

Estimation of udder cistern size in dairy ewes by ultrasonography

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Pavol Makovický^{1}, Milan Margetín², Michal Milerski³*¹J. Selye University, Pedagogical Faculty, Department of Biology, Hradná 21, 94501 Komárno, Slovak Republic²National Agricultural and Food Centre, Research Institute for Animal Production Nitra, Hlohovecká 2, 95141 Lužianky, Slovak Republic³Institute of Animal Science, Přátelství 815, Prague-Uhřetěves 10401, Czech Republic

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Abstract

We studied the size of mammary cistern in ewes of 9 genotypes (purebred Improved Valachian (IV), purebred Tsigai (T), purebred Lacaune (LC) and their crosses with genetic proportion of specialized dairy breeds Lacaune and East Friesian (EF) - (25 %, 50 % and 75 %) were evaluated. Data were evaluated using REML methodology and MIXED procedure (SAS/STAT). The effect of genotype showed the highest influence ($P < 0.001$) on the length and area of the left and right udder cisterns measurements. In purebred IV ewes, the average areas of the left and right udder cisterns sizes were obtained by using the side method were ($1519.39 \pm 77.212 \text{ mm}^2$ and $1558.45 \pm 74.480 \text{ mm}^2$). In purebred T ewes, the average areas of the left and right udder cisterns were ($1438.70 \pm 70.43 \text{ mm}^2$ and $1418.68 \pm 67.952 \text{ mm}^2$). These were significantly smaller than in purebred LC ($2694.44 \pm 71.95 \text{ mm}^2$ and $2693.48 \pm 69.340 \text{ mm}^2$). The udder cistern areas were significantly higher in crosses with 25 %, 50 % and 75 % genetic proportion of specialized dairy breeds LC and EF, than in purebred IV and T ewes. The analyses showed that crossbreeding of IV with LC and EF and T with LC and EF considerably increases ewe's cistern size.

Key words: ewes, mammary gland, udder cistern, ultrasonography

Introduction

Many scientific papers describe that milk production is influenced by mammary gland size and cistern dimension (Casu and Labussière, 1972; Labussière, 1988; Nudda et al., 2000). According to Wilde et al. (1996) and Stelwagen (2001), secreted milk in dairy ruminants can be divided extracellularly within two anatomical interconnected udder compartments. Each gland has its own secretory tissue and cisternal cavities, and each gland is drained by a separate teat (Bruckmaier and Blum, 1998). The cisternal milk fraction located in large mammary ducts and cisternal cavities is immediately

available for milking. The alveolar milk fraction is located in small milk ducts and alveoli is fixed by capillary forces and requires the milk ejection in order to be forcefully expelled into the cisternal cavities to be available for milking. Cisternal milk fraction in most of the dairy ewe breeds is usually larger than 50 % (Bruckmaier et al., 1994a; Caja et al., 1999; Marnet and McKusick, 2001). These larger cisterns play an important role in milk collection and storage and have a significant influence on milk ejection during milking. Milk in the udder of dairy cows is usually stored in the alveolar compartment (80 %), the cisternal milk fraction after an interval of 12 h from previous milking is 20 %

*Corresponding author/Dopisni autor: makovicky.pavol@gmail.com

(Pfeilsticker et al., 1996; Bruckmaier et al., 1994b; Bruckmaier, 2001). Cistern size is an important factor in determining the milkability (Labussière, 1988; Marnet and McKusick, 2001) and the adequate interval between milkings (Knight and Dewhurst, 1994; Salama et al., 2003). Animals that store a large proportion of milk in the gland cistern produce more milk, and are more able to tolerate extended milking intervals (Salama et al., 2004; Castillo et al., 2008b; Castillo et al., 2009). Moreover, cistern size plays an important role in controlling milk secretion because when the cistern reaches its full capacity, physical pressure and FIL concentration within alveoli are increased (Wilde et al., 1996).

The internal structure of the mammary gland can be studied by means of ultrasonography. Ultrasonography is a fast, accurate noninvasive means for investigation of mammary gland structures in dairy ewes (Hiepler et al., 2009; Olechnowicz and Jaśkowski, 2009; Alejandro et al., 2014b,c,d; Petridis et al., 2014), cows (Zwertvaegher et al., 2011; Fasulkov et al., 2014a), goats (Díaz et al., 2013; Alejandro et al., 2014a,e; Fasulkov et al., 2013; 2014b,c; Dar et al., 2014), mares (Ennen et al., 2011; Abshenas et al., 2014), camels (Abshenas et al., 2007) and buffaloes (Constante and Acorda, 2012; Kotb et al., 2014). The principle structures of the mammary gland, as cistern area and teat cistern, can easily be determined by the position and frequency of the transducer used for its exploration (Rovai et al., 2004). Nudda et al. (2000) proposed the volume of the mammary gland cistern as a selection objective to improve milk production and milking ability of dairy ewes. Makovický et al. (2014) made a recommendation for using sonographic measurements as a good indicator of the cistern size in dairy ewes. Mammary ultrasonography is therefore a very interesting method for studying ewe's mammary gland during lactation.

The goal of this research was to investigate the udder cistern size using ultrasonography technique in purebred Improved Valachian (IV), Tsigai (T), Lacaune (LC) ewes and their crosses with specialized dairy breeds Lacaune (LC) and East Friesian (EF). The analyses of non-genetic factors that are expected to influence the udder cistern size were also done.

Materials and methods

Purebred Improved Valachian (IV), purebred Tsigai (T) and purebred Lacaune (LC) ewes, and IV and T crosses with 25 %, 50 % and 75 % of genetically created breeds of specialized dairy breeds (SDB) Lacaune and East Friesian ewes (EF) were included in this seven year long experiment as follows: Improved Valachian (IV), $n=219$; IV \times SDB 25 %, $n=63$; IV \times SDB 50 %, $n=84$; IV \times SDB 75 %, $n=80$; Tsigai (T), $n=271$; T \times SDB 25 %, $n=17$; T \times SDB 50 %, $n=157$; T \times SDB 75 %, $n=46$; Lacaune (LC), $n=261$. In total, we compared the size of the udder cisterns in ewes of 9 genotypes (3 purebreds and 6 groups of crossbreds). About 378 ewes were diagnosed repeatedly using the method "from side" a total of 1198 measurements were performed. Ultrasound images of the left and right udder cisterns were recorded by portable ultrasonography with a 3.5 MHz convex sector probe following the method described in the works of Nudda et al. (2000). The procedure uses contact gel and places the probe directly against the upper part of the median suspensory ligament in the inguinal abdominal fold. The operator tried to scan equal axis of the opposite side of the udder in order to obtain an image with the largest cistern size. The images were taken once for each half udder, 12 hours after the last milking. For each scan a sonographic image was made. On the sonographic images the length of the left (LLC) and right (LRC) cisterns and the width of the left (WLC) and right (WRC) cisterns (in mm) were measured from the scan cross section. Specific numbers of observations in monitored indicators depending on the genotype, parity and lactation stage are shown in Table 3 and 4.

We monitor and evaluate the following rates of udder cisterns (a total of 7 indicators):

- Length of left and right cistern (LLC, respectively LRC) - mm.
- Width of left and right cistern (WLC, respectively WRC) - mm.
- Area of left and right cistern (ALC, respectively ARC) - mm².
- Sums of both cross-section areas (SLRC) - mm².

Data were processed by restricted maximum likelihood (REML) methodology using a MIXED procedure from the SAS statistical package v.9.2, 2002-2008. The following statistical model with fixed and random effects was applied:

$$y_{ijklm} = \mu + Y_i + LS_j + GEN_k + P_l + a_m + a * DIM_{ijklm} + e_{ijklm}$$

where:

y_{ijklm} = is the vector of observations for the investigated characteristics (see above for details); Y_i = year (fixed effect with 4 to 7 levels); LS_j = lactation stage, fixed effect with 4 levels (from 40th to 99th lactation day, from 100th to 129th lactation day, from 130th to 159th lactation day and from 160th to 210th lactation day); GEN_k = genotype (breed group; fixed effect with 9 levels; see above for detail characterization); P_l = parity (fixed effect with 3 levels; first, second, third and further parity); a_m = animal (random effect); DIM_{ijklm} = days in milk (covariate; 40 to 210 days in milk); e_{ijklm} = is the random error.

The differences were statistically significant at $P < 0.05$, or less.

Results and discussion

Sample statistics of parameters characterizing the size of the udder cisterns of ewes detected were from the side method for Improved Valachian, Tsiganai, Lacaune and their crosses with genetic proportion of Lacaune and East Friesian- 25 %, 50 % and 75 % are shown in Table 1. Animals were recorded repeatedly within and between lactations; therefore 1198 measurements were collected in total. The area of left cistern (ALC) and the area of right cistern (ARC) ranged from 133 mm² to 7560 mm², and from 10 mm² to 5799 mm², respectively. The sums of both cross-section areas (SLRC) ranged from 390 mm² up to 12900 mm² (mean = 3904.07 mm², $v = 44.78$ %). The average area of the left (ALC) and right cisterns (ARC) was 1933.35, and 1970.72 mm², respectively.

Results showed that genotype, parity and year (Table 2) had a significant effect ($P < 0.001$) on all surveyed indicators characterizing the size of ewes udder cisterns.

As shown in Table 3, the largest average size of the udder cisterns was found for all indicators in purebred LC ewes. Conversely, the smallest udder cistern was found practically in all indicators for purebred T ewes. The largest average of the left cistern's area (ALC) was found in purebreds LC ewes (2694.44 ± 71.95 mm²), and conversely, the smallest average of the left cistern's area (ALC) was found in purebred T ewes (1438.70 ± 70.43 mm²) also. The highest value of ARC was found in LC ewes (2693.48 ± 69.340 mm²) and conversely, the

Table 1. Basic statistical characteristics of the variation of selected parameters characterizing the udder cistern size of ewes

Measurement	N*1	Mean	SD	CV	min.	max.
Length of left cistern	1023	69.14	16.31	23.59	17	133
Width of left cistern	1023	36.39	11.19	30.75	5	104
Area of left cistern	1198	1933.35	929.15	48.06	133	7560
Length of right cistern	1023	69.94	15.86	22.68	20	118
Width of right cistern	1023	37.68	10.74	28.50	10	84
Area of right cistern	1198	1970.72	927.95	47.09	10	5799
Sums of both cross-section areas	1198	3904.07	1748.46	44.78	390	12900

*1n - number of sets of measurements

SD: standard deviation; CV: coefficient of variability

Table 2. Covariance analysis of traits describing udder cistern size of ewes diagnosed by the method “from side”

Source of variation	Measurement														
	LLC		WLC		ALC		LRC		WRC		ARC		SLRC		
	F value	P>F	F value	P>F	F value	P>F	F value	P>F	F value	P>F	F value	P>F	F value	P>F	
(df)															
Year	4	11.24	<0.0001	12.29	<0.0001	2.77	0.0113	6.37	<0.0001	3.78	0.0048	0.98	0.4373	1.58	0.1502
Lactation stage	3	0.88	0.4534	3.27	0.0208	4.02	0.0075	1.71	0.1634	0.71	0.5486	1.54	0.2026	3.47	0.0158
Genotype	8	19.37	<0.0001	23.43	<0.0001	25.09	<0.0001	19.33	<0.0001	26.12	<0.0001	27.00	<0.0001	29.25	<0.0001
Parity	2	10.47	<0.0001	10.78	<0.0001	11.57	<0.0001	15.35	<0.0001	20.44	<0.0001	22.83	<0.0001	20.03	<0.0001
Days in milk	1	12.31	0.0005	16.28	<0.0001	27.46	<0.0001	10.98	0.0010	11.17	0.0009	16.70	<0.0001	27.59	<0.0001

LLC1 = length of left cistern diagnosed by the method “from side”
 WLC1 = width of left cistern
 ALC1 = area of left cistern
 LRC1 = length of right cistern
 WRC1 = width of right cistern
 ARC1 = area of right cistern
 SLRC1 = sums of both cross-section areas

lowest mean value was found in purebred ewes T (1418.68±67.952 mm²). These results also show that the size of the udder cisterns was greater in crosses formed on the basis of specialized dairy breeds LC and EF (25 %, 50 %, 75 % LC and EF) compared with purebred ewes IV and T.

According to the Table 4, parity had a statistically significant influence (P<0.001) for all observed rates of the left and right udder cisterns. Although the differences between ewes in 1st, 2nd, and 3rd lactation were not in all indicators statistically significant, but in all indicators it was observed that tendency of increasing of the udder cisterns, depending on age (stage of lactation). Increasing of udder cisterns depending on parity were also reported by Marie et al. (1999). Regarding changes in the size of the udder cisterns during lactation, our results suggest that the basic level of the left and right udder cistern (length, width and area) of both cisterns increase during the lactation.

Scientific reports on the application of ultrasound devices in the diagnostics of the udder cistern size in dairy ewes have been scarce. In available scientific literature, cisternal area are detected in Ripollesa sheep (Caja et al., 1999; 5.6 cm²), East Friesian (Bruckmaier and Blum, 1992; Bruckmaier et al., 1997; from 19 to 40 cm²), Lacaune (Bruckmaier et al., 1997; 33 cm²; Rovai et al., 2008, 24 cm²; Castillo et al., 2008a, 31.36±1.00 cm²), Manchega (Castillo et al., 2008a, 15.01±1.00 cm²), Sarda (Nudda et al., 2000, 19 cm²). In goats, Bruckmaier and Blum (1992) reported the size of gland cistern in Saanen breed (15.9 cm²), Wójtowski et al. (2006) in Polish White Improved breed (35 to 38.91 cm²), Szymanowska et al. (2010) in Saanen and White Improved breed (56.48 cm² and 46.4 cm²) and Melo et al. (2012) in Canindé breed (5.23 to 11 cm²).

Table 3. Effect of genotype on traits describing udder cistern size of ewes diagnosed by the method "from side"

Source of variation	Measurement																	
	LLC (mm)*1	WLC (mm)*1	ALC (mm)*2	LRC (mm)*1	WRC (mm)*1	ARC (mm)*2	SLRC (mm)*2	LSM±SE	LSM±SE	LSM±SE								
	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE								
	Genotype																	
IV	(100)	189 ¹	219 ²	62.12	1.342	31.84	0.994	1519.39	77.212	63.43	1.338	33.02	0.944	1558.45	74.480	3072.64	142.94	
IV×SDB (25 %)	(125)	59	63	72.99	2.388	36.68	1.783	1999.24	139.68	72.70	2.384	38.50	1.693	2068.76	135.08	4088.12	258.08	
IV×SDB (50 %)	(150)	64	84	71.04	2.234	38.71	1.656	2115.82	116.58	73.36	2.228	40.91	1.573	2226.69	112.85	4333.72	215.30	
IV×SDB (75 %)	(175)	71	80	71.30	2.141	38.63	1.581	2120.08	120.64	70.58	2.134	39.77	1.503	2008.45	116.61	4123.34	223.05	
T	(200)	245	271	60.46	1.178	30.66	0.871	1438.70	70.43	61.04	1.174	31.36	0.828	1418.68	67.952	2858.67	130.38	
T×SDB (25 %)	(225)	11	17	69.75	5.325	36.39	3.915	1861.78	279.35	75.42	5.302	41.62	3.720	2237.89	268.80	4088.61	517.90	
T×SDB (50 %)	(250)	125	157	72.26	1.608	36.69	1.189	1974.72	88.97	72.06	1.604	37.19	1.130	1972.88	85.866	3944.97	164.64	
T×SDB (75 %)	(275)	36	46	72.90	3.104	40.08	2.336	2235.47	176.31	74.35	3.104	39.58	2.217	2197.36	169.35	4434.88	327.25	
LC	(300)	223	261	79.14	1.237	46.42	0.921	2694.44	71.95	80.11	1.234	47.24	0.875	2693.48	69.340	5396.70	133.29	
				100:125,150,175,250:300+++;		100:150,175,300+++;		100:150,175,250:275+++;		100:125,150,250:300+++;		100:125,150,175,250:275+++;		100:150,175,300+++;		125,150,175,250:275,300+++;		125,150,175,250:275,300+++;
				100:125+;		100:125+;		100:125+;		100:125+;		100:225+;		100:225+;		100:225+;		100:225+;
				125:300+++;		125:300+++;		125:200,300+++;		125:200,300+++;		125:200,300+++;		125:200,300+++;		125:200,300+++;		125:200,300+++;
				125:200+++;		150:200,300+++;		150:200,300+++;		125:300+++;		150:200,300+++;		150:200,300+++;		150:200,300+++;		150:200,300+++;
				150:200+++;		150:200,300+++;		175:200,300+++;		150:200+++;		175:200,300+++;		175:200,300+++;		175:200,300+++;		175:200,300+++;
				175:200+++;		200:250,275,300+++;		200:250,275,300+++;		200:250,275,300+++;		200:225+;		200:225+;		200:225+;		200:225+;
				175:300+++;		225:300+++;		225:300+++;		175:300+++;		200:225+;		200:225+;		200:225+;		200:225+;
				200:250,275,300+++;		250:300+++;		250:300+++;		200:250,275,300+++;		250:300+++;		250:300+++;		250:300+++;		250:300+++;
				250:300+++;		275:300+++;		275:300+++;		200:250,275,300+++;		275:300+++;		275:300+++;		275:300+++;		275:300+++;

+++ p < 0.001; ++ p < 0.01; + p < 0.05; ns - non significant

*1, *2 - Data represent the number of measurements, depending on the indicator

(100) - Improved Valachian,

(125) - crossbreds of Improved Valachian breed with 25 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(150) - crossbreds of Improved Valachian breed with 50 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(175) - crossbreds of Improved Valachian breed with 75 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(200) - Tsigai

(225) - crossbreds of Tsigai breed with 25 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(250) - crossbreds of Tsigai breed with 50 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(275) - crossbreds of Tsigai breed with 75 % genetic portion of specialized dairy breeds Lacaune and East Friesian

(300) - Lacaune

Table 4. Effect of parity and stage of lactation on traits describing udder cistern size of ewes diagnosed by the method “from side”

Source of variation	Measurement																
	LLC (mm)*1		WLC (mm)*1		ALC (mm²)*2		LRC (mm)*1		WRC (mm)*1		ARC (mm²)		SLRC (mm²)*2				
	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE			
Parity																	
1 st order of lactation	(1)	342 ¹	399 ²	67.46	1.028	35.48	0.734	1842.19	55.972	68.00	1.018	36.14	0.698	1820.24	54.498	3660.90	103.01
2 nd order of lactation	(2)	325	371	70.28	1.114	37.34	0.778	2004.63	55.879	71.92	1.099	39.35	0.741	2083.93	56.603	4088.03	106.21
3 rd and further order of lactation	(3)	356	428	72.92	1.163	39.21	0.824	2139.72	62.953	74.43	1.151	40.90	0.785	2223.38	61.421	4364.95	115.67
Level of significance				1:2+; 1:3+++; 2:3+;		1:2+++; 1:3++++; 2:3+;		1:3+++; 1:2+++;		1:2,3+++; 2:3+;		1:2,3+++; 2:3+;		1:2,3+++; 2:3+;		1:2,3++++; 2:3+++;	
Lactation stage																	
40 th - 99 th day	(1)	249	258	67.64	2.245	36.45	1.414	1891.54	112.20	69.14	2.179	37.91	1.354	1988.69	112.59	3878.83	202.37
100 th - 129 th day	(2)	319	367	68.91	1.188	36.44	0.807	1913.52	63.354	69.42	1.167	38.36	0.770	1981.36	62.454	3893.06	115.67
130 th - 159 th day	(3)	270	330	71.02	1.464	36.93	0.962	1968.23	68.805	73.01	1.431	38.80	0.919	2030.03	68.087	3994.90	125.30
160 th - 210 th day	(4)	185	243	73.31	2.351	39.55	1.474	2208.77	110.79	74.22	2.281	40.12	1.412	2169.98	111.21	4385.06	199.80
Level of significance				ns		3:4+++;		2:4+; 3:4+++;		2:3+;		ns		ns		2:4+; 3:4+++;	

+++ p<0.001; ++ p<0.01; + p<0.05; ns - non significant

*1, *2 - Data represent the number of measurements, depending on the indicator

Conclusion

In conclusion, based on our results, keeping line with trends in all countries with advanced sheep husbandry, we propose to use the ultrasonographic scanning technique for determination of ewes udder cisterns size, and then to use the obtained results in the selection of sheep with large cisterns, where it is a real potential for high milk production and milking ability. In practice, we consider it sufficient to make the diagnosis only in the ewes of first lactation and scan only the left cistern area (ALC) using the method "from side".

Ultrazvučna procjena veličine cisterne vimena u mliječnim ovaca

Sažetak

Istražena je veličina cisterne mliječne žlijezde ovaca kod 9 genotipova (čistokrvne Improved Valachian (IV), čistokrvne cigaje (T), čistokrvne Lacaune (LC) i njihovih križanaca s genetskim udjelom specijaliziranih mliječnih pasmina Lacaune i istočnofrizijske (EF) - (25 %, 50 % i 75 %). Podatci su analizirani korištenjem metodologije REML i MIXED procedure (SAS/STAT). Genotip je značajno utjecao ($p < 0.001$) na veličinu cisterni vimena. U čistokrvnih IV ovaca prosječna područja veličina lijevih i desnih cisterni vimena dobivena su pomoću bočne metode ($1519,39 \pm 77,212 \text{ mm}^2$ i $1558,45 \pm 74,480 \text{ mm}^2$). U čistokrvnih T ovaca prosječna područja lijeve i desne cisterne vimena bila su $1438,70 \pm 70,43 \text{ mm}^2$ i $1418,68 \pm 67,952 \text{ mm}^2$. Te su vrijednosti bile značajno manje nego u čistokrvnih LC ($2694,44 \pm 71,95 \text{ mm}^2$ i $2693,48 \pm 69,340 \text{ mm}^2$). Područja cisterne vimena bila su značajno veća u križanaca s 25 %, 50 % i 75 % genetskog udjela specijaliziranih mliječnih pasmina LC i EF, nego u čistokrvnih IV i T ovaca. Analize su pokazale da križanje IV s LC i EF i T s LC i EF znatno povećava veličinu ovčje cisterne vimena.

Ključne riječi: ovce, mliječna žlijezda, cisterna vimena, ultrazvuk

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