FATTY ACID COMPOSITION OF DIFFERENT ADIPOSE TISSUES IN HEAVY PIGS

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ABSTRACT

Forty-seven castrated male Duroc x (Landrace x Large White) pigs were used to determine fatty acids compositions from different adipose tissues. The outer subcutaneous backfat layer had a lower proportion of saturated and higher monounsaturated and polyunsaturated fatty acids than the inner layer. Liver fat had the highest proportion of polyunsaturated fatty acids. Intramuscular fat followed by subcutaneous backfat had the highest monounsaturation indexes. Moreover, omental and hepatic fat had the highest amount of n-3 fatty acids. In conclusion, the fatty acid profile was depended on fat location, with intramuscular and outer backfat the most beneficial from the point of view of nutrition and health.

1. INTRODUCTION

The fatty acid profile of adipose tissue in pigs is especially important since it relates to quality aspects in the process of preparing dry-cured products such as consistency, lipid oxidation, salt and water migration, curing duration, etc. (LOPEZ-BOTE, 2000; LOPEZ BOTE *et al.,* 2004) and the production of volatile compounds that are responsible for odor and flavor (BELITZ and GROSCH, 1997). Moreover, much attention is currently being paid to the fatty acid profile in animal products because of its association with consumer health (WOOD and ENSER, 1997; FAO, 2012). Hence a correlation has been found between saturated fatty acid consumption and cardiovascular diseases (FAO, 2012), whereas polyunsaturated fatty acids (mainly n-3 fatty acids) have been found to improve the status of the cardiovascular system and the immune response (WOOD and ENSER, 1997). However, high proportions of n-3 fatty acids in tissues reduce oxidative stability of meat (REY *et al.,* 2001).

In pigs, fatty acid composition of fat tissue is influenced by a wide range of factors such as genetics, sex, weight and age at slaughter, livestock production system, feed, environmental conditions, pre-slaughter management, etc. (MOUROT and HERMIER 2001; DE SMET *et al.,* 2004; LOPEZ-BOTE *et al.,* 2004). The effect of adipose tissue location on fatty acid profile has recently been studied in the Celta pig breed, which is a rustic breed from Spain (DOMINGUEZ *et al.* 2014; DOMINGUEZ & LORENZO 2014); however, there is a lack of informations in pigs of improved genotypes at heavy weights. Therefore, given the importance of the topic, the aim of this study was to investigate the fatty acid profile of fat from different locations (hepatic, intramuscular and omental) in heavy pigs.

2. MATERIALS AND METHODS

2.1 Animals, diet and sampling

All the experimental procedures used in the study were in compliance with the Spanish guidelines for the care and use of animals in research (BOLETÍN OFICIAL DEL ESTADO, 2007).

Forty-seven castrated male Duroc x (Landrace x Large White) pigs were used. All the animals received the same diet during the finishing period and before the beginning of the experiment all pigs were subjected to the same management and feeding conditions. The feedstuff consisted of a commercial diet based on barley, wheat and various vegetable protein sources (soybean, rapeseed, sunflower) that was administered *ad libitum*. The calculated energy (FEDNA, 2010) and composition (AOAC, 2000) of the diet are shown in Table 1. The pigs were slaughtered at 126±2.8 kg of live weight. On each carcass, fat thickness was measured by means of a graduated rule at the level of the *Gluteus medius* muscle. Individual samples of inner and outer subcutaneous backfat layers and *Longissimus dorsi* muscle at the level of the last rib from each left loin, and omental and hepatic fat samples were taken. Samples were vacuum-packaged in individual bags and stored at -20ºC for 3 weeks until subsequent analysis.

2.2 Fatty acid analysis of diet and fat

Fatty acids of the diet were extracted and quantified using the one-step procedure described by SUKHIJA and PALMQUIST (1988) in lyophilized samples. Pentadecenoic acid (C15:1) (Sigma, Alcobendas, Madrid, Spain) was used as internal standard. Previously, methylated fatty acids samples were identified according to REY *et al.* (1997)

using a gas chromatograph (Model HP6890; Hewlett Packard Co., Avondale, PA, USA) and a 30 m x 0.32 mm x 0.25 μm cross-linked polyethylene glycol capillary column (Hewlett Packard Innowax). A temperature program of 170ºC to 245ºC was used. The injector and detector were maintained at 250 ºC. The carrier gas (helium) flow rate was 3 ml/min.

Lipids from subcutaneous and omental fat were extracted using the procedure proposed by BLIGH and DYER (1959), whereas lipids from *Longissimus dorsi* and liver fat samples were extracted according to method developed by MARMER and MAXWELL (1981). Fat extracts were methylated in the presence of sulphuric acid and analysed as described above. Fatty acid percentages of the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were calculated. The indexes used to estimate thioesterase (Zhang et al., 2007) and elongase enzyme activity were C16:0/C14:0 and C18:0/C16:0 respectively. The Δ-9 desaturase activity was estimated by means of the following ratios: C16:1n-7/C16:0, C18:1n-9/C18:0 and MUFA/SFA.

Table 1. Calculated and determined analyses of the diet**.**

Calculated analysis	g/kg
Digestible energy (MJ/kg)	13.62
Determined analysis [1]	886.2
Crude protein (N x 6.25)	145.2
Crude fat	56.2
Crude fibre	35.1
Fatty acids (%)	
C14:0	0.29
C16:0	4.71
C18:0	1.61
C18:1 n-9	5.68
C18:2 n-6	16.23
C18:3 n-3	3.49

The ingredients composition of the diet in g/kg was: barley 430, wheat 301, Full fat soybean toasted 65.2, Rapeseed meal 84.0, Sunflower meal 54.2, Fat (lard) 31.0, Calcium carbonate 11.0, Sepiolite, 10.0, L - lysine 50% 5.5, Sodium chloride 4.1, Vitamin and mineral premix 4.0.
[1]According to Association of Official Analytical Chemists (2000).

2.3 Statistical analysis

The pig was the experimental unit for statistical analysis. Data were analyzed by means of variance analysis that included the adipose tissue location as the main effect. The Newman-Keuls test was used to assess differences between means. The relations between C18:2n-6 and C16:0 proportions in subcutaneous backfat layers were calculated by simple linear regression, comparing intercepts and slopes using "t" Student test. Correlation coefficients between *Gluteus medius* fat thickness and major fatty acid proportions from subcutaneous backfat, and correlation coefficients between intramuscular fat percentage from *Longissimus dorsi* and major fatty acid proportions of such tissue were calculated. All analyses were carried out using the GLM and CORR procedures of SAS (1999).

3. RESULTS

The omental fat had the highest proportion of SFA followed by the liver fat due to the high C18:0 proportion (Table 2). The outer subcutaneous backfat layer showed a lower saturation and higher mono and polyunsaturation than the inner layer. The C16:0 and C18:0 proportions were lower (P<0.0001) in the outer subcutaneous backfat layer than in the inner layer, whereas C16:1n-7, C18:1n-9, C18:1n-7, C18:2n-6 and C18:3n-3 were higher (P<0.0001) (Table 2).

Table 2. Major fatty acid composition (%) of different adipose tissues from heavy pigs.

Tissue	SFOL	SFIL	LDIF	OF	HF	sem	P <
n	47	47	47	47	46		
C16:0	23.78[c]	24.84[b]	24.43[b]	27.38[a]	21.97[d]	0.22	0.0001
C16:1 n-7	1.86[c]	1.67[d]	3.49[a]	1.58[d]	2.43[b]	0.061	0.0001
C18:0	14.56[d]	16.74[c]	13.29[e]	20.37[b]	23.18[a]	0.45	0.0001
C18:1 n-9	41.84[ab]	40.53[b]	43.05[a]	35.72[c]	30.72[d]	0.48	0.0001
C18:1 n-7	2.41[b]	2.16[c]	3.54[a]	1.50[d]	-	0.036	0.0001
C18:2 n-6	10.56[a]	9.36[b]	6.70[c]	8.98[b]	11.16[a]	0.24	0.0001
C18:3 n-3	0.79[a]	0.70[b]	0.044[d]	0.72[b]	0.44[c]	0.020	0.0001
C20:4 n-6	0.63[b]	0.58[b]	0.14[b]	0.42[b]	5.96[a]	0.18	0.0001
SFA	40.06[d]	43.26[c]	39.64[d]	49.66[a]	47.03[b]	0.37	0.0001
MUFA	47.81[b]	45.96[c]	51.57[a]	40.07[d]	34.14[e]	0.050	0.0001
PUFA	12.13[b]	10.77[c]	8.77[d]	10.27[c]	18.83[a]	0.47	0.0001
MUFA+PUFA	59.94[a]	56.73[b]	60.34[a]	50.34[d]	52.97[c]	0.47	0.0001
n-6	11.19[b]	9.93[c]	6.84[d]	9.41[c]	16.84[a]	0.54	0.0001
n-3	0.93[b]	0.83[b]	0.37[c]	0.86[b]	1.74[a]	0.42	0.0001

n = number of observations, SFOL= subcutaneous backfat outer layer, SFIL = subcutaneous backfat inner layer, LDIF = *Longissimus dorsi* intramuscular fat, OF = omental fat, HF = hepatic fat. sem = error standard of the mean. SFA, MUFA, PUFA, n-6, n-3 = sum of all saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-6 and n-3 fatty acids.

In the current experiment, the relations obtained between C18:2n-6 and C16:0 proportions in the outer and inner subcutaneous backfat layers were described by the following simple regression equations:

Outer layer: C18:2n-6 (%) = (28.40[a]±2.58) – (0.750[b]±0.11) C16:0 (%)
R^2 = 0.53, RSD = 0.98, P <0.0001 n = 47.

Inner layer: C18:2n-6 (%) = (29.76[a]±2.48) – (0.823[a]±0.10) C16:0 (%)
R^2 = 0.62, RSD = 0.88, P <0.0001, n = 47.

Table 2 also shows that C16:1n-7, C18:1n-9, C18:1n-7 and MUFA proportions were higher in the intramuscular fat of *Longissimus dorsi* muscle than in the other adipose tissues (P<0.0001).

The omental fat had lower monounsaturated and polyunsaturated fatty acids than subcutaneous backfat. The 18:2n-6, C20:4n-6, n-6, n-3 and PUFA proportions were higher in hepatic fat than in the other adipose tissues.

In this study, the highest unsaturation ranges (sum of MUFA and PUFA fatty acids) were obtained in intramuscular fat from *Longissimus dorsi* muscle and outer subcutaneous backfat layer followed by inner subcutaneous backfat layer, liver fat and omental fat.

The enzymatic activity indexes of different adipose tissues are shown in Table 3. The thioesterase index (C16:0/C14:0), that indicates catalysis of C16:0 synthesis from C14:0, and the elongase index, as an indicator of C18:0 synthesis from C16:0 (C18:0/C16:0), were higher ($p < 0.0001$) in liver fat than in the other adipose tissues. The C16:0/C14:0 and C18:0/C16:0 indexes in omental fat were higher than those in longissimus dorsi intramuscular fat ($p < 0.0001$). However, C16:0/C14:0 indexes were of similar magnitude in omental fat and inner backfat layer. Both indexes were higher in subcutaneous backfat than in intramuscular fat. The highest monounsaturation indexes C18:1 n-9/C18:0 and MUFA/SFA were found in intramuscular fat followed by outer the subcutaneous backfat layer, inner subcutaneous backfat layer, omental fat and liver fat. However, the C16:1 n-7/C16:0 index was higher in hepatic fat than in subcutaneous and omental fat.

The highest PUFA/SFA ratios were found in hepatic fat and the outer subcutaneous backfat layer and the lowest in intramuscular and omental fat. The lowest n-6/n-3 ratios were found in hepatic and omental fat and the highest in intramuscular fat from *Longissimus dorsi* muscle. The highest MUFA/SFA ratio was observed in intramuscular fat. The subcutaneous backfat thickness at the level of ham *Gluteus medius* muscle was 23.5±5.93 mm. This variable was positively correlated with C16:0 and SFA and negatively correlated with C18:1n-9, MUFA, C18:2n-6 and PUFA proportions of the outer and inner subcutaneous backfat layers (Table 4). The intramuscular fat percentage from *Longissimus dorsi* muscle was 4.02±0.90%. This variable was positively correlated with C16:0 ($r = 0.47$ $p < 0.001$) and SFA ($r = 0.30$ $p < 0.01$), and negatively correlated with C18:2n-6 ($r = -0.38$, $p < 0.01$) and PUFA ($r = -0.39$ $p < 0.01$) proportions found in *Longissimus dorsi* intramuscular fat. However, correlations were not found between *Longissimus dorsi* intramuscular fat and C18:1n-9 and MUFA proportions.

Table 3. Saturation and monounsaturation and quality index of different tissues from heavy pigs.

Tissue	SFOL	SFIL	LDIF	OF	HF	sem	P <
n	47	47	47	47	46		
C16:0/C14:0	20.15[c]	22.04[b]	16.59[d]	21.38[bc]	27.26[a]	0.57	0.0001
C18:0/C16:0	0.61[c]	0.68[bc]	0.54[d]	0.74[b]	1.08[a]	0.025	0.0001
C16:1 n-7/C16:0	0.078[c]	0.067[d]	0.14[a]	0.057[e]	0.11[b]	0.0024	0.0001
C18:1n-/C18:0	2.92[b]	2.41[c]	3.28[a]	1.77[d]	1.51[e]	0.072	0.0001
MUFA/SFA	1.20[b]	1.07[c]	1.31[a]	0.81[d]	0.75[e]	0.019	0.0001
PUFA/SFA	0.31[b]	0.25[c]	0.22[cd]	0.21[d]	0.41[a]	0.011	0.0001
n-6/n-3	12.52[b]	12.03[bc]	19.08[a]	11.00[cd]	10.32[d]	0.44	0.0001

n = number of observations, SFOL= subcutaneous backfat outer layer, SFIL = subcutaneous backfat inner layer, LDIF = *Longissimus dorsi* intramuscular fat, OF = omental fat, HF = hepatic fat. sem = error standard of the mean. SFA, MUFA, PUFA, n-6, n-3 = sum of all saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-6 and n-3 fatty acids.

Table 4. Correlation coefficients (r) between fat thickness (mm), at the level of *Gluteus medius* muscle, and major fatty acid proportions (%) in outer and inner backfat layers.

Fatty acid (outer layer)	r	Fatty acid (inner layer)	r
C16:0	0.64****	C16:0	0.55***
SFA	0.61****	SFA	0.58***
C18:1 n-9	- 0.41**	C18:1 n-9	- 0.40**
MUFA	- 0.44**	MUFA	- 0.45**
C18:2 n-6	- 0.53***	C18:2 n-6	-0.52***
PUFA	- 0.54***	PUFA	-0.52***

n = 47, ** p<0.01, ***p<0.001, ****p<0.0001. SFA, MUFA, PUFA = sum of all saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

4. DISCUSSIONS

The fact that omental and liver fat were more saturated than subcutaneous and intramuscular fat, is explained by the activity of lipogenic enzymes involved in *de novo* synthesis, which vary with tissue location (NARVAEZ-RIVAS *et al.*, 2009). Thus, in our experiment, the highest elongase index values (C18:0/C16:0) were detected in liver and omental fat whereas the thioesterase index (C16:0/C14:0) was higher in the liver, omental and inner subcutaneous backfat layer compared to the other fatty tissues. These results are in agreement with DOMINGUEZ *et al.* (2014), who observed in the Celta breed pigs higher values of elongase and thioesterase indexes in internal perirenal fat than in subcutaneous and intramuscular fat. According to BEE (2001), lipogenic activity of malic enzyme was higher in omental fat than inner and outer subcutaneous backfat layers, but fatty acid synthase activity was similar in these tissues, for different dietary fat types. On the other hand, the activities of both enzymes were similar in the inner and outer subcutaneous backfat layers when pigs received the same energy and fat content in feed as in this experiment. Moreover, BENITEZ *et al.* (2012) found in Iberian pigs fed saturated (5% hydrogenated lard), monounsaturated (5% high oleic sunflower) or polyunsaturated (5% sunflower) enriched diets that liver fat was more saturated than inner and outer subcutaneous backfat layers and *Longissimus dorsi* intramuscular fat. In our study, the outer subcutaneous backfat layer was more monounsaturated and polyunsaturated and less saturated than the inner layer, which is consistent with the results observed by IRIE and SAKIMOTO (1992), BEE *et al.* (2002), DAZA *et al.* (2007) and MONZIOLS *et al.* (2007). In a later study, BEE *et al.*, (2002) observed that the activity of the different lipogenic enzymes was not uniform in the outer and inner subcutaneous backfat layers. Hence, these authors reported that the activity of malic enzyme was lower in the inner than outer subcutaneous backfat layer, the activity of glucose 6-phosphate dehydrogenase was similar in both layers, whereas the fatty acid synthase enzyme activity was higher in the inner than in the outer layer.

The metabolic principles, on which the deposition of linoleic acid is higher in the outer than inner subcutaneous backfat layers in pigs, have not been sufficiently explained (MONZIOLS *et al.*, 2007). The higher *de novo* synthesis of SFA (especially C16:0) in the inner subcutaneous backfat layer resulted in a higher dilution of C18:2n-6 in the inner than outer subcutaneous backfat layer, in agreement with the results of the regression equations obtained between the proportions of C18:2n-6 and C16:0. The slope of the regression equation corresponding to the inner subcutaneous backfat layer was higher

than in the outer layer (0.823 *vs* 0.750, p<0.01). This means that as the C16:0 proportion increased in subcutaneous backfat, the decrease in the proportion of C18:2n-6 was higher in the inner than in the outer layer.

These results are in agreement with those obtained by CHRISTIE *et al.* (1972) and MONZIOLS *et al.* (2007). According to the earlier study of THOMPSON and ALLEN (1968), the degree of unsaturation of the fatty tissue in swine decreases from external to internal tissues, so that the high-to-low unsaturation gradation follows the series: outer subcutaneous layer, middle subcutaneous layer, inner subcutaneous layer, intramuscular fat and internal fat (omental, perirenal, etc.) (VILLEGAS *et al.*, 1973; MONZIOLS *et al.*, 2007). DEAN and HILDITCH (1933) reported that this gradation can be explained by a possible adaptation of adipose tissue to temperature in order to maintain adequate physical fluidity of lipids in different adipose tissues, so that the melting point of the fat would increase from subcutaneous fat to internal locations.

In this study, the unsaturation of *Longissimus dorsi* intramuscular fat was similar to the outer subcutaneous backfat layer and higher than the internal and omental fat, due to high proportions of C16:1n-7, C18:1n-9 and C18:1n-7 in intramuscular fat. Unsaturation indexes such as C16:1n-7/C16:0, C18:1n-9/C18: 0 and MUFA/SAT, which estimate the activity of the enzyme delta-9 desaturase (responsible for the formation of C18:1n-9 from C18:0), were higher in the *Longissimus dorsi* intramuscular fat than subcutaneous backfat, omental and hepatic fat. Also, LOPEZ-BOTE *et al.* (2002), BEE (2001), BEE *et al.* (2002) and BEE *et al.* (2008) in improved white pigs, DAZA *et al.* (2014) in Iberian pigs and DOMINGUEZ *et al.* (2014) and DOMINGUEZ and LORENZO (2014) in Celta breed pigs found that intramuscular fat was more monounsaturated than outer and inner subcutaneous backfat and omental fat. However, LLUCH *et al.* (1993) found that the intramuscular fat was less monounsaturated than subcutaneous fat, although this result was obtained from male and female heterogeneous samples. FRANCO *et al.* (2006) did not detect significant differences between the ratio of MUFA/SFA in intramuscular fat and subcutaneous backfat in Celta breed pigs, but observed lower concentrations of C18:1n-9 in intramuscular fat than subcutaneous backfat. MONZIOLS *et al.* (2007) found higher proportions of C18:1n-9 and MUFA in subcutaneous backfat than intermuscular fat from the loin, but these authors did not provide data on fatty acid profile of intramuscular fat.

Omental fat showed lower (p <0.05) proportions of C18:1n-9 and MUFA fatty acids than outer and inner subcutaneous backfat layers and intramuscular fat of *Longissimus dorsi.* These results agree with those obtained by BEE (2001) and BEE *et al.* (2002). Liver fat was the most polyunsaturated because the fatty acid profile in liver is largely influenced by diet. Pig liver has little ability to synthesize fatty acids, hence fatty acids used in the synthesis of lipids are mainly derived from circulating fatty acids provided by adipose tissue or feed (mainly C18:2n-6). Most triglycerides synthesized in the liver are esterified or stored, so that the composition is a reflection of the composition of plasma fatty acids, mainly, from the feed consumed (OTTEN *et al.* 1993). Also, DOMINGUEZ *et al.* (2014) found in Celta breed pigs, that liver fat was the most polyunsaturated when compared with the dorsal and ventral subcutaneous fat and *Longissimus dorsi* intramuscular fat.

The nutritional value of adipose tissues is related to high values of PUFA/SAT, MUFA/SAT ratios and low values for n-6/n-3 ratio. PUFA/SAT and n-6/n-3 values of the present study were similar to those obtained by DOMINGUEZ and LORENZO (2014) and DOMINGUEZ *et al.* (2014) in Celta breed pigs**.** In turn, in agreement with the present study, DOMINGUEZ *et al.* (2014) observed the highest value of PUFA/SFA in liver fat and the lowest in *Longissimus dorsi* intramuscular fat. WOOD and ENSER (1997), recommended values for PUFA/SAT higher than 0.45 and n-6:n-3 lower than 4.0 in contrast to the results found in the present study. The high n-6/n-3 fatty acid ratio of the present study is explained by the fact that pigs were fed a concentrate rich in

carbohydrates and C18:2 n-6. According to the results of the present study, the highest n-3 fatty acid proportion was found in omental and hepatic fat. These tissues would have lower lipid stability than the others (REY *et al.* 2001) and this may lead to more production of undesirable substances for consumers than potential benefits. Regarding the curing process of meat and in order to obtain adequate fat quality, MOUROT and HERMIER (2001) recommended that pig adipose tissue should contain no more than 12% of C18:2n-6 and exceed 12% of C18:0. All adipose tissues studied in the present research were within those ranges.

In the present study, we found positive correlations between backfat thickness and C16:0 and SFA proportions, whereas correlations were negative between thickness and C18:1n-9, MUFA, C18:2n-6 and PUFA proportions. MONZIOLS *et al.* (2007) found positive and negative correlation coefficients among PUFA and SFA ratios and muscle percentage of carcass, which is negatively correlated with backfat thickness at *Gluteus medius* level (IRTA, 2013). According to MOUROT and HERMIER (2001), a reduction of backfat thickness in pigs leads to an increase in C18:2n-6 proportion and a decrease in endogenous synthesis. In the present experiment, saturated and polyunsaturated fatty acids proportions and *Longissimus dorsi* intramuscular fat percentages were positively and negatively correlated, respectively. These results explain that *de novo* synthesis of fatty acids is reduced in the leanest pigs and, consequently, C18:2n-6 is less diluted which is related to a higher content of C18:2n -6 in adipose tissue.

5. CONCLUSIONS

Fatty acid profile varies according to fat tissue location probably due to variability of enzyme activity. In heavy pigs fed conventional feed, the omental and liver fats are the most saturated followed by the inner, outer subcutaneous backfat and intramuscular fat. The highest unsaturation corresponded to intramuscular fat, due to increased activity of the delta-9 desaturase enzyme, followed by outer, inner subcutaneous backfat, omental and liver. Negative correlations were detected between the backfat thickness and the percentage of intramuscular fat with respect to the proportion of PUFA, which is important given the effect of PUFA content on susceptibility to oxidation. The fatty acid profile of adipose tissue from heavy pigs is suitable for the preparation of cured products, and intramuscular and outer backfat are the most suitable from the point of view of nutrition and health.

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