies of Barkema *et al.* (1999) and Koç (2004). Erskine (2001) also reported that evening milking SCC could be higher than that of morning milking. The different SCC levels between milking times in this study could be mainly attributed to the dilution effect of shorter milking intervals for evening milking (Barkema *et al.*, 1999; Göncü and Özkütük, 2002) and it was found that increased SCC was associated with decreased milk yield. A negative association between DMY and SCC found in this study agreed with Omore *et al.* (1999) and Bielfeldt *et al.* (2004).

Statistically significant differences among the herds found in this study for SCC levels were agreed with some other studies conducted in Turkey (Uzmay *et al.*, 2002; Göncü and Özkütük, 2002). A significant difference was also determined for DMP per cow among the herds. The differences for SCC level and DMP among the herds were mainly originated from the different barn conditions, managerial practices, nutrition, milking management and hygiene on dairy farms in Turkey. A lower SCC level than some other studies in Turkey (Uzmay *et al.*, 2002; Eyduran, 2002; Göncü and Özkütük, 2002) found in this study for herds shows that there has been an increasing effort given by the farmers and other related organizations in the dairy sector in Turkey to increase the quality of raw milk produced on the farm to meet the EU criteria.

In conclusion, SCC in milk is always present in milk due to cow-side factors, naturally, and the level is increased by the result of inefficient application of managerial factors, lack of mastitis control measures, barn conditions. The SCC level in a herd can be kept lower by applying correct management principals, hygienic rules and providing suitable barn conditions and nutrition, continuously.

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