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Association between SREBP-1 gene expression in mammary gland and milk fat yield in Sarda breed sheep

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SUMMARY

The aim of this study was to examine the expression patterns of SREBP-1 gene in milk somatic cells and its association with milk fat yield during early lactation in Sarda breed sheep. A sample of 20 Sarda ewes, aged between 4 and 5 years, in their third to fourth lactation were chosen. From each ewe 28 days after lambing milk yield was measured, and a 160 ml milk sample for the RNA extraction and to test somatic cells count and lactose, fat and protein contents were collected. From the obtained RNA, total cDNA was synthesized and the quantitative PCR was performed. The fat, proteins and lactose content showed many differences among the animals, but these variations were no correlated with the milk yield. The SREBP-1 gene expression resulted higher in the high milk fat producing ewes. The correlation analysis showed that the SREBP-1 expression level is directly related to the amount of milk fat (g/die) ($P < 0.001$), while the total RNA obtained from each sample was found to be related to the somatic cells number ($P < 0.001$). Instead the expression of this gene showed no relations with the concentration of fat in milk. Our data highlight that in sheep SREBP-1 gene is expressed in the mammary gland during early lactation. Moreover, the positive relationship between SREBP-1 gene expression and the milk fat yield suggests that SREBP-1 gene is required for the lipid synthesis in the sheep mammary gland.

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1. Introduction

The synthesis of fatty acids and cholesterol, which in turn gives rise to multiple lipid compounds, can occur in any cell, but this biochemical process is particularly important in tissues such as liver, adipose tissue and mammary gland, organs specialized in lipogenesis or/and in lipolysis (Harvatine and Bauman, 2006; Hoashi et al., 2007). Both processes of the biosynthesis of cholesterol and fatty acids are controlled by a family of transcription factors (SREBPs) (Harvatine et al., 2009). These transcription factors play a central role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis. SREBPs belong to the original basic helixloop–helix–leucine zipper family of transcription factors (Eberle et al., 2004). SREBP are synthesized as ~125 kDa precursors that are bond to the membrane of the endoplasmic reticulum until proteolytically cleaved to release the amino terminal fragment of ~68 kDa that migrates to the nucleus to activate gene transcription (Brown and Goldstein, 1997; Horton et al., 2002). Two genes are accountable for the production of the 3 isoforms of SREBP: SREBP-1a and 1c are transcribed from a single gene through the use of alternative start sites and are associated with fatty acid metabolism, whereas SREBP-2 is transcribed from a distinct gene and controls primarily cholesterol metabolism (Horton et al., 2002). The two isoforms of SREBP-1 (a and c) can be expressed at different levels in tissues and differ by only 84 nucleotides at the first exon (Eberle et al., 2004). Then SREBP-1 gene is considered a candidate gene as it plays a role in the regulation of the synthesis of milk fat (Cecchinato et al., 2012; Hoashi et al., 2007), and controls the expression of more than 30 genes (McPherson and Gauthier, 2004). The expression of this gene in bovine mammary tissue plays a central role in milk fat synthesis regulation and highlights a pivotal function for a concerted action among PPARG, PPARGC1A, and INSIG1 genes (Bionaz and Loor, 2008). To date, there have been no published studies demonstrating the expression of SREBP-1 in ovine mammary gland, and an influence of this gene on the milk fat yield. The objective of the current study was to examine the expression patterns of SREBP-1 gene in milk somatic cells and its association with milk fat yield during early lactation in Sarda breed sheep.

2. Material and methods

2.1. Animals

The study was conducted on 20 Sarda breed sheep from a farm located in north Sardinia (40° 48′N). During the day the animals grazed on leguminous and gramineous grasses, and then they received each a supplement of 300 g/day of concentrate commercial food (crude protein 20.4% and 12.5 MJ ME/kg DM). The sheep were penned at night, and received hay (crude protein 11.1% and 7.2 MJ ME/kg DM) and water *ad libitum*. The chosen ewes (aged between 4 and 5 years and in their third to fourth lactation) lambed between 2010 November 01 and 03 and suckled their lambs until Day 21 after parturition.

2.2. Sampling

From each ewe, at Day 28 after lambing, milk yield was measured, and a 150 ml milk sample for the RNA extraction was collected. In the same date another 10 ml milk sample from each ewe was collected to test somatic cells count and some milk quality parameters (lactose, fat and protein contents). Milk samples were analyzed by using an infrared spectrophotometer (Milko-Scan 133B; Foss Electric, DK-3400 Hillerød, Denmark) to assess fat, lactose and protein percentage according to the International Dairy Federation standard (IDF 141C:2000). SCC was measured using an automatic cell counter (Fossomatic 90, Foss Electric) according to IDF 148A:1995.

2.3. Total RNA extraction and analysis

RNA extraction from whole milk was performed in accordance to Mura et al. (2012). Briefly the samples were centrifuged at 2000 rpm for 15 min at 6 °C. The supernatant was carefully removed by overturning the tubes and the remaining cell pellet was washed twice with 10 ml of PBS (Phosphate Buffered Saline), pH 7.2, and supplemented with 0.5 mM of EDTA (Ethylene-D-amine-Tetra-Acetic-Acid) and DEPC 0.1% (D-Ethyl-Pyro-Carbonate) to limit formation of casein micelles and fat globules and