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Effect of Ewes Entry Order into Milking Parlour on Milkability and Milk Composition

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ABSTRACT

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The aim of the present investigation was to evaluate how the order in which the ewes in a milking group enter the milking parlour affects their milkability and milk composition. Therefore, the order of entry into the milking parlour was evaluated in ewes of one flock (n = 353) during six evening milkings. In all, the sheep were milked in 15 milking groups. The sheep entering the milking parlour in the first milking group achieved 15 points and the sheep of each next milking group one point less, i.e. the sheep of the last group achieved 1 point. In the analysis, only the ewes with the highest and the lowest average number of points were included and assigned to the first (FG, n = 19) and the last group (LG, n = 29), respectively. After the last milking, the individual milk samples were collected from the jar to analyze the composition and somatic cell count. Machine milk yield in 30 s (0.15 ± 0.09 and 0.11 ± 0.05 l) and 60 s (0.26 ± 0.16 and 0.19 ± 0.10 l), peak flow rate (1.04 ± 0.39 and 0.77 ± 0.29 l/min), and latency time (14 ± 3 and 20 ± 13 s) significantly differed (P < 0.05) between FG and LG, respectively. Total milk yield (0.41 ± 0.17 and 0.35 ± 0.14 l) and machine milk yield (0.27 ± 0.15 and 0.22 ± 0.10 l) tended to be higher (P = 0.05 and P = 0.09) in FG than in LG, respectively. No significant differences were observed in milk composition between FG and LG. It seems that ewes which enter the milking parlour in early milking groups have better parameters of milkability than those milked in later groups.

Keywords: sheep; milking; milking order; milk flow

The effect of entry order into the milking parlour was studied according to animal species, breed, social status, health problems, age, stage of lactation, hornedness, body weight, milk productivity, etc. (Hopster et al. 1998; Margetinova et al. 2003; Villagra et al. 2007). The order of animal entering the milking parlours was reported as non-random in cattle (Stefanowska et al. 2000), sheep (Kes-

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zthelyi and Maros 1992; Wasilewski 1999), and goats (Donaldson et al. 1967; Margetinova et al. 2003). Polikarpus et al. (2015) found out that the milking order was stable within days and across days in dairy cows. Later entering of animals into the milking parlour can often be related to health problems or stress (if e.g. conditions of milking were changed) (Tancin et al. 2001; Polikarpus et al. 2015). If animals are stressed, cardiac rhythm increases (Hopster et al. 1998) and the release of oxytocin necessary for milking can be insufficient (Macuhova et al. 2002). During milking, the presence of the neuroendocrine milk ejection reflex is crucial for complete emptying of the udder because only 20% of the total milk in cows and above 40% in dairy sheep is located in cisternal compartment after a 12-hour milking interval (Pfeilsticker et al. 1996; Marnet and McKusick 2001). The extraction of milk stored in the alveoli is important to obtain maximum milk yield and content of fat also in sheep (Antonic et al. 2013; Ayadi et al. 2014).

The analysis of the milk flow curves shows that the milk ejection reflex does not occur every time during milking in sheep (Mayer et al. 1989; Bruckmaier et al. 1997; Marnet et al. 1998; Dzidic et al. 2004; Macuhova et al. 2008). One-peak milk flow curves can represent milk flow without (when only cisternal milk fraction is removed in response to machine milking and no notable milk flow is observed after 40 s of milking (Marnet et al. 1998)) or with alveolar milk (Bruckmaier et al. 1997; Marnet et al. 1998). The milk flow curves with two peaks show alveolar milk ejection after the cisternal milk is removed. In consequence of the genetic selection for higher milk production or decreased average milk flow rate, the occurrence of a two-peak milk flow curve has become rarer (Marnet et al. 1998), and a third type of milk flow with a plateau phase can be observed. Thus, the second peak is masked because the cistern fraction has not yet been completely removed at the time of milk ejection from the udder when alveolar fraction descends into cistern for removal (Marnet et al. 1998; Rovai et al. 2002; Tancin et al. 2011). Bruckmaier et al. (1997) observed another type of plateaued milk flow curve, but at a very low level milk flow. Milk flow kinetic could be a good indicator of stress load under different milking conditions (Bruckmaier et al. 1997; Tancin et al. 2015).

The somatic cell count (SCC) of milk is a representative of the udder health and can be used as an indicator of the potential presence of mastitis and as criterion for milk quality (Silanikove et al. 2014). Uncharacteristically late entry of animals into the milking parlour could often be related to health problems. Cows with mastitis entered the parlour later than when they were healthy (Rathore 1982; Polikarpus et al. 2015).

The aim of the trial was to study the effect of the order of ewes entry into milking parlour (in the first or the last group) on their milkability and milk compositions. In this study, we hypothesize that ewes entering the milking parlour first could have better milkability (parameters of milkability and milk flow patterns) than ewes entering with the last groups of the flock and their milk composition (milk richness) and SCC could differ.

MATERIAL AND METHODS

Animal and experimental design. The study was carried out in June in a flock of 353 mid-lactated (102 ± 5 days in lactation) ewes on their 1^{st}–7^{th} parity. The flock consisted of purebred Lacaune ewes (LC), Tsigai ewes crossbred with LC rams (TS × LC), and Improved Valachian ewes crossbred with LC rams (IV × LC) (with 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 places. The milking machine was set to provide 160 pulsations per min in a 50 : 50 ratio with a vacuum level of 39 kPa. During each milking, each ewe received 0.1 kg concentrate in the parlour. Ewes were milked routinely twice per day at 8:00 and 20:00 h without any udder preparation. At the end of milking, machine stripping was performed (machine stripping started immediately when milk flow rate declined to 0 l/min but not earlier than 70 s from the beginning of milking).

Ewes were selected based on the group number (batch) in which they entered the milking parlour. Therefore, during the six consecutive evening milkings, ewes of the whole flock (*n* = 353, milked in 14 batches of 24 ewes each and the last batch of 17 ewes) were rated between 1 and 15 according to the batch they joined while entering the parlour. Sheep entering the parlour in the first batch achieved 15 points and sheep of each next batch one point less, i.e. ewes in the last group achieved 1 point. Only ewes with the highest (15–13.7 points;

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predominantly entering parlour in the first two batches) and the lowest average number of points (1–2.3 points; predominantly entering parlour in the last two batches) were chosen for further investigation and were arranged to two milking groups – the animals with the highest score to the first milking group (FG, n = 19: TS × LC n = 8; LC n = 3; IV × LC n = 8) and the animals with the lowest score to the last milking group (LG, n = 29: TS × LC n =11; LC n = 7; IV × LC n = 11). There were no differences in the frequency of individual breed/crossbreeds neither between the milking groups (χ^2 test, df = 2, χ^2 value = 0.49, P = 0.78) nor within the milking groups (χ^2 test, df = 2, χ^2 value = 2.63 and 1.10, P = 0.27 and 0.58, by FG and LG, respectively). The number of primiparous and multiparous ewes was 3 and 16, 1 and 9, and 8 and 11 in TS × LC, LC, and IV × LC, respectively.

Milk flow recording and samples analysis. Milk flow kinetic was recorded using an electronic jar that collected the milk during additional three consecutive evening milkings. Within the jar, there was a 2-wire compact magnetostrictive level transmitter (NIVOTRACK, NIVELKO Ipari Elektronika Rt, Hungary) connected to a computer. The milk level was measured continuously by the 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 Macuhova et al. (2008):

$$\text{Milk flow rate (l/min)} = (L_n - L_{n-4}) \times 15$$

where:
L = recorded milk yield (l)
n = time (s)
15 = coefficient to correct milk yield increase in 4 s to milk flow (l/min)

The following milking characteristics 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)), latency time (i.e. time from attaching of cluster until the start of milk flow 0.006 l/min (s)), peak flow rate (l/min), time of peak flow rate (i.e. time the peak flow rate was reached (s)), milk yield in 30 s (l), and milk yield in 60 s (l). A short manual udder massage was performed by machine stripping.

Milk flow curves were evaluated according to Marnet et al. (1998), Rovai et al. (2002), and Macuhova et al. (2008) and divided into 4 types: 1 peak (1P; without notable milk flow after 40 s of milking), 2 peaks (2P), plateau (PL; represents milk flow by ewes with longer duration of steady phase and peak flow rate during plateau phase > 0.4 l/min at least for 20 s), and plateau low (PLII; represents also milk flow curves with steady milk flow during milking, but at peak milk flow rate at plateau phase 0.4 l/min or > 0.4 l/min shorter than for 20 s).

Individual milk samples were collected after the last experimental milking from the jar serving for composition analysis. Milk composition was analyzed for the percentage of fat, protein, lactose, solids, and solids-not-fat with MilkoScan FT120 (Foss, Denmark). Somatic cell count (SCC) was determined with a Somacount 150 analyzer (Bentley Instruments, lnc., USA).

Statistical analysis. The data for parameters of milkability, milk composition, and log SCC were not normally distributed. Consequently, to study the impact of the order of ewes' entry into the milking parlour on these parameters, the performed analysis of variance (ANOVA) was based on ranked data. Therefore, the means per animal were calculated from milkability data. Besides milking group, also parity (primiparous and multiparous), breed/crossbreed (LC, TS × LC, IV × LC), and interactions group × parity and group × breed/crossbreed were included in the analysis. The *post-hoc* group differences were evaluated using the Tukey adjustment for multiple comparisons. Proportions (e.g. to test the frequency of occurrence of individual breed/crossbreeds, milk flow types, and parity according to milking groups) were statistically evaluated by χ^2 test or exact χ^2 test. Statistical significance was set at $P \leq 0.05$. All statistical analyses were performed using the SAS software (Statistical Analysis System, Version 9.3, 2011).

RESULTS AND DISCUSSION

According to Keszthelyi and Maros (1992) and Wasilewski (1999), individual preferences for particular positions in milking groups seem to rule in sheep at least for short periods. The results of our study also support this statement. From the flock of 353 ewes, 19 entered the milking parlour predominantly as first (FG) and 29 as last (LG) dur-

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ing the six monitored evening milkings. Thereafter, milkability and milk composition were evaluated in these ewes to test whether the ewes of LG just prefer to enter the parlour as last (due to social rank and temperament) or whether their order of entry shows some differences in milk production (in occurrence of milk ejection, i.e. milk yield, milk composition, and mammary gland health) in comparison to ewes of FG.

Table 1 shows the data of observed milkability and milk composition parameters in these ewes. The milking group factor had a significant effect on latency time, peak flow rate, and milk yield in 30 s and 60 s. Though, the ewes of FG only tended to have a higher total milk yield and machine milk yield than ewes of LG ($P = 0.05$ and $P = 0.09$, respectively). No relation between milk yield and entry order into milking parlour was observed in Manchega sheep (Villagra et al. 2007). However, higher milk yield in animals entering the parlour early was observed in goats and cows (Margetinova

Table 1. Parameters of milkability and milk composition according to ewes milking group (parlour entry as the first (FG) and as the last group (LG))

Traits	Milking group	Mean	SD	Minimum	Maximum	*P*-value	Significance
Total milk yield (TMY) (l)	FG	0.41	0.17	0.18	0.91	0.05	ns
	LG	0.35	0.14	0.13	0.75		
Machine milk yield (l)	FG	0.27	0.15	0.09	0.74	0.09	ns
	LG	0.22	0.10	0.08	0.55		
Machine stripping yield (MS) (l)	FG	0.15	0.06	0.06	0.28	0.30	ns
	LG	0.13	0.06	0.03	0.33		
MS/TMY (%)	FG	37.36	13.14	18.52	60.42	0.59	ns
	LG	37.75	11.40	14.44	60.48		
Milking time (s)	FG	49	16	22	68	0.45	ns
	LG	51	22	15	91		
Latency time (s)	FG	14	3	9	19	< 0.01	++
	LG	20	13	7	78		
Peak flow rate (l/min)	FG	1.04	0.39	0.43	1.90	< 0.01	+++
	LG	0.77	0.29	0.42	1.51		
Time of peak flow rate (s)	FG	22	9	13	47	0.35	ns
	LG	27	14	11	71		
Milk yield in 30 s (l)	FG	0.15	0.09	0.05	0.36	< 0.01	++
	LG	0.11	0.05	0	0.19		
Milk yield in 60 s (l)	FG	0.26	0.16	0.05	0.78	0.02	+
	LG	0.19	0.10	0	0.58		
Fat (%)	FG	6.17	0.91	4.65	8.09	0.64	ns
	LG	6.29	0.84	4.85	8.03		
Protein (%)	FG	5.22	0.38	4.53	5.94	0.54	ns
	LG	5.38	0.42	4.67	6.61		
Lactose (%)	FG	4.80	0.22	4.40	5.09	0.38	ns
	LG	4.88	0.13	4.62	5.18		
Solids-not-fat (%)	FG	10.84	0.36	10.12	11.50	0.06	ns
	LG	11.09	0.40	10.33	12.23		
Solid (%)	FG	17.01	1.17	14.86	19.59	0.26	ns
	LG	17.38	1.03	15.78	20.26		
SCC (log)	FG	5.51	0.73	4.51	6.87	0.57	ns
	LG	5.49	0.64	3.90	6.35		

SCC = somatic cell count, ns = not significant, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$

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et al. 2003; Gorecki and Wojtowski 2004; Grasso et al. 2007).

Animals in FG had higher peak flow rate and needed shorter time to start milk flow (latency time) than LG ewes (1.04 vs 0.77 l/min and 14 vs 20 s, respectively ($P < 0.01$)). Milk yield in 30 and 60 s was higher in FG ewes than in ewes of LG (0.15 vs 0.11, $P < 0.01$ and 0.26 vs 0.19, $P < 0.05$, respectively). In contrast to LG ewes, none of FG ewes had zero value of milk yield in 30 and 60 s. No milk flow in first 30 s was observed mostly in ewes with PLII (in four of five); in one of them even during all three milkings. Now it can only be speculated if no milk flow in first 30 s and even in 60 s of milking in some ewes of LG was caused by unsuitable udder anatomy for machine milking, wrong cluster position, or other factors that could affect also the flow of cisternal milk. But these results could be related to further findings. The occurrence of individual milk flow types did not differ significantly ($P = 0.30$) between milking groups (Table 2). However, while the frequency of occurrence of individual milk flow types was similar ($P = 0.75$) in LG, this was not the case in FG ($P = 0.02$). In FG, the lowest occurrence (5.3%) was observed by PLII and therefore by the milk flow type mostly with the longest milking time according to Macuhova et al. (2008). Moreover, almost all of the ewes with PLII were in LG (7 out of 8; exact χ^2 test, χ^2 value = 4.50, df = 1, $P = 0.07$).

Table 2. Occurrence of milk flow types according to ewes milking groups (parlour entry as the first (FG) and as the last group (LG)) and breed/crossbreed (Lacaune (LC), LC × Tsigai (TS × LC), LC × Improved Valachian (IV × LC))

		Type of milk flow[6]				Total	*P*-value	
		1P	2P	PL	PLII		within group[1] and breed/crossbreed[3]	overall
Milking group								
FG	count	5	10	3	1	19	0.02[1]	0.30[2]
	% of total	10.4	20.8	6.3	2.1	39.6		
	% within group	26.3	52.6	15.8	5.3	100		
LG	count	8	9	5	7	29	0.75[1]	
	% of total	16.7	18.5	10.4	14.6	60.4		
	% within group	27.6	31.0	17.2	24.1	100		
Breed/crossbreed								
TS × LC[5]	count	7	9	2	1	19	0.02[3]	< 0.01[4]
	% of total	14.6	18.8	4.2	2.1	39.6		
	% within breed/crossbreed	36.8	47.4	10.5	5.3	100		
LC	count	0	0	5	5	10	0.02[3]	
	% of total	0	0	10.4	10.4	20.8		
	% within breed/crossbreed	0	0	50.0	50.0	100		
IV × LC[5]	count	6	10	1	2	19	0.01[3]	
	% of total	12.5	20.3	2.1	4.2	39.6		
	% within breed/crossbreed	31.6	52.6	5.3	10.5	100		
Total	count	13	19	8	8	48		

[1]*P*-value within milking group (by FG exact χ^2 test, df = 3, χ^2 value = 9.42; by LG χ^2 test, df = 3, χ^2 value = 1.21)

[2]overall *P*-value by milking group (exact χ^2 test, df = 3, χ^2 value = 3.83)

[3]*P*-value within breed/crossbreed (exact χ^2 test, df = 3, χ^2 value = 9.42, 10.00, and 10.68, respectively, by TS × LC, LC, and IV × LC)

[4]overall *P*-value by breed/crossbreed (exact χ^2 test, df = 6, χ^2 value = 25.74)

[5]no differences in the frequency when only crossbreeds are compared (exact χ^2 test, df = 3, χ^2 value = 0.80, $P = 1.00$)

[6]milk flow curve types: 1 peak (1P; without notable milk flow after 40 s of milking), 2 peaks (2P), plateau (PL; milk flow by ewes with longer duration of steady phase and peak flow rate during plateau phase > 0.4 l/min at least for 20 s), and plateau low (PLII; milk flow curves with steady milk flow during milking, but at peak milk flow rate at plateau phase 0.4 l/min or > 0.4 l/min shorter than for 20 s)

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Bruckmaier et al. (1997) supposed that the ewes with PLII milk flow have extremely weak or totally absent oxytocin release during milking. However, Macuhova et al. (2007) found out that the ewes with PLII type of milk flow had higher total milk yield than ewes with 1P type of milk flow (the ewes in which either no release or opportune alveolar milk ejection is presumed) and comparable with ewes with 2P type of milk flow. Therefore, it is possible that these ewes released oxytocin during the machine milking and low milk flow could be a result of a functional characteristic of teat sphincter (physiological or pathophysiological). McKusick (2000) demonstrated that ewes with low milk flow rate have significantly "tighter" teat sphincter and, conversely, ewes with high milk flow rate have sphincters that take less vacuum to open. This could be one reason for a significantly lower peak flow rate between milking groups. For ewes with unsuitable udder anatomy, it is supposed that milking without any milk flow could be painful as over-milking at the end of milk emission and, therefore, they were avoiding entering the milking parlour in the first milking groups.

Machine stripping yield, the percentage machine stripping yield from total milk yield, and milking time were not affected ($P > 0.05$) by the milking group (Table 1). Milking time can be influenced by the frequency of occurrence of particular types of milk flow (Macuhova et al. 2008). Ewes with 1P milk flow needed shorter time for machine milking than ewes with 2P or PL, PLII milk flow (Tancin et al. 2015). Moreover, Villagra et al. (2007) and Antonic et al. (2013) showed that animals with 1P milk flow had also a lower milk production and a higher machine stripping yield and residual milk yield from total milk yield than ewes with 2P milk flow. In the study of Antonic et al. (2013) (as in our study), the sheep were milked without any udder preparation and only milk flow curves whith one peak and time of machine milking up to 40 s were classified as 1P. Comparable findings were observed also in our study (data not shown). Milking time was significantly lower in ewes with 1P milk flow than in 2P and even in the other milk flow types (27 ± 14 vs 61 ± 10, 55 ± 11, and 56 ± 21 s in 2P, PL, and PLII, respectively; $P < 0.05$). Also total milk yield and the percentage of machine stripping yield from total milk yield were affected significantly ($P < 0.01$) by milk flow type. The lowest total milk yield was observed in 1P and the highest in PL (0.28 ± 0.10 vs 0.54 ± 0.21 l). Similar milk yield was observed in 2P and PLII (0.39 ± 0.12 and 0.36 ± 0.11 l, respectively). The lowest percentage of machine stripping yield from total milk yield was observed in PL and the highest in 1P and PLII (29 ± 10 vs 42 ± 12 and 46 ± 11%). But machine stripping yield was not affected by milk flow type ($P = 0.26$). These results support that only cisternal milk was removed during machine milking in ewes with 1P milk flow.

It is known that the occurrence of 1P milk flow type is higher during milking under stress (Tancin et al. 2015). Villagra et al. (2007) observed the higher occurrence of 1P milk flow in ewes entering the milking parlour in the last group. This was not observed in our study. The occurrence of ewes with 1P milk flow was similar in both groups (5 and 8 ewes in FG in LG, respectively (χ^2 test, χ^2 value = 0.69, df = 1, $P = 0.41$)). There was no significant difference in the number of animals with 2P and PL milk flow between FG and LG (10 and 3 ewes with 2P and PL in FG, respectively, 9 and 5 ewes with 2P and PL in LG, respectively

Table 3. Frequency of ewes parity according to milking group (parlour entry as the first (FG) and as the last group (LG))

Milking group		Parity		Total	*P*-value	
		primiparous	multiparous		within group	overall
FG	count	5	14	19	0.04[1]	0.55[2]
	% of total	10.4	29.2	39.6		
	% within group	26.3	73.7	100		
LG	count	10	19	29	0.09[1]	
	% of total	20.8	39.6	60.4		
	% within group	34.5	65.5	100		
Total	count	15	33	48		

[1]χ^2 test, df = 1, χ^2 value = 4.26 and 2.79, respectively by FG and LG

[2]χ^2 test, df = 1, χ^2 value = 0.36

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(exact χ^2 test, χ^2 value = 0.52, df = 1, P = 0.48)) in contrast to Villagra et al. (2007). In that study, Manchega ewes entering the parlour as first had higher occurrence of 2P milk flow than the ewes of the last group (83 vs 17%) (Villagra et al. 2007).

Unlike the studies in goats (Margetinova et al. 2003; Gorecki and Wojtowski 2004), where at least in one of three recording periods the relationship between milking order and age was observed, no relation was observed between the parity and milking order in this study. The number of primiparous and multiparous animals did not differ between milking groups (Table 3). Therefore, it is not possible to argue that animals in the first

Table 4. Effect of parity and ewes milking group (parlour entry as the first (FG) and the last group (LG)) on parameters of milkability and milk composition

Traits	Milking group	Mean ± SD per group and parity			Mean ± SD per parity		
		primiparous FG n = 5 LG n = 10	multiparous FG n = 14 LG n = 19	P-value, significance	primiparous n = 15	multiparous n = 33	P-value, significance
Total milk yield (TMY) (l)	FG	0.44 ± 0.08	0.40 ± 0.20	0.35	0.37 ± 0.15	0.38 ± 0.16	0.64
	LG	0.33 ± 0.17	0.37 ± 0.12	ns			ns
Machine milk yield (l)	FG	0.29 ± 0.10	0.26 ± 0.17	0.64	0.25 ± 0.13	0.24 ± 0.13	0.75
	LG	0.22 ± 0.14	0.22 ± 0.09	ns			ns
Machine stripping (MS) (l)	FG	0.14 ± 0.06	0.15 ± 0.06	0.48	0.12 ± 0.06	0.15 ± 0.06	0.95
	LG	0.11 ± 0.06	0.14 ± 0.06	ns			ns
MS/TMY (%)	FG	33.09 ± 13.95	38.89 ± 13.02	0.55	33.98 ± 12.29	39.24 ± 11.65	0.65
	LG	34.43 ± 12.16	39.50 ± 10.90	ns			ns
Milking time (s)	FG	41 ± 18	52 ± 15	0.78	44 ± 17	53 ± 20	0.25
	LG	46 ± 17	54 ± 24	ns			ns
Latency time (s)	FG	10 ± 2	15 ± 3	0.07	15 ± 7	18 ± 12	0.28
	LG	17 ± 7	21 ± 16	ns			ns
Peak flow rate (l/min)	FG	1.40 ± 0.42	0.91 ± 0.30	0.08	0.97 ± 0.41	0.83 ± 0.33	0.27
	LG	0.75 ± 0.18	0.78 ± 0.34	ns			ns
Time of peak flow rate (s)	FG	19 ± 7	23 ± 9	0.55	22 ± 8	26 ± 14	0.96
	LG	24 ± 8	28 ± 17	ns			ns
Milk yield in 30 s (l)	FG	0.21 ± 0.11	0.13 ± 0.07	0.37	0.14 ± 0.09	0.12 ± 0.06	0.69
	LG	0.11 ± 0.04	0.10 ± 0.06	ns			ns
Milk yield in 60 s (l)	FG	0.29 ± 0.10	0.24 ± 0.17	0.70	0.24 ± 0.13	0.20 ± 0.12	0.49
	LG	0.21 ± 0.13	0.18 ± 0.06	ns			ns
Fat (%)	FG	5.83 ± 1.03	6.30 ± 0.87	0.73	6.07 ± 0.84	6.32 ± 0.87	0.52
	LG	6.20 ± 0.75	6.34 ± 0.89	ns			ns
Protein (%)	FG	5.15 ± 0.48	5.24 ± 0.36	0.45	5.26 ± 0.48	5.34 ± 0.38	0.28
	LG	5.30 ± 0.50	5.42 ± 0.38	ns			ns
Lactose (%)	FG	4.75 ± 0.30	4.82 ± 0.19	0.77	4.85 ± 0.22	4.85 ± 0.16	0.40
	LG	4.90 ± 0.16	4.86 ± 0.12	ns			ns
Solids-not-fat (%)	FG	10.72 ± 0.31	10.88 ± 0.38	0.77	10.92 ± 0.40	11.02 ± 0.40	0.17
	LG	11.02 ± 0.42	11.12 ± 0.39	ns			ns
Solid (%)	FG	16.55 ± 1.23	17.17 ± 1.15	0.87	17.00 ± 1.07	17.34 ± 1.10	0.25
	LG	17.22 ± 0.97	17.46 ± 1.08	ns			ns
SCC (log)	FG	5.35 ± 0.71	5.57 ± 0.76	0.93	5.27 ± 0.73	5.60 ± 0.62	0.27
	LG	5.22 ± 0.77	5.63 ± 0.53	ns			ns

SCC = somatic cell count, ns = not significant

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Table 5. Parameters of milkability in dependence on ewes milking group (parlour entry as the first (FG) and as the last group (LG)) and breed/crossbreed (Lacaune (LC), LC × Tsigai (TS × LC), LC × Improved Valachian (IV × LC))

Traits	Milking group	Mean ± SD per group and breed/crossbreed				Mean ± SD per breed/crossbreed			
		LC FG n = 3 LG n = 7	TS × LC FG n = 8 LG n = 11	IV × LC FG n = 8 LG n = 11	P-value significance	LC n = 10	TS × LC n = 19	IV × LC n = 19	P-value significance
Total milk yield (TMY) (l)	FG	0.66 ± 0.22	0.31 ± 0.11	0.42 ± 0.10	0.44	0.49 ± 0.18^{a}	0.30 ± 0.10^{b}	0.39 ± 0.15^{ab}	< 0.01
	LG	0.42 ± 0.10	0.30 ± 0.10	0.37 ± 0.18	ns				++
Machine milk yield (l)	FG	0.49 ± 0.22	0.16 ± 0.05	0.28 ± 0.10	0.09	0.31 ± 0.17^{a}	0.18 ± 0.07^{b}	0.26 ± 0.12^{ab}	< 0.01
	LG	0.23 ± 0.06	0.19 ± 0.08	0.24 ± 0.14	ns				++
Machine stripping yield (MS) (l)	FG	0.17 ± 0.02	0.15 ± 0.08	0.13 ± 0.05	0.82	0.18 ± 0.07	0.12 ± 0.06	0.13 ± 0.05	0.15
	LG	0.18 ± 0.08	0.11 ± 0.04	0.12 ± 0.06	ns				ns
MS/TMY (%)	FG	26.73 ± 8.83	46.10 ± 11.37	32.61 ± 11.44	0.08	38.44 ± 13.20	40.89 ± 11.41	33.86 ± 11.45	0.10
	LG	43.46 ± 11.71	37.10 ± 10.31	34.76 ± 11.92	ns				ns
Milking time (s)	FG	54 ± 11	50 ± 18	46 ± 16	0.72	62 ± 15	47 ± 20	47 ± 20	0.59
	LG	65 ± 16	44 ± 21	48 ± 22	ns				ns
Latency time (s)	FG	15 ± 2	15 ± 3	12 ± 3	0.19	23 ± 8	15 ± 5	16 ± 15	0.06
	LG	26 ± 7	16 ± 6	20 ± 20	ns				ns
Peak flow rate (l/min)	FG	1.22 ± 0.16^{A}	0.78 ± 0.20	1.22 ± 0.46	< 0.01	0.73 ± 0.35	0.81 ± 0.25	1.02 ± 0.41	0.81
	LG	0.52 ± 0.08^{aB}	0.82 ± 0.28^{ab}	0.87 ± 0.31^{b}	++				ns
Time of peak flow rate (s)	FG	35 ± 13	20 ± 4	18 ± 6	0.91	35 ± 14^{a}	22 ± 7^{b}	22 ± 13^{b}	0.02
	LG	35 ± 15	23 ± 9	25 ± 17	ns				+
Milk yield in 30 s (l)	FG	0.21 ± 0.07^{A}	0.10 ± 0.03	0.19 ± 0.10	< 0.01	0.10 ± 0.09	0.11 ± 0.04	0.16 ± 0.08	0.39
	LG	0.05 ± 0.03^{aB}	0.11 ± 0.04^{ab}	0.13 ± 0.05^{b}	++				ns
Milk yield in 60 s (l)	FG	0.48 ± 0.22^{a}	0.15 ± 0.04^{b}	0.28 ± 0.10^{ab}	0.02	0.26 ± 0.19^{a}	0.16 ± 0.06^{b}	0.24 ± 0.11^{ab}	0.02
	LG	0.16 ± 0.05	0.17 ± 0.07	0.22 ± 0.12	+				+

ns = not significant, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$; $^{a,b}P \leq 0.05$ in row; $^{A,B}P \leq 0.05$ between groups

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lactation go to the milking parlour later than the older ones. Possibly younger ewes had sufficient time (mid stage of lactation) to get used to the milking process and milking was not so stressful for them at the time of the experiment. Also Dimitrov et al. (2012) did not find any influence of age (animals on the first and the second lactation) on their order of entry into the parlour during the evaluation of sheep temperament.

Besides milking group, the effect of parity, breed/crossbreed, and interactions milking group × parity and milking group × breed/crossbreed were evaluated on milkability parameters. Parity had no significant effect on any tested parameter (Table 4).

The breed/crossbreed (Table 5) had significant effect on total and machine milk yield, time of peak flow rate, and milk yield in 60 s. Thereby, only values of TS × LC and LC differed significantly. Considering the interaction group × breed/crossbreed, only peak flow rate and milk yield in 30 and 60 s were significantly affected. Interestingly, differences between milking groups were observed only by LC by these parameters (significant by peak flow rate and milk yield in 30 s (P < 0.01), in tendency by milk yield in 60 s (P = 0.07)). This could signalize some obstacles by milk removal in LC ewes of LG. This is supported also by the fact that other parameters were not affected and most of ewes with PLII milk flow type were LC (Table 2). Thereby, all these ewes with PLII (n = 5) belonged to LG (data not shown).

Overall, regarding the occurrence of milk flow types according to breed/crossbred, the relationship was significant (P < 0.01). However, this effect seems to be caused mostly by LC. No differences were observed between crossbreds in the occurrence of milk flow types (P = 1.00).

The effect of milking group on milk composition is shown in Table 1. The order of entry into milking parlour had no effect on milk components and SCC in this study; only solids-not-fat tended to differ. The parity and interactions group × parity (Table 4) and group × breed/crossbreed (Table 5) had no significant effect on any of these parameters. The breed/crossbreed had significant effect on fat and solid (P = 0.03 and P < 0.01, respectively) and tended to influence protein (P = 0.05) and solids-not-fat (P = 0.06) (Table 6). Thereby, the values of these parameters differed only between LC and TS × LC (fat: 5.85 vs 6.60% (P = 0.03); solid: 16.71 vs 17.75% (P < 0.01); protein: 5.10 vs 5.45%

Table 6. Milk composition according to ewes milking group (parlour entry as the first (FG) and the last group (LG)) and breed/crossbreed (Lacaune (LC), LC × Tsigai (TS × LC), LC × Improved Valachian (IV × LC))

Traits	Milking group	Mean ± SD per group and breed/crossbreed				Mean ± SD per breed/crossbreed			
		LC FG n = 3 LG n = 7	TS × LC FG n = 8 LG n = 11	IV × LC FG n = 8 LG n = 1	P-value significance	LC n = 10	TS × LC n = 19	IV × LC n = 19	P-value significance
Fat (%)	FG	5.74 ± 0.09	6.60 ± 0.82	5.91 ± 1.04	0.98	5.85 ± 0.63[a]	6.60 ± 0.76[b]	6.10 ± 0.95[ab]	0.03
	LG	5.90 ± 0.76	6.60 ± 0.75	6.23 ± 0.91	ns				+
Protein (%)	FG	4.96 ± 0.56	5.33 ± 0.30	5.20 ± 0.39	0.52	5.10 ± 0.34	5.45 ± 0.37	5.29 ± 0.44	> 0.05
	LG	5.15 ± 0.24	5.55 ± 0.40	5.35 ± 0.49	ns				ns
Lactose (%)	FG	4.88 ± 0.18	4.85 ± 0.14	4.73 ± 0.29	0.54	4.94 ± 0.15	4.85 ± 0.14	4.79 ± 0.20	0.34
	LG	4.97 ± 0.14	4.86 ± 0.14	4.84 ± 0.10	ns				ns
Solids-not-fat (%)	FG	10.65 ± 0.44	11.01 ± 0.27	10.73 ± 0.38	0.78	10.86 ± 0.35	11.14 ± 0.34	10.90 ± 0.44	0.06
	LG	10.95 ± 0.31	11.24 ± 0.36	11.03 ± 0.46	ns				ns
Solid (%)	FG	16.39 ± 0.44	17.62 ± 1.01	16.64 ± 1.31	0.78	16.71 ± 0.75[a]	17.75 ± 0.87[b]	17.00 ± 1.26[ab]	0.01
	LG	16.85 ± 0.84	17.84 ± 0.78	17.26 ± 1.22	ns				++
SCC (log)	FG	5.20 ± 1.00	5.60 ± 0.64	5.54 ± 0.79	0.56	5.50 ± 0.67	5.47 ± 0.75	5.53 ± 0.62	0.85
	LG	5.63 ± 0.53	5.37 ± 0.83	5.52 ± 0.50	ns				ns

SCC = somatic cell count, ns = not significant, + P < 0.05, ++ P < 0.01; [a,b]P ≤ 0.05 in row

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($P = 0.04$); solids-not-fat: 10.86 vs 11.15% ($P = 0.08$), respectively). It was shown that with increasing milk yield the percentage of some milk components (fat and protein content) can be negatively affected (Barillet 2007). This could be the reason for differences in this study. As already mentioned, LC and TS × LC differed significantly in total milk yield. No difference was observed in SCC between FG and LG ewes (Table 4). Moreover, SCC was at physiological level in all ewes in this study. Similar SCC values were found in crossbreeds TS × LC and IV × LC also by Margetin et al. (2013).

CONCLUSION

Some ewes prefer to enter the milking parlour predominantly early and some in the last groups. The order of the milking parlour group has no effect on milk composition and SCC. However, ewes entering the parlour first have better milk flow parameters (shorter latency time, higher peak flow rate, and higher milk yield in 30 s and 60 s) and tend to have higher milk production than ewes entering the milking parlour last. In FG, a higher occurrence of 2P and PL milk flow types, the most surely milk flow types with ejection reflex during milking, than of the other types was observed. Thus, ewes which enter the milking parlour in early milking groups have better parameters of milkability than those milked in later groups.

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