Spanish Journal of Agricultural Research
13(4), e0404, 8 pages (2015)
eISSN: 2171-9292
http://dx.doi.org/10.5424/sjar/2015134-8323
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

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

Eliana Henriquez-Rodriguez, Marc Tor, Ramona N. Pena and Joan Estany
Universitat de Lleida-Agrotecnio Centre, Departament de Producció Animal. Rovira Roure 191, 25198 Lleida, Spain

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.

Abbreviations used: B (barrows); CT (heterozygous genotype for the polymorphism *AY487830:g.2228T>C*); DM (dry matter); FA (fatty acids); G (gilts); IMF (intramuscular fat); MCMC (Markov chain Monte Carlo); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); *SCD* (stearoyl-CoA desaturase); SE (standard error); SNP (single nucleotide polymorphism); SFA (saturated fatty acids); TT (alternative homozygous genotype for the polymorphism *AY487830:g.2228T>C*).

Citation: Henriquez-Rodriguez, E.; Tor, M.; Pena, R. N.; Estany, J. (2015). A polymorphism in the stearoyl-CoA desaturase gene promoter influences monounsaturated fatty acid content of Duroc × Iberian hams. Spanish Journal of Agricultural Research, Volume 13, Issue 4, e0404, 8 pages. http://dx.doi.org/10.5424/sjar/2015134-8323.

Received: 16 Jul 2015. **Accepted:** 13 Nov 2015.

Funding: Spanish Ministry of Economy and Competitiveness (MINECO, grant AGL2012-33529). EHR is recipient of a PhD scholarship from the University of Lleida.

Competing interests: Three of the authors (JE, MT, and RNP) are named on the patent ES 2452791 B1 filed by University of Lleida on the use of markers of SCD for marker-assisted selective breeding in pigs.

Correspondence should be addressed to Joan Estany: jestany@prodan.udl.cat

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 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 (Narváez-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 using crossbred Duroc × Iberian females or barrows 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 previous 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 225ºC at 7ºC/min and injector and detector temperatures were both 250ºC. 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-7; C18:1n-9; 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

(LifeTechnologies, Grand Island, NY), 0.2 µM primer mix, 0.8 µM probe mix and 10 ng of DNA in a final volume of 5 µL.

The sex of the hams was determined using a modified protocol of Sembon *et al.* (2008) based on the amplification of the amelogenin (*AMEL*) gene. Using published sequences for the porcine amelogenin genes (EMBL/GenBank accession numbers, AB091791 [*AMELX*] and AB091792 [*AMELY*]), a set of PCR primers were designed (AMEL-F, 5′-TCATGAGGAATCTCTTTGGTA-3′; AMEL-R, 5′-CCAGAGGTTGTAACCTTACAG-3′) to amplify a portion of intron 2 that was expected to yield PCR products of different sizes between AMELX (450 bp) and AMELY (278 bp). The PCR reaction mix contained 1x buffer, 2.66 mM of $MgCl_2$, 0.13 mM dNTPs, 0.4 µM of each primer, 0.4 U of Taq DNA polymerase (Biotools, Madrid) and 40 ng of genomic DNA in a final volume of 15 µL. The amplification was carried out in a Veriti thermocycler (LifeTechnologies) with the following conditions: initial denaturation at 95°C for 5 min, 35 cycles of 96°C for 20 s, 54°C for 30 s and 72°C for 40 s and a final extension step of 72ºC × 5 min. PCR products were run in 1.5% agarose gels and visualized by ethidium bromide staining under UV illumination.

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:

$$y|b,\ \sigma_e^2 \sim N(Xb,\ \mathrm{I}\sigma_e^2),$$

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 σ_e^2 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, +∞), if D>0, or (–∞, 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 stand-

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

Gender	Number of samples		Allelic frequency	
	TT	CT	T	C
Barrows	20	18	0.76	0.24
Gilts	23	13	0.82	0.18
Total	43	31	0.79	0.21

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[1].

Trait[2],%	$Mean_{TT}$	$Mean_{CT}$	Mean D_{TT-CT}	$HPD_{95\%TT-CT}$	*p*	$k_{80\%}$
n	43	31				
Fat, % DM	27.77	27.20	0.57	–2.55; 3.79	0.64	
DM	62.11	62.11	0.00	–2.34; 2.40	0.51	
C14:0	1.41	1.41	0.00	–0.06; 0.05	0.48	
C16:0	24.80	24.93	–0.13	–0.63; 0.39	0.70	
C18:0	10.35	10.60	–0.24	–0.72; 0.24	0.83	–0.03
C20:0	0.18	0.21	–0.03	–0.09; 0.04	0.78	
SFA	36.76	37.10	–0.35	–1.27; 0.61	0.76	
C16:1n–9	3.16	3.05	0.11	–0.08; 0.31	0.87	0.03
C18:1n–9	45.62	44.93	0.69	–0.05; 1.46	**0.96**	0.37
C18:1n–7	3.82	3.68	0.13	–0.03; 0.31	**0.93**	0.06
C20:1n–9	0.90	0.88	0.02	–0.01; 0.05	**0.92**	0.01
MUFA	53.50	52.54	0.95	0.05; 1.91	**0.98**	0.56
C18:2n–6	8.33	8.81	–0.48	–0.88; –0.06	**0.99**	–0.31
C18:3n–3	0.47	0.48	–0.01	–0.08; 0.06	0.62	
C20:2n–6	0.36	0.37	–0.01	–0.03; 0.01	0.79	
C20:4n–6	0.59	0.68	–0.09	–0.20; 0.04	**0.91**	–0.03
PUFA	9.75	10.34	–0.59	–1.06; –0.08	**0.99**	–0.38
C18:1n–9/C18:0	4.46	4.27	0.20	–0.05; 0.45	**0.94**	0.09
C16:1n–9/C16:0	0.13	0.12	0.01	0.00; 0.01	0.89	0.00
MUFA/SFA	1.46	1.42	0.04	–0.02; 0.10	**0.92**	0.02
MUFA/PUFA	5.56	5.12	0.43	0.14; 0.74	**1.00**	0.31
SFA/PUFA	3.82	3.63	0.19	–0.03; 0.44	**0.95**	0.09

[1] Mean D_{TT-CT}: mean of the marginal posterior distribution of the difference between genotype TT and CT; $HPD_{95\%TT-CT}$: highest posterior density interval for D_{TT-CT} at 95%; *p*: posterior probability of D_{TT-CT} being greater (if $D_{TT-CT} > 0$) or lower (if $D_{TT-CT} < 0$) than zero; and $k_{80\%}$: limit of the interval [k, +∞) (if $D_{TT-CT} > 0$) or (-∞, k] (if $D_{TT-CT} < 0$) at 80% of probability (k is only displayed when D_{TT-CT} and $k_{80\%}$ 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 of 0.84 to 1.00), they showed lower values of C18:1n-9 (although only with *p* of 0.73). The effect of

Table 3. Features of estimated marginal posterior distribution of the difference between barrows (B) and gilts (G) for fat content (IMF) content and fatty acid composition in Duroc × Iberian dry-cured hams[1].

Trait[2], %	$Mean_B$	$Mean_G$	Mean D_{B-G}	$HPD_{95\%B-G}$	*p*	$k_{80\%}$
n	38	36				
Fat, % DM	27.56	27.42	0.14	–2.87; 3.31	0.53	
DM	62.27	61.95	0.32	–1.93; 2.69	0.60	
C14:0	1.44	1.39	0.06	0.01; 0.10	**0.99**	0.04
C16:0	25.06	24.67	0.40	–0.09; 0.88	**0.94**	0.18
C18:0	10.49	10.46	0.02	–0.47; 0.50	0.54	
C20:0	0.19	0.20	–0.01	–0.08; 0.06	0.59	
SFA	37.20	36.67	0.53	–0.36; 1.47	0.87	0.12
C16:1n–9	3.15	3.06	0.09	–0.09; 0.28	0.84	0.01
C18:1n–9	45.16	45.39	–0.24	–0.95; 0.52	0.73	
C18:1n–7	3.83	3.68	0.15	–0.01; 0.31	**0.96**	0.08
C20:1n–9	0.91	0.87	0.04	0.01; 0.07	**1.00**	0.03
MUFA	53.04	53.00	0.03	–0.86; 0.92	0.53	
C18:2n–6	8.32	8.82	–0.50	–0.90; –0.11	**0.99**	–0.33
C18:3n–3	0.46	0.49	–0.03	–0.10; 0.04	0.83	0.00
C20:2n–6	0.36	0.38	–0.01	–0.04; 0.01	**0.92**	–0.01
C20:4n–6	0.63	0.64	–0.01	–0.14; 0.11	0.57	
PUFA	9.77	10.33	–0.56	–1.03; –0.08	**0.99**	–0.36
C18:1n–9/C18:0	4.34	4.39	–0.05	–0.31; 0.19	0.66	
C16:1n–9/C16:0	0.13	0.12	0.00	–0.01; 0.01	0.65	
MUFA/SFA	1.43	1.45	–0.02	–0.08; 0.04	0.75	
MUFA/PUFA	5.48	5.20	0.28	0.00; 0.57	**0.97**	0.16
SFA/PUFA	3.84	3.60	0.24	0.00; 0.47	**0.98**	0.14

[1] Mean D_{B-G}: mean of the marginal posterior distribution of the difference between barrows and gilts; $HPD_{95\%B-G}$: highest posterior density interval for D_{B-G} at 95%; *p*: posterior probability of D_{B-G} being greater (if D_{B-G} >0) or lower (if D_{B-G} <0) than zero; and $k_{80\%}$: limit of the interval [k, +∞) (if D_{B-G} >0) or (-∞, k] (if D_{B-G} <0) at 80% of probability (k is only displayed when D_{B-G} and k80% are of the same sign). *p* >0.9 are written in bold. [2] See Table 2 for abbreviations.

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* of 0.98 and 0.97, respectively).

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 (Henriquez-Rodriguez *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; Henriquez-Rodriguez *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; Henriquez-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, Ramirez & 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

The authors thank Aleix Solé and Teresa Giró for their assistance in collecting the samples and in the laboratory analyses.

References

ANICE, 2014. El sector cárnico español. Asociación Nacional de Industrias de la carne de España. http://www.anice.es/v_portal/informacion/informacionver.asp?cod=9776&te=7&idage=11909 [03 March 2015].

Antequera T, López-Bote C, Córdoba J, García C, Asensio M, Ventanas J, García-Regueiro J, Díaz I, 1992. Lipid oxidative changes in the processing of Iberian pig hams. Food Chemistry 45(2): 105-110. http://dx.doi.org/10.1016/0308-8146(92)90018-W

AOAC, 2000. Official method 960.39 fat (crude) or ether extract inmeat. Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Belmonte S, 2006. El jamón ibérico: producción y consumo, situación en la Comunidad de Madrid y promoción de su calidad. Documentos Técnicos de Salud Pública, Nº 105. Dirección General de Salud Pública y Alimentación, Comunidad de Madrid (Spain).

BOE, 2014. Royal Decree 4/2014, of 10 January, which approves the norm of quality for pork, and cured hams, shoulders and loins from the Iberian pig. Boletín Oficial del estado Nº 10. pp: 1569-1585.

Bosch L, Tor M, Reixach J, Estany J, 2009. Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. Meat Sci 82(4): 432-437. http://dx.doi.org/10.1016/j.meatsci.2009.02.013

Cameron N, Enser M, Nute G, Whittington F, Penman J, Fisken A, Perry A, Wood J, 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci 55(2): 187-195. http://dx.doi.org/10.1016/S0309-1740(99)00142-4

Cava R, Ruiz J, Ventanas J, Antequera T, 1999. Oxidative and lipolytic changes during ripening of Iberian hams as affected by feeding regime: extensive feeding and alpha-tocopheryl acetate supplementation. Meat Sci 52(2): 165-172. http://dx.doi.org/10.1016/S0309-1740(98)00164-8

Chamorro A, Miranda F, Rubio S, Valero V, 2008. Análisis del proceso de compra del jamón ibérico en España. Distribución y Consumo 101: 50-58.

Cordero G, Isabel B, Menoyo D, Daza A, Morales J, Piñeiro C, Lopez-Bote C, 2010. Dietary CLA supplementation and gender modify fatty acid composition of subcutaneous and intramuscular fat in Iberian × Duroc finishing heavy pigs. Span J Agric Res 8(4): 962-970. http://dx.doi.org/10.5424/sjar/2010084-1244

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

Enge M, Furitsch H, Mili S, 1998. Hábitos de consumo y demanda de productos cárnicos en España. Revista Española de Economía Agraria 182: 131-166.

Estany J, Ros-Freixedes R, Tor M, Pena R, 2014. A functional variant in the stearoyl-CoA desaturase gene promoter enhances fatty acid desaturation in pork. PloS One 9(1): e86177. http://dx.doi.org/10.1371/journal.pone.0086177

Fernández M, Ordóñez J, Cambero I, Santos C, Pin C, de la Hoz L, 2007. Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications. Food Chem 101(1): 107-112. http://dx.doi.org/10.1016/j.foodchem.2006.01.006

Gandemer G, 2009. Dry cured ham quality as related to lipid quality of raw material and lipid changes during processing: a review. Grasas y Aceites 60(3): 297-307. http://dx.doi.org/10.3989/gya.130908

Garitano I, Liebana C, de Vargas E, Moreno A, Daza A, 2013. Effect of gender on growth performance, carcass characteristics, meat and fat composition of pigs slaughtered at 125 kg of live weight destined to Teruel (Spain) ham production. Ital J Anim Sci 12:e16, 95-100.

Gillingham L, Harris-Janz S, Jones P, 2011. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 46(3): 209-228. http://dx.doi.org/10.1007/s11745-010-3524-y

Gou P, Zhen Z, Hortós M, Arnau J, Diestre A, Robert N, Claret A, Čandek-Potokar M, Santé-Lhoutellier V, 2012. PRKAG3 and CAST genetic polymorphisms and quality traits of dry-cured hams--I. Associations in Spanish dry-cured ham Jamón Serrano. Meat Sci 92(4): 346-353. http://dx.doi.org/10.1016/j.meatsci.2012.06.018

Henriquez-Rodriguez E, Tor M, Pena R, Estany J, 2015. A polymorphism in the stearoyl-CoA desaturase gene promoter increases monounsaturated fatty acid content in dry-cured ham. Meat Sci 106: 38-43. http://dx.doi.org/10.1016/j.meatsci.2015.03.019

Lopez-Bote C, 1998. Sustained utilization of the Iberian pig breed. Meat Sci 49: S17-S27. http://dx.doi.org/10.1016/S0309-1740(98)00072-2

Lorido L, Estévez M, Ventanas J, Ventanas S, 2015. Salt and intramuscular fat modulate dynamic perception of flavour and texture in dry-cured hams. Meat Sci 107: 39-48. http://dx.doi.org/10.1016/j.meatsci.2015.03.025

Martín L, Córdoba J, Ventanas J, Antequera T, 1999. Changes in intramuscular lipids during ripening of Iberian dry-cured ham. Meat Sci 51(2): 129-134. http://dx.doi.org/10.1016/S0309-1740(98)00109-0

Muñoz G, Alcázar E, Fernández A, Barragán C, Carrasco A, de Pedro E, Silió L, Sánchez J, Rodríguez M, 2011. Effects of porcine MC4R and LEPR polymorphisms, gender and Duroc sire line on economic traits in Duroc × Iberian crossbred pigs. Meat Sci 88(1): 169-173. http://dx.doi.org/10.1016/j.meatsci.2010.12.018

Narváez-Rivas M, Vicario I, Constante E, León-Camacho M, 2008. Changes in the fatty acid and triacylglycerol profiles in the subcutaneous fat of Iberian ham during the dry-curing process. J Agr Food Chem 56 (16): 7131-7. http://dx.doi.org/10.1021/jf800990u

Pena R, Gallardo D, Guàrdia M, Reixach J, Arnau J, Amills M, Quintanilla R, 2013. Appearance, flavor, and texture attributes of pig dry-cured hams have a complex polygenic genomic architecture. J Anim Sci 91(3): 1051-1058. http://dx.doi.org/10.2527/jas.2012-5458

Ramírez R, Cava R, 2007. Carcass composition and meat quality of three different Iberian×Duroc genotype pigs.

Meat Sci 75(3): 388-396. http://dx.doi.org/10.1016/j.meatsci.2006.08.003

Reina R, López-Buesa P, Sánchez del Pulgar J, Ventanas J, García C, 2012. Effect of IGF-II (insulin-like growth factor-II) genotype on the quality of dry-cured hams and shoulders. Meat Sci 92(4): 562-568. http://dx.doi.org/10.1016/j.meatsci.2012.05.027

Reixach J, Tor M, Díaz M, Estany J, 2008. Between and within breed variation for lean growth and intramuscular fat content and fatty acid composition in pigs. Proc 54th Int Cong of Meat Science and Technology, ICoMST, Cape Town (South Africa), August 10-15. p. 9.13.

Renaville B, Piasentier E, Fan B, Vitale M, Prandi A, Rothschild M, 2010. Candidate gene markers involved in San Daniele ham quality. Meat Sci 85(3): 441-445. http://dx.doi.org/10.1016/j.meatsci.2010.02.013

Ros-Freixedes R, Estany J, 2013. On the compositional analysis of fatty acids in pork. J Agr Biol Environ Stat 19 (1): 136-155. http://dx.doi.org/10.1007/s13253-013-0162-x

Ros-Freixedes R, Reixach J, Tor M, Estany J, 2012. Expected genetic response for oleic acid content in pork. J Anim Sci 90(12): 4230-4238. http://dx.doi.org/10.2527/jas.2011-5063

Ros-Freixedes R, Reixach J, Bosch L, Tor M, Estany J, 2014. Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs. J Anim Sci 92(11): 5417-5425. http://dx.doi.org/10.2527/jas.2014-8202

Ruiz J, Ventanas J, Cava R, Andrés A, García C, 1999. Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. Meat Sci 52(1): 19-27. http://dx.doi.org/10.1016/S0309-1740(98)00144-2

Ruiz-Carrascal J, Ventanas J, Cava R, Andrés A, García C, 2000. Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. Food Res Int 33(2): 91-95. http://dx.doi.org/10.1016/S0963-9969(99)00153-2

Rule DC, 1997. Direct transesterification of total fatty acids of adipose tissue, and of freeze-dried muscle and liver with boron-trifluoride in methanol. Meat Sci 46(1): 23-32. http://dx.doi.org/10.1016/S0309-1740(97)00008-9

Sembon S, Suzuki S, Fuchimoto D, Iwamoto M, Kawarasaki T, Onishi A, 2008. Sex identification of pigs using polymerase chain reaction amplification of the amelogenin gene. Zygote 16(4): 327-332. http://dx.doi.org/10.1017/S0967199408004826

Serrano M, Valencia D, Fuentetaja A, Lázaro R, Mateos G, 2008. Effect of gender and castration of females and slaughter weight on performance and carcass and meat quality of Iberian pigs reared under intensive management systems. Meat Sci 80(4): 1122-1128. http://dx.doi.org/10.1016/j.meatsci.2008.05.005

Soriano A, Quiles R, Mariscal C, García A, 2005. Pig sire type and sex effects on carcass traits, meat quality and physicochemical and sensory characteristics of Serrano dry-cured ham. J Sci Food Agr 85(11): 1914-1924. http://dx.doi.org/10.1002/jsfa.2197

Stalder K, Rothschild M, Lonergan S, 2005. Associations between two gene markers and indicator traits affecting fresh and dry-cured ham processing quality. Meat Sci 69(3): 451-457. http://dx.doi.org/10.1016/j.meatsci.2004.08.014

Tejerina D, García-Torres S, Cabeza de Vaca M, Vázquez F, Cava R, 2012. Effect of production system on physical-chemical, antioxidant and fatty acids composition of Longissimus dorsi and Serratus ventralis muscles from Iberian pig. Food Chem 133(2): 293-299. http://dx.doi.org/10.1016/j.foodchem.2012.01.025

Tikk K, Tikk M, Aaslyng M, Karlsson A, Lindahl G, Andersen H, 2007. Significance of fat supplemented diets on pork quality - Connections between specific fatty acids and sensory attributes of pork. Meat Sci 77(2): 275-286. http://dx.doi.org/10.1016/j.meatsci.2007.03.019

Wood J, Nute G, Richardson R, Whittington F, Southwood O, Plastow G, Mansbridge R, da Costa N, Chang K, 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci 67(4): 651-667. http://dx.doi.org/10.1016/j.meatsci.2004.01.007

Wood J, Enser M, Fisher V, Nute G, Sheard P, Richardson R, Hughes S,Whittington F, 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci 78(4): 343-358. http://dx.doi.org/10.1016/j.meatsci.2007.07.019