Small Ruminant Research 137 (2016) 85–90

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Interrelationships among the length of milk stasis, tight junction permeability to lactose and monovalent cations, rate of milk secretion and composition in dairy goats traditionally milked once a day

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A R T I C L E I N F O

Article history:
Received 16 March 2016
Received in revised form 20 March 2016
Accepted 21 March 2016
Available online 22 March 2016

Keywords:
Dairy goat
Extended milking
Milk stasis
Tight junction

A B S T R A C T

Canarian goats are well adapted to extended milkings. To gain more information on this adaptation, 32 dairy goats in mid lactation belonging to Majorera and Palmera breeds were subjected to milk stasis of 10, 14, 24 (the normal routine), 28 and 32 h; and milk volumes, milk composition, concentrations of Na^+ and K^+ in milk and blood plasma and plasma lactose were measured at each interval. The major findings were: i. Milk stasis did not induce changes in milk fat and milk protein secretion rates. In contrast, the extension of the interval between milkings was associated with a decrease of milk lactose secretion rate, ii. Comparison of the relative changes of Na^+ and K^+ in milk with blood and plasma lactose content indicates that the tight junction (TJ) permeability to lactose was much higher than to monovalent cations. It was concluded that an important element in the adaptation of Canarian dairy goats to extended milking intervals may be related to maintenance TJ resistant to paracellular leakage, particularly to passage of monovalent ions. Thus, the present study increases the understanding on adaptation of small ruminant to extended milking interval, which has been related so far to their high cistern storage capacity.

1. Introduction

Conventional milking routine in small ruminants is based on twice daily milking (TDM). Once daily milking (ODM) is a management practice that can reduce production costs and improve the livelihood of dairy goats and sheep farmers compared with TDM (Marnet and Komara, 2008). It has been shown that dairy goats in the Canary Islands are adapted to ODM by accommodating large volumes of milk into their cisterns between milkings (Torres et al., 2013a, 2013b). Similar adaptation was found in Tunisian goats (Hammadi et al., 2012). The adaptation to ODM can be explained by the negative feedback model of Silanikove et al. (2010) for day-to-day regulation of milk yield, because large cisterns delay the effects of the intramammary feedback inhibitory signals, such as, chemical and/or intramammary pressure effects on the alveoli of the epithelial cells (Capote et al., 2008).

In the mammary gland, the tight junctions (TJ) are dynamic structures between the blood, or more precisely the interstitial fluid (basolateral side), and milk in the alveolar lumen (apical side), and thus prevent the passage of serum components into milk and vice versa (Stelwagen et al., 1995). In ruminants, TJ are sealed prior to onset of copious milk secretion and through lactation, and are leaky during the dry period (mammary involution) (Nguyen and Neville, 1998). During lactation, the TJ are highly impermeable to small molecules such as lactose or to ions such as Na^+ and Cl^-; whereas during the dry period, the epithelium is permeable to fairly large molecules, such as serum albumin and immunoglobulins (Rainard and Riollet, 2006; Silanikove, 2016).

The separation between high Na^+ – low K^+ concentration in blood serum and high K^+ – low Na^+ concentration in milk is controlled by TJ integrity. Disruption of TJ integrity results in sharp increase in Na^+ concentration in milk associated with reduction in K^+ content (Stelwagen et al., 1999; Shamay et al., 2002, 2003). Lactose is synthesized only in the mammary gland and is not secreted basolaterally in significant quantities (Kuhn and Linzell, 1970), so increases of plasma lactose concentration can only be explained by its movement from milk into blood via leaky TJ (Stelwagen et al., 1994; Castillo et al., 2008).

TJ switch to a leaky state after approximately 18 h of milk accumulation in cows (Stelwagen et al., 1997), after 20 h in sheep (Castillo et al., 2008), and after 21 h in goats (Stelwagen et al., 1994). Moreover, previous studies (Stelwagen et al., 1994; Delamaire and

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http://dx.doi.org/10.1016/j.smallrumres.2016.03.022
0921-4488/

Table 1
Milk volume and milk secretion rate in Canarian goats (n = 32) considering breed and parity factors at different milking intervals.[1,2]

	Group 1					Group 2				
	Milking interval (h)				SEM	Milking interval (h)				SEM
	10	14	24	28		10	14	24	32	
Milk volume (L)										
Majorera	1.03[a]	1.36[b]	1.92[c]	2.11[c]	0.116	1.09[a]	1.41[b]	2.23[c]	2.73[d]	0.142
Palmera	0.66[a]	0.87[a]	1.28[b]	1.56[c]	0.099	0.68[a]	0.90[b]	1.38[c]	1.63[d]	0.084
Primiparous	0.82[a]	1.06[b]	1.46[c]	1.70[c]	0.122	0.81[a]	1.06[b]	1.71[c]	2.08[d]	0.137
Multiparous	0.88[a]	1.17[b]	1.74[c]	1.97[d]	0.110	0.97[a]	1.25[b]	1.89[c]	2.28[d]	0.128
Milk secretion rate (mL/h)										
Majorera	102.75[b]	96.88[b] (−6%)	79.95[a] (−18%)	75.45[a] (−6%)	5.042	109.38[b]	100.45[b] (−8%)	92.97[ab] (−8%)	85.26[a] (−8%)	4.781
Palmera	66.25[b]	62.05[ab] (−7%)	53.39[a] (−14%)	55.58[a] (+4%)	4.128	68.06[b]	64.29[b] (−6%)	57.29[ab] (−11%)	50.78[a] (−11%)	2.918
Primiparous	81.50[b]	75.45[b] (−8%)	60.94[a] (−19%)	60.71[a] (−0.4%)	6.162	80.63[b]	75.45[b] (−6%)	71.35[ab] (−5%)	64.94[a] (−9%)	4.943
Multiparous	87.50[b]	83.48[b] (−5%)	72.40[a] (−13%)	70.31[a] (−3%)	4.151	96.81[b]	89.29[b] (−8%)	78.91[ab] (−12%)	71.10[a] (−10%)	5.195

[a–d]Means with different superscripts within the same row are different ($P < 0.05$).
[1] Values are least square means and standard error of means.
[2] In brackets, the percents of milk secretion reduction between consecutive milking intervals.

Guinard-Flament, 2006) have shown that a decrease in the rate of milk secretion is correlated with the leakiness of mammary TJ observed at extended milking intervals.

The objective of this study was to evaluate the interrelationships among the length of milk stasis, timing of TJ disruption, rate of milk secretion and milk composition in two Canarian goat breeds adapted to extended milking: Majorera and Palmera. The systematic effect of selection and migration, and specially the dispersive effect of the genetic drift (island effect) have acted on Canarian goats throughout five centuries resulting in animals with different ecological adaptations (Martínez et al., 2006), milk performance (Torres et al., 2013a), and characteristics in udder anatomy (Torres et al., 2013b).

2. Materials and methods

The experimental animal procedures were approved by the Ethical Committee of the Universidad de Las Palmas de Gran Canaria (Arucas, Spain).

The present study was performed in the experimental farm of the Instituto Canario de Investigaciones Agrarias (ICIA, Tenerife, Spain) on 32 dairy goats belonging to 2 different local breeds: Majorera (n = 16; 8 primiparous and 8 multiparous) and Palmera (n = 16; 8 primiparous and 8 multiparous). Average daily milk yields at the beginning of the experiment (99 ± 6 days in lactation) were: Majorera primiparous: 2.09 ± 0.53 L/d, Majorera multiparous: 2.11 ± 0.57 L/d, Palmera primiparous: 1.35 ± 0.39 L/d, and Palmera multiparous: 1.41 ± 0.20 L/d. The milking frequency before the start of the experimental period was once per day. The animals had *ad libitum* access to wheat straw and a vitamin-mineral block. The diet allowance was composed of 700 g/d of alfalfa and 1000 g/d of concentrate (CP = 13.76%; NDF = 24.12%; Net energy = 0.92 UFL/kg), which met the nutritional requirements in accordance with the guidelines issued for lactating goats by Institut National de la Recherche Agronomique (Jarrige, 1990).

Udder volumes were measured by water displacement at 24 h of milk stasis in accordance with Capote et al. (2006) and were performed one week before the start of the experimental period. The udder volumes recorded were: Majorera primiparous: 2.46 ± 0.79 L, Majorera multiparous: 3.49 ± 1.26 L, Palmera primiparous: 1.65 ± 0.55 L, and Palmera multiparous: 2.42 ± 0.49 L. The goats were divided into two groups with equal numbers of goats in each one. Within each group, the goats were distributed according to breed, parity, and milk yield. Four consecutive periods of milk stasis were imposed in each group: Group 1–10, 14, 24 and 28 h; Group 2–10, 14, 24, and 32 h. Any atypical behavior of goats was recorded during the experimental period (vocalization, abnormal gait, fatigue, loss of appetite, milk leakage). The goats were milked in a double 12-stall parallel milking parlor (Alfa Laval Iberia SA, Madrid, Spain) equipped with recording jars (4 L ± 5%) and a low-line milk pipeline. Milking conditions and milking routine were previously described by Capote et al. (2008).

Milk volumes were recorded by using the recording jars in the milking parlor after each milking interval. Representative milk samples (20 mL) were collected at each milking and were analyzed for fat, protein, and lactose fractions using a DMA2001 Milk Analyzer (Miris Inc., Uppsala, Sweden). In addition, 50 mL of milk were sampled at each milking and were sub-separated to aliquots and frozen at −20° C until analyzed for Na^+ and K^+ concentration by atomic absorption spectrometry (AAnalyst 200 spectrometer, Perkin-Elmer, Norwalk, USA).

Blood samples were taken immediately after each milking from the jugular vein into 2.5 mL tubes coated K-EDTA. The tubes were cooled at 4 °C, and blood plasma was separated by centrifugation at 490*g* for 15 min. Plasma was collected and stored at −20 °C. Na^+ and K^+ concentrations was determined by ion selective electrodes (Olympus AU2700 analyzer, Beckman Coulter, Tokyo, Japan). Lactose concentration was analyzed by enzymatic assay (Lactose/D-Galactose UV-method; Boehringer Mannheim/R-Biopharm, Darmstadt, Germany) according to the manufacturer instructions. All assays were done in duplicate.

The statistical analyses were performed by using SPSS 15.0 software (SPSS Inc., Chicago, USA). Repeated measures analysis of variance (ANOVA), with adjustments for sphericity (Greenhouse-Geisser correction used in repeated measurements to take into account when variances of the differences between all combinations of related groups are not equal) was applied to evaluate the milking interval effect on the studied parameters and was followed by the LSD post-hoc tests. Breed and parity effects were evaluated at each milking interval using a multiple comparison test by the Tukey method. Statistical differences were considered significant at $P < 0.05$. Data are presented as least squares means.

3. Results

Extended milking interval was associated with an increase in milk volume, which differed along with breed and parity (Table 1). No vocalization or other evidence that the goats were in pain was noticed. Milk leakage was found only in few goats at 32 h of milk stasis during the transfer from the pen to the milking parlor, but it was not recorded in animals at rest. Differences in milk volumes between Majorera and Palmera goats were found at each