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MILK YIELD AND SOMATIC CELLS IN DAIRY EWES WITH RESPECT TO THEIR MUTUAL RELATIONS

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ABSTRACT
The objective of this study was to analyze milk yield and somatic cell count (SCC) expressed as somatic cell sore (SCS) in Lacaune dairy breed. Data from milk performance testing recorded between 2016 and 2018 (farm in West Slovakia) were used. A total, 377 individual milk yield and SCC records of 61 ewes (first, second and third lacation, respectively) were analysed. Mixed model for milk yield included fixed factors: SCC class (lowest, low, middle, high and highest), year of measurement, lactation number, month in milk and interaction between month in milk and SCC class, and random factors of ewe and error. Mixed model for SCS included milk yield class (lowest, low, middle, high, highest), year of measurement, lactation number, month in milk and interaction between month in milk and milk yield class. Random factors of ewe and error were considered as well. Milk yield was significantly affected (p <0.05 or p <0.01) by all investigated factors. Except for interaction between month in milk and milk yield class, the remaining factors significantly affected (p <0.05 or p <0.01) also SCS. The analyses confirmed that SCC may be used as a useful indicator of udder health. It may help in identifying infected ewes, and thus, avoiding mammary infections to be spread throughout the whole flock.

Keywords: Lacaune; milk yield; somatic cell count and score; SCC; Slovakia

INTRODUCTION
Dairy sheep sector is a traditional branch of livestock in Slovakia. In order to be competitive, an increase of milk yield of good quality remains one of the most important goals od sheep farms. However, this aim may be a potential risk for udder health. Consumers, on the other hand, are more iterested in welfare of animals **(Tančin et al., 2019)**, when deciding which food to buy. Types of breeding systems and (also welfare) thus influence both ewe production abilities and health/desease conditions. Somatic cells are considered to be of a negative effect on health of mammary gland and are used for detection of udder infection in ewes **(Gonzalo et al., 1994; Gonzáles-Rodríguez et al., 1995; Tvarožková et al., 2019)**. The consequence of increased SCC is decreasing raw milk quality, which has further consequences for milk processing **(Hag, 2001)**. Mastitis is a costly health problem in dairy ewes **(Arias et al., 2012)**; mammary infections damage udder tissue **(Burriel, 1997)**.

Tvarožková et al. (2019) summarised knowledge about defining the physiological/pathological levels of somatic cell count (SCC) and of proposing the possible tresholds for healthy mammary gland in ewes **(Pengov, 2001; Berthelot et al., 2006; Sutera et al., 2018)**. These values vary among dairy sheep breeds and no single value to differentate between uninfected and infected udder was accepted **(Berthelot et al., 2006; Tančin et al., 2017)**. For example, tresholds for healthy udders in ewes may be as follows: 265×10^3 somatic cells.mL^{-1} **(Caboni et al., 2017)**, 300×10^3 somatic cells.mL^{-1} **(Kern et al., 2013)** or 500×10^3 somatic cells.mL^{-1} **(Sutera et al., 2018)**. **Gonzalo et al. (1994)** and **El-Saied, Carriedo and San Primitivo (1998)** recommended SCC values ranging from 2.5×10^5 to 3×10^5 cells.ml^{-1} as thresholds between healthy and infected udders. According to **Jaeggi et al. (2003)**, thresholds above 1000×10^3 somatic cells.mL^{-1} decrease the cheese yield and increase the development of rancid flavours in the cheese.

No routine determination of SCC in individual ewes is undertaken on national level in Slovakia; however, there are farms interested in SCC to be known due to fact that costs to cure infected individuals and the decrease of milk yield may affect the profitability. In Slovakia, reports aimed at investigation of SCC and distributions of ewes in respective SCC classes as well as their influence on milk yield and composition were published **(Idriss et al., 2015; Tančin et al., 2017)**; possible values that enable to distinguish between ewes infected/uninfected with mastitis were discussed **(Oravcová, Mačuhová and Tančin, 2018; Tvarožková et al., 2019)**.

In spite of fact that some analyses were done, this study was aimed at providing in-depth investigation of mutual relations between SCC and milk yield on a level of a single farm. Purebred Lacaune ewes were included in the analysis.

The hypothesis was as follows: SCC negatively influences amount of milk yield; vice versa amount of milk yield negatively influence SCC.

MATERIAL AND METHODOLOGY

Data were collected from the farm located in western Slovakia during the period of three years (from 2016 to 2018). Milk yield and somatic cell count (SCC) of Lacaune (LC) ewes were analysed. Test-day records were taken once per month (under the the guidance of certificated organisation for milk recording i.e. Plemenárske služby, š. p. SR Bratislava). Ewes were machine milked two times per day after lambs were weaned. However, only morning milkings were taken into account.

A total of 667 records of 61 ewes with 95 lactations i.e. 1.56 lactation per ewe) were included. Ewes were in their first, second and third lactation, respectively.

Ewes predominantly lambed in February and March. According to their lambing, ewes were on their second to sixth month in milk (MIM): MIM 2 (30 to 60 days after lambing), MIM 3 (61 to 90 days after lambing), MIM 4 (91 to 120 days after lambing), MIM 5 (121 to 150 days after lambing) and MIM 6 (151 and 180 days after lambing). Due to only six measurements taken between 181 and 194 days, these were included in MIM6. At least, ewes with three test-day records per lactation were considered.

According to SCC, five classes were formed: lowest SCC (under or equal to 200×10^3 cells.mL^{-1}), low SCC (between 200×10^3 and 400×10^3 cells.mL^{-1}) middle SCC (between 400×10^3 and 600×10^3 cells.mL^{-1}), high SCC between 600×10^3 and 1000×10^3 cells.mL^{-1}) and highest SCC (above 1000×10^3 cells.mL^{-1}). Because of non-normal distribution of SCC, these values were transformed and somatic cell score i.e. SCS= $\log_2$(SCC/100000)+3, as mentioned by **Riggio et al. (2007)**, was analysed. According to milk yield (MY), five classes were also formed: lowest MY (under or equal to 200 ml), low MY (between 200 and 400 ml) middle class MY (between 400 and 600 ml), high MY between 600 and 1000 ml) and highest MY (above 1000 ml).

The mixed model methodology using MIXED procedure **(SAS 9.2, 2009)** was applied to study the influence of factors affecting the variation of milk yield and SCS. Two different models were considered. The model equation (1) was used for milk yield:

$$y_{ijklmn} = \mu + Y_i + L_j + M_k + C_l + M_kC_l + u_m + e_{ijklmn} \quad (1)$$

where:

- y_{ijklmn} – individual observations of milk yield
- μ – general mean
- Y_i – fixed factor of year class (2016, 2017, 2018); $\sum_i Y = 0$
- L_j – fixed factor of lactation number (1, 2, 3); $\sum_j L = 0$
- M_k – fixed factor of month in milk (2, 3, 4, 5, 6); $\sum_k M = 0$
- C_l – fixed factor of SCC class (5 levels as mentioned above); $\sum_l C = 0$
- M_kC_l – fixed factor of interaction between month in milk and SCC class; $\sum_{kl} MC = 0$
- u_m – random factor of ewe (1, 2 to 61); $u_m \sim N(0, I\sigma_m^2)$
- e_{ijklmn} – random error; $e_{ijklmn} = N(0, I\sigma_e^2)$

The model equation (2) was used for SCS:

$$y_{ijklmn} = Y_i + L_j + M_k + C_l + M_kC_l + u_m + e_{ijklmn} \quad (2)$$

where:

- y_{ijklmn} – individual SCS
- μ – general mean
- Y_i – fixed factor of year (2016, 2017, 2018); $\sum_i Y = 0$
- L_j – fixed factor of lactation number (1, 2, 3); $\sum_j L = 0$
- M_k – fixed factor of month in milk (2, 3, 4, 5, 6); $\sum_j MIM = 0$
- C_l – fixed factor of milk yield class (5 levels as mentioned above); $\sum_l C = 0$
- M_kC_l – fixed factor of interaction between month in milk number and milk yield class; $\sum_{kl} LMIM = 0$
- u_m – random factor of ewe (1, 2 to 61); $u_m \sim N(0, I\sigma_m^2)$
- e_{ijklmn} – random error; $e_{ijklmn} = N(0, I\sigma_e^2)$

Fixed factors included in the models (1) and (2) were estimated using the Least Squares Means (LSM) method. Statistical significances of fixed factors were tested by Fischer's F-test; statistical significances of individual differences between estimated levels of fixed factors were tested by Scheffe's multiple-range tests. Differences were considered statistically significant when p <0.05 or p <0.01. Ewe and residual error variances were estimated using the Restricted Maximum Likelihood (REML) method. Estimated variances enable to estimate repeatability of MY and SCS and can be interpreted as the proportion of total variance attributable to within-individual variance:

$$r^2 = \frac{\sigma_m^2}{\sigma_m^2 + \sigma_e^2}.$$

RESULTS AND DISCUSSION

Analysis of variance of fixed factors affecting milk yield (MY) and (SCS) of Lacaune (LC) ewes is given in Table 1. The factors of year of measurement (three years included to increase number of observations), lactation number and month in milk (MIM) were significant (p <0.05 or p <0.01). Both, the factor of somatic cell count (SCC) class when MY as dependent variable was analysed and the factor of MY class when SCS as dependent variable was analysed, were significant (p <0.01). The factor of interaction between MIM and SCC class (model 1) was significant (p <0.01). The factor of interaction between MIM and MY class (model 2) was non-significant (p >0.05). Differences in studied traits with respect to individual levels of factors included in models are discussed below.

Table 1 Analyses of variance (statistical significance of Fisher F-test) for milk yield and somatic cell score.

	Traits	
Factor	**MY**	**SCS**
Year	++	+
Lactation number	+	+
MIM	++	+
SCC class	++	N.C.
MY class	N.C.	++
MIM*SCC class	+	N.C.
MIM*MY class	N.C.	−

Note: MY – milk yield, SCS – somatic cell score, MIM – month in milk, SCC – somatic cell count, N.C. – not considered, ^{++}p <0.01, ^{+}p <0.05, ^{-}p >0.05.

Table 2 Least squares means and standard errors for milk yield by somatic cell count class and for somatic cell score for somatic cell score by milk yield class.

	SCC class (10^3 cells.ml^{-1})				
	Lowest (1)	**Low (2)**	**Middle (3)**	**High (4)**	**Highest (5)**
	≤200	>200≤400	>400≤600	>600≤1000	>1000
	n=10	n=57	n=138	n=142	n=30
Trait	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$
MY (ml)	647 ±23	561 ±27	573 ±37	487 ±39	538 ±24
Scheffe's tests			1:2^{+},3^{++},4^{++}		
	MY class (ml)				
	Lowest (1)	**Low (2)**	**Middle (3)**	**High (4)**	**Highest (5)**
	≤200	>200≤400	>400≤600	>600≤1000	>1000
	n=10	n=57	n=138	n=142	n=30
Trait	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$
SCS	6.69 ±0.83	6.43 ±0.36	5.21 ±0.27	4.95 ±0.27	3.97 ±0.47
Scheffe's tests			2:3^{+},4^{++},5^{++}		

Note: MY – milk yield, SCS – somatic cell score, SCC – somatic cell count, ^{++}p <0.01, ^{+}p <0.05.

Table 3 Least squares means and standard errors for milk yield and somatic cell score by year of measurement, lactation number and month in milk.

		Traits	
Factor		**MY (ml)**	**SCS**
Year	n	$\mu \pm s_\mu$	$\mu \pm s_\mu$
2016 (1)	138	556 ±28	5.62 ±0.31
2017 (2)	138	602 ±24	4.91 ±0.31
2018 (3)	101	526 ±28	5.72 ±0.35
Scheffe's tests		2:3^{++}	2:3^{++}
Lactation number			
First (1)	145	517 ±26	5.90 ±0.33
Second (2)	127	584 ±25	4.80 ±0.30
Third (3)	105	583 ±29	5.64 ±0.35
Scheffe's tests		1:2^{+}	1:2^{+}
Month in milk			
30-60 days (2)	55	733 ±33	5.71 ±0.37
61-90 days (3)	81	667 ±27	6.19 ±0.33
91-120 days (4)	86	565 ±29	5.29 ±0.32
121-150 days (5)	86	454 ±28	4.99 ±0.33
>151 days (6)	69	389 ±29	5.07 ±0.34
Scheffe's tests		2:4^{++},5^{++},6^{++} 3:5^{++},6^{++} 4:5^{+},6^{+}	2:4^{+}

Note: MY – milk yield, SCS – somatic cell score, n – number of observations, ^{++}p <0.01, ^{+}p <0.05.

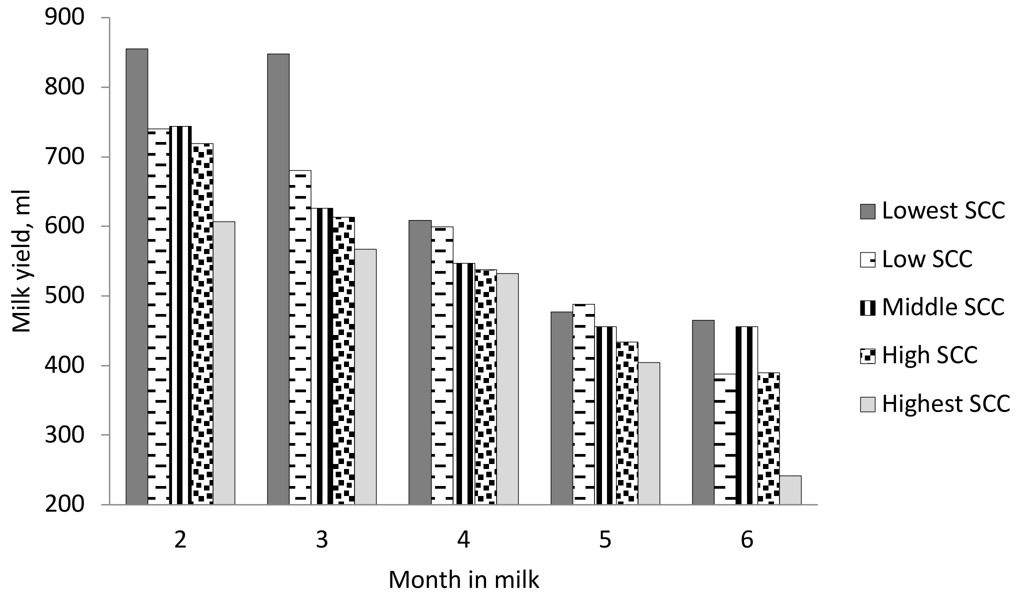

Figure 1 Milk yield in dependence of month in milk and somatic cell count class.

Least squres means (LSM) of MY and SCS confirmed negative relations between these traits (Table 2) i.e. the higher MY, the lower SCS is found. With increasing SCC (model 1), MY deacreased, with exception between classes with SCC>600≤1000 and >1000×10^3 cells.mL^{-1}. The differences between these classes, however, were found non-significant and respective LSM are probably affected by distribution of observations and their lower number (especially in highest SCC class). Accordingly, SCS increased with decreasing MY (model 2). Some differences between individual levels of MY class were also found non-signignicant. The proportion of highest SCC class of i.e. SCC above 1000×10^3 cells.mL^{-1} was 8 % of milk records. The proportion of records with SCC under or equal to 200×10^3 cells.mL^{-1} (lowest class of SCC) was only 3 %. The most of records fell in classes with SCC>400x10^3≤600x10^3 cells.mL^{-1} (middle SCC class) and SCC>600x10^3≤1000x10^3 cells.mL^{-1} (higher SCC class) i.e. 37 % per each. The remaing proportion (15 %) fell in class with SCC>200x10^3≤400x10^3 cells.mL^{-1} (lower SCC class). According to these findings, about 90 % of ewes had healthy udders (or may be of subclinical mastitis udders) as compared with report of **Gonzalo et al. (1994)**, who recommended SCC values ranging from 500×10^3 to 1000×10^3 cells.mL^{-1} as thresholds between healthy and infected udders. When comparing with reports **El-Saied, Carriedo and San Primitivo (1998)**, **Caboni et al. (2017) and Kern et al. (2013)** who recommended SCC values ranging from 250×10^3 to 300×10^3 cells.mL^{-1} as thresholds of healthy udders, the proportion of ewes those could suffer from subclinical mastitis icreased. Regarding distribution of ewes in dependence on SCC class, **Tvarožková et al., (2019)**, who analysed Tsigai, Lacaune and Slovak Dairy breed ewes, reported the following frequencies: about 88 % in lowest class of SCC (under or equal to 200×10^3 cells.mL^{-1}) and about 8 % in highest class of SCC (above 1000×10^3 cells.mL^{-1}) in 2017. Frequencies in 2018 were found to differ: about 21 % and 32 % in lowest and highest class, respectively. High changes between years were probably due to fact that more heterogeneous data were studied (various breeds and various flocks) than data analysed in this study. These also might indicate differences in managment between 2017 and 2018. **Idriss et al. (2015)**, reported highest proportion of ewes in lowest class of SCC and lowest proportion of ewes in highest class of SCC. These proportions slightly differed between breeds (Tsigai, Improved Valachian and Lacaune and their crossbreds, although the same pattern was was found in dependence of breed.

When comparing estimated changes in MY according to SCC class found in this study, these were between 12 and 25 %. **Tančin et al., (2019)**, who also investigated relationships between MY and SCC in Lacaune breed, estimated these changes between 10 to 18 %. When investigated these changes on farm level (five farms), these changes were higher **(Tančin et al., 2017)**. **Sutera et al. (2018)** reported that estimated losses in MY according to SCC level used were about 16% at maximum (in Valle del Belice ewes studied). Although no analyses of microrganisms in udders were done, the negative effect of increased SCC level on milk yield could be supposed when comparing with literature. For example, **Martí De Olives et al. (2013)**, who performed the bacteriological analysis in Manchega ewes, found that milk yield between healthy and infected ewes differred by about 17 % in favour of healthy ewes.

The fluctuation in LSM of MY (also of SCS) in dependence on year of measurement (Table 3) may indicate some problems in management practice of flock, especially when evaluating these traits between 2017 and 2018; probably worse conditions occurred in 2018. A rough increase of MY (and decrease of SCS) were found with increasing lactation number, although some differences were found non-significant (significant difference between first and second lactation was found). The effect of MIM showed significant influence on MY and SCS although variation of SCS was lower (less significant differences revealed) in comparison to variation of MY (more significant differences revealed). Finding about influence of lactation number partly agreed with previous studies **(Oravcová et al., 2006; Oravcová, Mačuhová and Tančin, 2018)**, who reported no significant differences in MY in dependence on lactation number when LC, Tsigai (TS) and Improved Valachian (IV) ewes and LCxTS and LCxIV crosses were analysed. For log_{10}SCC, the latter authors revealed all differences between invidual lactations to be significant. Detailed comparisons of MY in this study and study of **Oravcová, Mačuhová and Tančin (2018)** those related LC ewes in the same flocks showed worse levels of flock mangement, also in terms of mastitis control might be supposed: in earlier period (2010-2013) higher MY was observed. **El-Saied, Carriedo and San Primitivo (1998)** reported that lactation number and stage of lactation (could be considered as MIM) significantly affected SCS in Churra ewes. In contrast, **Othmane et al. (2002)** found age of ewe (could be considered as lactation number) and stage of lactation to be non-significant when SCC in ewes of the same breed were analysed later. According to the latter authors, no differences were a result of strict mastitis control (teat dip after milking, selective dry therapy and culling of ewes with chronic mastitis) and high levels of husbandry applied in flocks investigated. The lower variation of SCS and higher variation of MY in dependence on stage of lactation was reported for French Lacaune ewes **(Barillet et al., 2001)**.

The influence of interaction between MIM and SCC (Figure 1) was significant when MY (model 1) was analysed. Within individual months, some significant differences between SCC classes were revealed. However, most of differences were found between lower SCC classes (mostly MIM 2 and MIM 3) on the one hand and higher SCC classes (mostly MIM 4, MIM 5 and MIM 6) on the other hand. Comparisons with literature could not be done: to our best knowledge, no study which included interaction between MIM and SCC class in similar way was performed. However, a relationship between lactation stage and comatic cells showed that milk yield seemed to be of the higher influence on SCC at the end of lactation (MIM 6) than at the beginning, which is in accordance with findings of **Arias et al. (2012)**.

Figure 1

When interation between MIM and MY class was considered when SCS (model 2) was analysed, the differences were non-significant, although trends were similar to those found when individual MIM and MY classes were investigated (not shown).

CONCLUSION

The findings of this study confirmed fact that somatic cells were present in ewe milk and may used to indicate udder health and contribute to improve levels of management, in terms of preventing the mastitis to be spread. Because number of somatic cells increases when infectious agents enter the udder, further research aimed at relationships between somatic cells, microorganisms and quality of ewe milk is needed.

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