

**USE OF ULTRASONOGRAPHY
FOR DETERMINATION OF CISTERN SIZE
IN DIFFERENT GENOTYPES OF DAIRY SHEEP***

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Abstract

A total of 263 Improved Valachian (IV), Tsigai (*T*), Lacaune (LC) and crossbred lactating ewes were used to study milk cistern anatomy in dairy sheep bred in Slovakia, to compare two methods of ultrasound udder scanning and to evaluate relations between cistern size and milkability. Milkability traits recording, external measurements, linear assessments and ultrasonic scanning of sheep udders were done. Sums of both cistern cross-section areas were computed on the basis of two methods of udder ultrasonography – from the side of udder (SCA) and from below in a water bath (BCA). Between BCA and milk yield was found out slightly lower correlation ($r = 0.48$) than between SCA and milk yield ($r = 0.53$).

Introduction

Sheep milking has long and plentiful tradition in Slovakia. Nevertheless machine milking have been introduced more widely into dairy sheep husbandry only in the last two decades. The introduction of machine mil-

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king evokes the requirement to pay more attention on morphological and functional characteristics of sheep udders. One of the most interesting udder morphological characteristics from the machine milkability point of view is the size of glandular cistern (*Sinus lactiferus pars glandularis*), as the “cisternal milk” is available for milking before the oxytocine ejection, the large-cisterned animals being in general more efficient producers of milk and more tolerant to long milking intervals (WILDE et al. 1996).

There are large differences in the proportion of total milk stored within the cistern among ruminant dairy species. Specialized dairy cows store less than 30% of the total milk yield volume in the mammary gland cisterns (AYADI et al. 2003). Percentages of cisternal milk in sheep vary from 25% to 75% according to the breed but they are greater than 50% in most dairy sheep breeds (CAJA et al. 1999, ROVAI et al. 2000). *In vivo* scanning of the udder internal structures could be done by ultrasonography. Cisterns filled by milk are detectable very well as anechogenic structures in ultrasound scans. Different methods of sheep udder ultrasonography were proposed by BRUCKMAIER and BLUM (1992) and RUBERTE et al. (1994). The methods were used for cisternal measurements by BRUCKMAIER et al. (1997), CAJA et al. (1999), ROVAI et al. (2000), NUDDA et al. (2000), MAKOVICKÝ et al. 2015.

This investigation was aimed on the study of milk cistern anatomy in dairy sheep breeds and crossbreeds in Slovakia, the comparison of two methods of ultrasound udder scanning and the evaluation of relations between cistern size and milkability traits in dairy sheep.

Materials and Methods

Investigations were performed in the experimental flock of Research Institute of Animal Production in Nitra. Totally 263 lactating ewes of Tsigai (*T*; $n = 47$), Improved Valachian (*IV*; $n = 61$), Lacaune (*LC*; $n = 52$) and various crossbreeds between them ($n = 103$) were used. Six control days were organized in different stages of lactation and some animals were investigated repeatedly, so totally 590 sets of measurements were done. Control milkings were done in 1 x 24 side by side milking parlor, using vacuum level 40 kPa and pulsation rate 120 pulses/min. Ewes were milked without udder prestimulation and milk flow was recorded in 10 s intervals. After 60 s of machine milking and if no milk flow was detected for 20 s the machine milking with manual udder massage was performed. Then milk emission curves were constructed and total milk yield, machine milk yield, machine stripping milk yield and percentage of stripping milk

from total milk yield were computed. Subsequently 12 hours after control milking the external measurements (6 traits: using tape – udder length; using ruler – udder width, udder height, cistern height, teat length; using protractor – teat angle), linear assessments (7 traits: udder depth, cistern height, teats placement, teat length, udder attachment, udder cleft and overall subjective assessment of udder shape from the point of view of machine milking) and ultrasonic scanning of udders were done. Ultrasonography was carried out from the side of udder according to methodology of RUBERTE et al. (1994) and from below in a water bath as described by BRUCKMAIER and BLUM (1992). Scans were made with a digital ultrasound scanner Medison SonoVet2000 using a linear probe L2-5/170 CD. Acoustic coupling agent (Kerolan, Aveflor Kopidlno) was used to attach the probe to the skin in a case of scanning from the udder side. Images were recorded on memory card and later processed by the use of computer program Zodop32. Sums of cross section areas of both cisterns measured from the side (SCA) and from below (BCA) were measured. The statistical analysis of variance in the data set was performed using the GLM procedure of SAS. The model equation used for the data adjustment considered the effects of the day of control milking (fixed effect – 6 levels), breed or cross-breed combination fixed effect – 7 levels), parity (fixed effect – 3 levels), interaction between breed and parity and covariables days in milk (DIM) and square of DIM (DIM²). The CORR procedure SAS was used for the computing of partial correlation coefficients on residuals after the data adjustment by the above mentioned model equation.

Results and Discussion

In Table 1 the F-values of systematic effects obtained by analysis of variance of cistern areas are presented. Both measurements, from side (SCA) and from below (BCA), were significantly affected by all effects considered in model equation, anyway the effect of breed or crossbred combination was the strongest in both cases. Determination coefficients of used model equations of variance analysis were $R_2 = 0.531$ for BCA and $R_2 = 0.512$ for SCA. The least squares means and standard errors for the effect of breed or crossbred combination are listed in Table 2. There were statistically significant differences in cistern size and milk yield between purebred Improved Valachians (IV), Tsigai (*T*) and Lacaune (LC). LC imported to Slovakia in order to improve milk production had the highest cistern size and the highest milk yield and also hybrids between IV or *T* and LC had higher cisterns and milk yield than purebred animals of native

breeds. On the other hand purebred LC had significantly the highest stripping milk yield. Average percentage of stripping milk from total milk yield was 40.9% in LC while in other genotypes varied from 22.8% to 28.2%.

Table 1
Analysis of variance of sums of cistern cross-section areas measured from side (SCA) and from below (BCA)

Parameters	BCA	–	SCA	–
Effects	F-value	$P > F$	F-value	$P > F$
Control milking	5.02	0.0019	4.98	0.0020
Breed or crossbred combination	67.25	<0.0001	53.49	<0.0001
Parity	7.08	0.0009	3.91	0.0205
Breed-parity interaction	2.15	0.0086	2.10	0.0104
Days in milk (DIM)	5.71	0.0172	19.81	<0.0001
DIM ²	5.90	0.0154	15.85	<0.0001
–	$R_2 = 0.531$	–	$R_2 = 0.512$	–

DIM² – square of days in milk – in course to account of non-linearity of the relation between observed trait and day of lactation

Table 2
Effect of breed or crossbred combination on cistern size and milkability traits in sheep (LS-means±SE)

Breed	BCA cm ²	SCA cm ²	Total milk yield ml/milking	Stripping yield ml/milking
Improved Valachian (IV)	30.52±1.27 <i>b</i>	38.35±1.29 <i>b</i>	412.3±13.8 <i>b</i>	105.5±8.4 <i>a</i>
Tsigai (<i>T</i>)	25.13±1.07 <i>c</i>	30.08±1.09 <i>c</i>	293.1±11.5 <i>d</i>	79.3±6.8 <i>b</i>
Lacaune (LC)	58.55±1.19 <i>d</i>	60.98±1.21 <i>d</i>	544.9±12.8 <i>a</i>	222.9±7.5 <i>d</i>
IV x LC	43.04±2.42 <i>a</i>	46.80±2.46 <i>a</i>	510.2±26.1 <i>a</i>	134.3±15.3 <i>a</i>
(IV x LC) x LC	38.22±1.61 <i>a</i>	44.43±1.63 <i>a</i>	502.8±17.3 <i>a</i>	130.4±10.1 <i>a</i>
<i>T</i> x LC	37.81±1.72 <i>a</i>	41.91±1.74 <i>ab</i>	424.1±18.4 <i>bc</i>	119.7±10.8 <i>a</i>
(<i>T</i> x LC) x LC	42.67±4.81 <i>a</i>	45.37±4.88 <i>ab</i>	468.8±51.8 <i>ac</i>	107.1±30.2 <i>ab</i>

a, b, c – values with the same letters in the same column do not differ significantly ($P < 0.05$)

The sums of both cistern cross-section areas were higher for measurements from side (30.08–60.98 cm²) than from below (25.13–58.55 cm²) in all genotypes. Higher relative differences in cistern areas between scanning from below and from side was detected in native breeds (IV – 26.6%; *T* – 19.7%) while in LC the difference was only 4.2%. BRUCKMAIER et al. (1997) refer about total cisternal cross sections obtained by udder ultrasonography from below $33 \pm 7\text{cm}^2$ for LC.

However in response to oxytocin injection alveolar milk was ejected causing enlargement of the cisternal area by $45 \pm 8\%$. CAJA et al. (1999) detected in Ripollesa ewes 4 hours after milking average cistern area $5.6 \pm 0.5 \text{ cm}^2$ measured by ultrasonography from side of udder. Partial phenotypic correlations between BCA or SCA and other morphological and functional udder characteristics in purebred IV, T, LC and in all genotypes together are presented in Table 3. Correlations between cistern areas and

Table 3
Correlations between cistern areas and other udder traits in sheep

Breed	Improved Valachian BCA	SCA	Tsigai BCA	SCA	Lacaune BCA	SCA	All genotypes BCA	SCA
Ultrasound measurements SCA	0.71	–	0.76	–	0.84	–	0.79	–
Linear scoring of udders								
Udder depth	0.53	0.49	0.48	0.41	0.55	0.59	0.61	0.48
Cistern height	0.31	0.14	0.38	0.23	0.25	0.09	0.27	0.11
Teat placement	0.23	0.04	0.32	0.15	0.21	0.02	0.22	0.06
Teat length	-0.08	-0.06	0.21	0.12	0.26	0.17	0.13	0.09
Udder attachment	0.24	0.35	-0.06	0.11	0.12	0.16	0.11	0.18
Udder cleft	0.23	0.36	0.26	0.31	-0.04	0.06	0.10	0.18
Udder shape	0.47	0.46	0.42	0.43	0.16	0.26	0.27	0.32
External udder measurements								
Udder length	0.57	0.44	0.53	0.46	0.60	0.55	0.55	0.50
Udder width	0.43	0.50	0.40	0.38	0.40	0.47	0.41	0.45
Udder height	0.60	0.44	0.45	0.38	0.58	0.54	0.55	0.49
Cistern height	0.33	0.17	0.45	0.31	0.29	0.20	0.32	0.19
Teat length	-0.10	-0.09	0.04	0.03	0.21	0.06	0.03	0.00
Teat angle	0.14	0.01	0.24	0.05	0.15	0.06	0.17	0.03
Milkability traits								
Milk yield for 30 s	0.48	0.48	0.55	0.50	0.04	0.21	0.23	0.29
Machine milk yield	0.47	0.50	0.49	0.50	0.09	0.21	0.28	0.37
Total milk yield	0.53	0.58	0.49	0.52	0.45	0.53	0.48	0.53
Stripping yield	0.25	0.31	0.19	0.24	0.48	0.44	0.38	0.35
% of stripp. Yield	-0.07	-0.06	-0.15	-0.11	0.23	0.11	0.06	-0.02

external udder size represented by udder height, udder width and udder length were moderate ($r = 0.38\text{--}0.61$). Correlations between cistern areas and total milk yield were slightly higher in a case of measurements from

side ($r = 0.52\text{--}0.58$) than from below ($r = 0.45\text{--}0.53$). CAJA et al. (1999) found out similar correlation between SCA and milk yield $r = 0.46$. For purebred LC was characteristic low correlation between machine milk yield and cistern size and on the contrary higher correlation between stripping yield and cistern size. These facts resulted in slightly positive correlations between cistern size and percentage of stripped milk in LC, while in IV and *T* these correlations were rather negative. Also correlations between cistern size and linear score for the udder shape from the point of view of machine milking were in LC much lower ($r = 0.16\text{--}0.26$) than in IV ($r = 0.46\text{--}0.47$) or *T* ($r = 0.42\text{--}0.43$). Many LC ewes had baggy udders with big cisterns and horizontally placed teats. This fact was also documented by MILERSKI et al. (2006). Big part of cistern volume was located below the orifice into the teat canal and therefore part of cisternal milk could be reached rather by stripping than by machine milking. In this investigation the average percentage of stripping milk yield from total milk yield was 40.9%. Also MARGETÍN et al. (2013) found higher LSM for percentage of stripping milk in LC ewes 37.8% (SE 1.4%) compared to IV 24.8% (SE 1.5%) and TS 27.9% (SE 1.3%). TANČÍN et al. (2011) refere LSM 33.3% (SE 3.04%) for percentage of stripping milk in LC. FERNÁNDEZ et al. (1997) found out high positive genetic correlation between milk yield and udder depth ($r_g = 0.82$) and negative genetic correlation between milk yield and linear assessment of udder shape ($r_g = -0.26$). These correlations showed that selection for milk yield could produce worse udder morphology.

Conclusions

The results show that the use of Lacaune sheep breed in Slovakia for genetic improvement of native dairy sheep breeds or for creation of synthetic line will lead to improving of milk production, but on the other hand could turn to the worse udder morphology with negative impact on some aspects of milkability. Taking this fact into account the use of udder morphology traits in breeding programs for dairy sheep would be reasonable. Ultrasonography of udders, both from below and from side, could be used for cistern size evaluation. Correlations between cistern size and milkability traits could be utilized in breeding. Nevertheless some breed specificities have to be considered.

References

- AYADI M., CAJA G., SUCH X., KNIGHT C.H. 2003. *Use of ultrasonography to estimate cistern size and milk storage at different milking intervals in the udder of dairy cows*. J. Dairy Res., 70: 1–7.
- BRUCKMAIER R.M., BLUM J.W. 1992. *B-mode ultrasonography of mammary glands of cows, goats and sheep during alpha- and beta- adrenergic agonist and oxytocin administration*. J. Dairy Res., 59(2): 151–159.
- BRUCKMAIER R.M., PAUL G., MAYER H., SCHAMS D. 1997. *Machine milking of Ostfriesian and Lacaune dairy sheep: udder anatomy, milk ejection and milking characteristics*. J. Dairy Res., 64(2): 163–172.
- CAJA G., SUCH X., RUBERTE J., CARRETERO A., NAVARRO M. 1999. *The use of ultrasonography in the study of mammary gland cisterns during lactation in sheep*. In: *Milking and milk production of dairy sheep and goats*. EAAP Publication Nr 95. Ed. F. Barillet, N.P. Zervas, Wageningen Press, Wageningen, The Netherlands, pp. 91–93.
- FERNÁNDEZ G., BARÓ J.A., DE LA FUENTE L.F., SAN PRIMITIVO F. 1997. *Genetic parameters for linear udder traits in dairy ewes*. J. Dairy Sci., 78: 842–849.
- MAKOVICKÝ P., MARGETÍN M., MILERSKI M. 2015a. *Estimation of udder cistern size in dairy ewes by ultrasonography*. Mljekarstvo, 65(3): 210–218.
- MAKOVICKÝ P., MILERSKI M., MARGETÍN M., MAKOVICKÝ P., NAGY M. 2015b. *Genetic parameters for the size of udder cisterns in ewes diagnosed by ultrasonography among breeds. Improved Valachian, Tsigai, Lacaune and their crosses*. Arch. Zootec., 64(248): 403–408.
- MARGETÍN M., ORAVCOVÁ M., MAKOVICKÝ P., A D., DEBRECÉNI O. 2013. *Milkability of Improved Valachian, Tsigai and Lacaune purebred and crossbred ewes*. Slovak J. Anim. Sci., 46(3): 100–109.
- MILERSKI M., MARGETÍN M., ČAPISTRÁK A., APOLEN D., ŠPÁNIK J., ORAVCOVÁ M. 2006. *Relationships between external and internal udder measurements and the linear scores for udder morphology traits in dairy sheep*. Czech J. Anim. Sci., 51 (9): 383–390.
- NUDDA A., PULINA G., VALLEBELLA R., BENCINI R., ENNE G. 2000. *Ultrasound technique for measuring mammary cistern size of dairy ewes*. J. Dairy Res., 67: 101–106.
- ROVAI M., SUCH X., CAJA G., KNIGHT C.H. 2000. *Interbreed differences in cisternal and alveolar milk partitioning in the udder according to yield in dairy sheep*. J. Dairy Sci., 83(Suppl. 1): 166.
- RUBERTE J., CARRETERO A., FERNÁNDEZ M., NAVARRO M., CAJA G., KIRCHNER F., SUCH X. 1994. *Ultrasound mammography in the lactating ewe and its correspondence to anatomical section*. Small Rumin. Res., 13: 199–204.
- TANČÍN V., MAČUHOVÁ L., ORAVCOVÁ M., UHRINČÁČ M., KULINOVÁ K., ROYCHOUDHURY S., MARNET P.G. 2011. *Milkability assessment of Tsigai, Improved Valachian, Lacaune and F1 crossbred ewes (Tsigai x Lacaune, Improved Valchian x Lacaune) throughout lactation*. Small Rumin. Res., 97(1–3): 28–34.
- WILDE C.J., KNIGHT C.H., PEAKER M. 1996. *Autocrine regulation of milk secretion*. In: *Progress in dairy science*. Ed. C.J.C. Phillips. CAB International, Wallingford, Oxon, United Kingdom, pp. 311–332.

