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Short communication

Association study between variability in the *SCD* gene and the fatty acid profile in perirenal and intramuscular fat deposits from Spanish goat populations

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ABSTRACT

Despite the fact that goat meat is an important nutritive source throughout the world, aspects related to its characterization, and in particular genetic issues, have rarely been studied. Our objective was to assess the variability of the Stearoyl-CoA desaturase (*SCD*) gene in a sample of Spanish goat populations bred for both meat and dairy purposes and to look for possible associations between this genetic variability and the fatty acid composition in two fat deposits of industrial interest (perirenal and intramuscular). To do this, a goat population of 140 male suckling kids belonging to meat and dairy breeds was selected from farms in southern Spain. Four markers were detected in the *SCD* gene (*SCD2*, *SCD3 172*, *SCD3 181*, and *SCD3 231*) in the population, and significant associations were found between these markers and five individual fatty acids: C8:0, C11:0, C15:1, C16:1*cis*-9 and CLA 10 *trans*-12*cis* and two groups of fatty acids: SFA and MUFA. While significant associations in early (perirenal) fat depots for both dairy and meat purposes were observed, the only significant association in late (intramuscular) depots was found in goat kids bred for meat purposes. The *SCD* has been shown to be a useful gene to estimate the potential to show the different amounts of fatty acids in the fat of goat kids and use it in improvement programs which might provide an added value to goat meat and might help to produce a more competitive final product.

1. Introduction

Goats play an important socio-economic role in European Mediterranean countries (Castel et al., 2010). In 2013, despite having only 1.63% of the world's goat population, the EU produced 14.20% of the total goat milk and 4.05% of the total goat meat in the world (FAO, 2014). In general, EU goat herds are far more specialized in milk production than in meat production. However, in Mediterranean European countries, goat meat production has a recognized prestige that contributes to development of the meat industry and of rural areas. Although the vast majority of Spanish goats are used for dairy purposes (68.05% of the total number of heads in Spain), goat meat production is also an important economic resource in productive systems (Castel et al., 2010). In any case, whether the breed is intended for dairy (DP) or meat (MP) purposes, the kids are fed exclusively on their mothers' milk to produce suckling goat kids to be slaughtered at about 1-month-old.

Goat meat is considered an important nutritive source all over the world (Biswas et al., 2007). Apart from meat quality aspects such as tenderness, flavour, juiciness or colour, health concerns are of particular interest for consumers given the relationship found between incidence of coronary diseases and high ratios of $n-6/n-3$ fatty acids in meat or dietary intake of saturated fatty acids (SFA). There is interest in animal production practices to change the fatty acid profile of meat to make it more attractive for health reasons (Nuernberg et al., 2005). The chemical composition of fat is complex and is determined by many extrinsic and intrinsic factors. Among them, Horcada et al. (2012) reported that the purpose of the goat breed and the production system are two important factors that influence the fatty acid profile of goat kids.

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On the other hand, it is known that a certain percentage of the variability of fatty acid composition may be attributed to the genetic variants in the animal (Taniguchi et al., 2004).

Stearoyl-CoA desaturase (*SCD*), also called Δ-9 desaturase, is an endoplasmic reticulum enzyme not only responsible for the conversion of SFA into monounsaturated (MFA) fatty acids (MUFA) (Ntambi, 1999) but also involved in the desaturation of *trans*-vaccenic acid into conjugated linoleic acid (CLA) (Corl et al., 2001). The *SCD* gene is located in the caprine and bovine chromosome 26, and both species present high chromosomal homology (Bernard et al., 2001). There are many association studies between the *SCD* gene and the fatty acid profile of the different fat deposits developed mainly in dairy but also in beef cattle (Taniguchi et al., 2004; Li et al., 2012). However, not many studies of this kind have been carried out so far in goats, especially in meat breeds. Zidi et al. (2010) described an association of *TGT* deletion located in the untranslated region of the *SCD* gene and the fatty acid profile of the milk of Murciano–Granadina breed. Crepaldi et al. (2013) also reported an association between this deletion and average milk and protein yields in Alpine goats.

To sum up, there is little information about genetic variants in goats and the effect on fatty acid composition in meat. Therefore, the goal of the present work was to assess the variability of the *SCD* gene in a sample of Spanish goat populations used for both purposes and to look for possible associations between this genetic variability and the fatty acid composition in two fat deposits of industrial interest (perirenal and intramuscular). Knowledge of the variability of the *SCD* gene in goats may contribute to estimating the potential to control the different amount of fatty acids in the meat and may be a useful tool to make the improvements in the quality of the product demanded by the consumer.

2. Material and methods

One hundred and forty male suckling kids belonging to three goat meat breeds (MP): Blanca Andaluza ($n = 20$), Blanca Celtibérica ($n = 30$) and Negra Serrana ($n = 30$) and two dairy breeds (DP): Malagueña ($n = 30$) and Murciano–Granadina ($n = 30$) were selected from semi-extensive farms of southern Spain. In order to avoid different variation sources all the animals were selected when the availability of animals was higher: from the main kidding season (spring), main litter size (1 kid per birth) and not primiparous dams. Kid goats were reared on the farm of origin exclusively on their mother's milk until slaughter. In this study, all the mothers were reared under semi-extensive husbandry conditions and fed with forage and concentrates (supplement ingredients: maize grain, soybean meal, wheat grain, barley grain, gluten feed, sunflower meal, beans, peas, calcium carbonate and salt). Proximate average chemical composition of the concentrate supplements was as follows: Dry matter, 92.2 g/100 g; organic matter (93.1 g/100 g, DM basis); crude protein (21.2 g/100 g, DM basis) and ether extract (2.1 g/100 g, DM basis). According to the livestock's purpose, during suckling, supplementary feed concentrate was added at an approximate of 0.7 to 1.2 kg head-1day-1 for the meat and dairy breeds, respectively. Kid goats were chosen at each farm of origin and were slaughtered at the usual commercial weight, according to their usual production systems. When the animals reached the target live weight (in the range of 7–8 kg) at between 42 and 46 days of age, they were transported in accordance with welfare specifications and slaughtered in a slaughterhouse according to EU Council regulations (Directive, 1986). Next, total perirenal fat (PR) was extracted and weighed. After 24 h, *Longissimus dorsi* muscle was obtained from left carcass half. Both PR and *Longissimus dorsi* muscle were vacuum-packed and frozen at −18 °C for the analytical processes. Intramuscular fat from the *Longissimus dorsi* muscle (IM) and PR fat deposits were chosen for their high content of saturated and polyunsaturated fatty acids (PUFA), respectively (Zygoyiannis et al., 1985; Horcada et al., 2009).

Relative fat content of the IM depot was quantified in the *Longissimus dorsi* muscle of the left carcass half using the Ankom Procedure based on high temperature (90 °C) solvent extraction (AOCS, 2004) with an Ankomextractor (Model XT10, Ankom Technology, Madrid, Spain).

Total fatty acids from PR and IM were extracted, methylated and analyzed following an adaptation of the method described by Aldai et al. (2006). Separation and quantification of the fatty acid methyl esters was carried out using a gas-chromatograph (GC, Agilent 6890N, Agilent Technologies Spain, S.L., Madrid, Spain). Individual fatty acid methyl esters were identified by comparing their retention times with those of authenticated Sigma standards (Sigma Chemical Co., Ltd., Poole, UK). Identification of the CLA isomers *cis*-9 *trans*-11, and *trans*-10 *cis*-12 was achieved by comparing retention times with those of another authenticated standard mix (Sigma Chemical Co., Ltd., Poole, UK). Individual fatty acids were expressed as the percentage of total fatty acids identified and grouped as follows: SFA, MUFA, PUFA, $\sum n$-3 PUFA, $\sum n$-6 PUFA and total CLA. The lipid quality indices relating to human health were calculated: the atherogenic index, AI (Ulbricht and Southgate, 1991) and desirable fatty acids (Huerta-Leidenz et al., 1991). The Δ-9 desaturase activity indices were also estimated (Malau-Aduli et al., 1998).

Genomic DNA was isolated using a commercial kit (Canvax Biotech©, Spain). A subset of 25 samples (5 per breed) was selected to amplify four fragments of the *SCD* gene (Accession no. AF422167_69 and AF422171) in an Eppendorf thermocycler (Eppendorf© AG, Germany) using primers and PCR conditions reported by Zhang et al. (2010). Direct sequencing reactions were carried out in an ABI 3730 automatic sequencer (Applied Biosystems©, USA) and PCR products were analyzed with the Sequencher v.4.6 software (Gene Codes Corporation©, Ann Arbor, MI, USA 1991–2006). In order to check the variability of the putative SNPs detected in the population studied, a primer extension analysis was carried out with the remaining 115 samples. Six μl of PCR product was purified using the enzymatic method (Exo-SAP, New England Biolabs©) and the different SNPs were genotyped in the above-mentioned sequencer with the SNaPshot Multiplex kit (AB,Life Technologies©, USA) following the manufacturer's instructions. The sequences of the extension primers were: SCD2-E: 5′-TTT TCC AGA TCT CTA GCT CCT ACA C-3′; SCD3.1-E: 5′-TTT TTTTTT TTC TCC TCA CTC TTT AT-3′; SCD3.2-E: 5′-GAT CAC CTC CAT TCC ACC AC-3′ and SCD3.3-E: 5′-AGA GGG ACA GCA CCT GGA TA-3′.

Comparison of genic and genotypic frequencies between aptitude groups was performed using Fisher's method using Genepop software. The Hardy-Weinberg equilibrium was assessed with the Hardy–Weinberg exact test option from the same software. The D' value was estimated with a Chi-square method to evaluate the disequilibrium linkage between marker pairs.

The data to compare the fatty acid composition of each genotype of the markers in the *SCD* gene were tested using a linear mixed effect model using the SAS MIXED procedure (SAS Version 9.2, SAS Institute, Cary, NC, USA). In a preliminary model the breed, genotype and breed × genotype interaction were included as fixed effects. The five breeds were classified into two groups by their production purpose due to the lack of significance ($P > 0.05$) of the breed × genotype interaction. Separate analyses were carried out for each SNP, purpose and fatty acid or index in two different deposits (PR and IM fat) following this definitive model $y_j = \mu + G_i + \beta_{SW} + e_i$; where y_j was the fatty acid or index, μ was the general mean of the fatty acids or indices, G_i the genotype of the marker included as a fixed effect and β_{SW} the linear effect of the carcass weight as covariate. After adjustment by the Bonferroni method in those loci where the effect of the genotype was