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Transcriptomic analysis of two sheep breeds during lactation, using a new custom microarray platform

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Temporal changes of conjugated linoleic acid in milk from Sarda ewes with different milk fat secretion ability

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ABSTRACT - Individual milk samples were weekly collected from 48 ewes during eight weeks. Ewes were at the second month of lactation and they were fed the same dietary regimen. The animals were grouped according to the amount of daily milk fat yield: A) 38-57g/d, B) 58-63g/d, C) 64-73g/d, D) 75-110g/d. Milk from group D showed a higher content of FA from C4 to C10 and a lower content of monounsaturated FA (MUFA) and conjugated linoleic acids (CLA) than Group A (+12%, -11%, and -18%, for C4 to C10, MUFA and CLA, respectively), while Group B and C showed intermediate values. CLA content increased with days of lactation in a similar way for all groups. The mean correlation among all records within each individual lactation was 0.45 for CLA content. Six milk FA pair ratios representing a proxy for SCD activity were also evaluated: group A showed higher values of FA ratios than Group D (+26% on average for all FA pairs). In conclusion, increasing ability of daily milk fat yield was associated with lower milk content of MUFA and CLA and higher content of FA from C4 to C10.

Key words: Dairy sheep, CLA, Milk, Stage of lactation.

Introduction - In the last decade, several studies aimed at finding efficient strategies to improve the nutritional quality of milk fat concluded that feeding supplementation is the most efficient way to modify milk FA composition (Antongiovanni *et al.*, 2003). However, factors other than diet may significantly affect milk FA composition: stage of lactation, genetic level of the animals and polymorphisms of some genes involved in lipid metabolism (Mele *et al.*, 2007). Milk FA are synthesized either from FA which are taken up from the blood (60%) or by de novo synthesis in mammary gland (40%). Preformed FA arise either from plasma NEFA (especially when the energy balance of the lactating animal is negative) or from dietary FA. Stage of lactation and diet are the main factors which affect the availability of preformed FA. Milk fat represents the more than one half of the cost of milk synthesis (Bauman *et al.*, 1985), mainly due to energy requirements for FA neosynthesis and desaturation. The extent of mammary FA neo-synthesis may depend to the energy partitioning after parturition. Aim of the study was to evaluate how differences in yielding ability for milk fat affects milk FA composition in a group of Sarda dairy ewes fed the same diet, at the same lactation stage.

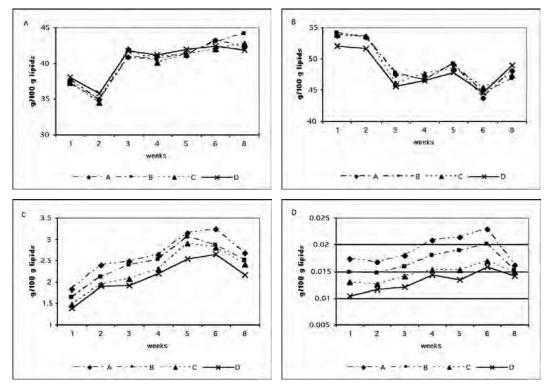
Material and methods - Individual milk samples were weekly collected from 48 Sarda ewes during eight weeks, from March to April. Ewes were at the second month of lactation and they were fed the same diet. The ewes grazed a mixture of *Avena sativa*, *Lolium italicum* and *Trifolium repens* for 6 hour/d on average. A commercial concentrate was also administrated during milking (500 g/d). Milk samples were analysed for protein and fat content by Milkoscan equipment, and for FA composition by gas-chromatograph. At the end of the experiment, the ewes were grouped according to the average amount of daily milk fat yield, taking into consideration data collected in the whole experiment. Four groups of 12 ewes were identified according to the average daily milk fat yield: A: 38-57 g/d; B: 58-63 g/d; C: 64-73 g/d; D: 75-110 g/d. Data of milk FA

composition and FA ratios were analysed by a repeated measure model including group of fat yield (4 levels), week of sampling (8 levels) as fixed factors and ewe nested group of fat yield as random factor. The interaction between group of fat yield and week of sampling was also tested.

Results and conclusions – As expected, group D produced more fat than the A group (46.66, 59.35, 68.10 and 90.60 for group A, B, C and D, respectively). Also milk yield (0.82, 1.06, 1.25 and 1.62 for group A, B, C and D, respectively) and protein (50.99, 62.78, 70.14, and 90.81 for group A, B, C, and D, respectively) were higher for group D, whereas milk fat and protein contents were higher for group A. In any case, groups B and C showed intermediate values. The temporal changes of preformed and neo-synthesized milk FA showed a similar pattern for all experimental groups (figure 1A and 1B). Neo-synthesized FA, in fact, tend to increase during lactation, while preformed FA tend to decrease (Kay *et al.*, 2005). The content of CLA increased during the first seven weeks and decreased at the eighth week for all groups, and the hierarchical distribution among groups was maintained across the 8 weeks of the experiment (figure 1C).

Increasing of CLA content in milk during lactation was previously reported by Signorelli *et al.* (2008) in sheep and by Secchiari *et al.* (2003) and Kay *et al.* (2005) in dairy cows. The ratio C14:1/C14, which is a proxy for SCD activity, showed a trend similar to CLA (figure 1D). Recently, Soyeurt *et al.* (2008) reported that C14:1/C14 ratio increased during lactation in dairy cows. The mean correlation among all records within each individual lactation was 0.45 for CLA content. This value felled in the range reported by Soyeurt *et al.* (2006) for the repeatability of FA in milk of dairy cows (from 0.4 to 0.6). No literature data are available about milk FA repeat-

Figure 1. Temporal changes of neo-synthesized FA (1A), preformed FA (1B), CLA (1C) and C14:1/C14:0 ratio (1D) in milk fat of the four experimental groups



	tios of the four experimental groups.								
	Groups			SEM					
	А	В	С	D	SEIVI				
(g/100g of lipids)	16.76 ^b	17.06 ^b	17.62 ab	18.72 ª	0.330				
(g/100g of lipids)	15.67 ª	15.29 ^a	14.71 ^b	13.99 ^b	0.320				
(g/100g of lipids)	2.58 ª	2.40 ab	2.29 ab	2.11 ^b	0.107				
	0.034 a	0.031 ^{ab}	0.028 ^{bc}	0.025 °	0.001				
	0.018 ª	0.017 ab	0.015 ^{bc}	0.013 ^c	0.001				
	0.036 a	0.034 ab	0.031 bc	0.029 ^c	0.001				
	0.66 ^a	0.64 ^a	0.63 ^{ab}	0.61 ^b	0.008				
	0.33 a	0.31 ^{ab}	0.29 bc	0.27 °	0.008				
	(g/100g of lipids)	(g/100g of lipids) 16.76 b (g/100g of lipids) 15.67 a (g/100g of lipids) 2.58 a 0.034 a 0.034 a 0.018 a 0.036 a 0.66 a 0.66 a	A B (g/100g of lipids) 16.76 b 17.06 b (g/100g of lipids) 15.67 a 15.29 a (g/100g of lipids) 2.58 a 2.40 ab 0.034 a 0.031 ab 0.017 ab 0.036 a 0.034 ab 0.034 ab 0.66 a 0.64 a 0.64 a	A B C (g/100g of lipids) 16.76 b 17.06 b 17.62 ab (g/100g of lipids) 15.67 a 15.29 a 14.71 b (g/100g of lipids) 2.58 a 2.40 ab 2.29 ab 0.034 a 0.031 ab 0.028 bc 0.015 bc 0.036 a 0.034 ab 0.031 bc 0.031 bc 0.66 a 0.64 a 0.63 ab 0.63 ab	A B C D (g/100g of lipids) 16.76 b 17.06 b 17.62 ab 18.72 a (g/100g of lipids) 15.67 a 15.29 a 14.71 b 13.99 b (g/100g of lipids) 2.58 a 2.40 ab 2.29 ab 2.11 b 0.034 a 0.031 ab 0.028 bc 0.025 c 0.018 a 0.017 ab 0.015 bc 0.013 c 0.036 a 0.034 ab 0.031 bc 0.029 c 0.66 a 0.64 a 0.63 ab 0.61 b				

Table 1.Selected milk fatty acid composition and fatty acid ra-
tios of the four experimental groups.

Different superscript on the same row $P \leq 0.05$.

ability in sheep. The average content of MUFA and CLA was higher in milk fat of low yielding ewes (group A), while short chain FA (C4 to C10) were higher in milk fat of high yielding ewes (group D). Group B and C showed intermediate values (table 1). Mean CLA content of

milk sheep linearly decreased with the increase of the mean fat yield (CLA=3.02-0.01 fat g/d; p<0.01). In a previous study, Signorelli *et al.* (2008) reported a negative correlation between CLA and fat content in three dairy sheep breeds. On the contrary, studies on dairy cows reported that milk fat yield and content had a little or not impact on individual variation for milk CLA content (Kelsey *et al.*, 2003). On the other hand, Kay *et al.* (2005) reported that Holstein cows selected for milk fat yield produced more milk than cows from control line, but with reduced 9-desaturase activity and lower MUFA content. In conclusion, increasing levels of milk fat secretion led to a decrease of MUFA, CLA content and of FA ratios which are a proxy of the mammary $\Delta 9$ desaturase enzyme. On the other hand, C4 to C10 FA content seemed to be positively affected by milk fat yield ability. This pattern could be a consequence of a different energy partition in the animals which higher milk fat secretion. Strategies aimed to improve FA composition of sheep milk should keep into consideration also individual ability for milk fat yield.

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