

# Breed differences in sheep milk fatty acid profiles: Opportunities for sustainable use of animal genetic resources

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## Abstract

Milk fatty acid profile of dairy sheep could be a clue for the sustainable use of local, endangered sheep breeds. We have determined milk quality parameters and fatty acid profiles in milk of three Italian breeds, Altamurana, Gentile di Puglia and Sarda. We found significant differences ( $P < 0.05$ ) between breeds in C4:0; C6:0; C12:0; C14:0; C16:0; C18:0. The Altamurana breed showed a higher content of caproic (C6:0), lauric (C12:0), myristic (C14:0) and palmitic (C16:0) than the Sarda. Compared to Gentile di Puglia, Altamurana milk had a higher content of butyric (C4:0), myristic (C14:0) and palmitic (C16:0). Butyric (C4:0) and stearic (C18:0) were highest in the Sarda breed. The Gentile di Puglia breed showed the lowest content of saturated fatty acids. No differences between breeds were evident for CLA and poly-unsaturated FAs (PUFAs), while MUFAs were lowest in the Altamurana.

We found that milk yield and fat content affected significantly milk fatty acid profiles ( $P < 0.05$ ); when milk yield increased, myristic fatty acid (FA) and total saturated FA were decreased, while PUFA were increased. Short- and medium-chain FA and CLA were negatively affected by fat percent, while stearic acid was positively affected.

The differences between breeds in milk fatty acid profiles, according to the reviewed literature on cheese characteristics, are likely to affect cheese quality and could be an indicator of typicity to launch specific types of cheese, to sustain the local, endangered Altamurana and Gentile di Puglia breeds.

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## 1. Introduction

Pressure to increase global food production and productivity has caused a dramatic shrinking of farm animal genetic resources. The most significant threat to genetic diversity is the marginalization of traditional produc-

tion systems and the associated local breeds, driven by the rapid spread of intensive livestock production systems. During the last decades, several indigenous breeds of sheep, in Italy, underwent a consistent decline in numbers, and were substituted by the Sarda breed. In particular, no more than 10,000 sheep are now left of the local multi-purpose Gentile di Puglia and Altamurana breeds, which represented up to 1963 the most important sheep resource in South-Eastern Italy, numbering to

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about 1 million head of each of the two breeds. On the other hand, numbers of the Sarda increased from about 2.5 million head in 1963 to over 5 million in 2000, and registered an average increase of lactation milk yield by 30% in 20 years (AIA, 1981–2005) thanks to sound milk recording systems and breeding programs.

For the endangered breeds, before proposing genetic improvement programs, some clues to their sustainable use need to be identified. The most important output of sheep farming in Italy is cheese production. Studies on the overall cheese making process have demonstrated that the fatty acid (FA) profile of raw milk influences cheese characteristics (Buchin et al., 1998), and Ha and Lindsay (1993) suggested that the qualitative presence and quantitative amounts of milk FA might contribute to differentiate new types of cheese. However, the number of studies on FA concentration in sheep milk are quite limited compared to cows and have mainly considered the widespread breeds. Moreover, such studies were mainly focused on the dietary sources of variation of those FA, that have a range of positive health effects, like poly-unsaturated FA (PUFA) and CLA in particular. Short- and medium-chain FA, that are produced by *de novo* synthesis in the mammary gland, are considered less important unjustifiably. The volatile flavour compounds in cheese originate during ripening particularly from degradation of lipids, therefore lipid milk composition is mostly important in traditional sheep cheeses that undergo several weeks of ripening (McSweneey, 2004). The major flavour of raw ewe milk cheeses, denoted by its high flavour intensity, comes from short- and medium-chain free fatty acids (FFA), which are characteristic odorous compounds of the volatile fraction and contribute to the cheesy, lipolyzed aroma (House and Acree, 2002; Curioni and Bosset, 2002; Fernandez-Garcia et al., 2006). Lipolysis is particularly important in sheep cheeses due to the high fat content and lipase activity (Carbonell et al., 2002a,b; Gomez-Ruiz et al., 2002; Larrayoz et al., 2002).

This work is based on the assumption that the evidence of a breed effect in the quality of milk could be a hint for the sustainable use of indigenous sheep breeds, and aims at identifying differences in milk FA composition between the Altamurana, Gentile di Puglia and Sarda breeds, independent of diet effects.

## 2. Materials and methods

The study was conducted on milk records of 94 sheep of three breeds, Altamurana, Gentile di Puglia and Sarda, raised in the same flock with traditional management system, consisting of lambing in November, suckling for 35–60 days, then regular

machine milking of the ewe twice a day. Adult weight of the ewes of the three breeds was similar, ranging between 40 and 45 kg. Milking ewes were grazed natural pasture with feeding addition in the shed of 250 g pellet concentrate, 150 g oat grains and 1.5 kg oat and vetch hay. Ewes were all at their second or third lambing.

Milk recording was performed three times along the lactation, on the same day for all the ewes, with collection of milk samples for analysis of milk quality, following the regulation of the International Committee for Animal Recording (ICAR), and for FA gas chromatographic analysis. Altamurana and Gentile di Puglia have shorter lactation compared to the Sarda, therefore milk sampling tests were designed so to cover the whole lactation of the local breeds, as follows: first record, 60–70 days after lambing (at removal of the lamb); second record, 100 days after lambing; third record, 140 days after lambing.

### 2.1. Milk fat extraction

Milk samples (50 ml) were digested with 10 ml of NH<sub>3</sub> (25%, v/v) and mixed with 40 ml of ethanol (96%, v/v). The extraction was performed with 100 ml of a mixture of diethyl ether-pentane (1:1 v/v). The solvent phase was filtered through 25 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. This procedure was based on the ISO method (2001).

### 2.2. Fatty acid methyl esters (FAME) analysis

Milk fat (100 mg) was diluted in 5 ml of hexane and derivatised as methylesters by addition of 0.25 ml KOH 2 N in methanol (ISO, 2002). One milliliter of the upper phase containing FAME was diluted with 7 ml of diethyl ether and 2 ml of hexane for the on-column injection.

Gas chromatography analysis of FAME was performed by HP6890 (Agilent Technologies, Palo Alto, CA) and DB23 low bleed (J&W, Agilent Technologies, Palo Alto, CA) capillary column (30-m length, 0.32-mm i.d., 0.25-μm film thickness). On-column injection was adopted and hydrogen (1 ml/min) was used as carrier gas. Temperature program was as follows: 40 °C for 3 min, 25 °C/min up to 120 °C for 1 min, 4 °C/min up to 162 °C for 2 min, 8 °C/min up to 220 °C for 3 min; FID detector used, held at 250 °C. The characteristics of the capillary column did not allow the *trans* fatty acids to be separated. As a consequence the peak indicated as C18:1 includes *trans*11, together with *cis*-9 and *cis*-7 isomers. The results were expressed as percentage on the total fatty acids, including known and unknown peaks.

### 2.3. Statistical analysis

The following variables ( $Y_{1-19}$ )  $Y_1$  = C4:0;  $Y_2$  = C6:0;  $Y_3$  = C8:0;  $Y_4$  = C10:0;  $Y_5$  = C10:1;  $Y_6$  = C12:0;  $Y_7$  = C14:0;  $Y_8$  = C14:1;  $Y_9$  = C16:0;  $Y_{10}$  = C16:1;  $Y_{11}$  = C18:0;  $Y_{12}$  = C18:1;  $Y_{13}$  = C18:2;  $Y_{14}$  = C18:3;  $Y_{15}$  = CLA;  $Y_{16}$  = saturated FA;  $Y_{17}$  = PUFA;  $Y_{18}$  = mono-unsaturated FA (MUFA) were anal-

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