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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 a 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

23 cooler season. In conclusion the lipid fraction of donkey milk did not show notable changes during  
24 lactation.

25 *Keywords:* Food analysis; Food composition; Amiata donkey; Donkey milk nutritional quality;  
26 Lactation; Seasonal changes in milk; Fatty acids; Milk fat globules

## 27 **1 Introduction**

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

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

46 globules. Thus, smaller globules provide a higher surface for digestive enzymes, and this surface is  
47 also rich in beneficial components.

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

## 54 **2 Materials and methods**

### 55 **2.1 Animals and sampling**

56 The study was performed on one farm with about 100 jennies reared outdoors with a rest area  
57 indoors. A key component of the jennies' diet was poliphita hay ad libitum and about 2.5  
58 kg/day/head of concentrate for dairy donkeys. For the study 31 Amiata pluriparous donkeys were  
59 selected. The animals delivered seven in winter, seven in autumn, nine in spring and eight in  
60 summer. Individual milk samples from the morning milking were carried out monthly starting from  
61 30 days of lactation until the 300th day. The jennies were routinely machine milked and the foals  
62 were separated 3-3.5h before the milking. Milk was refrigerated at 4°C immediately after the  
63 sampling and carried in tanks to the laboratory. No preservatives were added. Morphometric  
64 characteristics of the globules were performed on fresh milk in 2-3 hours after sampling, whereas an  
65 aliquot for each sample was stored at -20°C for seven days until the fatty acid analysis.

### 66 **2.2 Milk analysis**

67 A direct method, morphometric analysis of milk fat globules (Martini et al., 2013a), was used to  
68 determine the diameter ( $\mu\text{m}$ ) and the number of fat globules per mL of milk in each sample by  
69 fluorescence microscopy. The globules were grouped into three size categories: small globules (SG)

70 with a diameter  $< 2 \mu\text{m}$ , medium-sized globules (MG) with a diameter from 2 to  $5 \mu\text{m}$ , and large  
71 globules (LG) with a diameter  $> 5 \mu\text{m}$ .

### 72 **2.3 Milk fatty acid profile**

73 A total of 6 mL of each milk sample were subjected to milk fat extraction following Rose-Gottlieb's  
74 method, followed by methylation using methanolic sodium methoxide according to Christie (1982).  
75 A Perkin Elmer Auto System (Perkin Elmer, Norwalk, CT, USA) equipped with a flame ionization  
76 detector and a capillary column ( $30 \text{ m} \times 0.25 \text{ mm}$ ; film thickness  $0.25 \mu\text{m}$ ; FactorFour Varian,  
77 Middelburg, The Netherlands) were used. The helium carrier gas flow rate was  $1 \text{ mL} \cdot \text{min}^{-1}$ . The  
78 oven temperature program was as follows: level 1,  $50^\circ\text{C}$  held for 2 min, level 2,  $50$  to  $180^\circ\text{C}$  at  
79  $2^\circ\text{C} \cdot \text{min}^{-1}$  then held for 20 min, level 3,  $180$  to  $200^\circ\text{C}$  at  $1^\circ\text{C} \cdot \text{min}^{-1}$  then held for 15 min, and finally  
80 level 4,  $200$  to  $220^\circ\text{C}$  at  $1^\circ\text{C} \cdot \text{min}^{-1}$  then held for 30 min. The injector and detector temperatures  
81 were set at  $270^\circ\text{C}$  and  $300^\circ\text{C}$ , respectively. Individual fatty acids were identified by comparing their  
82 retention times with those of an authenticated standard FA FIM\_FAME mix (Restek Corporation,  
83 110 Benner Circle, Bellefonte, PA, USA) and quantified as a percentage of the total FA. The  
84 desaturase index was calculated for three pairs of fatty acids representing the products and  
85 substrates for  $\Delta 9$ -desaturase: cis-9 14:1/14:0, cis-9 16:1/16:0, cis-9 18:1/18:0 as reported by Kelsey  
86 et al. (2003).

### 87 **2.4 Statistical analysis**

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

### 92 **3 Results and discussion**

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

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

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

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

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

118 The results showed that in winter the milk fat globules were significantly larger due to a decrease (P  
119 <0.01) in globules smaller than 2 microns (SG), and an increase (P <0.05) in those larger than 2  
120 microns (MG and LG).

121 Regarding classes of fatty acids, there were more variations in winter compared to the other  
122 seasons. Table 4 shows that lower percentages of short chains and saturated fatty acids (P <0.01)  
123 and higher long chains and monounsaturated fatty acids were found in winter milk (P <0.01).

124 According to some authors, increases in monounsaturated vs saturated fatty acids are desirable for  
125 human health (Nicklas et al., 2004; Ohlsson, 2010). The changes in the saturated fatty acids were  
126 mostly due to the lower amount of short chain fatty acids. Of the medium chains, the decrease in  
127 C12: 0 and C14: 0 is considered positive for the milk nutritional value. In fact C12: 0 and C14: 0  
128 are notoriously considered hypercholesterolemic (Ohlsson, 2010). In addition in winter there were  
129 higher amounts of C16: 0. In any case, milk palmitic acid made up a significant proportion of the  
130 saturated medium chain followed by 14: 0, both in donkey and in mare milk (Pikul et al., 2008).

131 In the colder season significantly lower percentages of C18: 0 were also observed. Stearic acid  
132 improves the plasma profile by decreasing total cholesterol/HDL cholesterol ratio compared to  
133 other SFA, while palmitic acid increases plasma cholesterol and LDL more than HDL cholesterol.

134 To our knowledge there have been no studies in donkeys on the effect of season on the donkey milk  
135 fatty acid profile. If we also consider the studies on ruminants, the saturated fatty acids are higher in  
136 autumn and winter both in cow and sheep milk (Martini et al., 2008; Lopez et al., 2014).

137 During the winter, increases were detected mainly for palmitoleic, oleic and vaccenic acids. Oleic  
138 and vaccenic acids are reported to have beneficial effects for human health. In fact, oleic acid has a

139 cholesterol- and triglycerides-lowering effect compared with SFA, whereas vaccenic acid (VA) is a  
140 positional and geometric isomer of oleic acid. VA is also the major trans fatty acid in milk and the  
141 only known dietary precursor of c9, t11 conjugated linoleic acid (CLA). Scientific data suggest that  
142 VA consumption from dairy products is as beneficial to human health as CLA (Field et al., 2009).  
143 Delta 9 desaturase indices were in line with these observations, and higher C16 and C18 indices  
144 were observed in the colder season.

#### 145 **4 Conclusions**

146 In our study, the lipid fraction of Amiata donkey milk did not show notable changes during  
147 lactation. The significant changes happened in the winter period. These findings could help milk  
148 producers to obtain a stable product for the market. Further studies are needed to evaluate the  
149 possibility of changing the quality of milk lipids by acting on other factors, such as diet, which is  
150 known to strongly influence the fatty acid composition of non-ruminant milk. Given the low fat and  
151 the low saturated fatty acids, donkey milk has an additional important benefit: it may help prevent  
152 the onset of obesity and chronic diseases, with a consequent significant economic and social impact.

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#### 156 **References**

- 157 Andrews, F. M., Buchanan, B. R., Elliot, S. B., Clariday, N. A., Edwards, L. H. (2005). Gastric  
158 ulcers in horses. *Journal of Animal Science*, 83, 18–21.
- 159 Chiofalo, B., Polidori, M., Costa, R., Salimei, E. (2005). Fresh forage in dairy ass's ration effect on  
160 milk fatty acid composition and flavours. *Italian Journal of Animal Science*, 4, 433- 435.



- 161 Christie, W. W. (1982). A simple procedure of rapid transmethylation of glycerolipids and  
162 cholesteryl esters. *Journal of Lipid Research*, 23, 1072-1075.
- 163 Couvreur, S., Hurtaud, C., Marnet, P. G., Faverdin, P., Peyraud, J. L. (2007). Composition of milk  
164 fat from cows selected for milk fat globule size and offered either fresh pasture or a corn  
165 silage-based diet. *Journal of Dairy Science*, 90, 392–403.
- 166 EFSA (European Food Safety Authority) (2010). Scientific Opinion on Dietary Reference Values  
167 for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty  
168 acids, trans fatty acids, and cholesterol. *EFSA Journal*, 8, 1461.
- 169 Field, C. J., Blewett, H. H., Proctor, S., Vine, D. (2009). Human health benefits of vaccenic acid.  
170 *Applied Physiology, Nutrition, and Metabolism*, 34, 979–991.
- 171 JMP, 2002. User's guide, version 5.0. SAS. Inst. Inc., Cary, NC, USA.
- 172 Kelsey, J. A., Corl, B. A., Collier, R. J., Bauman, D.E. (2003). The Effect of Breed, Parity, and  
173 Stage of Lactation on Conjugated Linoleic Acid (CLA) in Milk Fat from Dairy Cows.  
174 *Journal of Dairy Science*, 86, 2588-2597.
- 175 Lionetti, L., Cavaliere, G., Bergamo, P., Trinchese, G., De Filippo, C., Gifuni, G., Gaita, M.,  
176 Pignalosa, A., Donizzetti, I., Putti, R., Di Palo, R., Barletta, A., Mollica, M. P. (2012). Diet  
177 supplementation with donkey milk upregulates liver mitochondrial uncoupling, reduces  
178 energy efficiency and improves antioxidant and antiinflammatory defences in rats. *Molecular  
179 nutrition & food research*, 56, 1596–1600.
- 180 Lopez, C., Briard-Bion V., Ménard, O. (2014). Polar lipids, sphingomyelin and long-chain  
181 unsaturated fatty acids from the milk fat globule membrane are increased in milks produced  
182 by cows fed fresh pasture based diet during spring. *Food Research International*, 58, 59–68.

- 183 Martemucci, G., D'Alessandro, A. G. (2012). Fat content, energy value and fatty acid profile of  
184 donkey milk during lactation and implications for human nutrition. *Lipids in Health and*  
185 *Disease, 11*, 113.
- 186 Martini, M., Altomonte, I., Salari, F. (2012). Relationship between the nutritional value of fatty acid  
187 profile and the morphometric characteristics of milk fat globules in ewe's milk. *Small*  
188 *Ruminant Research, 105*, 33-37.
- 189 Martini, M., Salari, F., Altomonte, I. (2013a) The macrostructure of milk lipids: the fat globules.  
190 *Critical Reviews in Food Science and Nutrition* 10.1080/10408398.2012.758626
- 191 Martini, M., Salari, F., Altomonte, I. (2013b). Evaluation of the fatty acid profile from the core and  
192 membrane of fat globules in ewe's milk during lactation. *Food Science and Technology, 50*,  
193 253-258.
- 194 Martini, M., Altomonte, I., Salari, F. (2014). Amiata donkeys: fat globule characteristics, milk gross  
195 composition and fatty acids. *Italian Journal of Animal Science, 13*, 123-126.
- 196 Melanson, E. L., Astrup, A., Donahoo, W. T. (2009). The Relationship between Dietary Fat and  
197 Fatty Acid Intake and Body Weight, Diabetes, and the Metabolic Syndrome. *Annals of*  
198 *Nutrition and Metabolism, 55*, 229-243.
- 199 Monti, G., Bertino, E., Muratore, M. C., Coscia, A., Cresi, F., Silvestro, L., Fabris, C., Fortunato,  
200 D., Giuffrida, M. G., Conti, A. (2007). Efficacy of donkey's milk in treating highly  
201 problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatric Allergy and*  
202 *Immunology, 18*, 258-264.
- 203 Nicklas, T. A., Demory-Luce, D., Yang, S. J., Baranowski, T., Zakeri, I., Berenson, G. (2004).  
204 Children's food consumption patterns have changed over two decades (1973-1994): The  
205 Bogalusa heart study. *Journal of the American Dietetic Association, 104*, 1127-1140.

- 206 Ohlsson, L. (2010). Dairy products and plasma cholesterol levels. *Food & Nutrition Research*, 54,  
207 5124.
- 208 Pereira, S. L., Leonard, A. E., Mukerji, P. (2003). Recent advances in the study of fatty acid  
209 desaturases from animals and lower eukaryotes. *Prostaglandins, Leukotrienes and Essential*  
210 *Fatty Acids*, 68, 97–106
- 211 Pikul, J., Wójtowski, J., Danków, R., Kuczyńska, B., Łojek, J. (2008). Fat content and fatty acids  
212 profile of colostrum and milk of primitive Konik horses (*Equus caballus gmelini* Ant.) during  
213 six months of lactation. *Journal of Dairy Research*, 75, 302–309.
- 214 Vincenzetti, S., Foghini, L., Pucciarelli, S., Polzonetti, V., Cammertoni, N., Beghelli, D., Polidori,  
215 P. (2014). Hypoallergenic properties of donkey milk: a preliminary study. *Veterinaria*  
216 *Italiana*, 50, 99-107.
- 217 WHO (2012). Obesity and overweight. Fact sheet N°311 <http://www.who.int>
- 218 Vlaeminck, B., Fievez, V., Tamminga, S., Dewhurst, R. J., Van Vuuren, A. M., De Brabander, D.,  
219 Demeyer, D. (2006). Milk odd- and branched-chain fatty acids in relation to the rumen  
220 fermentation pattern. *Journal of Dairy Science*, 89, 3954–3964.

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	(n*10 <sup>9</sup> )	2.43 <sup>A</sup>	1.76 <sup>A</sup>	2.32 <sup>A</sup>	1.78 <sup>A</sup>	2.01 <sup>A</sup>	1.27 <sup>AB</sup>	1.14 <sup>AB</sup>	1.08 <sup>AB</sup>	0.71 <sup>B</sup>	0.67 <sup>B</sup>	1.254
Mean Diameter	( $\mu$ m)	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 row sharing a common superscript number are not significantly different ( $P < 0.01$ )

Abbreviations : SG: small globules ( $<2 \mu$ m); MG: medium globules (between 2 and 5  $\mu$ m); LG: large globules ( $>5 \mu$ m).

Table 2- Effect of lactation on donkey milk fatty acids

	Days in Milk										SEM
	30	60	90	120	150	180	210	240	270	300	
C4:0	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.031
C6:0	0.26	0.27	0.27	0.25	0.23	0.23	0.21	0.22	0.23	0.20	0.086
C8:0	4.03 <sup>A</sup>	4.08 <sup>A</sup>	3.78 <sup>A</sup>	3.36 <sup>AB</sup>	3.01 <sup>AB</sup>	2.89 <sup>B</sup>	2.54 <sup>B</sup>	2.64 <sup>B</sup>	3.06 <sup>AB</sup>	2.45 <sup>B</sup>	1.027
C10:0	9.46 <sup>a</sup>	9.80 <sup>a</sup>	8.49 <sup>ab</sup>	7.05 <sup>ab</sup>	6.32 <sup>b</sup>	6.10 <sup>b</sup>	6.10 <sup>b</sup>	6.08 <sup>b</sup>	6.51 <sup>ab</sup>	5.99 <sup>b</sup>	2.604
C11:0	1.22	0.91	0.73	0.78	0.74	0.83	1.04	0.88	0.99	1.03	0.500
C12:0	8.26	8.61	7.01	6.10	5.34	5.30	5.70	5.86	6.17	5.98	2.594
C13:0	0.02	0.03	0.03	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.022
C14:0	6.36	6.54	5.36	5.01	4.61	4.73	4.58	5.32	5.41	5.75	1.683
C14:1	0.36 <sup>a</sup>	0.28 <sup>b</sup>	0.23 <sup>c</sup>	0.28 <sup>b</sup>	0.27 <sup>b</sup>	0.30 <sup>b</sup>	0.34 <sup>b</sup>	0.40 <sup>a</sup>	0.36 <sup>a</sup>	0.42 <sup>a</sup>	0.134
C15:0	0.28	0.28	0.30	0.34	0.35	0.36	0.35	0.61	0.39	0.52	0.378
C15:1	0.11 <sup>C</sup>	0.13 <sup>BC</sup>	0.13 <sup>BC</sup>	0.16 <sup>B</sup>	0.16 <sup>B</sup>	0.16 <sup>B</sup>	0.17 <sup>B</sup>	0.18 <sup>AB</sup>	0.15 <sup>B</sup>	0.21 <sup>A</sup>	0.059
C16:0	21.25	20.76	20.65	20.87	20.68	22.07	21.12	22.50	20.75	22.82	2.941
C16:1	3.94 <sup>a</sup>	2.96 <sup>ab</sup>	2.78 <sup>b</sup>	3.71 <sup>ab</sup>	4.18 <sup>a</sup>	4.45 <sup>a</sup>	4.03 <sup>a</sup>	4.85 <sup>a</sup>	3.81 <sup>ab</sup>	4.13 <sup>a</sup>	1.709
C17:0	0.23 <sup>B</sup>	0.25 <sup>B</sup>	0.24 <sup>B</sup>	0.22 <sup>B</sup>	0.22 <sup>B</sup>	0.22 <sup>B</sup>	0.20 <sup>B</sup>	0.22 <sup>B</sup>	0.21 <sup>B</sup>	0.30 <sup>A</sup>	0.072
C17:1	0.37 <sup>B</sup>	0.35 <sup>B</sup>	0.31 <sup>B</sup>	0.36 <sup>B</sup>	0.40 <sup>B</sup>	0.43 <sup>B</sup>	0.39 <sup>B</sup>	0.44 <sup>B</sup>	0.36 <sup>B</sup>	0.53 <sup>A</sup>	0.133
C18:0	1.85	1.90	2.06	1.94	1.84	1.74	1.73	1.58	1.66	1.78	0.39
C18:1 <i>trans</i> -9	0.03	0.02	0.02	0.03	0.04	0.03	0.03	0.11	0.03	0.03	0.125
C18:1 <i>trans</i> -11	1.24	0.93	1.09	1.15	1.23	1.31	1.12	1.28	1.14	1.27	0.417
C18:1 <i>cis</i> -9	20.67	22.14	24.61	25.41	26.13	26.53	23.88	25.96	25.95	23.26	4.500
C18:2 <i>trans</i> -9,12	0.03	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.027
C18:2 <i>cis</i> -9,12	11.52	12.79	14.80	14.73	13.79	13.30	13.16	12.97	15.26	13.80	4.54
C18:3n3	0.28	0.30	0.33	0.28	0.30	0.30	0.26	0.28	0.27	0.26	0.07
C18:3 n6	0.03 <sup>B</sup>	0.03 <sup>B</sup>	0.03 <sup>B</sup>	0.03 <sup>B</sup>	0.04 <sup>AB</sup>	0.03 <sup>B</sup>	0.04 <sup>AB</sup>	0.04 <sup>AB</sup>	0.04 <sup>AB</sup>	0.05 <sup>A</sup>	0.021
C20:0	0.03	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.022
C20:1	7.53 <sup>b</sup>	5.80 <sup>b</sup>	5.86 <sup>b</sup>	7.13 <sup>b</sup>	9.20 <sup>a</sup>	7.79 <sup>ab</sup>	7.98 <sup>a</sup>	6.38 <sup>b</sup>	6.44 <sup>b</sup>	8.09 <sup>a</sup>	4.225
C21:0	0.03	0.04	0.05	0.04	0.09	0.05	0.04	0.05	0.04	0.04	0.081
C20:2	0.15	0.17	0.17	0.17	0.172	0.16	0.17	0.16	0.18	0.15	0.063
C20:3n3	0.19	0.22	0.15	0.19	0.21	0.18	0.18	0.18	0.16	0.18	0.134
C20:3 n6	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.015
C22:0	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.02	0.017
C22:1	0.03	0.03	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.024
C20:4	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.03	0.019
C23:0	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.012
C22:2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.008
C20:5	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.010
C24:0	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.014
C24:1	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.015
C22:5	0.01	0.01	0.02	0.01	0.02	0.03	0.04	0.04	0.02	0.05	0.044
C22:6	0.03	0.04	0.05	0.07	0.05	0.06	0.08	0.06	0.05	0.07	0.041
SCFA ( $\leq$ C10)	13.81 <sup>ab</sup>	14.21 <sup>a</sup>	12.68 <sup>ab</sup>	10.69 <sup>ab</sup>	9.65 <sup>b</sup>	9.32 <sup>b</sup>	9.23 <sup>b</sup>	9.23 <sup>b</sup>	9.85 <sup>b</sup>	8.98 <sup>b</sup>	3.628
MCFA ( $\geq$ C11 $\leq$ C17)	42.41	41.15	37.78	37.84	36.98	38.88	41.81	41.45	38.62	41.70	5.756
LCFA ( $\geq$ C18)	43.78	44.64	49.54	51.47	53.37	51.80	48.95	49.32	51.52	49.32	7.054
SFA	53.38	53.67	49.21	46.11	43.64	44.74	47.98	46.52	45.59	47.30	7.499
MUFA	34.32	32.67	35.11	38.29	41.68	41.08	38.01	39.67	38.31	38.02	7.344
PUFA	12.30	13.65	15.68	15.60	14.69	14.18	14.01	13.81	16.11	14.68	4.587
UFA/SFA	0.90	0.94	1.09	1.20	1.34	1.29	1.22	1.25	1.23	1.16	0.320
n3/n6	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.05	0.04	0.05	0.018
Desaturase C14 index	0.05	0.04	0.05	0.05	0.06	0.07	0.08	0.07	0.06	0.07	0.034
Desaturase C16 index	0.15 <sup>A</sup>	0.11 <sup>AB</sup>	0.11 <sup>B</sup>	0.14 <sup>A</sup>	0.16 <sup>A</sup>	0.16 <sup>A</sup>	0.15 <sup>A</sup>	0.17 <sup>A</sup>	0.14 <sup>A</sup>	0.15 <sup>A</sup>	0.046
Desaturase C18 index	0.90	0.92	0.92	0.92	0.93	0.94	0.93	0.94	0.94	0.93	0.028

A, B. Values within row sharing a common superscript number are not significantly different ( $P < 0.01$ )

a, c. Values within row sharing a common superscript number are not significantly different ( $P < 0.05$ )

Abbreviations : SCFA : Short Chain Fatty Acids; MCFA: Medium Chain Fatty Acids; LCFA: Long Chain Fatty Acids; SFA: Saturated Fatty Acids; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids; UFA/SFA: unsaturated fatty acids/saturated fatty acids; Desaturase C14 index:  $[C14:1]/[C14:1+C14:0]$ ; Desaturase C16 index:  $[C16:1]/[C16:1+C16:0]$ ; Desaturase C18 index:  $[C18:1c9]/[C18:1c9+C18:0]$ .

Table 3- Effect of the season on the morphometric characteristics of donkey milk fat globules

		Season				SEM
		Autumn	Winter	Spring	Summer	
Fat	%	0.43	0.56	0.53	0.39	0.301
Globules/ml	(n <sup>o</sup> *10 <sup>9</sup> )	2.03 <sup>A</sup>	0.62 <sup>B</sup>	1.36 <sup>A</sup>	2.08 <sup>A</sup>	1.250
Mean Diameter	( $\mu$ m)	1.90 <sup>B</sup>	2.44 <sup>A</sup>	2.12 <sup>B</sup>	1.84 <sup>B</sup>	0.669
SG	(%)	71.71 <sup>A</sup>	54.43 <sup>B</sup>	63.09 <sup>A</sup>	73.09 <sup>A</sup>	18.228
MG	(%)	25.09 <sup>B</sup>	36.68 <sup>Aa</sup>	31.17 <sup>ABb</sup>	25.21 <sup>ABb</sup>	14.762
LG	(%)	3.20 <sup>b</sup>	7.37 <sup>a</sup>	5.74 <sup>b</sup>	1.70 <sup>b</sup>	8.405

A, B. Values within row sharing a common superscript number are not significantly different (P<0.01)

a, b. Values within row sharing a common superscript number are not significantly different (P<0.05)

Abbreviations : SG: small globules (<2  $\mu$ m); MG: medium globules (between 2 and 5  $\mu$ m); LG: large globules (>5  $\mu$ m).

Table 4- Effect of the season on donkey milk fatty acids

	Season				SEM
	Autumn	Winter	Spring	Summer	
C4:0	0.03 <sup>B</sup>	0.03 <sup>B</sup>	0.05 <sup>A</sup>	0.04 <sup>B</sup>	0.025
C6:0	0.25 <sup>Ab</sup>	0.20 <sup>B</sup>	0.27 <sup>Aa</sup>	0.27 <sup>Aa</sup>	0.075
C8:0	3.39 <sup>Ab</sup>	2.60 <sup>B</sup>	3.53 <sup>Aab</sup>	4.35 <sup>A</sup>	1.021
C10:0	7.14 <sup>B</sup>	5.28 <sup>C</sup>	7.90 <sup>AB</sup>	10.32 <sup>A</sup>	2.603
C11:0	0.94 <sup>a</sup>	0.77 <sup>b</sup>	1.03 <sup>a</sup>	0.95 <sup>a</sup>	0.470
C12:0	6.27 <sup>B</sup>	4.75 <sup>C</sup>	7.33 <sup>AB</sup>	8.97 <sup>A</sup>	2.594
C13:0	0.024	0.02	0.03	0.02	0.019
C14:0	5.05 <sup>A</sup>	4.50 <sup>B</sup>	6.10 <sup>A</sup>	6.63 <sup>A</sup>	1.676
C14:1	0.30	0.34	0.34	0.27	0.128
C15:0	0.32	0.44	0.41	0.29	0.369
C15:1	0.16	0.17	0.17	0.13	0.045
C16:0	20.52 <sup>B</sup>	22.54 <sup>A</sup>	21.18 <sup>B</sup>	20.18 <sup>B</sup>	2.941
C16:1	3.74 <sup>B</sup>	5.01 <sup>A</sup>	3.10 <sup>C</sup>	2.29 <sup>C</sup>	1.697
C17:0	0.22 <sup>Ba</sup>	0.20 <sup>Bb</sup>	0.28 <sup>A</sup>	0.25 <sup>A</sup>	0.067
C17:1	0.38 <sup>B</sup>	0.45 <sup>A</sup>	0.39 <sup>B</sup>	0.29 <sup>B</sup>	0.126
C18:0	1.89 <sup>A</sup>	1.63 <sup>B</sup>	1.92 <sup>A</sup>	2.02 <sup>A</sup>	0.388
C18:1 <i>trans</i> -9	0.03	0.05	0.03	0.03	0.122
C18:1 <i>trans</i> -11	1.17 <sup>AB</sup>	1.40 <sup>A</sup>	1.09 <sup>B</sup>	0.79 <sup>B</sup>	0.411
C18:1 <i>cis</i> -9	25.56 <sup>B</sup>	27.52 <sup>A</sup>	21.75 <sup>C</sup>	20.07 <sup>C</sup>	4.500
C18:2 <i>trans</i> -9,12	0.02	0.02	0.01	0.01	0.020
C18:2 <i>cis</i> -9,12	13.33	14.39	12.72	13.65	4.54
C18:3n3	0.31 <sup>a</sup>	0.28 <sup>a</sup>	0.25 <sup>b</sup>	0.29 <sup>a</sup>	0.068
C18:3 n6	0.03	0.04	0.03	0.03	0.015
C20:0	0.037	0.03	0.04	0.04	0.016
C20:1	6.80 <sup>A</sup>	6.51 <sup>B</sup>	9.38 <sup>A</sup>	7.05 <sup>A</sup>	4.224
C21:0	0.06	0.04	0.04	0.04	0.084
C20:2	0.16 <sup>b</sup>	0.16 <sup>b</sup>	0.16 <sup>b</sup>	0.19 <sup>a</sup>	0.061
C20:3n3	0.17	0.18	0.20	0.20	0.135
C20:3 n6	0.03	0.04	0.03	0.03	0.015
C22:0	0.03	0.02	0.03	0.03	0.017
C22:1	0.04	0.05	0.04	0.04	0.024
C20:4	0.04	0.03	0.04	0.05	0.02
C23:0	0.02	0.02	0.02	0.02	0.012
C22:2	0.01	0.01	0.01	0.01	0.008
C20:5	0.01 <sup>B</sup>	0.01 <sup>B</sup>	0.01 <sup>B</sup>	0.02 <sup>A</sup>	0.010
C24:0	0.01	0.01	0.02	0.02	0.014
C24:1	0.02 <sup>B</sup>	0.02 <sup>B</sup>	0.02 <sup>B</sup>	0.03 <sup>A</sup>	0.015
C22:5	0.01	0.04	0.03	0.01	0.044
C22:6	0.06	0.07	0.04	0.05	0.041
SCFA ( $\leq$ C10)	10.80 <sup>B</sup>	8.11 <sup>C</sup>	11.76 <sup>AB</sup>	14.98 <sup>A</sup>	3.628
MCFA ( $\geq$ C11 $\leq$ C17)	39.39	39.31	40.34	40.30	5.756
LCFA ( $\geq$ C18)	49.80 <sup>A</sup>	52.58 <sup>A</sup>	47.90 <sup>B</sup>	44.72 <sup>B</sup>	7.055
SFA	47.63 <sup>A</sup>	43.14 <sup>B</sup>	50.16 <sup>A</sup>	54.47 <sup>A</sup>	7.500
MUFA	38.19 <sup>AB</sup>	41.59 <sup>A</sup>	36.29 <sup>B</sup>	30.99 <sup>B</sup>	7.344
PUFA	14.18	15.27	13.55	14.54	4.588
UFA/SFA	1.17 <sup>B</sup>	1.38 <sup>A</sup>	1.03 <sup>B</sup>	0.89 <sup>B</sup>	0.320
n3/n6	0.04	0.05	0.04	0.04	0.019
Desaturase C14 index	0.06	0.07	0.05	0.04	0.034
Desaturase C16 index	0.15 <sup>A</sup>	0.17 <sup>A</sup>	0.12 <sup>B</sup>	0.10 <sup>B</sup>	0.046
Desaturase C18 index	0.93 <sup>B</sup>	0.94 <sup>A</sup>	0.92 <sup>BC</sup>	0.90 <sup>C</sup>	0.028

A-C. Values within raw sharing a common superscript number are not significantly different ( $P < 0.01$ )

a, b. Values within raw sharing a common superscript number are not significantly different ( $P < 0.05$ )

Abbreviations: SCFA: Short Chain Fatty Acids; MCFA: Medium Chain Fatty Acids; LCFA: Long Chain Fatty Acids; SFA: Saturated Fatty Acids; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids; UFA/SFA: unsaturated fatty acids/saturated fatty acids; ; Desaturase C14 index:  $[C14:1]/[C14:1+C14:0]$ ; Desaturase C16 index:  $[C16:1]/[C16:1+C16:0]$ ; Desaturase C18 index:  $[C18:1c9]/[C18:1cis\ 9+C18:0]$ .

## Highlights

- Donkey milk quality during lactation and production season was investigated
- Fat percentage and the diameter of milk fat globules were stable during lactation.
- Short chain fatty acids decreased whereas monounsaturated increased during lactation
- In winter milk, a larger diameter of the globules, and a lower number/mL were found
- Lower short chains and saturated were found in winter

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