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EFFECT OF WEANING SYSTEM AND TYPE OF MILK FLOW ON MILK PRODUCTION OF CROSSBRED EWES IMPROVED VALACHIAN AND TSIGAI WITH LACAUNE

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ABSTRACT
Improved Valachian (IV x LC; n = 41) and Tsigai (TS x LC; n = 44) crossbred ewes with Lacaune were used to study the effects of three weaning systems on milk production. Prior to parturition, ewes were assigned to one of the following three treatments for the first 53 day of lactation: 1) ewes weaned from their lambs at 24 h postpartum and afterwards machine milked twice daily (MTD), 2) ewes, beginning 24 h postpartum, kept during the daytime with their lambs and allowed them to suckle for 12 h, nights separated from their lambs for 12 h and machine milked once daily in the morning (MIX), and 3) ewes exclusively suckled by their lambs (ES). After the treatment period, lambs were weaned from MIX and ES ewes, and all three groups were machine milked twice daily. Furthermore, ewes were evaluated according to number of live-born and weaned lambs (with one (n = 35) or with two lambs (n = 50)). The measurements of milk yield and milk flow were performed on 110 ±5 day of lactation by the equipment for graduated electronic recording of the milk level in a jar in one-second intervals. No significant differences were observed in the measured values (total milk yield, machine milk yield, latency time, milking time, machine stripping, milk flow rate, and machine milk yield in 30 and 60 s) among weaning treatments and between ewes with one or two lambs and evaluated breeds too. The highest occurrence of one peak milk flow (milk flow without milk ejection) was found out in MTD ewes (50%) compared to MIX (19%) and ES (17%). In conclusion, the different systems of weaning did not influence the milk yield and milk flow parameters in the mid-lactation.

Keywords: ewe; weaning system; milk flow curve

INTRODUCTION
The mammary glands serve to nourish the new-born young in all mammalian species. However, in dairy animals such as the cows, the sheep, and goats, through genetic selection and breeding advances in milking technology, the mammary glands yield far more milk than a new-born young requirement for normal growth and far greater quantities than the original organ was designed to accommodate **(Marnet and Negrao, 2000; Nickerson, 2011)**. Approximately 25% of the total milk yield of a dairy ewe is produced during the first 30 day of lactation **(Folman, Volcani and Eyal, 1966)**; it is the period when lambs are typically allowed to suckle their dams. There are several lambs' weaning systems applied on dairy sheep farms. A mixed-management weaning system of suckling and milking (MIX, allowing suckling only during the day hours and performing once daily machine milking at mornings) is an option for the farmers to obtain milk, which lamb does not need for normal grow **(McKusick, Thomas and Berger, 2001; Dikmen et al., 2007)**. The main disadvantage of the MIX system is low milk fat content **(McKusick, Thomas and Berger, 2001)**. **McKusick et al. (2002)** observed the inhibition of milk ejection during machine milking (i.e. only cisternal milk was obtained) in ewes with MIX system. Moreover, also the inhibition of transfer milk fat was found out, whereas the transfer of milk protein from alveoli to cistern during the separation of MIX ewes from their lambs **(McKusick et al., 2002)**. Another system of weaning are exclusively milking (MTD; (where lambs are weaned at 24 h postpartum and the ewes are machine milked twice daily) and exclusively suckling (ES; during the first 30 to 60 d ewes are only suckled and no milking is performed) **(Marnet and Negrao, 2000; McKusick, Thomas and Berger, 2001; McKusick, 2002; Dikmen et al., 2007; Thomas et al., 2014)**. In Slovakia, the lambs are traditionally suckled until the weaning age of 40 to 60 days without any milking during this period. Due to intense crossing Tsigai and Improved Valachian with Lacaune, the milk production is growing **(Mačuhová et al., 2008; Tančin et al., 2011; Margetín et al., 2013)**. Therefore, it is necessary to optimize the weaning systems of lamb so that the market milk production is as high as possible while maintaining good milk quality. Whether the milk

ejection reflex during milking occurred can be found out by invasive detection of oxytocin release **(Bruckmaier et al., 1997; Marnet, Negrao and Labussière, 1998; Marnet and Negrao, 2000)** or by non-invasive method of recording milk flow during machine milking **(Bruckmaier et al., 1997; Marnet, Negrao and Labussière, 1998; Mačuhová et al., 2012)**. Milk flow kinetic could be a good indicator of stress load under different milking conditions **(Bruckmaier et al. 1997; Tančin et al. 2015)**.

The aim of the trial was to study the effect of three weaning systems on the milkability of ewes. Possible effect of number of lambs, mil flow type and breed was evaluated too.

Scientific hypothesis

In this study, we hypothesized that ewes, which were not suckled by lamb during the first 53 days of lactation, would have a higher milk production at the middle stage of lactation. The second hypothesis was that ewes with two lambs would have a higher production of milk at the middle stage of lactation than ewes with one lamb. The third hypothesis was that breed did not affect the production parameters. The milk flow type affects the production parameters was the fourth hypothesis.

MATERIAL AND METHODOLOGY

The experiment was conducted at the research farm of NPPC- Research Institute for Animal Production Nitra in Trenčianska Teplá. 85 animals of crossbreds Tsigai (50% TS x LC; n = 40) and Improved Valachian (50% IV x LC, n = 45) with Lacaune were included in the experiment. Prior to parturition, ewes were assigned to one of the following three weaning systems for the first day of lactation: 1) ewes weaned from their lambs at 24 h postpartum and afterwards machine milked twice daily (MTD), 2) ewes, beginning 24 h postpartum, kept during the daytime with their lambs and allowed them to suckle for 12 h, nights separated from their lambs for 12 h and machine milked once daily in the morning (MIX), or 3) ewes exclusively suckled by their lambs (ES). After the treatment period of 38 days, lambs were weaned from MIX and ES ewes, and all three groups were machine milked twice daily. Furthermore, the ewes were evaluated according to live - born and weaned lambs (with one (n = 35) or with two lambs (n = 50)). The measurements of milk yield and milk flow were performed on 110 ±5 day of lactation. The ewes were milked in one-platform milking parlour with 24 stalls. The milking machine was set to provide 160 pulsations per minute in a 50:50 ratio with 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 7:00 and 19:00 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).

Milk flow recording and samples analysis

Milk flow kinetic was recorded using an electronic jar. 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)**. Milk flow rate ($L.min^{-1}$) = (L_n-L_{n-4}) x 15 (where L = recorded milk yield in L, n = time in s, 15 = coefficient to correct milk yield increase in 4 s to milk flow in $L.min^{-1}$). The following milking characteristics were evaluated: total milk yield (L), machine milk yield (L), machine stripping yield (L), milking time (i.e. time from attaching of clusters until the milk flow ceased before stripping; s), milk flow latency (i.e. time from attaching of cluster until start of milk flow 0.006 $L.min^{-1}$; s), peak flow rate ($L.min^{-1}$), machine milk yield in 30 s (l), and machine milk yield in 60 s (L). Milk flow curves were evaluated according to **Marnet, Negrao and Labussière, (1998)** and **Mačuhová et al. (2008)** into 4 milk flow types: one peak (no significant milk flow after 40 s of milking; 1P), 2 peaks (bimodal; 2P), plateau (represents milk flow by ewes with longer duration of steady phase and peak flow rate >0.4 $L.min^{-1}$ without clear differences between peaks 1 and 2; PLI), and plateau low (represents also milk flow curves with steady milk flow during milking but at peak flow rate ≤0.4 $L.min^{-1}$; PLII). In 6 animals, the curve of milk flow was not evaluated due to zero machine milk yield.

Statistic analysis

Data from evening milkings were available for statistical evaluation. The data set consisted of 85 measurements belonging to 85 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 (milk production and milk emission parameters). The experimental measurements were performed during two years. Therefore, factor YEAR included 2 groups of ewes in model: data obtained during 2012 (n = 52) and 2013 (n = 33). Other factors included: FLOW represented 4 groups of ewes divided according to milk flow type (1P, n = 21 ewes; 2P n = 30 ewes; PLI n = 21 ewes; PLII n = 7 ewes), system of WEANING (only milking n = 22 ewes (MTD); milking/suckling n = 38 ewes (MIX); only suckling n = 25 ewes (ES)), number of LAMBs (single n = 35 ewes, twins = 50 ewes), BREED (Tsigai x Lacaune, (CxLC) n = 40 ewes, Improved Valachian x Lacaune, (IVxLC) n = 45 ewes).

$$y_{ijklm} = \mu + YEAR_i + LAMB_j + WEANING_k + FLOW_l + BREED_m + e_{ijklm}$$

where: y_{ijklm} – individual observations of studied parameters: total milk yield (L), machine milk yield (L), machine stripping (L), machine milking time (s), latency time (s), peak flow rate ($L.min^{-1}$), proportion of machine stripping from total milk yield (%), machine milk yield in 30 s and 60 s.

y_{ijklm} = the measurements of the studied parameters, μ = overall mean, $YEAR_i$ = the fixed effects of year (i = 2012, 2013), $LAMB_j$ = fixed effect of lambs (j = 1 to 2), $WEANING_k$ = fixed effect of weaning systems (k = 1 to 3), $FLOW_l$ = fixed effect of milk flow type (l = 1 to 4), $BREED_m$ = fixed effect of breed (m = 1 to 2.), e_{ijklm} = random error, assuming $e_{ijklm} \sim N(0, I\ \sigma_e^2)$.

Fixed effects of the model were estimated using the LSM (Least Squares Means) method. Statistical significance at the 5% level 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 a basic statistics of studied traits. The year of measurement did not affect the evaluated parameters (Table 2, Table 3). The factor breeds did not influence the evaluated parameters except of the machine milk yield. The machine milk yield was significantly higher in IV x LC than TS x LC (0.217 ±0.015 and 0.170 ±0.016 l, resp.; p <0.0260). However, in previous studies **(Mačuhová et al., 2008, 2017)**, there were not found out any significant differences in the machine milk yield. Moreover, high proportion of the machine stripping from total milk yield was recorded in tested crossbreds IV x LC and TS x LC (50 ±3%; and 47 ±3%; resp.). 33% of the animals had a higher proportion machine striping yield from total milk yield than 50% (from 100 to 75% – 11% of animals; from 74.99 to 50% – 22% of animals; from 49.99 to 25% – 43.5% of animals; from 24.99 to 0% – 23.5% of animals).

Table 1 Characteristics of statistical file of studied traits.

Variable	N	Minimum	Maximum	Mean	Std Error
Total milk yield (TMY), L	85	0.055	0.710	0.362	0.015
Machine milk yield (MMY), L	85	0	0.504	0.216	0.013
Machine stripping (MS), L	85	0.03	0.511	0.146	0.010
MS/TMY, %	85	8	100	44	2.480
Milking time, s	85	23	132	66	2.678
Milk flow latency, s	85	8	113	22	2.252
Peak flow rate, $L.min^{-1}$	85	0	2.205	0.732	0.045
MMY in 30 s, L	85	0	0.42	0.102	0.009
MMY in 60 s, L	85	0	0.417	0.179	0.012

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

	Year	Breed	Weaning system	Number of lamb	Milk flow type
Total milk yield (TMY), L	0.7081	0.1885	0.5495	0.4601	0.0007
Machine milk yield (MMY), L	0.5055	0.0260	0.4421	0.6102	<0.0001
Machine stripping (MS), L	0.8827	0.6682	0.5751	0.5982	0.2963
MS/TMY, %	0.3237	0.3277	0.7323	0.9608	<0.0001
Milking time, s	0.3772	0.7915	0.6748	0.9747	<0.0001
Milk flow latency, s	0.376	0.9438	0.6877	0.9116	<0.0001
Peak flow rate, $L.min^{-1}$	0.5913	0.1574	0.4631	0.2428	<0.0001
MMY in 30 s, L	0.9189	0.0812	0.8635	0.9927	<0.0001
MMY in 60 s, L	0.6053	0.0514	0.7991	0.8099	<0.0001

Table 3 Parameters of milkability of different year evaluation breed, weaning systems and number of lambs.

	Year		Breed		Weaning system			Number of lambs	
	2012	2013	TS x LC	IV x LC	MTD	MIX	ES	One	Two
N	52	33	40	45	22	38	25	35	50
Total milk yield (TMY), L	0.353 ±0.019	0.341 ±0.023	0.328 ±0.022	0.366 ±0.020	0.330 ±0.030	0.341 ±0.021	0.370 ±0.027	0.336 ±0.023	0.353 ±0.019
Machine milk yield (MMY), L	0.201 ±0.014	0.186 ±0.017	0.170 ±0.016[a]	0.217 ±0.015[b]	0.172 ±0.021	0.201 ±0.015	0.207 ±0.019	0.188 ±0.017	0.199 ±0.013
Machine stripping (MS), L	0.152 ±0.014	0.155 ±0.017	0.158 ±0.015	0.149 ±0.04	0.157 ±0.021	0.140 ±0.015	0.163 ±0.019	0.148 ±0.016	0.159 ±0.013
MS/TMY, %	46 ±3	50 ±3	67±3	47 ±3	50 ±4	46 ±3	49 ±3	48 ±3	48 ±2
Milking time, s	70 ±3	663	50±3	68 ±3	66 ±4	67 ±3	70 ±4	68 ±3	68 ±3
Milk flow latency, s	24 ±2	28 ±3	26±3	26 ±3	27 ±4	24 ±3	27 ±3	26 ±2	27 ±3
Peak flow rate, $L.min^{-1}$	0.659 ±0.049	0.618 ±0.059	0.587 ±0.055	0.690 ±0.051	0.573 ±0.075	0.688 ±0.053	0.654 ±0.067	0.600 ±0.058	0.682 ±0.047
MY30, L	0.088 ±0.010	0.089 ±0.012	0.076 ±0.011	0.101 ±0.010	0.083 ±0.015	0.089 ±0.011	0.094 ±0.013	0.088 ±0.012	0.089 ±0.009
MY60, L	0.165 ±0.018	0.154 ±0.015	0.141 ±0.014	0.178 ±0.013	0.150 ±0.019	0.166 ±0.014	0.163 ±0.018	0.157 ±0.015	0.162 ±0.012

Table 4 The effect of milk flow types on milkability of ewes

	Milk flow type			
	Bimodal	One peak	Plateau	Plateau low
N	30	21	21	7
Total milk yield (TMY), l	0.395 ±0.026[a]	0.316 ±0.029[ab]	0.424 ±0.030[a]	0.252 ±0.035[b]
Machine milk yield (MMY), l	0.266 ±0.019[a]	0.172 ±0.021[b]	0.271 ±0.021[a]	0.064 ±0.052[b]
Machine stripping (MS), l	0.129 ±0.019	0.144 ±0.021	0.153 ±0.021	0.188 ±0.025
MS/TMY, %	34 ±3[a]	46 ±4[a]	36 ±4[a]	78 ±5[b]
Milking time, s	72 ±4[a]	39 ±4[b]	65 ±4[a]	94 ±5[c]
Milk flow latency, s	14 ±3[a]	17 ±4[a]	20 ±4[a]	53 ±4[b]
Peak flow rate, $L.min^{-1}$	0.881 ±0.066	0.878 ±0.073	0.639 ±0.075	0.157 ±0.089
MMY in 30 s, l	0.141 ±0.013[a]	0.129 ±0.014[ab]	0.078 ±0.015[b]	0.006 ±0.018[c]
MMY in 60 s, l	0.220 ±0.017[a]	0.155 ±0.019[a]	0.229 ±0.019[b]	0.034 ±0.023[c]

Note: [a,b,c] The means in the same line without same letter were significantly different at $p \leq 0.05$.

Table 5 Frequency of milk flow types according to weaning system.

Weaning system		Type of milk flow				Total
		Bimodal	One peak	Plateau	Plateau low	
MTD	count	4	10	5	1	20
	% within group	20	50	25	5	
MIX	count	15	7	9	5	36
	% within group	41.67	19.44	25	13.89	
ES	count	11	4	7	1	23
	% within group	47.83	17.39	30.43	4.35	
Total	count	30	21	21	7	79
	%	37.97	26.58	26.58	8.86	

So high values of proportion of the machine stripping from total milk yield have not been detected in these crossbreds so far **(Mačuhová et al., 2008, 2017; Margetín et al., 2013)**. High machine stripping could be due to improper teat position of ewes **(Marnet et al., 1998)**, but this parameter was not evaluated in this study. The impact of the weaning system and the number of lambs on the performance parameters observed is shown in the Table 3. Unlike previous studies **(Dikmen et al., 2007; Thomas et al., 2014)**, it was not found any significant differences in the machine milk yield between different weaning systems. No machine milk yield was observed in 6 animals. Therefore, only 79 milk flow curves were evaluated. All four types of milk flow curves could be observed in the present study as in previous studies testing these crossbreds **(Mačuhová et al., 2012; Mačuhová et al., 2017; Tančin et al., 2011)**. The number and the frequency of occurrence of particular milk flow types are shown in Table 4. The highest occurrence of one peak milk flow was found out in MTD ewes (50%) compared to MIX (19%) and ES (17%). One peak milk flow curves are supposed to represent milk flow without alveolar milk ejection when only cisternal milk fraction is removed in response to machine milking **(Mayer et al., 1989; Bruckmaier et al., 1997)**. On the other side, the proportion of bimodal milk flow in MTD ewes was lower than in ewes of other systems. The milk flow curves with two peaks (bimodal) 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 bimodal 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 at the time of milk ejection, the cistern fraction has not yet been completely removed from the udder when alveolar fraction descends into cistern for removal **(Marnet et al., 1998)**. Even the second peak is not observed, it is supposed that milk ejection occurs in ewes with this milk flow **(Marnet et al., 1998; Mačuhová et al., 2012; Tančin et al., 2011)**.

The proportion of this milk flow type was quiet similar in all weaning system. Ewes with bimodal and plateau milk flows had the highest machine milk yield (0.266 ±0.019, 0.271 ±0.021, 0.172 ±0.021, 0.064 ±0.052 L in bimodal, plateau, one peak, plateau low; p <0.0001; Table 5). According to **Labussiere (1988)** when ewes are not exclusively machine milked immediately post-partum, the longer they remain in contact with their lambs during the suckling period, the more difficult it is for them to adapt to exclusive machine milking following weaning. And whereas ewes with bimodal and plateau milk flow belong to well-adapted to machine milking **(Marnet et al. 2001)**, it is surprising that, ES ewes had the highest incidence of bimodal and plateau milk flows (Table 5). On the other hand, the release of oxytocin takes longer time during suckling compared to milking **(Marnet and Negrao, 2000)** what can support milk production, and probably the weaning took place at a time when there was no such great the mother-young bond. So, ewes were very well prepared for machine milking. When the milking machine parameters are optimized, and the ewes had time to adapt to the milking routine, oxytocin release patterns are similar during milking as during suckling **(Marnet and Negrao, 2000)**. The fourth, but least occurring milk flow type, was plateau low (9%). This type of milk flow was associated with the longest milking time (94 ± 5 in plateu low, 39 ±4 in one peak, 65 ±4 in plateau and 72 ±4 s in bimodal; p <0.0001, Table 5). According to **Bruckmaier et al. (1997)** this type of milk flow was obviously associated with extremely weak or totally absent oxytocin release during milking. This shape of milk flow in our study was probably due to uneven milk flow distribution in two udder halves, and it cannot be excluded that the milk ejection occurred also in ewes with this type of milk flow.

CONCLUSION

In conclusion, the application of different weaning systems (MTD, MIX, and ES) and the number of lambs had not effect on total milk yield, machine milk yield, machine stripping, and milking time in the middle of lactation. The relatively high ocurrence of bimodal and plateau milk flow curves was observed in ES system. Both milk flow types characterize better-adapted animals to machine milking, because it is assumed that the ewes with these milk flow types achieve milk ejection during milking.

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