MASTER INTERUNIVERSITARIO EN MEJORA GENÉTICA
ANIMAL Y BIOTECNOLOGÍA DE LA REPRODUCCIÓN

Mejora genética de la composición de la grasa en porcino

(Breeding for fat composition in pig)

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Autor: Eliana Henriquez Rodriguez

Director:
Joan Estany Illa
Universitat de Lleida

Resumen

El contenido y la composición de la grasa, en particular el contenido de ácido oleico (C18:1), son dos aspectos que influencian la calidad de la carne. La síntesis endógena de C18:1 depende en gran medida de la vía enzimática que implica a Elovl6 (E), que cataliza la elongación del ácido palmítico (C16:0) a esteárico (C18:0) y la estearoil-CoA desaturasa (SCD), que desatura C18:0 a C18:1. La actividad SCD y E puede ser explicada indirectamente por los ratios C18:1/C18:0 y C18:0/C16:0 respectivamente. Hay evidencia de que estas dos enzimas, pero particularmente SCD, se comportan de manera diferente a través de los tejidos e indicaciones de que, porque existe variación genética en las concentraciones de sus sustratos y productos, C18:1/C18:0 y C18:0/C16:0 están sujetos a variación genética dentro de tejido. Por otro lado, recientemente ha sido reportado un polimorfismo de un solo nucleótido (SNP) en la región promotora del gen *SCD (AY487830:g.2281A>G)* que afecta el ratio C18:1/C18:0. Por lo tanto, los objetivos de esta tesis fueron (1) determinar si los ratios C18:1/C18:0 y C18:0/C16:0 difieren a través de los tejidos; (2) estimar la correlación genética entre SCD y E entre músculos y grasa subcutánea, y con caracteres productivos, y (3) evaluar el efecto del SNP sobre la variabilidad genética y las correlaciones de SCD y E entre tejidos. Todos los datos utilizados en los experimentos pertenecen a una línea cerrada de cerdos Duroc. Los ácidos grasos utilizados para calcular los ratios se determinaron por cromatografía de gases. Se concluye que la actividad SCD es tejido-específica, siendo mayor en músculo *(gluteus medius* y *longissimus dorsi)* en comparación con la grasa subcutánea y el hígado. La correlación genética entre SCD en *gluteus medius* y SCD en *longissimus dorsi* fue lo suficientemente alta (0.76) para sugerir que SCD se expresa de manera similar en los músculos. Por el contrario, la correlación genética de SCD en *gluteus medius* y SCD en grasa subcutánea (0,41) sugiere que SCD en grasa subcutánea puede no ser un buen indicador de SCD en músculo. La actividad SCD se correlacionó negativamente con E y está incorrelacionada con peso corporal y espesor de grasa dorsal. La correlación negativa entre SCD y E se puede atribuir a una mayor eficiencia en la desaturación de C18:0 que en la de C16:0. El SNP *AY487830: g.2281A> G* juega un papel en la actividad diferenciada de esta enzima, explicando hasta 46,3% de la varianza genética aditiva del ratio C18:1/C18:0. Los resultados de esta tesis confirman que el contenido de C18:1 se puede mejorar genéticamente mediante el uso tanto de selección asistida por marcadores como de selección directa sobre la composición de la grasa.

Palabras clave: elongasa, composición de la grasa, grasa intramuscular, cerdo, estearoil-CoA desaturasa.

Summary

Fat content and composition, particularly oleic acid (C18:1), are two aspects that influence meat quality. The endogenous synthesis of C18:1 greatly depends on the enzymatic pathway involving Elovl6 (E), which catalyzes the elongation of palmitic (C16:0) to stearic (C18:0) and the stearoyl-CoA desaturase (SCD), which desaturates C18:0 to C18:1. The SCD and E activity can be indirectly accounted for by the C18:1/C18:0 and C18:0/C16:0 ratios, respectively. There is evidence that these two enzymes, but particularly SCD, behave differently across tissues and indications that, because there exists genetic variation in their substrate and product concentrations, the C181:/C18:0 and C18:0/C16:0 ratios are subject to genetic variation within tissue. On other hand, there is a single nucleotide polymorphism (SNP) in the promoter region of the *SCD* gene (*AY487830:g.2281A>G)* that affects the C18:1/C18:0 ratio. Therefore, the objectives of this thesis were (1) to determine whether the C18:1/C18:0 and C18:0/C16:0 ratios differ across tissues; (2) to estimate the genetic correlation between them among muscles and subcutaneous fat, and with production traits; and (3) to assess the effect of the above SNP on the genetic variability and correlations of SCD and E among tissues. All data used in the experiments belong to a closed line of Duroc pigs. The fatty acids used to calculate the ratios were determined by gas chromatography. It is concluded that the activity of the SCD is tissue-specific, being higher in muscle (*gluteus medius* and *longissimus dorsi*) as compared to subcutaneous fat and liver. The genetic correlation between SCD in *gluteus medius* and SCD in *longissimus dorsi* was high enough (0.76) to suggest that SCD is similarly expressed in muscle. In contrast, the genetic correlation of SCD in *gluteus medius* and SCD in subcutaneous fat (0.41) suggests that SCD in backfat may not be a good indicator of SCD in muscle. The SCD activity was negatively correlated to E and uncorrelated to body weight and backfat thickness. The negative correlation between SCD and E may be attributed to a greater efficiency in the desaturation of C18:0 than of C16:0. The SNP *AY487830:g.2281A>G* plays a role in the differential activity of this enzyme, explaining up to 46.3% of the additive genetic variance of the C181:/C18:0 ratio. The results of this thesis confirm that content of C18:1 can be genetically improved by using both marker assisted selection and direct selection on fat composition.

Keywords: elongase, fat composition, intramuscular fat, pig, stearoyl-CoA desaturase.

General introduction

According to data from FAO (2003), pig meat is the most consumed meat in the world with an average of 14.6 kg per capita/year between 1997/99 and a projection of 15.3 for 2015. Spain is the fourth worldwide producer of pork meat and the second in Europe (FAOSTAT, 2011), with the autonomous community of Catalonia being the region with the largest pig census (MAGRAMA, 2012). Given that pork is an important source of protein for a significant part of the population, it becomes fundamental to ensure consumer satisfaction fulfilling a high quality standard.

In terms of pork quality, fat tissue firmness (hardness), shelf life (lipid and pigment oxidation) and flavor are aspects influenced by fatty acid composition (Wood et al., 2003), while several studies have reported that intramuscular fat content (IMF) affects characteristics such as tenderness (DeVolt et al., 1988), juiciness (Cannata et al., 2010), overall acceptability and meat taste intensity (Font-i-Furnols et al., 2012). The oleic acid (C18:1) is the major component of the lipid fraction, with approximately 45% of the total fatty acids (Zhang et al., 2006), and has been positively correlated with pork flavor, flavor liking and overall acceptability (Cameron et al., 2000). With regard to health, has been reported (among other benefits) that C18:1 reduces coronary heart disease risk (Kris-Etherton, 1999) and that has a protective effect against breast cancer (Menendez et al., 2005).

The content of C18:1 is genetically determined (Sellier et al., 2010; Ros-Freixedes et al, 2012), and therefore it can be improved by selection. In the biosynthesis pathway of C18:1, elongase Elovl6 and the stearoyl-CoA desaturase (SCD) are the two main enzymes involved: Elovl6 catalyzes the chain elongation of palmitic (C16:0) to stearic (C18:0) (Matsuzaka and Shimano, 2009) and the SCD catalyzes the conversion of stearic to oleic (Guillou et al., 2010). For the study of desaturase and elongase activity, different indices relating substrate and product have been calculated (Smith et al., 2002; Pitchford et al., 2002; Ntawubizi et al., 2009); however, these indices should be interpreted not as a measure of absolute enzyme activity but rather as indirect estimators of the SCD activity (Smith et al., 2002).
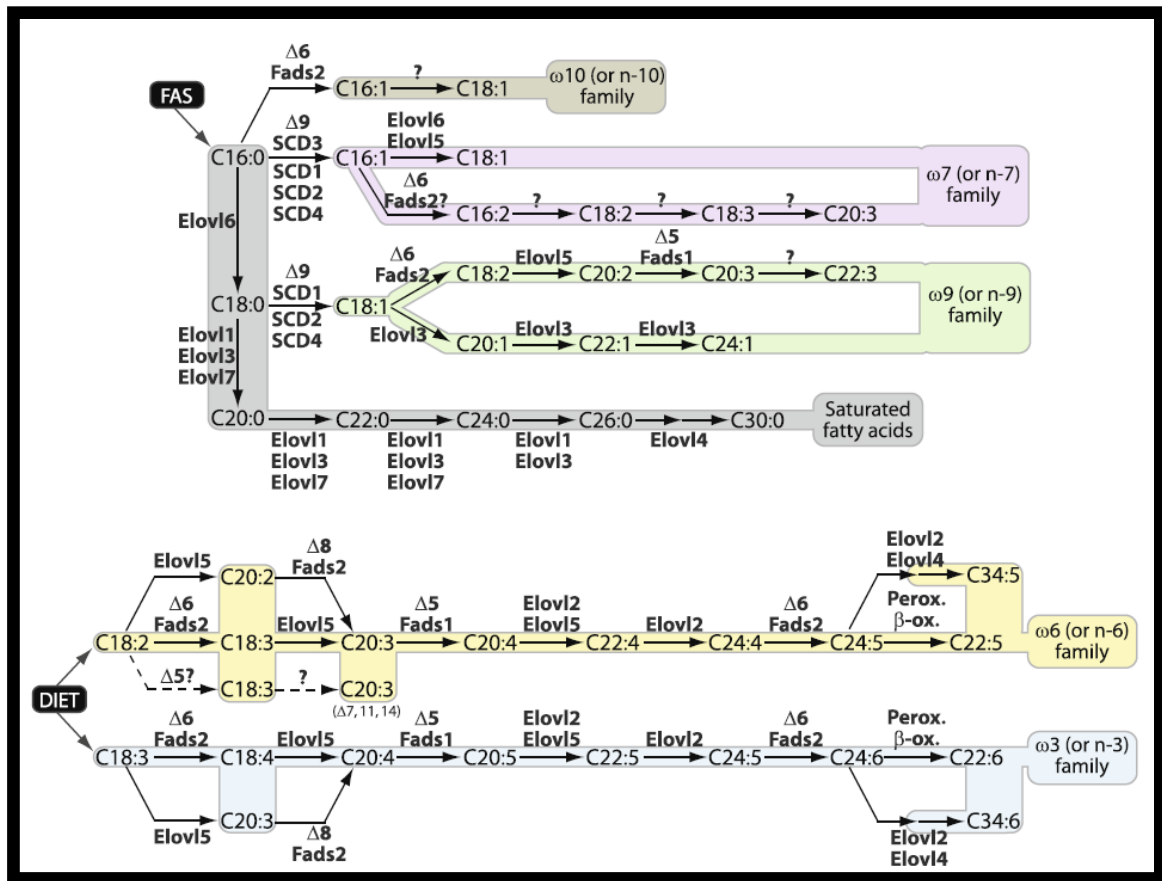
Figure 1. Long chain and very long-chain fatty acid biosynthesis in mammals (Guillou et al., 2010).

Several studies have been searching for quantitative trait loci associated to fatty acid composition. Ros-Freixedes et al. (2013) have recently reported the existence of a single nucleotide polymorphism (SNP) in the promoter region of the *SCD* gene that affected the C18:1/C18:0 ratio. The presence of this SNP segregating in a population can change the genetic correlation structure among SCD and Elovl6 between tissues.

There is evidence that the SCD behave differently across tissues (Doran et al., 2006; Cánovas et al., 2009), however there are not estimates in the literature on genetic correlations between the ratios indicators of SCD and Elovl6 activity at different tissues.

Therefore, the objectives of this thesis are:

1. To determine whether the desaturase and elongase activity differ across tissues.
2. To estimate the genetic correlation of SCD and Elovl6 activity among muscles and subcutaneous fat, and with production traits.
3. To assess the effect of the SNP described in the promoter region of the *SCD* gene (Ros-Freixedes et al., 2012) on the genetic variability and genetic correlations of SCD and Elovl6 activity among tissues.

All data used in this thesis are from a closed line of Duroc pigs from Selección Batallé. Duroc pigs have greater IMF or marbling fat than high lean-growth commercial breeds, like Landrace (Lo et al., 1992) or Large White, but also more than some of the traditional or less selected breeds, like Berkshire (Suzuki et al., 2003) or Tamworth (Wood et al., 2004). An experiment comparing Large White x Landrace, Iberian, and Duroc lines concluded that the Duroc used in this thesis, as compared to these genetic types, has a higher level of IMF and oleic acid content (Reixach et al., 2008). These features are relevant because the major purpose of this line has been to produce high-quality pork but particularly dry-cured hams. The IMF content and composition is important in their manufacturing because they affect the aspect of slices, the color of ham slices, the texture of hams and the intensity and persistence of aroma (Gilles, 2009).

CHAPTER I: Desaturase and elongase activity across tissues and genotypes in Duroc pigs.

Henriquez E, Ros-Freixedes R, Pena RN, Tor, M y Estany, J.

Universitat de Lleida, Departament de Producció Animal, 25198 Lleida.

ABSTRACT: Fat content and composition, particularly oleic acid (C18:1n-9), are two aspects that influence meat quality. The biosynthesis of C18:1n-9 depends on the stearoyl-CoA desaturase (SCD) and elongase (E) enzymes. The aim of the present study was to determine whether the activity of these enzymes differs across tissues and genotypes in Duroc pigs. Genotypes were based on the *AY487830:g.2281A>G* polymorphism of the *SCD* gene. The SCD related-ratios {SCD1=C16:1n-7/C16:0; SCD2=C18:1n-9/C18:0} were greater in muscle than in subcutaneous fat and liver, confirming that SCD activity is tissue-specific. The elongase ratios {E1=C18:0/C16:0; E2=C18:1n-7/C16:1n-7} were greater in liver and subcutaneous fat than in muscle. The AA genotype had higher activity for SCD but not for E. The correlations between SCD ratios, as well as those between E ratios, were positive in all tissues. By contrast, the correlations between SCD and E ratios were negative.

KEYWORDS: intramuscular fat; fat composition; pigs; stearoyl-CoA desaturase.

INTRODUCTION

The fat composition, most particularly the oleic acid content, has become increasingly important in relation with the quality of meat, since fatty acids influence technological aspects such as hardness, the shelf life and flavor (Wood et al., 2003). Moreover, in pigs oleic acid is the major monounsaturated fatty acids found in fat depots (Ren et al., 2004) and has been positively correlated with pork flavor, taste preference and overall acceptability (Cameron et al., 2000). Ros-Freixedes et al. (2012) showed that the content of C18:1n-9 in muscle is genetically determined and thus it

can be improved by selection. It is known that desaturase and elongase enzymes are involved in the synthesis routes of C18:1n-9. The activity of these enzymes may vary between tissues and may be influenced by structural variants of the encoding genes, being the stearoyl-CoA desaturase the key enzyme that catalyzes the biosynthesis of oleic acid (Miyazaki and Ntambi, 2003). The experiment described in the present study was designed to test for differences in the desaturase (SCD) and elongase (E) activity between tissues and genotypes for the *SCD* gene in Duroc pigs.

MATERIAL AND METHODS

An experimental population of 48 Duroc barrows was used. The animals were born from matings between 19 males and 48 females and were bred in 3 rearing batches as explained in Ros-Freixedes et al (2012). At 205 days of age, pigs were slaughtered in a commercial abattoir. For each of them samples of the muscle *semimembranosus* (SM), subcutaneous fat (GS) between the third and fourth last rib, and liver (L) were collected immediately after culling and snap frozen in liquid nitrogen. Additionally, samples of muscle *gluteus medius* (GM) were taken after refrigerating the carcass at 2°C for 24 hours. All the samples were stored at -80ºC until analysis. For fatty acid determination, samples were allowed to thaw, and then lyophilized and pulverized. A representative aliquot of each was used to determine, in duplicates, fatty acid composition according to Bosch et al. (2009), while a modification of the method was used for quantifying C18:1n-7. The 192 samples were distributed in a factorial design of 4 tissues × 3 genotypes. **Table 1** indicates the average percentage, regarding to the total fatty acids determined, of the fatty acids considered in this work.

Table 1. Number of pigs and mean percentage (standard deviation) of fatty acids considered in this paper according to tissues

Fatty acid %	gluteus medius	semimembranosus	Subcutaneous fat	Liver
n	48	48	48	48
C16:0	24.6 (0.84)	22.44 (1.03)	22.41 (1.21)	18.81 (2.25)
C16:1n-7	3.56 (0.45)	3.53 (0.49)	2.13 (0.29)	1.34 (0.46)
C18:0	11.61 (0.95)	10.68 (0.84)	11.66 (1.04)	20.88 (3.56)
C18:1n-9	39.65 (1.33)	40.04 (2.57)	40.58 (2.66)	20.12 (4.70)
C18:1n-7	5.71 (0.62)	6.51 (0.59)	2.61 (2.03)	3.01 (0.60)

Data on fatty acid content (**Table 1**) was used to calculate, for each pig and tissue, the following indices: $SCD1 = C16{:}1n{-}7/C16{:}0$ and $SCD2 = C18{:}1n{-}9/C18{:}0$, as indicators of the activity of the enzyme stearoyl-CoA desaturase, as well as $E1 = C18{:}0/C16{:}0$ and $E2 = C18{:}1n{-}7/C16{:}1n{-}7$ as indicators of elongase activity. In parallel, a DNA sample was extracted from each pig in order to genotype the *AY487830:g.2281A>G* polymorphism described in the *SCD* gene promoter region by Ros-Freixedes et al. (2013) in the present workshop.

The differences among genotypes and tissue for indices SCD1, SCD2, E1 and E2 were tested using an animal model which included, as fixed effects, the batch (batch 1 to 3), the tissue (GM, SM, GS and L) and genotype (AA, AG and GG), while the animal was considered as a random effect . The effects of tissue and genotype were contrasted with Kenward-Roger test and the difference between levels within factor with the Tukey-HSD test. The phenotypic correlations between indexes based on tissue were also computed with the REML method. Statistical analyzes were performed using the statistical package JMP 8 (SAS Institute Inc, Cary, NC).

RESULTS AND DISCUSSION

Table 2 shows the differences between indices according to tissue and genotype. Desaturase indices SCD1 and SCD2 are higher in muscle, especially in SM, than in GS and L. This differential behavior of the stearoil-CoA desaturase agrees with the results of Doran et al. (2006), who showed that, in animals fed a protein-restricted diets, the SCD protein expression increased in muscle *(longissimus thoracis et lumborum)* but not in subcutaneous adipose tissue. In our experimental population (which was selected for reduced backfat thickness at constant intramuscular fat) Cánovas et al. (2009) reported a significant decrease in SCD protein expression in subcutaneous adipose tissue but not in muscle (SM), while Muñoz et al. (2013) did not find a significant effect of genetic selection in the hepatic expression of SCD. These results confirm that the SCD activity behaves in a tissue-specific manner. In contrast, elongase activity is much higher in GS and L, particularly E2. Animals with AA genotype, in line with the results of Ros-Freixedes et al. (2013), present higher SCD activity,

particularly evident in SCD2. By contrast, no differences were observed among genotypes for elongase activity.

Table 2. Least square means (± standard error) of desaturase and elongase activity (×100) by tissue and genotype of the promoter of the SCD gen

	Indices			
	SCD1	SCD2	E1	E2
Tissue				
SM	15.66 ± 0.25^a	377.91 ± 5.49^a	48.88 ± 2.49^b	188.48 ± 6.17^b
GM	14.38 ± 0.25^b	344.14 ± 5.49^b	48.53 ± 2.49^b	164.12 ± 6.17^c
GS	9.45 ± 0.25^c	329.45 ± 5.49^b	53.30 ± 2.49^b	257.76 ± 6.17^a
Liver	6.87 ± 0.25^d	102.56 ± 5.49^c	116.09 ± 2.49^a	245.68 ± 6.17^a
SNP g.2281A>G				
AA	12.10 ± 0.25^a	300.50 ± 5.32^a	67.30 ± 1.98^a	209.75 ± 5.91^a
AG	11.68 ± 0.25^a	286.21 ± 5.32^{ab}	64.80 ± 1.98^a	209.59 ± 5.91^a
GG	10.99 ± 0.25^b	278.82 ± 5.32^b	68.01 ± 1.98^a	222.68 ± 5.91^a

Indices SCD1 = C16:1n-7/C16:0, SCD2 = C18:1n-9/C18:0, E1 = C18:0/C16:0, E2 = C18:1n-7/C16:1n-7
[a, b, c, d] Within a column and factor, means with different superscripts differ significantly ($P < 0.05$)

Table 3 shows the phenotypic correlation between desaturase and elongase indices according to tissue. SCD1 and SCD2 indices are positively correlated in all tissues, in the same manner as E1 and E2. By contrast, the correlations between SCD and E indices are negative. It is interesting to note the unfavorable high correlation between SCD2 and E1, which suggests that an increase in desaturase activity in the step from stearic acid (C18:0) to oleic acid (C18:1n-9), may be limited by the biosynthesis of stearic (C18:0) from palmitic (C16:0).

Table 3. Phenotypic correlations between indices desaturase and elongase according to tissue

		Tissues			
		Liver	GM	GS	SM
Indices					
SCD2	SCD1	0.89*	0.49*	0.71*	0.44*
E1	SCD1	-0.88*	-0.63*	-0.89*	-0.59*
E1	SCD2	-0.93*	-0.87*	-0.75*	-0.74*
E2	SCD1	-0.79*	-0.63*	-0.66*	-0.63*
E2	SCD2	-0.71*	-0.45*	-0.09	0.2
E2	E1	0.78*	0.69*	0.59*	0.29

*($P < 0.05$)

REFERENCES

Bosch, L., M. Tor, J. Reixach, and J. Estany. 2009. Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. Meat Sci. 82:432–437.

Cameron N.D., M. Enser, G.R. Nute, F.M. Whittington, J.C. Penman, A.C. Fisken, A.M. Perry, J.D. Wood. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci. 55:187-195.

Cánovas, A., J. Estany, M. Tor, R. N. Pena and O. Doran. 2009. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905–3914.

Doran, O., S.K. Moule, G.A. Teye, F.M. Hallett, K.G. Whittington and J.D. Wood. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. Br. J. Nutr. 95:609–617.

Miyazaki, M. and M. Ntambi. 2003. Role of stearoyl-conenzyme A desaturase in lipid metabolism. Prostaglandins Leukot. Essent. Fatty Acids 68:113–121.

Muñoz, R., J. Estany, M. Tor, O. Doran. 2013. Hepatic lipogenic enzyme expression in pigs is affected by selection for decreased backfat thickness at constant intramuscular fat content. Meat Sci. 93:746-751.

Ren, J., C. Knorr, L. Huang, and B. Brenig. 2004. Isolation and molecular characterisation of the porcine stearoyl-CoA desaturase gene. Gene 340:19–30.

Ros-Freixedes, R., J. Reixach, M. Tor and J. Estany. 2012. Expected genetic response for oleic acid content in pork. J. Anim. Sci. 90:4230-4238.

Ros-Freixedes, R., R.N. Pena, M. Tor and J. Estany. 2013. Breeding for high oleic acid content in pork using direct selection and molecular markers. 2013. XV Jornadas sobre Producción Animal, edited by Asociación Interprofesional para el Desarrollo Agrario. Volume II, 580-582.

Wood J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard, M. Enser. 2003. Effects of fatty acids on meat quality: a review. Meat Sci. 66:21-32.

CHAPTER II: Genetic correlation among desaturase and elongase activity across muscles and subcutaenous fat.

Henriquez E, Ros-Freixedes R, Pena RN, Tor, M y Estany, J.

Universitat de Lleida, Departament de Producció Animal, 25198 Lleida.

ABSTRACT: The oleic acid (C18:1) content is a key factor determining the quality of dry-cured hams. The biosynthesis of C18:1 depends on the elongase Elovl6 enzyme, which elongates palmitic (C16:0) to stearic (C18:0), and on the stearoyl-CoA desaturase, which desaturates C18:0 to C18:1. The genetic variance of C18:1/C18:0 (SCD) and C18:0/C16:0 (E) in different muscles (GM: *gluteus medius*; n=1204; LD: *longissimus dorsi*; n=318), and in the subcutaneous fat (BF; n=333), along with their genetic correlations, were estimated. The fatty acids used to calculate the indices are expressed as percentage of total fatty acids and were determined by gas chromatography. Genetic parameters were estimated using a full-pedigree Duroc line (n=111,305 pigs) under a Bayesian 4-trait animal model, in which the first two traits were body weight (BW; n= 102,325) and backfat thickness (BT; n=98,397) at 180 d. Pigs with data on C18:1 were genotyped for *AY487830:g.2281A>G* and the genotype included or not in the model so as to assess its effect on the genetic variability of SCD and E. The heritability for SCD was higher in muscle (0.47 in GM and 0.52 in LD) than in BF (0.34) but similar to E (0.58 in GM). The genetic correlation of SCD in GM with SCD in LD was also greater (0.76) than that with SCD in BF (0.41) while that of SCD and E in GM was high and negative (-0.79). The genetic correlations of SCD and E with BW and BT were negligible, with values ranging from -0.17 to 0.00. The proportion of the additive genetic variance explained by the genotype *AY487830:g.2281A>G* was 46.3%, 27.4%, and 2.3% for SCD in GM, SCD in LD, SCD in BF, respectively. The results indicate that SCD behave differently between BF and muscles but not across muscles, and that SCD is not expected to change after selection for BW or BT. Rather than a direct effect, it is hypothesized that the negative correlation between SCD and E is the result of a differential efficiency in the desaturation of C16:0 and C18:0. The genotype for

AY487830:g.2281A>G affects SCD increasing both the genetic variance and the genetic correlation of SCD at different tissues.

KEYWORDS: elongase, fat composition, intramuscular fat, pig, stearoyl-CoA desaturase.

INTRODUCTION

Fat tissue firmness, shelf life and flavor are components of pork quality influenced by fatty acid composition (Wood et al., 2003). The oleic acid (C18:1) is the major component of the lipid fraction, with approximately 45% of the total fatty acids (Zhang et al., 2007), and has been positively correlated with pork flavor, flavor liking and overall acceptability (Cameron et al., 2000). The endogenous synthesis of C18:1 greatly depends on the enzymatic pathway involving the elongase Elovl6 (E), which catalyzes the elongation of palmitic (C16:0) to stearic (C18:0) (Matsuzaka and Shimano, 2009), and the stearoyl-CoA desaturase (SCD), which desaturates C18:0 to C18:1 (Guillou et al., 2010). The SCD and E activity can be indirectly accounted for by the C18:1/C18:0 and C18:0/C16:0 ratios, respectively. There is evidence that these two enzymes, but particularly SCD, behave differently across tissues (Doran et al., 2006; Cánovas et al., 2009), and indications that, because there exists genetic variation in their substrate and product concentrations (Ros-Freixedes et al., 2012), that the C181:/C18:0 and C18:0/C16:0 ratios are subject to genetic variation within tissue (Ntawubizi et al., 2009). However, there are not estimates in the literature on genetic correlations involving the above ratios at different fat tissues.

Several studies have been searching for quantitative trait loci associated to fatty acid composition. Ros-Freixedes et al. (2013) have recently reported the existence of a single nucleotide polymorphism (SNP) in the promoter region of the *SCD* gene that affected the C18:1/C18:0 ratio. The presence of this SNP segregating can change the genetic variability of C18:1/C18:0 and C18:0/C16:0 and their genetic correlation structure among tissues. The purpose of this study was, using the C18:1/C18:0 and C18:0/C16:0 ratios as indirect measures of the SCD and the E activity, respectively, to determine the genetic correlation of these ratios among different muscles and subcutaneous fat, and with production traits. The effects of the SNP on the genetic parameters associated to them are also discussed.

MATERIAL AND METHODS

All experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida.

Animals and Sample Collection

Data from a purebred Duroc line were used for the analyses. The line was completely closed in 1991 and since then it has been selected for an index including body weight (BW), backfat thickness (BT), and intramuscular fat (IMF) (Solanes et al., 2009; Ros-Freixedes et al., 2012). The data set used for the estimation of the genetic parameters consisted of 111,305 pigs, from which 102,325 had at least one recorded trait. Pigs with records were born from 1996 to 2012. At about 75 d of age piglets were moved to the fattening units, where they were penned by sex (8 to 12 pigs per pen) until slaughter. All pigs were performance-tested at an average age of 180 d for BW and BT. Backfat thickness was ultrasonically measured at 5 cm off the midline at the position of the last rib (Piglog 105, Herlev, Denmark). During the test period pigs had ad libitum access to commercial diets. Since 2002 a sample of the purebred barrows used for producing dry-cured ham was taken for recording IMF and fatty acid profile. Two barrows per litter were taken from fixed litters. These barrows were raised in 15 batches until slaughter at around 205 d. From 160 d onwards barrows were fed a commercial pelleted finishing diet (Esporc, Riudarenes, Girona, Spain) with an average composition of 17.5% crude protein, 5.94% fiber, and 6.41% fat (C16:0: 20.4%; C18:0: 6.9%; C18:1: 33.9%; C18:2: 30.0%). Feed in each batch was analysed in triplicate as described in Cánovas et al. (2009). At the end of the finishing period the barrows were slaughtered in a commercial slaughterhouse. There, a sample of the muscle *gluteus medius* (GM) was collected from the left ham of all pigs and, in randomly chosen subgroups of them, additional samples of the muscle *longissimus dorsi* (at the level of the third and fourth last ribs; LD) were taken. Finally, one sample of subcutaneous backfat (BF) was obtained at the position where the LD muscle sample was taken. The sample of BF was collected immediately after slaughter and frozen in liquid nitrogen until required for analyses. The samples of GM and LD were collected after chilling for about 24 h at 2ºC, vacuum packaged and stored in deep freeze until analysis. A

summary of the population characteristics and number of records, sires, dams and litters used for each analyzed trait is given in **Table 1**.

Table 1. Description of the data set used in the analyses

	No of pigs	No of sires	No of dams	No of barrows	No of litters	Mean	SD
Pedigree	111305	832	22635		40658		
Fatty acids (%)							
C18:0 GM	1204			1204		11.21	1.68
C18:1 GM	1204			1204		44.91	2.87
C18:0 LD	318			318		11.94	1.89
C18:1 LD	318			318		45.75	2.67
C18:0 BF	333			333		11.10	2.24
C18:1 BF	333			333		44.10	3.74
C16:0 GM	1204			1204		23.28	2.08
Traits							
SCD GM	1204			1204		41.30[a]	8.82[a]
SCD LD	318			318		39.52[a]	7.95[a]
SCD BF	333			333		42.50[a]	14.66[a]
E GM	1204			1204		4.80[a]	0.46[a]
BW at test (kg)	102325	95791	5332	1202		104.76	12.31
BT at test (cm)	98397	92010	5186	1201		15.58	3.46
Covariates							
Age at test (d)	102915	96341	5371	1203		179.26	10.65
Age at slaughter (d)	4317	1510	94	2713		207.20	16.10

[a] Values have been multiplied by 10

SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD; SCD BF=C18:1BF/C18:0BF

E GM=C18:0GM/C16:0GM

Fat Analysis

Once defrosted, a representative aliquot from pulverized freeze-dried samples was used for fat analysis. The IMF content and FA composition were determined in duplicate by quantitative determination of the individual FA by gas chromatography (Bosch et al., 2009). Fatty acid methyl esters were directly obtained by transesterification using a solution of 20% boron trifluoride in methanol (Rule et al., 1997). Methyl esters were determined by gas chromatography using a capillary column SP2330 (30 m × 0.25 mm; Supelco, Bellefonte, PA) and a flame ionization detector with helium as carrier gas at 1 ml/min. 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 (Sigma, Tres Cantos, Madrid). The internal standard was 1,2,3-tripentadecanoylglycerol. 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; C16:1, C18:1, and C20:1), and polyunsaturated (PUFA; 18:2, C18:3, C20:2, and C20:4) FA. The IMF content in the four muscles was calculated as the sum of the individual FA expressed as triglyceride equivalents (AOAC, 1997) on a dry tissue basis. The ratio of C18:1/C18:0 was used to measure the SCD activity while the C18:0/C16:0 ratio to measure the Elovl6 (E) activity.

Isolation of genomic DNA and genotyping

The extractions of genomic DNA were carried out from muscle tissue samples stored at -80ºc. The samples were lysed in the presence of proteinase K and the DNA was purified through extraction with phenol:chloroform, followed by ethanol precipitation. Finally, the 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 *AY487830:g.2281A>G* polymorphism (Ros-Freixedes et al., 2013) was genotyped using an allelic discrimination assay. This is a quantitative PCR-based technique that uses allele-specific fluorescently labeled TaqMan probes to

discriminate between the two possible alleles. The reaction mix contained 1x Universal TaqMan master mix (Applied Biosystems), 0.2 uM Primer mix, 0.8 uM Probe mix and 10 ng of DNA in a finals volum of 5 ul. A total of 915 animals were genotyped with the following results: AA=176, AG=462, GG=277.

Estimation of Genetic Parameters

Genetic parameters for SCD and E were estimated fitting a 4-trait multivariate animal model. The first three traits were always BW, BT, and SCD in GM while the last one was, alternatively, SCD in LM, SCD in BF, and E in GM. In matrix notation, the model was:

$$\mathbf{y}_i = \mathbf{X}_i\,\mathbf{b}_i + \mathbf{Z}_i\,\mathbf{a}_i + \mathbf{W}_i\,\mathbf{c}_i + \mathbf{e}_i,$$

where $\mathbf{y}_i$ is the vector of observations for trait i (BW, BT, SCD and E); $\mathbf{b}_i$, $\mathbf{a}_i$, $\mathbf{c}_i$, and $\mathbf{e}_i$ are the vectors of systematic, additive genetic, litter, and residual effects, respectively; and $\mathbf{X}_i$, $\mathbf{Z}_i$, and $\mathbf{W}_i$, the known incidence matrices that relate $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{c}_i$ with $\mathbf{y}_i$, respectively. Systematic effects for BW and BT were the batch (1226 levels), gender (3 levels; males, females and castrates), and age at measurement as a covariate. Pigs tested at the same time and in the same unit were considered as one batch. Systematic effects for SCD and E were the batch (15 levels) and the age at slaughter as covariate. Because there were only 1.7 piglets per litter with records on SCD and E, the litter was dropped from the model for these two traits. To test the effect of the SNP the same models were solved but including the SNP (4 levels; AA, AG, GG, and without genotype) as systematic effect.

Genetic parameters were estimated in a Bayesian framework using Gibbs sampling with the TM software (Legarra et al., 2008). The traits were assumed to be conditionally normally distributed as follows (Ros-Freixedes et al., 2012):

$$
\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} \mid b_1, b_2, b_3, b_4, a_1, a_2, a_3, a_4, c_1, c_2, R \sim N\left(\mathbf{X}\begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix} + \mathbf{Z}\begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ a_4 \end{bmatrix} + \mathbf{W}\begin{bmatrix} c_1 \\ c_2 \end{bmatrix}, R\right),
$$

where **R** was the (co)variance matrix. Sorting records by pig, and trait within pig, **R** could be written as $\mathbf{R}_0 \otimes \mathbf{I}$, with $\mathbf{R}_0$ being the 4×4 residual (co)variance matrix between the four traits analyzed and **I** an identity matrix of appropriate order. Flat priors were used for $\mathbf{b}_i$ and residual (co)variance components. Additive genetic and litter values, conditionally on the associated (co)variance components, were both assumed multivariate normally distributed with mean zero and with (co)variance $\mathbf{G} \otimes \mathbf{A}$ and $\mathbf{C} \otimes \mathbf{I}$, respectively, where **A** was the numerator relationship matrix, **G** was the 4×4 genetic relationship matrix between the four traits, and **C** was the 2×2 (co)variance matrix between litter effects of BW and BT. The matrix **A** was calculated using all the pedigree information summarised in **Table 1**. Flat priors were used for additive and litter (co)variance components. Statistical inferences were derived from the samples of the marginal posterior distribution using a unique chain of 1,500,000 iterations, where the first 500,000 were discarded and one sample out of 100 iterations retained. Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the BOA package (Smith, 2005). Convergence was tested using the *Z*-criterion of Geweke and visual inspection of convergence plots. For every genetic parameter the highest posterior density interval at 95% (HDP95%) and the limit for the interval [k, +∞) having a probability of 95% were calculated.

RESULTS AND DISCUSSION

The heritabilities for SCD in GM, LM, and BF, as well as that for E in GM, are showed in **Table 2**. The features of their posterior distribution are given in **Table 3**. The heritability of SCD is moderate, ranging from 0.47-0.52 in muscle to 0.34 in BF, and resulted higher that those reported by Ntawubizi et al. (2009). The HDP95 associated to SCD in LD and BF were wider than in GM because of the smaller number of samples available, but in any case the minimum value of the heritability for SCD having a probability of 95% is 0.35 in muscle and 0.21 in BF. This result will confirm that SCD behaves differently across tissues, in line with what was observed in Henriquez et al. (2013). The heritability of E was similar to that of SCD. Brooks et al. (2011) have showed, in Angus steers, that the proportions of MUFA in muscle and adipose tissue were dictated by SCD activity rather than by diet.

Table 2. Posterior means (SD) of heritabilities (bolded diagonal), genetic correlations (above diagonal), residual correlations (under diagonal), additive genetic variance (σ^2a) and residual variance (σ^2e) for SCD GM, SCD LD, SCD BF and E GM

Traits	Model without SNP				Model with SNP			
	SCD GM	SCD LD	SCD BF	E GM	SCD GM	SCD LD	SCD BF	E GM
SCD GM	**0.47 (0.07)**	0.76 (0.09)	0.41 (0.19)	-0.79 (0.05)	**0.30 (0.07)**	0.61 (0.13)	0.26 (0.23)	-0.63 (0.11)
SCD LD	0.48 (0.09)	**0.52 (0.10)**			0.49 (0.08)	**0.45 (0.09)**		
SCD BF	0.32 (0.12)		**0.34 (0.08)**		0.29 (0.11)		**0.34 (0.11)**	
E GM	-0.57 (0.07)			**0.58 (0.08)**	-0.61 (0.05)			**0.41 (0.09)**
Variance								
σ^2a^b	14.76 (2.73)	13.98 (3.23)	22.51 (6.25)	0.09 (0.01)	7.92 (2.16)	10.15 (2.44)	21.99 (8.08)	0.05 (0.01)
σ^2e^b	16.54 (2.06)	12.78 (2.56)	43.19 (6.22)	0.06 (0.01)	18.45 (1.78)	12.19 (2.10)	41.75 (6.81)	0.07 (0.01)

^{b} Values have been multiplied by 100

SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD; SCD BF=C18:1BF/C18:0BF

E GM=C18:0GM/C16:0GM

Table 3. Features of the posterior distribution of the heritability of SCD GM, SCD LD, SCD BF y E GM

	Trait	Mean	Median	HDP95[1]		k^2
Model without SNP	SCD GM	0.47	0.47	0.33	0.62	0.35
	SCD LD	0.52	0.52	0.35	0.71	0.36
	SCD BF	0.34	0.34	0.18	0.51	0.21
	E GM	0.58	0.58	0.42	0.72	0.45
Model with SNP	SCD GM	0.30	0.29	0.16	0.45	0.18
	SCD LD	0.45	0.44	0.29	0.63	0.32
	SCD BF	0.34	0.33	0.15	0.56	0.18
	E GM	0.41	0.40	0.26	0.58	0.28

[1]HDP95: highest posterior density interval at 95%

[2]k: limit for the interval $[k, +\infty)$ having a probability of 95%

SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD; SCD BF=C18:1BF/C18:0BF

E GM=C18:0GM/C16:0GM

The genetic correlations among SCD in different tissues and with E are showed in **Table 2,** with the features of their respective posterior distributions in **Table 4**. The genetic correlation between SCD in GM and SCD in LD was high (0.76), with an associated HDP95 [0.57;-0.90], indicating that SCD is relatively similar expressed in these two relevant muscles. However, the genetic correlation of SCD in GM and BF is lower (0.41), with a HDP95 [0.04,-0.78]. This result only allows for positive correlation between SCD in GM and BF, and little more, but clearly put in evidence that the activity of SCD is different in muscle and in subcutaneous fat. The genetic correlation of the SCD with E in GM is high and negative (-0.79). The first interpretation to this result is that the expression of one of the two enzymes repressed the other. But there is not any experimental evidence confirming it. Results from Bosch et al. (2012) indicate that at the end of the fattening period C16:0 and C18:1 increase with age while C16:1 and C18:0 remain stable or even decrease. This leads to a more plausible interpretation: the negative correlation between SCD and E may be attributed to a differential efficiency in the desaturation of C16:0 and C18:0. This result stresses the spurious effects that may arise in the interpretation of compositional data (Ros-Freixedes and Estany, 2013). The genetic correlations of SCD and E with BW and BT are described in **Table 5** and the features of their posterior distribution in **Table 6**. All of these correlations were close to zero, with HDP95 evenly distributed around it, suggesting that SCD and E activity are not expected to change with BW and BT.

Table 4. Features of the posterior distribution of the genetic correlations among SCD indices and with the E index

	Traits	Mean	Median	HDP95[1]		k^2
Model without SNP	SCD GM-SCD LD	0.76	0.77	0.57	0.90	0.59
	SCD GM-SCD BF	0.41	0.41	0.04	0.78	0.08
	SCD GM-E GM	-0.79	-0.79	-0.88	-0.69	-0.87
Model with SNP	SCD GM-SCD LD	0.61	0.63	0.35	0.84	0.37
	SCD GM-SCD BF	0.26	0.27	-0.16	0.66	-0.13
	SCD GM-E GM	-0.63	-0.65	-0.84	-0.41	-0.80

[1]HDP95: highest posterior density interval at 95%

[2]k: limit for the interval [k, +∞) having a probability of 95%

SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD; SCD BF=C18:1BF/C18:0BF

E GM=C18:0GM/C16:0GM

Table 5. Genetic correlations (SD) of BW and BT with SCD GM, SCD LD, SCD BF and E GM

Traits	Model without SNP		Model with SNP	
	BW	BT	BW	BT
SCD GM	-0.05 (0.11)	0.00 (0.11)	-0.07 (0.14)	-0.04 (0.14)
SCD LD	-0.08 (0.14)	-0.09 (0.15)	-0.10 (0.16)	-0.16 (0.16)
SCD BF	-0.03 (0.18)	-0.17 (0.18)	-0.03 (0.20)	-0.20 (0.19)
E GM	-0.09 (0.10)	-0.12 (0.10)	-0.11 (0.12)	-0.13 (0.11)

SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD;
SCD BF=C18:1BF/C18:0BF
E GM=C18:0GM/C16:0GM

Table 6 . Features of the posterior distribution of the genetic correlations of BW and BT with SCD indices and the E index

	Traits	Mean	Median	HDP95[1]		k^{2}
Model without SNP	SCD GM-BW	-0.05	-0.05	-0.26	0.16	-0.23
	SCD GM-BT	0.00	0.00	-0.20	0.23	-0.17
	SCD LD-BW	-0.08	-0.08	-0.35	0.18	-0.31
	SCD LD-BT	-0.09	-0.08	-0.38	0.18	-0.33
	SCD BF-BW	-0.03	-0.01	-0.41	0.27	-0.35
	SCD BF-BT	-0.17	-0.16	-0.50	0.15	-0.46
	E GM-BW	-0.09	-0.09	-0.27	0.10	-0.25
	E GM-BT	-0.12	-0.12	-0.31	0.07	-0.29
Model with SNP	SCD GM-BW	-0.07	-0.07	-0.35	0.19	-0.30
	SCD GM-BT	-0.04	-0.03	-0.29	0.25	-0.27
	SCD LD-BW	-0.10	-0.10	-0.41	0.21	-0.36
	SCD LD-BT	-0.16	-0.15	-0.47	0.11	-0.42
	SCD BF-BW	-0.03	-0.03	-0.41	0.34	-0.35
	SCD BF-BT	-0.20	-0.21	-0.56	0.17	-0.49
	E GM-BW	-0.11	-0.11	-0.33	0.13	-0.30
	E GM-BT	-0.13	-0.13	-0.34	0.09	-0.30

[1]HDP95: highest posterior density interval at 95%

[2]k: limit for the interval [k, +∞) having a probability of 95%
SCD GM=C18:1GM/C18:0GM; SCD LD=C18:1LD/C18:0LD; SCD BF=C18:1BF/C18:0BF
E GM=C18:0GM/C16:0GM

All the genetic parameters indicated above were also estimated including the SNP *AY487830:g.2281A>G* in the model. As expected, the SNP explained affected SCD. The percentage of the additive genetic variance explained by the SNP for SCD in GM, SCD in LD, SCD in BF, and E in GM were 46.3%, 27.4%, 2.3% and 41.8%, respectively. The effect of the SNP on SCD was greater in GM than in LD, which was less variable and more poorly estimated. The values of the heritabilities for SCD in muscle and E, along with the correlations among them, decrease accordingly. In GM the heritability associated to the SNP is 17% while it is negligible for SCD in BF. This would confirm again that the activity of SCD differs between muscle and subcutaneous fat, and that the SNP *AY487830:g.2281A>G* is playing a role in such specificity, with a more remarkable effect on muscle in accordance with Henriquez et al. (2013). That the SNP had an effect on E may be also an indirect result of a more efficient desaturation of C18:0 than C16:0, which prompt to a greater clearance of C18:0 than C16:0.

General discussion

The means of SCD in different muscles, subcutaneous fat, and liver, along with the estimated heritability in GM, LM, and BF, and the genetic correlations among them confirm that SCD behaves differently across tissues, in line with previous results (Doran et al., 2006; Cánovas et al., 2009; and Muñoz et al., 2013). The liver shows this distinct behavior likely because, in pig, the liver is the main organ synthesizing long PUFA but it is the adipose tissue the main site for *de novo* fat synthesis (Duran-Montgé et al., 2009). However, differences among muscles and particularly between GM and LD, the two economically most important, do not involve dramatic differences. In contrast, SCD in BF may not be a good indicator of the SCD activity in muscle. For a breeding program, this has the practical implications that selection for increased C18:1 (or C18:1/C18:0) in hams and loins should be done from records taken on muscle and not in BF. The muscle sampled, GM or LD, is not so relevant. Therefore, because it is easier and less costly, sample from GM, the current procedure, is a good option. Adjusting for the effect of the SNP *AY487830:g.2281A>G* decrease both the genetic variability of SCD and the genetic correlation of SCD at different tissues. The pigs with genotype AA have higher SCD activity and content of C18:1 than the GG. Selection for genotype AA is a good alternative for increasing C18:1 in the population analyzed in this thesis. Then, because the remaining genetic variance will be lower, note that the additional direct response based on phenotypic records will be lower too. The genetic correlation among SCD with BW and BT suggest that SCD activity is not expected to change with BW and BT.

The results of this thesis also put in evidence that the elongase index varied among tissues. The negative genetic correlation between the indices E (C18:0/C16:0) and SCD (C18:1/C18:0), as well as the fact that the SNP has a negative effect upon the E activity, can be indirectly indicating that C18:0 is a more preferred substrate for SCD than C16:0. This side-effect may be a cause of biological misinterpretations, and need to be further assessed. On the other hand, in chapter II the value for oleic acid was the sum of C18:1n-9 (oleic) and C18:1n-7 (vaccenic), while in chapter I these two fatty acids were individually separated. The oleic acid is much more abundant (almost 7

times more) that the vaccenic acid and for this reason it is not determined in routine determination. The commercial pattern used to identify the fatty acids in the process of transesterification does not identify the vaccenic acid. To evaluate the impact of using the sum of both C18:1n-9 and C18:1n-7 instead of C18:1n-9 alone on the estimates of the SCD activity, the correlation between (C18:1n-9 + C18:1n-7)/C18:0 and C18:1n-9/C18:0 ratios were calculated. This correlation was always greater than 0.99 in GM, SM and liver while in BF is >0.98, showing that for practical purposes the distinction between the oleic and the vaccenic acids is not determinant for measuring the SCD activity.

The results shown in this thesis raise some new questions. It is not clear whether the effect of the SNP *AY487830:g.2281A>G* on the SCD activity changes with ages or whether its impact is maintained on dry-cured products rather on raw meat. It is known that the SCD activity increase with age and therefore it might be expected that the effect of SNP increases as the pig gets older. Also, it is known that fatty acid composition change during the manufacturing of dry-cured hams. It would be interesting to prove that the effect of the SNP is maintained in the commercial product of highest added-value. These two aspects of interest will be addressed in future research.

Conclusions

1. The activity of the SCD is tissue-specific, being higher in muscle and lower in subcutaneous fat and liver.

2. The genetic correlation between SCD in GM and in LD was high enough to suggest that SCD is similarly expressed in muscle. However, the genetic correlation with SCD in BF is much lower, suggesting that SCD in BF may not be a good indicator of the SCD activity in muscle. The SCD activity is not expected to be modified selecting for BW and BT. The negative correlation between SCD and E may be attributed to a greater efficiency in the desaturation of C18:0 than of C16:0. This result stresses the spurious effects that may arise in the interpretation of compositional data.

3. The SNP *AY487830:g.2281A>G* affects SCD and plays a role in the differential activity of this enzyme. Adjusting for the effect of the SNP decrease both the genetic variability of SCD and the genetic correlation of SCD at different tissues.

References

Bosch, L., M. Tor, J. Reixach, and J. Estany. 2009. Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. Meat Sci. 82:432–437.

Brooks, M.A., C.W. Choi, D.K. Lunt, H. Kawachi and S.B. Smith. 2011. Subcutaneous and intramuscular adipose tissue stearoyl-coenzyme A desaturase gene expression and fatty acid composition in calf- and yearling-fed Angus steers. J. Anim. Sci. 89:2556-2570.

Cameron N.D., M. Enser, G.R. Nute, F.M. Whittington, J.C. Penman, A.C. Fisken, A.M. Perry, J.D. Wood. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci. 55:187-195.

Cannata, S., T.E. Engle, S.J. Moellerc, H.N. Zerbyc, A.E. Radunzc, M.D. Greenb, P.D. Bassb, K.E. Belkb. Effect of visual marbling on sensory properties and quality traits of pork loin. Meat Sci. 2010. 85:428-434.

Cánovas, A., J. Estany, M. Tor, R. N. Pena and O. Doran. 2009. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905–3914.

DeVol, D.K., F.K. McKeith, P.J. Bechtel, J. Novakofski, R.D. Shanks and T.R. Carr. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. J. Anim. Sci. 66, 385–395.

Doran, O., S.K. Moule, G.A. Teye, F.M. Hallett, K.G. Whittington and J.D. Wood. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. Br. J. Nutr. 95:609–617.

Duran-Montgé, P., P.K. Theil, C. Lauridsen and E. Esteve-Garcia. 2009. Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulatory factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period. Animal. 3:11, 1580–1590.

FAO. 2003. World agriculture: towards 2015/2030, an FAO perspective. Earthscan Publications Ltd. London.

FAOSTAT. 2011. Producción de productos alimenticios y agrícolas. Available at http://faostat.fao.org/site/339/default.aspx [Accessed June 19, 2013; verified June 26, 2013].

Font-i-Furnols, M., N. Tous, E. Esteve-Garcia, M. Gispert. 2012. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. Meat Sci. 91: 448–453.

Gilles, G. 2009. Dry cured ham quality as related to lipid quality of raw material and lipid changes during processing: a review. Grasas Aceites. 60:297-307.

Guillou, H., D. Zadravec, P.G.P. Martin, A. Jacobsson. 2010. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog. Lipid. Res. 49:186–199.

Henriquez, E., R. Ros-Freixedes, R. N. Pena, M. Tor and J. Estany. 2013. Desaturase and elongase activity across tissues and genotypes in Duroc pigs. XV Jornadas sobre Producción Animal, edited by Asociación Interprofesional para el Desarrollo Agrario. Volume II, 517-519.

Kris-Etherton PM. 1999. Monounsaturated fatty acids and risk of cardiovascular disease. Circulation. 100:1253–1258.

Lo, L. L., D.G. McLaren, F.K. McKeith, R.L. Fernando, and J. Novakofski. 1992. Genetic analyses of growth, real-time ultrasound, carcass, and pork quality traits in Duroc and Landrace pigs: I. Breed effects. J. Anim. Sci. 70:2373–2386.

Matsuzaka, T. and H. Shimano. 2009. Elovl6: a new player in fatty acid metabolism and insulin sensitivity. J. Mol. Med. 87:379–384.

Menéndez, J.A., L. Vellon, R. Colomer and R. Lupu. 2005. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erb B-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptine) in breast cancer cells with Her-2/neu oncogene amplification. Ann. Oncol. 16: 359–371.

Ministerio de Agricultura, Alimentación y Medioambiente (MAGRAMA). 2012. Encuestas ganaderas. Available at

http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/PDF__tcm7-286216.pdf [Accessed June 19, 2013; verified June 26, 2013]. Spain.

Miyazaki, M. and M. Ntambi. 2003. Role of stearoyl-conenzyme A desaturase in lipid metabolism. Prostaglandins Leukot. Essent. Fatty Acids 68:113–121.

Muñoz, R., J. Estany, M. Tor, O. Doran. 2013. Hepatic lipogenic enzyme expression in pigs is affected by selection for decreased backfat thickness at constant intramuscular fat content. Meat Sci. 93:746-751.

Ntawubizi, M., K. Raes, N. Buys, S. De Smet. 2009. Effect of sire and sex on the intramuscular fatty acid profile and indices for enzyme activities in pigs. Livest. Sci. 122:264–270.

Reixach, J., M. Tor, M. Díaz and J. Estany. 2008. Between and within breed variation for lean growth and intramuscular fat content and fatty acid composition in pigs. 54th International Congress of Meat Science and Technology, ICoMST, Helsinki, Finland, 1-3.

Ren, J., C. Knorr, L. Huang, and B. Brenig. 2004. Isolation and molecular characterisation of the porcine stearoyl-CoA desaturase gene. Gene 340:19–30.

Ros-Freixedes and J. Estany. 2013. On the compositional analysis of fatty acids in pork. (Paper submitted for assessment).

Ros-Freixedes, R., J. Reixach, M. Tor and J. Estany. 2012. Expected genetic response for oleic acid content in pork. J. Anim. Sci. 90:4230-4238.

Ros-Freixedes, R., R.N. Pena, M. Tor and J. Estany. 2013. Breeding for high oleic acid content in pork using direct selection and molecular markers. XV Jornadas sobre Producción Animal, edited by Asociación Interprofesional para el Desarrollo Agrario. Volume II, 580-582.

Sellier, P., L. Maignel and J. P. Bidanel. 2010. Genetic parameters for tissue and fatty acid composition of backfat, perirenal fat and longissimus muscle in Large White and Landrace pigs. Animal. 4:4, 497–504.

Smith, S.B., T.S. Hively, G.M. Cortese, J.J. Han, K.Y. Chung, P. Casteñada, C.D. Gilbert, V.L. Adams and H.J. Mersmann. 2002. Conjugated linoleic acid depresses the d9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J. Anim. Sci. 80:2110-2115.

Suzuki, K., T. Shibata, H. Kadowaki, H. Abe, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64:35–42.

Wood J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard, M. Enser. 2003. Effects of fatty acids on meat quality: a review. Meat Sci. 66:21-32.

Wood, J.D., G.R. Nute, R.I. Richardson, F.M. Whittington, O. Southwood, G. Plastow, R. Mansbridge, N. da Costa, K.C. Chang. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci. 67:651–667.

Zhang, S., T.J. Knight, K.J. Stalder, R.N. Goodwin, S.M. Lonergan and D.C. Beitz. 2007. Effects of breed, sex, and halothane genotype on fatty acid composition of pork longissimus muscle. J. Anim. Sci. 85:583-591.