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Effect of increasing amounts of a linoleic-rich dietary fat on the fat composition of four pig breeds. Part III: Triacylglycerol composition in muscle and fat tissues

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Abstract

Here, we study the triacylglycerol (TAG) profile of four different tissues of the pig (backfat, abdominal fat, and muscles *trapezius* and *longissimus thoracis et lumborum*). For this purpose, 48 pigs of four breeds (Landrace, Large White, Duroc and a crossbreed Landrace × Duroc) were given one of four diets containing increasing amounts (0%, 2%, 4% and 8%) of a fat blend rich in linoleic acid. The effects of dietary fat and breed on TAG were tested separately for each tissue, and the results are presented using five TAG composition markers, PLL, PStO, PStL, PStSt and OOO. The increasing linoleic acid content provided by diets 1–4 showed a positive effect on the levels of TAG related to this dietary supply (here represented by PLL), and on those of PStL. This increase was at the expense of TAGs containing mainly fatty acids from *de novo* synthesis (represented by PStO) and in PStSt and OOO. A comparison of the relative % of change for the five selected TAG markers in the distinct tissues indicates that PLL and PStL show much higher increases in muscle than in adipose tissues, whereas PStO and PStSt show similar percentages of decrease in all tissues. OOO showed a higher % of decrease only in *trapezius*. Results indicate that the breed has a null or scarce effect on the levels of PLL and PStO. For the remaining TAG markers, Large White showed a higher synthesis of saturated TAG (PStSt and PStL) in fat tissues, but not in muscle. Large White also had the lowest levels of OOO in all tissues, being the breed most susceptible to the changes in dietary linoleic acid content. Moreover, the Landrace showed enhanced deposition of monounsaturated TAG in *trapezius* muscle and abdominal fat.

Keywords: Pig tissues; Triacylglycerols; Dietary fat; Breed; Metabolic markers

1. Introduction

As discussed in the two former papers of this series (Part I and II), the composition of pork fat is modified by dietary fat, which can have several effects on metabolic pathways. Many studies have addressed the influence of dietary unsaturated fatty acids (FA) on lipid composition of pork tissues. However, few have focused on the triacylglycerol (TAG) composition of these tissues, in relation to alterations in dietary FA. In contrast to human and most other animals, the adipose tissue of pigs contains palmitic acid (C16:0) preferentially esterified at the 2-position of the

Abbreviations used: TAG, triacylglycerols; FA, fatty acids; PLL, palmitoyl-dilinoleoylglycerol; POL, palmitoyl-oleoyl-linoleoylglycerol; POLn, palmitoyl-oleoyl-linolenoylglycerol; PStO, palmitoyl-stearoyl-oleoylglycerol; POO, palmitoyl-dioleoylglycerol; PPO, dipalmitoyl-oleoylglycerol; PPSt, dipalmitoyl-stearoylglycerol; PStL, palmitoyl-stearoyl-linoleoylglycerol; PPL, dipalmitoyl-linoleoylglycerol; PStSt, palmitoyl-distearoylglycerol; PoOO, palmitoleoyl-dioleoylglycerol; OOO, trioleoylglycerol; OOL, dioleoyl-linoleoylglycerol; OOLn, dioleoyl-linolenoylglycerol; OLL, oleoyl-dilinoleoylglycerol; StOL, stearoyl-oleoyl-linoleoylglycerol; StOO, stearoyl-dioleoylglycerol; StStO, distearoyl-oleoylglycerol; LW, Large White; D, Duroc; L, Landrace; F1, crossbreed Landrace × Duroc; muscle LTL, *longissimus thoracis et lumborum*.

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TAG rather than at the 1,3 position (Breckenridge, Marai, & Kuksis, 1969; Brockerhoff, Hoyle, & Wolmark, 1966; Parodi, 1982). Piglets fed sow milk or palm oil have the same proportion of TAG with palmitic acid in the 2-position, although these diets had distinct proportions of this FA in this position (Innis, Dyer, Quinlan, & Diersen-Schade, 1996). This observation could be attributed to TAG hydrolysis during the transport of lipids to the tissues and to the continuous hydrolysis and re-esterification of FA in the adipose cells (Scheeder, Gumy, Messikommer, Wenk, & Lambelet, 2003). Therefore, diets with a low content of palmitic acid, such as those containing sunflower or safflower oils, lead to a decrease in adipose tissue, compared with diets which include animal fat (Miller, Shackelford, Hayden, & Reagan, 1990). In Spain, several studies have examined the TAG composition of the Iberian pig and commercial breeds. So, Díaz, García-Regueiro, Casillas, and de Pedro (1996) reported seven main triglycerides in fresh ham from Iberian pigs, among which the increase in trioleoylglycerol (OOO) content in *montanera* Iberian pigs was the most significant difference, with respect to pigs of other breeds. In addition, *montanera* pigs showed greater increases of palmitoyl-dioleoylglycerol (POO), palmitoyl-stearoyl-oleoylglycerol (PStO) and trioleoylglycerol (OOO), in comparison to pigs fed a commercial feed (Tejeda, Gandemer, Antequera, Viau, & García, 2002). TAG analysis may be a useful approach to study fat composition, since it is an easy and fast method and shows a similar or higher discriminant capacity, compared with the analysis of FA composition. Furthermore, several authors (Davenel, Riaublanc, Marchal, & Gandemer, 1999) also found that the levels of PStO and other disaturated TAG show an excellent correlation ($R^2 = 0.92$) with the solid fat content (%), in fat coming from pig backfat. Therefore, determination of these TAG can provide a good marker of the quality of lard and of possible "soft fat" defects in pig carcasses.

Here, we examined the effect of the addition of increasing dietary amounts of a polyunsaturated fat on the TAG composition of muscle and adipose tissues of pigs, following a similar study on FA composition presented in a former paper (Part II). We also assessed the differences in TAG composition between four pig breeds in relation to these dietary changes. This information may be of great interest to the pig farming industry, in order to improve several aspects of pork fat quality.

2. Materials and methods

2.1. Samples and experimental design

The experimental work was based on a 4×4 complete factorial design of two factors, diet and breed, with 4 levels per factor. The sample size was 48, with 3 animals for each of the 16 diet-breed combinations. Castrated male pigs of four breeds were used: Large White (LW), Duroc (D), Landrace (L) and a commercial crossbreed Landrace × Duroc (F1). Animals of each breed were distributed uniformly according to their weight and original litter (avoiding littermates in the same group) and were fed a conventional adaptation diet for 7 days before the start of the experiment. The diet given to controls (diet 1) was a mixture comprised mainly of wheat, barley and soya meal. The three other diets were obtained by adding increasing amounts of fat as follows: 2%, 4% and 8%, respectively, for diets 2, 3 and 4. The fat added to the feed was a commercial mixture of 50% soybean/sunflower acid oil and 50% of animal fat. Diets were formulated to achieve minimal differences in energy and protein content. A complete description of the ingredients and the composition of the four diets is given in Part I (Pascual et al., 2006) . The experiment was carried out under controlled conditions of temperature, light and ventilation. The pigs fed *ad libitum* throughout the experiment, and animal weight and feed consumption were recorded every 15 days until the end of the experiment. Also, *daily feed intake* (kg feed/day), *average daily gain* (kg live weight/day) and *feed conversion ratio* were calculated. After slaughter, which took place at a live weight of 85–90 kg, the following carcass measurements were taken: *carcass weight*, *carcass yield*, *backfat thickness* measured at the 4th and last ribs (Fat-O-Meter, SFK Ltd., Denmark), and *percentage of lean,* calculated using the method of Oliver, Gispert, Tibau, and Diestre (1991). After slaughter, samples from four tissues were taken from each animal: two adipose tissues, backfat and abdominal fat; and two muscle tissues, *longissimus thoracis et lumborum* (*muscle LTL*) and *trapezius*. Backfat and *longissimus* samples were taken at the level of the 10th rib. Samples were then vacuum stored in plastic bags and kept at −20 °C until analysis.

2.2. Reagents

Chloroform and methanol (ACS grade) were from Panreac (Montplet & Esteban, Barcelona, Spain), HPLC grade chloroform was from Romil (Teknokroma, San Cugat del Vallés, Barcelona), and HPLC grade propionitrile was from Fluka (Sigma–Aldrich Química S.A., Alcobendas, Madrid). Anhydrous sodium sulfate and sodium chloride (analysis) were from Panreac. All the homogeneous TAG standards used were from SIGMA (Alcobendas, Madrid).

2.3. Triacylglycerol determination

The lipid fraction was obtained by extraction, using the method of Folch, Lees, and Stanley (1957), but with slight modifications. Muscle tissue (5 g) or adipose tissue (5 g) was weighed and homogenised with 30 ml of chloroform/methanol mixture (2:1 v/v), at 20,000 rpm for 30 s, using a Polytron (PT 2000, Kinematica AG, Lucerne, Switzerland). The solid residue was re-extracted with 30 ml of the solvent mixture, and the combined organic fractions were washed with aqueous NaCl solution (0.58%). The chloroform phase was then filtered through

anhydrous sodium sulphate and evaporated to dryness. A proportion of this fat extract (200 mg) was diluted to 10% (p/v) with HPLC grade chloroform and filtered through a nylon filter with a pore size of 0.45 mm (Lida, Kenosha, WI, USA). The chloroform solutions of TAG were injected into an Perkin–Elmer HPLC system (Norwalk, CO, USA) with a pump (series 10) and a refractive index (RI) detector (LC-25), fitted with a Rheodyne Loop (150 μl) injector and coupled to a Hewlett–Packard HP 3396A integrator (Avondale, PA, USA). The chromatograph was equipped with an octadecylsilane pre-column (2.5 cm × 4.6 mm i.d.) and an octadecylsilane column (25 cm × 4.6 mm i.d.) with 5 mm particle size (Spherisorb ODS-2, Teknokroma, Sant Cugat del Vallès, Spain). The chromatographic conditions were as follows: mobile phase was propionitrile (GC distilled after the addition of phosphorus pentoxide (approx. 4 g/l)), and the elution was isocratic, at a flow rate of 1 ml/min. Measurements were made at room temperature. Injection volume was 10 μl. TAG identification was performed on the basis of the equivalent carbon number (ECN) system. The peaks were identified using the logarithm of TAG retention time, relative to OOO (Goiffon, Reminiac, & Olle, 1981), and retention values were confirmed by calculations made after the injection of homogeneous TAG standards. Extra confirmation of TAG identity was achieved by injecting various oil samples (olive, soybean and sunflower oils). The results were expressed as compensated area normalization (CAN). The repeatability and intermediate precision of the TAG quantification were evaluated separately for adipose and muscle tissue. In the repeatability assessment, 6 aliquots of a sample were extracted and injected in triplicate on the same day, following the design: 1 day × 6 samples × 3 replicates. In the intermediate precision determination, 12 aliquots of a sample were used, following the design: 4 days × 3 samples × 3 replicates. The repeatability and intermediate precision estimates for the five TAG, expressed as % RSD (relative standard deviations in percentage scale) ranged between 0.75% and 10.68%. The repeatability values were not significantly different from those of intermediate precision. For the non-reported TAG, the results obtained were similar.

2.4. Selection of TAG markers

To reduce the number of variables, we classified the TAG as a function of their accumulation pattern in tissues.

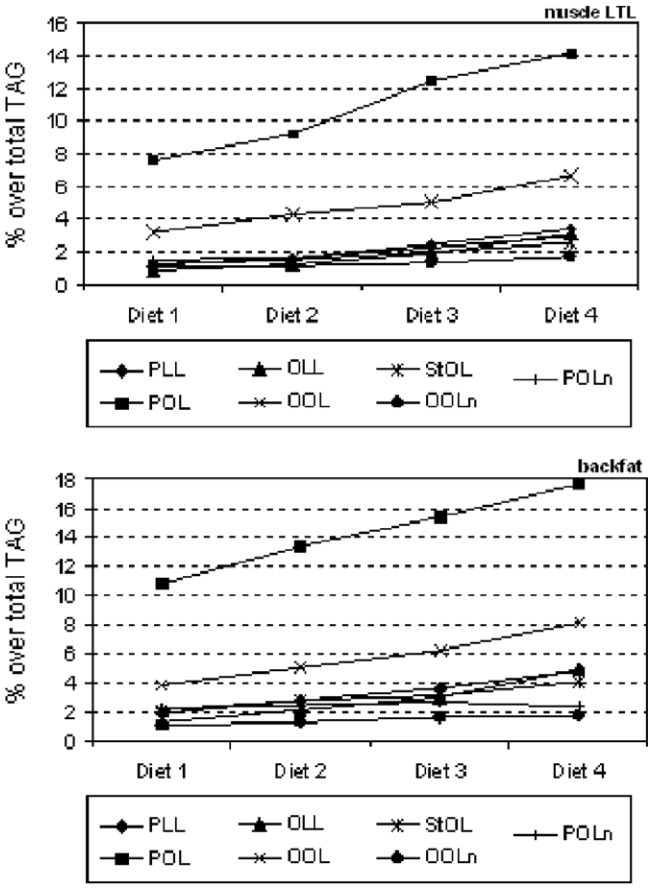

Fig. 1. Levels of TAG markers showing an effect of dietary fat on TAG synthesis, in *muscle LTL* (*longissimus thoracis et lumborum*) and backfat (diets 1–4 correspond to 0%, 2%, 4% and 8% of added dietary fat, respectively).

We measured 16 TAG, but the presentation of the results is restricted to five selected TAG (PLL, PStO, PStL, PStSt and OOO). A previous study (Pascual, 2000), using principal components analysis, shows that the remaining ones are highly correlated to one of the five markers chosen. These five TAG are sufficient to study the effects of the diet on TAG profile, and the strong correlations observed support extrapolation of the results to the remaining TAG. Four TAG groups were defined (Table 1). An initial group corresponded to those whose synthesis is regulated mainly by the dietary fat and which are not highly dependent on endogenous FA synthesis. This group included PLL, POL, OLL, OOL, StOL, OOLn and POLn. PLL is the most representative TAG, because linoleic acid is the most characteristic FA of the fat added to our diets. Fig. 1 shows

Table 1
Classification of TAG in pork tissues, according to their internal correlations (bold letters indicate the markers selected)

Effect	TAG markers
Markers of a predominant effect of the FA dietary supply on TAG synthesis	**PLL**, POL, OLL, OOL, StOL, OOLn, POLn
Markers of a predominant effect of the *de novo* FA synthesis on TAG synthesis	**PStO**, POO, StOO, PPO, PPSt, StStO
Markers of the activation/inhibition of saturated TAG synthesis	**PStL**, PPL, **PStSt**
Markers of the activation/inhibition of monounsaturated TAG synthesis	**OOO**, PoOO

the increasing curves of these TAG according to the diet. More detailed correlations between these TAG in the four tissues analysed are discussed below. The second group comprised TAG whose deposition depends mainly on *de novo* FA synthesis, namely PStO, POO, StOO, PPO, PPSt and StStO. Fig. 2 shows the curves of these TAG decreasing according to the diet. More detailed correlations between these TAG in the four tissues analysed are discussed below. Three TAG rich in palmitic and stearic acids, that showed a distinct pattern of deposition were classified in a third group. These were PStL, PStSt and PPL, all markers of the priority synthesis of saturated TAG. The correlation between these three TAG indicates a distinct dietary influence. Therefore, since PPL and PStL are well correlated, we selected two markers, PStL and PStSt (Fig. 3). Finally, the fourth group included TAG that contain only monounsaturated FA, and which could be markers of the priority synthesis of monounsaturated TAG. This group included OOO and PoOO and their curves of change according to the diet are shown in Fig. 4.

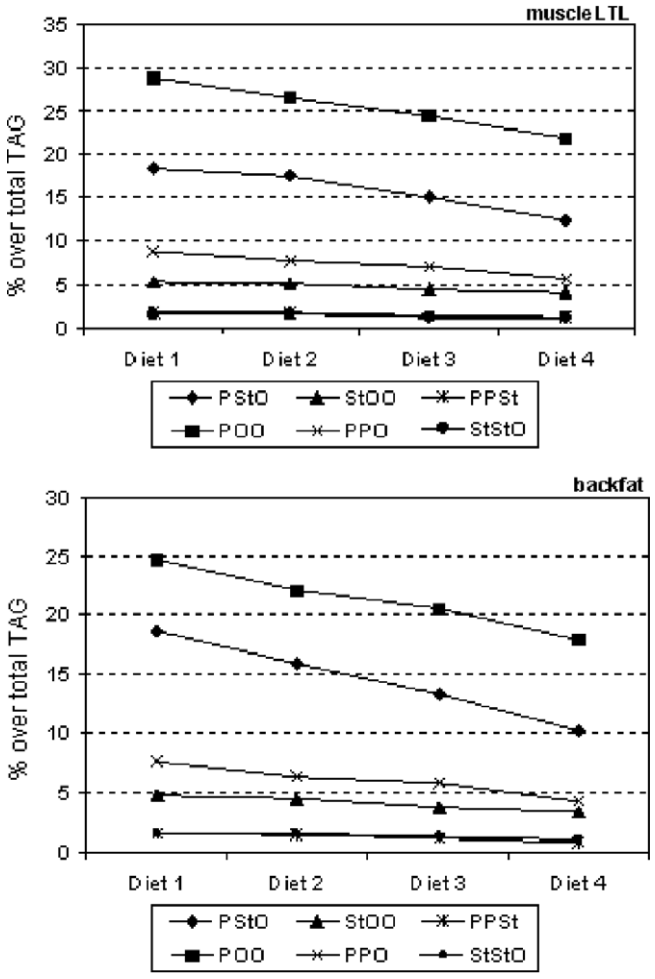

Fig. 2. Levels of TAG markers showing an effect of *de novo* FA synthesis on TAG synthesis, in *muscle LTL* (*longissimus thoracis et lumborum*) and backfat (diets 1–4 correspond to 0%, 2%, 4% and 8% of added dietary fat, respectively).

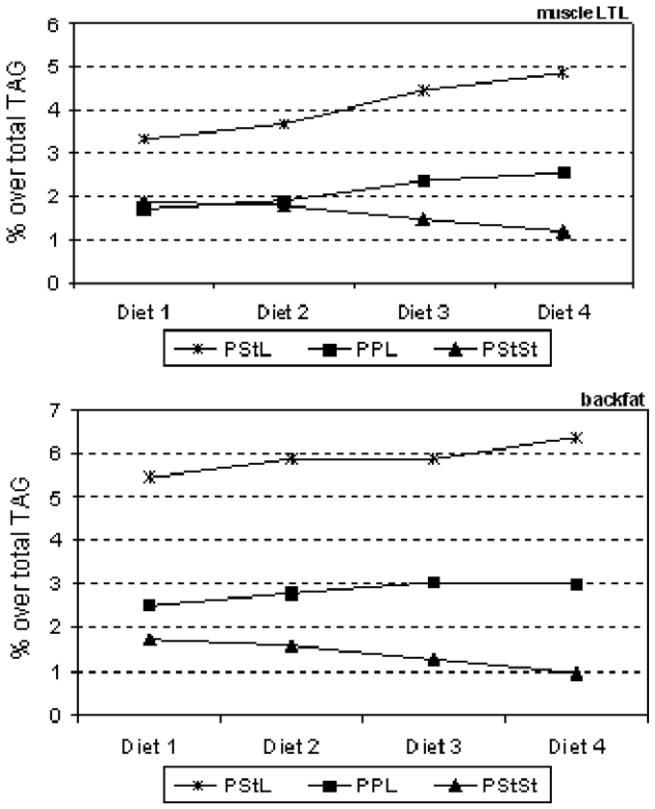

Fig. 3. Levels of TAG markers of activation/inhibition of saturated TAG synthesis, in *muscle LTL* (*longissimus thoracis et lumborum*) and backfat (diets 1–4 correspond to 0%, 2%, 4% and 8% of added dietary fat, respectively).

2.5. Statistical methods

A two-factor analysis of variance (ANOVA) was used to test the effect of diet and breed. *F*-tests were used to test the overall significance, and Bonferroni tests for pair-wise

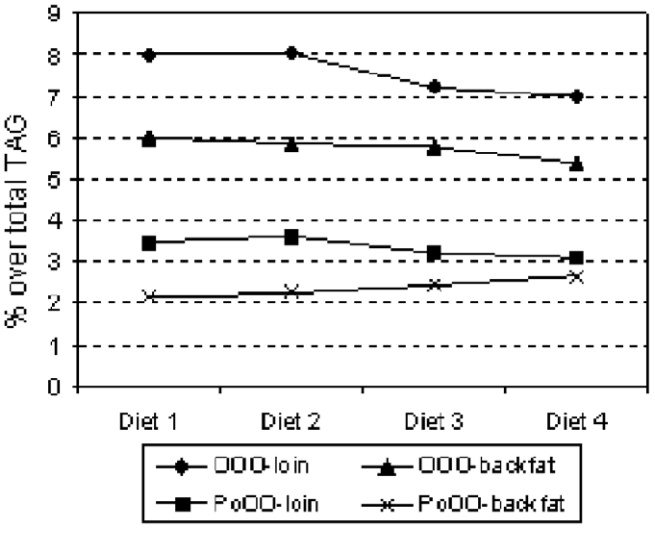

Fig. 4. Levels of TAG markers of activation/inhibition of monounsaturated TAG synthesis, in *muscle LTL* (*longissimus thoracis et lumborum*) and backfat (diets 1–4 correspond to 0%, 2%, 4% and 8% of added dietary fat, respectively).

comparisons. The analysis was carried out with the SPSS 11.0 statistical package (2001).

3. Results and discussion

3.1. General

No significant differences were observed for any of the production parameters, evaluated with respect to diet. In contrast, significant differences were observed in relation to breed, but only for two parameters (Table 2). Mean values of *daily feed intake* were significantly lower for LW (1.72 kg feed/day) and D (1.58) than for L (1.88) and F1 (1.89), and values of *average daily gain* were also lower for LW (0.58 kg weight/day) and D (0.56) than for L (0.67) and F1 (0.67). However, the *feed conversion ratio* did not show significant differences between breeds at the end of the experiment. No significant differences were observed for the carcass measurements, with a similar mean percentage of lean for the four diets (52.09, 52.81, 51.94 and 52.69), nor were differences found between breeds (54.08 for LW, 51.99 for D, 52.24 for L, and 51.27 for F1). All these results are shown in Table 2. These results are consistent with the results of other authors, e.g. when comparing a concentrate diet *versus* a chestnut diet (rich in oleic and linoleic acids) no differences were found in carcass fatness, live weight or carcass weight at slaughter (Coutron-Gambotti, Gandemer, & Casabianca, 1998). However, significant differences were detected when Corsican pigs were compared with crossbreed Corsican × Large White pigs.

3.2. Effect of breed on TAG composition

Results for the five TAG markers content in the four tissues are given in Tables 3–7. In general, the differences in the TAG composition due to breed were lower than those induced by diet. The effect of breed was not significant (Table 3) for PLL in an overall ANOVA *F*-test. The mean differences between breeds for PStO (Table 4) were also very small, and the differences for breed were almost significant in backfat and *muscle LTL* (overall significance level close to 0.08). In contrast, the breed effect on PStL (Table 5) was significant (overall level close to 0.01), except in *trapezius*, and PStL levels were higher in LW and F1. The overall significance level for the diet-breed interaction was around 0.05 in backfat, but much higher in the other tissues. For PStSt (Table 6), the breed effect was highly significant in adipose tissues (overall level 0.005 or lower), being higher in LW and F1 than in the other two breeds. The effect of breed was not significant for PStSt in muscle tissues, while the influence of the diet–breed interaction was not significant in the four tissues for this TAG. The breed effect on OOO levels (Table 7) was also significant, except in backfat. The overall significance level fell below 0.001 in abdominal fat and *trapezius* and was close to 0.01 in *muscle LTL*. Landrace showed the highest levels of OOO in all tissues, compared with the other breeds. The overall significance level for the diet–breed interaction was 0.05 in *trapezius*, due to the weaker effect of diet on LW, compared with the high effect observed for the other three breeds. In conclusion, LW and F1 showed a higher activation of the synthesis of the most saturated TAG, particularly PStL,

Table 2
Results corresponding to the productive parameters and caracass measurements (28 animals per group)

	Large White	Duroc	Landrace × Duroc	Landrace
Daily feed intake (kg/day)	1.72[ab]	1.58[a]	1.89[b]	1.88[a]
Average daily gain (kg/day)	0.58[a]	0.56[a]	0.67[b]	0.67[b]
Feed conversion ratio	3.27	2.88	3.01	2.91
Final live weight (kg)	88.46	85.95	88.97	89.18
Carcass weight (kg)	65.20	63.20	65.38	66.09
Carcass yield (%)	73.70	73.52	73.46	74.08
Backfat thickness 3–4th rib (mm)	16.51	17.93	19.37	18.58
Backfat thickness 10th rib (mm)	15.61	16.47	17.40	16.47
Loin diameter (mm)	44.67[b]	39.30[a]	42.50[ab]	44.52[b]
Percentage of lean (%)	54.08	51.99	51.27	52.24
	[A]Diet 1	Diet 2	Diet 3	Diet 4
Daily feed intake (kg/day)	1.72	1.68	1.92	1.75
Average daily gain (kg/day)	0.59[a]	0.60[a]	0.68[b]	0.62[a]
Feed conversion ratio	3.03	3.00	2.87	3.18
Final live weight (kg)	87.93	87.46	88.31	88.86
Carcass weight (kg)	65.37	64.52	65.04	64.93
Carcass yield (%)	74.30	73.78	73.65	73.03
Backfat thickness 3–4th rib (mm)	18.47	17.75	18.47	17.69
Backfat thickness 10th rib (mm)	16.45	16.43	17.09	15.96
Loin diameter (mm)	42.98	43.57	41.98	42.45
Percentage of lean (%)	52.09	52.81	51.94	52.69

Values in the same row with different superscript letters were significantly different ($p < 0.05$).
[A] Diet 1, no added fat; Diet 2, 2% added fat; Diet 3, 4% added fat; Diet 4, 8% added fat.

in fat tissues. Furthermore, L showed enhanced deposition of monounsaturated TAG in *trapezius* muscle and abdominal fat, compared with the other breeds. Therefore, LW is the most susceptible breed to modifications in the levels of dietary linoleic acid. This breed tends to have lower levels of OOO in all tissues, and higher levels of PStL in adipose tissues, than the other three breeds. Finally, the greatest effect of breed on TAG composition was detected in the abdominal fat. In contrast, *trapezius* muscle was the least affected.

3.3. Effect of diet on TAG composition

3.3.1. General

The results on the composition of the five TAG markers in the four tissues are summarised in Tables 3–7. TAG content is expressed as a mean value. To simplify the tables we do not report the usual measures of dispersion. The effect of the diet is observed as a shift towards the TAG related to dietary supply (PLL, POL, OLL, OOL, StOL, OOLn, POLn), mainly at the expense of disaturated and trisaturated TAG (PStO, StOO, PPO, PPSt, StStO), and of monounsaturated TAG (POO, OOO). The addition of increasing percentages of dietary fat tends to balance the contents of TAG related to dietary supply. An increasing amount of dietary fat percentage enhanced PLL and PStL, and decreased PStO, PStSt and OOO levels. A similar effect was observed in Iberian pigs (Díaz et al., 1996; Tejeda et al., 2002). A *montanera* diet (rich in linoleic acid) increases OOL and decreases PStO compared with a commercial feed treatment. In this case, OOL would be the best marker of the dietary effect (instead PLL), since it shows the highest increase. In the previous publication of this series (Part II) it was reported that linoleic acid and palmitic acid, markers of the effect of diet and endogenous synthesis, respectively, have a strong negative correlation. In our study, the same pattern of correlation between PLL and PStO, TAG markers representing the same effects, was observed (Table 8). This pattern indicates the contribution of increased dietary linoleic acid to a reduction in endogenous synthesis, which was detected for the four tissues (correlation −0.944 and −0.938 in fat tissues, and −0.859 and −0.904 in muscle tissues). This 4-fold correlation matrix for the five TAG markers highlights other relevant results. The correlation was fairly consistent across tissues for certain pairs of TAG, such as PLL–PStO, PLL–PStSt, PLL–OOO, PStO–PStSt and PStL–OOO. In contrast, in other pairs, such as PStO–PStL and PStSt–PStL, marked differences between tissues were detected, revealing a correlation only in muscle tissues. The synthesis and deposition of the most saturated TAG showed the most complex pattern. We need two saturated TAG to

Table 3
PLL content of the four tissues, expressed as compensated area normalization (%) (mean of three animals)

	Diet 1	Diet 2	Diet 3	Diet 4	Mean
Abdominal fat					
Large White	2.33	3.77	4.26	5.67	4.01
Duroc	1.79	2.58	4.17	5.83	3.59
F1	1.97	3.74	4.19	5.64	3.89
Landrace	2.75	3.34	5.55	5.14	4.19
Mean	2.21[a]	3.36[b]	4.54[c]	5.57[d]	3.92
Backfat					
Large White	2.14	2.93	2.94	4.62	3.16
Duroc	1.90	2.83	4.2	4.87	3.45
F1	1.62	3.06	3.51	5.55	3.43
Landrace	2.14	2.33	3.79	4.33	3.15
Mean	1.95[a]	2.78[b]	3.61[c]	4.84[d]	3.30
Muscle LTL					
Large White	1.40	1.46	2.46	3.12	2.11
Duroc	1.14	1.53	2.43	3.42	2.13
F1	1.20	1.78	2.32	4.08	2.34
Landrace	1.30	1.76	2.58	3.06	2.18
Mean	1.26[a]	1.63[a]	2.45[b]	3.42[c]	2.19
Trapezius					
Large White	1.31	2.62	2.79	4.14	2.72
Duroc	1.03	1.74	3.42	4.18	2.60
F1	1.31	1.92	3.37	4.42	2.75
Landrace	1.34	1.71	2.66	4.24	2.48
Mean	1.25[a]	2.00[b]	3.06[c]	4.24[d]	2.64

[*] Results are expressed as percentage of total TAG (compensated area normalization).
[a–d] Means with different letters are significantly different ($p < 0.05$).

Table 4
PStO content of the four tissues, expressed as compensated area normalization (%) (mean of three animals)

	Diet 1	Diet 2	Diet 3	Diet 4	Mean
Abdominal fat					
Large White	18.84	16.36	14.25	10.73	15.04
Duroc	20.65	17.82	12.6	10.47	15.39
F1	20.97	15.64	14.49	10.74	15.46
Landrace	16.70	14.91	10.37	11.74	13.43
Mean	19.29[d]	16.18[c]	12.93[b]	10.92[a]	14.83
Backfat					
Large White	18.10	16.05	14.28	10.92	14.84
Duroc	18.02	14.63	11.58	9.85	13.52
F1	19.50	15.94	14.84	9.74	15.00
Landrace	18.72	17.19	12.70	10.35	14.74
Mean	18.58[d]	15.95[c]	13.35[b]	10.22[a]	14.53
Muscle LTL					
Large White	18.51	19.22	16.36	13.87	16.99
Duroc	18.28	17.83	14.17	11.36	15.41
F1	18.28	17.47	15.96	12.63	16.09
Landrace	18.77	15.65	13.46	11.76	14.91
Mean	18.46[c]	17.54[c]	14.99[b]	12.41[a]	15.85
Trapezius					
Large White	21.28	17.95	16.60	11.85	16.92
Duroc	19.23	17.11	13.53	11.62	15.37
F1	19.54	17.37	14.43	10.48	15.46
Landrace	19.80	17.29	13.78	11.01	15.47
Mean	19.97[d]	17.43[c]	14.58[b]	11.24[a]	15.80

[*] Results are expressed as percentage of total TAG (compensated area nor- malization).
[a–d] Means with different letters are significantly different ($p < 0.05$).

Table 5
PStL content of the four tissues, expressed as compensated area normalization (%) (mean of three animals)

	Diet 1	Diet 2	Diet 3	Diet 4	Mean
Abdominal fat					
Large White	7.16	8.23	8.52	8.76	8.16^{c}
Duroc	6.49	6.72	6.45	7.29	6.74^{a,b}
F1	6.22	7.96	7.57	7.18	7.23^{b}
Landrace	3.86	6.33	7.09	7.29	6.14^{a}
Mean	5.93^{a}	7.31^{b}	7.41^{b}	7.63^{b}	7.07
Backfat					
Large White	6.4	7.28	5.31	6.91	6.48^{b}
Duroc	5.23	4.77	5.72	6.14	5.46^{a}
F1	5.00	6.22	6.50	6.45	6.04^{ab}
Landrace	5.15	5.17	5.96	5.92	5.55^{a}
Mean	5.45^{a}	5.86^{ab}	5.87^{ab}	6.35^{b}	5.88
Muscle LTL					
Large White	3.46	3.71	5.09	4.88	4.28^{bc}
Duroc	3.03	3.49	3.83	4.38	3.68^{a}
F1	3.70	4.00	4.78	5.65	4.53^{c}
Landrace	3.06	3.54	4.23	4.49	3.83^{ab}
Mean	3.31^{a}	3.69^{a}	4.48^{b}	4.85^{b}	4.08
Trapezius					
Large White	3.80	5.23	5.10	6.26	5.10
Duroc	3.53	4.43	5.40	6.53	4.97
F1	3.04	4.47	6.05	7.29	5.21
Landrace	3.25	4.18	4.95	6.04	4.60
Mean	3.40^{a}	4.58^{b}	5.37^{c}	6.53^{d}	4.97

[*] Results are expressed as percentage of total TAG (compensated area normalization).
[a–d] Means with different letters are significantly different ($p < 0.05$).

Table 6
PStSt content of the four tissues, expressed as compensated area normalization (%) (mean of three animals)

	Diet 1	Diet 2	Diet 3	Diet 4	Mean
Abdominal fat					
Large White	2.71	2.39	1.85	1.00	1.99^{c}
Duroc	1.67	1.70	1.26	1.17	1.45^{ab}
F1	2.54	1.88	1.82	1.06	1.82^{bc}
Landrace	1.33	1.61	0.80	1.04	1.19^{a}
Mean	2.06^{b}	1.90^{b}	1.43^{a}	1.07^{a}	1.61
Backfat					
Large White	2.25	1.80	1.27	1.18	1.63^{b}
Duroc	1.57	1.53	0.92	0.78	1.20^{a}
F1	1.73	1.52	2.05	1.05	1.59^{b}
Landrace	1.31	1.54	0.86	0.89	1.15^{a}
Mean	1.72^{c}	1.60^{bc}	1.28^{ab}	0.97^{a}	1.39
Muscle LTL					
Large White	2.18	1.85	1.25	1.31	1.65
Duroc	1.38	1.94	1.17	1.18	1.42
F1	1.79	1.64	2.03	1.29	1.69
Landrace	2.04	1.71	1.39	0.96	1.53
Mean	1.85^{c}	1.79^{bc}	1.46^{ab}	1.19^{a}	1.57
Trapezius					
Large White	2.02	2.11	1.42	1.23	1.70
Duroc	2.16	1.67	1.04	1.35	1.56
F1	1.99	1.59	1.44	1.13	1.54
Landrace	1.69	1.40	1.44	0.79	1.33
Mean	1.96^{c}	1.69^{bc}	1.34^{ab}	1.12^{a}	1.53

[*] Results are expressed as percentage of total TAG (compensated area normalization).
[a–d] Means with different letters are significantly different ($p < 0.05$).

explain this deposition. First, PStSt, which shows a clear positive correlation with PStO and a negative correlation with PLL, and second, PStL, which shows a certain positive correlation with PLL, and suggests a higher influence of the diet in its accumulation pattern. Next, we discuss the effects of the experimental diets on the TAG composition of the four tissues, in relation to the markers selected.

3.3.2. Palmitoyl-dilinoleoylglycerol (PLL)

A large positive diet effect was observed for PLL (more than a 100% increase) in the four tissues. The overall significance level fell below 0.001 in all cases, and all the pair-wise comparisons between diets (Table 3) gave significant differences. Except for abdominal fat, the largest increase was found between diet 3 and diet 4. This is consistent with the observation that the greatest increase in the linoleic acid supplied by feed occurred changing from diet 3 to 4. The PLL levels were higher in adipose tissue than in muscle, but the relative increase from diet 1 to 4 showed differences between the tissue type. Thus, while the increase in PLL was similar for the two fat tissues (150%), it was a little higher in *muscle LTL* (170%) and much higher in *trapezius* (240%). The effects observed for PLL can be extrapolated to a group of TAG, which have a high positive correlation with PLL in the four tissues: POL (minimum correlation 0.912), OLL (min. 0.926), OOL (min. 0.758, in *muscle LTL*, but above 0.900 in the other three tissues), StOL (min. 0.782, in abdominal fat, and max. 0.918, in *trapezius*) and OOLn (between 0.800 and 0.850). POLn can also be included in this group, but a separate analysis was required in backfat, where the correlation was quite low (0.217). Finally, increasing amounts of dietary linoleic acid tended to reduce the differences in the TAG levels of this group (PLL and others).

3.3.3. Palmitoyl-stearoyl-oleoylglycerol (PStO)

In contrast to PLL, there was a clear negative diet effect on PStO (between 5% and 9% units) in the four tissues ($p < 0.001$); however, in relative terms, the diet effect on PStO was weaker than that observed on PLL. The pair-wise comparisons between diets (Table 4) gave significant differences, and the effect was slightly weaker in *muscle LTL* (33% decrease from diet 1 to 4), but similar in the other three tissues (44% decrease). Therefore, modifications in dietary linoleic acid content have a lower relative effect on PStO levels (mean decrease, 42% from diet 1 to 4) than on PLL (mean increase, 177% from diet 1 to 4). The effects observed for PStO can be extrapolated to other TAG that are positively correlated with it in the four tissues, such as POO (minimum correlation 0.615 in abdominal fat, maximum 0.792 in *trapezius*), StOO (minimum 0.772 in abdominal fat, maximum 0.814 in backfat), PPO (min. 0.763 in backfat, maxi-

Table 7
OOO content of the four tissues, expressed as compensated area normalization (%) (mean of three animals)

	Diet 1	Diet 2	Diet 3	Diet 4	Mean
Abdominal fat					
Large White	4.50	3.87	3.75	3.62	3.94[a]
Duroc	5.63	4.96	5.24	4.49	5.08[c]
F1	5.13	4.50	4.27	4.21	4.53[b]
Landrace	5.51	5.55	5.02	4.53	5.15[c]
Mean	5.19[c]	4.72[bc]	4.57[ab]	4.21[a]	4.68
Backfat					
Large White	5.67	5.56	6.35	5.31	5.72
Duroc	5.86	6.18	5.45	5.39	5.72
F1	6.47	5.52	5.24	4.83	5.52
Landrace	6.04	6.19	6.10	6.03	6.09
Mean	6.01	5.86	5.79	5.39	5.76
Muscle LTL					
Large White	7.75	7.55	6.54	6.89	7.18[a]
Duroc	8.56	8.07	7.47	7.43	7.88[b]
F1	8.32	7.79	6.75	6.01	7.22[a]
Landrace	7.45	8.88	8.12	7.57	8.01[b]
Mean	8.02[b]	8.06[b]	7.22[a]	6.98[a]	7.57
Trapezius					
Large White	6.83	6.51	5.63	5.89	6.21[a]
Duroc	7.89	7.45	6.42	5.85	6.90[b]
F1	8.39	7.29	6.19	5.47	6.84[b]
Landrace	8.25	8.30	8.80	6.00	7.84[c]
Mean	7.84[c]	7.39[c]	6.76[b]	5.80[a]	6.95

[*] Results are expressed as percentage of total TAG (compensated area normalization).
[a–d] Means with different letters are significantly different ($p < 0.05$).

mum 0.842 in abdominal fat), PPSt (minimum 0.650 in *muscle LTL*, maximum 0.825 in *trapezius*) and StStO (minimum 0.603 in *muscle LTL*, maximum 0.731 in *trapezius*).

3.3.4. Palmitoyl-distearoylglycerol (PStSt) and palmitoyl-stearoyl-linoleoylglycerol (PStL)

In the third group, which includes markers of saturated TAG synthesis (PStSt, PPL and PStL), tissue deposition was explained by two different patterns. PStL and PPL were well correlated and their levels increased from diet 1 to 4, while the levels of PStSt decreased. Therefore, PStL and PStSt can be used as markers to explain the effect of diet on the synthesis of the most saturated TAG. The influence of diet on PStL levels was slightly stronger (<0.001) in muscle than in fat tissues (0.001 in abdominal fat and around 0.05 in backfat). Pair-wise comparisons between diets (Table 5) indicated that the dietary fat increase has a greater positive effect on the synthesis of PStL and PPL in muscle tissues (46% increase from diet 1 to 4 for *muscle LTL*, and 92% for *trapezius*), than in adipose tissues (16% increase for backfat, and 29% for abdominal fat). As for PStL and PPL, levels of PStSt were also higher in adipose tissues than in muscle (Table 6), but the diet effect in the four tissues was negative (around 45% mean decrease from diet 1 to 4), with an overall level of significance of 0.005 or lower. Comparing PStL and PStSt patterns, it is also relevant to remark that, while the PStL content in fat was much less affected by the diet than in muscle, the decrease in PStSt content is much more balanced among the four tissues. This clear divergence could be explained by the presence of linoleic acid in the saturated TAG. Therefore, as linoleic acid increases (particularly in pig muscle tissues), as a function of the percentage of this FA in the diet (see Part II of this work), the synthesis of PPL and PStL is also favoured in these tissues. In contrast, as palmitic and stearic acid contents decrease (particularly in fat tissues), as a function of the percentage of dietary linoleic acid, the synthesis of PStSt follows a similar pattern to these two saturated FA. This divergence indicates that the marker of the dietary effect on saturated TAG synthesis differs for the distinct types of tissue.

3.3.5. Trioleoylglycerol (OOO)

Tissue levels of this TAG were the least affected by the increasing amounts of dietary linoleic acid (16% mean decrease, from diet 1 to 4). This negative and significant dietary effect (overall level <0.01) was stronger in *trapezius* (26% decrease) and abdominal fat (19%) than in *muscle LTL* (13%) and backfat (10%) (Table 7). In contrast to our findings, results obtained by other authors (Díaz et al., 1996; Tejeda et al., 2002), show a higher capacity of the *montanera* diets, with respect to commercial feedings, to increase OOO levels in Iberian pigs. This could be explained by the particular composition of the *montanera* diet (based on acorns), which has a much higher oleic/linoleic ratio than the experimental diets used in our study. The minor dietary effect observed in our study could explain the observation (Table 8) that the correlations between OOO and the remaining TAG markers present a low level of significance. Thus, there was no correlation with the levels of

Table 8
Correlation matrix between the levels of the TAG markers (from left to right and from top to bottom: abdominal fat, backfat, *longissimus thoracis et lumborum*, and *trapezius*)

	PLL		PStO		PStL		PStSt	
PstO	−0.944	−0.938						
	−0.859	−0.904						
PStL	0.369	0.372	−0.234	−0.206				
	0.739	0.834	−0.437	−0.753				
PStSt	−0.611	−0.591	0.710	0.693	0.070	0.198		
	−0.486	−0.634	0.559	0.688	−0.329	−0.483		
OOO	−0.453	−0.427	0.232	0.176	−0.706	−0.723	−0.234	−0.079
	−0.547	−0.699	0.119	0.439	−0.748	−0.752	−0.004	0.334

PStO, although a slight positive correlation value was observed in *trapezius* (0.439), which is consistent with the finding that this muscle was the only tissue with a clear decrease in OOO from diet 1 to 4. In contrast, levels of OOO showed high negative correlation values with those of PStL in all tissues (from −0.706 in abdominal fat to 0.752 in trapezius), but they correlated weakly with PLL (from −0.453 in abdominal fat to 0.699 in trapezius). These findings indicate that OOO deposition is reduced by PUFA-rich dietary fat, but in a distinct way to the most saturated TAG, which are more dependent on *de novo* FA synthesis.

4. Conclusions

As a result of our findings we can conclude that determination of TAG composition in pig tissues can be a good method to assess the influence of dietary fat and its interaction with breed. We classified TAG in pig tissues into four groups, represented by five TAG markers, the levels of which indicate the influence on the different processes involved in TAG synthesis and deposit. The information given by TAG values cannot replace that supplied by FA composition. Similar information can be obtained from changes in linoleic acid levels to that obtained from changes in PLL levels. Moreover, complementary information can be obtained from other TAG level changes, e.g., PStL, PPL or PStSt, indicating a preferential synthesis and deposition of disaturated and trisaturated TAG. Another interesting conclusion is that TAG composition of pig tissues shows a higher sensitivity than FA composition in assessing the influence of the diet, since the rates of increase of some TAG markers are higher than the corresponding FA markers. Thus, comparing the rate of linoleic acid incorporation in pig tissues with that of PLL, respectively, the most representative FA and TAG markers of tissue linoleic uptake, the relative increase in linoleic acid levels was around 140% from diet 1 to 4, while the increase in PLL was around 240% (mean values for the four tissues). Similar effect was found for the percentage of decrease from diet 1 to 4, for the markers related with *de novo* synthesis. Thus, palmitic acid value decreases 20%, while PStO or PPSt decrease 75–100%. Moreover, for almost all of the TAG and FA, the muscle *trapezius* showed the highest rate of change, in response to diet. This higher susceptibility of *trapezius* to dietary FA is accomplished by our findings showing that breed has a small effect on TAG composition in this tissue. In contrast, abdominal fat was the most affected by breed. For this reason *trapezius* muscle could be a good target tissue to study dietary influences, since it is scarcely dependent on the breed. Regarding breed, from our results it can be concluded that PStSt is the most variable marker in adipose tissues, while OOO is the most variable in muscle tissues. Finally, Large White was the breed showing the highest susceptibility to change in TAG composition, with regard to to the dietary linoleic supply.

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