



Morphometric analysis of fat globules in ewe's milk and correlation with qualitative parameters

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Paper received July 10, 2003; accepted November 11, 2003

ABSTRACT

In this study the morphometric characteristics of fat globules from ewe's milk have been correlated with the chemical and technological parameters of the milk.

Milk samples were taken from twenty-five Massese ewes, subdivided according to the parity; the animals were homogeneous for lactation phase and diet. The morphometric analysis of fat globules (n./ml, diameter, surface area/volume), the standard chemical analysis, SCC and determination of the rheological parameters were performed on milk samples obtained during the morning milking.

The mean number of fat globules/ml was 3.09×10^9 , with a mean diameter of 3.93 μm , ranging from 1.20 μm to 12.30 μm . For all parities, a fat globule diameter ranging from 3.21 to 4.20 μm was found most frequently. Animals in the first lambing order showed a significantly lower percentage (5.26%) of large globules ($>5.21\mu\text{m}$), while animals in the fifth lambing order showed a higher percentage (20.75%). The number of globules/ml was negatively correlated to milk production ($P \leq 0.01$) and curd firmness at 45 min ($P \leq 0.05$); whereas it was positively correlated to protein content, non-fat dry matter, and curd firming time ($P \leq 0.05$). Fat globule dimensions varied according to the parity of the animals and influenced various qualitative parameters of the milk.

Key words: Milk fat globules, Ewe, Morphometric parameters, Milk quality.

RIASSUNTO

ANALISI MORFOMETRICA DEI GLOBULI DI GRASSO DEL LATTE OVINO E CORRELAZIONI CON I PARAMETRI QUALITATIVI

In questo studio vengono correlate le caratteristiche morfometriche dei globuli di grasso del latte di pecora con i parametri chimico-nutrizionali e tecnologici del latte. Sul latte individuale di 25 pecore di razza Massese di diverso ordine di parto sono state effettuate le analisi morfometriche dei globuli di grasso (n. globuli/ml, diametro, superficie/volume), l'analisi chimica standard, la determinazione del contenuto di cellule somatiche (SCC) e l'analisi tromboelastografica. Il numero medio dei globuli di grasso visualizzati per ml di latte sul totale dei campioni esaminati, è stato di $3,09 \times 10^9$ e il diametro è risultato mediamente pari a 3,93 μm , con un range di variabilità compreso tra 1,20 μm e 12,30 μm . Per tutti gli ordini di parto considerati, la classe maggiormente rappresentata è risultata quella con diametro dei globuli compreso tra 3,21 e 4,20 μm . Gli animali di primo parto presentano una percentuale significativamente minore (5,26%) di globuli grandi (diametro $>5,21 \mu\text{m}$), mentre gli animali di quinto parto ne hanno fatto registrare la percentuale più elevata (20,75%). Il numero di globuli/ml risulta correlato negativamente con la produzione di latte ($P \leq 0.01$) e con la consi-

stenza del coagulo valutato a 45 minuti ($P \leq 0,05$), positivamente ($P \leq 0,05$) con le proteine, il residuo secco magro ed il tempo di formazione del coagulo. Le dimensioni dei globuli di grasso misurati variano in funzione dell'età dell'animale e influiscono su alcuni parametri qualitativi del latte.

Parole chiave: *Globuli di grasso, Latte ovino, Qualità, Morfometria.*

Introduction

Fat is present in milk as spherical globules, mainly composed of triacylglycerol and surrounded by a portion of the mammary cell membrane (Keenan *et al.*, 1988; Danthine *et al.*, 2000).

Previous studies carried out in the livestock breeding field have been concerned with globule chemical composition and membrane structure (Mather and Keenan, 1998; Danthine *et al.*, 2000), while correlations between morphometric characteristics of fat globules and milk quality have been less frequently investigated. A correlation between the chemical-physical and the rheological parameters of milk with fat globules has been hypothesized by some authors (Walstra and Jenness, 1984; Mehaia M.A. 1995), but literature on the subject remains scanty.

In the previously studied species, milk fat globule diameters ranged from less than 1 to 20 μm (Timmen and Patton, 1988; Walstra, 1994). Data obtained from the evaluation of human milk (Simonin *et al.*, 1984) showed that the numerical distribution of fat globules was influenced by physiological factors such as gestational age (*i.e.*, full-term or preterm) and time elapsed since delivery. According to Walstra (1969), fat globule distribution in the different species studied might also depend on the measurement techniques employed: temperature, homogenization, conservation and contact with reagents all can alter the fat globule membrane structure, leading to breakdown or aggregation of the globules (Danthine *et al.*, 2000; Scolozzi *et al.*, 2002). Since the size of the fat globule appears to influence both chemical composition (Timmen and Patton, 1988; Jensen *et al.*, 1991), and milk digestibility, the study of the relative amount of core and membrane of variously-sized fat globules may prove to be a useful aid in improving the qualitative production of milk,

thus leading to alleviation of various biomedical problems associated with the accumulation of dietary fats.

The purpose of this study is to evaluate the morphometric characteristics of ewe's milk fat globules according to different parities and to correlate the data with the chemical and technological aspects of milk production.

Material and methods

Subjects

The trial was carried out on 25 Massese ewes (5 animals per lambing order: 1st, 2nd, 3rd, 4th, 5th and up), homogeneous for age within the lactation order and phase of lactation (approximately two months after delivery). The animals all came from a single herd located in the province of Pisa; all partook of the same diet consisting of grass and polyphyte hay.

For the quantitative/qualitative evaluation, milk samples were taken individually during the morning milking and refrigerated until use.

Morphometric analysis

The number of fat globules/ml, diameter, surface area and volume were calculated as previously described (Scolozzi *et al.*, 2003): briefly, fresh milk, diluted 1:100 with distilled water was added to an Acridine Orange solution 0.1% in phosphate buffer pH 6.8 and placed in a Burkner chamber for observation with a fluorescent microscope and the Quantimet 500 image analyzer system (Leica Ortomat Microsystem, S.P.A., Milan, Italy). Since the lowest detection limit of the microscope and image analyzer apparatus was 1 μm , the number and volume frequencies of particles with a diameter of less than 1 μm were estimated by extrapolation from the cumulative volume of milk fat.

Chemical and clotting analysis

Milk samples were analyzed for fat, protein and lactose content by infrared analysis (Milkoscan, Italian Foss Electric); somatic cell count (SCC) (Fossomatic 360) and pH were also determined; non-fat dry matter was calculated to be the difference between dry matter and fat content.

Rheological parameters were measured according to ASPA, 1995: rennet clotting time (r), rate of curd firming (k20), and curd firmness 30 and 45 min after rennet addition (a30 and a45) were measured (Formagraph, Foss Electric).

Statistical Analysis

Frequency distribution of milk fat globules was evaluated according to their size and histogram step: fat globule diameters were divided into eight size classes of 1- μ m class widths (from 1.20 μ m to 12.30 μ m). Class widths were further utilized to define small, medium and large globules: medium-sized globules included the three classes occurring most frequently (from 2.21 to 5.20 μ m in diameter); small and large classes included globules with dimensions smaller than 2.21 and greater than 5.20 μ m, respectively. The association between the three above-mentioned classes and parity was investigated testing the differences with the following model:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where: y_{ij} = considered parameters (morphometric, chemical and technological),

μ = overall mean, α_i = fixed effect of i^{th} parity ($i = 1, \dots, 5$); ε_{ij} = random error.

The relationships between morphometric and qualitative parameters were evaluated by single pair correlations. Values for globules with diameter <1 μ m were estimated by extrapolation from the cumulative volume distribution curve as previously described (Scolozzi et al., 2003).

Statistical evaluation was performed by JMP computer software version. 3.1.6.2 (1996).

Results and discussion

Morphometric analysis

The overall mean number of fat globules/ml

was 3.09×10^9 , in accordance with data previously obtained from ewe's milk by other authors (Mehaia, 1995; Scolozzi et al., 2003).

The values reported for other species (Ruegg and Blanc, 1981; Hood, 1981; Mehaia, 1995; Timmen and Patton, 1998; Mather and Keenan, 1998) reflect the fact that cattle have been the most frequently studied species up until now, and the values are not homogeneous, perhaps due to the wide variability in breed, lactation phase and diet plan. The mean diameter of fat globules was 3.93 μ m (ranging from 1.20 to 12.30 μ m) in accordance with Mehaia's findings on sheep's milk (1995).

Fat globules with a diameter of less than 1 μ m and therefore undetectable by microscope represented 80% of the total number of globules, but only about 1 % of the total fat volume. Similar results were obtained by other authors evaluating cow's milk (Walstra, 1969; Mehaia, 1995) and human milk (Ruegg and Blanc, 1981).

The distribution of fat globules according to size (Figure 1) shows a similar trend in all parities, with a nearly normal shape, where the third class (globules with a diameter of 3.21-4.20 μ m) is the most represented. The milk of primipara ewes showed the highest percentage of globules of the third class (48.06%), compared to the lowest percentage observed for the quintipara ewes (30.63%).

On the other hand, the primipara ewes, compared to those belonging to other parities, had fewer fat globules with a diameter greater than 4.21 μ m (25.65%), whereas the quintipara ewes had the highest percentage of globules with diameter greater than 4.21 μ m (48.3%); these data suggest a relationship between the diameter of globules secreted by the mammary gland and the age of animals. This result was further confirmed when the mean value of the globule diameter was compared in the different groups of ewes divided according to parity: a statistically significant difference ($P \leq 0.01$) was observed between ewes of the fifth lambing order and all others except for the second, especially the lambing ewes (Table 1). Consequently, the surface/volume ratio was statistically lower ($P \leq 0.01$) in older animals' milk, in which the average globule size was larger; thus in larger globules the influence of the globule's mem-

brane compared to the core is lower (Jensen, 1991; Walstra, 1994); this factor may influence the lipid quality of the milk.

When the distribution of the three classes of globules was compared in the different parity groups, again the highest percentage (20.75%) and the lowest percentage (5.26%) of large globules were found in animals of the 5th and the 1st lambing orders, respectively. Medium-sized globules presented the opposite trend; i.e., statistically higher in the primipara ewes (89.72%) and lower in the more mature animals (72.06%).

Chemical and rheological analysis and correlations with the morphometric data.

Significant correlations between the morphometrical characteristics of fat globules, quanti/qualitative milk production and rheological parameters exist: the number of globules/ml was negatively correlated to milk production ($P \leq 0.01$) and positively correlated to protein and non-fat dry matter percentage ($P \leq 0.05$); regarding clotting aptitude, a positive correlation ($P \leq 0.05$) was observed between the number of globules/ml and curd firming time (k_{20}) and a negative correlation ($P \leq 0.05$) with curd firmness (a_{45}). In Table 2, the correlations between qualitative and quantitative characteristics of milk and the three classes of globules are shown: the positive correlation between small globules and number of globules/ml ($P \leq 0.01$) indicates that the small-

Table 1. Percentage and morphometric analysis of milk fat globules within the parity.

Parity		I		II		III		IV		V and up	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Globules	n./ml ($\times 10^9$)	3.00	0.640	2.26	0.640	3.84	0.640	3.44	0.640	2.87	0.640
Average diameter	μm	3.61 A	0.181	4.07 AB	0.181	3.82 A	0.181	3.69 A	0.181	4.46 B	0.181
Surface/volume	$\mu\text{m}^2/\mu\text{m}^3$	1.67 B	0.067	1.48 AB	0.067	1.59 B	0.067	1.64 B	0.067	1.36 A	0.067
Small globules	%	5.02	2.545	6.93	2.545	8.27	2.545	9.98	2.545	7.20	2.545
Medium globules	"	89.72 B	3.045	77.89 Aa	3.045	82.25 ABb	3.045	80.38 ABb	3.045	72.05 Aa	3.045
Large globules	"	5.26 A	2.386	15.18 BC	2.386	9.48 B	2.386	9.64 B	2.386	20.75 C	2.386

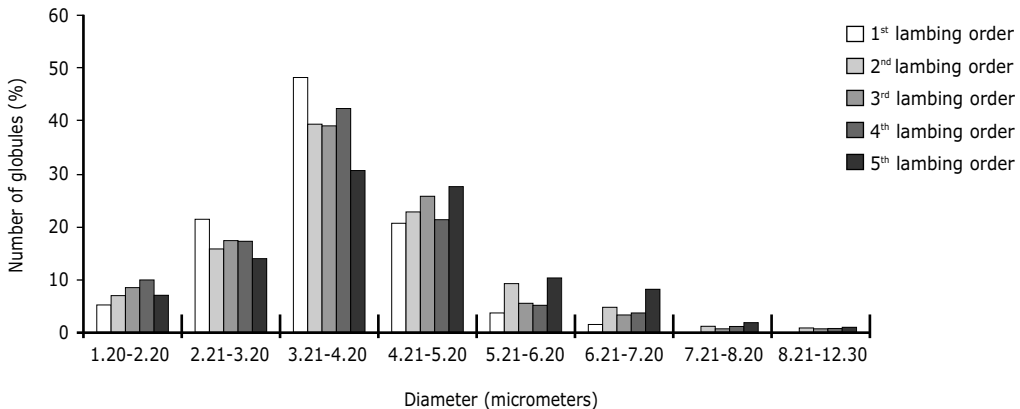
A, B: $P \leq 0.01$; a, b: $P \leq 0.05$.

Table 2. Correlations between size of globules and quantitative/qualitative characteristics of the milk.

		Small globules (%)	Medium globules (%)	Large globules (%)
Globules	(n./ml) $\times 10^9$	0.571**	-0.173	-0.278
Production	%	0.344	-0.389	0.198
Dry matter	"	-0.658**	0.560**	-0.165
Non-fat dry matter	"	-0.648**	0.531**	-0.112
Protein	"	-0.486*	0.477*	-0.196
Fat	"	-0.546**	0.463*	-0.135
Lactose	"	-0.382	0.171	0.084
SCC		0.361	-0.417*	0.219
pH		0.504**	-0.175	-0.171
r	min	0.133	-0.044	-0.042
k_{20}	"	0.168	0.055	-0.182
a_{30}	mm	-0.287	0.239	0.081
a_{45}	"	-0.416*	0.245	0.002

** $P \leq 0.01$; * $P \leq 0.05$; SCC: somatic cell count

Figure 1. Distribution of globules according to size and parity.



ler the globules, the more numerous they are, suggesting that a physiological process exists to maintain a constant fat production from alveolar mammary cells. Regarding milk quality, small globules are negatively correlated with dry matter ($P \leq 0.01$), protein content ($P \leq 0.05$), total fat ($P \leq 0.01$), non-fat dry matter and curd firmness at 45 min ($P \leq 0.01$); the only positive correlation was observed with pH values ($P \leq 0.01$).

These results suggest that a greater number of small globules may determine a decrease in the main milk chemical components that, together with increased pH values, can negatively influence the rheological properties of milk. In fact, a worsening of curd quality after 45 minutes from enzyme addition occurs: milk of this type is less suitable for the transformation process. Correlations of milk with a higher percentage of medium-sized globules showed an opposite trend for the above parameters, as well as a negative correlation with SCC ($P \leq 0.05$).

Conclusions

The age of the ewes appeared to be an important factor in the variability of the fat globule size: younger animals' milk contained a higher percentage of medium-sized globules whereas older ewes showed a higher percentage of large-sized globules. The fat globule dimensions were also correlated to the chemical properties of milk: milk with a lower percentage of its principal chemical com-

ponents appeared to contain a higher percentage of small globules while the best quality milk contained a higher percentage of medium-sized globules. Regarding milk coagulation aptitude, an increased number of globules slows down clot formation (k_{20}) and inhibits clot consistency after 45 min from rennet addition.

These results show that fat globules are not homogeneously represented in milk from animals of different ages and that they can influence milk quality.

The paper must be attributed equally to the authors.

Research supported by MURST (grant 40%, 2001-2002) and ARSIA (local funds for research).

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