The level of udder emptying and milk flow stability in Tsigai, Improved Valachian, and Lacaune ewes during machine milking

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ABSTRACT: The objective of this investigation was to evaluate the stability of milk flow curves and the volume of residual milk in relation to milk flow curves in breeds Tsigai, Improved Valachian, and Lacaune (n = 16 heads per breed) milked twice daily. Milk flow type stability was evaluated at morning milking (stabile milkings) during three successive days in the middle of two months (June, July). After the following fourth morning milking (OT milkings) in both months, oxytocin was injected intravenously at a dose of 2 UI. Afterwards the ewes were milked again in order to remove residual milk. The milk flow curves were classified into four types: 1 peak (1P), 2 peaks (2P), plateau I (maximal milk flow over 0.4 l/min) (PLI), plateau II (maximal milk flow less than 0.4 l/min) (PLII). If all milk flow curves of one animal were of the same type within one month and within both months, respectively, the animal was characterized as the ewe with the stabile type of milk flow. Frequency of occurrence of single milk flow types (1P: 2P: PLI: PLII) during stabile milkings was 42:49:9:0% in June and 51:37.5:11.5:0% in July. 33 ewes (i.e. 69%) had the stabile type of milk flow within both months. But, more than 51% of them had 1P type of milk flow. Milk production varied according to milk flow curve during OT milkings (0.427 ± 0.015, 0.498 ± 0.024, and 0.655 ± 0.035 l for 1P, 2P, and PLI, respectively). The highest percentage of residual milk from total milk yield (RM/TMY) was observed in ewes with 1P (20.29 \pm 0.85%), followed by ewes with PLI (12.31 \pm 1.99%) and 2P (9.72 \pm 1.36%) (P < 0.0001). Lacaune ewes had the lowest amount of residual milk (0.054 ± 0.006 l) and RM/TMY $(9.86 \pm 1.16\%)$ compared to breeds Tsigai $(0.088 \pm 0.008 \text{ l}; 16.47 \pm 1.44\%)$ and Improved Valachian $(0.069 \pm 0.008 \text{ l}; 16.47 \pm 0.008 \text{ l})$ 0.010 l; $15.99 \pm 1.78\%$) indicating their better udder emptying.

Keywords: ewe; milk flow curves; stability of milk flow types; residual milk; breeds

Milkability of ewes during machine milking could be evaluated by partitioning of milk collected during milking (machine milking, machine stripping), amount of residual milk (milk which remains in the udder and is only removed after administration of supraphysiological doses of oxytocin) or by the analysis of milk flow kinetics (Labussière, 1988; Bruckmaier et al., 1997; Marnet and McKusick, 2001; Mačuhová et al., 2008b; Tančin et al., 2011).

Milk flow kinetics is mainly influenced by physiological response of ewes to machine stimulation

(milk ejection occurrence), udder and teat morphology, and distribution of milk within udder. Thus milk flow varies according to species, breed, age, stage of lactation, and milking interval (Bruckmaier et al., 1997; Davis et al., 1998; Castillo et al., 2008).

The milk emission typology considers curves of different shape (Labussière, 1988; Bruckmaier et al., 1997; Marnet et al., 1998; Rovai et al., 2002a). The milk flow curves of ewes can be classified as one peak (1P), two peaks (2P), plateau I(PLI), and plateau II (PLII). 1P milk flow type could represent

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milk flow without alveolar milk ejection when only cisternal milk fraction is removed in response to machine milking. On the other hand, the milk flow curves with two separated peaks (2P) show alveolar milk ejection after the cisternal milk is removed (Labussière, 1988). The PLI milk flow type refers to ewes with larger emission curve and did not show clear differences between peaks (Marnet et al., 1998; Rovai et al., 2002a). Ewes with PLII have a very low milk flow. It is supposed that they have extremely weak or totally absent oxytocin release during milking (Bruckmaier et al., 1997).

Higher amounts of residual milk and increased occurrence of 1P curves may result from incomplete milk ejection associated with poor milking routines, frightened or nervous animals or uncomfortable milking equipment (Mačuhová et al., 2002, 2008a). Thus the effect of different factors on milk removal can be studied through evaluation of milk flow kinetics. For that, it is important to know if the milk flow curves varied within short and/or long periods of lactation.

There were three main hypotheses tested in this work – firstly, that ewes with different milk flow kinetics and stage of lactation also differ in residual milk volume; secondly, that breeds Tsigai and Improved Valachian had higher residual milk volume than Lacaune; and, lastly, that physiological response of ewes to machine stimulation represented by milk flow kinetics is relatively stable within short and also longer periods of lactation. The objective of this investigation was to evaluate the volume of residual milk affected by breeds, milk flow type and also to evaluate the stability of milk flow type within short and longer periods of lactation.

MATERIAL AND METHODS

Animals and routine milking

A total of 48 ewes of three breeds (16 heads Tsigai, 16 heads Improved Valachian, 16 heads Lacaune) were used in the experiment. The ewes were in their first to fourth lactation. The animals were kept on the pasture and they were brought into the stable only at milking time. Each ewe received 0.1 kg of concentrate during milking.

The ewes were routinely milked twice a day (at 7.30 a.m. and 7.30 p.m.) in a one-side milking parlour, designed for 24 animals and equipped with 12 standard milking units. The milking machine was set to provide 180 pulsations per min in

a 50:50 ratio with a vacuum level of 38 kPa. Milking was performed without any contact of hand with the udder before cluster attachment (no stimulation and fore-stripping). The machine stripping was performed after ceasing of milk flow but not earlier than 70 s from cluster attachment, to be able to detect the second emission of milk in a case of milk ejection occurrence.

The experimental milkings were performed in the middle of June and July. In the first part of the experiment the kinetics of milk flows was measured during three consecutive morning milkings (stabile milkings) to evaluate the stability of milk flow curve (stability milking) in individual ewes within a month as well as within both months.

In the second part of the experiment, the volume of residual milk in relation to milk flow kinetics was evaluated at the fourth morning milking (OT milkings). Oxytocin (OT) was injected intravenously at a dose of 2 UI to ewes after milking with usual milking routine at the milking place. The ewes were milked again after 40 s from OT injection in order to remove residual milk. Therefore, milking was performed with only four ewes per one parlour turn to reduce the waiting time in the parlour for the other ewes.

Data recording and evaluation

Milk flow was recorded continuously by graduated electronic milk collection jar designed for ewe placed between the claw and milk line. Milk flow curves were evaluated according to Bruckmaier et al. (1997), Rovai et al. (2002a), and Mačuhová et al. (2008b) and divided into four types: 1 peak (1P), 2 peaks (2P), plateau I (PLI), and plateau II (PLII). 1P represents milk flow curves with one peak of milk flow before stripping. 2P type of milk flow has two clearly separated milk flow peaks, i.e. after the first peak (peak 1) transient decreasing followed by the second increasing of milk flow (peak 2) can be observed prior to stripping. PLI represents milk flow by ewes with steady milk flow during milking and larger emission curves with maximal milk flow rate over 0.4 l/min without clear differences between peaks 1 and 2. PLII represents also milk flow curves with steady milk flow during milking, but at very low milk flow level (maximal milk flow rate ≤ 0.4 l/min). This type of milk flow occurred only during one milking, therefore these data were not included in statistical evaluation of the effect of milk flow on milkability.

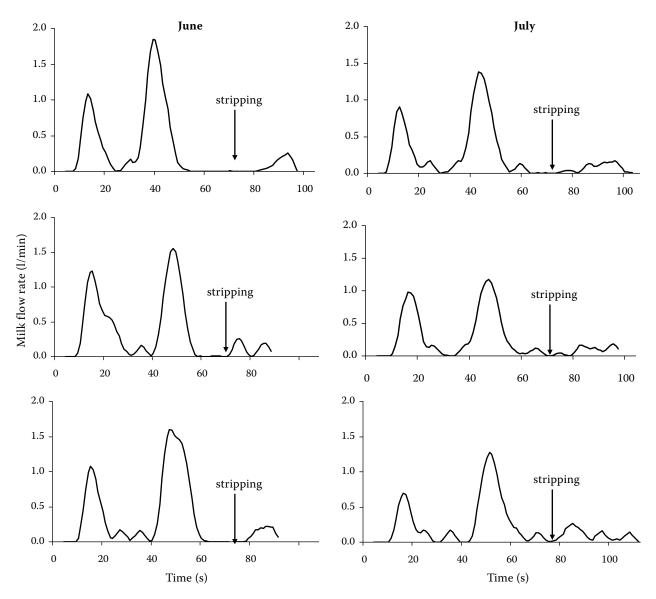


Figure 1. Example of Improved Valachian ewe with stabile milk flow curves (2P) during three consecutive milkings in two months of experiment

After classification of milk flow type of recorded milk flow curves, stability of milk flow curves in individual ewes was evaluated. If the ewe had all 3 and 6 milk flow curves of the same type within one month and within both months respectively, it was characterized as the ewe with the stabile milk flow type (Figure 1).

In the second part of the experiment (i.e. during OT milkings), the following milking characteristics were evaluated: machine milk yield (MMY) and milking time (the amount of milk obtained by the machine and time from the attachment of cluster (time 0) to milk flow ceased), machine stripping (MS; the amount of milk obtained by milker during machine stripping), milk yield (MY = MMY + MS), residual milk (RM; amount

of milk removed after administration of OT), total milk yield (TMY = MY + RM), maximal milk flow rate (maximum flow rate recorded during machine milking).

The milk flow kinetics was calculated according the following model:

$$Yn = [yield in time (n) - yield in time (n - 4)] \times 15, n > 3$$

Statistical analysis

The data set consisted of 95 measurements belonging to 48 ewes during OT milkings in both months. Mixed model (Mixed procedure; SAS/

Milk flow type Month 2P PLI PLII 1P stabile OT stabile OT stabile OT stabile OT June 42 54.0 49 31 9.0 13.0 0 2 July 51 64.5 37.5 25 11.5 10.5 0 0 0 June + July 46 59.5 44 2.8 10.0 11.5 1

Table 1. Frequency (in %) of particular types of milk flow within evaluated months during stabile and OT milkings

1P = one peak, 2P = two peaks, PLI = plateau I, PLII = plateau II

STAT 9.1, 2002–2003) was applied to study the influence of the sources of variation in studied traits (milk production and milk emission/milkability).

The model equation was the following:

$$y_{iiklm} = \mu + T_i + G_l + M_i + u_m + e_{iiklm}$$

where:

y_{ijklm} = individual observations of studied traits: TMY (l), MY
(l), MMY (l), MS (l), RM (l), milking time (s), maximal milk flow rate (l/min), MS/MY (%), RM/TMY (%)

μ = intercept

 T_i = fixed effect of milk flow type (2P, PLI, 1P); $\Sigma T_i = 0$

 G_l = fixed effect of genotype (breeds Tsigai, Improved Valachian, Lacaune); $\sum g_i = 0$

 M_j = fixed effect of month of measurement (June, July); $\Sigma M_i = 0$

 u_m = random effect of ewe (1, 2 to 61); $u_m = N(0, I\sigma_w^2)$

 e_{iiklm} = random error; e_{iiklm} = N(0, $I\sigma_w^2$)

Fixed effects included in 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 fixed effects were tested by Scheffe's multiple range tests. The effect of ewes and residual error variances were estimated using the REML (Restricted Maximum Likelihood) method.

RESULTS

Evaluation of stability and frequency of milk flow type occurrence

The total of 288 morning milk flow curves were recorded during stabile milkings. Out of these milk flows, 46% were 1P, 44% 2P, and 10% PLI. As shown in Table 1, the frequency of distribution was influ-

enced by the month and OT treatment before OT injection.

33 ewes (i.e. 69%) had the stabile type of milk flow within both months. But, more than 51% of them had 1P type of milk flow. There were 41 ewes with stabile type of milk flow in the first month and 39 ones during the next month. Distribution of the number of ewes with stabile type of milk flow during the evaluated months is shown in Figure 2.

During OT milkings, the decrease in the number of ewes with 2P or PLI type of milk flow was observed (59.5% 1P, 28% 2P, 11.5% PLI, 1% PLII) when compared with stabile milkings (46, 44, 10, 0%) during both months. The percentage of ewes with 1P type of milk flow increased during OT milking in both months.

Evaluation of udder emptying

The amount of milk obtained in response to OT injection ranged from 2.3 to 41.0% of the TMY in individual ewes. Volume of RM was similar during

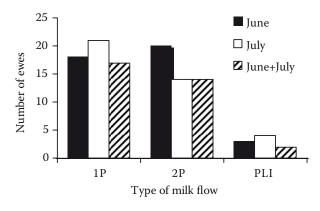


Figure 2. Distribution of number of ewes with stabile type of milk flow within evaluated months

1P = one peak, 2P =two peaks, PLI = plateau I

Table 2. Effect of month measurement on milkability of ewes

Traits	June	July	P
Total milk yield (TMY) (l)	0.551 ± 0.018^{a}	0.502 ± 0.020^{b}	0.0308
Milk yield (MY) (l)	0.487 ± 0.016^{a}	0.426 ± 0.018^{b}	0.0033
Machine milk yield (MMY) (l)	0.361 ± 0.017	0.319 ± 0.019	0.0571
Machine stripping (MS) (l)	0.127 ± 0.009	0.106 ± 0.011	0.0899
MS/TMY (%)	24.61 ± 1.83	21.23 ± 2.05	0.1508
Milking time (s)	57 ± 2	55 ± 2	0.3816
Maximal milk flow rate (l/min)	0.857 ± 0.061	0.731 ± 0.069	0.1112
Residual milk (RM) (l)	0.064 ± 0.006	0.076 ± 0.006	0.0973
RM/TMY (%)	12.09 ± 1.01^{a}	16.13 ± 1.13^{b}	0.0024

^{a,b}differences significant at P < 0.05

both months (0.064 and 0.076 l). Proportion of RM/TMY was lower in June than in July (12.09 \pm 1.01% vs. 16.13 \pm 1.13%; Table 2).

The effect of type of milk flow on milkability and its relation to RM volume is shown in Table 3. The type of milk flow had a significant effect on all tested parameters besides MS and maximal milk flow rate. TMY, MY, and MMY were the highest in PLI, followed by 2P and the lowest in 1P. The lowest volumes of RM and RM/TMY (9.72%) were in ewes with 2P type of milk flow. The highest RM/TMY was in ewes with 1P (20.29%) followed by PLI (12.31%) type of milk flow.

The effect of breed on tested parameters of milkability is shown in Table 4. The TMY was one of tested parameters significantly affected by breed. The significant difference was observed between breed Lacaune with the highest and Improved Valachian with the lowest TMY. Values of fractional milk (MY: MS: RM) were 62: 21.5: 16.5%; 61: 29: 10%, and 66: 18: 16% for Tsigai, Lacaune, and Improved Valachian, respectively. Additionally, in Lacaune the lowest percentage of RM, however, the highest percentage of MS/TMY was observed.

DISCUSSION

Milk flow kinetics

On the basis of our results we could find out animals that show stabile milk flow curves during short (within few days) and long (within two months) periods of lactation. Stability during short periods was

Table 3. Effect of milk flow types on milkability of ewes

Traits -	Milk flow curve type			
	1P	2P	PLI	P
Total milk yield (TMY) (l)	0.427 ± 0.015^{a}	0.498 ± 0.024^{b}	0.655 ± 0.035^{c}	< 0.0001
Milk yield (MY) (l)	0.343 ± 0.013^{a}	0.451 ± 0.021^{b}	0.575 ± 0.031^{c}	< 0.0001
Machine milk yield (MMY) (l)	0.221 ± 0.014^{a}	0.345 ± 0.023^{b}	0.454 ± 0.034^{c}	< 0.0001
Machine stripping (MS) (l)	0.123 ± 0.008	0.107 ± 0.013	0.121 ± 0.019	0.5574
MS/TMY (%)	29.29 ± 1.54^{a}	21.34 ± 2.47^{b}	18.13 ± 3.61^{b}	0.0029
Milking time (s)	35 ± 2^a	72 ± 3^{b}	62 ± 3^{b}	0.0228
Maximal milk flow rate (l/min)	0.735 ± 0.051	0.865 ± 0.083	0.781 ± 0.121	0.4166
RM/TMY (%)	20.29 ± 0.85^{a}	9.72 ± 1.36^{b}	12.31 ± 1.99^{b}	< 0.0001

^{a, b, c}differences significant at P < 0.05

Table 4. Parameters of milkability of different breeds

Traits -	Breed			<i>F</i> -test
	Tsigai	Lacaune	Improved Valachian	P
Total milk yield (TMY) (l)	0.528 ± 0.025^{ab}	0.588 ± 0.020^{b}	0.464 ± 0,031 ^a	0.0041
Milk yield (MY) (l)	0.440 ± 0.023^{a}	0.534 ± 0.019^{b}	0.395 ± 0.028^a	< 0.0001
Machine milk yield (MMY) (l)	0.328 ± 0.024	0.375 ± 0.020	0.317 ± 0.030	0.1594
Machine stripping (MS) (l)	0.112 ± 0.013^{a}	0.160 ± 0.011^{b}	0.078 ± 0.017^{a}	0.0002
MS/TMY (%)	21.41 ± 2.61^{ab}	29.10 ± 2.10^{a}	18.26 ± 3.23^{b}	0.0092
Milking time (s)	55 ± 3^a	62 ± 2^{b}	51 ± 4^{c}	0.0403
Maximal milk flow rate (l/min)	0.834 ± 0.087	0.742 ± 0.070	0.806 ± 0.108	0.6968
Residual milk (RM) (l)	0.088 ± 0.008^{a}	0.054 ± 0.006^{b}	0.069 ± 0.010^{ab}	0.0055
RM/TMY (%)	16.47 ± 1.44^{a}	9.86 ± 1.16^{b}	15.99 ± 1.78^{a}	0.0007

^{a, b, c}differences significant at P < 0.05

higher than that over long time periods. As it was expected, the highest stability was found in ewes with 1P milk flow type. But from the physiological point of view, stability of 2P milk flow is the most important. The ewes with constant 2P type of milk flow from day to day could be good experimental animals to study the effect of milking management on milkability without blood collection to measure OT. The second emission (peak 2) is related to the release of OT in response to milking stimuli (Bruckmaier et al., 1997) and thus some disturbing of OT release due to milking management changes may result in changing the time of the second peak occurrence (e.g. to cause its delay) or in the lack of the second emission (i.e. change in 1P milk flow type) (Negrao et al., 1999; Tančin et al., 2006). The presented data showed that changing of milking management due to OT injection and milking of only 4 ewes in one turn during the second part of the experiment has resulted in lesser occurrence of 2P milk flow and increased occurrence of 1P milk flow type. Recent investigations revealed that also the presence of unknown person in front of ewes' heads increased the occurrence of 1P milk flows as a result of reducing percentage of 2P (Kulinová et al., 2012). Thus the selection of ewes with 2P milk flow for experiment could be a good approach to study the effect of milking management on physiological response of ewes.

Relatively high percentage of 1P milk flow curves is not surprising due to the breeds involved in the experiment. We have published several times the higher occurrence of 1P milk flow in autochthonous and locally adapted breeds raised in Slovakia

- Tsigai and Improved Valachian (Mačuhová et al., 2007, 2008b; Tančin et al., 2011).

Tančin et al. (2011) found out reduction of 2P and PLI curves throughout the lactation. This was observed also in Manchega and Lacaune breeds (Rovai et al., 2002a). The machine milkability of dairy ewes tends to worsen with the advancing stage of lactation (Casu et al., 2008). However, it need not mean that the ewes exhibiting change of milk flow did not release OT during milking. The reason could be that due to the decrease of the alveolar milk fraction in later lactation stage (Such et al., 1999; Rovai et al., 2002b), the milk ejection occurred less frequently in this stage before stripping was started (Bruckmaier et al., 1997).

Residual milk volume

Presented data on milkability during OT milking in relation to the breed, stage of lactation, and type of milk flow (Tables 2–4) are similar as we published earlier in our breeds (Mačuhová et al., 2007, 2008b; Kulinová et al., 2010; Tančin et al., 2011) or as published by other authors (Rovai et al., 2002a; Villagrá et al., 2007). Less or no information on RM in these breeds is available and therefore our investigation was concentrated more on RM. The RM volume and its percentage were influenced by milk flow kinetics, breeds, and stage of lactation.

There was no difference in RM volume between the months of June and July. However, RM/TMY increased significantly from June to July. This increase can be explained by the decrease in MY. Decreased MY in July could result in the increased occurrence of 1P milk flow. Similar observations were published by Heap et al. (1986). In their study, percentage of residual milk remained steady, however, only in months with higher milk production of ewes, and it declined when the yields were less than 1 kg per day.

Lacaune breed had the lowest volume of RM and RM/TMY, therefore it could be assumed that this breed retained less milk in the secretory system of the udder than autochthonous and locally adapted breeds Tsigai and Improved Valachian. Because of high milk production, breed Lacaune has already been bred for many decades. Lacaune ewes have a large cistern and low residual milk and proved to be excellent producers when submitted to one daily milking without machine stripping (Labussière, 1988). In our conditions, breed Lacaune had almost double cisternal volume than Tsigai and Improved Valachian (Milerski et al., 2006). High-yielding Lacaune ewes presented greater cisternal milk percentages and more cisternal milk than mediumyielding ewes (Castillo et al., 2008).

Tsigai and Improved Valachian (Table 4) had comparable RM/TMY (16%) to Manchega (Such et al., 1999) and lesser RM/TMY than the ewes of Sicilo-Sarde breed (up to 38%) (Mohamed et al., 2008). However, compared to our study, Labussière (1988) observed slightly lower values of RM/TMY (13%) in breed Tsigai.

CONCLUSION

During ordinary milkings in June and July, about 69% of ewes had the stabile type of milk flow curve. The type of milk flow curve changed in some ewes with advancing lactation (assuming due to decreased milk production). The change of the milk flow type was mostly observed in ewes with 2P type in the course of lactation. The ewes with 1P had the lowest milk production, the highest MS/TMY, amount of RM and RM/TMY, and the shortest milking time. The ewes of breed Lacaune had the lowest RM/TMY as compared with Tsigai and Improved Valachian. Under the same milking, feeding, and housing conditions Lacaune showed better milk production, milkability, and better emptying of the udder during milking than our autochthonous breeds Tsigai and Improved Valachian. However, regarding that milk production of Tsigai and Improved Valachian breeds has attracted increased attention only in the last decades, these differences are not so pronounced. Milkability of the mentioned breeds may also be improved by purebred selection.

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