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Investigation of Milk Somatic Cell Counts During Early Lactation Stages in Awassi Ewes

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ABSTRACT This study aimed to investigate the change of somatic cells counts (SCC) and to determine its normal value during the early lactation period in healthy Awassi ewes. The study was conducted on a total of 75 healthy Awassi ewes. The animals were divided into three groups as those in the second week of lactation (Group 1; n= 25), those in the fourth week of lactation (Group 2; n= 25), and those in the sixth weeks of lactation (Group 3; n= 25). California Mastitis Test (CMT) negative animals were included in the study and, milk samples were collected for SCC analysis. In addition, blood samples were taken from all animals for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gama glutamil transferaz analysis. SCC was significantly reduced in Group 2 compared to Group 1 ($p<0.05$). However, it was observed that Group 3 had a higher rate of SCC than the other groups ($p<0.05$). In conclusion, milk SCC was lowest in the fourth week of lactation, but reached the highest level in the sixth week. Since the milk yield was similar between the groups, it was thought that the SCC was not related to milk yield.

Keywords: *Ewe, Lactation, Milk, Somatic cell.*

ÖZ

İvesi Koyunlarında Erken Laktasyon Dönemindeki Süt Somatik Hücre Sayılarının Araştırılması

Sunulan çalışma, sağlıklı İvesi koyunlarda erken laktasyon dönemindeki somatik hücre sayısının (SCC) değişimini araştırmayı ve normal değerini belirlemeyi amaçlamıştır. Çalışma toplam 75 baş sağlıklı İvesi koyunu üzerinde yürütüldü. Hayvanlar, laktasyonun ikinci haftasında olanlar (Grup 1; n= 25), laktasyonun dördüncü haftasında olanlar (Grup 2; n= 25) ve laktasyonun altıncı haftasında olanlar (Grup 3; n= 25) olmak üzere üç gruba ayrıldı. California Mastitis Testi (CMT) negatif olan hayvanlar çalışmaya dahil edildi ve SCC analizi için süt örnekleri alındı. Ayrıca tüm hayvanlardan alanin aminotransferaz, aspartat aminotransferaz, alkalin fosfataz ve gama glutamil transferaz analizleri için kan örnekleri alındı. SCC, Grup 1'e kıyasla Grup 2'de önemli ölçüde azaldı ($p<0.05$). Bununla birlikte diğer gruplara kıyasla Grup 3'ün daha yüksek SCC oranına sahip olduğu tespit edildi ($p<0.05$). Sonuç olarak, süt SCC laktasyonun dördüncü haftasında en düşük düzeyde iken altıncı haftada en yüksek seviyeye ulaştı. Süt verimi gruplar arasında benzer olduğu için SCC'nin süt verimi ile ilişkili olmadığı düşünüldü.

Anahtar Kelimeler: *Koyun, laktasyon, Somatik hücre, Süt.*

INTRODUCTION

The somatic cell value is the total number of the different cells types such as leukocytes and epithelial cells in milk (Lafi 2006; Souza et al. 2012). Healthy milk has a moderate number of species-specific somatic cells counts (SCC). However, an increase in milk SCC is considered as an indicator of mammary gland inflammation (Lafi 2006). Therefore, milk SCC value is closely related to udder health (Harmon 2001; Stocco et al. 2020) and is accepted as a standard tool at the international level in the evaluation of milk quality (Costa et al. 2020; Podhorecká et al. 2021). However, in order to evaluate milk quality and udder health with SCC, its normal limit should be known first. The milk secretion of ewes is largely apocrine, and the milk contains cytoplasmic particles similar in size to somatic cells, and these particles are considered as normal components of their milk (Souza et al. 2012). On the other hand, although these particles contain large amounts of RNA and protein, they are not considered cells because they do not contain nuclei or DNA. For this reason, there are significant differences in SCC among ruminants, and udder health studies with SCC in small ruminants should be approached from this perspective (Souza et al. 2012).

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However, the SCC threshold value in sheep milk is still a controversial issue (Albenzio et al. 2019). Moreover, it is known that determining the legal limit of milk SCC value in sheep is a complex situation and has not been clarified yet (Paape et al. 2007; Albenzio et al. 2019). It is known that factors such as environment, lactation stage, age, and the number of born lambs are among the parameters that affect the SCC value in healthy sheep (Arias et al. 2012). It is also known that liver function can affect some parameters of milk assessment (Mordak et al. 2020). It has been stated that parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) can be used to determine liver function (Puppel and Kuczyńska 2016). The presented study hypothesized that the number of SCC may change during the early lactation period in ewes with similar characteristics. Therefore, in this study, it was aimed to investigate the change of SCC during the early lactation period in healthy Awassi ewes. In addition, ALT, AST, ALP, and GGT levels were measured to evaluate the health status of the animals and to determine whether there were differences between the groups. So, it is aimed that the results obtained can be used to determine the normal SCC value and to characterize milk quality in Awassi ewes.

MATERIAL AND METHODS

In order to carry out this study, approval was obtained from the Ethics Committee of Cukurova University Ceyhan Veterinary Faculty (15/01 and 30.12.2021). The present study was conducted on a total of 75 healthy Awassi ewes during the early lactation period. The ewes used in the study were selected from among animals that were housed and fed under similar conditions and were identical in terms of age, yield and parity. In addition, the animals were included in the study after the completion of the colostrogenesis stage in the postpartum period.

Group and Study Plan

The animals were divided into three groups as those in the second week of lactation (Group 1; n= 25), those in the fourth week of lactation (Group 2; n= 25), and those in the sixth weeks of lactation (Group 3; n= 25). The ewes in each group were randomly selected from among animals with similar characteristics.

Milk and blood samples

Before collecting milk samples, animals in all groups subjected to the California Mastitis Test (CMT) as previously described (Baştan et al. 2008) and according to the manufacturer's instructions (California mastitis test kit, ImmuCell). CMT reactions were recorded as negative (0), trace, 1 (+), 2 (+) and 3 (+) as previously defined (Baştan et al. 2008; Kandeel et al., 2018). The result was considered negative when 0 value was obtained. Then, CMT negative animals were included in the study and, milk samples were equally collected (15 mL) from 2 udder lobes of each animals into a sterile falcon tube (Isolab®, Germany). Milk samples were transferred to the laboratory under the cold chain (+ 4°C) for SCC analysis. Blood samples (5 mL) were taken from the jugular vein into serum tubes (Hema & Tube®) with clot activator. Blood samples were centrifuged (1500 x g during 10 min), serum was harvested and stored at (-20°C) until analyzes.

Laboratory Analyzes

Analysis of blood biochemistry

Blood serum ALT, AST, ALP and GGT levels were analyzed by electrochemiluminescence immunoassay (ECLIA) method using commercial kits (Cobas®, Roche, Germany).

Determination of milk somatic cell counts

The milk SCC was analyzed using an automatic somatic cell counting device (DeLaval Cell Counter DCC®, Sweden) and a counting cassette (DeLaval Cell Counter Cassettes: 92865881). For the measurement of SCC in ewes milk, device's the extra sucking time was activated as stated in the instruction book for the DCC. All steps of the analysis were carried out according to the manufacturer's instructions (DeLaval 2009).

Statistical Analysis

The results were analyzed using the SPSS package program (version: 16.0; IBM, USA). Kolmogorov-Smirnov test was performed as normality test. As a result of the normality test, continuous measurements showed a normal distribution. The two- factor analysis of variance (ANOVA) was used to analyze the differences between groups. When significant effects were detected, differences between study groups were determined with the Tukey test. The significance level was accepted as $p<0.05$ and the results were given as Mean ± SEM.

RESULTS

It was found that ALT, AST, ALP, GGT levels were similar between all groups ($p>0.05$). The results of biochemical parameters obtained from three groups are given in detail in Table 1. There was no difference between the groups in terms of milk yield ($p>0.05$). SCC was significantly reduced in Group 2 compared to Group 1 ($p<0.05$). However, it was observed that group 3 had a higher rate of SCC than the other groups ($p<0.05$). SCC and milk yield values are presented in Table 2.

Table 1: Biochemical parameters obtained from all groups.

Parameters	Groups			p value
	Group 1 (n:25) (Mean ± SEM)	Group 2 (n:25) (Mean ± SEM)	Group 3 (n:25) (Mean ± SEM)	
ALP (U/l)	89.20±2.98	91.00±4.02	92.48±4.55	NS
ALT (U/l)	18.72±0.57	19.84±0.95	20.68±1.19	NS
AST (U/l)	95.36±1.46	97.72±3.79	93.76±1.46	NS
GGT (U/l)	29.44±1.62	32.32±2.67	27.96±1.32	NS

ALP: Alkaline phosphatase, ALT: Alanine aminotransferease, AST: Aspartate aminotransferase, GGT: Gama glutamil transferaz, SEM: Standart Error of Mean, NS: Not Significant.

Table 2: Somatic cell counts and milk yield findings of all groups.

Parameters	Groups			p value
	Group 1 (n:25) (Mean ± SEM)	Group 2 (n:25) (Mean ± SEM)	Group 3 (n:25) (Mean ± SEM)	
SCC x 10^3(cells/mL)	85.72±1.31[a]	58.40±3.35[b]	122.92±6.08[c]	p<0.05
Milk Yield (L)	0.81±0.03	0.89±0.07	0.93±0.08	NS

SEM: Standart Error of Mean, NS: Not Significant.

DISCUSSION AND CONCLUSION

In this study, it was investigated that the change of SCC level during early lactation in Awassi ewes with similar characteristics. To clarify this, three groups were created from healthy ewes in the second, fourth and sixth weeks of lactation.

In dairy sheep, SCC analysis is a tool used as an indicator of milk quality (Schukken et al. 2003) and udder health (Bouvier-Muller et al. 2018; Hofmannova et al. 2018). Moreover, it is considered the cornerstone of abnormal milk control programs in ruminant such as sheep, cows and goats (Paape et al. 2007). However, the acceptable SCC level for healthy sheep of different breeds has not been clarified, and is still controversial (Leitner et al. 2003; Paape et al. 2007; Albenzio et al. 2019).

In the European Union, it has been declared that the legal limit for hygienic cow milk production criteria is 400.000 cells/ml, but for other milking types, no legal limit has been declared (Albenzio et al. 2019). It has been reported that sheep milks with somatic cell counts <500.000 and between 500.000 to 1.000.000 cells/mL are of good quality and average quality, respectively (Albenzio et al. 2019). Sevi et al. (1999) reported that the SCC threshold value of hygienic sheep milk is 700.000 cells/mL. On the other hand, many different results have been reported regarding the milk SCC level of healthy sheep (Paape et al. 2001; Leitner et al. 2003; Alekish et al. 2014; Riggio and Portolano 2015). In addition, it has been reported that many factors such as lactation number, stage of lactation, management factors (Leitner et al. 2003), twinning, parity and breed (Lafi 2006), milking method and feed affect the SCC level (Sevi et al. 1999). Most importantly, it is known that udder infection affects milk quality (Kurt et al. 2019) and significantly increases SCC level (Leitner et al. 2003; Souza et al. 2012). Therefore, in the presented study, ewes with the same characteristics were used.

In the presented study, it can be thought that the results were negatively affected since the scc numbers of different ewes were followed at 2, 4 and 6 weeks. The fact that the ewes in this study are healthy and have similar characteristics minimizes this assumption. However, we think that following the SCC change of the same animals during the lactation can give more precise results. In this study, animals were first subjected to the CMT test, and then those with healthy udders were included in the study. In addition, some important general health parameters within ALT, AST, ALP and GGT were examined to determine whether there was a difference between the groups. It was found that these parameters were similar between all groups. Thus, it was revealed that the groups were identical in terms of general health. When previous reports are examined (Sevi et al. 1999; Albenzio et al. 2019), it is understood that the SCC value of all groups is acceptable level for healthy udders.

In the presented study, the highest SCC was determined as 122.92±6.08 x 103 cells/mL. However, significant differences were observed between groups in terms of SCC. The SCC decreased in Group 2 compared to Group 1, and it increased in Group 3 compared to the other groups. This revealed that mik SCC in Awassi ewes was lowest at the fourth week compared to second and sixth weeks of lactation. Sevi et al. (2004) stated that SCC increased in the late lactation period compared to the early and middle lactation periods. In this study, although SCC was measured only in the early lactation period, it is thought that the lactation day may have affected the SCC level. Similarly, the SCC increased in Group 3 parallel to lactation day. However, there is no similar relationship between Group 1 and Group 2. It is also thought that there may be a relationship between milk yield and the milk SCC. However, since milk yield was similar between all groups in this study, such a comparison could not be made.

In conclusion, in this study it was investigated SCC variation during early lactation in healthy Awassi ewes. The milk SCC was lowest in the fourth week of lactation, but reached the highest level in the sixth week. Since the milk yield was similar between the groups, it was thought that the SCC was not related to milk yield. In addition, we think that future studies involving larger numbers of animals should investigate SCC change and the relationship between milk yield and SCC throughout the whole lactation period in healthy Awassi ewes.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: SK, FE
Supervision / Consultancy: AB, SS
Data Collection and / or Processing: SK, FE
Analysis and / or Interpretation: SK, FE
Writing the Article: SK, FE
Critical Review: AB, SS

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