Evaluation of Udder Cisterns and Effects on Milk Yield of Dairy Ewes

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ABSTRACT

Nine Manchega (0.94 L/d) and 10 Lacaune (2.07 L/d) ewes at the same stage of lactation (90 d in milk) were used to study the interbreed differences in milk yield, mammary morphological traits, and machine-milking ability. Udder traits were measured after 6 h of udder filling before the start of the experiment. Cisternal area (by ultrasonography), cisternal milk (by teat cannula drainage), and alveolar milk (by machine milking after an intravenous oxytocin injection) were randomly measured 8 h after milking for 2 wk consecutively either with an intravenous injection of an oxytocin receptor blocking agent (atosiban, AT) or without (control, C) to avoid the occurrence of milk letdown before milking. Lacaune ewes had greater udder depth (22.5 ± 0.9 vs. 19.6 ± 0.9 cm) and cistern height (27.1 ± 3.8 vs. 15.6 ± 3.5 mm), whereas Manchega ewes had longer (42.7 ± 1.5 vs. 32.7 ± 1.5 mm) and wider teats (17.4 ± 0.5 vs. 13.9 ± 0.5 mm). Values per half udder for Manchega and Lacaune ewes differed in cisternal area (12.8 ± 0.7 and 23.7 ± 0.6 cm²) and cisternal milk (120 ± 0.6 and 269 ± 0.5 mL), but not in alveolar milk (95 ± 0.5 and 102 ± 0.4 mL), respectively. Cisternal area and cisternal milk were positively correlated ($r = 0.79$). Ratios between cisternal and alveolar milk were 56:44 and 73:27 for Manchega and Lacaune ewes, respectively. Cisternal milk volumes obtained with the AT or C treatment were similar in Manchega (111 ± 10 vs. 122 ± 8 mL) but differed in Lacaune ewes (239 ± 8 vs. 299 ± 8 mL), respectively. Consequently, alveolar milk with AT vs. C was similar in Manchega (104 ± 8 vs. 86 ± 7 mL) but different in Lacaune ewes (115 ± 7 vs. 89 ± 7 mL). Results of this experiment confirm the need for the use of an oxytocin-blocking agent for accurate evaluation of milk contained in the udder of dairy ewes. Moreover, despite the differences in daily milk yield, alveolar milk did not vary between breeds, emphasizing the role of

the cisternal more than the alveolar compartment for maximizing daily milk secretion in dairy sheep.

Key words: dairy sheep, cisternal milk, oxytocin, milking ability

INTRODUCTION

Mammary morphology is a key factor for optimizing machine-milking ability in ruminants, and its inclusion in dairy sheep improvement programs has been widely recommended (Labussière, 1988; De la Fuente et al., 1996; Caja et al., 2000). Milk fractions collected during milking (machine milk and stripping milk), residual milk (obtained after oxytocin injection), and milk flow curves during machine milking were used to evaluate machine-milking ability in dairy sheep (Labussière, 1988; Caja et al., 2000; Díaz et al., 2004). Moreover, milk partitioning between cisternal and alveolar compartments may influence milk secretion and milk yield response to extended milking intervals (Salama et al., 2004; Castillo et al., 2008). Large differences between species and breeds exist with regard to the proportion of total milk that can be stored within the cisternal compartment (Bruckmaier and Blum, 1992; Ayadi et al., 2003b; Salama et al., 2004). In sheep, high variation in cisternal milk was reported, ranging from <30% for meat breeds (Caja et al., 1999) to >50% for dairy breeds (Caja et al., 2000; Nudda et al., 2000; McKusick et al., 2002), suggesting that selection for greater milk yield also resulted in larger cisternal udders to accommodate the greater milk volumes secreted.

The ratio between cisternal and alveolar milk may be affected by the methodology used for measuring milk partitioning in the udder because of occasional oxytocin release during udder manipulation. The release of oxytocin can be avoided by using oxytocin antagonists to temporarily block spontaneous milk letdown, as reported in ewes (Rovai, 2001; McKusick et al., 2002) and goats (Salama et al., 2004). Ultrasonography is used as a noninvasive method to explore the internal structure of the mammary gland in sheep (Ruberte et al., 1994; Caja et al., 1999; Nudda et al., 2000) and goats (Bruckmaier and Blum, 1992; Salama et al., 2004), as well as to measure the cisternal milk storage capacity within

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the udder without the interference of milk drainage (Ayadi et al., 2004; Caja et al., 2004).

The aim of this study was to analyze the differences in milk partitioning between the udder compartments (cisternal and alveolar) of 2 dairy sheep breeds (Manchega and Lacaune) of different genetic merit and productive milk yield to understand the differences observed in daily milk secretion and milking ability. A secondary objective was to relate external (udder morphology) and internal (udder compartments) mammary traits with machine-milking ability for both breeds of dairy sheep.

MATERIALS AND METHODS

The experimental procedures and animal care conditions were approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (Reference CEEAH 410).

Animals, Feeding, and Routine Milking

A total of 19 multiparous ewes (parity, 3.7 ± 0.4) in mid lactation (90 ± 20 DIM) of Manchega ($n = 9$; 0.94 ± 0.42 L/d and 72.0 ± 8.2 kg of BW) and Lacaune ($n = 10$; 2.07 ± 0.21 L/d and 75.6 ± 6.7 kg of BW) dairy breeds from the Servei de Granges i Camps Experimentals (S1GCE) of the Universitat Autònoma de Barcelona (Bellaterra, Spain) were used. Both groups of ewes weaned their lambs (1.6 ± 0.2 lambs/ewe) at 35 d of age and had representative milk yields and BW for their breeds according to DIM. Ewes were kept in a semiconfinement system grazing on natural pastures for 6 h daily and supplemented indoors with a chopped dehydrated forage mixture ad libitum (50% alfalfa hay and 50% corn whole plant) and 0.5 kg/d of a commercial concentrate (60.7% barley, 14.1% fish meal, 20.2% soybean meal, 3.0% salt, and 2.0% vitamin and minerals supplement; as fed) individually fed in the milking parlor.

Machine milking took place at 0800 and 1730 h in a 2- × 12-stall parallel milking parlor (Westfalia Surge Ibérica, Granollers, Spain) equipped with recording jars ($2 \text{ L} \pm 5\%$) and a low-line milk pipeline at a vacuum level of 42 kPa. Machine milking was set to provide 120 pulsations/min and a 50:50 ratio. Milking routine included machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-cide plus, Henkel Hygiene, Madrid, Spain).

Experimental Procedures

Treatments. The experiment consisted of a 2 × 2 factorial in which udder cisternal area of both dairy

breeds was evaluated before milking. Milk partitioning (cisternal and alveolar compartments) at machine milking was measured by half udders for an 8-h milking interval. Treatments included intravenous injection of 10 µg/kg of BW of an oxytocin receptor blocking agent (**AT**, atosiban; Tractocile, 7.5 mg/mL, Ferring, Madrid, Spain) or a control (**C**) with no treatment to avoid the occurrence of spontaneous milk letdown because of conditioned stimulation and udder manipulation. Atosiban was administered individually when the ewes were in the pens, approximately 8 h after the a.m. milking (1700 h) and immediately before ewes went to the milking parlor for the p.m. milking. Only 4 ewes were processed daily at 10-min intervals to avoid delays in the scheduled milking (1720 to 1750 h).

Cisternal Area, Cisternal Milk, and Alveolar Milk. Measurements were made at random in the p.m. milking of different days in 2 consecutive weeks (91 to 115 DIM), and a minimum of 1 wk between treatments was maintained for the same ewe to avoid carryover effects on the milk yield and milk partitioning in the udder.

Cisternal area was measured by udder half in duplicate as indicated by Ruberte et al. (1994) and Rovai (2001) using a real-time B-mode ultrasonograph (Ultra Scan 900, Ami Alliance Medical Inc., Montreal, Canada) with a 5-MHz sectorial probe. An inclined sagittal imaging plane, from the upper part of the proximal intermammary groove toward the teat and with the teat as the scanning axis, was used. Images were transferred to a portable computer and analyzed by using specific software (MIP4 Advanced System, Microm España, Barcelona, Spain) to estimate the cisternal area of the scans in triplicate.

After scanning, cisternal milk was drained from each udder half by using a teat cannula (2 mm o.d. × 80 mm; Hauptner-Heberholtz, Inprosa Ibérica, Madrid, Spain) and milk volume was recorded separately. Time between the application of the oxytocin antagonist and the extraction of cisternal milk was ≤ 20 min (Wellnitz et al., 1999). A supraphysiological dose of synthetic oxytocin (4 IU; Veterín Lobulor, Laboratorios Andreu, Barcelona, Spain) was injected i.v. to each ewe, and alveolar milk was machine milked and collected separately by udder half to abolish the oxytocin receptor blockage and to remove the alveolar milk completely from the udder.

Milk Recording and Milk Composition Analyses. Average milk yield and milk composition for each ewe were calculated by using the daily milk records and the samples collected on the test day of the week before (80 DIM) and after (120 DIM) the experimental period to avoid interferences. For the analysis of cisternal and alveolar milk composition during the experimental pe-

Table 1. Milk partitioning in the udders of dairy ewes by breed and use of an oxytocin-blocking agent¹

Item	Manchega		Lacaune		SEM	Effect ($P < $)	
	Control	Atosiban	Control	Atosiban		Breed	Atosiban
No. of observations	9	9	10	10			
Milk yield, L/d	0.94		2.07		0.10	0.001	—
Milk partitioning ²							
Cisternal, mL	122 ^c	111 ^c	299 ^a	239 ^b	8	0.001	0.010
Alveolar, mL	86 ^b	104 ^a	89 ^b	115 ^a	7	0.699	0.001
Total, mL	208 ^b	215 ^b	388 ^a	354 ^a	11	0.001	0.307
Cisternal: alveolar, %	59:41 ^c	52:48 ^d	77:23 ^a	68:32 ^b	—	0.001	0.001
Cisternal area, cm ²	12.4 ^b	13.1 ^b	24.0 ^a	23.3 ^a	9	0.001	0.961

^{a-d}Means within a row with different superscripts differ at $P < 0.05$.

¹Atosiban (i.v. 10 µg/kg of BW) before udder scanning and milking.

²Values are the mean of udder halves after 8 h of udder filling.

riod, a representative sample was collected from each milk fraction, preserved with potassium dichromate (0.3 g/L), and stored at 4°C until analysis. Fat, protein ($N \times 6.38$), total solids, and casein contents of milk samples were analyzed by direct near infrared spectroscopy of reflectance (InfraAlyzer 450, Bran+Luebbe, Norderstedt, Germany; Albanell et al., 1999).

For SCC determination, a parallel representative milk sample was collected, preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Micro-Tabs II, D&F Control Systems, San Ramon, CA), and kept at 4°C until analysis in the DHI Laboratory of Catalonia (Allic, Cabriels, Barcelona, Spain) using an automatic cell counter analyzer (Fossomatic 250, Foss-Electric, Hillerød, Denmark).

Mammary Morphological Traits. Mammary morphology traits were evaluated once before the start of the experiment in both groups of ewes. Udder measurements were taken 2 h before the p.m. milking (6-h udder filling) while the ewes were locked in the milking parlor. The methodology used was based on Labusière (1988) and the traits recorded were udder depth (vertical distance from rear attachment to udder floor), udder length (horizontal distance from the rear to the fore udder attachment), distance between teats (at the teat base), teat angle (degrees from vertical, measured in a rear view), teat length, teat width (measured at the middle of the teat), and cistern heights (distance between teat insertion and udder floor per half udder).

Statistical Analysis

A MIXED model procedure (v. 8.1; SAS Inst. Inc., Cary, NC) was used. The statistical model included the fixed effects of breed (Manchega and Lacaune), udder half (left and right), and atosiban treatment (C and AT), the random effect of the animal nested into the breed, the respective interactions, and the residual er-

ror. Logarithmic transformations ($\log_{10}$) of SCC values were made before statistical analysis. When the probability of the interaction term was not significant ($P > 0.20$), it was deleted from the model. Differences between least squares means were localized with the PDIFF test and significance was declared at $P < 0.05$. Pearson correlation coefficients between cistern area and cistern milk were also calculated.

RESULTS AND DISCUSSION

Milk Yield and Composition According to Breed

Manchega and Lacaune ewes differed in milk yield during the experiment (Table 1; $P < 0.001$) in accordance with the previous information on the performance of the dairy sheep breeds used (Marie et al., 2002). Calculated total milk yield for 150 DIM after the weaning of the lambs was 126 ± 8 L and 264 ± 13 L ($P < 0.001$) for Manchega and Lacaune ewes, respectively. Significant differences according to breed were observed for milk composition ($P < 0.01$), and the Manchega ewes had a greater content in cheese-yielding milk components than the Lacaune ewes. Average milk composition for the experimental period was as follows: total milk solids (21.3 vs. 18.4%), milk fat (9.07 vs. 7.03%), and protein (6.60 vs. 5.81%) for Manchega versus Lacaune, respectively. Milk casein values were 4.99 vs. 4.44% ($P < 0.01$), representing 75.6 and 76.4% ($P = 0.897$) of total milk protein, respectively. No differences ($P = 0.632$) between breeds were observed for milk SCC values, being $84,450 \pm 5,200$ cells/mL ($\log_{10}$ SCC = 4.93 ± 3.72).

Milk Partitioning in the Udder Under Regular Milking Conditions

Despite the milk yield difference between Manchega and Lacaune ewes (difference = 1.13 L/d; $P < 0.001$),

the volume of alveolar milk under regular milking conditions (control treatment without AT) in both ewe breeds was similar ($P > 0.05$; Table 1). This result was unexpected due to the relationship between the mammary gland secretory tissue (alveolar population) and daily milk yield (Capuco and Akers, 1990). In contrast, significant differences in the volume of cisternal milk ($P < 0.001$; Table 1) were found between breeds, the Lacaune ewes having a greater volume than the Manchega ewes. Cisternal:alveolar milk percentages were 59:41 and 77:23 for Manchega and Lacaune ewes, respectively, which was in accordance with the known machine-milking ability of each breed (Caja et al., 2000). Results agree with the typical udder morphology and selection aims of each breed and suggest that selection for milk yield in dairy sheep has modified the anatomical structure of the udder, increasing the size of the cisternal compartment in high-yielding ewes.

Nevertheless, milk partitioning in the udder is usually measured in the milking parlor under regular milking conditions and, as a consequence, it can be conditioned by spontaneous milk letdown produced as a result of prestimulation (milking under familiar surroundings). Data obtained in these conditions showed marked differences between sheep breeds (Caja et al., 1999; Nudda et al., 2000). Percentages of cisternal milk for Manchega and Lacaune dairy breeds in our study (59 and 77%, respectively) were lower than those reported in Sarda dairy ewes (82%; Nudda et al., 2000), but the hand milking performed in the latter study may have overestimated the Sarda value.

Moreover, short-term autocrine inhibition of milk secretion in the mammary gland was related to cisternal size, with the large-cisternal animals generally being more efficient producers of milk and more tolerant to long milking intervals and simplified milking routines (Ayadi et al., 2003a). In our results, the correlation coefficient between daily milk yield and cisternal milk was high ($r = 0.87$; $P < 0.001$), whereas the coefficient between daily milk yield and alveolar milk was lower ($r = 0.32$; $P < 0.05$).

Milk Partitioning in the Udder by Using an Oxytocin-Blocking Agent

Use of AT produced marked differences in milk partitioning between udder compartments by breed (Table 1). Cisternal milk was similar for AT and C Manchega ewes ($P > 0.05$), but was different in Lacaune ewes ($P < 0.01$), in which cisternal milk was lower and alveolar milk greater ($P < 0.001$) in the AT than in the C ewes. Bruckmaier et al. (1997) used AT to inhibit oxytocin-induced milk letdown in dairy cows and showed that the cisternal milk obtained by oxytocin receptor block-

age under regular milking conditions was similar to the cisternal milk measured when the inhibition of the milk ejection takes place in unfamiliar surroundings by effect of natural catecholamines.

Despite the difference in average milk yield between Manchega and Lacaune ewes at the same stage of lactation (Table 1), values for alveolar milk in both breeds were similar for C (difference = 3 mL; $P > 0.05$) and AT treatments (difference = 11 mL; $P > 0.05$). In contrast, differences between breeds in cisternal milk ($P < 0.05$) were greater in C than in AT treatments (177 vs. 128 mL; $P > 0.05$).

Cisternal:alveolar milk percentages measured under the effect of AT were 52:48 and 68:32 for Manchega and Lacaune ewes, respectively, which were lower than the values for the C treatments. Percentages of milk in udder compartments differed by breed ($P < 0.001$) and treatment ($P < 0.01$), the cisterns being greater in the case of Lacaune and C ewes (Table 1). In contrast, Manchega and Lacaune AT-treated ewes had a greater percentage of alveolar milk. In practice, volumes of cisternal milk were similar for Manchega ewes with or without the use of an oxytocin receptor blocking agent. However, for Lacaune ewes, the use of an oxytocin antagonist prevented spontaneous milk letdown and its use was considered necessary for an accurate determination of milk partitioning between udder compartments. Lacaune ewes had spontaneous milk ejection while entering the milking parlor (concentrate was available).

A high correlation coefficient was found between daily milk yield and volume of cisternal milk ($r = 0.91$; $P < 0.001$) in the AT ewes (Figure 1a), which were greater than those under regular milking conditions. The correlation was low for milk yield and alveolar milk ($r = 0.37$; $P < 0.05$) in the same group of ewes (Figure 1b). Our results suggest that cistern size is a limiting factor for milk production in dairy sheep, its role being more important than the amount of secretory tissue under the milking conditions and sheep breeds used. No similar results have been reported previously in dairy ruminants.

The use of AT did not modify ($P > 0.05$) milk composition in Manchega or Lacaune dairy ewes (Figure 2a and b). Only a tendency to increase $\log_{10}$ SCC was observed when milk samples from C or AT ewes were compared (4.85 vs. 5.05, respectively; $P = 0.08$), probably because of an effect of concentration from the smaller amount of milk stored in the cistern when the antagonist was used. Results for milk composition differed from those of McKusick et al. (2002), who reported greater cisternal milk fat content and greater alveolar SCC in AT-treated East Friesian crossbred dairy ewes.

Taking into account the milk partitioning values for the whole udder in the AT groups, a mean ratio of 4.3 mL of daily milk yield per 1 mL of cisternal milk was obtained for both Manchega and Lacaune ewes ($P > 0.05$). On the other hand, daily milk yield to alveolar milk ratios in Manchega and Lacaune ewes were 4.5 and 8.7 mL of milk yield per 1 mL of alveolar milk, respectively, with the difference being significant between breeds ($P < 0.05$).

The greater milking ability of Lacaune ewes can be explained by the progress in breeding for improving amount of milk production, which has yielded animals with quiet temperament at the milking parlor, more spontaneous milk ejection reflex, and improvement of dairy sheep management without the need for a pre-milking udder preparation as used for dairy cows.

Evaluation of Udder Cisterns by Scanning

Cisternal scans of the experimental ewes are shown in Figure 3. No significant differences were observed by udder halves for both breeds and, as a result, they were averaged. Our results agree with those of Salama et al. (2004) in dairy goats, but disagree with those obtained by Nudda et al. (2000) in Sarda dairy ewes, in which the right udder half was larger than the left half because of differences in suckling and hand milking. In addition, differences in the ultrasound technique used by Nudda et al. (2000) for measuring cisternal area may also explain these differences.

Area of cisternal udder (expressed per udder half) differed between breeds in our study, with the Lacaune showing a greater cisternal area than the Manchega ewes (24 vs. 13 cm², respectively; $P < 0.001$; Table 1), in accordance with the milking ability of each breed (Caja et al., 2000).

Correlation between cisternal area and cisternal milk volume was $r = 0.79$ ($P < 0.05$), and breed differences were reported (Manchega, $r = 0.83$; Lacaune, $r = 0.50$; $P < 0.05$). The lower correlation coefficient observed for Lacaune ewes, whose volume of cisternal milk was significantly greater, was probably due to the size limitations for visualizing the entire cisternal area when using a 5-MHz ultrasound transducer. No significant correlation coefficient was found between cisternal area and alveolar milk, agreeing with the results of Ayadi et al. (2003b) in cows and Salama et al. (2004) in goats. Moreover, Manchega ewes had a positive correlation ($r = 0.40$; $P < 0.05$) between cisternal area and alveolar milk, whereas Lacaune ewes showed no relationship. This discrepancy was probably related to differences observed in the cistern storage capacities between both breeds.

Figure 1. Relationship between a) cisternal, and b) alveolar milk fractions with daily milk yield in Manchega (○; $n = 9$) and Lacaune (●; $n = 10$) ewes treated with an oxytocin-blocking agent (atosiban). Each point is the mean of the udder halves of each ewe used.

Cisternal area measured by ultrasonography varied by breed, but did not show changes by effect of AT treatment ($P > 0.05$; Figure 3). The lack of differences in the cisternal area of Manchega ewes by treatment agreed with the results observed in the volume of cisternal milk (Table 1). On the contrary, Lacaune ewes showed differences by treatment in the volume of cisternal milk, but not in the cisternal area, which was probably a consequence of the technical limits (exploration depth) of the ultrasound probe used (Ruberte et al., 1994; Rovai, 2001). Correlations between volume and area of cisternal milk differed ($P < 0.05$) by treatment, being greater for the AT group (Manchega, $r = 0.89$; Lacaune, $r = 0.57$) than for the C group (Manchega, $r = 0.75$; Lacaune, $r = 0.51$). The difference observed between treatments could be explained by the occurrence or absence of partial milk letdown because of udder prestimulation.

Figure 2. Changes in milk components for cisternal and alveolar udder compartments in a) Manchega, and b) Lacaune dairy ewes without (control; white bars) and with the use of an oxytocin-blocking agent (atosiban; gray bars).

The positive relationship found between the area of udder cisterns by ultrasonography and the amount of milk produced in both breeds allow us to consider this method as a useful tool for evaluating the capacity of the sheep udder.

Mammary Morphological Traits

Averaged values of udder and teat size obtained (Table 2) were in the range of other dairy sheep breeds (Labussière, 1988; Fernández et al., 1995). Lacaune ewes had udders with greater udder depth and cistern height ($P < 0.001$) than Manchega ewes, in accordance with their greater daily milk yield. On the other hand, Manchega ewes had longer and wider teats ($P < 0.01$; Table 2). However, udder length and distance between teats did not differ between breeds indicating that dairy sheep increase udder capacity by vertical enlargement according to milk yield. Teat angle correlated positively ($P < 0.01$) with the distance between teats ($r = 0.75$) and cistern height ($r = 0.71$), indicating that wide udders present a more horizontal teat insertion, which is unfavorable for machine milking. Cistern height was positively correlated ($P < 0.001$) with udder depth (r

$= 0.66$), teat angle ($r = 0.77$), and distance between teats ($r = 0.73$). Moreover, cisternal area correlated positively with udder depth and cistern height ($r = 0.53$ and $r = 0.77$, respectively; $P < 0.05$) in Lacaune ewes. Thus, udders with higher cisterns were deeper. Correlations between morphological udder traits and milk yield were positive and significant ($r = 0.44$ to 0.65 ; $P < 0.05$) in both breeds, showing that milk yield was related to udder size.

CONCLUSIONS

Despite the differences in milk yield between both breeds, the amount of alveolar milk was approximately the same for Manchega and Lacaune ewes. In contrast, Lacaune ewes had greater volumes and larger areas of the cisternal compartment related to their greater milk yield. These results reduce the importance of the alveolar compartment for high-yielding dairy sheep and emphasize the role of the udder cisterns to allow a little-downregulated milk secretion and greater storage of milk between milkings. For an accurate evaluation of udder compartments, milk partitioning should be done by using an oxytocin receptor blocking agent, aiming

Figure 3. Cistern ultrasonography in Manchega and Lacaune dairy ewes without (control) or with the use of an oxytocin-blocking agent (atosiban) after 8 h of udder filling. Udder cisterns full of milk appear as dark areas and udder parenchyma as gray-white areas. Scans in the same column are from the same ewes.

to prevent spontaneous milk ejection while entering the milking parlor. This was the case for high-yielding and easy-milking ewes such as the Lacaune dairy ewes. For ewes less adapted to milking (such as the Manchega ewes) milk partitioning could be done without the use of the blocking agent or, alternatively, in unfamiliar surroundings. Use of the blocking agent did not alter alveolar or cisternal milk composition. Ultrasonography was an easy and noninvasive method for evaluating the size of udder cisterns and the milk storage capacity of the udders in dairy ewes, which may lead to increase milk yield and milking ability simultaneously.

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