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Exploring polymorphisms and effects of candidate genes on milk fat quality in dairy sheep

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ABSTRACT

The aim of the present study was to investigate the genetic control of the fatty acid (FA) composition in milk from 3 breeds of sheep: Altamurana, Gentile di Puglia, and Sarda. Single nucleotide polymorphisms within genes, encoding enzymes putatively involved in the synthesis and metabolism of milk fat, were selected for analysis, and the allele substitution effects were determined for 16 genes, which were polymorphic in the 3 sheep breeds, upon the milk fat composition. Four genes (α-1-antichymotrypsin-2; diacylglycerol O-acyltransferase homolog-2; propionyl Coenzyme A carboxylase, β polypeptide; and insulin-like growth factor-I) play a role in the desaturation of stearic FA into polyunsaturated fatty acids. Furthermore, 2 genes (growth hormone receptor and zona pellucida glycoprotein-2) affect the variability of the total fat content in addition to the butyric and stearic FA profile, and the fatty acid synthetase gene has an influence on the medium-chain FA. Milk FA profiles play an important role in dairy sheep farming because they have a large effect on cheese characteristics and also because sheep milk may be marketed as a source of nutraceuticals because it contains higher levels of conjugated linoleic acid than milk from other ruminants. The current study evaluated the global effects of a large number of single nucleotide polymorphisms and haplotypes on traits that are not commonly investigated in sheep but that are potentially very useful for improving milk quality.
Key words: fatty acid, single nucleotide polymorphism, dairy sheep, milk composition

INTRODUCTION

Milk fat triglycerides are synthesized in the mammary epithelial cells but the fatty acids (**FA**) used to synthesize them may arise either from the breakdown of blood lipids or via de novo synthesis within the mammary epithelial cells. Between 40 and 60% of FA come from the blood and are primarily derived from very low density lipoproteins (**VLDL**), which are synthesized in the intestine or liver. Triglycerides in the VLDL are hydrolyzed in the mammary capillaries by lipoprotein lipase (**LPL**) (Fielding and Frayn, 1998).

The FA contained in VLDL are dependent on dietary lipids and on mobilized fat from body adipose tissue. The 2 key enzymes involved in FA synthesis in the mammary gland are acetyl-CoA carboxylase (ACACA), which is the rate-limiting step, and fatty acid synthetase (**FASN**), a large complex of enzyme activities responsible for the chain elongation of FA (Bionaz and Loor, 2008).

Most of the previous research on milk composition, both in cows and sheep, has focused on the dietary sources of FA variation (Firkins et al., 2006; Tsiplakou et al., 2008) and in particular on those FA that have a range of positive health effects [i.e., polyunsaturated FA (**PUFA**) and conjugated linoleic acid (**CLA**); Tsiplakou et al., 2006; Mele et al., 2007]. Other authors have investigated the effects of specific genes, acyl CoA:diacylglycerol acyltransferase (***DGAT1***) and stearoyl CoA desaturase (*SCD*), that are known to directly affect FA desaturation (Mele et al., 2007; Moioli et al., 2007; Schennink et al., 2008). Recently, genome-wide screens for bovine milk fat composition (Schennink et al., 2009; Stoop et al., 2009) revealed several QTL for short-, medium- and long-chain FA and demonstrated that the QTL for BTA14 and BTA26 are associated with polymorphisms in the *DGAT1* and stearoyl CoA desaturase genes, respectively. The composition of sheep milk, both between and within breeds, can differ by between 6 and 9% for fat, 4 and 7% for protein, and 17 and 21% for TS (Haenlein, 2001). Short- and medium-chain FA are the most important sources of fat during the processing of cheeses that undergo several weeks of ripening (McSweneey, 2004). The principal flavors and cheesy and lipolyzed aromas of raw ewe milk cheeses

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are derived from short- and medium-chain FFA, which originate from the degradation of lipids (Curioni and Bosset, 2002; House and Acree, 2002; Fernandez-Garcia et al., 2006). Moreover, sheep milk contains higher levels than cow or goat milk of CLA (Jahreis et al., 1999), an FA that is considered to have beneficial effects on human health (Bhattacharya et al., 2006).

The main objectives of the present study were to evaluate the association of different genotypes with milk fat composition in an experimental dairy sheep population comprising 3 breeds: Altamurana, Gentile di Puglia, and Sarda.

MATERIALS AND METHODS

Animals, Sampling, and Diet

The study was conducted using 94 sheep of 3 breeds: Altamurana (n = 36), Gentile di Puglia (n = 24), and Sarda (n = 34). Altamurana is a dairy sheep belonging to the subgroup of south European milk sheep (Pieragostini and Dario, 1996). Altamurana is a local breed from Apulia (southeastern Italy) that lives in a rather harsh environment. Gentile di Puglia is a historical triple-purpose Merino-type breed whose origin may be traced back to the Roman times (Altobella and Muscio, 1995). Until about the mid 1960s, Altamurana and Gentile di Puglia were the most important sheep breeds in southern Italy, numbering about 1 million head each. During recent decades, however, they have undergone a consistent decline in numbers, largely being replaced by the Sarda breed so that now no more than 5,000 head of each breed remain. The Sarda sheep, a specialized dairy breed native to Sardinia, is rapidly spreading outside of its island of origin so that its numbers have increased from about 2.5 million head in 1963 to more than 5 million in 2000.

The experimental farm on which the present trials were conducted maintains the local breeds with the aim of conservation and sustainable use of animal genetic resources. Sheep were raised using a traditional management system consisting of lambing in November, suckling for 35 to 60 d, and then regular machine milking of the ewe twice daily. Adult weights of ewes from the 3 breeds were similar, ranging between 40 and 45 kg. Milking ewes were allowed to graze on natural pastures and were additionally fed 250 g of pellet concentrate, 150 g of oat grains, and 1.5 kg of oat and vetch hay. The ewes analyzed were all at their second or third lambing. Milk recording was performed 3 times during the lactation [i.e., the first record at 60–70 d after lambing (at removal of the lamb), the second at 100 d after lambing, and the third at 140 d after lambing]. Milk samples were collected for analysis of milk quality in accordance with the regulations of the International Committee for Animal Recording (Rome, Italy).

Milk and FA Analysis

The following 22 traits were assessed for each animal: daily milk yield, milk fat content, and milk fat composition with regard to the following FA methyl esters (**FAME**): C4:0, C6:0, C8:0, C10:0, C10:1, C12:0, C14:0, C14:1, C15:0, C15 *aiso*, C15 *iso*, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, and CLA. The FAME were obtained by digesting milk samples (50 mL) with 10 mL of NH_3 (25% vol/vol) followed by mixing with 40 mL of ethanol (96% vol/vol). The extraction was then performed with a 100-mL mixture of diethyl ether-pentane (1:1 vol/vol). The solvent phase was filtered through 25 g of anhydrous Na_2SO_4 and evaporated under a vacuum. This procedure is based on the ISO (2001) method. The obtained milk fat (100 mg) was diluted in 5 mL of hexane and derivatized as methyl esters by the addition of 0.25 mL of KOH 2 *N* in methanol (ISO, 2002). One milliliter of the upper phase containing the FAME was then diluted with 7 mL of diethyl ether and 2 mL of hexane for on-column injection. Gas chromatography analysis of the FAME was performed using an HP6890 device (Agilent Technologies, Palo Alto, CA) and a DB23 low bleed capillary column (30 m length, 0.32 mm i.d., 0.25 μm film thickness; Agilent Technologies). On-column injections were adopted and hydrogen (1 mL/min) was used as carrier gas. The 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, and 8°C/min up to 220°C for 3 min. A flame ionization detector was used and 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.

Genes, Genotyping, and In Silico Analysis

Sixteen candidate genes were selected for their potential effect on milk fat quality based on their previous functional descriptions (Pariset et al., 2006; Marchitelli et al., 2007; Williams et al., 2009) and involvement at various level in lipid metabolism (i.e., as regulators, receptors, and encoding enzymes involved in fatty acid biosynthesis and desaturation). The first group of genes encoded enzymes of the somatotropic axis, which consists of growth hormone-releasing hormone receptor (**GHRHR**), growth hormone receptor (**GHR**), and IGF1, all of which play a key role in the metabolism and physiology of mammalian growth (Akers, 2002). Growth hormone receptor is considered to be a strong