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 (Mehaia, 1995; Couvreur, Hurtaud, Marnet, Faverdin & Peyraud, 2007; Sanz Sampelayo, Chilliard, Schmidely, & Boza, 2007; Salari, Altomonte, & Martini, 2010).

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 et al., 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, Rombaut, Thienpont, Le Messens, Van Camp, 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 (Palmquist & Schanbacher, 1991; Jensen & Nielsen, 1996), while relatively little is known about the core and membrane composition in ewe’s milk (Scolozzi, Martini, & Salari, 2006).

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.

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.

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 100,000xg for two hours 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, & Stanley (1957), using a chloroform-methanol mixture. Membrane fat extraction was performed using HCL, methanol and toluene according to Ichihara & 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 $mL \cdot min^{-1}$. The oven temperature program was as follows: level 1, 50°C held for 2 min, level 2, 50 to 180°C at $2°C \cdot min^{-1}$ then held for 20 min, level 3, 180 to 200°C at $1°C \cdot min^{-1}$ then held for 15 min, and finally level 4, 200 to 220°C at $1°C \cdot min^{-1}$ 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, & 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 analysed 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 characterised 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 < 2µm 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 analysed 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 the average diameter compared to the later time. The increase in the number of MFGs and the decrease in the diameter with advancing lactation had already been observed in our previous study (Salari et al., 2010). The trends observed in the number and the diameter of MFGs are mainly linked to the decrease ($P \leq 0.01$) in the percentage of LGs and to the increase in SGs ($P \leq 0.01$). In addition, these trends led to a significant increase ($P \leq 0.01$) in the content of the membrane after the 30th day of lactation, thus affecting the content of the fatty acids deriving from the membrane, as suggested by Briard et al. (2003) for bovine milk.

The average content of membrane lipids (mg/g of milk), assessed during the lactation was 1.64± 0.71, which was 4.5 times greater than that of cow's milk. Indeed, the content of lipid membrane reported in the literature in cow’s milk is 0.36 (mg/g of milk) (Fong, Norris, & Mac Gibbon, 2007). Our findings are consistent with the fact that the average content of fat in cow's milk is approximately half that of sheep's milk, and the average size of the fat globules is larger in cow's milk compared to ewe’s milk (Mehaia, 1995), which leads to a lower contribution of membrane lipids in cow's milk. In addition, given the bioactive molecules of the membrane (Dewettinck et al., 2008) the higher contribution of the membrane in ewe's milk could be significant in terms of the nutritional functionality of this milk.

Regarding the trend of milk fat content during lactation (Table 1), there was an increase in its secretion from the 45th day. The amount of fat secreted corresponds to a decrease in the average size of the MFGs and to an increase in the content of the membrane and in the number of MFGs. These findings highlight the fact that in sheep, an increased fat secretion is linked to smaller fat globules and not the other way round as suggested by Wiking, Stagsted, Björck, & Nielsen (2004) in cows. The opposite trend between fat content and diameter could be caused by the energy deficit that animals selected for milk production usually have during the first

phase of lactation. Since fat secretion involves an expenditure of resources because parts of the mammary epithelial cell membrane are sacrificed to envelope the fat globule (Argov, Lemay, & German, 2008), the energy balance could affect the availability of the membrane during the first lactation phase. Thus larger fat globules may be secreted to reduce the amount of membrane used by the cell.

The difference between cows and sheep could be due to the effects on the mammary metabolism of the energetic balance and the availability of nutrients (including related endocrine changes) that vary between species, thus changing their relative responses in fat, lactose, and protein secretions as observed by Chilliard, Ferlay, Rouel & Lamberet, (2003). The effects of the energy balance on mammary gland metabolism have not yet been completely understood.

During lactation, changes in the morphometric characteristics of the MFGs are followed by changes in the composition of the milk fatty acids (Table 2).

The short-chain fatty acids (SCFAs) showed higher values ($P \leq 0.05$) at 60 days of lactation, while the incidence of short chains was lower ($P \leq 0.05$) at 30 and 120 days. The trend in SCFAs was particularly evident in C8:0 and C10:0, which is in agreement with findings reported by other authors in the decreasing phase of the lactation curve in sheep (De La Fuente et al., 2009; Salari et al., 2010). SCFAs (Table 3) were found mainly in the core (11.60 vs. 1.81 g/100 g of fat), as shown in other studies on cow's milk (Jensen & Nielsen, 1996) and in our preliminary study on sheep (Scolozzi et al., 2006). In any case, in the latter the findings refer to bulk milk whereas changes occurring in the membrane and in the core during lactation were not investigated.

Figure 1 shows that the amount of SCFA in the membrane remained almost unchanged during lactation, thus the changes occurring in this class of fatty acids in milk were mainly due to the core.

The importance of SCFAs in the human diet stems from the fact that they are an interesting class of fatty acids from a therapeutic point of view, as they form medium chain triglycerides with a particular metabolism and are used in certain cases of metabolic illness (Haenlein, 2004).

With the advance in lactation, there was also an increase in the medium chain fatty acids (MCFAs).

The increasing trends found in the SCFAs and in MCFAs were probably linked to an increase in fat endogenous synthesis in the intermediate phase of lactation (Lock & Bauman, 2004). The changes in these classes of fatty acids seem to confirm the link between the energetic balance and the lower diameter of

globules (Argov et al., 2008). An increase in MCFAs has been found especially in some saturated fatty acids that are considered to be hypercholesterolemic (Clark, Frost, Collins, Appleby, & Peto, 1997) such as C12:0, C14:0 and C16:0. The increase in C14:0 and C16:0 was in agreement with previous studies on sheep's milk (Salari et al., 2010; De la Fuente et al., 2009) and occurred both in the core and in the membrane (Figure 1). In addition, the membrane showed a higher percentage of MCFAs (Table 3) mainly due to the incidence of C16:0 (+21.5% compared to the core), which has also been found in large quantities in the bovine membrane (Palmquist & Schanbacher, 1991). In fact, C16:0 has been reported to have a role in the conservation of membrane rigidity and stability together with C18:0 (Jensen & Nielsen, 1996), as well as an antibacterial action (Isaacs, 2001).

Although the core had lower amounts of MCFAs, it contained significantly higher amounts of C12:0 (+65.37%) and C14:0 (+12.49%), in agreement with the findings observed by other authors in ewe's and cow's milk (Scolozzi et al., 2006; Jensen & Nielsen, 1996).

As reported in the literature for both cow's and sheep's milk (De La Fuente et al., 2009; Bitman & Wood, 1990), a significant decrease occurred in the long-chain fatty acids during lactation (Table 2). C18:0 decreased gradually and C18:1 cis 9, C18:2 cis 9,12 and C20:4 decreased from 60 days, while C18:1 trans 9 and C18:3 w3 decreased at the 120th day. The decrease in the long chains in the whole milk was also detectable in the core and in the membrane (Figure 1).

Membrane lipids were characterized overall by higher percentages of long chains than the core (+17.5%), and in particular by C18:0 (+67.64%), C18:1 trans 9 (+176.84%), CLA cis 9 trans 11 (+227.30%), C18:3 w3 (+245%), C20:4 (+89.19%), C22:6 (+306.67%) and C24:0 (+ 1475%) fatty acids; the latter coming mostly from membrane sphingolipids (Snow et al., 2010).

Some of these fatty acids are of particular interest from a nutritional point of view. In fact, isomers of CLA showed anti-obesity, anti-diabetic and antiatherogenic effects in animals, although an excessive intake of CLA was found to induce insulin resistance in humans (Melanson, Astrup, & Donahoo, 2009); C18:3 w 3 (alpha-linolenic acid) and C22: 6 (DHA) are w3 fatty acids, which are regulators of gene expression in the body (Kaur, Smith, Barr, Garg, & Sinclair, 2011). C18:3 w 3 ingested with food could have an anti-adipogenenic action because of the competition with linoleic acid for the delta 6 desaturase enzyme in the synthesis of C20:4 (Kaur et al., 2011).

Indeed, C20:4 is an essential fatty acid, however an excessive synthesis or ingestion influences adipose tissue development, and increases the synthesis of pro inflammatory and vasoconstrictor prostaglandins and leukotrienes, as well as the synthesis of endocannabinoids. The latter are involved in increasing a sense of hunger, and affect the energy balance and glucose homeostasis (Gaillard et al., 1989). On the other hand, C22:6 is an important constituent of the retina and of the nervous system (Kaur et al., 2011).

A higher w3/w6 ratio ($P \leq 0.01$) was found in the membrane than in the core (0.163 vs 0.075). The w3/w6 ratio increased significantly after 45 days of lactation, mainly due to a decrease in w6 fatty acids. A recent scientific panel of the European Food Safety Authority (EFSA) proposed not to set specific values for the intake of w3 and w6, since the biochemical and clinical data in humans are not sufficient to recommend a ratio independently of the levels of total intake (EFSA, 2010). In spite of this, a general decrease in w6 fatty acids in human food could be positive as currently there is an excessive intake of these fatty acids (EFSA, 2010) and modifying the amount of membrane could balance the uptake of these two families of fatty acids in milk.

Of the long chain fatty acids, C18:1 cis9 and C18:2 cis9,12 have been detected in greater percentages in the core than in the membrane, unlike what has been observed in cows (Jensen & Nielsen, 1996).

In our study, the content of saturated fatty acids (SFA) increased during lactation, both in the core and in the membrane (Figure 2). In the membrane (Table 3), the average percentage of SFA was significantly higher (+2.88%). The families of monounsaturated fatty acids (MUFAs) and polyunsaturated (PUFAs) on the other hand, tended to decrease in the decreasing lactation phase and the same trend was observed in both the core and in the membrane (Figure 2).

The trends of MUFAs and PUFAs during lactation were the opposite to those reported by De La Fuente et al. (2009). This difference could be due to the different farming systems used in each study. In fact, the trials of De la Fuente et al. (2009) included periods of grazing, and as is well known, pasture leads to larger amounts of MUFAs and PUFAs in milk (Couvreur, Hurtaud, Lopez, Delaby, & Peyraud, 2006).

Given the same weight for the core and membrane, the core was richer in MUFAs (+12.33%), while in the membrane there were 48.66% more PUFAs (Table 3). However, higher percentages of PUFAs have also been found in bovine species (Jensen & Nielsen, 1996).

Despite this, in ewes the ratio of unsaturated/saturated fatty acids was higher ($P \leq 0.05$) in the core of MFGs than in the membrane (0.437 vs. 0.386).

4. Conclusions

In this study the membrane and core of fat globules in ewe' s milk showed remarkable differences in fatty acid composition, and the fatty acid profile from both lipid fractions changed during lactation probably due to the energy metabolism of the mammary gland. The changes in the fatty acids occurred in similar ways in both the substrates analyzed, with the exception of the short chains.

Despite the higher presence of C16:0, the membrane contained higher percentages of beneficial fatty acids. Therefore, the greater contribution of the membrane in milk could be significant for nutritional functionality.

In conclusion, membrane–enrichment or modulating the size of the fat globules and changing the membrane/core could provide a way to adjust the content of some fatty acids in whole milk in order to develop dairy products with better health properties.

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References

AOAC (1995). *Official Methods of Analysis Association of Official Agricultural Chemists*. Eds. AOAC, Washington D.C.

Argov, N., Lemay, D.G., & German, J.B. (2008). Milk fat globule structure and function: nanoscience comes to milk production. *Trends in Food Science and Technology, 19,* 617-623.

Bitman, J., & Wood, D.L. (1990). Changes in milk fat phospholipids during lactation. *Journal of Dairy Science, 73,* 1208-1216.

Briard, V., Leconte, N., Michel, F., & Michalski, M.C. (2003). The fatty acid composition of small and large naturally occurring milk fat globules. *European Journal of Lipid Science and Technology, 105,* 677-682.

Christie, W.W. (1982). A simple procedure of rapid transmethylation of glycerolipids and cholesteryl esters. *Journal of Lipid Research, 23,* 1072-1075.

Clark, R., Frost, C., Collins, R., Appleby, P., & Peto, R. (1997). Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *British Medical Journal, 314,* 122.

Chilliard,Y., Ferlay, A., Rouel, J., & Lamberet, G. (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science, 86,* 1751–1770.

Couvreur, S., Hurtaud, C., Marnet, P.G, Faverdin P., & Peyraud, J.L. (2007) Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or a corn silage-based diet. *Journal of Dairy Science, 90,* 392-403.

Couvreur, S., Hurtaud, C., Lopez, C., Delaby, L., & Peyraud, J.L. (2006). The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition and butter properties. *Journal of Dairy Science, 89,* 1956-1969.

De La Fuente, L.F., Barbosa, E., Carriedo, J.A., Gonzalo, C., Arenas, R., Fresno, J.M., & San primitive, F. (2009). Factors influencing variation of fatty acid content in ovine milk. *Journal of Dairy Science, 92,* 3791–3799.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T.T., Messens, K., & Van Camp, J. (2008). Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal,* 18, 436–457.

EFSA (European Food Safety Authority) (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal, 8,* 1461.

Evers, J.M. (2004)- The milk fat globule membrane-compositional and structural changes post secretion by the mammary secretory cell. *International Dairy Journal, 14,* 661-674.

Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226,* 497–509.

Fong, B.Y., Norris, C.S., & Mac Gibbon, A.K.H. (2007). Protein and lipid composition of bovine milk-fat-globule membrane. *International Dairy Journal, 17,* 275-288.

Gaillard, D., Negrel, R., Lagarde, M., & Ailhaud, G. (1989). Requirement and role of arachidonic acid in the differentiation of pre-adipose cells. *Biochemistry Journal, 257,* 389-397.

Haenlein, G.F.W. (2004). Goat milk in human nutrition. *Small Ruminant Research, 51***,** 155-163.

Ichihara, K., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research, 51,* 635-640.

Isaacs, C.E. (2001). The antimicrobial function of milk lipids. *Advances in Food and Nutrition Research, 10,* 271-285.

J.M.P. (2002). *User's Guide* ver.5.0. S.A.S. Institute Inc, Ed. Cary (NC), U.S.A.

Jensen, S.K., & Nielsen, K.N. (1996). Tocopherols, retinol, b-carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. *Journal of Dairy Research, 63,* 565-574.

Kaur, G., Smith, D.C., Barr, D., Garg, M., & Sinclair, A.J. (2011). Docosapentaenoic acid (22:5n-3): A review of its biological effects. *Progress in Lipid Research, 50,* 28-34.

Lock, A.L., & Bauman, D.E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids, 39,* 1197-1206.

Martini M., Cecchi F., & Scolozzi C. (2006). Relationship between fat globules size and chemical and fatty acid composition of cow's milk in mid lactation. *Italian Journal of Animal Science, 5,* 215-227.

Martini, M., Salari F., Pesi, R., & Tozzi, M.G. (2010). Relationship between activity of some fat globule membrane enzymes and the lipidic fraction in ewes' milk: Preliminary studies. *International Dairy Journal, 20,* 61–64.

Mather, I.H., & Keenan, T.W. (1998). Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia, 3,* 259-273.

Mehaia, M.A. (1995). The fat globule size distribution in camel, goat, ewe and cow milk. *Milchwissenschaft, 50,* 260-263.

Melanson, E.L., Astrup, A., & Donahoo, W.T. (2009). The relationship between dietary fat and fatty acid intake and body weight, diabetes, and the metabolic syndrome. *Annals of Nutrition and Metabolism, 55,* 229-243.

Palmquist, D.L., & Schanbacher, F.L. (1991). Dietary fat composition influences fatty acid composition of milk fat globule membrane in lactating cows. *Lipids, 26,* 718-722.

Patton, S., & Huston, G.E. (1986). A method for isolation of milk fat globules. *Lipids, 21,* 170-174.

Salari, F., Altomonte, I., & Martini M. (2010). Effect of lactation phase on the lipidic fraction of ewe's milk, especially milk fat globules characteristics and fatty acids composition. In: Proceedings of XVIII Fe.Me.S.P.Rum. Durres (Albania), 43-48.

Sanz Sampelayo, M.R., Chilliard, Y., Schmidely, Ph, & Boza, J. (2007). Influence of type of diet on the fat constituents of goat and sheep milk. *Small Ruminant Research, 68,* 42–63.

Scolozzi, C., Martini, M., & Abramo, F. (2003). A method for identification and characterization of ewe's milk fat globules. *Milchwissenschaft, 58,* 490-493.

Scolozzi, C., Martini, M., & Salari, F. (2006). Different fatty acids composition of fat globule core and membrane in ewe's milk. *Milchwissenschaft, 61,* 397-400.

Secchiari, P., Antongiovanni, M., Mele, M., Serra, A., Buccioni, A., Ferruzzi, G., Paoletti, F., & Petacchi, F. (2003). Effect of kind of dietary fat on the quality of milk fat from Italian Fresian cows. *Livestock Production Science, 83,* 43-52.

Snow, D.R., Jimenez-Flores, R., Ward, R.E., Campbell, J., Young, M.J., Nemere, I., & Hintze, K.J. (2010). Dietary milk fat globule membrane reduces the incidence of aberrant crypt foci in fisher-344 rats. *Journal of Agriculture and Food Chemistry, 58,* 2157-2163.

Wiking, L., Stagsted, J., Björck, L., & Nielsen, J.H. (2004). Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal, 14,* 909-913.

Wiking, L., Nielsen, J.H., Båvius, A.-K., Edvardsson, A., & Svennersten-Sjaunja, K., (2006). Milk Fat Globule Stability. Impact of Milking Frequencies on the Level of Free Fatty Acids. *Journal of. Dairy Science, 89,* 1004-1009.

Figure 1

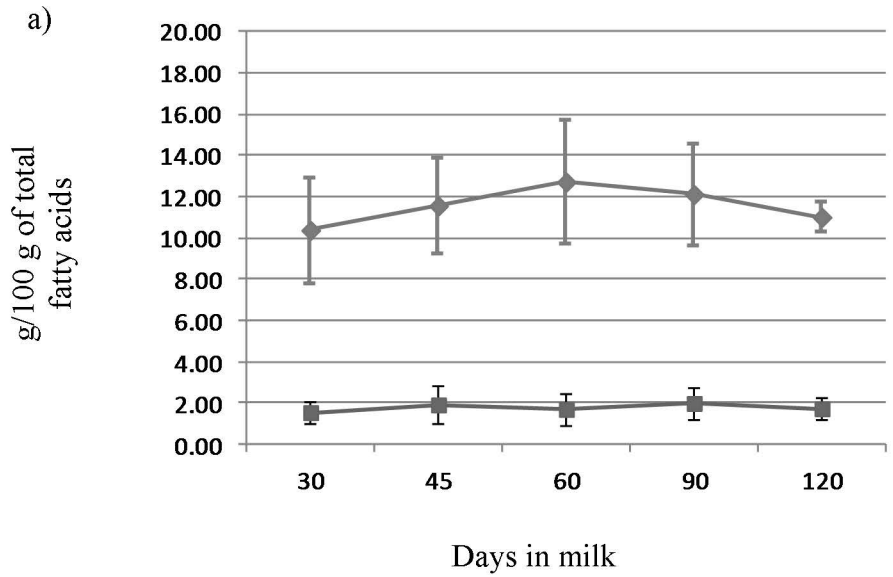
Trends of the short (a), medium (b) and long chain fatty acids (c) in membrane and core of milk fat globules during lactation (g/100 g of total fatty acids) (Mean of the measured values for the subjects at each sampling day).

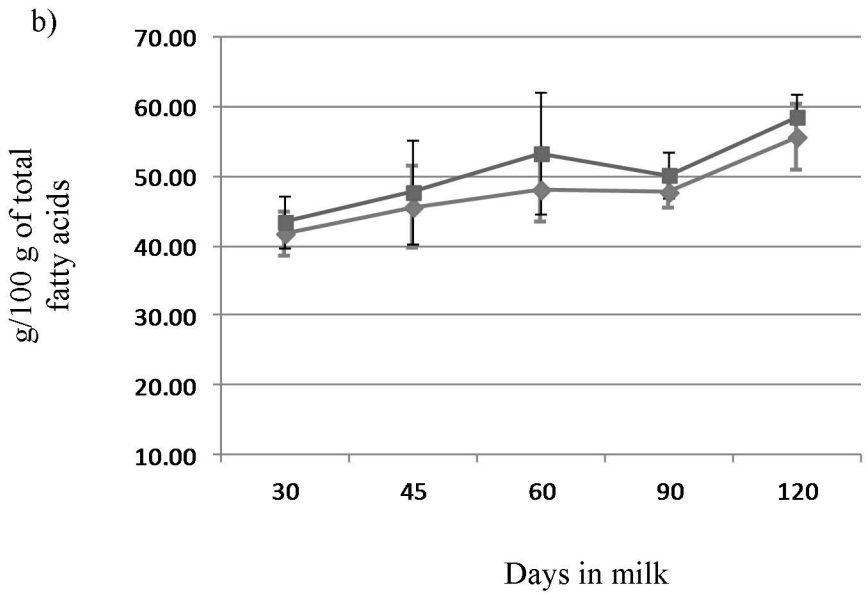
Footnote:

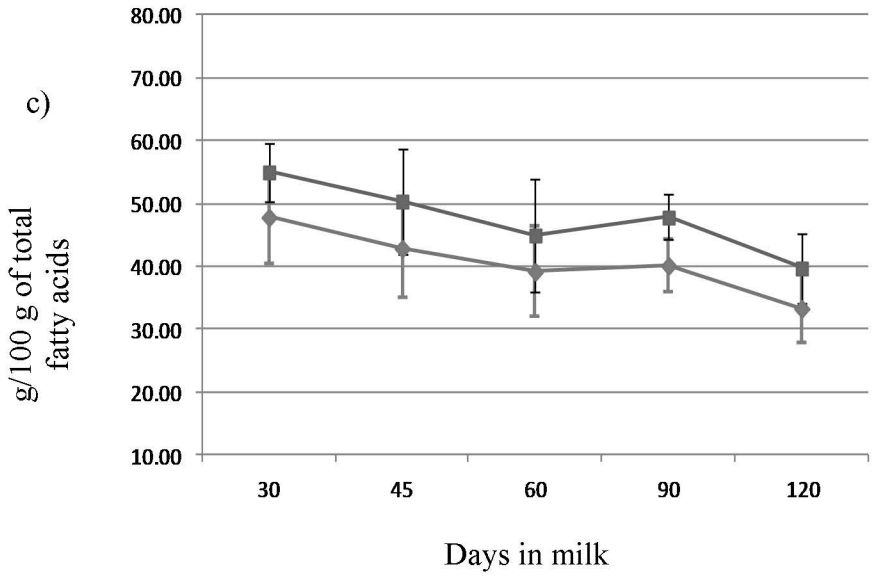
short chain fatty acids (chain length from 4 to 10 C); medium chain fatty acids (chain length from 11 to 17 C); long chain fatty acids (chain length from 18 to 24 C)

Figure 2

Trends of saturated (a), monounsaturated (b) and polyunsaturated fatty acids (c) in membrane and core of milk fat globules during lactation (g/100 g of total fatty acids) (Mean of the measured values for the subjects at each sampling day).


a)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk


b)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk


c)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk

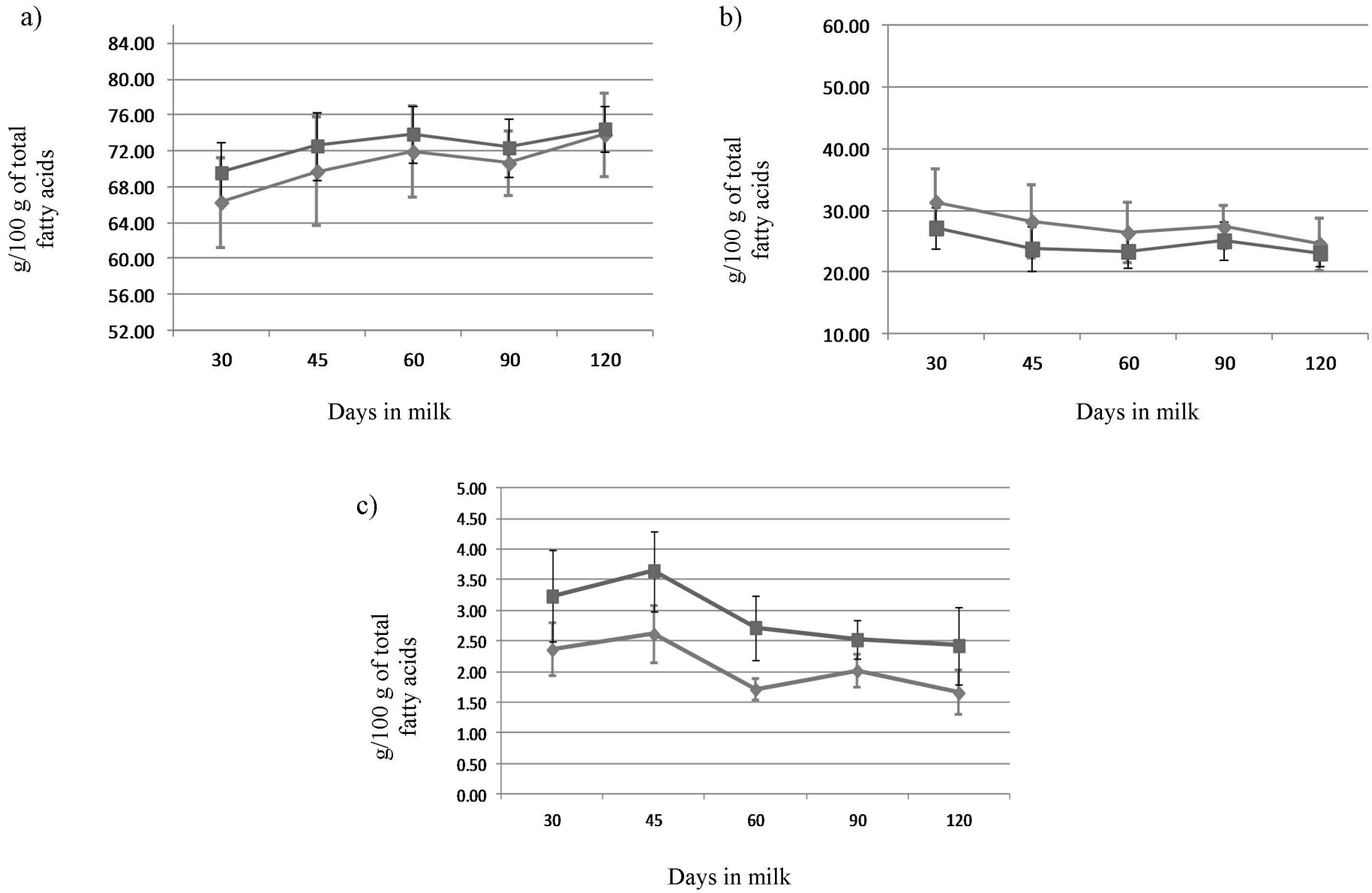

a)
84.00
80.00
76.00
72.00
68.00
64.00
60.00
56.00
52.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk
b)
60.00
50.00
40.00
30.00
20.00
10.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk
c)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
g/100 g of total fatty acids
30
45
60
90
120
Days in milk

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[1]	%	30.3^{B}	41.3^{A}	39.9^{A}	40.7^{A}	37.2^{A}	9.004
MG[1]	%	48.7^{B}	41.8^{C}	42.4^{C}	44.3^{BC}	58.2^{A}	5.229
LG[1]	%	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[1]	g/100 g of fat	2.58^{B}	3.26^{A}	3.17^{A}	3.02^{A}	3.57^{A}	0.602
MFGM lipids[1]	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)

[1]Abbreviations: SG: small globules (diameter <2µm); MG: medium globules (diameter between 2 and 5µm); LG: large globules (diameter >5µm); MFGM: Milk fat globules membrane; SEM: standard error of the model

Table 2- Composition of milk fatty acids (g/100g total milk fatty acids) during lactation (mean of the measured values for the subjects at each sampling day).

	Days in milk					
	30	45	60	90	120	SEM
C4:0	1.15^{a}	1.09^{ab}	1.04^{ab}	0.94^{b}	0.97^{b}	0.203
C8:0	1.13^{b}	1.35^{ab}	1.47^{a}	1.33^{ab}	1.01^{b}	0.420
C10:0	4.10^{b}	5.02^{ab}	5.64^{a}	5.37^{a}	4.68^{ab}	1.570
C12:0	2.93^{B}	3.58^{A}	3.98^{A}	3.97^{A}	3.66^{A}	0.850
C14:0	9.36^{C}	10.86^{B}	12.05^{AB}	11.66^{B}	13.16^{A}	1.568
C14:1	0.34^{B}	0.36^{B}	0.46^{A}	0.43^{AB}	0.41^{AB}	0.102
C15:0	0.93^{C}	1.00^{C}	1.14^{BC}	1.16^{B}	1.42^{A}	0.197
C15:1	0.19^{B}	0.19^{B}	0.22^{B}	0.23^{AB}	0.28^{A}	0.051
C16:0	27.85^{C}	28.42^{C}	30.52^{B}	29.17^{BC}	37.46^{A}	2.764
C16:1	0.76^{C}	0.72^{C}	0.78^{C}	0.98^{B}	1.47^{A}	0.207
C17:0	0.88^{a}	0.78^{ab}	0.76^{b}	0.77^{b}	0.92^{a}	0.187
C18:0	18.15^{A}	16.10^{AB}	13.61^{BC}	14.24^{B}	10.41^{C}	3.667
C18:1c9	24.20^{A}	22.45^{AB}	19.26^{B}	21.73^{AB}	18.75^{B}	4.682
C18:1 t9	0.88^{B}	1.03^{AB}	1.12^{AB}	1.234^{A}	0.327^{C}	0.452
C18:2 t9,12	0.40^{AB}	0.45^{AB}	0.32^{B}	0.47^{A}	0.16^{B}	0.177
C18:2 c9,12	0.98^{A}	0.95^{AB}	0.69^{B}	0.77^{B}	0.67^{B}	0.289
C18:3 n3	0.12^{AB}	0.16^{A}	0.14^{A}	0.12^{A}	0.06^{B}	0.065
C20:4	0.20^{A}	0.19^{A}	0.05^{B}	0.02^{B}	0.02^{B}	0.131
SCFA[1]	7.37^{b}	8.58^{ab}	9.35^{a}	8.70^{ab}	7.57^{b}	2.291
MCFA[1]	43.52^{C}	46.21^{C}	50.16^{B}	48.69^{BC}	59.20^{A}	4.401
LCFA[1]	49.10^{A}	45.20^{AB}	40.49^{B}	42.61^{B}	33.23^{C}	5.684
SFA[1]	68.87^{B}	70.70^{B}	73.03^{AB}	71.31^{B}	75.42^{A}	4.163
MUFA[1]	28.45^{A}	26.63^{AB}	24.86^{B}	26.42^{AB}	22.72^{B}	4.197
PUFA[1]	2.67^{A}	2.67^{A}	2.11^{B}	2.27^{AB}	1.85^{B}	0.570
w6	2.08^{A}	2.05^{A}	1.56^{B}	1.68^{B}	1.28^{C}	0.421
w3/w6	0.09^{b}	0.10^{ab}	0.12^{a}	0.13^{a}	0.13^{a}	0.049
UNS[1]/SFA	0.46^{A}	0.42^{AB}	0.37^{B}	0.40^{B}	0.35^{B}	0.084

In the table are shown only the significant differences
Different superscript letters indicate statistical differences across a row at P <0.01 (A, B, C) and P<0.05 (a, b)

[1]Abbreviations: SCFA: short chain fatty acids (chain length from 4 to 10 C); MCFA: medium chain fatty acids (chain length from 11 to 17 C); LCFA: long chain fatty acids (chain length from 18 to 24 C); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UNS: unsaturated fatty acids; SEM: standard error of the model

Table 3- Fatty acid composition of whole milk (g/100 g of milk total fatty acids), isolated core (g/100g of fat from core) and membrane (g/100g of fat from membrane) (mean of the repetitions)

	Whole milk	Core	Membrane	SEM
C4:0	1.51^{A}	1.58^{A}	0.01^{B}	0.203
C6:0	1.551^{A}	1.622^{A}	0.002^{B}	0.298
C8:0	1.88^{A}	1.87^{A}	0.03^{B}	0.420
C10:0	6.59^{A}	6.52^{A}	1.77^{B}	1.570
C11:0	0.05^{A}	0.05^{A}	0.02^{B}	0.017
C12:0	4.24^{A}	4.14^{A}	2.50^{B}	0.850
C13:0	0.07^{A}	0.07^{A}	0.04^{B}	0.019
C14:0	11.95^{A}	11.81^{A}	10.49^{B}	1.568
C14:1	0.41^{B}	0.49^{A}	0.29^{C}	0.102
C15:1	0.23^{A}	0.25^{A}	0.19^{B}	0.051
C16:0	29.10^{B}	28.42^{B}	34.53^{A}	2.764
C16:1	1.01^{A}	1.01^{A}	0.80^{B}	0.207
C17:0	0.78^{B}	0.73^{B}	0.95^{A}	0.187
C17:1	0.27^{A}	0.23^{A}	0.14^{B}	0.131
C18:0	11.88^{B}	11.82^{B}	19.81^{A}	3.667
C18:1c9	22.23^{a}	21.98^{a}	19.62^{b}	4.682

	Whole milk	Core	Membrane	SEM
C18:1 t9	0.72^{B}	0.73^{B}	1.30^{A}	0.452
CLAc9.t12	0.36^{B}	0.26^{B}	0.59^{A}	0.219
C18:2t9.12	0.41^{A}	0.41^{A}	0.26^{B}	0.177
C18:2c9.12	0.91^{A}	0.97^{A}	0.56^{B}	0.289
C18:3 n3	0.11^{B}	0.07^{C}	0.17^{A}	0.065
C20:0	0.94^{A}	0.89^{A}	0.76^{B}	0.238
C20:1	0.72^{B}	0.86^{A}	0.57^{C}	0.201
C20:2	0.02^{B}	0.02^{B}	0.03^{A}	0.017
C20:3 n6	0.01^{B}	0.01^{B}	0.07^{A}	0.062
C20:4	0.06^{b}	0.07^{b}	0.14^{a}	0.131
C21:0	0.08^{B}	0.08^{B}	0.13^{A}	0.055
C22:1	0.21^{B}	0.25^{B}	1.05^{A}	1.018
C22:2	0.05^{B}	0.05^{B}	0.91^{A}	0.218
C22:6	0.04^{B}	0.04^{B}	0.14^{A}	0.054
C24:0	0.05^{B}	0.05^{B}	0.66^{A}	0.151
C24:1	0.02^{B}	0.02^{B}	0.16^{A}	0.048

	Whole milk	Core	Membrane	SEM
SCFA[1]	11.54^{A}	11.60^{A}	1.81^{B}	2.291
MCFA[1]	49.24^{ab}	48.33^{b}	51.10^{a}	4.401
LCFA[1]	39.22^{B}	40.07^{B}	47.09^{A}	5.684
SFA[1]	71.85^{ab}	70.85^{b}	72.89^{a}	4.163
MUFA[1]	26.04^{ab}	27.20^{a}	24.21^{b}	4.197
PUFA[1]	2.10^{B}	1.95^{B}	2.89^{A}	0.570
w3	0.16^{B}	0.12^{B}	0.32^{A}	0.097
w6	1.65^{B}	1.64^{B}	2.06^{A}	0.421
w3/w6	0.10^{B}	0.07^{B}	0.16^{A}	0.049
UNS[1]/SFA	0.41^{ab}	0.44^{a}	0.37^{b}	0.084

Different superscript letters indicate statistical differences across a row at P <0.01 (A, B, C) and P<0.05 (a, b)

[1]Abbreviations: SCFA: short chain fatty acids (chain length from 4 to 10 C); MCFA: medium chain fatty acids (chain length from 11 to 17 C); LCFA: long chain fatty acids (chain length from 18 to 24 C); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UNS: unsaturated fatty acids; SEM: standard error of the model.