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Evaluation of the fatty acid profile from the core and membrane of fat globules in ewe's milk during lactation

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ABSTRACT

The aim of this study was to investigate the differences in the fatty acid composition between the core and the membrane of the fat globules (MFGM) in sheep's milk during lactation.

Individual milk samples were collected from seven Massese ewes and analyzed for fatty acids from whole milk, from the core and from the MFGM.

The MFGM showed more saturated fatty acids (SFAs) than the core, specifically C16:0 (+21.5%) and C18:0 (+67.64%), and more polyunsaturated fatty acids (PUFAs) (+48.66%). The core had a higher content of monounsaturated (MUFAs) (+12.36%) and short chain fatty acids (SCFAs) (+640.42%).

SCFAs showed higher values ($P \leq 0.05$) in milk at 60 days of lactation and lower values ($P \leq 0.05$) at 30 and 120 days. These changes in the SCFAs occurred mainly in the core, whereas the amount of SCFAs in the MFGM remained almost unchanged.

The medium chain fatty acids increased with advancing lactation in the whole milk, in the core and in the MFGM; the long chain fatty acids on the other hand decreased.

In addition, the SFAs increased during lactation, while MUFAs and PUFAs tended to decrease in the decreasing lactation phase; the same trends were observed the core and in the MFGM.

1. Introduction

Milk fat is scattered as milk fat globules (MFGs) of different sizes in a liquid phase (Mather & Keenan, 1998). Research on dairy cows and ewes has shown that the size of MFGs affects the fatty acid profile of milk (Briard, Leconte, Michel, & Michalski, 2003; Martini, Cecchi, & Scolozzi, 2006) and the size itself is affected by genetic, physiological and environmental factors (Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007; Mehaia, 1995; Salari, Altomonte, & Martini, 2010; Sanz Sampelayo, Chilliard, Schmidely, & Boza, 2007).

MFGs are made up of a core of triglycerides enveloped by a triple membrane, composed of a single inner layer originating from the endoplasmic reticulum and a double layer from the membrane of the secretory cell (Mather & Keenan, 1998). Thus during fat secretion, part of the apical membrane is sacrificed for secretion, resulting in a consumption of resources for the organism (Argov, Lemay, & German, 2008), but also contributing to the nutritional characteristics of the lipid fraction of milk (Jensen & Nielsen, 1996).

In fact, scientific evidence on the nutritional benefits of milk fat globule membranes (MFGMs) is accumulating (Dewettinck et al., 2008) due to the various bioactive protein and lipid components of the MFGM that act as defense mechanisms in newborns and have health-enhancing functions.

Although the fat globule membranes (MFGMs) are minor components of fat compared to the triglycerides of the core (approximately 98% of fat), the membrane content in milk changes depending on the number and diameter of globules (Martini, Salari, Pesi, & Tozzi, 2010) and thus on the changes in the core/membrane ratio (Briard et al., 2003; Martini et al., 2010).

Nevertheless, studies on the fatty acid composition of the core and MFGMs have been carried out mainly on cow's milk (Jensen & Nielsen, 1996; Palmquist & Schanbacher, 1991), while relatively little is known about the core and membrane composition in ewe's milk (Scolozzi, Martini, & Salari, 2006).

Focus on the changes in the fatty acid profile of the core and MFGM and on the morphometric characteristics of MFGs contributes to the knowledge of the nutritional characteristics of milk fat and the variability in the nutritional quality of fat during lactation.

Abbreviations: MFG, milk fat globules; MFGM, milk fat globules membrane; SG, small globules; MG, medium globules; LG, large globules; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UNS, unsaturated fatty acids; FAME, methyl esters of fatty acids.

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The aim of this paper was to investigate the differences in the fatty acid composition between the core and membrane of the fat globules in sheep's milk during lactation.

2. Materials and methods

2.1. Animals and sampling

The trial was carried out on seven dairy ewes (Massese breed). All ewes were reared on the same farm and kept indoors at 10th day before partum. The experiment lasted 90 days, from 30 to 120 days in milk. All the ewes lambed over a period of six days and were homogeneous in terms of parity, average live weight and feed.

2.2. Milk analysis

Individual milk samples from the morning milking were collected at 30, 45, 60, 90 and 120 days post partum for a total of 35 samples ($N = 35$). All the samples were refrigerated at 4 °C. For each sample collected, three aliquots ($n = 3$) were taken.

The first aliquot of each fresh milk sample was analyzed in terms of milk fat globule characteristics (number of globules/mL and average diameter). The second aliquot of each fresh milk sample was used to isolate the MFGM from the core according to the macroversion of Patton & Huston's method (1986) followed by centrifugation of the cream at 100,000×*g* for 2 h at 10 °C. The core and the membrane of the milk fat globules were stored at −20 °C until analysis. The third aliquot of each whole milk sample was stored at −20 °C until fat extraction and fatty acid analysis. Each analysis was carried out in duplicate.

2.3. Fatty acid analysis

Whole milk fat extraction was performed using hexane and ethanol, according to Rose–Gottlieb's method (AOAC, 1995), modified by Secchiari et al. (2003). Methyl esters of fatty acids (FAME) were obtained after transesterification with sodium methoxide (Christie, 1982). Core lipids were extracted according to Folch, Lees, and Stanley (1957), using a chloroform-methanol mixture. Membrane fat extraction was performed using HCL, methanol and toluene according to Ichihara and Fukubayashi (2010).

The composition of the fatty acids extracted from the whole milk, core, and membrane, was determined by gas chromatography using a Perkin Elmer Auto System (Norwolk, CT, USA) equipped with a flame ionization detector (FID) and a capillary column (FactorFour Varian, 30 m × 0.25 mm; film thickness 0.25 μm, Middelburg, Netherlands). The helium carrier gas flow rate was 1 mL min^{-1}. The oven temperature program was as follows: level 1, 50 °C held for 2 min, level 2, 50–180 °C at 2 °C min^{-1} then held for 20 min, level 3, 180–200 °C at 1 °C min^{-1} then held for 15 min, and finally level 4, 200–220 °C at 1 °C min^{-1} then held for 30 min. The injector and detector temperatures were set at 270 and 300 °C, respectively.

2.4. Morphometric analysis of milk fat globules

The number of fat globules per mL of milk and the diameter (μm) were measured by florescence microscopy according to Scolozzi, Martini, and Abramo (2003). This is a simple method for the identification and morphometrical assessment of MFGs, and means that the diameter of each visible native globule from fresh milk can be analyzed directly using the image analyzer system. Other methods use the refractive index in order to carry out an indirect analysis of the standard parameters of milk fat globules using software applications. Our method enables the fat globules to be characterized without handling the milk too much. In fact, it has been demonstrated that changes in the MFGM (Evers, 2004) and in the size of MFGs can result from milk-handling practices (Wiking, Nielsen, Båvius, Edvardsson, & Svennersten-Sjaunja, 2006).

2.5. Statistical analysis

The frequency distribution of the total counted and measured MFGs was evaluated according to their size: fat globule diameters were divided into ten classes of 1 μm class width, from 0 to > 9 μm. For each milk sample, the percentage of MFGs within each size class was calculated. All ten classes were represented in all the milk samples evaluated. Each milk sample was thus characterized by a different percentage of MFG, for each diameter size class. Subsequently, the ten classes were arbitrarily grouped into three sizes of fat globules: small globules (SG) with a < 2 μm diameter, medium-sized globules (MG) with a diameter from 2 to 5 μm, and large globules (LG) with a > 5 μm diameter.

The results of the fatty acid composition and of the morphometric characteristics of the MGFs were analyzed by ANOVA for repeated measurements, where sampling time and fat source (whole milk, core and membrane of MFGs) were fixed effects. Significant differences were considered at the level $P \leq 0.05$. The statistical analysis was carried out using JMP (2002) software.

3. Results and discussion

In the milk of the first lactation phase (30 days), the number of MFGs per mL (Table 1) was lower ($P \leq 0.01$) than the following period, while at 30 days, higher values ($P \leq 0.01$) were recorded for

Table 1
Morphometric characteristics of sheep milk fat globules, fat yield, and amount of MFGM lipids during lactation (mean of the measured values for the subjects at each sampling day).

		Days in milk					
		30	45	60	90	120	SEM
Mean diameter	μm	3.15^{A}	2.79^{B}	2.71^{B}	2.63^{B}	2.68^{B}	0.341
Number of globules	N°/mL*10^{9}	2.16^{B}	2.56^{A}	2.54^{A}	2.70^{A}	2.62^{A}	0.699
SG	%	30.3^{B}	41.3^{A}	39.9^{A}	40.7^{A}	37.2^{A}	9.004
MG	%	48.7^{B}	41.8^{C}	42.4^{C}	44.3^{BC}	58.2^{A}	5.229
LG	%	21.0^{A}	16.8^{B}	17.7^{B}	15.1^{B}	4.6^{C}	7.145
Fat	g/mL of milk	0.049^{C}	0.061^{B}	0.056^{BC}	0.077^{A}	0.082^{A}	0.016
MFGM lipids	g/100 g of fat	2.58^{B}	3.26^{A}	3.17^{A}	3.02^{A}	3.57^{A}	0.602
MFGM lipids	mg/mL of milk	1.44^{B}	1.92^{A}	2.01^{A}	2.05^{A}	1.93^{A}	0.511

Different superscript letters indicate statistical differences across a row at $P \leq 0.01$ (A, B, C).
Abbreviations: SG: small globules (diameter <2 μm); MG: medium globules (diameter between 2 and 5 μm); LG: large globules (diameter >5 μm); MFGM: Milk fat globules membrane; SEM: standard error of the model.