A polymorphism in the stearoyl-CoA desaturase gene promoter influences monounsaturated fatty acid content of Duroc × Iberian hams

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Abstract

Data on 74 dry-cured hams from Duroc × Iberian pigs were used to examine whether the tag polymorphism AY487830.g.2228T>C in the promoter region of the stearoyl-CoA desaturase [SCD] gene affect fat desaturation and monounsaturated fatty acid (MUFA) as previously described in purebred Duroc hams. Samples were taken from sliced trays of dry-cured hams marketed as Jamón Ibérico de cebo, which were randomly purchased from the same supplier in different stores of the same supermarket chain. Genomic DNA was isolated from each sample to genotype for SCD and gender. Also, a sample of two slices was used to determine fat content and fatty acid (FA) composition by gas chromatography. The effect of the genotype (TT and CT) and gender (barrows and gilts) was estimated under a Bayesian setting. Results showed that the SCD polymorphism was associated to fat composition but not to fat content, with TT hams showing increased C18:1n-7, C18:1n-9, C20:1n-9 and MUFA (probability between 0.92-0.98) and decreased C18:2n-6, C20:4n-6 and polyunsaturated FA (PUFA) (probability between 0.91-0.99) as compared to the CT. As a result, the TT hams had more MUFA (0.95%) and a higher MUFA/PUFA ratio (0.43) than the CT. Barrows had more saturated FA (SFA) and less PUFA than gilts. No differences in MUFA content were found between genders. The SCD polymorphism had a greater impact on MUFA than using hams from barrows instead of gilts. It is concluded that the SCD polymorphism is a good tool to increase MUFA and MUFA/PUFA ratio in Duroc crossbred dry-cured hams.

Additional key words: dry-cured ham; fatty acid composition; genetic marker; meat quality; pigs.

Introduction

The dry-cured ham industry is a traditional sector with an enormous economic importance in the Mediterranean area, particularly in Spain, where it is the pork product most preferred by consumers (Enge et al., 1998; ANICE, 2014). The intramuscular fat content (IMF) and fatty acid (FA) composition, especially the monounsaturated fatty acid (MUFA) content, influence the final quality of the dry-cured products, since they affect color, aspect and texture of ham slices, as well as the intensity and persistence of aroma (Ruiz-Carrascal et al., 2000; Gandemer, 2009; Lorido et al., 2015). Moreover, the consumption of dietary MUFA promotes healthy blood lipid profiles, mediates blood pressure, improves insulin sensitivity and regulates glucose levels (Gillingham et al., 2011).

MUFA represent the most abundant fatty acids in pork and oleic acid (C18:1n-9) is its major component, accounting for approximately 35-50% of total fatty acids (López-Bote, 1998; Wood et al., 2008; Tejerina et al., 2012). It has been shown that the oleic acid,
acid content is positively correlated with pork flavor, flavor liking and overall acceptability (Cameron et al., 2000; Tikk et al., 2007). A relevant part of the variation in MUFA and oleic acid has a genetic origin and thus can be attributed to differences between breeds (Wood et al., 2004; Reixach et al., 2008) and individuals within breed (Ros-Freixedes et al., 2012). A recent research of Estany et al. (2014) has shown that a haplotype in the promoter region of the stearoyl-CoA desaturase (SCD) gene, which enhances fat desaturation and, as a result, MUFA and oleic acid in muscle and subcutaneous fat, segregates in Duroc. This beneficial effect is maintained in purebred dry-cured Duroc hams (Henriquez-Rodriguez et al., 2015), despite the fatty acid compositional changes occurring through lipolysis and oxidation in the curing process (Navráz-Rivas et al., 2008; Gandemer, 2009).

The Iberian hams represent about 20% of total turnover of Spain’s dry-cured ham industry (Belmonte, 2006; Chamorro et al., 2008; Cruz, 2013). The Spanish Ministry of Agriculture guidelines (BOE, 2014) establishes two types of labelling for Iberian hams according to their genetic background: 100% Iberian (produced from purebred Iberian pigs) and Iberian (produced using Iberian crossbred pigs with a maximum 50% of Duroc). Nowadays, however, most of the Iberian marketed hams, around 94%, are based on crosses of Iberian sows with Duroc boars (Cruz, 2013). The objective of this study was to assess whether the haplotype segregating at the SCD gene in Duroc also affects the MUFA content of marketed Duroc × Iberian dry-cured hams.

**Material and methods**

**Sample collection**

Seventy four dry-cured ham sliced packs of ‘Jamón Ibérico de Cebo’ (fodder fed Iberian ham) from the same supplier were randomly purchased in 15 different franchised stores of the same supermarket chain in the city of Lleida (Catalonia, Spain) and surroundings between July 2013 and January 2014. The supplier is known to produce the Iberian dry-cured hams from their own nucleus herds, farms, and manufacturing facilities. To randomize unidentified effects, each pack was taken from a different production batch. All packs were vacuum-packaged and kept in refrigerated shelves. Two standard entire slices per pack were freeze-dried and pulverized previously to fat analysis.

**Determination of fat content and composition**

A representative aliquot from the pulverized freeze-dried sample was used to determine, in duplicate, the individual FA by gas chromatography (Bosch et al., 2009). In brief, FA methyl esters were directly obtained by transesterification using a solution of 20% boron trifluoride in methanol (Rule, 1997). Methyl esters were determined by gas chromatography using a capillary column SP2330 (30 m × 0.25 mm; Supelco, Bellefonte, PA, USA) and a flame ionization detector with helium as carrier gas. Runs were made with a constant column-head pressure of 172 kPa. The oven temperature program increased from 150 to 225°C at 7°C/min and injector and detector temperatures were both 250°C. The quantification was carried out through area normalization with an external mixture of FA methyl esters (Supelco® 37 Component FAME Mix, Sigma, Tres Cantos, Madrid). The internal standard was 1,2,3-tripentadecanoylglycerol (Tripentadecanoin, Sigma, Tres Cantos, Madrid). Then, the FA composition was expressed as the percentage of each individual FA relative to total FA. The complete profile for each sample included saturated (SFA: C14:0; C16:0; C18:0; and C20:0); monounsaturated (MUFA: C16:1n-9; C18:1n-9; C18:1n-7; and C20:1n-9); and polyunsaturated (PUFA: C18:2n-6; C18:3n-3; C20:2n-6; and C20:4n-6) FA. The identification and quantification of the C18:1n-7 isomer was made by using a commercial methyl ester mixture (FAME Column Evaluation Mix, Sigma, Tres Cantos, Madrid) and was confirmed by mass spectrometry. The fat content was calculated as the sum of the individual FA expressed as triglyceride equivalents (AOAC, 2000) on a dry tissue basis.

**Isolation of genomic DNA and genotyping**

The isolation of genomic DNA was carried out from refrigerated Iberian hams. Samples were lysed in the presence of proteinase K and DNA was purified through extraction with phenol:chloroform, followed by ethanol precipitation. Finally, DNA was re-suspended and stored in TE buffer. The quantification and estimation of the quality and purity of genomic DNA was performed using a Nanodrop N-1000 spectrophotometer; DNA integrity was tested through electrophoresis in a 1% agarose gel.

The single nucleotide polymorphism AY487830: g.2228T>C, which was selected as a tag SNP to evaluate the effect of alternate haplotypes, was genotyped using an allelic discrimination assay with the primers and probes indicated in Estany et al. (2014). The reaction mix contained 1x Universal TaqMan master mix.
SCD affects MUFA content in ham

Statistical analyses

The effect of the SCD genotype on fatty acid content and composition was estimated under a Bayesian approach by fitting a model which included the genotype (TT and CT) and the gender (barrows and gilts). Each trait was assumed to be conditionally distributed as follows:

\[ y \mid b, \sigma^2_e \sim N(Xb, I\sigma^2_e) \]

where \( b \) is the vector including the effects of genotype and gender; \( X \) is the known incidence matrix relating the observations with the systematic effects; \( I \) is the identity matrix; and \( \sigma^2_e \) is the residual variance. Bounded uniform priors were used to represent vague previous knowledge of \( b \) and for the residual variance. Marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Chains of 60,000 samples with a burn-in period of 10,000 were used. One sample of each 10 was saved to avoid high correlations between consecutive samples. The Monte Carlo SE was estimated using effective sample size to take into account the autocorrelation in the MCMC samples. Convergence was tested using the Z-criterion of Geweke. Inferences were done from the marginal posterior distributions of the differences between two genotypes or genders. In particular, the following parameters were calculated: the mean of the marginal posterior distribution of the difference (D); the highest posterior density interval for D at 95% (HPD 95%); the probability (\( p \)) of D being greater (if D > 0) or lower (if D < 0) than zero; and the limit k of the interval \([k, +\infty)\), if D > 0, or \((-\infty, k]\), if D < 0, at 80% of probability. All the analyses were performed using the statistical package Rabbit developed by Institute for Animal Science and Technology (Valencia, Spain; http://www.dcam.upv.es/dcia/ablasco/Publi.htm).

Results

For all the analyzed traits, the Monte Carlo SE was very small and the Geweke test did not detect lack of convergence.

Effect of SCD genotype

Allelic frequencies of the SCD gene by gender are presented in Table 1. The allele frequencies were 0.79 and 0.21, for allele T and C, respectively. Because only one sample with genotype CC was found, it was discarded for further analyses. Features of estimated marginal posterior distributions of the difference between TT and CT genotypes for fat content and fatty acid composition in Duroc × Iberian hams are shown in Table 2. Results provided no evidence for an association between the SCD genotype and fat content. However, they clearly support the hypothesis that the investigated polymorphism affects fatty acid composition. Thus, the hams produced by TT pigs showed greater values for C18:1n-9, C18:1n-7, C20:1n-9 and MUFA (with \( p \) from 0.92 to 0.98) and lower values for C18:2n-6, C20:4n-6 and PUFA (with \( p \) ranging 0.91 to 0.99) as compared to the CT. Differences between genotypes were much lower for SFA, the most evident being for C18:0 (with \( p \) of 0.83). The effect of the polymorphism was higher than one-third of the standard deviation.

Table 1. Number of samples by gender and genotype at the SCD gene in sampled Duroc × Iberian hams.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of samples</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>CT</td>
</tr>
<tr>
<td>Barrows</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Gilts</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>31</td>
</tr>
</tbody>
</table>
Table 2. Features of the estimated marginal posterior distribution of the difference between SCD genotypes for fat content (IMF) and fatty acid composition in Duroc × Iberian dry-cured hams.

<table>
<thead>
<tr>
<th>Trait²,%</th>
<th>MeanTT</th>
<th>MeanCT</th>
<th>Mean ΔTT–CT</th>
<th>HPD95%TT–CT</th>
<th>p</th>
<th>k80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, % DM</td>
<td>27.77</td>
<td>27.20</td>
<td>0.57</td>
<td>–2.55; 3.79</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>62.11</td>
<td>62.11</td>
<td>0.00</td>
<td>–2.34; 2.40</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>1.41</td>
<td>1.41</td>
<td>0.00</td>
<td>–0.06; 0.05</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>24.80</td>
<td>24.93</td>
<td>–0.13</td>
<td>–0.63; 0.39</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td>10.35</td>
<td>10.60</td>
<td>–0.24</td>
<td>–0.72; 0.24</td>
<td>0.83</td>
<td>–0.03</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.18</td>
<td>0.21</td>
<td>–0.03</td>
<td>–0.09; 0.04</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>36.76</td>
<td>37.10</td>
<td>–0.35</td>
<td>–1.27; 0.61</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>C16:1n–9</td>
<td>3.16</td>
<td>3.05</td>
<td>0.11</td>
<td>–0.08; 0.31</td>
<td>0.87</td>
<td>0.03</td>
</tr>
<tr>
<td>C18:1n–9</td>
<td>45.62</td>
<td>44.93</td>
<td>0.69</td>
<td>–0.05; 1.46</td>
<td>0.96</td>
<td>0.37</td>
</tr>
<tr>
<td>C18:1n–7</td>
<td>3.82</td>
<td>3.68</td>
<td>0.13</td>
<td>–0.03; 0.31</td>
<td>0.93</td>
<td>0.06</td>
</tr>
<tr>
<td>C20:1n–9</td>
<td>0.90</td>
<td>0.88</td>
<td>0.02</td>
<td>–0.01; 0.05</td>
<td>0.92</td>
<td>0.01</td>
</tr>
<tr>
<td>MUFA</td>
<td>53.50</td>
<td>52.54</td>
<td>0.95</td>
<td>0.05; 1.91</td>
<td>0.98</td>
<td>0.56</td>
</tr>
<tr>
<td>C18:2n–6</td>
<td>8.33</td>
<td>8.81</td>
<td>–0.48</td>
<td>–0.88; –0.06</td>
<td>0.99</td>
<td>–0.31</td>
</tr>
<tr>
<td>C18:3n–3</td>
<td>0.47</td>
<td>0.48</td>
<td>–0.01</td>
<td>–0.08; 0.06</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>C20:2n–6</td>
<td>0.36</td>
<td>0.37</td>
<td>–0.01</td>
<td>–0.03; 0.01</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>C20:4n–6</td>
<td>0.59</td>
<td>0.68</td>
<td>–0.09</td>
<td>–0.20; 0.04</td>
<td>0.91</td>
<td>–0.03</td>
</tr>
<tr>
<td>PUFA</td>
<td>9.75</td>
<td>10.34</td>
<td>–0.59</td>
<td>–1.06; –0.08</td>
<td>0.99</td>
<td>–0.38</td>
</tr>
<tr>
<td>C18:1n–9/C18:0</td>
<td>4.46</td>
<td>4.27</td>
<td>0.20</td>
<td>–0.05; 0.45</td>
<td>0.94</td>
<td>0.09</td>
</tr>
<tr>
<td>C16:1n–9/C16:0</td>
<td>0.13</td>
<td>0.12</td>
<td>0.01</td>
<td>0.00; 0.01</td>
<td>0.89</td>
<td>0.00</td>
</tr>
<tr>
<td>MUFA/SFA</td>
<td>1.46</td>
<td>1.42</td>
<td>0.04</td>
<td>–0.02; 0.10</td>
<td>0.92</td>
<td>0.02</td>
</tr>
<tr>
<td>MUFA/PUFA</td>
<td>5.56</td>
<td>5.12</td>
<td>0.43</td>
<td>0.14; 0.74</td>
<td>1.00</td>
<td>0.31</td>
</tr>
<tr>
<td>SFA/PUFA</td>
<td>3.82</td>
<td>3.63</td>
<td>0.19</td>
<td>–0.03; 0.44</td>
<td>0.95</td>
<td>0.09</td>
</tr>
</tbody>
</table>

¹ Mean DTT–CT: mean of the marginal posterior distribution of the difference between genotype TT and CT; HPD95%TT–CT: highest posterior density interval for DTT–CT at 95%; p: posterior probability of DTT–CT being greater (if DTT–CT >0) or lower (if DTT–CT <0) than zero; and k80%: limit of the interval [k, +∞) (if DTT–CT >0) or (-∞, k] (if DTT–CT <0) at 80% of probability (k is only displayed when DTT–CT and k80% are of the same sign). p >0.9 are written in bold. ² DM, dry matter; SFA, saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA, monounsaturated fatty acids (C16:1n-9+C18:1n-9 + C18:1n-7+ C20:1n-9); PUFA, polyunsaturated fatty acids (C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6).

ard deviation of the trait for C18:1n-9, C18:1n-7, C20:1n-9, MUFA, C18:2n-6, C20:4n-6 and PUFA. The TT hams had a probability of 80% of having at least 0.37% more C18:1n-9 and 0.56% more MUFA than the CT. As a result, the 18:1n-9/C18:0, MUFA/SFA, MUFA/PUFA, and SFA/PUFA ratios were higher in the TT hams. In particular, the MUFA/PUFA ratio was 0.43 higher in the TT than in the CT hams (with p of 1.00).

Effect of the gender

Features of estimated marginal posterior distribution of the difference between barrows and gilts for fat content and fatty acid composition in Duroc × Iberian hams are given in Table 3. The hams were evenly distributed by gender, with 51.1% from barrows and 48.6% from gilts, suggesting, because packs were randomly taken, that males and gilts are indistinctly used for producing dry-cured hams.

The barrows did not differ from gilts for fat content (the probability of barrows showing higher fat content than gilts was 0.53), but they showed greater values for SFA (C14:0, and C16:0, with p of 0.99 and 0.94 respectively), and lower for PUFA (C18:2n-6 and PUFA with p of 0.99). Results concerning MUFA were more disparate. Thus, while barrows exhibited higher levels of C16:1n-9, C18:1n-7 and C20:1n-9 (with p ranging from 0.84 to 1.00), they showed lower values of C18:1n-9 (although only with p of 0.73). The effect of
the gender was higher than one-third of the standard deviation of the trait for C14:0, C16:0, C18:1n-7, C20:1n-9, C18:2n-6, and PUFA. As a result, the ratios SFA/PUFA and MUFA/PUFA were, respectively, 0.24 and 0.28 higher in barrows than in gilts (with \( p < 0.9 \) are written in bold. 2 See Table 2 for abbreviations.

### Discussion

Iberian hams, including Duroc × Iberian crossbreds, are characterized by a high level of fat infiltration, which is very appreciated by consumers and provides a high degree of marbling, a firm texture and an intense, delicate and very special flavor (Fernández et al., 2007). The official regulation concerning the required genetic types that can be used under the designation Iberian (BOE, 2014) are not always perfectly fulfilled by some producers and therefore it might be possible to find some Duroc × Iberian hams with a proportion of Duroc alleles greater than 50%. However, this is certainly not the case here because, as indicated above, all sampled packs were from pigs of identified origin. Moreover, the observed genotypic frequencies (0.58, for TT, and 0.42, for CT genotypes, with only 1 CC) is in line with the hypothesis that allele T is almost fixed in Iberian and segregating at an intermediate frequency in Duroc (Estany et al., 2014).

The IMF content has a positive influence on texture and appearance traits of hams, such as oiliness, bright-
ness and juiciness (Ruiz-Carrascal et al., 2000). In this study the SCD genotype did not affect fat content, in agreement with what has been reported in Estany et al. (2014) for IMF in three distinct raw muscles. Similarly, the fat content was not affected by the gender, which is in accordance with what has been observed by some authors using Duroc × Iberian crossbred barrows and castrated and intact females (Serrano et al., 2008; Cordero et al., 2010), but not all. In particular, Muñoz et al. (2011) found that barrows had more IMF than intact females. However, these works were carried out with raw rather than dry-cured samples. Using samples of dry-cured Serrano hams, Soriano et al. (2005) did not find an effect of sex on IMF. However, because we analyzed entire slices, samples also included intermuscular fat and the untrimmed subcutaneous fat. Therefore, as determined, fat content here should be considered as total edible fat rather than IMF. Nevertheless, taking together, these results suggest that both the SCD polymorphism and the use of barrows instead of gilts do not exert a relevant influence on the edible fat content.

The main purpose of this study was to investigate the effect of the SCD polymorphism on the fatty acid profile of the edible fat in an entire slice. This approach was used for its simplicity but also because, from previous studies, we know that the correlation of fatty acid profile between fat tissues is relatively high (Ros-Freixedes et al., 2014) and the effect of the polymorphism is consistent across muscles and fat tissues (Estany et al., 2014). Fatty acid composition affects eating and meat quality traits of pork, particularly flavor, tenderness and juiciness (Antequera et al., 1992; Ruiz et al., 1999; Ruiz-Carrascal et al., 2000). In contrast to fat content, we found that both the SCD polymorphism and the gender influenced the fatty acid profile of fat. In line with previous results in purebred Duroc, either with raw meat (Estany et al., 2014) or with dry-cured hams (Henríquez-Rodríguez et al., 2015), the T allele increased MUFA (+0.95%). However, here, contrarily to these previous works, the increase in MUFA was at expense of PUFA (-0.59%), especially C18:2n-6 (-0.48%), rather than SFA (-0.35%). The side-effect of the marker on PUFA content is not obvious to explain, although it might be influenced by the sampling scheme used, which includes fat sources other than IMF, or by differing ripening conditions. It is known that the subcutaneous fat contains more PUFA than IMF (Ros-Freixedes & Estany, 2013) and that PUFA decrease across ripening due to oxidative reactions (Martín et al., 1999; Narváez-Rivas et al., 2008; Henríquez-Rodríguez et al., 2015). Therefore, changes in PUFA are expected to be more marked in samples from subcutaneous fat subjected to long dry-curing periods. The substitution of MUFA for PUFA is a side effect that indirectly may benefit the quality of the ham, since it is known that PUFA increase susceptibility to oxidative processes and off-flavors (Cava et al., 1999).

The substitution effect of the T allele for C18:1n-9 and MUFA (0.69% and 0.95%) was very close to the values obtained by Estany et al. (2014) for C18:1 (C18:1n-9+C18:1n-7, 0.70%) and MUFA (1.02%), thereby confirming that the favorable effect of the T allele on fat desaturation observed in purebred Duroc raw muscles is maintained in Duroc × Iberian dry-cured hams. The favorable effect of allele T on MUFA in Duroc × Iberian had already been reported in raw muscle (Estany et al., 2014). Although quality attributes of dry-cured ham are subjected to complex polygenic inheritance patterns (Pena et al., 2013), a number of associated genetic markers have been reported to use in pig breeding programs (Stalder et al., 2005; Renaville et al., 2010; Gou et al., 2012). However, only one of them, and likely as a result of a correlated change in overall fatness, affected the fatty acid composition of dry-cured hams (Reina et al., 2012). In practical terms, the results obtained indicate that the SCD polymorphism can be a good tool to discriminate among Duroc boars by their transmittance ability for increased MUFA content in ham. In fact, we have proved that its effects can even be detected in the total edible fat of dry-cured ham slices from commercial packs randomly sampled.

Barrows, as compared to gilts, increased SFA and decreased PUFA. It is a generalized result that castration decreases PUFA (Garitano et al., 2013; Henríquez-Rodriguez et al., 2015), but it is not clear whether this is mainly caused as a result of increased SFA, MUFA, or both. While in our experiment barrows had a greater proportion of SFA (but not MUFA) than gilts, in that of Garitano et al. (2013), based on Duroc × (Landrace × Large White) crossbreds, the barrows had more MUFA (but not SFA). On the other hand, although using raw muscle samples, Ramírez & Cava (2007) did not find differences for SFA, MUFA and PUFA between barrows and gilts in different crossbreds of Duroc × Iberian. However, interestingly, our findings proved that, in commercial Duroc × Iberian dry-cured hams, the investigated SCD polymorphism can have a greater impact on MUFA than using hams from barrows instead of gilts.

Acknowledgments

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References


Cruz J, 2013. Los productores de jamón curado buscan superar con las exportaciones la caída de las ventas en España. Eurocarne 216: 35-44.


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