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Original Research Article

# Changes in donkey milk lipids in relation to season and lactation

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ABSTRACT

In this study the fatty acid profile and morphometric characteristics of fat globules in Amiata donkey milk in relation to the lactation phase and production season have been evaluated. Individual donkey milk samplings were carried out monthly starting from day 30 of lactation until day 300. The amount of fat and the diameter of the milk fat globules were fairly stable during lactation, whereas the number of globules/mL of milk decreased significantly only in the last phase of lactation. The fatty acid composition showed only few changes during lactation, which consisted in a progressive decrease in the short chain fatty acids and an increasing trend in the monounsaturated fatty acids. Winter milk showed a significantly larger average diameter, a lower number of fat globules/mL, lower ($P < 0.01$) percentages of short-chain saturated fatty acids and more ($P < 0.01$) long-chain and monounsaturated fatty acids. In addition, significantly lower percentages of C18:0 and higher of palmitoleic, oleic and vaccenic acids were detected in the cooler season. In conclusion the lipid fraction of donkey milk did not show notable changes during lactation.



## 1. Introduction

Lipids have traditionally been considered to play a role in diet-related diseases such as overweight, obesity and other metabolic diseases (diabetes, ischemia, heart disease), which are increasingly widespread nowadays. Appropriate lifestyle and diet play an essential role in the prevention of metabolic diseases (WHO, 2012). However, the optimal amount and type of fat in the diet for the maintenance of good health have not yet been clarified (Melanson et al., 2009). The European Food Safety Authority (EFSA, 2010) recommends in terms of daily intake, a quantity of lipids ranging from 20% and 35% of the energy in the diet and that the intake of saturated fatty acids should be as low as possible. Several milk components such as proteins, calcium, and lactose may affect the lipid metabolism directly or indirectly, however the strongest impact on plasma lipids emerges from the intake of milk fat (Ohlsson, 2010).

Donkey milk is of particular interest in pediatric cases of food allergies (Monti et al., 2007; Vincenzetti et al., 2014), and in mice, the ingestion of donkey milk vs. cow milk helps to maintain a normal weight and normal levels of cholesterol and triglycerides (Lionetti et al., 2012). The diameter of the native fat globules in donkey milk is considerably lower compared to the globules in other milk traditionally used for direct human consumption (Martini et al., 2014). Studies carried out in cows and sheep (Couvreur et al., 2007; Martini et al., 2012) have highlighted relationships between the dimensions of the milk fat globules and the nutritional quality of the milk. In fact, smaller globules have a larger amount of membrane per volume of fat compared to the larger globules. Thus, smaller globules provide a higher surface for digestive enzymes, and this surface is also rich in beneficial components.

The changes in the fatty acid profile of donkey milk as a result of physiological factors such as distance from delivery have been poorly investigated. Nothing is known about the changes that occur in the macrostructure of lipids during lactation. The aim of this study was to evaluate the fatty acid profile and morphometric characteristics of fat globules in Amiata donkey milk, in relation to the lactation phase and the season of production in order to better understand the variability and to study plans to improve the nutritional quality.

## 2. Materials and methods

### 2.1. Animals and sampling

The study was performed on one farm with about 100 jennies reared outdoors with a rest area indoors. A key component of the

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jennies' diet was poliphita hay ad libitum and about 2.5 kg/day/head of concentrate for dairy donkeys. For the study 31 Amiata pluriparous donkeys were selected. The animals delivered seven in winter, seven in autumn, nine in spring and eight in summer. Individual milk samples from the morning milking were carried out monthly starting from 30 days of lactation until the 300th day. The jennies were routinely machine milked and the foals were separated 3–3.5 h before the milking. Milk was refrigerated at 4 °C immediately after the sampling and carried in tanks to the laboratory. No preservatives were added. Morphometric characteristics of the globules were performed on fresh milk in 2–3 h after sampling, whereas an aliquot for each sample was stored at −20 °C for seven days until the fatty acid analysis.

### 2.2. Milk analysis

A direct method, morphometric analysis of milk fat globules (Martini et al., 2013a), was used to determine the diameter (μm) and the number of fat globules per mL of milk in each sample by florescence microscopy. The globules were grouped into three size categories: small globules (SG) with a diameter <2 μm, medium-sized globules (MG) with a diameter from 2 to 5 μm, and large globules (LG) with a diameter >5 μm.

### 2.3. Milk fatty acid profile

A total of 6 mL of each milk sample were subjected to milk fat extraction following Rose-Gottlieb's method, followed by methylation using methanolic sodium methoxide according to Christie (1982). A Perkin Elmer Auto System (Perkin Elmer, Norwolk, CT, USA) equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm; film thickness 0.25 mm; FactorFour Varian, Middelburg, The Netherlands) were used. 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 °C and 300 °C, respectively. Individual fatty acids were identified by comparing their retention times with those of an authenticated standard FA FIM_FAME mix (Restek Corporation, 110 Benner Circle, Bellefonte, PA, USA) and quantified as a percentage of the total FA. The desaturase index was calculated for three pairs of fatty acids representing the products and substrates for Δ9-desaturase: cis-9 14:1/14:0, cis-9 16:1/16:0, cis-9 18:1/18:0 as reported by Kelsey et al. (2003).

### 2.4. Statistical analysis

Milk composition data were analyzed by ANOVA for repeated measurements using JMP software (JMP, 2002), regarding the sampling time (30, 60, 90, 120, 150, 180, 210, 240, 270, 300 days in milk) and the production season (autumn, winter, spring, summer) as fixed effects, and the subject as a random effect. All the stages of lactation were represented in each season.

## 3. Results and discussion

Table 1 shows the changes in the morphometry of the fat globules from Amiata donkey milk during lactation. There are no studies regarding the effect of lactation and production season on the morphometry of the fat globules in donkey milk till today. Despite the findings in ruminants (Martini et al., 2012), in donkey milk the fat percentage and the diameter of fat globules were fairly stable during lactation and the number of globules/mL of milk decreased significantly only at the end of lactation.

Like the macro-structure of lipids, the fatty acid composition showed only few changes during lactation (Table 2). This result is in agreement with the findings of Chiofalo et al. (2005) on Ragusana donkey milk. The only change highlighted in milk fatty acids was the progressive decrease ($P < 0.05$) in the short chain fatty acids, mostly due to the simultaneous decrease in caprylic and capric acids (C8:0–C10:0). A decrease of C8:0–C10:0 during lactation has also been observed in horse and donkey milk (Pikul et al., 2008; Martemucci and D'Alessandro, 2012).

In the last month of lactation there was a significant increase in C17:0. In equidae C17:0 synthesis is assumed to take place in the stomach (Andrews et al., 2005), whereas in ruminants it is synthesized by bacteria in the rumen (Vlaeminck et al., 2006).

Regarding the monounsaturated fatty acids, increasing trends were highlighted for C14:1, C15:1, C16:1, C17:1 with advancing lactation. These trends are associated with significant increases in C16 delta 9 desaturase index after 90 days and have also been observed in donkey milk by other authors (Martemucci and D'Alessandro, 2012). Delta 9 desaturase indexes evaluate the activity of stearoyl-CoA desaturase enzyme (or delta 9 desaturase enzyme) which desaturates the saturated fatty acids by catalyzing the insertion of a double bond between carbon atoms 9 and 10 of a fatty acid (Pereira et al., 2003).

Table 3 shows that the fat percentage did not change during the year, however a similar inverse relation between the diameter and the number of fat globules was found to that reported in ruminants (Martini et al., 2013b).

The results showed that in winter the milk fat globules were significantly larger due to a decrease ($P < 0.01$) in globules smaller than 2 μm (SG), and an increase ($P < 0.05$) in those larger than 2 μm (MG and LG).

Regarding classes of fatty acids, there were more variations in winter compared to the other seasons. Table 4 shows that lower percentages of short chains and saturated fatty acids ($P < 0.01$) and higher long chains and monounsaturated fatty acids were found in winter milk ($P < 0.01$). According to some authors, increases in monounsaturated vs. saturated fatty acids are desirable for human health (Nicklas et al., 2004; Ohlsson, 2010). The changes in the

**Table 1**
Effect of lactation on the morphometric characteristics of donkey milk fat globules.

| | Days in milk | | | | | | | | | | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | |
| Fat (%) | 0.42 | 0.35 | 0.34 | 0.42 | 0.43 | 0.41 | 0.44 | 0.46 | 0.44 | 0.35 | 0.301 |
| Globules/mL (No.*$10^9$) | $2.43^{A}$ | $1.76^{A}$ | $2.32^{A}$ | $1.78^{A}$ | $2.01^{A}$ | $1.27^{AB}$ | $1.14^{AB}$ | $1.08^{AB}$ | $0.71^{B}$ | $0.67^{B}$ | 1.254 |
| Mean diameter (μm) | 2.16 | 1.92 | 2.00 | 1.91 | 1.97 | 2.10 | 2.10 | 2.27 | 2.38 | 2.62 | 0.669 |
| SG (%) | 60.84 | 70.91 | 69.68 | 69.682 | 70.10 | 63.46 | 61.29 | 58.45 | 58.90 | 54.13 | 18.228 |
| MG (%) | 34.07 | 25.78 | 26.45 | 27.55 | 25.57 | 31.27 | 34.72 | 34.75 | 32.25 | 33.56 | 14.762 |
| LG (%) | 5.09 | 3.310 | 3.87 | 2.78 | 4.33 | 5.26 | 3.99 | 6.80 | 8.85 | 12.31 | 8.405 |

A, B: values within raw sharing a common superscript number are not significantly different ($P < 0.01$). *Abbreviations*: SG, small globules (<2 μm); MG, medium globules (between 2 and 5 μm); LG, large globules (>5 μm).