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B-MODE ULTRASONOGRAPHY OF MAMMARY GLANDS IN DAIRY EWES DURING THE LACTATION PERIOD

Ultrasonografia Modo B en glándula mamaria

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ABSTRACT

Ultrasound is a powerful tool for the evaluation of morphological characteristics of the ewes udder and teats. It was studied the size of mammary cistern in ewes of nine genotypes (purebred Improved Valachian (IV), purebred Tsigai (T), purebred Lacaune (LC) and their crosses of IV and T with specialized dairy breeds Lacaune and East-Friesian (EF). Data were evaluated using restricted maximum likelihood (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 size obtained using the method “from bottom” were (1078.81 ± 78.9 mm^2 and 1033.68 ± 79.63 mm^2). In T ewes (813.56 ± 71.06 mm^2 and 810.85 ± 71.66 mm^2). These were significantly smaller than in LC ewes (1941.64 ± 74.73 mm^2 and 1989.46 ± 75.39 mm^2). The obtained analyses showed that crossbreeding of IV and T with specialized dairy breeds considerably increases ewe‘s cistern size. In conclusion, it was proposed to use the ultrasonographic scanning technique for determination of ewes udder cisterns size, and then use the obtained results in the selection of sheep with large cisterns, where it is a real potential for high milk production and fast milking speed.

Key words: Ultrasonography; measurement; milk production; sheep.

RESUMEN

El ultrasonido es una poderosa herramienta para la evaluación de las características morfológicas de la ubre y las tetas de las ovejas. Se estudió el tamaño de la cisterna mamaria en ovejas de nueve genotipos (de raza pura Valachian (IV), de raza pura Tsigai (T), de raza pura Lacaune (LC) y sus cruces de IV y T con razas lecheras especializadas Lacaune y East Friesian (EF). Oriental el efecto del genotipo mostró la mayor influencia ($P <0.001$) en la longitud y el área de las mediciones de cisternas de ubres izquierdas y derechas (1078.81 ± 78.9 mm^2 y 1033.68 ± 79.63 mm^2), en las ovejas (813.56 ± 71.06 mm^2 y 810.85 ± 71.66 mm^2), las áreas medias del tamaño de las cisternas de ubres izquierdas y derechas obtenidas utilizando el método „desde abajo“. Los análisis obtenidos mostraron que el cruzamiento de las variedades IV y T con las razas lecheras especializadas aumenta considerablemente el tamaño de las cisternas de las ovejas y, en conclusión, se propuso utilizar el escáner ultrasonográfico tec para la determinación del tamaño de las cisternas de ubres de oveja, y luego utilizar los resultados obtenidos en la selección de ovejas con grandes cisternas, donde es un potencial real para alta producción de leche y velocidad de ordeño rápido.

Palabras clave: Ultrassonografia; medición; producción de leche; oveja.

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INTRODUCTION

The anatomical characteristics of the mammary gland in the ewe *(Ovis aries L.)* have importance for milk production and the aptitude of the udder to machine milking. A number of authors [26, 28-33] have studied udder cisterns size milking ewes in terms of the importance of quality milk production and suitability of udders for machine milking. Animals with large cisterns produce more milk, are milked faster and are more tolerant to longer milking intervals [8, 17, 18, 27, 41, 45].

The mammary gland ultrasound imaging was first performed for the teat area [15, 16, 47]. Udder and teat scanning is generally performed for the diagnosis of milk flow disturbances and also for examination and measurement of different anatomical structures [36, 46]. A number of studies outline the advantages of ultrasonography for diagnostics of physiological and pathological changes in the mammary gland in ruminants [11, 24]. B-mode, also known as real-time ultrasonography, is most widely used as the dynamics of the processes is shown in real time on the screen as a twodimensional greyscale image. This technique was proven to be reliable for determining the anatomic features of the udder and measuring of the teat parameters of all dairy animals. Ultrasonographic scanning of the udder and teat is generally performed to diagnose milk flow disorders but is increasingly used as alternative method to measure teat morphology and teat tissue changes [22].

Recent literature describe the use of ultrasonography for estimating the size of udder cisterns in recent domestic species. This method makes it also possible to assess changes in the cisternal size in different dairy species during lactation [2, 6, 12, 25]. The ultrasound is very good, precise and modern method for studying machine-induced changes of the mammary gland [6, 7, 24, 25, 44], which provides important data for genetic selection and mastitis prevention [21, 37-39]. This method makes it also possible to assess changes in the cisternal size in different dairy species during lactation [9, 10, 14, 19, 20, 40, 41, 43]. According to Milerski et al. [35], the results obtained using ultrasonographic techniques are also useful for indirect selection on marks milkability and sheep milk yield.

The goal of this study was to investigate the udder cistern size using ultrasound technique in purebred Improved Valachian (IV), Tsigai (T), Lacaune (LC) ewes, and their crosses with specialized dairy breeds Lacaune (LC) and East Friesian (EF).

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% genetic proportion of specialized dairy breeds (SDB) Lacaune and East Friesian were included in this seven year long experiment experiment (IVxSDB 25%; IVxSDB 50%; IVxSDB75 %; TxSDB25 %; TxSDB 50%; TxSDB 75%). In total, it was investigated the size of the udder cisterns in ewes of nine genotypes (3 purebreds and 6 groups of crossbreds). For the whole period the method “from bottom“ was used to investigate the size of the udder cisterns at 265 ewes. Each year, the ewes were kept within the same flock and were milked twice a day (d). Machine milking was carried out in a 1x24 low-line, side by side milking parlour. Milking machine was set to provide 140-160 pulsations per minute (min) (1:1 ratio with a vacuum level of 38 kPa). Sonographic measurements of the left and right udder cistern (length, width and area) were taken. Two apparatuses were used: an ultrasonograph ALOKA 250 (Hyogo, Japan) with a linear probe with the frequency 3.5 MHz (early years of the experiment) and ultrasonograph SonoVet2000 (Medison Co., Ltd., South Korea) with a 170 mm linear probe with the frequency 2 to 5 MHz (late years of the experiment). B-mode (brightness) ultrasonography generates cross sections of body tissues according to the amplitude of the reflected signal. B-mode ultrasound images in a water bath taken vertically with the axis longitudinally through the teat canal of the total mammary cisternal area in cows *(Bos taurus L.)*, goats *(Capra hircus L.)* and sheeps were performed by Bruckmaier and Blum [12]. The ewe udder was dipped into the water-filled bucket before the evening milking once in mid-lactation. The water displacement was measured for each of the ewes. Specific numbers of observations in monitored indicators depending on the genotype, parity and lactation stage are shown in TABLES II and III.

TABLE I

COVARIANCE ANALYSIS OF TRAITS DESCRIBING UDDER CISTERN SIZE OF EWES DIAGNOSED BY THE METHOD “FROM BOTTOM”

Source of variation	(df)	Trait													
		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
Year	5	336.41	<0.0001	228.86	<0.0001	118.32	<0.0001	341.07	<0.0001	221.36	<0.0001	116.73	<0.0001	128.86	<0.0001
Lactation stage	3	4.90	0.0022	4.76	0.0027	8.11	<0.0001	4.02	0.0075	4.54	0.0037	4.81	0.0025	7.58	<0.0001
Genotype	8	13.95	<0.0001	11.39	<0.0001	17.77	<0.0001	15.51	<0.0001	12.42	<0.0001	19.09	<0.0001	20.06	<0.0001
Parity	2	10.89	<0.0001	8.43	0.0002	8.65	0.0002	11.78	<0.0001	7.02	0.0010	12.57	<0.0001	11.16	<0.0001
Days in milk	1	8.16	0.0044	8.27	0.0042	10.68	0.0011	6.55	0.0107	11.06	0.0009	15.72	<0.0001	15.13	0.0001

LLC = length of left cistern; WLC = width of left cistern; ALC = area of left cistern; LRC = length of right cistern; WRC = width of right cistern; ARC = area of right cistern; SLRC = sums of both cross-section are

It was monitored and evaluated 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) - mm^2.
Sums of both cross-section areas (SLRC) - mm^2.

TABLE II
EFFECT OF GENOTYPE ON TRAITS DESCRIBING UDDER CISTERN SIZE OF EWES DIAGNOSED BY THE METHOD "FROM BOTTOM"

Source of variation		Trait													
		LLC (mm)		WLC (mm)		ALC (mm²)		LRC (mm)		WRC (mm)		ARC (mm²)		SLRC (mm²)	
		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE	
			Genotype												
Improved Valachian (100)	135*	42.97	1.682	19.78	0.931	1078.81	78.9	41.63	1.675	19.48	0.950	1033.68	79.63	2107.11	151.94
IVxSDB (25 %) (125)	40	54.20	3.280	24.05	1.821	1544.97	155.37	54.05	3.272	22.47	1.860	1443.93	156.69	2995.20	299.15
IVxSDB (50 %) (150)	41	52.30	2.689	24.86	1.478	1535.81	124.48	52.90	2.672	24.39	1.510	1571.64	125.47	3105.36	239.17
IVxSDB (75 %) (175)	67	54.87	2.587	24,56	1.432	1574.12	121.61	53.97	2.577	24.45	1.462	1545.57	122.62	3121.42	233.99
Tsigai (200)	194	38.50	1.508	16.76	0.835	813.56	71.06	37.56	1.503	16.91	0.853	810.85	71.66	1623.34	136.80
TxSDB (25 %) (225)	3	41.02	6.975	19.19	3.831	1089.14	321.76	44.02	6.926	20.29	3.908	1251.79	324.31	2341.47	618.24
TxSDB (50 %) (250)	88	52.38	1.943	22.90	1.074	1371.33	90.857	52.11	1.934	22.68	1.096	1408.58	91.601	2778.06	174.73
TxSDB (75 %) (275)	23	55.49	3.888	25.29	2.157	1579.07	183.89	54.71	3.877	23.53	2.202	1520.58	185.46	3099.48	354.12
Lacaune (300)	162	57.92	1.565	27.04	0.871	1941.64	74.73	57.97	1.563	28.22	0.890	1989.46	75.39	3933.62	144.06
Significant differences		100:125,150,275++; 100:175,250,300+++; 125:200+++; 150:200+++; 175:200+++; 200:250,275,300+++; 225:300+; 250:300+;		100:150,175++; 100:300+++; 100:125,200,250,275+; 125:200+++; 150:200+++; 175:200+++; 200:250,275,300+++; 225:300+; 250:300++;		100:125,150++; 100:175,300+++; 100:200,250,275+; 125:200+++; 125:300+; 150:200+++; 150:300++; 175:200+++; 175:300++; 200:250,275,300+++; 225:300++; 250:300+++;		100:125,150,175,250,300+++; 100:275++; 125:200+++; 150:200+++; 175:200+++; 200:250,275,300+++; 225:300+; 250:300+;		100:150,175++; 100:200,250+; 100:300+++; 125:200,300++; 150:200+++; 150:300+; 175:200+++; 175:300+; 200:250,300+++; 200:275++; 225:300+; 250:300+++;		100:125,200,275+; 100:150,175,300+++; 100:250++; 125:200+++; 125:300++; 150:200+++; 150:300++; 175:200+++; 175:300++; 200:250,275,300+++; 225:300+; 250:300+++; 275:300+;		100:125,250++; 100:150,175,300+++; 100:200,275+; 125:200+++; 125:300++; 150:200+++; 150:300++; 175:200+++;175:300++; 200:250,275,300+++;225:300+; 250:300+++; 275:300+;	

+++ P<0,001; ++P<0,01; +P<0,05; ns – non significant
* No. of measurements
(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 III
EFFECT OF PARITY AND STAGE OF LACTATION ON TRAITS DESCRIBING UDDER CISTERN SIZE OF EWES DIAGNOSED BY THE METHOD „FROM BOTTOM"

Source of variation		Trait													
		LLC (mm)		WLC (mm)		ALC (mm^2)		LRC (mm)		WRC (mm)		ARC (mm^2)		SLRC2 (mm^2)	
		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE		LSM±SE	
			Parity												
1st parity (1)	263*	46.49	1.394	20.87	0.755	1253.75	61.322	45.77	1.377	20.66	0.769	1211.89	61.692	2465.90	116.98
2nd parity (2)	234	49.29	1.470	22.87	0.793	1386.80	63.556	50.48	1.445	23.17	0.807	1475.94	63.881	2864.04	120.80
3+ (3rd and next order of lactation) (3)	256	54.09	1.549	24.41	0.841	1535.59	68.514	53.40	1.532	23.64	0.857	1504.20	68.919	3038.41	130.62
Significant differences		1:3+++; 2:3++;		1:2+; 1:3+++;		1:2+; 1:3+++; 2:3+		1:2++; 1:3+++;		1:2++; 1:3+++;		1:2+++; 1:3+++;		1:2,3+++;	
			Lactation stage												
40th – 99th day (1)	165	47.24	3.504	22.31	1.826	1375.86	134.01	48.55	3.411	21.32	1.853	1297.51	133.94	2678.95	249.18
100th –129th day (2)	250	45.58	1.844	20.80	0.975	1235.18	74.883	46.32	1.805	20.34	0.991	1253.06	75.081	2489.25	140.99
130th –159th day (3)	200	50.05	1.788	22.27	0.950	1326.14	73.554	49.47	1.753	22.45	0.966	1385.38	73.778	2704.71	138.70
160th –210th day (4)	138	56.97	3.164	25.48	1.651	1631.02	121.68	55.19	3.082	25.85	1.676	1653.42	121.66	3284.89	226.52
Significant differences		2:4++; 3:4++;		2:4+; 3:4+;		2:4+; 3:4+++;		2:4+; 3:4+;		2:4+; 3:4++;		2:4+; 3:4++		2:4++; 3:4+++;	

+++P<0.001; ++P<0.01; +P<0.05; ns – non significant
*No. of measurements

Statistical methods

Data were processed by REML methodology using a MIXED procedure from the SAS statistical package [42]. The following statistical model with fixed and random effects was applied:

$$yijklm = \mu + Y_i + LSj + GEN_k + P_l + an_m + a^*DIM_{ijklm} + e_{ijklm}$$

where:

y_{ijklm}=is an observed trait (see above for details); Y_i=year (fixed effect with 4 to 7 levels); LSj = 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_1= parity (fixed effect with 3 levels; first, second, third and further parity); an_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

Recent studies also suggest that udder anatomy (mainly size of mammary cisterns) in terms of milk storage may be an important factor in determining reduced yield associated with extended milking intervals in dairy species [1, 3-5, 8-10, 23, 34]. Bruckmaier and Blum [12] conducted B-mode ultrasonography of teat and cisternal parts of the mammary glands of dairy cows, goats and sheep with 5-MHz linear array transducer in a water bath during alpha and beta–adrenergic agonist and oxytocin administration. The authors concluded that water bath technique was an excellent method for continuos observation of mammary cistern cavities in these species and the cross sectional images of the cisternal areas revealed excellent information about the form and volume of these cavities. Bruckmaier et al. [13] refer about total cisternal cross sections obtained by udder ultrasonography from below 33 ± 7cm^2 for LC ewes. The obtained results show (TABLE I) that the factor genotype, parity and year had a statistically significant effect (P<0.001) for all surveyed indicators characterizing the size of ewes udder cisterns. As shown in TABLE II, the largest average size of the udder cisterns are found in all indicators for LC ewes. The smallest udder cistern was found practically in all indicators for T ewes. The largest average of the left cistern's area (ALC) are found in LC ewes (1941.64 ± 74.725 mm^2), and the smallest average of the left cistern's area was found in T ewes (813.56 ± 71.061 mm^2). With regard to the indicator area of right cistern (ARC), again the highest average value for this indicator reached LC ewes (1989.46 ± 75.385 mm^2), and conversely, the lowest mean value was found in T ewes (810.85 ± 71.662 mm^2). The results of this work 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 IV and T ewes. TABLE III shows that the influence of "parity" had a significant effect (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 was observed tendency of increasing of the udder cisterns, depending on age (stage of lactation). Regarding changes in the size of the udder cisterns during lactation, results suggest that the basic level of the left and right udder cistern (length, width and area) of the cisterns during the lactation increase.

CONCLUSION

Ultrasonography of the mammary gland is a non-invasive technique that is easily performed. Using appropriate equipment, the teat canal and gland cisterns and udder parenchyma can be visualized. Based on the obtained results, keeping line with trends in all countries with advanced sheep husbandry, it was propose to use the ultrasonographic scanning technique for determination of ewes udder cisterns size, and then use the obtained results in the selection of sheep with large cisterns, where it is a real potential for high milk production and fast milking speed.

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