Effect of dietary oleic acid content: Different genetic regulation of fatty acid metabolism on muscle and fat of Iberian pigs

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Abstract. Feeding, genetics and their interactions influence animal tissue composition, thus affecting meat products quality. These influences are especially relevant in Iberian pig production. The goal of this work was the evaluation of the dietary oleic acid content effect on Iberian pigs muscle and fat composition and on δ9-desaturase (*SCD*) gene expression. Two groups of 13 castrated Iberian males were fed with two isocaloric diets differing on high-oleic sunflower oil content (0 *vs* 6%), from 28 to 110 kg live weight. Fatty acid profile of subcutaneous fat showed great differences between both groups. Nevertheless, intramuscular fat quantity and composition were not affected by the treatment. Results of qPCR show a great stability of *SCD* gene expression on adipose tissue. Contrarily, results obtained for muscle indicate a higher *SCD* gene expression (2x) on animals fed with 0% oleic diet (P <0.054), thus suggesting a different metabolic control of fat deposition on both studied tissues.

Keywords. Iberian pig – Oleic acid – *SCD* – Feeding.

Effets de la teneur en acide oléique de la ration : Différents contrôles géniques du métabolisme des acides gras du lard et de la longe chez les porcs Ibériques

Résumé. *L'alimentation, la génétique et leurs interactions influent sur la composition des tissus animaux et en conséquence sur la qualité de la viande. Cette influence est particulièrement importante chez un porc charcutier comme l'Ibérique. Dans ce travail, les effets de la teneur en acide oléique de la ration sur la composition lipidique et sur l'expression du gène SCD (δ9 désaturase) ont été étudiés dans la graisse de la longe et du lard des porcs Ibériques. Deux groupes de 13 mâles castrés furent nourris, de 28 jusqu'à 110 kg, avec des rations iso-caloriques avec différents pourcentages (0* vs *6%) d'huile de tournesol riche en acide oléique. Des différences significatives entre les deux groupes furent détectées dans le profil en acides gras du lard, mais la quantité et la composition de la graisse intramusculaire de la longe furent similaires. Les données obtenues pour l'analyse du transcriptome par qPCR montrent une grande stabilité de l'expression du gène SCD dans le tissu adipeux (lard). Les données obtenues dans le tissu musculaire (longe) indiquent cependant une plus grande expression (2x) de ce gène chez les porcs nourris avec l'aliment riche en acide oléique (P < 0,054). Le contrôle génique de la composition lipidique peut être différent entre les deux tissus.*

Mots-clés. *Porc Ibérique – Acide oléique – SCD – Alimentation.*

I – Introduction

Monounsaturated fatty acids (MUFA) enriched diets, through the inclusion of high oleic acid sunflower oil, are being used to fed Iberian-type pigs in order to mimic the profile of fatty acids (FA) characteristic of pigs fattened with the traditional system based on acorns from evergreen oaks and pasture. The effects of these enriched diets on the fatty acid composition of subcutaneous and intramuscular fat have been studied (Daza *et al*., 2005; Ventanas *et al*., 2008; Pérez-Palacios *et al*., 2009). In spite of the practical utilization of MUFA enriched diets in the production of Iberian pigs, their effect on the transcription of genes related to lipid metabolism has not been studied in this breed.

Stearoyl-CoA desaturase (*SCD*) gene has been proposed as a potential biomarker for fat deposition due to its key role in lipid metabolism and evidences of association between its expression and intramuscular fat content in pigs (Cánovas *et al.*, 2009; Zhao *et al.*, 2009). SCD is a key enzyme required for the biosynthesis of MUFA from saturated fatty acids (SFA) that are either synthesized de novo or derived from the diet. This enzyme catalizes the Δ^9-*cis* desaturation of a range of fatty acyl-CoA substrates, mainly palmitoyl and stearoyl-CoA, which are converted into palmitoleoyl- and oleoyl-CoA respectively. These products are the most abundant MUFA and serve as substrates for the synthesis of various kinds of lipids. Also, MUFA have been implicated as mediators in signal transduction and cellular differentiation. Thus, given the multiple roles of MUFA, variation in Stearoyl CoA desaturase expression or activity would be expected to affect a variety of key physiological variables in mammals. Also, in Iberian pig production, the influence of oleic acid content of meat products on the sensorial and technological quality is an added key variable that could be dependent on SCD function. For this reason this gene is considered a candidate to influence FA composition and pig meat and fat quality.

SCD is a highly regulated enzyme, which expression is known to be regulated by several dietary, hormonal and environmental factors (Paton and Ntambi, 2009). Recently, *SCD* expression has been shown to be down-regulated in response to linoleic and oleic acid treatment in vitro, in several species (Zulkifli *et al.*, 2010). This effect varies in intensity between tissues, cell types, species and fatty acids; and is a consequence of a reduction of *SCD* promoter activity in response to the fatty acid treatment. Specifically, the fatty acids are known to bind a highly conserved PUFA response region (PUFA-RR) of the *SCD* gene promoter, which contains several transcription factor binding sites as a sterol response element (SRE). Binding of FA to the PUFA-RR inhibits *SCD* transcription, with linoleic acid showing a more potent effect than oleic acid.

In live animals, changes in dietary fat composition have different impacts on the expression of genes related to lipid metabolism, and these impacts are highly dependent on the specie, age of the animal and studied tissue (Ding *et al*, 2003; Duran-Montge *et al*, 2009). *SCD* gene expression has been observed to be influenced by the FA dietary content (and specifically by dietary oleic acid) in adipose tissue and liver, but not in *semimembranosus* muscle of Duroc x Landrace female pigs of 100 Kg BW (Duran-Montge *et al*, 2009). Other tissue–specific responses of porcine *SCD* function have also been previously reported in dietary trials of protein reduction (Doran *et al*, 2006).

The objective of this work was to evaluate the effect of the oleic acid content of diet on fatty acid composition and transcription of *SCD* gene both in fat and muscle tissues of Iberian pigs. Also differences due to age, tissue and feeding status were studied.

II – Material and methods

1. Animals, dietary treatments and recorded traits

The experiment was performed at CIA *Dehesón del Encinar*. Twenty seven castrated males of the *Torbiscal* Iberian strain were used. At 28 kg of body weight the animals were housed individually, distributed in two groups fed with two different diets, one of them with the inclusion of high-oleic sunflower oil. Feeds composition is shown in Table 1. The energy content per kg of the feeds was: 3,130 Kcal crude energy for the control (C) diet, and 3,360 Kcal for the high oleic (HO) diet. Animals were fed twice a day. The higher energy content of the HO diet was compensated with a 10% increase in the feed quantity received by the animals of the control group. Water was provided *ad libitum*. Dietary treatment was maintained during 24 weeks until the pigs reached 110 kg of average live weight , when animals were slaughtered.

Live weight was recorded on live animals each two weeks during the treatment. Ham subcutaneous fat samples were obtained *in vivo* by shot-biopsies at different ages. One week before the sacrifice, fat biopsies were obtained immediately before and three hours after feeding. Animals were stunned and slaughtered at a local slaughterhouse (Alcaudete de la Jara, Toledo, Spain). At slaughter tissue samples were collected for gene expression analyses and stored at -80ºC. Backfat samples were taken at the level of the last rib and separated into outer and inner layers which were separately analyzed for fatty acid composition.

Table 1. Chemical and fatty acid composition of feeds

Diet	Control (C)	High oleic (HO)
Chemical composition, g/kg of feed		
Moisture	129.99	118.60
Lipids	15.33	74.98
Crude protein	127.00	140.00
Crude fiber	37.54	60.00
Nitrogen-free Extractives	505.56	399.38
Ash	41.60	48.10
Main Fatty acids, g/kg of feed		
C14:0	0.03	0.20
C16:0	2.19	5.85
C18:0	0.12	2.46
C18:1 n-9	1.52	44.78
C18:2	5.97	15.54

The extraction of total lipids from subcutaneous fat (inner and outer layers) and from *Longissimus dorsi*, and the analysis of fatty acid methyl esters by gas chromatography were performed according the procedures reported by Rey *et al*. (2006).

2. Gene expression analyses

RNA was extracted with Ribopure kit (Ambion), from 50-100 mg samples of different tissues from 13 animals of each experimental group: (i) ham subcutaneous fat biopsies obtained before and after feeding; (ii) inner layer of backfat samples from carcasses; and (iii) *Longissimus dorsi* muscle. RNA obtained was quantified with Nanodrop and evaluated with an Agilent Bioanalyzer. The quality of the obtained samples measured as 28S/18S ratio and RIN (RNA integrity number) was high.

SCD gene expression was quantified by qPCR with SYBR Green (Takara) in a Stratagene Real Time PCR (MxPro 3000). Two different primer pairs for quantification were designed using software QuickPri; in order to detect possible splice variants previously described for humans. The porcine mRNA sequence available was used for the primer design. These primer sets amplify fragments of 205bp and 218 bp and cover exons 4 to 5 and 5 to 6, respectively. Relative quantification was performed using *GADPH* as endogenous gene. All the reactions were run in triplets.

3. Statistical analyses

The influence of diet on fatty acid composition was separately analyzed for each fatty acid and tissue sample with a linear model fitting the mean, diet treatment and residual effects. A similar model was used for analysing *SCD* gene expression data fitting as covariable the expression

values of the above quoted endogenous gene. All the analyses were performed using the GLM procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

III – Results

1. Effects of diet on fatty acid composition

Both groups showed similar weights during all the experiment. The C group grew slightly faster than HO group, although this difference was not significant. At slaughter, mean carcass weight was 86.9 kg for the C group and 80.4 kg for the HO group.

To study the effect of diet on tissue composition, fatty acid profile of subcutaneous fat was analysed from samples obtained at slaughter (Table 2) and from fat biopsies obtained from live animals at 45 and 70 kg BW (results not shown). FA composition of subcutaneous fat showed significant differences between groups in all the samplings performed. Differences in FA composition of fat vary with sampling time and fat layer. Higher differences were obtained at slaughter on outer layer, with the HO diet group showing a greater percentage of MUFA (63.6 *vs* 54.8%; *P*<0.0001) and PUFA (9.2 *vs* 8.4%; *P*<0.007), and lower percentage of SFA (27.2 *vs* 36.7%; *P*<0.0001).

Table 2. Significant differences between Iberian pigs fed with Control (C) and High oleic (HO) diets for FA composition of outer and inner layers of subcutaneous fat samples obtained at slaughter

Backfat layer	Outer layer			Inner layer		
Fatty acid	**C diet**	**HO diet**	***P***	**C diet**	**HO diet**	***P***
C12:0	0.05±0.002	0.04±0.002	***	0.04±0.002	0.04±0.002	ns
C14:0	1.24±0.031	1.04±0.030	***	1.14±0.029	0.98±0.028	***
C16:0	23.85±0.224	18.83±0.216	***	25.11±0.225	20.49±0.217	***
C16:1	2.19±0.062	1.87±0.060	**	1.98±0.085	1.64±0.082	**
C17:0	0.40±0.019	0.31±0.018	**	0.38±0.020	0.26±0.019	***
C17:1	0.42±0.021	0.28±0.020	***	0.32±0.016	0.19±0.015	***
C18:0	10.84±0.228	6.67±0.219	***	13.53±0.384	9.15±0.370	***
C18:1	50.63±0.302	59.88±0.291	***	49.01±0.442	58.01±0.426	***
C18:2	6.88±0.149	7.65±0.144	**	5.72±0.117	6.48±0.113	***
C20:0	0.22±0.008	0.17±0.007	***	0.20±0.017	0.17±0.016	ns
C20:4	0.14±0.006	0.16±0.006	**	0.09±0.008	0.10±0.007	ns
C22:5	0.05±0.003	0.03±0.003	**	0.02±0.003	0.01±0.003	**
C22:6	0.02±0.001	0.03±0.001	**	0.01±0.003	0.01±0.003	ns

*** = *P*<0.001; ** = *P*<0.01; * = *P*<0.05; ns = not significant.

Fatty acids profile was also analysed in *Longissimus dorsi* muscle samples obtained at slaughter. In contrast to the backfat samples, the fatty acid profile of intramuscular fat was not affected by the dietary treatment (Table 3). Also total IMF content in this muscle was not different between both dietary groups.

Table 3. Least-squares means and standard errors of FA percentages on intramuscular fat of Longissimus dorsi muscle samples from Iberian pigs fed with Control (C) and High oleic (HO) diets

Fatty acid	C diet	HO diet	*P*	Fatty acid	C diet	HO diet	*P*
C10:0	0.10±0.017	0.09±0.016	ns	C18:3	0.22±0.009	0.22±0.009	ns
C12:0	0.07±0.002	0.08±0.002	ns	C18:4	0.05±0.002	0.05±0.002	ns
C14:0	1.26±0.032	1.28±0.031	ns	C20:0	0.16±0.007	0.16±0.007	ns
C16:0	24.03±0.297	24.23±0.286	ns	C20:1	0.72±0.018	0.72±0.017	ns
C16:1	3.93±0.242	3.78±0.234	ns	C20:3	0.16±0.009	0.16±0.009	ns
C17:0	0.25±0.014	0.25±0.013	ns	C20:4	1.70±0.137	1.54±0.132	ns
C17:1	0.22±0.012	0.22±0.012	ns	C20:5	0.21±0.018	0.20±0.017	ns
C18:0	10.57±0.288	10.95±0.277	ns	C22:5	0.15±0.016	0.15±0.015	ns
C18:1	50.40±0.606	50.33±0.584	ns	C22:6	0.07±0.006	0.05±0.006	*
C18:2	5.72±0.287	5.54±0.277	ns	% IMF	3.54±0.277	3.56±0.267	ns

** = *P*<0.01; * = *P*<0.05; ns = not significant

2. Effects of diet on gene expression: Fat and muscular tissues

The *SCD* gene expression was quantified in all backfat and loin samples taken from carcasses, and also in subcutaneous fat biopsies obtained before and three hours after feeding, one week before the slaughter of experimental pigs. Quantification was performed by using two different primer sets in the qPCR reactions, in order to account for possible expression differences specific of splice variants previously described for humans. The *SCD* quantity values obtained for both amplicons were highly correlated, indicating either the existence of only one transcript or more than one but with a highly correlated expression level. Thus, statistical analyses of the diet effect on *SCD* gene expression were performed using the expression values obtained with the primer set covering exons 4 to 5, which potentially include all the possible transcript variants.

The *SCD* gene expression in fat tissues was not affected by the dietary fat neither by the feeding status of the animal (Figure 1). The expression level of this gene was higher in the biopsies obtained one week before slaughter, than in the sampling performed from the carcasses. This could be probably due to the effect of the sampling region or the fat layer used for the RNA extraction, backfat inner layer at slaughter, and mainly ham outer layer on the biopsies.

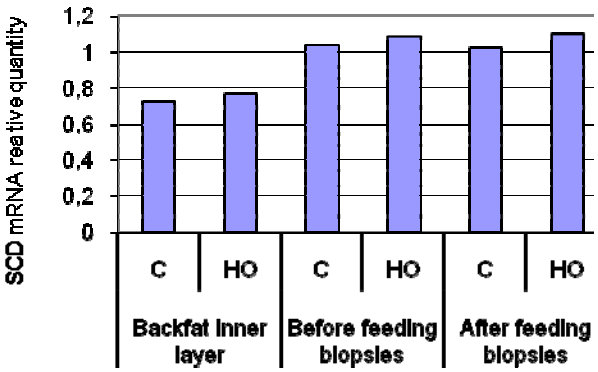

Fig. 1. Mean SCD gene expression in subcutaneous fat samples from different samplings of control (C) and high oleic (HO) groups.

SCD gene expression in muscle was significantly affected by the dietary treatment (Figure 2). A higher expression (2x) was observed on animals fed with control diet. The mean expression values were 0.249 ± 0.040 and 0.126 ± 0.039 for C and HO groups, respectively (P <0.054).

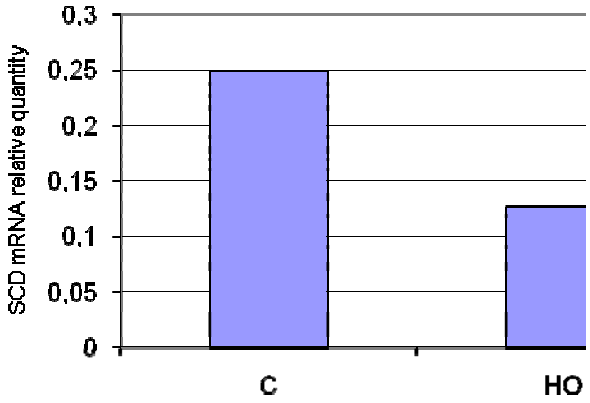

Fig. 2. Mean SCD gene expression in Longissimus dorsi samples of pigs from control (C) and high oleic (HO) diet treatments.

IV – Discussion

Feeding, genetics and their interactions influence animal tissue composition, thus affecting meat products quality. These influences are especially relevant in Iberian pig production, in which diet composition and genetic type are the strongest determinants in their meat and fat high quality and ability for processing. In fact, the highest quality and prize of Iberian pig meat products are obtained from pure animals reared outdoors, implying the consumption of acorns and grass (López-Bote, 1998). Due to the restricted acorn production and the high demand of Iberian pig products, the use of MUFA enriched concentrates is becoming a usual practice in order to achieve FA profiles in animal tissues similar to that obtained with the consumption of acorns, as the fatty acid profiles of animals tissues are expected to reflect the fatty acid composition of the feeds received (Daza *et al*., 2005; Ventanas *et al*., 2008; Pérez-Palacios *et al*., 2009).

Nevertheless, the effects of feeding with MUFA enriched diets on the different animal tissues composition are not clear. Previous studies show non consistent and tissue-specific effects although their comparison is difficult due to the different dietary treatments (high or moderate inclusion of MUFA in enriched diet) or tissue (muscle type) and sampling. Moreover, the relative roles of direct deposition of dietary FA and endogenous synthesis and the regulation of FA synthesis by diet components are key factors in pig lipid metabolism and production, which are not well understood.

In the present work, we studied the effect of a long-term moderate MUFA inclusion in the diet on loin muscle and subcutaneous fat FA composition. Animal tissue analyses showed no effect of dietary fat on muscle fatty acid composition. Both C and HO groups showed similar intramuscular FA pattern instead of the very different FA composition of their diets. In contrast, subcutaneous fat FA composition clearly reflected the dietary FA composition, with much higher MUFA and lower SFA content in the HO group. Slightly higher PUFA content was observed in the HO group which may be attributed to the positive effect of dietary fat on C18:2 content. This increase of C18:2 percentage has been reported as a potentially undesirable secondary effect

of MUFA enriched diets by Ventanas *et al*. (2008), although their MUFA inclusion level and the corresponding C18:2 increase were much higher than those of the present study.

The different response of adipose and muscular tissues to the dietary influence has been previously observed with a similar pattern in other works, that is, adipose tissue reflect to a greater extent the dietary modifications in terms of total tissue FA content, but the influence of dietary treatment on muscle FA is not so evident (Duran-Montge *et al.*, 2009). In Iberian pigs, Pérez-Palacios *et al*. (2009) reported significant effects on backfat and *Semimembranosus* muscle but not on *B. femoris*.

Regarding the effect of the diet on *SCD* gene expression, no difference on mRNA abundance was observed between fat samples of C and HO animals. Previous results of differential gene expression obtained from this experimental material with a microarray approach indicated that changes in backfat gene expression induced by fatty acid composition of the diet are small in number and magnitude (Óvilo *et al.*, 2009). This result indicate a low genetic responsiveness of fat tissues to dietary treatments which could be particularly relevant in obese genetic types, as a consequence of a dilution effect, as it has been described in humans (Van Erk *et al.*, 2008).

In contrast to fat tissue, muscles show a much different response to the dietary treatment not only at the tissue composition but also at *SCD* transcriptional levels. Loin samples are not affected in this study by the dietary fat, showing identical FA and total IMF composition between groups. This fact would reflect an effect at the transcriptional or enzymatic activity levels that lead to an increase in MUFA endogenous synthesis or desaturation thus compensating the direct deposition of dietary FA. The results of *SCD* gene expression quantification agree with this hypothesis, as the C animals show twice *SCD* gene expression than HO animals. Although there are abundant evidences of tissue-specific responses of lipogenic enzymes expression in response to dietary treatments, the reasons of these changes are not clear. One possible explanation could be the different levels and/or classes of transcription factors regulating the enzyme expression, which accompany the morphological and metabolic differences between both intramuscular and subcutaneous fat depots (Gardan *et al.*, 2006; Gondret *et al.*, 2008).

Our work support previous evidences indicating the modulation of *SCD* gene expression by dietary fatty acids and also the tissue-specificity of this effect. Nevertheless, the higher influence would be expected to appear in the most lipogenic tissues, which in pigs are liver and adipose tissue (Duran-Montge *et al.*, 2009). The higher transcriptional response obtained in muscle in our work could be related to the higher lipogenic potential of Iberian muscle tissue in comparison with other breeds. The study of these dietary effects in other genetic backgrounds is indicated in order to understand the effects detected.

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