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Effect of increasing amounts of a linoleic-rich dietary fat on the fat composition of four pig breeds. Part I: Backfat fatty acid evolution

J.V. Pascual [a], M. Rafecas [a], M.A. Canela [b], J. Boatella [a], R. Bou [a], M.D. Baucells [c], R. Codony [a,*]

[a] Department of Nutrition and Food Science-CeRTA, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n. 08028-Barcelona, Spain
[b] Department of Applied Mathematics and Analysis, University of Barcelona. Gran Via Corts Catalanes 585. 08007-Barcelona, Spain
[c] Department of Nutrition and Animal Feeding, Faculty of Veterinary, Universitat Autònoma of Barcelona, 08193-Bellaterra-Cerdanyola, Spain

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Abstract

Four diets prepared, respectively, with 0%, 2%, 4% and 8% of a high-linoleic added fat were administered (76 days of treatment) to a sample of 112 pigs of four breeds (Landrace, Large White, Duroc and a crossbreed Landrace>Duroc/>×>Duroc/>Duroc). The effects of diet and breed on the evolution of the fatty acid composition of backfat were examined by taking biopsies. Over time, a continuous increase in stearic, palmitic and oleic acids throughout the pig's life was observed. Oleic acid showed the smallest differences among the four diets at the end of the experiment, while stearic and palmitic acid showed higher differences according to the increase in the percentage of dietary fat. Stearic acid showed the highest rate of increase over time, according to the increasing intake of linoleic acid (diets 1–4). These increases were compensated by a decrease in linoleic acid, although this decrease tended to stabilize according to a higher percentage of added fat and also, for diet 4 (8% fat), an increase in linoleic acid was observed at the end of the experiment. Among the minor fatty acids, arachidonic acid showed a clear decrease over time, although higher levels at the end of the experiment were observed for diets including 4% and 8% of added fat, compared to the other two diets including lower amounts of linoleic acid. Moreover, a significant effect was observed for the factor breed. So, Duroc pigs showed the highest rate of deposit of linoleic acid and the lowest of stearic acid, while the other three breeds showed similar rates.

Keywords: Pig; Backfat biopsy; Fatty acids; Linoleic acid intake; Dietary fat; Breed

1. Introduction

The effect of dietary fats on the fatty acid (FA) compositions of pork adipose and muscle tissues and the interactions between this factor and others, such as breed or feed energy level, is well known (Averette Gatlin, See, Hansen, Sutton, & Odle, 2002; Wood, Buxton, Whittington, & Enser, 1986). An increase in dietary linoleic acid leads to a higher content of this FA in the loin, but not to a significant increase in arachidonic acid (Ahn, Lutz, & Sim, 1996; Eder, Nonn, & Kluge, 2001; Scheeder, Gläser, Eichenberger, & Wenk, 2000) and some data are also available on the parallel increase in linoleic and arachidonic acids in fat tissues in response

Abbreviations: FA, fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; FAME, fatty acid methyl esters; F1, crossbreed Landrace×Duroc.

* Corresponding author. Tel.: +34 934024514; fax: +34934035931.
E-mail address: rafaelcodony@ub.edu (R. Codony).

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to an increase in dietary linoleic acid (D'Arrigo et al., 2002). Moreover, a few studies report results on repeated measures of FA during pig life (Camoes, Mourot, Kouba, Cherot, & Mounier, 1995; Fontanillas, Barroeta, Baucells, & Guardiola, 1998; Irie & Sakimoto, 1992; Warnants, Van Oeckel, & Boucqué, 1999). These studies have used various diets, some rich in *cis*-monounsaturated, others in *trans*-monounsaturated, and others in n-3 or n-6 polyunsaturated fatty acids. Their respective rate of deposit in backfat over time was quite variable and dependent on a number of factors, such as the FA concentration in feed, possible metabolisation of the FAs (arachidonic, eicosapentaenoic or docosahexaenoic acids), and the possibility of being synthesized by the pig (oleic acid). The best markers of diet influence seem to be linoleic and linolenic acids, because they are major FA and, also, *trans* FA because they are scarcely affected by lipid metabolism. But a minimum level of fat addition is always necessary to reach a significant deposit of these dietary FAs in the pig's backfat. Therefore, these kinds of studies can be useful for the formulation of diets aimed to control pig fat composition.

In this paper, we present the results and conclusions of the analysis of longitudinal data on the FA profile of pigs subjected to fat-enriched diets in order to examine progressive changes. Four diets with increasing amounts of added fat (0%, 2%, 4% and 8% of a high-linoleic acid fat blend) were administered to a sample of 112 pigs of four breeds and the effects of diet and breed on FA profile were examined. We studied the incorporation rate of dietary FA into backfat against time in order to determine an adequate level of a polyunsaturated dietary fat, and also the influence of weight and breed. We chose a high polyunsaturated diet, specifically a high-linoleic diet, to obtain a desirable amount of PUFA in backfat, from a human nutritional point of view. The detailed presentation of results in this paper is mainly focussed on the weight and the four main components of the FA profile, linoleic acid (C18:2 n-6) palmitic acid (C16:0), oleic acid (C18:1 n-9) and stearic acid (C18:0), since, on average, they accounted for 88% of the total FAs at the end of the experiment. Nevertheless, we also include relevant results about the effects on some minor FAs. The development curve of each variable, for the different breeds and diets, was also examined. In two further papers we will give results of fatty acid and triacylglycerol composition of four different tissues, obtained from the same animals after slaughter.

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 four levels per factor. The sample size was 112, i.e., seven pigs for each of the 16 diet-breed combinations. Castrated male pigs of four breeds were used: Large White, Duroc, Landrace and a commercial crossbreed Landrace×Duroc (F1). Duroc and F1 pigs came from the same farm, while Large White and Landrace pigs were from two other farms. At the beginning, mean weight of Duroc pigs was 15.40 ± 1.60 kg (age, 62.18 ± 3.01 days), that of F1 pigs was 16.10 ± 1.57 (age, 60.93 ± 2.11 days), that of Large White was 24.74 ± 2.12 kg (age, 69.33 ± 4.07 days), and that of Landrace was 21.11 ± 2.24 (age, 70.32 ± 6.24). Animals of each breed were distributed uniformly according to their weight and original litter (avoiding littermates inside the same group) and they were fed a conventional adaptation diet during a 7-d period, before the start of the experiment. The control group (diet 1) was fed a diet that consisted of a mixture of wheat, barley and soy flour. The other three diets were obtained by adding increasing amounts of fat as follows: 2% for diet 2 and 4% for diet 3 and 8% for diet 4. The additional fat was a commercial mixture of 50% animal fat and 50% soy/sunflower acid oil. Diets were formulated to obtain minimum differences in energy and protein. A complete description of the ingredients and the composition of the four diets is given in Table 1. The experiment was carried out under controlled conditions of temperature, light, and ventilation. Animals were given ad libitum access to feed throughout the experiment, and animal weight and feed consumption were recorded with the same periodicity that biopsies were taken, and at 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, 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 as proposed by Oliver, Gispert, Tibau, and Diestre (1991).

2.2. Biopsy procedure

Samples of subcutaneous adipose tissue were taken from the area between the 3rd and 4th dorsal vertebrae, on the level of the 10th rib. To take the biopsy, we used a Czech gun with an adapted cannula (PPB-2 Biotech, Nitra, Slovakia). Measurements were always taken from whole fat thickness. All the necessary measures were taken to prevent animal discomfort during and after the process. The first biopsy (day 0) was performed at the end of the adaptation period (start of the experiment) and successively on days 22, 37, 55 and 76. All samples were stored in plastic bags at −80 °C prior to analysis.

Table 1
Ingredients and composition, including FA, of the four experimental diets

Ingredients (g/100 g)	Diet 1	Diet 2	Diet 3	Diet 4
Wheat	61.86	48.06	27.76	10.00
Barley	20.00	25.00	34.00	45.94
Wheat bran	3.00	5.12	15.00	15.00
47% Soy meal	9.50	14.50	13.94	15.44
50% Meat meal	2.00	2.00	2.00	2.00
Added fat[a]	0.00	2.00	4.00	8.00
Salt	0.50	0.50	0.50	0.58
Calcium carbonate	0.88	0.78	0.74	0.94
Bicalcium carbonate	1.10	1.16	1.20	1.28
78% Methionine	0.04	0.02	0.02	0.01
78% Lysine	0.40	0.24	0.22	0.18
Vit-mineral premix	0.07	0.07	0.63	0.63
Composition (g/100 g)				
Digestible energy[b] (kcal/kg)	3579	3579	3579	3733.9
Dry matter	88.48	88.82	89.51	90.55
Crude protein	16.4	17.5	17.9	17.5
Ash	4.78	5.56	6.18	6.88
Ether extract	2.71	4.34	7.35	10.56
Crude fibre	3.50	3.40	4.26	4.17
Fatty acids (mg/100 g)				
C14:0	16	38	66	133
C16:0	334	668	946	1729
C18:0	101	286	470	848
C16:1 n-7	12	36	59	128
C18:1 n-9 *cis*	392	917	1454	2575
C18:1 *trans*[c]	18	61	105	202
C18:2 n-6	897	1526	2140	3208
C20:4 n-6	2	4	5	12
C18:3 n-3	79	117	189	199
C20:5 n-3	5	11	13	25

[a] 50% Acid oil (soy and sunflower) and 50% animal fat.
[b] Estimated values.
[c] Total C18:1 *trans* isomers.

2.3. Reagents

All solvents were of ACS grade. Chloroform methanol and diethylether were from Panreac (Montplet & Esteban, Barcelona, Spain) and *n*-hexane was from E. Merck (Darmstadt, Germany). The other reagents were anhydrous sodium sulfate and sodium chloride (both for analysis) from Panreac and sodium (for synthesis), phenolphthalein (ACS) and boron trifluoride in methanol (14% p/v for synthesis) were from E. Merck. All the standards of fatty acid methyl esters (FAME) were supplied by Sigma Chemical Co. (St. Louis, MO), except for the dodecanoic acid methyl ester from Altech Associates Inc. (Deerfield, IL). A mixture of some FAME (PUFA-2) was supplied by Supelco Inc. (Bellefonte, PA). Except for the C20:2 n-6 methyl ester (98%), all the standards were of 99% purity.

2.4. Analytical methods

All feed samples taken were analysed for crude energy, dry matter, crude protein, ether extract, crude fibre and ash (AOAC methods 15th ed., 1990). Mean values for each diet are given in Table 1.

The determination of the FA profile was done in the lipid fraction obtained by the method of Folch, Lees, and Stanley (1957), with a few modifications. Homogeneous sample (0.5 g) was weighed and homogenized with 30 ml of chloroform/methanol mixture (2:1 v/v) by using a Polytron (PT 2000, Kinematica AG, Lucerne, Switzerland), at 20,000 rpm, for 30 s. The solid residue was re-extracted with 30 ml of the solvent mixture, and the combined organic fractions were washed with a NaCl aqueous solution (0.58%, p/v). The chloroform phase was then filtered through anhydrous sodium sulfate and evaporated to dryness. The fatty acids were determined in the fat extract, by obtaining their methyl esters (FAME) and by gas chromatographic determination according to the method of Guardiola, Codony, Rafecas, Boatella, and López (1994). The FAMEs were prepared by a double reaction, first with BF3/methanol solution and, after that, by a sodium methoxyde/methanol solution. FAMEs were then extracted with hexane and injected into a Hewlett Packard Series II 5890 gas chromatograph (FID detector), equipped with a glass precolumn (2.5 m × 0.25 mm i.d.) coated with deactivated cyanopropyl/phenyl/methyl silicone, and a glass capillary column (50 m × 0.25 mm i.d.) coated with 0.2 mm of stationary phase of 100% cyanopropyl silicone (CP Sil 88 Chrompack, Middleburg, Holland). The chromatographic conditions were: oven temperature programme, a first period of 11.2 min at a constant temperature of 177 °C, followed by a second period, increasing the temperature from 177 to 225 °C, at 7 °C/min, and by a third period of 11 min at 225 °C; injector temperature of 270 °C; detector temperature of 300 °C; inlet pressure of 24 psi; and split ratio equal to 1:30. The injected sample volumes ranged from 0.2 to 0.5 μl. A PE Nelson 1020 personal integrator was used for the acquisition and treatment of the chromatographic data. The FAs were quantified by applying relative response factors and the results were expressed as compensated area normalization. The repeatability and intermediate precision of the FA analysis were evaluated. In the repeatability assessment, six aliquots of a sample were extracted, and their FAMEs were 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 four reported FAs, expressed as %RSD (relative standard deviations in percentage scale), ranged between 1.27% and 4.16% for the repeatability, and between 0.52% and 4.45% for the intermediate precision. For the non-reported FAs, the values were similar.

2.5. Statistical methods

We applied a two-factor test of parallelism (Davis, 2002) to the five variables studied, and examined the significance given by the Wilks lambda statistic for the effects of diet and breed, and also for the interaction, diet-breed. The effects on FA variation for the four periods of the study were tested with an ordinary two-factor ANOVA *F* test. The analysis was performed with R, the public domain version implementation of the S statistical language (Venables & Ripley, 2002). A description of the methods used for the analysis of repeated measures can be found in Davis (2002).

3. Results and discussion

3.1. General

No significant differences were observed for any of the productive parameters evaluated, with respect to the factor diet. In contrast, significant differences were observed related to the breed, but only for two parameters. So, 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, *feed conversion ratio* did not show significant differences at the end of the experiment. No significant differences were observed for any of the carcass measurements.

3.2. Weight

We observed some differences in initial weight, which can be attributed to breed and farm (Fig. 1). At the beginning of the study, Large White pigs were the heaviest, but were exceeded by Landrace pigs between days 37 and 55 of treatment. Comparing the whole increase, from start to the end, the effects of breed and diet on the weight were highly significant ($p = 0.000$), whereas the interaction diet × breed was not significant ($p = 0.104$). Overall, the highest and lowest weight increases were registered in diets 3 and 1, respectively. For all diets, Duroc showed the lowest mean weight increase (from 34 kg in diet 1 to 49 kg in diet 3) and Landrace the highest (from 49 kg in diet 1 to 56 kg in diet 4). The range of the final mean weights across diets within the same breed was maximum for Landrace (18 kg) and minimum for F1 (5.5 kg). On the other hand, the analysis of the individual curves of weight variation, for each diet and breed (Fig. 1), showed that diet × breed was significant ($p = 0.025$). This interaction is represented by the higher rate of weight increase of Landrace on diet 3 with respect to the other diets, while F1 showed a similar curve for the four diets. Duroc and Large White pigs gave similar response for diets 1 and 2, and similar for diets 3 and 4, showing, for the two diet pairs, a clear difference in weight response. In addition, a correlation study, at time four (day 76), between the values of weight and the four fatty acid contents in backfat, revealed that there was a significant positive correlation ($p = 0.000$) between the weight of the animal and the backfat levels of oleic acid (0.364) and of stearic acid (0.458), and a significant negative correlation between the weight and the linoleic acid level (−0.453). Palmitic acid did not show significant correlation with the weight. These correlations were calculated after subtracting the diet and breed effects. Thus, for instance, it can be concluded that, among those of the same breed following the same diet, those with higher weight had lower linoleic acid levels and higher stearic and oleic acid levels in backfat. This higher weight must be related to a higher *de novo* synthesis in this tissue, and our results show that the increase is more due to a higher accumulation of stearic and oleic acids than to the deposit of palmitic acid.

3.3. Fatty acid composition

Results on the evolution of the four major FA can be summarized as a continuous increase of stearic acid during pig life, an increase in oleic acid in the first stages and of palmitic acid in the last stages, and all this compensated by a decrease in linoleic acid (mainly in the last stages). This competition is clear throughout the growth period, and the effect of the percentage of added dietary fat is highly relevant. Stearic and palmitic acid levels were more affected by the increase in the percentage of added dietary fat while levels of oleic acid showed a smaller change. The effect of an increase in palmitic acid was reversed as added dietary fat increased (diet with 8% added fat showed a slight decrease over time), but this effect of reversion, according to the percentage of added fat, was not observed for the increase in stearic and oleic acids levels. Finally, the levels changing most significantly corresponded to linoleic acid. In accordance with the increase in the percentage of added dietary fat (which involves increasing intakes of linoleic acid), the decrease in backfat linoleic acid levels with time tended to stabilize and, at 8% of added fat (diet 4), a slight increase was observed. In respect of the effect on Δ6-desaturase activity, the level of arachidonic acid in backfat was the best marker. Changes in the levels of this FA across time were characterised by a relevant increase during the first weeks and a clear decrease (parallel to that of linoleic acid) during the last weeks of treatment. However, when the most-fat-enriched diet (8%) was given to the pigs, the levels of arachidonic acid were maintained during the last weeks. This was confirmed by the analyses done in the carcass samples

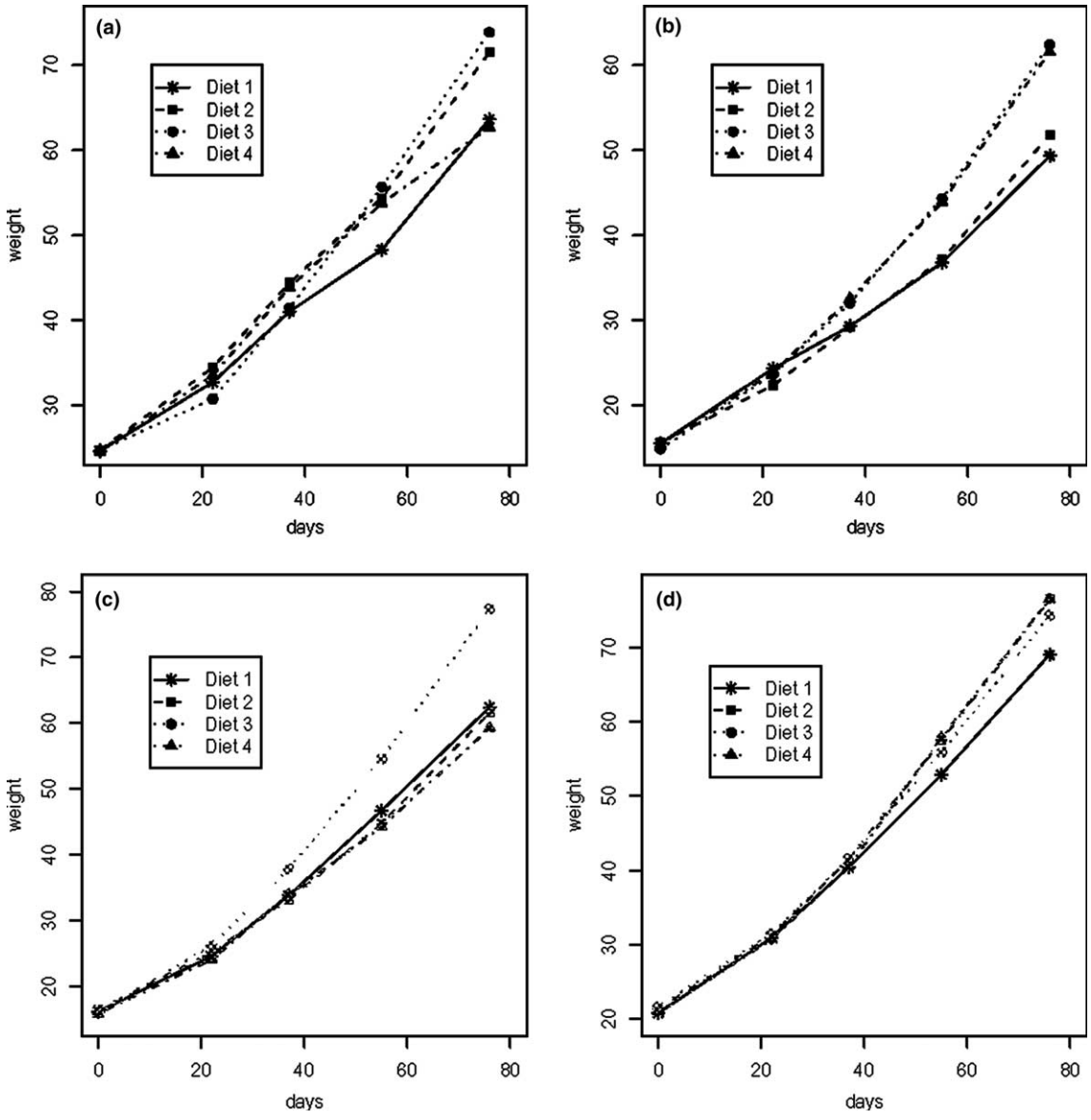

Fig. 1. Curves of weight increase (kg), corresponding to the four experimental diets (0%, 2%, 4% and 8% high-linoleic added fat), for the four breeds (a, Large White; b, Duroc; c, Landrace; d, F1).

from the same animals (Part II of this paper), where there was a statistically significant correlation between arachidonic and linoleic levels in adipose tissues.

In general, the different FA development across time were linear for diets 1 and 2. In contrast, clear curvilinear patterns occurred in most cases for diets 3 and 4 (Figs. 2(a)–(d)). Compared to diet, the factor breed showed a smaller effect. Overall, the changes in the FA profile related to breed were minimum for Duroc pigs and maximum for F1 and Landrace (Figs. 3(a)–(d)). Diet 3 curve was usually coupled to diet 4, except for Large White pigs (in order to simplify the graphical presentation, individual curves for each diet and breed are not shown, but main interaction effects are widely commented upon in detail in the text).

3.4. Linoleic acid

The effects of diet and breed on the linoleic acid profile (Figs. 2(a) and 3(a)) were highly significant ($p = 0.000$), whereas those of diet-breed interaction were moderately significant ($p = 0.030$). Diet 1 led to a decrease of linoleic acid levels (on average i.d. 6%), but this decrease was slightly compensated by diet 2, while no variation was observed in diet 3. Pigs on diet 4 showed an increase in linoleic acid levels. Non-linear behaviour was observed in diets 3 and 4, with an initial increase, followed by a decrease in the last days of the experiment. The decrease of linoleic acid levels in diets 1 and 2 was compensated by an increase in palmitic and stearic acids. Camoes et al. (1995) reported similar observations in backfat biopsies

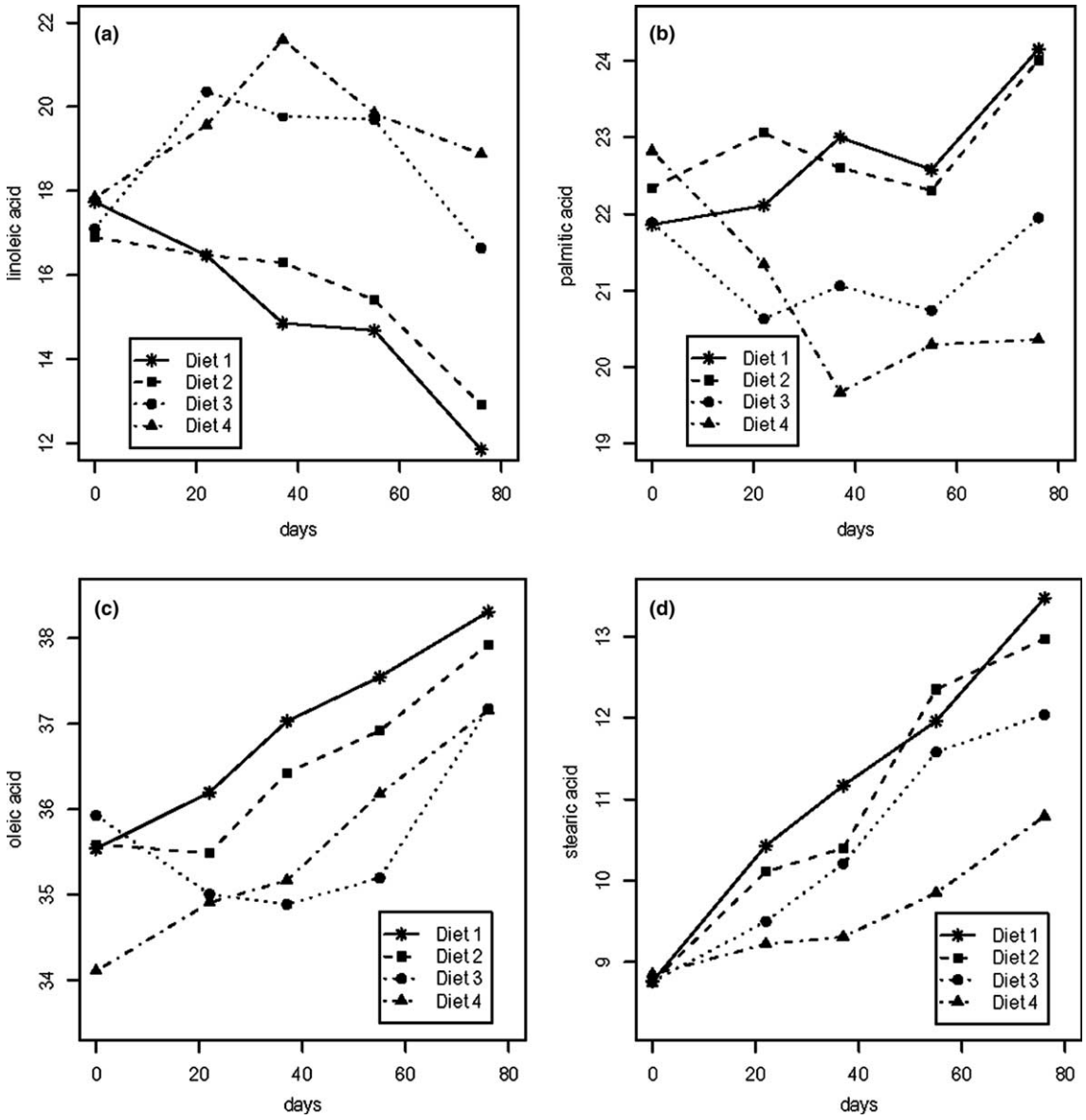

Fig. 2. Changes in backfat FA levels (a, Linoleic acid; b, Palmitic acid; c, Oleic acid; d, Stearic acid), corresponding to the four experimental diets: 0%, 2%, 4% and 8% high-linoleic added fat (data expressed as percentage of total FA).

from pigs fed linoleic acid-rich diets, followed by a period with a saturated fat diet. In this case, the levels of this acid increased and decreased with time, depending on the type of dietary fat. When a diet rich in n-3 PUFA is administered, these FAs replace linoleic acid as markers of dietary effect on the FA composition of pig tissues. This observation is supported by results from other authors (Fontanillas et al., 1998; Irie & Sakimoto, 1992; Romans, Wulf, Johnson, Libal, & Costello, 1995), who assayed dietary treatments containing linseed, linseed oil or fish oil. Pigs administered a 15% flaxseed diet, during a range of periods before slaughter, show significant differences, between all periods, in the levels of linoleic acid in backfat level, and also in other n-3 PUFAs derived from linoleic, mainly in C20:5 and C22:6 (Romans et al., 1995). After the addition of 4% of linseed oil to the feed, the linoleic and eicosapentaenoic acid levels in backfat biopsies increase greatly over time, which is mainly compensated by the decrease in n-6 PUFA levels, because SFA slightly increases and MUFA does not show significant changes (Fontanillas et al., 1998). For diets supplemented with fish oil, the best dietary markers are eicosapentaenoic and docosahexaenoic acids. In a four-week treatment with increasing percentages of fish oil, the levels of these two long-chain PUFAs in backfat biopsies clearly increase over time (Irie & Sakimoto, 1992). This increase is significant for diets containing 4% and 6% fish oil, but less so with 2% added oil. In addition, our results showed that Duroc pigs have the highest linoleic acid levels and F1 the

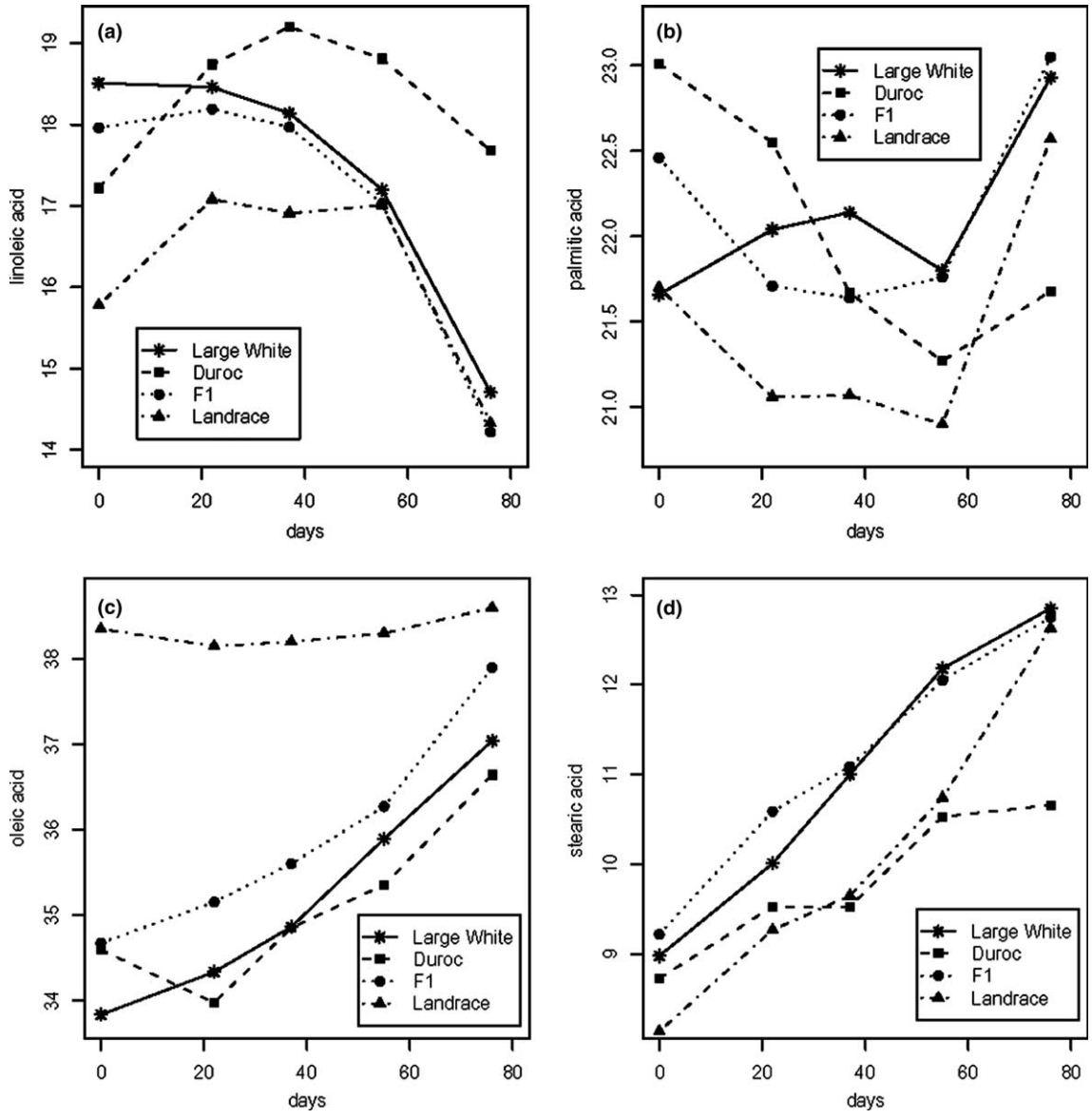

Fig. 3. Changes in backfat FA levels (a, Linoleic acid; b, Palmitic acid; c, Oleic acid; d, Stearic acid) corresponding to the four breeds: Large White, Duroc, F1 and Landrace (data expressed as percentage of total FA).

lowest ones (Fig. 3). Moreover, D and LD showed more balanced levels of linoleic acid across time, since levels started to fall between days 37 and 55. In contrast, LW and F1 seem to be less able to maintain backfat linoleic acid levels, that started to fall around day 22 of treatment. Also, an interesting diet × breed interaction effect was observed. When breed individual curves corresponding to diet 4 (the only diet able to increase the backfat final levels of linoleic acid) were analysed, only LW and D showed this increase, while LD and F1 showed flat curves without significant increases.

3.5. Palmitic acid

Like linoleic acid, the effects of diet and breed were significant for palmitic acid (Figs. 2(b) and 3(b)), but the interaction effect was non-significant ($p = 0.063$). In general, the levels of palmitic acid were negatively correlated with those of linoleic acid ($p < 0.001$), when a correlation analysis was performed, point by point. In general, we observed that curves differed mainly during the first 56 days (diets 1 and 2 increased while diets 3 and 4 decreased), but from this point all curves showed an increase, proportional to the percentage of added fat. However, except for the diet 4 curve, there were lower changes for palmitic acid than for linoleic acid levels over time. Thus, we detected decreases of 6% and 4% of linoleic acid for diets 1 and 2, respectively, against increases of 2.3% and 1.7% of palmitic acid. For diet 3, changes in these two FA levels were balanced while, for diet 4, the relative change in palmitic levels was greater than that for linoleic. These results are consistent

with those reported in another longitudinal study (Fontanillas et al., 1998). In addition, no significant changes in palmitic acid levels for three types of added dietary fat (linseed oil, olive pomace oil and hydrogenated fat) were reported by these authors. In contrast, the levels of polyunsaturated, total *trans*, stearic and oleic acids changed significantly. Similar results, showing the opposition of palmitic acid to n-3 PUFA levels, have been reported by another authors (Irie & Sakimoto, 1992), who worked with diets supplemented with flaxseed and linseed oils. Significant differences were also observed in our results for breed with respect to the evolution of palmitic acid. Duroc pigs showed a decrease in the levels of this FA, while the other three breeds showed a more balanced and similar evolution (Fig. 3(b)). In comparison with Duroc curves, for the other main FAs, this breed also shows a higher propensity to accumulate stearic acid, and these lower levels of both saturated FAs are compensated by the higher levels of linoleic acid.

3.6. Oleic acid

For oleic acid (Figs. 2(c) and 3(c)), the effects of diet and breed were moderately significant ($p = 0.018$ and 0.022, respectively), whereas the interaction effect was non-significant ($p = 0.090$). There was a general increase in oleic acid during pig life, but the increase was lower in relative terms compared to that of stearic acid. Oleic acid levels in diet 1 were the highest and also showed the maximum rate of increase, while the curves tended to a smaller increase when the percentage of added fat increased. This fact is quite interesting since from diet 1 to 4, there is an increase in the linoleic supply but also in the oleic supply. However, there is a higher effect of the diet on backfat linoleic acid deposit than on oleic acid deposit. This observation indicates that the inhibition of the Δ9-desaturase activity rose when increasing amounts of linoleic fatty acids were given in the diet, although similar increases in oleic acid intake occurred simultaneously. This inhibition of the Δ9-desaturase activity was supported by the palmitoleic acid curves (Fig. 4(b)) which also showed lower backfat levels for diets 3 and 4, and included higher linoleic acid concentrations. Some authors (Díaz, García-Regueiro, Casillas, & de Pedro, 1996; Fontanillas et al., 1998; Tejeda, Gandemer, Antequera, Viau, & García, 2002) showed that diets, particularly rich in oleic acid and low in PUFA, can increase the levels of oleic acid in backfat, while slight decreases or no significant changes are observed upon giving polyunsaturated diets (Fontanillas et al., 1998; Irie & Sakimoto, 1992). Oleic acid levels slightly increased in all breeds over time, but our results showed significant differences in oleic acid levels among breeds. Landrace showed higher levels than the rest (D, LW and F1), among which there were no significant differences. The levels of oleic acid in Landrace pigs were already much higher at the beginning of the experimental treatment than levels observed in the other breeds. But the fact that the high oleic acid levels in Landrace also increase over time can only be explained by a higher activity of the Δ9-desaturase, related to the genetic type. The relevance of the factor breed in the control of oleic acid level in backfat is also supported by our results showing that differences among breeds (Fig. 3(c)) were higher than differences between diets (Fig. 2(c)).

3.7. Stearic acid

For stearic acid, both effects of diet (Fig. 2(d)) and breed (Fig. 3(d)) were highly significant ($p = 0.000$), whereas interaction was only moderately significant ($p = 0.040$). A consistent linear increase in levels of this acid was observed during pig life. In relative terms, at the end of the experiment, there were similar differences between diet 1 and diet 4 for stearic acid levels as for palmitic acid levels (4–5%). However, development curves were quite different, because stearic acid curves always increased over time, while palmitic acid showed increasing curves for diets 1 and 2, but decreasing curves for diets 3 and 4 (Fig. 2). This effect is in accordance with the observation (commented upon above) that increasing percentages of polyunsaturated fats are able to inhibit Δ9-desaturase, which could lead to accumulation of stearic acid as the substrate of this enzyme. Moreover, while pigs on diet 4 (8% added fat) showed a development curve similar to that of diet 3 for palmitic and oleic acids, the stearic acid curve for diet 4 clearly diverged from the other three curves and showed a much lower rate of increase. This indicates that a critical level of added fat existed between diets 3 and 4, which induced a higher activation in the *de novo* synthesis. In other studies (Fontanillas et al., 1998; Irie & Sakimoto, 1992), a similar effect was observed, since stearic acid always shows a higher rate of increase in backfat with time than did other FAs. However, this increase was greater for high-polyunsaturated and saturated diets than for monounsaturated ones (olive pomace oil), where the rise in oleic acid compensated for part of the increase in stearic acid (Fontanillas et al., 1998). Furthermore, there was a lower increase in stearic acid for the Duroc breed at the end of the experiment with respect to the other three breeds, which showed similar development curves (Fig. 3(d)). The significant interaction found between diet and breed indicates that, for LW, the stearic acid curves were not different according to the diet, while the D, LD and F1 diet curves showed a higher rate of increase for diet 4 than for the other three diets.

3.8. Minor fatty acids

For the minor components of the FA profile, variation was much higher in relative terms but, in most

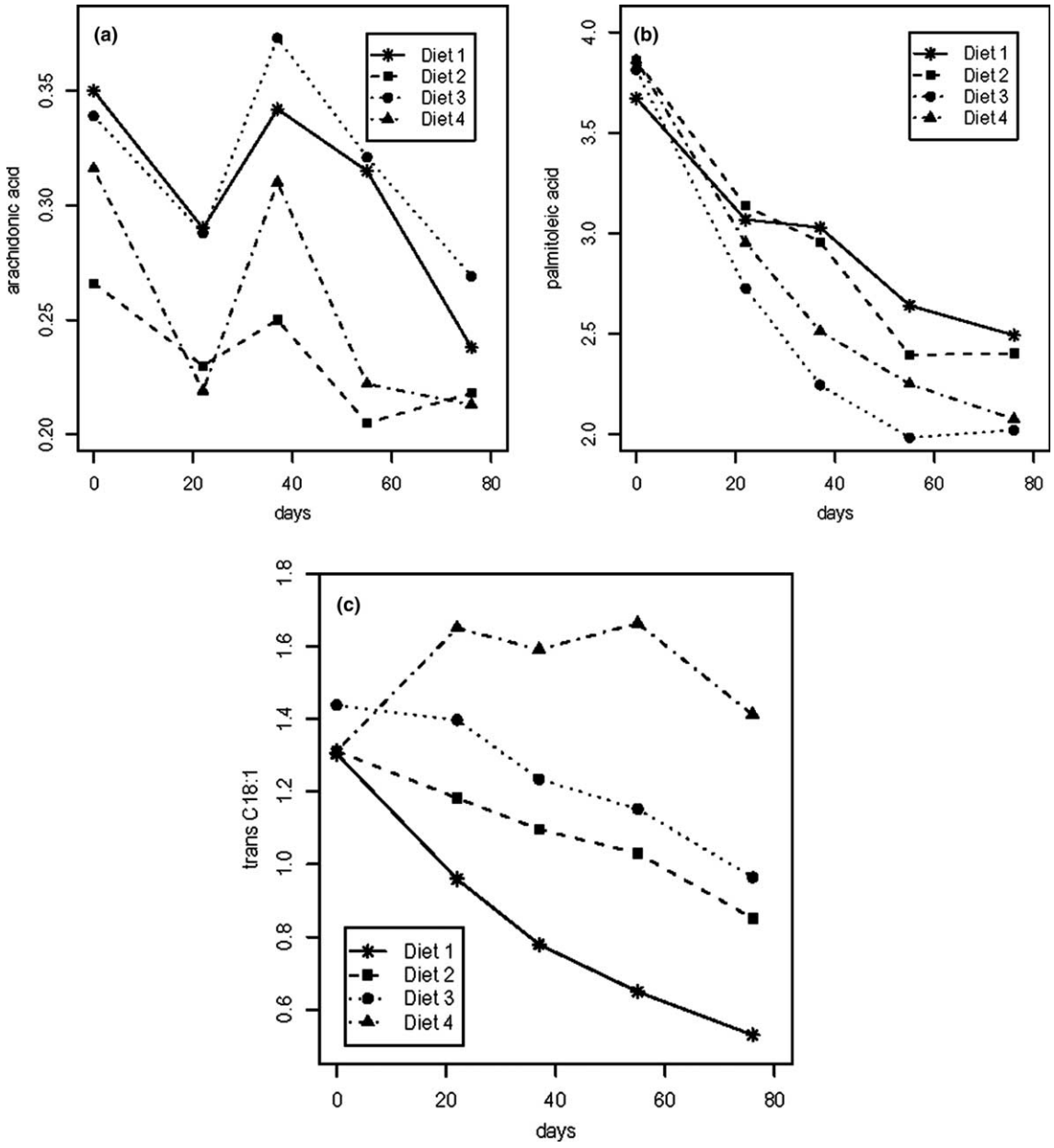

Fig. 4. Changes in backfat levels of arachidonic acid (a), palmitoleic acid (b) and total *trans* C18:1, corresponding to the four experimental diets: 0%, 2%, 4% and 8% high-linoleic added fat (data expressed as percentage of total FA).

cases, there were no clear differences between diets, with the exception of palmitoleic, and arachidonic acids, and *trans* FA (Fig. 4). So, the levels of palmitoleic acid presented a consistent linear decrease during the pig's life and the range of the group (breed × diet) means was from 4.4 at the start to 1.8% at the end. This trend was increased by fat-enriched diets, showing the above-mentioned inhibitory effect on the Δ9-desaturase. Moreover, Duroc pigs showed significantly higher levels of palmitoleic acid than the other breeds at the end of the experiment. Arachidonic acid showed a particular profile, since it increased during the first 22 days, peaked at day 37, and then decreased at a low rate. This last decrease was almost totally compensated by the administration of the most fat-enriched diet (8%) in a similar way to that observed for linoleic acid levels. This parallelism indicates the obvious dependence of arachidonic acid levels on its synthesis from linoleic acid, since the four diets supply very small amounts of arachidonic acid. Differences related to the breed can be summarized as a higher rate of arachidonic acid synthesis throughout the growth period for Large White, which is the only breed not showing decreasing backfat levels at the end of the experiment. Finally, a pronounced dietary effect was also observed for *trans* FA levels (Fig. 4(c)). These levels decreased consistently to less than one half of

the initial value in diet 1 while, for diets 2 and 3, they showed more moderate decreases. For diet 4, a slight increase was obtained at day 76. The intensity of this effect is not relevant since our diets have low *trans* FA levels but, in a study carried out in pigs fed hydrogenated fat-enriched diets, the level of total *trans* MUFA showed a very high and constant increase, compared with the slight decrease observed when polyunsaturated or monounsaturated diets were given to animals (Fontanillas et al., 1998).

4. Conclusions

The aim to find the most adequate diet and breed for a higher linoleic and less saturated FA pork meat, shows that diet 3 (supplying 2 g C18:2 per 100 g feed) marks a reference point. First, pigs on diet 3 in all cases showed the highest rate of weight increase. Moreover, with respect to backfat FA deposit, diets 1 and 2 showed similar curves, while diet 3 showed clear separate curves for all FAs. This change from diet 2 to 3 is the most relevant when all FA are compared, since the change observed between diets 3 and 4 is only relevant in some cases. However, the higher linoleic increase rate for diet 4 than for 3, indicates that diet 3 is not enough to avoid a certain linoleic acid decrease in backfat during pig life. This difference between diets 3 and 4 is also clear for stearic and palmitic acids, which showed a higher rate of decrease for diet 4, compensating for the higher increase observed for the linoleic acid. Another interesting conclusion is that, in spite of the breed, there is a significant inverse correlation between the weight and the backfat linoleic content. This suggests that it is necessary to undertake studies reaching higher weights, in order to quantify the level of decrease in backfat linoleic acid according to the weight. Also interesting are the effects of the factor breed, particularly on the weight and the linoleic acid deposit curves. The experimental diets, on the four breeds studied, showed a clear differentiation for the Landrace curves, and diet 3 showed the highest weight increase rate. In contrast, F1 crossbreed is the least affected by the diet, since its four diet curves were quite coincident. Duroc is the breed showing the highest rate of linoleic increase, while the other three breeds showed similar and quite lower rates. Obviously, this higher linoleic increase for Duroc is compensated by a significantly higher decrease in palmitic and stearic acid levels for this breed than for the others, while oleic acid levels are less variable.

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References

Ahn, D. U., Lutz, S., & Sim, J. S. (1996). Effects of dietary α-linoleic acid on the fatty acid composition, storage stability and sensory characteristics of pork loin. *Meat Science, 43*, 291–299.

A.O.A.C. (1990). *Official methods of analysis of the AOAC* (15th ed.). Arlington, VA: AOAC International.

Averette Gatlin, L., See, M. T., Hansen, J. A., Sutton, D., & Odle, J. (2002). The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *Journal of Animal Science, 80*, 1606–1615.

Camoes, J., Mourot, J., Kouba, M., Cherot, P., & Mounier, A. (1995). Effets de régimes à teneurs variables en acide linoléique sur les caractéristiques des tissus adipeux. *Journées de la Recherche Porcine in France, 27*, 291–296.

D'Arrigo, M., Hoz, L., López-Bote, C. J., Cambero, I., Pin, C., Rey, A. I., et al. (2002). Effect of dietary linseed oil and α-tocopherol on selected properties of pig fat. *Canadian Journal of Animal Science, 82*, 339–346.

Davis, C. S. (2002). *Statistical methods for the analysis of repeated measurements*. NY, USA: Springer.

Díaz, I., García-Regueiro, J. A., Casillas, M., & de Pedro, E. (1996). Triglyceride composition of fresh ham fat from Iberian pigs produced with different systems of animal nutrition. *Food Chemistry, 55*, 383–387.

Eder, K., Nonn, H., & Kluge, H. (2001). The fatty acid composition of lipids from muscle and adipose tissues of pigs fed various oil mixtures differing in their ratio between oleic acid and linoleic acid. *European Journal of Lipid Science and Technology, 103*, 668–676.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Fontanillas, R., Barroeta, A., Baucells, M. D., & Guardiola, F. (1998). Backfat fatty acid evolution in swine fed diets high in either *cis*-monounsaturated, trans, or (n-3) fats. *Journal of Animal Science, 76*, 1045–1055.

Guardiola, F., Codony, R., Rafecas, M., Boatella, J., & López, A. (1994). Fatty acid composition and nutritional value of fresh eggs, from large- and small-scale farms. *Journal of Food Composition and Analysis, 7*, 171–178.

Irie, M., & Sakimoto, M. (1992). Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *Journal of Animal Science, 70*, 470–477.

Oliver, M. A., Gispert, M., Tibau, J., & Diestre, A. (1991). The measurement of light scattering and electrical conductivity for the prediction of PSE pig meat at various times post mortem. *Meat Science, 29*, 141–151.

Romans, J. R., Wulf, D. M., Johnson, R. C., Libal, G. W., & Costello, W. J. (1995). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II: duration of 15% dietary flaxseed. *Journal of Animal Science, 73*, 1987–1999.

Scheeder, M. R. L., Gläser, K. R., Eichenberger, B., & Wenk, C. (2000). Influence of different fats in pig feed on fatty acid composition of phospholipids and physical meat quality. *European Journal of Lipid Science and Technology, 102*, 391–401.

Tejeda, J. F., Gandemer, G., Antequera, T., Viau, M., & García, C. (2002). Lipid traits of muscle as related to genotype and fattening diet in Iberian pigs: total intramuscular lipids and triacylglycerols. *Meat Science, 60*, 357–363.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. NY, USA: Springer.

Warnants, N., Van Oeckel, M. J., & Boucqué, C. V. (1999). Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. *Journal of Animal Science, 77*, 2478–2490.

Wood, J. D., Buxton, P. S., Whittington, F. M., & Enser, M. (1986). The chemical composition of fat tissues in the pig: effect of castration and feeding treatment. *Livestock Production Science, 15*, 73–82.