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Correlation of somatic cell count in sheep milk with the total amount of milk obtained by machine milking, the total milk yield and the percentage of milk stripped by machine

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Summary

***Mastitis* is an inflammation of the mammary gland (udder). It can be caused by physical injury or by infection, leading to the growth of microorganisms which invade the mammary gland. *Mastitis* is a major problem for farmers of dairy sheep and other dairy animals leading to economic losses. Different breeds have different traits and are therefore are differently susceptible to developing *mastitis*. It is therefore important to study and compare different breeds (Tsigai and Improved Valachian breeds) and their crosses in terms of milk yield and other various traits that are determinants of the likelihood of *mastitis*. The results of the study indicate that there is a statistically significant ($P < 0.001$) effect of genotype on the amount of milk obtained at machine milking in 30 seconds, the source of variation, and the total milk yield. There are notable differences between crossbreeds and purebreeds, particularly in the incidence of subclinical *mastitis* in favour of purebreds. The results demonstrated that the order of lactation had a statistically highly demonstrable effect on LOG SCC ($P < 0.001$). Ewes in their first lactation exhibited significantly lower somatic cell counts. Therefore, selecting ewes for lower somatic cell counts could also leads to less sickness and better ewe performance.**

Keywords: dairy sheep, *mastitis*, milk, somatic cell count, udder

Mastitis is an inflammation of the udder, generally caused by bacteria, and it leads to economic loss, mainly consisting of discarded milk, reduced milk production and quality, and increased health costs.

Bergonier and Berthelot (10) reported that annual incidence of clinical mastitis in sheep is generally lower than 5%, whereas the incidence of subclinical *mastitis* ranges from less than 10 to 50% or more. Moreover, Legarra et al. (26) reported that susceptibility to *mastitis* is one of the reasons for culling in sheep. Generally, the incidence of clinical *mastitis* varies between 20 and 40% per cow/year (23); whereas the annual incidence of clinical *mastitis* in small ruminants is generally lower than 5% (15). The incidence of subclinical *mastitis* in sheep and goats has been estimated at 5-30% per lactation or even higher (10, 14). *Mastitis* in dairy sheep results mainly from bacterial infections whose reservoir is generally in the udder or teat and transmission between ewes is increased by milking (25). Poor milking practices and equipment cleanliness can introduce bacteria into the udder, increasing the risk of infections and thus SCC. The following environmental factors influence somatic cell count (SCC) in sheep milk, in addition to milking hygiene: nutrition and stress. Stressful conditions, including poor handling during milking, uncomfortable living conditions, nutritional deficiencies,

and extreme weather, can impair immune function and increase susceptibility to infections (31). Furthermore, the age of the animal and stage of lactation can also affect SCC. Older ewes frequently exhibit elevated SCC levels, which can be attributed to diminished immunity and the cumulative impact of udder infections over time. Somatic cells occur normally in milk of both cattle and small ruminants. Somatic cells consist of many types of cells, including polymorphonuclear leukocytes, macrophages, lymphocytes, eosinophils, and various epithelial cells from the mammary gland. Albenzio et al. (1) reported a reduction in fat and casein content in ewes infected by *mastitis*. When a ewe suffers from *mastitis*, the infection can damage the mammary epithelial cells, which are responsible for synthesizing milk components such as fat and casein. Barillet et al. (7) reported a 5% frequency of culling for clinical *mastitis* and a 9.7% frequency for subclinical mastitis as predicted by SCC. In cattle, the incidence of *mastitis* has an important effect on culling decisions (30), particularly *mastitis* that occurs before the time of peak milk yield (9). Selection for improved resistance to *mastitis* can be done directly, by selecting against *mastitis* itself or indirectly by selecting for a trait correlated with *mastitis* (21).

Moreover, *mastitis* data are difficult and expensive to collect, whereas SCC is currently recorded in several milk recording schemes in both dairy sheep (5) and cattle (11). Therefore, SCC is promoted as an indirect method of predicting mammary infections and as a selection criterion to improve mastitis resistance (6, 23). However, Legarra et al. (26) considered the measure of SCC as an indicator of subclinical *mastitis*. Whereas clinical *mastitis* is generally identified by evident signs, subclinical *mastitis* is usually inferred from SCC (10).

Milk quality, with respect of hygiene, is an important aspect in dairy sheep farming. Where quality payment systems are applied, SCC is one of the parameters considered when determining premiums or penalties on milk price (33). As a consequence, the somatic cell count has a great impact on the economy of the farmer and of the dairy industry. In sheep industries where meat rather than milk is the predominant focus of production, a reduction in milk yield from mastitis is of commercial importance because of its negative effect on lamb growth rate.

Because there is a strong relationship between udder health and the amount of somatic cells in milk, limits have been set for SCC in milk in many countries. However, there are no limits to SCC in sheep's milk as it is in cow's milk in Slovakia.

In the present study, we wanted to investigate to what extent the number of somatic cells in ewes reared in Slovakia is influenced by the amount of milk obtained by machine milking (MM), the total milk yield (TMY) and the percentage of machine stripped milk (PMSM), and which factors influence the overall variability of these parameters.

Material and methods

In the conditions of one breeding, we monitored milk production and milk yield in ewes of 5 genotypes created on the basis of purebred Tsigai (T) and Improved Valachian (IV) sheep under the conditions of machine milking (row milking parlour 1 × 24, vacuum pressure 38 kPa, 120 pulses, ratio 1 : 1). At the same time, the number of somatic cells – SCC – was determined in milk samples taken from the same ewes (Bentley 500 apparatus).

The number of control measurements for somatic cell count (SCC), decadal logarithm of SCC, milk yield per 30 seconds, machine milk yield, total milk yield and percentage of machine-stripped milk exhibited a range of 159 to 191 (Tab. 1). A normality test was conducted for each group and each dependent variable. The distribution of SCC is not normal; whereas, conventional statistical methods usually assume normally distributed data. In order to obtain a distribution which closely resembles a normal distribution, the SCC is log-transformed to somatic cell score (SCS). The formula widely used is: $SCS = \log2(SCC/100) + 3$ (2).

Besides purebred T and IV ewes, IV × LC ewes with 50%, 62.5% and 75% of the improving breed and T × LC crossbred ewes with 50% of LC were included in the experiment. Ewes of all genotypes were on the first to third lactation and 2 control measurements or milk sampling were made during the milking period. Among the observed parameters characterizing milk yield, in the present study we evaluate the amount of milk obtained by machine milking in 30 s (MY30s), followed by machine milk yield (MMY), total milk yield (TMY) and the percentage of machine stripped milk (PMSM). We used multivariate analysis of covariance to evaluate the influence of the observed genetic and non-genetic factors (lactation order, control measurement and days in milk) and Pearson correlation coefficient (phenotypic and residual correlations) was used to determine the level of dependence between the observed traits. The mathematical-statistical software package SAS-ver was used in the calculations. 8.2, GLM method and Corr.

Results and discussion

Table 1 shows the influence of genetic makeup and lactation stages on milk production traits in sheep. This is of significant importance for breeding and management decisions in dairy production. The genotypes analysed include Improved Valachian (IV), crosses of IV with Lacaune at 50% and 75% (IV × LC), Tsigai, and Tsigai crossed with 50% Lacaune (T × LC). The data set includes information on the number of control measurements, averages, standard errors, coefficients of variation, and minimum to maximum values for each trait. Additionally, the table presents the F test values, which indicate the significance of variance among genotypes and lactation order. The analysis reveals significant differences between genotypes in terms of somatic cell count (SCC), milk yield per 30 seconds (MY30s), machine milk yield (MMY), and total milk yield (TMY) and percentage of machine stripped milk (PMSM). For instance, the T × LC (50% LC) genotype exhibits elevated values in numerous milk yield param-

eters in comparison to other genotypes. Lactation order also exerts an influence on these parameters, with first lactation generally demonstrating disparate outcomes in comparison to subsequent lactations. Table 1 denotes statistical significance levels with annotations such as ns (not significant), +, ++, and +++ (denoting increasing levels of significance), helping to identify which differences are statistically robust.

Table 1 shows that the number of somatic cells transformed by the decadic logarithm was statistically highly significantly influenced by genotype ($P < 0.001$). Purebred IV (1.90) and purebred T (1.96) ewes had the lowest LOG SCC. The differences between these breeds were not statistically significant. Thus, it appears that our most abundantly represented dairy sheep breeds are equally susceptible to mastitis. However, the data presented in Table 1 also suggest that crossbred Tsigai and Improved Valachian sheep with the Lacaune breed, which were reared under the same conditions as purebred T and IV ewes, are more likely to have subclinical *mastitis*, also manifested by a higher SCC. The differences between T and T × LC crossbreeds with a 50% genetic proportion of LC were statistically significant, as was the case for the Improved Valachian ewes. The highest somatic cell counts (428.8×10^3/ml) were observed in IV × LC crossbreeds with 75% and 62.5% LC, respectively. Whether the observed condition is only a result of the influence of the genotype of the improving breed or whether the obtained results are also related to the different morphological and functional characteristics of the udder of crossbred ewes will require further study. However, the decisive factor influencing the somatic cell count is the udder inflammation itself, i.e. the crossbred ewes had a higher incidence of subclinical *mastitis*.

The results presented in Table 1 show that genotype had a statistically highly significant effect ($P < 0.001$) on the amount of milk obtained at machine milking in 30 s, on SV and TMY, with significant differences especially when comparing crossbreeds and purebreeds. According to our results, the order of lactation had a statistically highly demonstrable effect on LOG SCC

Tab. 1. Results of analysis of variance of somatic cell count and selected parameters characterizing milk letdown in ewes of different genotypes

Source of variation	Measurement					
	SCC ($\times 10^3$/ml)	LOG SCC	MY30s ml	MMY ml	TMY ml	PMSM%
Number of control measurements	159	159	190	190	191	191
Total average	246.70	2.03	228.95	335.94	448.12	27.23
Standard error	471.84	0.422	83.94	130.93	147.09	14.62
Coefficient of variation	192.1	20.78	36.66	38.97	32.83	53.71
Min.	10.0	1.0	10.0	10.0	30.0	5.0
Max.	370.60	3.57	560.0	880.0	1080.0	100.0
Genotype						
IV	184.1	1.90^{bc}	262.7^{a}	390.6^{b}	507.5^{bc}	23.9
IV × LC (50% LC)	176.1	2.00^{abc}	255.4^{ad}	433.5^{b}	581.4^{c}	25.7
IV x LC (75 and 62.5% LC)	428.8	2.33^{a}	217.2^{bcd}	422.5^{b}	560.3^{bc}	28.8
Tsigai	223.3	1.96^{bc}	193.2^{bc}	229.4^{a}	317.1^{a}	28.6
T × LC (50% LC)	283.4	2.20^{a}	248.8^{ad}	360.5^{b}	482.2^{b}	27.4
F test value	1.07^{ns}	5.00^{+++}	5.80^{+++}	18.74^{+++}	23.16^{+++}	0.83^{ns}
Lactation order						
1.	124.80	1.87^{a}	244.53	392.91	510.4	23.38
2.	326.04	2.16^{b}	236.26	354.1	475.56	28.02
3.	326.54	2.22^{b}	225.54	353.97	483.18	29.24
F test value	2.86^{ns}	8.71^{+++}	0.69^{ns}	1.54^{ns}	0.85^{ns}	2.39^{ns}
Control measurements						
1.	437.17	2.10	277.48	435.44	564.94	25.95
2.	81.08	2.06	193.40	299.14	414.47	27.81
F test value	1.58^{ns}	0.03^{ns}	3.53^{ns}	3.81^{ns}	3.69^{ns}	0.06^{ns}
Covariance						
Days in milk (F value)	1.64^{ns}	6.07^{+}	0.04^{ns}	0.16^{ns}	0.44^{ns}	0.01^{ns}

Explanations: SCC – somatic cell count; LOGSCC – decadal logarithm of SCC; MY30s – milk yield per 30 seconds; MMY – machine milk yield; TMY – total milk yield; PMSM percentage of machine stripped milk +++ $P < 0.001$; ++ $P < 0.01$; +++ $P < 0.05$; ns – non significant (non-significant effect); a, b, c – differences in means marked with an unequal letter are statistically significant (also applies to Table 2)

($P < 0.001$), when ewes on 1st lactation had significantly lower somatic cell counts.

In Table 2, we present the phenotypic and residual correlations between somatic cells and selected parameters characterizing the milk yield of ewes. The results obtained show that the amount of milk yield in 30 s, machine milk yield and total milk yield are in negative correlation with the number of somatic cells. However, the results are not conclusive. Based on phenotypic correlations, the dependence between LOG SCC and MY30s, SV and TMY was statistically significant ($P < 0.01$ to 0.001). It seems that ewes with higher SCC, i.e. with subclinical *mastitis*, are likely to have worse milk let-down at machine milking and overall milk yield. If based on residual correlations, adjusted for the influence of analysing genetic and non-genetic factors, then the correlations were also negative but not statistically significant.

Tab. 2. Correlation coefficients (phenotypic lower corner, residual right corner) between somatic cell count and selected traits characterizing milk yield of ewes

	SCC	LOGSCC	MY30s	MMY	TMY	PMSM	
SCC	–	0.835^{+++}	–0.153^{ns}	–0.152^{ns}	–0.075^{ns}	0.203^{+}	residual
LOGSCC	0.705^{+++}	–	–0.098^{ns}	–0.108^{ns}	–0.015^{ns}	0.175^{+}	
MY30s	–0.134^{ns}	–0.266^{+++}	–	0.572^{+++}	0.452^{+++}	–0.519^{+++}	
MMY	–0.100^{ns}	–0.277^{+++}	0.676^{+++}	–	0.890^{+++}	–0.613^{+++}	
TMY	–0.032^{ns}	–0.234^{++}	0.599^{+++}	0.941^{+++}	–	–0.260^{+++}	
PMSM	0.234^{++}	0.236^{++}	–0.507^{+++}	–0.506^{+++}	–0.265^{+++}	–	
	phenotype						

Explanations: as in Tab. 1

The finding of a positive relationship between SCC or LOG SCC and the percentage of machine stripped milk is quite remarkable. The correlation coefficients ranged in values from 0.175 to 0.236 and were significant ($P < 0.05$ and $P < 0.001$ for residual and phenotypic correlations, respectively). This indicates that the percentage of machine stripped milk to total milk yield increased with increasing somatic cell number. It is logical that udders with subclinical *mastitis* have worse milk let-down with machine milking, and that a significant proportion of milk is obtained from such udders only by machine milking.

Subclinical *mastitis* decreases milk production of dairy sheep (19, 38). The different percentage of infected animals does not seem sufficient to explain the difference in SCC suggesting that other management factors are involved (3). In this species a relationship is often deduced indirectly from the fact that infected ewes show a higher SCC and contemporarily a lower milk yield (16, 28, 37). Some authors (13, 20, 24) studied the effect of dilution effect, e.g. low milk yield corresponds to higher percentages either for fat and protein or somatic cells, in cattle and goat milk. This is a crucial point for the farmer which may be induced to incorrectly conclude that there is a negative effect of SCC on milk yield and vice versa a positive one on contents. Moreover, farmers involved in selection schemes are debating the inclusion of SCC as selection criterion. The genetic relationship between SCC and milk yield antagonistic in dairy cattle, are quite inconsistent across dairy sheep studies. Genetic correlation estimates with milk yield ranged from antagonistic (0.08 to 0.23) to favourable (–0.15 to –0.30) (35).

Evidence has been published that healthy ewes normally have higher SCC than healthy cows (18). Bufano et al. (12) have shown that high SCC (> 1 million/mL) do occur in healthy sheep's milk, especially towards the end of lactation. Therefore, whereas in cattle SCC is widely recognized as an indicator of mastitis, results on the efficiency of SCC as an indicator trait are inconsistent in dairy sheep studies. However, Ariznabarreta et al. (4) and Gonzalo et al. (19) have demonstrated that for around 70% of mammary pathogens isolated from ewes with subclinical *mastitis*, their presence in ewe milk is associated with high SCC. Therefore, published evidence exists that mastitis does accompany an increase in SCC in sheep (27). Moreover, Leitner et al. (28) have suggested that because sheep have only two mammary glands, dilution effects due to the mixing of milk with high SCC from an infected gland, and milk with low SCC from a healthy gland, will be relatively small at the animal level. Besides, in dairy cows, subclinical *mastitis*, with a frequency ranging from 20-50% (32, 39) may be less apparent because the increase in SCC in an infected gland is modest (about 300-500 $\times 10^3$ cells/mL) and the mixing with the milk from uninfected quarters is sufficient in most cases to appreciably lower the effect of SCC at the cow level (17).

The heritability estimates for overall SCS and SCS in apparently healthy animals were generally in the range reported in the literature for repeatability test-day models: i.e., 0.04 to 0.16 (8, 22, 34). Other studies have reported higher heritability estimates for the average SCS during lactation, from 0.11 to 0.18 (7, 29, 36). However, the heritability for SCS in infected ewes (0.03) was at the low end of published values. It is important to stress that the similarity between the heritability for bacteria negative SCS and that usually observed for SCS is probably due to the fact that the former refers to a mix of repeatable healthy animals, animals that have recovered from infection, and infected animals with incorrect diagnosis. In contrast, SCS in infected animals are mostly truly positive samples, and the low heritability actually reflects that most of the variation in these samples is non-genetic. The high environmental variance for the bacteria positive SCS is possibly due to the nature of the pathogens (i.e., hosts may respond differently to infection by a pathogen or another) and

the sinusoidal variation of SCC after infection, both of which would increase variation in the dataset.

The results of the study indicate that there is a statistically significant ($P < 0.001$) effect of genotype on the amount of milk obtained at machine milking in 30 seconds, the source of variation, and the total milk yield. There are notable differences between crossbreeds and purebreeds, particularly in the incidence of subclinical *mastitis* in favour of purebreds. The results demonstrated that the order of lactation had a statistically highly demonstrable effect on LOG SCC ($P < 0.001$). Ewes in their first lactation exhibited significantly lower somatic cell counts. Based on the results we obtained, it can be assumed that SCC is one of the factors influencing the proportion of machine milking. Therefore, selecting ewes for lower SCC could also result in better milkability in ewes, but this needs to be confirmed by further studies in this area.

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