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in

De Pedro E.J. (ed.), Cabezas A.B. (ed.).
7th International Symposium on the Mediterranean Pig

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 101

2012
pages 47-52

Δ9-desaturase polymorphism association with fatty acid profile of Italian PDO dry cured hams

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Abstract. PDO dry-cured ham (prosciutto) is the main product of the Italian pig industry. Fatty acid profile is of major importance for the technological properties of the fresh thigh and for the dietary and sensory quality of the ham. Δ9-desaturase (SCD) is a microsomal enzyme required for the biosynthesis of unsaturated fatty acids and therefore a potential candidate gene to explain the differences in the MUFA content in meat and fat. Through sequencing, a SNP was observed in the SCD promoter. Forty-six pigs of Italian Landrace x Italian Large White cross breed ("reference hybrids") and 32 Goland, a commercial hybrid, were genotyped by PCR-RFLP for this mutation. Fatty acid profiles were determined by gas chromatography in thigh subcutaneous fat. Single locus genotype association analysis was carried out using the General Linear Model in SPSS. The genotypic frequencies of the SCD polymorphism were different between the two pig hybrids. However, independently of the pig genetic type, significant associations ($P < 0.05$) were found between the SCD polymorphism and the ratios of palmitoleic to palmitic acids and of oleic to stearic acids, as well as the ratio of MUFA to SFA. These findings confirm our previous results and suggest that the SCD promoter polymorphism is associated with the quality of PDO dry cured Italian hams.

Keywords. Italian heavy pig – SCD – Fatty acid profile – Association – Dry-cured ham.

Association entre un polymorphisme de la Δ9-désaturase et le profil en acides gras des jambons crus AOC italiens

Résumé. *Le principal produit de l'industrie porcine italienne est le jambon cru AOC. Le profil en acides gras est d'importance majeure pour les propriétés technologiques de la cuisse et pour la qualité diététique et sensorielle du jambon. La Δ9-désaturase (SCD) est une enzyme microsomale impliquée dans la biosynthèse des acides gras insaturés ce qui en fait un gène candidat potentiel pour interpréter les différences dans le contenu en MUFA de la viande et de la graisse. Un SNP a été identifié par séquençage dans le promoteur de la SCD. Quarante-six porcs Landrace Italien x Large White Italien (hybrides de référence) ainsi que 32 porcs Goland, un hybride commercial, ont été génotypés pour cette mutation par PCR-RFLP. La composition en acides gras dans la graisse sous-cutanée de la cuisse a été déterminée par chromatographie gazeuse. L'analyse d'association entre le génotype et le profil en acides gras a été réalisée par le General Linear Model de SPSS. La fréquence génotypique pour cette mutation de la SCD était différente entre les deux groupes de porc étudiés. De plus, indépendamment de la souche porcine, des associations significatives ont été observées (P<0,05) entre le polymorphisme du promoteur de la SCD et les ratios entre acides palmitoléique et palmitique, entre acides oléique et stéarique, ainsi qu'entre MUFA et SFA. Ces résultats confirment nos observations antérieures et suggèrent que le polymorphisme du promoteur de la SCD est associé à la qualité du jambon cru italien AOC.*

Mots-clès. *Porc lourd italien – SCD – Profil en acides gras – Association – Jambon cru.*

I – Introduction

PDO dry-cured ham (prosciutto) is the main product of the Italian pig industry and more than

80% of the pig production is destined to the PDO traditional Italian ham market (Renaville *et al.*, 2010). To obtain high quality PDO dry-cured hams, the production is subjected to rules fixed by several Consortia (Bosi and Russo, 2004) concerning the characteristics of fresh thighs, genetic type, feedstuff, age and slaughtering weight of animals. Hams are obtained from pigs of at least 9 months of age and 160 kg of minimum live weight. Heavy pigs have two main origins: the first is a specific Italian selection obtained from the "traditional breeds" genetically improved by the Italian Breeders Association, in particular the crossing breeds used are Italian Large White, Italian Landrance, Italian Duroc and their crosses are called "reference crosses". The second one regards commercial hybrids produced by international breeding companies in agreement with the objectives of the Italian heavy pig selection.

It is widely accepted that the genetic type can influence ham quality (Vitale *et al.,* 2009) and fatty acids profile (Lo Fiego *et al.*, 2005; Wood *et al.*, 2008; Piasentier *et al.*, 2009). In particular unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids and they can influence the sensory quality and acceptability of dry cured hams (Musella *et al.,* 2009).

Δ9 desaturase, also called Stearoyl-CoA desaturase (SCD), is a rate-limiting enzyme located in the membrane of the endoplasmic reticulum and it is responsible for the insertion of a double bond between carbons 9 and 10 into saturated fatty acids (Ntambi and Miyazaki, 2004). Previously Ramos *et al.* (2008), considering another SNP, showed that a SCD polymorphism was associated with colour, weight and yield of cured hams.

The purpose of this work was to estimate the effect of the SCD polymorphisms on fatty acid composition of subcutaneous adipose tissue of thighs destined to the production of PDO Italian dry-cured hams.

II – Materials and methods

Forty six thighs of Italian Landrace x Italian Large White cross pigs ("reference hybrids", IL x LW) and 32 thighs of Goland pigs (commercial hybrid) were considered. Pigs were reared in the same farm and fed with a standard cereals-soybean based meal as described in Minelli *et al.* (2010). All the thighs were destined to the production of PDO Italian dry-cured hams.

Fatty acid profile of subcutaneous fat was determined using a TRACE™ GC Ultra (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with the Ultra Fast Module (UFM), a Fast Flame Ionization Detector and a UFM-Carbovax column, 5 m long, 0.1 mm i.d, 0.2 µm film thickness as described in a previous paper (Ficarra *et al*., 2010). The FAME were identified by comparison of each retention time with the known retention times of the corresponding pure standards (Supelco 37 Component FAME mix and PUFA standard n. 2, animal source, Supelco, Bellafonte, PA, USA). For quantification purposes, the response factor was calculated and the method of the internal standard was used. Results were expressed as g of each fatty acid methyl ester/100 g of total lipids.

DNA was extracted from muscle samples using a standard DNA extraction method. The genotype procedure was as follows. The PCR amplifications were performed using 12.5 ng of porcine DNA, 1 x PCR buffer, 0.125 mM each dNTP, 0.3 mM of each primer and 0.35 U Taq polymerase (Promega, Madison, WI, USA). The primers used were: forward: CTCTGTCTCCTCCCCTCTCC; reverse: GATCACTTTCCCAGGGATGA. PCR products were sequenced using an ABI automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). A C/T polymorphism was found in the fragment and a PCR-RFLP test was designed using the specific restriction enzyme PflF1 following the recommendations of the manufacturer. The fragment size was 322 bp for allele 1 and 212 + 110 bp for allele 2. With the aim of estimating the SCD enzyme activity Δ9 desaturase index was assessed and calculated as proposed by Smith *et al.* (2002), (16:1 + 18:1) / (14:0 + 16:0 + 18:0 + 16:1 + 18:1).

The statistical analyses were done using the package SPSS vers. 17 (SPSS Inc., Illinois). The

difference in SCD genotypic frequencies between hybrids was tested by chi-square tests. Fatty acids profiles were subjected to analysis of variance using a two way factorial design with hybrids and SCD genotype as fixed effects. Interaction between hybrids and SCD genotype was considered, but the value was not reported in Table 2 because it never reached the level of significance ($P > 0.05$). Post hoc pairwise comparisons were analyzed by the Tukey-Kramer tests.

III – Results and discussion

The SCD promoter genotypes were different between hybrids (Table 1). In particular Goland commercial hybrid pigs had a higher frequency of genotype 22 than IL x LW pigs (84.3% *vs*. 50.0%). Conversely the IL x LW pigs presented a higher frequency of genotype 12 than Goland pig (37.0% *vs*. 15.6%) in which the genotype 11 were not found. Renaville *et al.* (2010) reported similar frequencies (89% for 22 and 11% for 12) in a Goland population (n = 149). Overall, these authors reported a frequency of genotype 22, 12 and 11 of 76, 22 and 2% respectively, considering 615 pigs from four different commercial hybrids destined to the PDO traditional Italian ham market. Ramos *et al.* (2008) in a study that took into account 321 US country hams derived from commercially crossbred pigs found a frequency of SCD markers genotype 22, 12 and 11 of 50, 39 and 11% respectively. Although, the polymorphism of these 2 studies was different, it is in completely linked with our mutation in the promoter allowing to compare the results.

Table 1. Genotypic frequencies of Stearoyl Co-A desaturase (SCD) markers in different pig hybrid - Italian Landrance x Italian Large White (IL x LW) and Goland.

Marker	Hybrids		Total	*P*-value
	IL x LW	Goland		
No of thighs	46	32	78	
SCD marker				0.01
11	6	0	7.7 %	
12	17	5	28.2 %	
22	23	27	64.1 %	

The estimated marginal means, i.e. the mean response for each factor adjusted for the other variables in the model, of the fatty acid composition of the subcutaneous adipose tissue of thighs are reported in Table 2. A significant effect of genetic type was observed. The IL x LW "reference hybrid" had significantly higher proportion of C18:0 ($P < 0.01$), C20:0 ($P < 0.01$), C18:1 ($P < 0.01$), C20:1 ($P < 0.01$) and had, consequently, higher levels of saturated fatty acids (SFA) ($P < 0.01$) and monounsaturated fatty acids (MUFA) ($P < 0.01$) than Goland commercial pigs. The MUFA / SFA ratio was not affected by the hybrid ($P > 0.05$). Goland commercial hybrids showed significantly higher proportion of C18:2 ($P < 0.01$), C18:3 ($P < 0.01$), C20:2 ($P < 0.01$), and had, consequently, higher level of polyunsaturated fatty acids (PUFA) ($P < 0.01$) than "reference hybrids". In particular Goland hybrids had a content of linoleic acid (C18:2), 12.38%, near to the threshold value set by the Consortia for this fatty acid (15%) and higher than those proposed by Girard *et al.* (1988) (12%). This value was introduced by the Consortia with the aim to limit the content of PUFA of the subcutaneous adipose tissue of fresh thighs in order to guarantee an adequate firmness of fat and to limit its oxidability (Bosi and Russo, 2004). Our results are consistent with the results obtained by Lo Fiego *et al.* (2005) that considered 112 pig, 56 "traditional pigs" (Italian Landrace x Large White cross) and 56 animals from a commercial hybrid, destined to the production of PDO traditional dry-cured ham. The above

cited authors suggested that the genetic selection of the commercial hybrids have significantly increased pigs' performance reducing the quantity of carcass fat with an increasing in PUFA content. Consequently, it makes the thighs less suitable for curing.

Table 2. Effect of the different pig hybrid –Italian Landrance x Italian Large White cross (IL x LW) and Goland– and of Stearoyl Co-A desaturase (SCD) markers on thickness (mm) and on fatty acids composition (g/100g of total lipids) of subcutaneous adipose tissue of thighs (estimated marginal means).

	Hybrids		SCD markers			RMSE	P – value	
	IL x LW	Goland	11	12	22		H	SG
Fat thickness	25.41	23.95	27.00	25.71	22.85	5.513	0.713	0.171
C14:0	1.21	1.22	1.17	1.25	1.20	0.146	0.759	0.366
C16:0	21.90	21.12	21.76	21.74	21.34	1.447	0.072	0.666
C16:1	2.01	2.28	1.82	2.17	2.22	0.294	0.075	0.078
C17:0	0.43	0.38	0.39	0.40	0.42	0.116	0.078	0.522
C17:1	0.41	0.38	0.36	0.39	0.43	0.113	0.163	0.172
C18:0	13.49^{A}	10.99^{B}	14.21^{a}	12.27^{b}	11.84^{b}	0.933	0.000	0.014
C18:1	41.72^{A}	38.05^{B}	41.03	39.44	40.67	2.285	0.000	0.148
C18:2	8.56^{B}	12.38^{A}	7.95	10.44	10.80	1.490	0.000	0.287
C18:3n-6	0.13	0.12	0.12^{AB}	0.10^{B}	0.15^{A}	0.054	0.345	0.005
C18:3	0.40^{B}	0.55^{A}	0.37	0.46	0.50	0.077	0.000	0.084
C20:0	0.20^{A}	0.16^{B}	0.21	0.17	0.18	0.028	0.001	0.080
C20:1	0.94^{A}	0.73^{B}	1.04^{A}	0.75^{C}	0.87^{B}	0.143	0.001	0.005
C20:2	0.44^{B}	0.54^{A}	0.45	0.46	0.52	0.076	0.000	0.063
C20:3	0.08	0.08	0.08	0.08	0.09	0.022	0.476	0.273
C20:4	0.13	0.15	0.12	0.14	0.15	0.032	0.057	0.429
SFA[†]	37.22^{A}	33.86^{B}	37.74	35.83	34.99	2.151	0.000	0.337
MUFA[††]	45.08^{A}	41.44^{B}	44.25	42.75	44.19	2.424	0.000	0.095
PUFA[†††]	9.73^{B}	13.82^{A}	9.08	11.67	12.21	1.622	0.000	0.229
C16:1 / C16:0	0.09^{B}	0.11^{A}	0.08^{b}	0.10^{a}	0.10^{a}	0.011	0.001	0.025
C17:1 / C17:0	0.96	1.03	0.91	0.99	1.03	0.107	0.217	0.156
C18:1 / C18:0	3.11^{b}	3.51^{a}	2.90^{b}	3.27^{a}	3.46^{a}	0.336	0.010	0.021
C20:1 / C20:0	4.80	4.72	4.89	4.62	4.87	0.714	0.858	0.522
Δ9 desaturase index[††††]	0.545	0.548	0.536^{b}	0.542^{ab}	0.556^{a}	0.018	0.813	0.025
MUFA / SFA	1.21	1.23	1.17^{b}	1.20^{b}	1.27^{a}	0.097	0.878	0.035

RMSE: root mean standard error; H: effect of hybrid; SG: effect of SCD markers; ^{A,B,C} Means within the same row with unlike letters differ significantly at $P \leq 0.01$; ^{a,b,c} Means within the same row with unlike letters differ significantly at $P \leq 0.05$.

[†]SFA (saturated fatty acids) = C14:0 + C16:0 + C17:0 + C18:0 + C20:0.

[††]MUFA (monounsaturated fatty acids) = C16:1 + C17:1 + C18:1 + C20:1.

[†††]PUFA (polyunsaturated fatty acids) = C18:2 + C18:3n-6 + C18:3 + C20:2 + C20:3 + C20:4.

[††††]Δ9 desaturase index = (C16:1 + C18:1) / (C14:0 + C16:0 + C18:0 + C16:1 + C18:1)

The "reference hybrids" obtained from the "traditional breeds" had lower C18:1 / C18:0 ($P < 0.05$) and C16:1 / C16:0 ratio ($P < 0.01$) than Goland hybrid, however the Δ9 desaturase index was similar between breeds ($P > 0.05$).

Stearoyl-CoA Desaturase or Δ9-desaturase is a key enzyme in lipid metabolism, indeed, it is involved in the conversion of SFA in MUFA. In particular SCD enzyme converts C16:0 and C18:0 to C16:1 and C18:1 respectively. Moreover Cánovas *et al.* (2009) suggested that SCD might be an effective potential biomarker for fat deposition in pigs. Renaville *et al.* (2010) found an association between SCD polymorphism and ham fat thickness and the lowest value was recorded by the genotype 11.

In our trial the effect of the SCD promoter genotype on ham fat thickness did not reach the threshold of significance ($P > 0.05$) likely due to the little number of pigs considered. Significant associations were found between the SCD markers and the levels of C18:3n-6 and C20:1. Genotype 12 showed the lowest level of C20:1 ($P < 0.01$) which is probably synthesized by elongation of oleic acid (Durant-Montgé *et al.,* 2010). Genotype 22 presented significantly the highest C18:3n-6 level ($P < 0.01$) although the SCD enzyme is not directly involved in the metabolism of C18:3n-6, the effect observed might be due to an indirect effect of SCD. Indeed, SCD is involved in the packaging of fatty acids in the Very Low Density Lipoprotein (Stefan *et al.*, 2008) and therefore in their transport to the adipose tissue. An increase activity of SCD might increase the trafficking of C18:3. The content of linoleic acid (C18:2) in thigh subcutaneous fat was not affected by SCD marker genotype, and it is lower than the value fixed in the rules set by the Consortia (15%).

Moreover genotype 11 presented the highest C18:0 ($P < 0.05$) content and the lowest C16:1 / C16:0 ($P < 0.05$) and C18:1 / C18:0 ratio ($P < 0.05$). In general, these results highlighted a lower SCD enzyme activity of genotype 11. This statement seems to be confirmed by the $\Delta 9$ desaturase index that was lower in genotype 11 than genotype 22 ($P < 0.05$). This index was previously used as an estimator of SCD enzyme activity in cattle and in pig (Corl *et al.,* 2001; Smith *et al.,* 2002). The total content of SFA, MUFA and PUFA were not affected by SCD genotype, however the genotype 22 presented higher MUFA / SFA ratio than the other groups ($P < 0.05$) probably in consequence of its higher SCD enzyme activity. This result suggests that the lipids in the ham of pig with a SCD promoter genotype 22 have a higher nutritional quality (WHO, 2003) than those of the other genotypes.

IV – Conclusions

In summary we can be concluded that there are differences in the genotypic frequencies of the SCD promoter polymorphism between the two pig hybrids. In particular, the “reference hybrids” have lower frequencies of genotype 22 and higher frequencies of genotypes 11 and 12 than Goland commercial hybrids. However, independently of the pig genetic type, SCD promoter genotype 11 had the lowest ratios of palmitoleic to palmitic acid and of oleic to stearic acid. Accordingly the genotype 22 had the highest ratio of MUFA to SFA and $\Delta 9$ desaturase index. These findings suggest, in agreement with our previous results, that the SCD promoter polymorphism is associated with the quality of PDO Italian dry cured hams probably reflecting a different SCD enzyme activity.

Acknowledgements

The authors would like to thank Dr. Elena Saccà for her technical assistance. The research was supported by MIUR- PRIN 2007 (Prof. E. Piasentier).

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