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Evaluation of the morphometric characteristics of ewe milk fat globules, cheese yield and ripening in the intermediate lactation phase

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ABSTRACT – The study was carried out on twenty ewes kept indoors at 25 days post partum. The experiment lasted 60 days, from 40 to 100 days post partum. The animals were homogeneous in terms of parity, lactation phase and feed. The results show that, as the lactation phase progresses, there is a gradual increase in the percentage of fat and a decrease in the average diameter of the fat globules. In particular, before the 55 days of lactation the milk quality and the cheese yield are lower and the weight loss at 10 days of ripening is higher.

Key words: Ewe, Milk fat globule, Cheese yield, Cheese ripening.

Introduction – Current knowledge on the physiological variability of the physico-chemical characteristics of ewe milk during lactation is above all in terms of its principal constituents. The aim of this study was to assess the influence of the lactation phase on milk characteristics, with particular reference to the morphometric characteristics of the milk fat globules, the cheese yield and the weight loss of cheese during ripening.

Material and methods – Twenty Massese ewes, reared in the same farm, all lambed during a period of six days and homogeneous in terms of parity (terziparous), average weight, milk yield, were kept indoors at 25 days post partum, after the lambs had been weaned. The animals were fed the same diet based on alfalfa hay and mixed feeds which conformed to milk production requirements (INRA, 1988). The experiment lasted 60 days, from 40 to 100 days post partum and it was preceded by an adaptation period of 14 days. Every 15 days, the milk yield of morning milking was measured and bulk milk samples were analyzed; for each milk sampling 10kg of milk were used to produce 2 caciotta loaves of 1 kg each. The cheese-making process was repeated for each sampling cycle under the same conditions by the same dairyman. The yields at 24h were calculated from the total milk produced and expressed as g cheese/100g milk. To estimate the decrease in weight of the cheeses, the caciotta loaves were ripened at 8-10°C and 85% relative moisture and weighed after 10 and 30 days. Chemical analyses: percentage of dry matter (DM), protein, fat and lactose (Milko-Scan, Italian Foss Electric, Padova, Italy); percentage of casein, ash, phosphorus and calcium (A.O.A.C., 1995), somatic cell count (SCC) (Fossomatic 360, Padova, Italy); non fat dry matter (N.F.D.M.) was calculated as the difference between dry matter and fat content. The following ratios were also calculated: Casein/Fat (C/F); Protein/Fat (Pr/F); Calcium/Phosphorus (Ca/P) and Casein Number (C.N.) expressed as caseinic nitrogen/total nitrogen*100. Rheological parameters: r (min), k_{20} (min), a_{30} (mm) were performed by Formagraph (Italian Foss Electric, Padova, Italy). Morphometric characteristics: the number of fat globules per mL of milk and diameter were measured according to Scolozzi *et al.* (2003). Each analysis was carried out in duplicate. Statistical analysis: the frequency distribution of the total counted and measured milk fat globules was evaluated according to their size:

Table 1. Effect of the lactation phase on the quantity and quality characteristics of bulk milk.

		Days in Milk					SEM
		40	55	70	85	100	
Milk Yield	L	25.6 ^A	26.1 ^A	20.95 ^B	17.55 ^C	15.20 ^D	0.331
DM	%	16.24 ^D	17.61 ^{Cb}	17.77 ^{Ca}	18.07 ^B	18.50 ^A	0.081
N.F.D.M.	%	11.13 ^C	11.45 ^B	11.62 ^{Ab}	11.64 ^A	11.70 ^{Aa}	0.050
Fat	%	6.61 ^{CD}	6.40 ^D	6.39 ^D	6.79 ^B	7.12 ^A	0.128
Protein	%	5.02 ^E	5.38 ^D	5.59 ^C	5.77 ^B	5.89 ^A	0.050
Casein	%	3.82 ^D	4.32 ^C	4.47 ^{Bcb}	4.64 ^{ABa}	4.74 ^A	0.106
Lactose	%	4.85 ^A	4.75 ^{Ba}	4.72 ^{Bb}	4.68 ^C	4.62 ^D	0.020
Ash	%	0.93 ^B	0.96 ^A	0.92 ^B	0.86 ^C	0.85 ^C	0.018
Ca	%	0.20 ^B	0.23 ^{Aa}	0.21 ^{Abb}	0.22 ^A	0.21 ^B	0.008
P	%	0.18 ^C	0.19 ^B	0.20 ^A	0.19 ^B	0.17 ^D	0.002
SCC	n°*1000/mL	317.50 ^B	522.75 ^{AB}	334.00 ^B	565.00 ^A	333.50 ^B	190.330
C/F		0.68	0.68	0.70	0.69	0.67	0.048
Pr/F		0.90 ^A	0.84 ^{Bb}	0.87 ^{ABa}	0.86 ^B	0.83 ^{Bc}	0.020
C.N.	%	75.44 ^{Bb}	76.24 ^{AB}	76.32 ^{ABa}	76.69 ^A	76.35 ^A	0.438
Ca/P		1.14 ^C	1.28 ^{Ab}	1.29 ^A	1.21 ^B	1.33 ^{Aa}	0.035

^{A, B}= $P \leq 0.01$; ^{a, b}= $P \leq 0.05$.

Table 2. Effect of the lactation phase on the rheological characteristics of the bulk milk.

		Days in Milk					SEM
		40	55	70	85	100	
r	minute	21.26 ^A	15.00 ^B	15.15 ^B	14.69 ^B	14.15 ^B	1.546
k ₂₀	minute	1.51 ^B	2.08 ^{Aa}	1.80 ^{Ab}	1.45 ^B	2.00 ^A	0.156
a ₃₀	mm	42.28 ^B	50.90 ^A	55.32 ^A	54.53 ^A	52.96 ^A	3.047

^{A, B}= $P \leq 0.01$; ^{a, b}= $P \leq 0.05$.

curd, such as protein, casein and calcium revealed the lowest percentages ($P \leq 0.01$), whereas the percentage of fat remained constant until 70 days and subsequently increased ($P \leq 0.01$). The C/F ratio, which is important to ensure that the incorporation between fat and casein is at its maximum (Sinclair *et al.*, 2007), remained constant during the considered period, whereas significantly higher values of the casein number (C.N.) were found after 55 days of lactation, indicating, in the first phase of lactation, a higher presence of non caseinic nitrogen. Before 55 days of lactation there was the lowest Ca/P ($P \leq 0.01$) ratio. The lactation phase significantly influences the rheological parameters as well (Table 2).

In fact, before 55 days in milking, these parameters showed a higher clotting time (r) and a lower curd firmness (a₃₀) ($P \leq 0.01$). The trend of the morphometry of the fat globules during lactation has rarely been investigated (Martini *et al.*, 2004; 2006). This study shows that the average diameter of milk fat globules increased until 55 days in milk ($P \leq 0.01$) and then gradually decreased (Table 3). In particular, after 55 days of lactation, SG increased significantly, while MG and LG decreased ($P \leq 0.01$). As in previous study (Martini *et al.*, 2008), the number of globules per ml generally had an inverse trend with respect

each milk sample was thus characterised by a different percentage of fat globules. The globules were grouped into three size categories: small globules (SG) with a <2µm diameter, medium-sized globules (MG) with a diameter from 2 to 5µm, and large globules (LG) with a >5µm diameter. The estimated parameters were analysed using the following model: $y_i = \mu + \alpha_i + \epsilon_i$ where: y_i =parameters considered; μ =overall mean; α_i =fixed effect of sampling time ($i=1, \dots, 5$); ϵ_i =residual error. The statistical analysis was carried out using JMP software, Ver.5.0 for PC, SAS Institute (2002).

Results and conclusions – The quantity and quality characteristics of milk (Table 1) show, as expected, some variability during the considered period (Pugliese, 2000; Bianchi *et al.*, 2004).

Maximum milk production was at 55 days of milking ($P \leq 0.01$); before this period, the dry matter and the milk components needed for the formation of the

Table 3. Effect of lactation phase on morphometric characteristics of fat globules of the bulk milk.

		Days in Milk					SEM
		40	55	70	85	100	
Globules/ml	n°*10 ⁹	2.58 ^A	1.46 ^D	1.81 ^C	2.42 ^{ABa}	2.05 ^{Bb}	0.213
Average Diameter	µm	3.19 ^B	3.54 ^A	2.73 ^C	2.50 ^{Da}	2.39 ^{Db}	0.321
SG	%	30.70 ^D	23.32 ^E	43.45 ^C	50.01 ^B	55.95 ^A	7.163
MG	%	50.89 ^A	51.73 ^A	43.82 ^{Ba}	40.25 ^{Bb}	35.42 ^C	3.884
LG	%	18.41 ^B	24.94 ^A	12.73 ^C	9.74 ^D	8.63 ^D	5.62

^{A, B}= $P \leq 0.01$; ^{a, b}= $P \leq 0.05$.

Table 4. Effect of the lactation phase on cheese yields and ripening of the bulk milk.

		Days in Milk					SEM
		40	55	70	85	100	
Yield to 24h	%	17.54 ^C	20.90 ^A	20.69 ^A	20.67 ^A	18.83 ^B	0.578
Weight loss at 10 days	%	24.61 ^A	17.88 ^{Ba}	14.25 ^{Bcb}	14.11 ^{Bcb}	10.99 ^{Cc}	1.953
Weight loss at 30 days	%	33.04 ^A	33.49 ^A	25.47 ^{Ba}	24.27 ^{Ba}	21.08 ^{Bb}	2.892

^{A, B}= $P \leq 0.01$; ^{a, b}= $P \leq 0.05$.

at 100 days of lactation which had the lowest average diameter of globules. In conclusion, before 55 days of lactation, ewe's milk is low in quality, leading to a lower cheese yield and a higher weight loss of cheese during ripening.

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to the average diameter. The best cheese yields ($P \leq 0.05$) were between 55 and 85 days of lactation, while before and after this period there were significantly lower values (Table 4). The weight losses of cheese during ripening were lower ($P \leq 0.01$) after 55 days of lactation. This is in relation to the size of milk fat globules, since large milk fat globules have a lower membrane surface than the small globules and have a lesser ability to bind water, thus favouring the production of less soft cheeses (Goudéranche *et al.*, 2000; Wiking *et al.*, 2004) and higher weight losses during ripening, as observed in previous studies (Martini *et al.*, 2008).

In fact, in this study we found that the lowest weight losses of cheese during ripening was with milk produced