



Effect of genotype on fatty acid composition of intramuscular and subcutaneous fat of Celta pig breed

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SUMMARY: A total of 45 Celta breed pigs were used to investigate the effect of genotype (Barcina, Carballiña and Santiaguesa lines) on the fatty acid composition of intramuscular (IMF) and subcutaneous fat (SF). The total IMF content was influenced by genotype ($P<0.05$), since the Barcina line had the highest levels (5.21% vs 1.99 and 3.59% for Santiaguesa and Carballiña lines, respectively). The total and neutral lipids from the IMF of the Santiaguesa line displayed higher contents of PUFA than the other two lines. The nutritional indices were also affected by genotype, since the Santiaguesa line presented the lowest atherogenic (AI) and thrombogenic (TI) indices and the highest hypocholesterolemic/hypercholesterolemic ratio (h/H) ($P<0.05$). The IMF total and neutral lipids presented higher ($P<0.05$) values of MUFA than SF, while the PUFA content was greatest in SF ($P<0.05$). Regarding the polar fraction, samples from IMF presented the highest values of PUFA (between 37–44%). Finally, SF showed higher percentages of MUFA and SFA than IMF ($P<0.05$). The differences in IMF content and back fat thickness imply that the deposition of IMF and SF may be regulated by different mechanism among the three lines.

KEYWORDS: *Celta pig breed; Fatty acid profile; Genotype; Location in the carcass*

RESUMEN: *Efecto del genotipo sobre la composición de ácidos grasos de la grasa intramuscular y subcutánea de cerdos de raza Celta.* Un total de 45 cerdos de raza Celta fueron usados para estudiar el efecto del genotipo (líneas Barcina, Carballiña y Santiaguesa) sobre la composición de ácidos grasos de la grasa intramuscular y subcutánea. El contenido en grasa intramuscular estuvo influenciado por el genotipo ($P<0.05$); la Barcina presentó los mayores valores (5.21% vs 1.99 y 3.59 para las líneas Santiaguesa y Carballiña respectivamente). Los lípidos totales y neutros de la grasa intramuscular de la línea Santiaguesa mostraron mayores contenidos de PUFA que las otras dos líneas. Los índices nutricionales también se vieron influenciados por el genotipo; la línea Santiaguesa presentó los menores valores de los índices aterogénico y trombogénico y los mayores de la relación entre ácidos grasos hipo e hipercolesterolémicos. En los lípidos totales y neutros de la grasa intramuscular se obtuvieron mayores valores de MUFA y menores de PUFA ($P<0.05$) que en la grasa subcutánea. En los lípidos polares, las muestras de grasa intramuscular presentaron los mayores valores de PUFA (entre 37–44%). Finalmente, la grasa subcutánea mostró valores superiores de MUFA y SFA que la intramuscular ($P<0.05$). Las diferencias en el contenido de grasa intramuscular y de espesor de grasa subcutánea implica que su deposición puede estar regulada por diferentes mecanismos.

PALABRAS CLAVE: *Cerdo de raza Celta; Genotipo; Localización en la canal; Perfil de ácidos grasos*

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1. INTRODUCTION

The Celta pig is an autochthonous breed reared in the north- west of the Iberian Peninsula. These pigs are reared under extensive production systems and fed with balanced diets and natural sources, being slaughtered at >140 kg live weight.

The Celta pig breed has a high content of intramuscular fat (Franco and Lorenzo, 2013). Previous studies have shown that muscles from rustic pig breeds, in contrast to the muscle from selected pig breeds, contain higher amounts of intramuscular lipids (Morales *et al.*, 2002; Cava *et al.*, 2003). Moreover, the amount of intramuscular fat influences the fatty acid profiles of the lipid fractions. This may have important consequences on the oxidative stability of meat and meat products (Cava *et al.*, 2004).

Previous studies have evaluated the influence of carcass location (Martínez *et al.*, 2007; Lorenzo *et al.*, 2012), sex (Lorenzo *et al.*, 2012), crossbreed (Franco *et al.*, 2014) and diet (Franco *et al.*, 2006; Bermúdez *et al.*, 2012) on the fatty acid profile in Celta pigs, but there are no studies about the differences in fatty acid compositions among Celta pig breed lines.

It is well known that within the Celta pig breed, three genotypes differ significantly (Santiaguesa, Barcina and Carballiña). The three lines are morphologically identical, and only differ in the absence or presence of pigmentation and in place of origin (Carril *et al.*, 2012). Thus, the aim of this research was to study the influence of genotype on the fatty acid profile from different fat locations (intramuscular, subcutaneous dorsal and subcutaneous ventral) of the Celta pig breed.

2. MATERIAL AND METHODS

2.1. Experimental design and animal management

For this study, 45 castrated pigs (males and females) from the Celta breed (15 Barcina line, 15 Carballiña line and 15 Santiaguesa line) were used. All specimens, registered in the Record of Births of the Stud-Book were obtained from ASOPORCEL. All the animals were reared under the extensive system. The pigs were fed *ad libitum* with a commercial concentrate suited to the nutritive needs of the animals. Table 1 shows the chemical composition and fatty acid profile of the commercial feed. The animals were slaughtered at 16 months of age. The day before slaughter, the animals were weighed and transported to the abattoir trying to minimize stress. The pigs were slaughtered in an accredited abattoir (Lugo, Spain), using carbon dioxide to stun the animals.

After 45 min *post-mortem*, the dorsal fat thickness was measured with a flexible tape at the level

TABLE 1. Chemical composition and fatty acid profile of the commercial feed

Chemical composition (%)	
Crude Protein	15.3
Ash	5.5
Fat	3.5
Celulose	3.5
Lysine	0.7
Methionine	0.2
Phosphate	0.5
Ca	1.1
Na	0.1
Fatty acid profile (%)	
C16:0	15.56
C16:1	0.12
C18:0	2.63
C18:1n9c	25.24
C18:2n6c	48.89
C20:0	0.42
C18:3n3	6.23
C22:0	0.45
C20:5n3	0.11
C24:1	0.19
SFA	19.15
MUFA	25.56
PUFA	55.28
P/S	0.24
Σn-6/n-3	7.70

The concentrate was formulated using the following ingredients (%): 40 wheat, 25.5 barley, 15 soybean flour, 14.6 corn, 1.5 soybean oil, 2 calcium carbonate, 1 dicalcium phosphate and 0.20 sodium chloride.

of the first rib. Carcasses were chilled at 4 °C in a cold chamber for 24 h and cold carcass weight was recorded. The samples of fat, one per deposit, were taken from three different deposits (intramuscular from *longissimus dorsi* muscle, subcutaneous ventral and subcutaneous dorsal). The samples were transported to the laboratory under refrigeration (<4 °C) and analyzed on the day of collection.

2.2. Reagents

Fatty acid methyl ester (FAMES) standard mixtures and nonadecanoic acid were acquired from Supelco Inc. (Bellefonte, PA, USA). Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Boron trifluoride (14% solution in methanol) was obtained from Panreac

(Castellar del Vallès, Barcelona, Spain). NH₂-SPE columns (1 mL×100 mg) were acquired from Waters (Cerdanyola del Vallès, Spain).

2.3. Analysis of fatty acid methyl esters

IMF was extracted using chloroform/methanol (2/1;v/v) according to the method proposed by Folch *et al.* (1957) whereas the adipose fat was extracted following the procedure described by De Pedro *et al.* (1997) and stored at -80 °C until analysis by preparation of FAMES.

Lipids were trans-esterified with a solution of boron trifluoride (14%) in methanol (Carreau and Dubacq, 1978). For the total fatty acid analysis, 50 mg of the extracted lipids were esterified while the neutral and polar fractions were separated using NH₂-SPE columns according to the procedure developed by Kaluzny *et al.* (1985). FAMES were stored at -80 °C until chromatographic analysis. Separation and quantification of the FAMES was carried out using a gas chromatograph (GC-Agilent 6890N; Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector and an automatic sample injector HP 7683, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness). The chromatographic conditions were as follows: initial column temperature 120 °C, maintaining this temperature for 5 min, programmed to increase at a rate of 5 °C·min⁻¹ up to 200 °C, maintaining this temperature for 2 min, then at 1 °C·min⁻¹ up to 230 °C, maintaining this temperature for 3 min. The injector and detector were maintained at 260 and 280 °C, respectively. Helium was used as the carrier gas at a constant flow-rate of 1.1 mL·min⁻¹, with the column head pressure set at 35.56 psi. The split ratio was 1:50 and 1 µL of solution was injected. Nonadecanoic acid (C19:0) at 0.3 mg·mL⁻¹ was used as internal standard and added to the samples prior to fat extraction and methylation. Individual FAMES were identified by comparing their retention times with those of authenticated standards (Supelco 37 component FAME Mix). Data regarding FAME composition were expressed in percentage according to the weight of the total identified FAMES. The proportion of polyunsaturated (PUFA) (C18:2 n6; C18:3 n3; C20:2 n6; and C20:4 n6), monounsaturated (MUFA) (C16:1 n7; C18:1 c-n9; and C20:1 n9) and saturated (SFA) (C14:0; C16:0; C18:0; and C20:0) fatty acid contents and PUFA/SFA ratio (P/S) were calculated. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht and Sauthgate, (1991): AI=[C12:0+(4*C14:0)+C16:0]/[(ΣPUFA)+(ΣMUFA)];

TI=[C14:0+C16:0+C18:0]/[(0.5*ΣMUFA)+(0.5*n-6)+(3*n-3)+(n-3/n-6)].

The hypocholesterolemic/Hypercholesterolemic ratio (h/H) was calculated according to Fernández

et al. (2007): h/H = [(sum of C18:1 c-n9, C18:1 n7, C18:2 n6, C18:3 n6, C18:3 n3, C20:3 n6, C20:4 n6, C20:5 n3, C22:4 n6, C22:5 n3 and C22:6 n3)/(sum of C14:0 and C16:0)].

2.4. Statistical analysis

An analysis of variance (ANOVA), using the General Linear Model (GLM) procedure of the SPSS package (SPSS 19.0, Chicago, IL, USA) was performed for all variables considered in the study. When we studied the effect of genotype and location of the fat in the carcass on fatty acid composition, fixed the effects of anatomical location and genotype were included in the model. The model used was: $Y_{ij} = \mu + S_i + A_j + \epsilon_{ij}$; where: Y_{ij} is the observation of dependent variables, μ is the overall mean, S_i is the effect of genotype, A_j is the effect of location in the carcass, and ϵ_{ij} is the residual random error associated with the observation. Correlations between variables ($P < 0.05$) were determined using the Pearson's linear correlation coefficient.

3. RESULTS

3.1. Effect of location

Pork fat is mainly located in the subcutaneous area, perirenal area, between muscles (intermuscular) or between muscle fibers (intramuscular). The fatty acid composition of the total and neutral lipid fractions (from the three locations) are shown in Tables 3 and 4, respectively. The most abundant fatty acids were monounsaturated fatty acids (MUFA) (mainly C18:1 c-n9, about 40–45% of total fatty acids in total lipids and 45–51% in neutral lipids), followed by saturated fatty acids (SFA) (approximately 38–43% in total lipids and 35–38% in neutral lipids) and finally polyunsaturated fatty acids (PUFA) (approximately 7–14% in both types of lipids).

The total and neutral lipids of the intramuscular fat (IMF) showed significantly ($P < 0.05$) higher values of MUFA and lower values of PUFA than the subcutaneous fat (SF), while SFA showed no significant differences ($P > 0.05$) among the three locations. The greater MUFA content in IMF could be related to the higher contents of C18:1c-n9 ($r = 0.965$, $P < 0.01$) and C16:1 n7 ($r = 0.741$, $P < 0.01$), while the lower PUFA content in this location could be linked to the smaller values of C18:2 n6 ($r = 0.979$, $P < 0.01$) and, to a lesser extent, to the contents of C18:3 n3 ($r = 0.593$, $P < 0.01$) and C20:2 n6 ($r = 0.789$, $P < 0.01$).

The nutritional indices (IA, IT and h/H) presented significant differences ($P < 0.05$) among locations, but did not show a clear trend (see Table 3). However, the polyunsaturated/saturated fatty acid (P/S) and $\Sigma n6/\Sigma n3$ ratio presented significant differences ($P < 0.05$) among locations, since the lowest

values of the P/S ratio were found in samples from IMF, while the lowest values of $\Sigma n6/\Sigma n3$ ratio were obtained in samples from SF.

The fatty acid composition of polar lipids (from the three depots) is shown in Table 5. IMF presented a fatty acid profile totally different from that observed in SF. IMF polar lipids presented higher values of PUFA (between 37 and 44%) than SF (between 12 and 17%). However, SF showed higher percentages of MUFA (43–45%) and SFA (39–44%) than IMF (18–22% MUFA and 36–38% SFA) (see Table 5). These differences were due to IMF having higher contents of C18:2 *n6* and C20:4 *n6* (between 10–19% higher than SF), and a lower percentages of C16:0, C18:0 and C18:1c-*n9* than SF. On the other hand, the content of C18:3 *n3* was greater in the subcutaneous locations than in IMF.

3.2. Effect of genotype

Table 2 shows the live weight, carcass weight, IMF and backfat thickness of the three genotypes of the Celta pig breed. Live weight ($P<0.05$), carcass weight ($P<0.05$) and IMF contents ($P<0.001$) were significantly different among the three lines. However, no significant differences ($P>0.05$) were observed in back fat thickness (Table 2). Although the effect of genotype on back fat thickness was not significant, some fatty acid from SF, as those from IMF, were still significantly influenced by genotype, suggesting the possible existence of a common mechanism for the regulation of some individual fatty acids from different locations. Regarding IMF, genotype also showed significant differences, since the highest values were obtained for Barcina (5.21%), followed by the Carballiña (3.59%) and Santiaguesa line (1.99%).

The total lipids from IMF (Table 3) showed higher percentages of PUFA and MUFA and lower percentages of SFA in the Santiaguesa line than in the Barcina and Carballiña genotypes. These values are due to the fact that the Santiaguesa line had higher

contents ($P<0.05$) of C16:1 *n7*, C18:1c-*n9* and C20:4 *n6* than the other two lines, while the lowest values ($P<0.05$) of C14:0, C16:0 and C20:0 were obtained for the Santiaguesa line. On the other hand, SF showed greater differences due to genotype in the dorsal fat location than in the ventral fat. In the dorsal fat, Carballiña and Santiaguesa genotypes had the highest values of PUFA, while the lowest values of SFA were obtained for the Santiaguesa line. In the ventral fat, the SFA content showed significant differences ($P<0.05$), and was also lower in the Santiaguesa line. The effects of genotype were different in the 3 locations, as indicated by significant genotype \times location interactions for the percentages of C14:0, C16:0, C16:1 *n7*, C17:0, C17:1, C20:0 and C20:4 *n6*.

The nutritional indices also showed differences ($P<0.05$) by genotype. The Santiaguesa line showed higher values of h/H ratio and lower values of AI and TI (in IMF and ventral fat) indices than the other two lines.

The neutral lipids (Table 4) showed a similar trend to the total lipids. The santiaguesa line presented higher ($P<0.05$) values of PUFA than the other two genotypes. These differences were mainly due to the fact that the Santiaguesa line pigs had the highest content of C18:2 *n6*. In fact, the Pearson correlation test indicated that PUFA content was positively correlated with C18:2 *n6* ($r=0.968$, $P<0.001$). On the contrary, neutral lipids in the Santiaguesa genotype presented the lowest values of MUFA in the IMF and dorsal fat, while the SFA content did not show significant differences ($P>0.05$) among the three lines. The highest content of MUFA in the Barcina and Carballiña lines was due to the higher values of C18:1c-*n9* ($r=0.998$ and $r=0.997$; $P<0.001$, for the Barcina and Carballiña lines) which was higher in these genotypes compared to the Santiaguesa line. There were significant interactions between the effects of genotype and location for the percentages of C20:2 *n6* and C20:4 *n6*.

TABLE 2. Number of pigs, live weight and carcass traits (mean \pm standard deviation values)

Trait	Genotype			Significance
	Barcina	Carballiña	Santiaguesa	
No of pigs	15	15	15	
Live Weight (kg)	171 \pm 15.5 ^b	184 \pm 17.9 ^a	178 \pm 25.5 ^{ab}	*
Carcass Weight (kg)	136 \pm 12.4 ^b	146 \pm 14.1 ^a	140 \pm 22.5 ^{ab}	*
Loin IMF (g·100 g ⁻¹)	5.21 \pm 1.72 ^a	3.59 \pm 1.11 ^b	1.99 \pm 0.72 ^c	***
Backfat thickness (cm)	5.89 \pm 0.90	5.75 \pm 1.02	6.37 \pm 1.56	ns

^{a-c}Means within the same row not followed by the same letter differ significantly (influence by genotype) ($P<0.05$); Significance: significantly different values as influenced by genotype * ($P<0.05$); ** ($P<0.01$); *** ($P<0.001$); ns: no significant difference.

TABLE 3. Fatty acid composition (% of total fatty acids) of total lipids in *longissimus dorsi* and dorsal and ventral subcutaneous fat

	<i>Longissimus dorsi</i>					Dorsal fat					Ventral fat					Significance		
	BL	CL	SL	SEM	SEM	BL	CL	SL	SEM	SEM	BL	CL	SL	SEM	SEM	G	L	G×L
C14:0	1.37 ^b	1.69 ^c	1.22 ¹	0.039	0.039	1.40 _b	1.46 ¹	1.20 ¹	0.031	0.031	1.45 _a	1.72 ²	1.32 ²	0.037	0.037	***	**	*
C16:0	27.42 ²	28.39 ^c	24.10 ¹	0.331	0.331	26.08 ¹	25.59 ¹	24.49 ¹	0.231	0.231	26.62 ¹	27.69 ^c	25.53 ²	0.218	0.218	***	***	***
C16:1 n7	3.79 ³	3.70 ³	4.26 ³	0.071	0.071	2.54 ¹	2.57 ¹	2.03 ¹	0.059	0.059	2.81 ²	3.09 ²	2.79 ²	0.061	0.061	ns	***	***
C17:0	0.23 ^b	0.15 ¹	0.32 ¹	0.013	0.013	0.48 ²	0.35 ²	0.41 ²	0.016	0.016	0.42 ²	0.33 ²	0.41 ²	0.016	0.016	***	***	*
C17:1	0.29 ¹	0.26 ¹	0.52 ²	0.025	0.025	0.43 ²	0.35 ²	0.27 ¹	0.016	0.016	0.42 ²	0.40 ²	0.29 ¹	0.018	0.018	ns	ns	***
C18:0	13.09 ¹	12.38 ^{ab}	11.87 ¹	0.182	0.182	14.46 ²	14.17 ²	14.46 ³	0.220	0.220	13.87 ¹	13.55 ²	13.19 ²	0.241	0.241	*	***	ns
C18:1 c n9	44.73 ²	44.15 ²	46.96 ²	0.317	0.317	40.59 ¹	40.17 ¹	41.97 ¹	0.343	0.343	41.29 ¹	40.60 ¹	42.81 ¹	0.476	0.476	***	***	ns
C18:2 n6	6.34 ¹	6.61 ¹	7.00 ¹	0.160	0.160	10.84 ²	12.20 ³	12.21 ³	0.194	0.194	10.47 ^{ab}	9.89 ²	11.31 ²	0.242	0.242	*	***	ns
C20:0	0.15 ²	0.14 ¹	0.08 ¹	0.010	0.010	0.19 ³	0.19 ²	0.19 ²	0.007	0.007	0.11 ¹	0.15 ¹	0.11 ¹	0.005	0.005	**	***	*
C20:1 n9	0.92 ¹	0.84 ¹	0.83 ¹	0.015	0.015	1.27 ²	1.12 ²	1.10 ²	0.029	0.029	0.96 ¹	0.93 ^{ab}	0.84 ¹	0.021	0.021	**	***	ns
C18:3 n3	0.38 ¹	0.40 ¹	0.38 ¹	0.008	0.008	0.75 ²	0.90 ²	0.80 ²	0.020	0.020	0.74 ²	0.85 ²	0.79 ²	0.021	0.021	**	***	ns
C20:2 n6	0.23 ¹	0.19 ¹	0.24 ¹	0.007	0.007	0.60 ³	0.56 ³	0.62 ³	0.015	0.015	0.45 ²	0.39 ²	0.43 ²	0.011	0.011	**	***	ns
C20:4 n6	0.91 ²	0.74 ²	1.27 ²	0.066	0.066	0.19 ¹	0.12 ¹	0.14 ¹	0.007	0.007	0.25 ¹	0.20 ^{ab}	0.19 ¹	0.011	0.011	***	***	***
SFA	42.64 ^b	42.72 ^b	38.30 ¹	0.400	0.400	42.61 ^b	41.76 ^{ab}	40.75 ²	0.301	0.301	42.47 ^b	43.44 ^b	40.54 ²	0.396	0.396	***	ns	*
MUFA	49.77 ²	48.88 ²	52.70 ²	0.376	0.376	44.84 ¹	44.21 ¹	45.37 ¹	0.342	0.342	45.50 ¹	45.02 ¹	46.73 ¹	0.494	0.494	***	***	ns
PUFA	7.55 ¹	8.15 ¹	9.00 ¹	0.226	0.226	12.55 ²	14.03 ³	13.87 ³	0.215	0.215	12.02 ²	11.54 ²	12.73 ²	0.250	0.250	*	***	ns
Σn3	0.42 ¹	0.60 ¹	0.38 ¹	0.020	0.020	0.91 ²	1.15 ²	0.92 ³	0.026	0.026	0.86 ²	1.06b ²	0.83 ²	0.029	0.029	***	***	ns
Σn6	7.13 ¹	7.54 ¹	8.62 ¹	0.223	0.223	11.64 ²	12.88 ³	12.96 ³	0.199	0.199	11.16 ^{ab}	10.48 ²	11.90 ²	0.241	0.241	**	***	ns
Σn6/Σn3	17.40 ²	18.85 ²	22.94 ²	0.777	0.777	12.93 ¹	11.38 ²	14.19 ¹	0.276	0.276	13.37 ¹	10.01 ¹	14.46 ¹	0.406	0.406	***	***	***
P/S	0.18 ¹	0.19 ¹	0.24 ¹	0.007	0.007	0.29 ²	0.34 ³	0.34 ²	0.006	0.006	0.28 ²	0.27 ²	0.31 ²	0.006	0.006	***	***	*
AI	0.57 ^b	0.62 ^c	0.47 ¹	0.012	0.012	0.55 ^b	0.54 ¹	0.50 ¹²	0.007	0.007	0.56 ^b	0.61 ^c	0.52 ²	0.009	0.009	***	**	***
TI	1.41 ^b	1.41 ^b	1.17 ¹	0.023	0.023	1.35 ^b	1.29 ^{ab}	1.26 ²	0.016	0.016	1.35 ^b	1.39 ²	1.26 ²	0.019	0.019	***	ns	**
h/H	1.78 ¹	1.69 ¹	2.14 ²	0.035	0.035	1.87 ²	1.95 ²	2.11 ¹²	0.027	0.027	1.85 ¹²	1.72 ¹	2.02 ^c	0.029	0.029	***	***	**

BL: Barcina line; CL: Carballiña line; SL: Santiagueta line; SFA: sum of saturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; AI: atherogenic index; TI: thrombogenic index; h/H: ratio hypocholesterolemic/hypercholesterolemic fatty acids; SEM: standard error of the mean; G: significantly different values as influenced by genotype *(P<0.05); ***(P<0.001); ns: no significant difference; L: significantly different values as influenced by location *(P<0.05); ***(P<0.01); ****(P<0.001); ns: no significant difference; G×L: interaction of genotype and location *(P<0.05); ***(P<0.001); ns: no significant difference; ¹⁻³Means within the same row not followed by the same number differ significantly (influence of location) (P<0.05); ^{a-c}Means within the same row not followed by the same letter differ significantly (influence of genotype) (P<0.05).

TABLE 4. Fatty acid composition (% of total fatty acids) of neutral lipids in *longissimus dorsi* and dorsal and ventral subcutaneous fat

	<i>Longissimus dorsi</i>						Dorsal fat						Ventral fat						Significance						
	BL		SL		SEM		BL		CL		SL		SEM		BL		CL		SL		SEM		G	L	G×L
	CL	SL	CL	SL	SEM	BL	CL	SL	SEM	BL	CL	SL	SEM	BL	CL	SL	SEM	BL	CL	SL	SEM	G	L	G×L	
C14:0	1.11	1.25 ^{1,2}	1.11 ^{ab}	1.19 ^b	0.033	1.11 ^{ab}	1.19 ^b	0.94 ^a	0.043	1.18 ^a	1.33 ^b	1.14 ^a	0.030	1.18 ^a	1.33 ^b	1.14 ^a	0.030	1.18 ^a	1.33 ^b	1.14 ^a	0.030	*	*	ns	
C15:0	0.17	0.26 ²	0.07	0.00 ¹	0.071	0.07	0.01 ¹	0.015	0.015	0.01	0.02 ¹	0.004	0.015	0.01	0.02 ¹	0.004	0.015	0.01	0.02 ¹	0.004	ns	***	ns		
C16:0	22.87	23.28	23.03	22.72	0.203	23.03	22.72	22.83	0.209	23.74	23.65	23.82	0.196	23.74	23.65	23.82	0.196	23.74	23.65	23.82	0.196	ns	ns	ns	
C16:1 n7	3.64 ²	5.21 ³	2.68 ¹	2.58 ^b	0.189	2.68 ¹	2.58 ^b	2.03 ¹	0.103	2.98 ^{1,2}	3.56 ^b	2.77 ²	0.109	2.98 ^{1,2}	3.56 ^b	2.77 ²	0.109	2.98 ^{1,2}	3.56 ^b	2.77 ²	0.109	***	***	ns	
C17:0	0.43	0.48	0.48	0.47	0.032	0.48	0.47	0.48	0.017	0.44	0.51	0.51	0.021	0.44	0.51	0.51	0.021	0.44	0.51	0.51	0.021	ns	ns	ns	
C17:1	0.22 ¹	0.24 ^a	0.33 ²	0.33	0.044	0.33 ²	0.33	0.33	0.023	0.36 ²	0.31	0.31	0.019	0.36 ²	0.31	0.31	0.019	0.36 ²	0.31	0.31	0.019	ns	**	ns	
C18:0	11.07 ¹	10.12 ¹	12.59 ²	12.42 ³	0.220	12.59 ²	12.42 ³	13.96 ²	0.252	12.06 ^{ab,12}	11.23 ^a	12.95 ²	0.249	12.06 ^{ab,12}	11.23 ^a	12.95 ²	0.249	12.06 ^{ab,12}	11.23 ^a	12.95 ²	0.249	***	***	ns	
C18:1 c n9	50.03 ²	51.01 ²	47.91 ¹	46.75 ^{ab}	0.428	47.91 ¹	46.75 ^{ab}	45.50 ^a	0.427	48.17 ^{1,2}	47.07 ¹	45.87	0.477	48.17 ^{1,2}	47.07 ¹	45.87	0.477	48.17 ^{1,2}	47.07 ¹	45.87	0.477	***	***	ns	
C18:2 n6	6.48 ¹	5.52 ¹	9.03 ²	9.76 ²	0.391	9.03 ²	9.76 ²	11.32 ^c	0.199	8.71 ²	9.08 ²	10.42 ²	0.189	8.71 ²	9.08 ²	10.42 ²	0.189	8.71 ²	9.08 ²	10.42 ²	0.189	***	***	ns	
C20:0	0.26 ²	0.21	0.16 ¹	0.22 ^a	0.014	0.16 ¹	0.22 ^a	0.33 ^b	0.015	0.16 ¹	0.21 ^b	0.27 ^{1,2}	0.017	0.16 ¹	0.21 ^b	0.27 ^{1,2}	0.017	0.16 ¹	0.21 ^b	0.27 ^{1,2}	0.017	*	ns	ns	
C20:1 n9	0.70 ¹	0.71 ¹	0.95 ²	1.01 ³	0.032	0.95 ²	1.01 ³	1.06 ²	0.026	0.80 ^{1,2}	0.79 ²	0.94 ^{1,2}	0.043	0.80 ^{1,2}	0.79 ²	0.94 ^{1,2}	0.043	0.80 ^{1,2}	0.79 ²	0.94 ^{1,2}	0.043	*	***	ns	
C18:3 n3	0.53	0.53 ¹	0.72	0.862	0.047	0.72	0.862	0.75	0.044	0.73 ^a	0.95 ^b	0.94 ^b	0.042	0.73 ^a	0.95 ^b	0.94 ^b	0.042	0.73 ^a	0.95 ^b	0.94 ^b	0.042	*	***	ns	
C20:2 n6	0.28 ¹	0.20 ¹	0.45 ²	0.51 ³	0.015	0.45 ²	0.51 ³	0.61 ²	0.022	0.38 ^{1,2}	0.37 ²	0.40 ¹	0.012	0.38 ^{1,2}	0.37 ²	0.40 ¹	0.012	0.38 ^{1,2}	0.37 ²	0.40 ¹	0.012	***	***	*	
C20:4 n6	0.75 ²	0.42 ²	0.15 ¹	0.12 ¹	0.091	0.15 ¹	0.12 ¹	0.14 ¹	0.005	0.20 ¹	0.18 ¹	0.18 ¹	0.008	0.20 ¹	0.18 ¹	0.18 ¹	0.008	0.20 ¹	0.18 ¹	0.18 ¹	0.008	**	***	**	
SFA	35.91	35.63	37.42	37.07	0.336	37.42	37.07	38.29	0.402	37.54	36.95	38.23	0.390	37.54	36.95	38.23	0.390	37.54	36.95	38.23	0.390	ns	ns	ns	
MUFA	54.66 ²	57.26 ²	51.62 ¹	50.73 ^{ab}	0.529	51.62 ¹	50.73 ^{ab}	48.85 ^a	0.475	52.32 ^{1,2}	51.80 ¹	49.83 ¹	0.508	52.32 ^{1,2}	51.80 ¹	49.83 ¹	0.508	52.32 ^{1,2}	51.80 ¹	49.83 ¹	0.508	***	***	ns	
PUFA	7.99 ¹	6.89 ¹	10.54 ²	11.63 ^b	0.443	10.54 ²	11.63 ^b	12.78 ^c	0.219	10.15 ²	10.94 ^a	11.83 ^b	0.198	10.15 ²	10.94 ^a	11.83 ^b	0.198	10.15 ²	10.94 ^a	11.83 ^b	0.198	***	***	ns	
Σn3	0.60 ¹	0.59 ¹	0.84 ²	1.04 ²	0.049	0.84 ²	1.04 ²	0.81	0.050	0.82 ²	1.09 ^b	1.00 ^{ab}	0.043	0.82 ²	1.09 ^b	1.00 ^{ab}	0.043	0.82 ²	1.09 ^b	1.00 ^{ab}	0.043	*	***	ns	
Σn6	7.31 ¹	6.21 ¹	9.65 ²	10.54 ²	0.415	9.65 ²	10.54 ²	11.95 ^c	0.207	9.31 ²	9.79 ²	10.82 ²	0.184	9.31 ²	9.79 ²	10.82 ²	0.184	9.31 ²	9.79 ²	10.82 ²	0.184	***	***	ns	

BL: Barcina line; CL: Carballiña line; SL: Santiaguense line; SFA: sum of saturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; SEM: standard error of the mean; G: significantly different values as influenced by genotype *(P<0.05); ***(P<0.001); ns: no significant difference; L: significantly different values as influenced by location *(P<0.05); ***(P<0.001); ns: no significant difference; G×L: interaction of genotype and location *(P<0.05); ***(P<0.001); ns: no significant difference; ¹⁻³Means within the same row not followed by the same number differ significantly (influence of location) (P<0.05); ^{a-c}Means within the same row not followed by the same letter differ significantly (influence of genotype) (P<0.05).

TABLE 5. Fatty acid composition (% of total fatty acids) of polar lipids in *longissimus dorsi* and dorsal and ventral subcutaneous fat

	<i>Longissimus dorsi</i>												Significance					
	Dorsal fat						Ventral fat						G	L	G×L			
	BL	CL	SL	SEM	BL	CL	SL	SEM	BL	CL	SL	SEM						
C14:0	nd	nd	nd	1.004	1.66 _b ²	1.54 _b ²	0.28 _a ¹	0.208	1.47 ²	1.36 ²	1.72 ²	0.197	ns	ns	ns			
C16:0	21.52 ¹	22.17	22.25 ¹	1.004	26.04 _b ²	24.32 _{ab} ²	22.90 _a ¹	0.504	24.32 ¹²	24.22	25.52 ²	0.467	ns	ns	ns			
C16:1 n7	0.99 ¹	1.17 ¹	1.47 ¹	0.095	1.95 ²	2.74 ²	2.55 ²	0.182	2.77 ²	3.04 ²	2.40 ₂	0.146	ns	**	ns			
C18:0	13.74 ¹	13.38 ¹	13.23 ¹	0.313	16.83 ²	16.03 ²	16.36 ²	0.459	16.79 _{ab} ²	14.63 _a ¹²	17.92 _c ²	0.526	ns	ns	ns			
C18:1 c n9	20.18 _b ¹	16.12 _a ¹	19.66 _b ¹	1.116	40.58 ²	42.27 ²	41.84 ²	0.668	41.78 ²	41.70 ²	40.76 ²	0.727	ns	***	ns			
C18:2 n6	23.94 _a ²	30.93 _b ²	23.99 _a ²	0.875	11.19 _{ab} ¹	11.03 _a ¹	12.82 _b ¹	0.354	11.09 ¹	12.24 ¹	10.91 ¹	0.36	ns	***	ns			
C18:3 n3	1.12 ¹	1.02	1.32 ¹	0.152	2.58 ²	1.62	2.47 ²	0.213	2.28 ¹²	1.51	1.82 ¹²	0.179	ns	ns	ns			
C20:4 n6	13.89 _{b2}	12.36 _{ab} ²	11.62 _a ²	0.592	1.86 _{ab} ¹	1.16 _a ¹	2.58 _b ¹	0.265	1.981	1.26 ¹	1.13 ¹	0.176	ns	***	ns			
SFA	38.39 ¹	39.78	36.36 ¹	1.468	43.62 _b ²	40.51 _{ab} ²	39.54 _a ¹	0.750	41.99 _{ab} ²	39.97 _a ²	44.13 _b ²	0.587	ns	ns	ns			
MUFA	22.23 ¹	18.30 ¹	22.06 ¹	1.199	43.19 ²	45.82 ²	44.01 ²	0.698	44.80 ²	44.81 ²	43.09 ²	0.604	ns	***	ns			
PUFA	41.53 ²	44.28 ²	37.22 ³	1.967	13.12 _a ¹	13.55 _a ¹	17.28 _b ²	0.586	13.21 ¹	14.73 ¹	12.66 ¹	0.479	ns	***	ns			
Σn3	2.28	1.98	2.75 ²	0.308	1.26	2.22	1.67 ¹	0.277	2.28	1.85	1.82 ¹	0.176	ns	*	ns			
Σn6	35.18 ²	37.44 ²	33.81 ²	2.559	11.86 _a ¹	11.77 _a ¹	14.58 _b ¹	0.437	12.07 ¹	12.22 ¹	11.63 ¹	0.505	ns	***	ns			

BL: Barcina line; CL: Carballiña line; SL: Santiaguesa line; SFA: sum of saturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; SEM: standard error of the mean; G: significantly different values as influenced by genotype *(P<0.05); ***(P<0.001); ns: no significant difference; L: significantly different values as influenced by location *(P<0.05); ***(P<0.001); ns: no significant difference; G×L: interaction of genotype and location *(P<0.05); ***(P<0.001); ns: no significant difference; ¹⁻³Means within the same row not followed by the same number differ significantly (influence of location) (P<0.05); ^{a-b}Means within the same row not followed by the same letter differ significantly (influence of genotype) (P<0.05); nd: not detected.

In the polar lipid fraction, there was a different behavior between intramuscular and subcutaneous fat. The IMF from the Santiaguesa line showed the lowest values ($P > 0.05$) of PUFA (37.2%). Regarding ventral fat, there were observed significant ($P < 0.05$) differences among the three genotypes in SFA contents since the highest values were obtained for the Santiaguesa line and this fact could be related to the greater amount of C18:0 ($r = 0.498$; $P < 0.01$). On the other hand, in the dorsal fat location, the C18:0 content did not show significant ($P > 0.05$) differences among genotypes, although the percentage of C16:0 and SFA contents presented significant ($P < 0.05$) differences, since the lowest values were observed in the Santiaguesa line. In this location, the Santiaguesa genotype showed the highest values of PUFA, which also occurred in the neutral and total lipids, mainly due to the higher values of C18:2n6 and C20:4n6. In this case, there were no significant interactions between the effects of genotype and location.

4. DISCUSSION

4.1. Effect of location

The fatty acid compositions found in this study are consistent with those previously reported for the Celta pig breed (Franco *et al.*, 2006; Lorenzo *et al.*, 2012) and other rustic pig breeds such as Iberian (Daza *et al.*, 2008), Chato Murciano (Galián *et al.*, 2008; Peinado *et al.*, 2009) and Cinta Senese (Franci *et al.*, 2005; Pugliese *et al.*, 2005).

On the other hand, the higher content of triglycerides than phospholipids in the IMF and SF locations (Estévez *et al.*, 2003) causes the fatty acid profile of the neutral lipid fraction to be very similar to that found in the total lipid fraction.

The higher content of MUFA and lower PUFA in IMF than in SF were previously described in the literature (Raj *et al.*, 2010; Bosch *et al.*, 2012; Lorenzo *et al.*, 2012).

Sellier (1998) and Estévez *et al.* (2003) described how the amount of fat influences the fatty acid composition of different fat deposits. These authors found that carcass leanness is inversely related to the proportion of SFA and MUFA in SF and IMF, whereas it is positively related to the content of most PUFA. Thus, an increase in PUFA content has been reported as fat deposition decreased (Raj *et al.*, 2010; Bosch *et al.*, 2012). In our study, a negative correlation was observed between IMF and PUFA content ($r = -0.541$; $P < 0.01$), as well as a positive correlation between IMF content and SFA ($r = 0.594$; $P < 0.01$) in the intramuscular location and a negative correlation between backfat thickness and PUFA content ($r = -0.351$; $P = 0.021$) in the dorsal fat.

The differences found in the fatty acid composition in the different adipose tissues could be due to specific development and metabolism (Monziols

et al., 2007). It is well known that muscles with an oxidative metabolism have a higher proportion of polar lipids, which are rich in PUFA and, therefore, have higher contents of PUFA than muscles with a glycolytic metabolism (Muriel *et al.*, 2004). On the other hand, Monziols *et al.* (2007) noticed that the different fatty acid composition of adipose tissue could be due to an adipose tissue adaptation to the temperature in order to maintain the physical fluidity of lipids in the different deposits. C18:1c-n9, the most abundant fatty acid in neutral and total lipids, is synthesized by $\Delta 9$ -desaturase. Monziols *et al.* (2007) and Cánovas *et al.* (2009) explained that the activity of stearoyl Co-A desaturase and acetyl Co-A carboxylase varies depending on the location, which would explain the differences found in the present study for the content of MUFA.

Regarding nutritional indices, our P/S ratio was lower than the limit set by international institutions (Department of Health, 1994) and those reported by other authors (Cava *et al.*, 2003; Raj *et al.*, 2010). On the other hand, the $\Sigma n6/\Sigma n3$ ratio was similar to that described by several authors for Iberian pigs (Estévez *et al.*, 2003; Muriel *et al.*, 2004; Juárez *et al.*, 2009). Finally, the AI and TI indices were slightly higher than those reported by Franci *et al.*, (2005) who found values ranging from 0.46 to 0.47 and from 1.09 to 1.16 for AI and TI indices, respectively.

The fatty acid compositions of the polar lipids found in this study are in agreement with those described for different lines of Iberian pigs (Estévez *et al.*, 2003; Cava *et al.*, 2004). The higher PUFA content of IMF would be related to the muscle, being rich in cell membranes with a higher proportion of phospholipids, which are rich in PUFA (Alasnier *et al.*, 1996), while SF has a higher proportion of triglycerides. This may explain the differences in the contents of PUFA, SFA and MUFA from the polar lipid fraction among the three locations.

4.2. Effect of genotype

The effect of genotype on live weight have been previously described by Franco *et al.* (2011) who noticed a higher growth rate in the Carballiña than in the Santiaguesa line.

Regarding IMF content it is well known that the occurrence of intramuscular fat deposition has a strong genetic component (Gispert *et al.*, 1997; Suzuki *et al.*, 2009). In this case, the three genotypes of Celta pig showed a markedly different adipogenic capacity, since the Barcina line had more IMF compared to the other ones. This implies that the deposition of IMF and SF (total lipids) may be regulated by different mechanisms among the three lines. The activity of enzymes responsible for fatty acid metabolism and fat deposition depends on the genetic component. Ntawubizi *et al.* (2009) reported that the desaturase and elongase activity in muscles was

significantly ($P < 0.05$) related to the IMF content. Moreover, differences in fat infiltration have been reported to be mainly due to genetic influence (Poto *et al.*, 2007; Juárez *et al.*, 2009). Each 1% genetic increase in lean content may reduce IMF by around 0.07% (Webb, 1998).

It is well known that traditional breeds are fatter than industrial ones. They have more adipose tissues thickness and more intramuscular fat (Tejeda *et al.*, 2002; Gandemer, 2009). The differences in IMF are mainly due to higher triglyceride accumulation while the polar lipid fraction is similar between traditional and industrial genotypes of pigs. According to Gandemer (2009) this fact is due to the fact that traditional pigs have a low capacity to deposit muscles in carcass and deposit a large amount of the feed energy as fat in both adipose and intramuscular depots.

The differences in nutritional indices among the three lines could be due to different amounts of SFA, which were lower in the Santiaguesa genotype. In fact, the Pearson correlation test indicated that SFA were significantly ($P < 0.01$) related to the IA ($r = 0.803$), IT ($r = 0.961$) and h/H ratio ($r = -0.833$).

There is controversy about the effect that genotype has on the fatty acid composition. Gandemer and Viau (1992) and Tejeda *et al.* (2002) have reported that genotype slightly affected the triglycerides and fatty acid composition, while Ventanas *et al.* (2006) have reported significant differences in fatty acids between purebred and crossbred, even for animals fed on the same diet. In agreement with this, Vieira-Alcaide *et al.* (2008) concluded that the triglyceride composition of the subcutaneous fat of Iberian pigs is affected by genotype. These authors suggested that the different activities of stearoyl CoA desaturase between genotypes are the main cause of the differences in the fatty acid and triglyceride contents.

It is well known that *de novo* synthesis causes an increase in the content of SFA and/or MUFA, while it decreases the values of the PUFA (Warnants *et al.*, 1999; Kloareg *et al.*, 2005; Gandemer, 2009). Fatty acids from *de novo* synthesis are deposited mainly in the neutral lipid fraction, thus the dilution effect is more intense in this fraction than in polar lipids (Muriel *et al.*, 2004). In our case, we observed a strong dilution of the PUFA content in total and neutral lipid fractions as the result of increased fat content from the IMF and SF locations, however this effect was not observed on polar lipids. These results are in agreement with those reported by Gandemer (2009), who found that genotype slightly affected the fatty acid composition from polar lipids.

Some experiments focused on the relationship between fatty acid composition and genotypes demonstrate that this parameter has a low effect when animals are compared at the same degree of fatness (Gandemer, 2009). Therefore, differences in fatty

acid composition are mainly explained by differences in the fatness of the carcasses and the muscles. However, according to Gandemer and Viau (1992) any variation in the relative proportions of endogenous and dietary lipids stored in the muscle or in the elongation and desaturation abilities of fatty acids according to genotype would lead to differences in the fatty acid composition.

Thus, our results suggest that differences in lipid content may be due, as mentioned above, to the different enzyme activity and the differences in fatness, and differences in fatty acid profile could be a consequence of differences in *de novo* synthesis and turnover between the three lines of Celta pig breed, in agreement with values reported by Franci *et al.* (2005).

5. CONCLUSIONS

There were very evident differences in IMF content and fatty acid profile among the three Celta pig lines. The differences may be due to a genetic component that regulates the metabolism of fatty acids and the amount of fat deposited in the animal tissues. The effect of genotype on fatness was mainly due to differences in the proportion of triglycerides. In fact, the fatty acid composition from polar lipids was hardly affected by genotype. Further studies are needed to clarify the mechanisms that differentiate fat deposition and fatty acid metabolism among the Celta pig lines.

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