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EFFECT OF SOMATIC CELL COUNT ON MILKABILITY AND MILK COMPOSITION OF EWES

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ABSTRACT
The trial aimed to study the effect of somatic cell count, breed, milk flow type, and parity on the milkability and milk composition of ewes. The flock consists of purebred Lacaune ewes (LC; n = 29) and crossbreds ewes of Improved Valachian (IV x LC; n = 35) and Tsigai (TS x LC; n = 37) with LC (with a genetic portion of Lacaune 25 and 50%). Ewes were assigned according to somatic cell count (SCC) to one of the following three groups: SCC ≤300,000 cells per mL (SCC Group 300,000), SCC between 300,000 and 1000,000 cells per mL (SCC Group 300,000 – 1000,000), SCC ≥1000,000 cells per mL (SCC Group 1000,000). 56% of evaluated ewes had less than 300,000 cells per mL and 29% more than 1000,000 cells per mL in milk. No significant differences were observed between different groups of SCC in total, machine milk yield, and the proportion of milk yield in 30 s and 60 s. The significant differences were observed between SCC Group 300,000 and SCC Group 300,000 – 1000,000 in the proportion of machine stripping from total milk yield (41 ±2 vs. 57 ±4%). Milk flow type had a significant effect on all evaluated milkability parameters but not on milk composition. Ewes on fourth parity had the highest proportion of machine stripping from total milk yield then ewes on third, fifth, and sixth and higher (60% vs. 47, 45, 46%; resp.). Effect of SCC Group on milk composition manifested only in solids not fat. The significant differences were observed between SCC Group 1000,000 to SCC Group 300,000 and SCC Group 300,000 – 1000,000 (10.75 ±0.08% vs. 11.05 ±0.06 and 11.15 ±0.11%, $p \leq 0.0004$).

Keywords: ewe; somatic cell count; milk composition; SCC

INTRODUCTION
In ewes and other dairy animals, somatic cells are an important natural component of milk. Their number is used as an indirect predictor of udder health status and milk quality as they are involved in the protection of the mammary gland from infection as part of the innate immune system **(Rupp and Boichard, 2000; Guzel et al., 2017; Tančin et al., 2006; Tančin et al., 2007; Tančin, Ipema and Hogewerf, 2007)**. Somatic cell count (SCC) in milk is influenced by several factors: animal species, production level, physiological processes (such as estrus or stage of lactation), animal individuality, and also environmental factors and farm management **(Rupp and Boichard, 2000; Tančin et al., 2016; Tančin et al., 2017; Paape et al., 2007; Margetín et al., 2013)**. According to **Ranucci and Morgante (1996)** and **Bergonier et al. (2003)**, somatic cells in healthy ewe milk consist of macrophages, polymorphonuclear neutrophil leukocytes (which have an important biological function of phagocytic activity), lymphocytes, and, to a lesser extent other cell types (eosinophils, epithelial cells, and unidentifiable cells). As in dairy cows, also in goats or sheep, an increase in SCC has been reported as a consequence of infection **(Poutrel et al., 1997; Paape et al., 2001; Paape et al., 2007; Bergonier and Berthelot, 2003; Bergonier et al., 2003; Luengo et al., 2004; Moroni et al., 2005; Koop et al., 2010)**. During mastitis, milk SCC increases mainly as a result of increased migration of leukocytes from blood to mammary tissue **(Leitner et al., 2003; Le Roux, Laurent, and Moussaoui, 2003)**. 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. These limits determine which milk can be on the market or what penalties in the contractual terms of payment are imposed by the milk producer when the milk does not meet the required criteria **(Berger et al., 2004; Haenlein, 2002; Kalantzopoulos et al., 2004; Raynal-Ljutovac, Gaborit and Lauret, 2005)**. However, there are no limits to SCC in sheep's milk as it is in cow's milk in Slovakia **(Tančin et al., 2017)**. The major income from dairy animals is derived from milk; therefore, factors that reduce milk quantity and quality can cause high economic losses to the farmers **(Sutera et al., 2018)**.

The trial aimed to study the effect of SCC on the milkability and milk composition of ewes. Possible effects of breed, milk flow type, and parity were evaluated too.

Scientific hypothesis

In this study, we hypothesized that ewes, which had lower SCC than 300,000 mL^{-1} (SCC Group 300,000), would have better parameters of milkability and milk composition than ewes with the higher SCC. The second hypothesis was that ewes with two peaks and plateau milk flow types would have a higher milk production and milk fat content than ewes with one peak and plateau II. The third hypothesis was that breed affects the production parameters. The fourth hypothesis was that parity affects the production parameters and SCC.

MATERIAL AND METHODOLOGY

Animal and experimental design

The study was carried out in June in the flock of 101 mid-lactated ewes (102 ±5 days in lactation) their 3rd – 9th parity at one evening milking. The flock consists of purebred Lacaune ewes (LC; n = 29) and crossbreds ewes of Improved Valachian (IV x LC; 35) and Tsigai (TS x LC; 37) with LC (with a genetic portion of Lacaune 25 and 50%). The ewes were milked in a one-platform milking parlour with 24 stalls and one milking unit per 2 milking stalls. The milking machine was set to provide 160 pulsations per minute in a 50:50 ratio and a vacuum level of 39 kPa. During each milking, ewes received 0.1 kg concentrate per head in the parlour. Ewes were milked routinely twice daily at 8:00 and 20:00 without any udder preparation. At the end of milking, machine stripping was performed (machine stripping started when milk flow rate declined to 0 $L.min^{-1}$ but not earlier than 70 s from the beginning of milking). Short manual udder massage was performed by machine stripping.

Milk flow recording and samples analysis

Milk flow kinetic was recorded using an electronic jar that collected the milk during the next three consecutive evening milkings. Within the jar, there was a 2-wire compact magnetostrictive level transmitter (NIVO-TRACK, NIVELKO Ipari Elektronika Rt, Budapest Hungary) connected to a computer. The milk level was continuously measured by a transmitter that recorded the position of the float in the jar on a computer once per second. The milk flow patterns were drawn by using a formula by **Mačuhová et al. (2008)**. The following parameters of milkability were evaluated: total milk yield (L), machine milk yield (L), machine stripping yield (L), machine stripping yield from total milk yield (%), milking time (i.e. time from attaching of clusters until the milk flow ceased before stripping; s), milk yield in 30 s (L), and milk yield 60 s (L).

According to SCC, ewes were assigned to one of the following three groups: SCC ≤300,000 cells per mL (SCC Group 300,000), SCC between 300,000 and 1000,000 cells per mL (SCC Group 300,000 – 1000,000), SCC ≥1000,000 cells per mL (SCC Group 1000,000).

Milk flow curves were evaluated according to **Marnet, Negrao and Labussière (1998)**, **Rovai et al. (2002)** and **Mačuhová et al. (2008)** into 4 milk flow types; 1 peak (1P; without notable milk flow after 40 s of milking), 2 peaks (2P; two separate milk emissions), plateau (PL; milk flow with longer duration of steady phase and milk flow rate during plateau phase >0.4 $L.min^{-1}$ at least for 20 s), and plateau low (PLII; milk flow curves with steady milk flow during milking for 20 s but with milk flow rate ≤0.4 $L.min^{-1}$ or >0.4 $L.min^{-1}$ shorter than for 20 s at plateau phase). In 1 animal no milk flow occurred, and the curve of milk flow type could not be identified.

Individual milk samples were collected after milking from the jar for composition analysis. Milk composition was analyzed for the percentage of fat, protein, lactose, solids, and solids-not-fat with MilkoScan FT120 (Foss, Hillerød, Denmark). SCC was analyzed with Somacount 150 analyzer (Bentley Instruments, lnc, Chaska, Minnesota).

Statistical analysis

The data set consisted of 101 measurements belonging to 101 ewes. Mixed model (Mixed procedure; SAS/STAT 9.1, 2002 – 2003) was applied to study the influence of the sources of variation in studied traits (parameters of milkability and milk composition).

$$y_{ijkl} = \mu + \text{SCC GROUP}_i + \text{FLOW}_j + \text{BREED}_k + \text{PARITY}_l + e_{ijkl}$$

where: y_{ijkl} – individual observations of studied parameters of milkability and milk composition, μ = overall mean, SCC GROUP_i = fixed effect of SCC group (i = 1 to 3; ≤300,000, between 300,000 and 1000,000, ≥1000,000 cells per mL), FLOW_j = fixed effect of milk flow type (j = 1 to 4; 2P, 1P, PL, PLII) + BREED_k = fixed effect of Breed (k = 1 to 3; TS x LC, LC, IV x LC) + PARITY_l = fixed effect of parity (l = 1 to 4; 3, 4, 5, ≥6), e_{ijkl} = random error, assuming $e$ijkl ~ N(0, I σ_e^2).

The fixed effects of the model were estimated using the LSM (Least Squares Means) method. Statistical significance was tested by Fischer's F-test and differences between the estimated levels of effects were tested by Scheffe's multiple range tests.

RESULTS AND DISCUSSION

In Table 1, there are presented basic statistics of studied traits and in Table 2, p–values for the statistical significance of tested factors on evaluated parameters. SCC has been described in numerous studies as a useful method for diagnosing intramammary infection in monitoring udder health. In this study, animals were classified according to SCC in three groups (SCC Group ≤300,000 cells per mL; SCC Group between 300,000 and 1000,000 cells per mL; SCC Group ≥1000,000 cells per mL). 56% of evaluated ewes had SCC lower than 300,000 cells per mL and 29% more than 1000,000 cells per mL. SCC Group had no significant effect on parameters of milkability (except machine stripping yield from total milk yield; Table 3) or parameters of milk composition (except for solids not fat (%)) (Table 5). There is a discussion on the SCC threshold level for diagnostic purposes **(Raynal-Ljutovac et al., 2007; Albenzio et al., 2012; Rovai et al., 2015; Tvarožková et al., 2019)**.

the SCC over 600,000 cells per mL is considered as high.

Table 1 Characteristics of statistical file of studied traits.

Label	N	Minimum	Maximum	Mean	Std Error
Total milk yield (TMY), L	101	0.112	1.001	0.392	0.017
Machine milk yield (MMY), L	101	0.067	0.781	0.25	0.015
Milking time, s	101	15	98	50	2
Milk flow latency, s	101	2	78	18	1
Milk yield in 30 s, L	101	0	0.399	0.127	0.008
Milk yield in60 s, L	101	0.024	0.781	0.224	0.014
Machine stripping yield/TMY, %	101	5.22	84.56	37.4	1.59
log SCC	101	4.908	7.874	6.473	0.064
Fat, %	101	4.65	8.9	6.23	0.08
Protein, %	101	4.35	6.61	5.27	0.04
Lactose, %	101	4.4	5.18	4.87	0.02
Solids, %	101	14.72	19.97	16.99	0.1
Solids not fat, %	101	10.12	12.23	10.96	0.037

Table 2 Statistical significance (*p*-values) of tested factors on evaluated parameters.

	Total milk yield (TMY), L	Machine milk yield, L	Milking time, s	Milk flow latency, s	Milk yield in 30 s, L	Milk yield in 60 s, L	Machine stripping yield/TMY, %	Fat, %	Lactose, %	Solids, %	Solids not fat, %
SCC	0.7795	0.4299	0.0713	0.2366	0.0644	0.1418	0.0013	0.2565	0.1318	0.4442	0.0004
Milk flow type	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3524	0.9828	0.2968	0.1011
Breed	0.051	0.0025	0.0014	0.605	0.0002	0.0002	0.0188	0.3962	0.0042	0.1313	0.0271
Parity	0.9251	0.8362	0.2847	0.0963	0.4726	0.6640	0.0124	0.0578	0.5411	0.0373	0.0151

Table 3 Parameters of milkability according to Somatic cell cound (SCC) and milk flow type.

	SCC Group			Milk flow type			
Parameters	**≤300,000**	**300,000 – 1000,000**	**≥1000,000**	**2P**	**1P**	**PL**	**PL II**
N	57	15	29	37	27	23	13
Total milk yield (TMY), L	0.363 ±0.027	0.399 ±0.050	0.367 ±0.036	0.416 ±0.032^{a}	0.289 ±0.032^{b}	0.541 ±0.038^{a}	0.367 ±0.048^{b}
Machine milk yield, L	0.212 ±0.022	0.159 ±0.039	0.196 ±0.029	0.250 ±0.025^{a}	0.164 ±0.025^{a}	0.382 ±0.030^{b}	0.152 ±0.038^{a}
Milking time, s	50 ±3	59 ±5	47 ±3	67 ±3^{ac}	29 ±3^{b}	54 ±4^{a}	66 ±4^{c}
Milk flow latency, s	23 ±2	20 ±3	26 ±2	13 ±2^{a}	11 ±2^{a}	18 ±2^{a}	34 ±3^{b}
Milk yield in 30 s, L	0.105 ±0.014	0.045 ±0.025	0.081 ±0.018	0.099 ±0.016^{ab}	0.144 ±0.016^{a}	0.154 ±0.019^{a}	0.0303 ±0.024^{b}
Milk yield in 60 s, L	0.187 ±0.020	0.112 ±0.037	0.165 ±0.027	0.208 ±0.023^{a}	0.151 ±0.023^{a}	0.356 ±0.028^{b}	0.114 ±0.036^{a}
Machine stripping/TMY, %	41.27 ±2.43^{a}	57.23 ±4.43^{b}	49.92 ±3.22^{b}	39.92 ±2.81^{ab}	42.52 ±2.77^{ab}	31.17 ±3.41^{a}	55.30 ±4.30^{b}
log SCC	-	-	-	6.297 ±0.111	6.666 ±0.125	6.346 ±0.148	6.827 ±0.186

Note: ^{a,b} The means in the same line without same letter were significantly different at $p \leq 0.05$.

However, in most studies, 300,000 or even 400,000 cells per mL are considered as normal values in sheep's and goat's milk **(Kern et al., 2013; Tančin, 2017; Oravcová, Mačuhová and Tančin, 2018)**. On the other hand, whereas **Kern et al. (2013)** indicate already 400,000 cells per mL in meat breeds and 300,000 cells per mL in dairy breeds as a threshold level to assist the farmer in detecting of the udder health problems, in the studies of **Tančin et al. (2017)** and **Oravcová, Mačuhová and Tančin (2018)**, Only by 1000,000 cells per mL is milk considered as mastitis milk **(Tančin et al., 2017)** or ewe as infected **(Berthelot et al., 2006)**.

The significant differences were observed between SCC Group ≤300,000 and SCC Group 300 – 1000,000 in the proportion of machine stripping from total milk yield (41 ±2 vs. 57 ±4%). The tendency of the longest milking time was observed in SCC Group 300,000 and 1000,000 in comparison to SCC Group ≥1000,000 (59 vs. 47 s).

Table 4 Parameters of milkability according to breed, and parity.

	Breed			Parity			
Parameters	**TS x LC**	**LC**	**IV x LC**	**3**	**4**	**5**	**≥ 6**
N	37	29	35	29	20	25	27
Total milk yield (TMY), L	0.317 ±0.041	0.409 ±0.043	0.406 ±0.034	0.383 ±0.039	0.385 ±0.048	0.382 ±0.037	0.358 ±0.036
Machine milk yield (MMY), L	0.127 ±0.032^{a}	0.206 ±0.034^{ab}	0.234 ±0.027^{b}	0.193 ±0.031	0.166 ±0.038	0.191 ±0.029	0.206 ±0.028
Milking time, s	52 ±4^{ab}	61 ±4^{a}	43 ±3^{b}	49 ±3	49 ±5	56 ±3	49 ±3
Milk flow latency, s	23 ±3	21 ±3	24 ±2	21 ±2	24 ±3	20 ±2	26 ±2
Milk yield in 30 s, L	0.045 ±0.024^{a}	0.060 ±0.02^{ab}	0.125 ±0.017^{b}	0.074 ±0.019	0.054 ±0.024	0.081 ±0.019	0.098 ±0.018
Milk yield in 60 s, L	0.094 ±0.030^{a}	0.151 ±0.03^{ab}	0.218 ±0.025^{b}	0.159 ±0.029	0.139 ±0.036	0.141 ±0.027	0.179 ±0.027
Machine stripping/TMY, %	54.72 ±3.63^{a}	48.82 ±3.78^{ab}	44.87 ±3.03^{b}	47.38 ±3.44^{ab}	59.67 ±4.31^{a}	44.64 ±3.30^{b}	46.19 ±3.21^{ab}
log SCC	6.367 ±0.144	6.482 ±0.166	6.462 ±0.133	6.182 ±0.138^{a}	6.211 ±0.170^{ab}	6.695 ±0.137^{b}	6.661 ±0.146^{b}

Note: ^{a,b} The means in the same line without same letter were significantly different at $p \leq 0.05$.

Table 5 Milk composition according to Somatic cell count (SCC) and milk flow type.

	SCC Group			Milk flow type			
Parameters	**≤300,000**	**300,000 – 1000,000**	**≥1000,000**	**B**	**N**	**PL**	**PLN**
N	57	15	29	37	27	23	13
Fat, %	6.26 ±0.15	5.82 ±0.27	6.26 ±0.20	6.36 ±0.17	6.36 ±0.17	5.79 ±0.21	5.87 ±0.26
Lactose, %	4.91 ±0.03	4.90 ±0.06	4.82 ±0.04	4.87 ±0.04	4.86 ±0.03	4.86 ±0.04	4.85 ±0.05
Solids, %	17.10 ±0.18	16.78 ±0.32	16.82 ±0.23	17.07 ±0.21	17.22 ±0.20	16.48 ±0.25	16.58 ±0.31
Solids not fat, %	11.05 ±0.06^{a}	11.15 ±0.11^{a}	10.75 ±0.08^{b}	10.92 ±0.07	11.12 ±0.07	10.87 ±0.08	10.89 ±0.11
log SCC	-	-	-	6.297 ±0.111	6.666 ±0.125	6.346 ±0.148	6.827 ±0.186

Note: ^{a,b} The means in the same line without same letter were significantly different at $p \leq 0.05$.

Table 6 Milk composition according to breed and milk flow type.

	Breed			Parity			
Parameters	**TS x LC**	**LC**	**IV x LC**	**3**	**4**	**5**	**≥6**
N	37	29	35	29	20	25	27
Fat, %	6.32 ±0.22	5.96 ±0.23	6.05 ±0.19	5.82 ±0.21	6.29 ±0.27	5.94 ±0.20	6.39 ±0.20
Lactose, %	4.8 ±0.05^{ab}	4.97 ±0.05^{a}	4.78 ±0.04^{b}	4.91 ±0.04	4.85 ±0.05	4.89 ±0.042	4.85 ±0.05
Solids, %	17.22 ±0.26	16.74 ±0.28	16.742 ±0.22	16.48 ±0.25^{a}	16.95 ±0.31^{ab}	16.85 ±0.24^{ab}	17.32 ±0.23^{b}
Solids not fat %	11.10 ±0.09^{a}	10.99 ±0.08^{ab}	10.87 ±0.075^{b}	10.85 ±0.09	10.87 ±0.11	11.13 ±0.08	11.10 ±0.08
log SCC	6.367 ±0.144	6.482 ±0.166	6.462 ±0.133	6.182 ±0.138^{a}	6.211 ±0.170^{ab}	6.695 ±0.137^{b}	6.661 ±0.146^{b}

Note: ^{a,b} The means in the same line without same letter were significantly different at $p \leq 0.05$.

Rovai et al. (1999) found out that the height of cisterns correlated with teat angle and distance between teats. Thus, udders with higher cisterns have deeper and show bigger teat angles. Consequently, the udder emptying can be negatively affected during machine milk, and a higher stripping fraction is observed in ewes with this udder morphology. Moreover, they found out that parity had a significant effect on cistern height **(Rovai et al., 1999)**. In SCC Group 300,000, only 14% of ewes were observed on sixth or higher lactation, but 73% in group 300,000 – 1000,000.

Table 4 shows parameters of milkability and Table 5 parameters of milk composition according to milk flow type, breed, and parity. The milk flow type had a significant effect on all tested parameters of milkability, but non on milk composition parameters. The total milk yield was lower in ewes with 1P and PL II milk flow type than in ewes with 2P and PL type in this study (Table 4). The analysis of the milk flow curves shows that the milk ejection reflex does not occur every time during milking in ewes **(Bruckmaier et al., 1997; Dzidic, Kaps and Bruckmaier, 2004; Mačuhová et al., 2008; Mačuhová et al., 2012; Tančin et al., 2011)**. 1P flow type is supposed to represent milk flow without alveolar milk ejection when only cisternal milk fraction is removed in response to machine milking **(Marnet, Negrao and Labusière, 1998; Mačuhová et al., 2008)**. This support also significantly shorter milking time ewes with 1P type of milk flow than in ewes with 2P, PL, and PL II (29 vs. 67, 54, and 66 s; resp.) as observed also in previous studies of **Mačuhová et al. (2008)** and **Mačuhová et al. (2012)** and **Tančin et al. (2011)**. 2P and in most cases also PL represent the milk flow types with milk ejection **(Mačuhová et al., 2012)**. Even the second peak is not observed in PL type of milk flow, it is supposed that milk ejection occurs in ewes with this milk flow **(Marnet, Negrao and Labussière, 1998; Rovai et al., 2002; Tančin et al., 2011)**. According to **Marnet, Negrao and Labussière (1998)**, the occurrence of the PL type of milk flow rises in consequence of the genetic selection for higher milk production or decreased

average milk flow rate. Thus, this type of milk flow can be observed when the second peak (i.e. the removal of an alveolar fraction) is masked because the cistern fraction has not yet been completely removed from the udder at the time of milk ejection **(Marnet, Negrao and Labussière, 1998)**. In the study of **Bruckmaier et al. (1997)**, high machine stripping yield was observed in ewes with 1P milk flow is possibly caused by a late response to milking and oxytocin release after the end of milking. This does not seem to be the case in this study. Machine stripping yield from total milk yield (%) was highest in PL II, whereas it did not differ among other milk flow types. However, whereas the data of this study support that no oxytocin was released during machine milking or stripping in ewes with 1P milk flow type, it is possible that oxytocin was released during milking or machine stripping in ewes with PL II milk flow type. Ewes with PL II had the lowest milk yield in 30 s (significantly) 60 s (in tendency) (Table 3). Low milk yield in 30 and 60 s could be caused by some health problems or deformity of the teat canal **(Mačuhová et al., 2008)**.

The breed influenced significantly machine milk yield, milking time, milk yield in 30 and 60 s, and machine stripping yield from total milk yield (%), and also lactose and solids not fat (Table 4 and Table 6). The machine milk yield and milk yield in 30 and 60 s were significantly higher in crossbreds IV x LC than in TS x LC (Table 4). This does not correspond to results in previous studies **(Mačuhová et al., 2008; Mačuhová et al., 2017)** where no differences were found in these parameters and also machine stripping yield from total milk yield. However, whereas the total milk yield did not differ between crossbreds in this study, machine stripping from total milk yield was higher in TS x LC than IV x LC. The reason for it could be possible worse udder morphology for milking in TS x LC ewes in comparison to IV x LC ewes of the same crossbreds observed previously.

The parity (Table 4, Table 5 and Table 6) had a significant effect only on machine stripping yield from total milk yield (%), log SCC, and solids (%). The ewes on the fourth parity have significantly higher machine stripping from total milk yield than ewes on lower or higher parities (60% vs. 47, 45, 46%). This could signalize that udder worsened with increasing parity (fourth vs. third parity). However, on higher parities (≥5) only "better" ewes (healthy, with good udder morphology, and adequate milk production) stayed in the flock.

CONCLUSION

Surprisingly, SCC Group did not affect evaluated parameters of milkability except for machine stripping yield from total milk yield (%). Milk flow type and the breed had mainly effect on the parameter of milkability. Parity had a significant effect on machine stripping yield from total milk yield (%), log SCC, and solids (%). Concerning the composition of milk, fat (%) was influenced by none of the tested parameters, lactose (%) and solids (%) only by one of the tested parameters, and only solids not fat was influenced by three (SCC group, breed, and parity) of four tested factors.

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