Fatty Acid Content in Chicken Thigh and Breast as Affected by Dietary Polyunsaturation Level

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ABSTRACT One hundred ninety-two female broiler chickens were randomly distributed into 16 experimental treatments as a result of the combination of 4 levels of dietary polyunsaturated fatty acids (PUFA) (15, 34, 45, and 61 g/kg) and 4 levels of supplementation with α-tocopheryl acetate (α-TA) (0, 100, 200, and 400 mg/kg), to determine the modification of the amount and type of fatty acids (FA) deposited in raw and cooked chicken tissues. At 44 d, quantified FA of thighs and breasts were not affected by dietary supplementation with α-TA. Total FA content of breast was less than 15% of the total FA content of thigh. However, increasing the PUFA content of the diet by 46 g, from 15 to 61 g/kg, decreased total FA of thigh 17%, but did not affect FA content in breast meat. Monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) content of thigh (y) decreased linearly as the inclusion of dietary PUFA (x) increased (MUFA: $y = 89.34 - 0.92x$, $R^2 = 0.70$; SFA: $y = 53.81 - 0.43x$, $R^2 = 0.57$), whereas the relationship between PUFA content of feed (x) and thighs (y) was exponential ($y = 92.03 - 92.03e^{(-0.0155x)}$, $R^2 = 0.75$). A similar response was observed in breast, with less variation and more incorporation of PUFA than thigh. Cooking of thigh meat led to a reduction in total FA content that affected SFA, MUFA, and PUFA in a similar proportion.

(*Key words*: polyunsaturated fat, thigh and breast, α-tocopherol, cooking process, chicken)

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INTRODUCTION

There are 2 reasons for the increasing level of polyunsaturation in chicken meat. First, human nutritionists recommend reducing the intake of saturated fatty acids (SFA) because of its relationship with the development of cardiovascular diseases (Krauss et al., 2001). Secondly, the use of animal fats has been reduced in Europe, in favor of vegetable oils that are more polyunsaturated.

Many authors have studied how the inclusion of different fat sources in the broiler's diet affect 1) the proportion of fatty acids (FA), mainly polyunsaturated fatty acids (PUFA), in meat (Scaife et al., 1994; Hrdinka et al., 1996; López-Ferrer et al., 1999a,b), and 2) the amount of fat deposited by the birds (Sanz et al., 1999, 2000a; Crespo and Esteve-García, 2001, 2002a,b). However, there are few reports on the effect of increasing levels of dietary PUFA on the amount and type of FA deposited in chicken tissues, especially in the edible portions.

An increase in the degree of polyunsaturation of meat may enhance the development of organoleptic problems (Ajuyah et al., 1993; González-Esquerra and Leeson, 2000; Bou et al., 2001) and lead to an increased susceptibility to lipid oxidation (Klaus et al., 1995; Cortinas et al., 2001; Grau et al., 2001a,b). Supplementation with α-tocopherol has proven to be an effective measure to prevent lipid oxidation (Lin et al., 1989; Ahn et al., 1995; Cortinas et al., 2001; Grau et al., 2001a,b) and to improve sensory quality (O'Neill et al., 1998, Bou et al., 2001) of poultry meat. Because α-tocopherol protects PUFA from lipid oxidation, its inclusion in the birds' diet may result in a higher deposition of PUFA in poultry tissues. However, the effect of tocopherol supplementation on FA in poultry has rarely been studied and is rather controversial (Bou et al., 2004).

Cooking of meat may lead to loss or alteration of FA, especially PUFA. However, data regarding the effect of cooking processes of chicken meat on the amount and type of FA are inconsistent (Dawson et al., 1990; López-Ferrer et al., 1999c; Grau et al., 2001a,b). Most of the reports do not express the FA composition of chicken meat as amount but rather as profile (percentage of total FA), which may not allow observation of the real FA losses produced by cooking.

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Abbreviation Key: EPA = eicosapentanoic acid; DHA = docosahexaenoic acid; FA = fatty acids; LNA = linolenic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; α-TA = α-tocopherol acetate.

TABLE 1. Composition and chemical analysis of the diets[1]

Ingredients	%
Wheat	39.30
Soybean meal 48% CP	34.09
Barley	13.39
Added fat	9.00
Bicalcium phosphate	2.17
Calcium carbonate	0.98
Salt	0.45
Vitamin-mineral mix[2]	0.40
DL-Methionine	0.28
L-Lysine	0.04
Chemical analysis	
Dry matter	90.78
Crude protein	22.98
Crude fat	10.17
Crude fiber	3.47
Ash content	6.08
Crude energy (kcal/kg)	4,481
Metabolizable energy[3] (kcal/kg)	3,100

[1]Values given in this table are means of 16 dietary treatments result of a 4 × 4 factorial design with 4 different proportions of tallow, linseed, and fish oil, and 4 different levels of dietary supplementation with α-tocopheryl acetate (0, 100, 200, and 400 mg/kg).

[2]Vitamin and mineral mix per kilogram of feed: vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin K_3, 3 mg; vitamin B_1, 2.2 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 11 μg; folic acid, 1.5 mg; biotin, 150 μg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg.

[3]Estimated value.

The objective of the present study was to determine the effects of diets containing increasing amounts of PUFA and different levels of supplementation with α-tocopheryl acetate (α-TA) on the amount and type of FA deposited in marketable raw and cooked chicken meat.

MATERIALS AND METHODS

Birds and Diets

The experiment received prior approval from the Animal Protocol Review Committee of the Universitat Autònoma of Barcelona, and all animal housing and husbandry conformed to European Union guidelines.

One hundred and ninety-two Ross female broilers at 1 d of age were randomly distributed into 16 dietary treatments with 3 replicates each. The birds were housed in groups of 4 in 48 cages under standard conditions of temperature, humidity, and ventilation. The diet was formulated according to requirements recommended by the NRC (1994) on the basis of 39% wheat, 34% soy, and 13% barley (Table 1). The 16 experimental treatments resulted from a 4 × 4 factorial arrangement, where the 2 variation factors were the level of dietary PUFA: 15 (PU15), 34 (PU34), 45 (PU45), and 61 (PU61) g of PUFA/kg of feed, and the level of supplementation with α-TA:[2] 0 (E0), 100 (E1), 200 (E2), and 400 (E4) mg of α-TA/kg of feed. The gradient of dietary PUFA was achieved by blending different quantities of tallow,[3] linseed oil[3] and fish oil[4] [eicosapentanoic acid (EPA), 20%; docosahexanoic acid (DHA), 7%], keeping the added fat content of the diets constant (9%) (Table 2).

Feed and water were provided ad libitum. Body weight and feed consumption were measured 3 times during the experimental period. Feed samples were taken 3 times during the experiment for analysis.

Sample Collection

After 44 d of age, 2 animals per cage were randomly selected and killed in a commercial slaughterhouse. The usual edible portions of thighs and breasts were removed and weighed individually. Thighs were deboned and ground with skin. Two homogeneous 30-g samples were packed in Cryovac CN300 bags.[5] One portion of thigh was analyzed raw, and the other was cooked in a water bath under gentle agitation using a Unitronic 320 OR,[6] at 80°C for 30 min. Internal temperature was measured by placing a thermometer in the center of a portion before cooking. Breast portions were ground without skin. Tissue samples were freeze-dried, ground, and stored at –20°C until further analyses.

Fatty Acid Content

Fatty acid content of feeds was determined following the methodology described by Sukhija and Palmquist (1988). Thigh and breast samples were analyzed as described previously by Carrapiso et al. (2000). Nonadecanoic acid (C19)[7] was used as internal standard. The FA content was determined using a gas chromatograph HP6890[8] equipped with a flame ionization detector and an HP 19091 to 136 capillary column[9] (60 m × 0.25 mm internal diameter) with a film thickness (0.25 μm) of stationary phase. Helium was used as carrier gas. Oven temperature was programmed as follows: from 140 to 160°C at 1.50°C/min; from 160 to 180°C at 0.50°C/min; and from 180 to 230°C at 2.50°C/min. The other chromatographic conditions were: injector and detector temperatures, 280°C; sample volume injected, 1 μL. Fatty acids were identified by matching their retention times with those of their relative standards, as well as by mass spectrometry (HP5973)[8] of each peak.

α-Tocopherol

α-Tocopherol from feeds was extracted as described previously by Jensen et al. (1999) starting from 2 g of feed sample.

Statistics

Multifactorial ANOVA with repeated measures (n = 96) was performed to determine whether the input factors

[2]Rovimix E-50 Adsorbate, F. Hoffmann-La Roche Ltd., Basel, Switzerland.
[3]Cailá-Parés S.A., Barcelona, Spain.
[4]Agrupación de Fabricantes de Aceites Marinos, S.A., Vigo, Spain.
[5]Cryovac, Sant Boi de Liobregat, Spain.
[6]J. P. Selecta, S.A., Abrera, Spain.
[7]Sigma-Aldrich Chemical Co., St. Louis, MO.
[8]Agilent, Waldbronn, Germany.
[9]Hewlett Packard, Newtown, PA.

TABLE 2. Inclusion levels of dietary fat and oils in experimental diets (expressed as g/kg of feed) and dietary polyunsaturation level achieved [expressed as g of polyunsaturated fatty acids (PUFA)/kg of feed]

Dietary treatment[1]	Fat or oil source (g/kg of feed)			Dietary polyunsaturation level (g of PUFA/kg of feed)
	Tallow	Linseed oil	Fish oil	
PU15	90	0	0	15
PU34	55	30	5	34
PU45	35	45	10	45
PU61	0	70	20	61

[1]PU15 = 15 g PUFA/kg of feed; PU34 = 34 g PUFA/kg of feed; PU45 = 45 g PUFA/kg of feed; PU61 = 61g PUFA/kg of feed.

(dietary PUFA and α-TA) affected the FA content of chicken thighs and breasts. ANOVA (n = 192) was also used to determine whether the processing (cooking) of thighs affected its FA content. Data were treated using the PROC MIXED procedure of SAS (SAS Institute, 2000). Differences between treatment means were evaluated using Tukey's correction for multiple comparisons. The evolution of total SFA and monounsaturated fatty acids (MUFA) (n = 96) in thigh and breast was fitted by linear regression analysis. The evolution of total PUFA (n = 96) in thigh and breast was fitted by an exponential equation of type $y = a - ae^{(-bx)}$, where a is the maximum level that can be reached and b is the accretion fractional rate. The fit was performed using nonlinear regressions by means of the NLIN procedure of SAS software (SAS Institute, 2000). The comparative response of PUFA content between thigh and breast to variation in dietary PUFA content was assessed by the likelihood ratio test (n = 192). In all cases, $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Diet Composition

The FA composition of the experimental diets is shown in Table 3. The greatest differences among treatments were found for the PUFA content. Increasing the polyunsaturation level of a diet and keeping the total FA content constant was achieved by reducing its SFA and MUFA. Thus, dietary PUFA to SFA ratio (PUFA:SFA) increased with the dietary polyunsaturation level.

Supplementation with 100, 200, or 400 mg of α-TA/kg of feed resulted in dietary levels of α-tocopherol that matched the amounts added. Thus, α-tocopherol content of diets were 6 ± 0.6, 136 ± 1.5, 236 ± 14.5, and 451 ± 18.1 g/kg for E0, E1, E2, and E4 treatments, respectively.

Effect of Dietary α-TA

Body weight (2,340 ± 54.4 g), dressing percentage (82 ± 0.5%) and thigh (481 ± 13.9 g) and breast (377 ± 15.9 g) weights were not affected by the dietary supplementation with different levels of α-TA. Similarly, the FA contents of thigh and breast were not modified by α-TA level (Tables 4 and 5). The effect of α-TA supplementation on FA composition of chicken tissues is rather controversial. In most published reports, results are expressed as percentages (area normalization), which makes comparison of the results difficult. Some authors observed a higher proportion of some long-chain ω-3 PUFA in thighs (Ahn et al., 1995; Surai and Sparks, 2000) and breasts (Ajuyah et al., 1993; Cherian et al., 1996; Nam et al., 1997; Zanini et al., 2003), as well as in other chicken tissues such as testes, heart, and cerebellum (Surai and Sparks, 2000) when chicken diets were supplemented with tocopherols. It may be because α-tocopherol protects PUFA from oxidation, avoiding their loss. Furthermore, α-tocopherol promotes FA synthesis, because α-tocopherol quinone, a product of tocopherol metabolism, has been suggested as an essential enzyme cofactor for the FA desaturases (Infante, 1999). Other authors have reported a lower proportion of some ω-3 PUFA, such as linolenic acid (LNA), in thighs when α-TA was added to the diet (Nam et al., 1997). It has been suggested that there may be interference in the intestinal absorption between PUFA and α-tocopherol (Gallo-Torres et al., 1971). However, recent studies have shown that α-tocopherol does not interfere with intestinal absorption of PUFA (Tijburg et al., 1997; Villaverde et al., 2004). Some authors have also found no variation in FA composition of chicken thighs when broiler diets were supplemented with tocopherols (Lin et al., 1989; Ajuyah et al., 1993; Cherian et al., 1996; O'Neill et al., 1998; Bou et al., 2004). In the present study, FA were quantified with the aid of an internal standard to avoid the inaccuracy associated with area normalization; no differences were found on FA content of thigh (60.0 vs. 53.9 g of PUFA/kg of thigh for treatments PU61 supplemented with 0 and 400 mg of α-TA/kg of feed, respectively, $P \geq 0.05$) and breast (7.5 vs. 8.0 g of PUFA/kg of breast for treatments PU61 supplemented with 0 and 400 mg of α-TA/kg of feed, respectively, $P \geq 0.05$) depending on the level of supplementation with α-TA. Using this methodology, it was concluded that the effect of supplementing broiler diets with α-TA up to 400 mg/kg of feed did not have any effect on thigh and breast FA content.

Effect of Dietary Polyunsaturation

Body weight, dressing percentage, and thigh and breast weights were not modified by dietary polyunsaturation level. However, when thigh weight was expressed as percentage of the carcass, it showed a tendency to increase

TABLE 3. Fatty acid composition of the experimental diets (expressed as g/kg)[1]

	Polyunsaturation level[3]			
Fatty acid[2]	PU15	PU34	PU45	PU61
Total FA	100.45	98.81	99.57	96.89
Total SFA	43.75	32.38	26.22	15.74
C10:0	0.05	0.03	0.02	0.00
C14:0	2.72	2.01	1.79	1.45
C15:0	0.44	0.30	0.23	0.11
C16:0	23.80	18.15	15.25	10.31
C17:0	1.19	0.77	0.53	0.14
C18:0	14.64	10.23	7.68	3.33
C20:0	0.12	0.16	0.17	0.17
Total MUFA	41.30	32.55	28.32	20.31
C16:1t	0.20	0.15	0.12	0.07
C16:1	2.25	1.73	1.65	1.52
C18:1 ω9[4]	35.62	27.76	23.59	15.69
C18:1 ω7t	1.60	1.37	1.29	1.12
C20:1	0.28	0.29	0.31	0.35
C24:1	0.09	0.46	0.81	1.46
Total PUFA	15.40	33.77	45.03	60.84
C18:2 ω6	13.16	16.23	17.98	20.17
C18:3 ω3	1.55	16.45	24.62	36.27
C18:4 ω3	0.27	0.11	0.23	0.43
C20:4 ω6	ND[5]	ND	0.13	0.19
C20:5 ω3	ND	0.81	1.77	3.35
C22:6 ω3	ND	0.07	0.18	0.33
PUFA:SFA	0.35	1.04	1.72	3.87

[1]Values given in this table are means of 4 dietary treatments with different levels of supplementation with α-tocopheryl acetate: 0, 100, 200, and 400 mg/kg.

[2]FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[3]PU15 = 15 g of PUFA/kg of feed; PU34 = 34 g of PUFA/kg of feed; PU45 = 45 g of PUFA/kg of feed; PU61 = 61 g of PUFA/kg of feed.

[4]C18:1 ω9 includes sum of *cis* and *trans* forms.

[5]ND = Not detected.

as dietary polyunsaturation increased (PU15: 24.7, PU34: 24.6, PU45: 25.2 and PU61: 25.4 g thigh/100 g carcass, $P = 0.076$). A similar difference in thigh proportion was reported by López-Ferrer et al. (1999b, 2001) who observed increases of 2.7 and 2.3% in thigh proportion when fish oil replaced rapeseed oil or tallow, respectively. Other authors did not find any differences in thigh proportion between chickens fed tallow or different vegetable oils with a lower content of very long chain ω-3 PUFA (Olomu and Baracos, 1991; Crespo and Esteve-García, 2001).

Total FA content in thigh meat, which included subcutaneous fat, reduced as the dietary polyunsaturation level increased (from 141.2 to 116.8g/kg, in PU15 and PU61 treatments, respectively, $P \leq 0.05$) (Table 4). These results agree with other studies showing that separable fat depots (Crespo and Esteve-García, 2002a), and specifically abdominal fat (Vilà and Esteve-García, 1996; Sanz et al., 1999; Crespo and Esteve-García, 2001, 2002a,b; Villaverde et al., 2003a), are reduced with the addition of unsaturated oils to the diets. It seems that fat deposits (subcutaneous and abdominal) may easily be influenced by the polyunsaturation level of the diet. The mechanism by which dietary polyunsaturation modifies body fat deposition is not completely understood. Some authors have suggested that the lower fat deposition in broilers fed polyunsaturated fats compared with those fed saturated fats was, in part, explained by an increased rate of lipid catabolism and by a decrease of FA synthesis (Sanz et al., 2000b). Similarly, other authors reported significantly lower metabolic oxidation of lipids and consequently a lower thermogenesis in tissues of rats fed saturated fats than in rats fed unsaturated fats (Shimomura et al., 1990; Wilson et al., 1990). Nevertheless, further studies are needed on why body fat deposition is reduced as dietary polyunsaturation increases.

Total FA content of breast (18.1 g/kg) was not affected by dietary polyunsaturation level (Table 5). There are contradictory reports on lipid content in breast. Some authors showed that the dietary polyunsaturation level of fat does not influence intramuscular lipid content of breast (Scaife et al., 1994; Crespo and Esteve-García, 2001), but Kirchgessner et al. (1993) and Ajuyah et al. (1991) found a higher fat content in breast muscle with increasing levels of PUFA in the diet. However, other authors found lower lipid content of breast of chickens fed diets enriched with polyunsaturated oils (Sanz et al., 1999). Such discrepant findings in intramuscular fat content of breast muscles may be attributed to several factors, such as the analytical procedure used to extract fat from samples. Recent studies showed that fat content of tissues in more polyunsaturated treatments was underestimated when lipid contents were analyzed using AOAC (1995) methodology, suggesting total FA content as an estimator of crude fat in highly polyunsaturated samples (Vil-

TABLE 4. Effect of dietary polyunsaturation and α-tocopheryl acetate (α-TA) supplementation on the fatty acid (FA) content of thighs with skin (expressed as g/kg)[1]

Fatty acid[2]	Polyunsaturation level[3]				Significance			
	PU15	PU34	PU45	PU61	PUFA	α-TA	PUFA × α-TA	SE
Total FA	141.22[a]	132.74[ab]	132.48[ab]	116.80[b]	*	NS	NS	4.932
Total SFA	46.79[a]	38.95[b]	35.68[b]	27.12[c]	***	NS	NS	1.559
C10:0	0.03	0.02	0.02	0.02	NS	NS	NS	0.002
C12:0	0.07[a]	0.06[b]	0.05[b]	0.04[c]	***	NS	NS	0.002
C14:0	2.93[a]	2.14[b]	1.93[b]	1.51[c]	***	NS	NS	0.075
C15:0	0.46[a]	0.35[b]	0.30[c]	0.19[d]	***	NS	NS	0.012
C16:0	29.97[a]	24.73[b]	23.00[b]	17.29[c]	***	NS	NS	1.038
C17:0	0.93[a]	0.75[b]	0.61[c]	0.31[d]	***	NS	NS	0.028
C18:0	11.22[a]	10.19[ab]	9.23[b]	7.33[c]	***	NS	NS	0.430
C20:0	0.16[a]	0.09[b]	0.09[b]	0.09[b]	***	*	NS	0.008
Total MUFA	76.37[a]	55.29[b]	49.21[b]	34.04[c]	***	NS	NS	2.254
C14:1	0.69[a]	0.42[b]	0.33[c]	0.14[d]	***	NS	*	0.020
C16:1t	0.90[a]	0.67[b]	0.62[b]	0.44[c]	***	NS	NS	0.026
C16:1	6.41[a]	4.78[b]	4.39[b]	3.27[c]	***	NS	*	0.233
C18:1 ω9[4]	61.39[a]	43.73[b]	39.19[b]	26.56[c]	***	NS	NS	1.859
C18:1 ω7t	3.55[a]	2.19[b]	2.06[b]	1.64[c]	***	NS	NS	0.108
C20:1	0.52[a]	0.35[b]	0.33[b]	0.31[b]	***	NS	NS	0.015
C24:1	0.18[d]	0.83[c]	1.00[b]	1.17[a]	***	NS	NS	0.043
Total PUFA	17.91[a]	38.54[b]	47.70[c]	55.66[d]	***	NS	NS	2.212
C18:2tt	0.20[a]	0.14[b]	0.12[b]	0.05[c]	***	NS	NS	0.009
C18:2 ω6	13.72[c]	17.00[b]	18.41[ab]	19.09[a]	***	NS	NS	0.560
C18:3 ω3	1.97[d]	18.19[c]	25.25[b]	31.43[a]	***	NS	NS	1.580
C18:4 ω3	0.50[a]	0.30[b]	0.19[c]	0.48[a]	***	NS	NS	0.027
C20:2 ω6	0.12	0.12	0.12	0.12	NS	NS	NS	0.007
C20:3 ω6	0.16	0.15	0.15	0.15	NS	NS	NS	0.005
C20:4 ω6	0.75[a]	0.58[b]	0.52[b]	0.51[b]	***	NS	NS	0.018
C20:5 ω3	0.07[d]	1.06[c]	1.69[b]	2.39[a]	***	NS	NS	0.129
C22:4 ω6	0.06[a]	0.01[b]	0.03[ab]	0.06[ab]	*	NS	NS	0.011
C22:6 ω3	0.17[d]	0.73[c]	0.92[b]	1.13[a]	***	NS	NS	0.046
PUFA:SFA	0.39[d]	1.01[c]	1.34[b]	2.09[a]	***	NS	NS	0.072

[a–d]Values in the same row with no common superscripts are significantly different.

[1]Values given in this table are means of 4 dietary treatments with different levels of supplementation with α-TA (0, 100, 200, and 400 mg/kg) and correspond to least-squares means obtained from ANOVA (n = 24) and their pooled SE.

[2]PU15 = 15 g PUFA/kg of feed; PU34 = 34 g PUFA/kg of feed; PU45 = 45 g PUFA/kg of feed; PU61 = 61 g PUFA/kg of feed.

[3]FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[4]C18:1 ω9 includes sum of *cis* and *trans* forms.

*$P \leq 0.05$; *** $P \leq 0.001$.

laverde et al., 2003b). In general, modification of FA composition of intramuscular fat seems to be more limited (Pan and Storlien, 1993; López-Bote et al., 1997). It may be due to the fact that FA in intramuscular fat are used mainly as components of cellular membranes, and the cell has to maintain its physical characteristics to ensure fluidity and permeability of different compounds.

As expected, when the dietary polyunsaturation level increased, PUFA content in the tissues also increased (Tables 4 and 5). The 46-g increase in dietary PUFA (from 15 to 61 g/kg diet) resulted in levels of PUFA that were 3.1 and 2.4 times higher in thigh and breast, respectively, than those in the most saturated diet. A similar response was observed in certain PUFA, particularly in LNA, linoleic acid, EPA, and DHA. However, the SFA and MUFA contents of thigh and breast were reduced as the dietary degree of polyunsaturation increased. This reduction was more marked in MUFA (55 and 46% in thigh and breast, respectively, from PU15 to PU61) than in SFA (42 and 24% in thigh and breast, respectively, from PU15 to PU61).

Increasing the level of dietary polyunsaturation caused an increase in the accumulation of PUFA in thigh and breast. Depending on dietary polyunsaturation level, EPA and DHA proportions ranged from 0.05 to 2.05% and from 0.12 to 0.99% in thigh, respectively, and from 0.18 to 3.17% and from 0.56 to 2.79% in breast, respectively. Thus, the predominant long-chain PUFA in breast was DHA and in thigh, EPA. Thigh deposition of LNA varied from 1.4 to 26.7% and breast deposition ranged from 0.98 to 22.2%. Furthermore, both tissues accumulated a similar proportion of linoleic acid (C18:2 ω6: 10.3, 12.7, 13.7, and 16.0% for PU15, PU34, PU45, and PU61, respectively). Similar results have previously been reported by other authors who found a higher deposition of long-chain PUFA in breast muscle compared with thigh (Hulan et al., 1988; López-Ferrer et al., 1999a; González-

TABLE 5. Effect of dietary polyunsaturation and α-tocopheryl acetate (α-TA) supplementation on the fatty acid content of breasts without skin (expressed as g/kg)[1]

Fatty acid[2]	Polyunsaturation level[3]				Significance			
	PU15	PU34	PU45	PU61	PUFA	α-TA	PUFA × α-TA	SE
Total FA	18.85	18.33	17.01	18.09	NS	NS	NS	0.977
Total SFA	6.24^{a}	5.74^{ab}	5.10^{bc}	4.71^{c}	**	NS	NS	0.264
C10:0	0.16	0.15	0.16	0.15	NS	NS	NS	0.016
C12:0	0.01	0.01	0.01	0.01	NS	NS	NS	0.007
C14:0	0.32^{a}	0.25^{b}	0.21^{bc}	0.19^{c}	***	NS	NS	0.015
C15:0	0.05^{a}	0.04^{b}	0.04^{c}	0.03^{d}	***	NS	NS	0.002
C16:0	3.78^{a}	3.39^{ab}	3.01^{b}	2.74^{b}	**	NS	NS	0.185
C17:0	0.12^{a}	0.11^{a}	0.08^{b}	0.05^{c}	***	NS	NS	0.004
C18:0	1.70	1.74	1.56	1.51	†	NS	NS	0.071
C20:0	0.01	0.01	0.01	0.01	NS	NS	NS	0.002
Total MUFA	9.13^{a}	7.20^{b}	5.95^{bc}	4.91^{c}	***	NS	NS	0.416
C14:1	0.07^{a}	0.05^{b}	0.03^{c}	0.01^{d}	***	NS	NS	0.004
C16:1t	0.10^{a}	0.08^{b}	0.06^{bc}	0.05^{c}	***	NS	NS	0.005
C16:1	0.69^{a}	0.52^{b}	0.44^{b}	0.38^{b}	***	NS	NS	0.041
C18:1 ω9[4]	7.40^{a}	5.66^{b}	4.56^{bc}	3.57^{c}	***	NS	NS	0.327
C18:1 ω7t	0.54^{a}	0.38^{b}	0.32^{c}	0.30^{c}	***	NS	NS	0.017
C20:1	0.06^{a}	0.04^{b}	0.03^{b}	0.03^{b}	***	NS	NS	0.005
C24:1	0.12^{a}	0.40^{b}	0.44^{b}	0.52^{c}	***	NS	NS	0.010
Total PUFA	3.48^{c}	5.39^{b}	5.98^{b}	8.48^{a}	***	NS	NS	0.371
C18:2tt	0.03^{a}	0.01^{b}	0.01^{b}	0.01^{b}	***	NS	NS	0.003
C18:2 ω6	1.99^{b}	2.28^{b}	2.29^{b}	2.82^{a}	NS	NS	NS	0.121
C18:3 ω3	0.18^{c}	1.89^{b}	2.49^{b}	4.10^{a}	***	NS	NS	0.183
C18:4 ω3	0.04^{b}	0.02^{b}	0.04^{b}	0.06^{a}	***	NS	NS	0.005
C20:2 ω6	0.04^{a}	0.02^{b}	0.02^{b}	0.03^{ab}	**	NS	NS	0.004
C20:3 ω6	0.09^{a}	0.07^{b}	0.05^{b}	0.06^{b}	***	NS	NS	0.005
C20:4 ω6	0.41^{a}	0.28^{b}	0.23^{c}	0.22^{c}	***	NS	NS	0.010
C20:5 ω3	0.03^{d}	0.30^{c}	0.40^{b}	0.57^{a}	***	NS	NS	0.015
C22:4 ω6	0.071^{a}	0.013^{b}	0.002^{bc}	0.001^{c}	***	NS	NS	0.003
C22:6 ω3	0.10^{c}	0.40^{b}	0.42^{b}	0.48^{a}	***	NS	NS	0.010
PUFA:SFA	0.56^{d}	0.94^{c}	1.17^{b}	1.78^{a}	***	NS	NS	0.035

[a–d]Values in the same row with no common superscripts are significantly different.

[1]Values given in this table are means of 4 dietary treatments with different levels of supplementation with α-TA (0, 100, 200, and 400 mg/kg) and correspond to least-squares means obtained from ANOVA (n = 24) and their pooled SE.

[2]FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[3]PU15 = 15 g of PUFA/kg of feed; PU34 = 34 g of PUFA/kg of feed; PU45 = 45 g of PUFA/kg of feed; PU61 = 61 g of PUFA/kg of feed.

[4]C18:1 ω9 includes sum of *cis* and *trans* forms.

*$P \leq 0.05$; ** $P \leq 0.001$; ***$P \leq 0.001$; †$P \leq 0.10$.

Esquerra and Leeson, 2000; Crespo and Esteve-García, 2001). Differences in tissue FA profile could be attributed to different roles of FA in these tissues or to their different phospholipid contents. The PUFA are preferentially incorporated into phospholipids (Hulan et al., 1988) and phospholipids are in a higher proportion in breast than in thigh muscles (Ratnayake et al., 1989).

Relationships between different families of FA (PUFA, MUFA, and SFA) in feed and tissues were studied. Regression analysis showed a relationship between PUFA content of diets and FA (MUFA and SFA) content of thighs and breasts (Tables 6 and 7). This relationship was linear, whereas the relationship between PUFA content of feed and PUFA content of tissues was exponential. The relationship between dietary PUFA content and the content of the different families of FA in chicken tissues supports the idea that the FA composition in chicken tissues is a combination of direct deposition from dietary FA and endogenous fat synthesis. All the variability in the content of PUFA, MUFA, and SFA in the studied tissues, 75, 70, and 57%, respectively for thigh, and 48, 44, and 19%, respectively for breast, can be attributed to the PUFA content in the diet. These results agree with other authors who observed that the PUFA content in chicken tissues depends more on the variation in dietary FA content than the SFA and MUFA contents in these tissues (López-Ferrer et al., 2001). However, Hrdinka et al. (1996) observed a poor relationship between dietary PUFA content and the percentage of these FA in meat. However, their dietary treatments used a low range of PUFA variation.

Incorporation rates of PUFA in the more polyunsaturated treatments (PU61) reached 60% of the maximum of 92.0 g/kg in thigh and 66% of the maximum of 13.3 g/kg in breast (Tables 6 and 7). The accretion fractional rate of PUFA in thigh was 1.55% and was not significantly different from that in breast. As explained previously, the behavior in the deposition of MUFA and SFA content

TABLE 6. Multiple regression equations: y = fatty acid content of thigh with skin (expressed as g/kg); x = polyunsaturated fatty acid content of feeds (expressed as g/kg)

Fatty acid dependent variable	Equation	R^2	*P*
Total PUFA	$y = 92.03 - 92.03e^{(-0.0155x)}$	0.75	***
Total MUFA	$y = 89.34 - 0.92x$	0.70	***
Total SFA	$y = 53.81 - 0.43x$	0.57	***

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
*** $P \leq 0.001$.

in tissues showed an inverse relationship to the one shown for PUFA when dietary PUFA was modified. Monounsaturated fatty acid content decreased by 0.92 g/kg in thigh and 0.09 g/kg in breast when dietary PUFA was increased by 1 g/kg of feed. The decrease in rates of SFA in thigh and breast were 0.4 and 0.04 g/kg, respectively, for an increase in dietary PUFA of 1 g/kg of feed. These results agree with those of other authors who observed an inverse relationship between the accumulation of total MUFA and PUFA, mainly as ω-3 FA, and to a lesser extent in SFA deposition, as dietary FA were modified (Ajuyah et al., 1991; Olomu and Baracos, 1991; López-Ferrer et al., 1999b, 2001).

Effect of Cooking

The FA content in raw and cooked thighs (expressed as gram per kilogram of raw and cooked thigh on a dry matter basis; water content: 64.93 and 64.74% in raw and cooked thigh, respectively) are shown in Table 8. Cooking of thigh meat led to a reduction in FA content that affected all 3 families of FA in a similar proportion. This reduction was 6.2% for SFA, 6.8% for MUFA, and 5.7% for PUFA. Thus, PUFA to SFA ratio was not significantly modified by heat processing. These results agree with other authors who quantified some FA of thighs and observed a similar reduction in FA (Myers and Harris, 1975). When thighs were cooked at 106°C for 77 min in a conventional oven, the reduction observed was 7.0% for SFA, 5.3% for MUFA, and 6.6% for PUFA. Other authors (Dawson et al., 1990; Grau et al., 2001a,b) did not observe differences in FA profile between raw and cooked thighs when they were cooked at lower temperatures (80 to 90°C). Nevertheless, researchers who processed thigh meat at higher temperatures (200°C) found PUFA proportion reduced by 12.4% (López-Ferrer et al., 1999c). These results reveal that cooking at low temperatures can cause fat losses with similar magnitude in all families of FA, although quantification of FA is necessary. On the other hand, more aggressive thermal processes may cause harsh PUFA losses, which can be observed in an altered FA profile.

Dietary supplementation with α-TA had no significant effect on FA content of cooked thighs. Although PUFA are the most sensitive FA to oxidation, it seems that they have a high durability and low susceptibility to thermal oxidative processes at mild temperatures. This theory is supported by the results of other authors (Regulska-Ilow and Ilow, 2002) who processed herring meat rich in long-chain ω-3 PUFA by culinary methods and did not observe any variation in the FA profile that would indicate PUFA losses.

The fact that cooking seems to affect the different families of FA in a similar way may indicate that this reduction is due to an alteration of the samples during cooking. Therefore, cooking the meat results in exudates that may have homogeneous FA contents. Unfortunately, we did not analyze the FA composition of the exudates from the samples. It would be expected that high temperatures during cooking increase lipid oxidation, which may result in a higher reduction of PUFA compared with the other FA families. However, from our results, it seems that the melting point of fat has a more marked effect on FA losses than lipid oxidation.

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TABLE 7. Multiple regression equations: y = fatty acid content of breast without skin (expressed as g/kg); x = polyunsaturated fatty acid content of feeds (expressed as g/kg)

Fatty acid dependent variable	Equation	R^2	*P*
Total PUFA	$y = 13.29 - 13.29e^{(-0.0155x)}$	0.48	***
Total MUFA	$y = 10.43 - 0.09x$	0.44	***
Total SFA	$y = 6.82 - 0.04x$	0.19	***

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
***$P \leq 0.001$.

TABLE 8. Effect of cooking process, dietary polyunsaturation level, and α-tocopheryl acetate (α-TA) supplementation on the fatty acid content of thigh with skin (expressed as g/kg of raw and cooked thigh on a dry matter basis)[1]

	Process			Polyunsaturation level[3]					
Fatty acid[2]	Raw	Cooked	*P*	PU15	PU34	PU45	PU61	*P*	SE
Total FA	374.91	351.36	***	385.93[a]	366.31[a]	361.17[a]	339.12[b]	***	7.057
Total SFA	106.14	99.61	***	128.19[a]	108.09[b]	97.71[c]	77.49[d]	***	2.430
C10:0	0.06	0.06	NS	0.07[a]	0.06[b]	0.06[b]	0.06[b]	***	0.003
C12:0	0.16	0.16	NS	0.20[a]	0.16[b]	0.15[c]	0.12[d]	***	0.003
C14:0	6.08	5.73	***	8.05[a]	5.96[b]	5.27[c]	4.34[d]	***	0.101
C15:0	0.93	0.88	***	1.27[a]	0.98[b]	0.82[c]	0.53[d]	***	0.017
C16:0	67.87	63.55	***	82.06[a]	68.46[b]	62.73[b]	49.57[c]	***	1.667
C17:0	1.86	1.75	**	2.57[a]	2.11[b]	1.67[c]	0.86[d]	***	0.046
C18:0	27.16	25.79	***	30.82[a]	28.44[b]	25.62[c]	21.01[d]	***	0.737
C20:0	0.31	0.26	***	0.42[a]	0.26[b]	0.22[b]	0.23[b]	***	0.013
Total MUFA	153.52	143.02	***	208.30[a]	153.20[b]	133.79[c]	97.79[d]	***	3.398
C14:1	1.13	1.06	*	1.90[a]	1.19[b]	0.89[c]	0.40[d]	***	0.033
C16:1t	1.88	1.76	***	2.47[a]	1.87[b]	1.69[b]	1.25[c]	***	0.048
C16:1	13.49	12.51	**	17.66[a]	12.89[b]	12.00[b]	9.45[c]	***	0.467
C18:1 ω9[4]	124.67	116.30	***	171.45[a]	125.53[b]	108.28[c]	76.68[d]	***	2.805
C18:1 ω7t	6.75	6.19	***	9.69[a]	6.13[b]	5.36[c]	4.69[c]	***	0.189
C20:1	1.09	0.98	***	1.43[a]	0.98[b]	0.90[bc]	0.82[c]	***	0.024
C24:1	2.30	2.14	***	0.50[d]	2.28[c]	2.76[b]	3.35[a]	***	0.073
Total PUFA	115.07	108.48	***	49.06[d]	104.82[c]	129.51[b]	163.72[a]	***	3.552
C18:2tt	0.37	0.37	NS	0.55[a]	0.40[b]	0.34[c]	0.18[d]	***	0.012
C18:2 ω6	49.06	46.07	***	37.62[d]	46.89[c]	50.29[b]	55.47[a]	***	0.843
C18:3 ω3	55.38	52.58	†	5.32[d]	48.80[c]	68.23[b]	93.57[a]	***	2.551
C18:4 ω3	1.06	0.81	***	1.40[a]	0.84[b]	0.53[c]	0.98[b]	***	0.043
C20:2 ω6	0.35	0.32	***	0.27[b]	0.36[a]	0.34[a]	0.36[a]	***	0.011
C20:3 ω6	0.44	0.42	*	0.44	0.44	0.43	0.42	NS	0.009
C20:4 ω6	1.70	1.53	***	2.08[a]	1.58[b]	1.40[b]	1.40[b]	***	0.046
C20:5 ω3	3.77	3.64	NS	0.24[d]	2.82[c]	4.61[b]	7.14[a]	***	0.238
C22:4 ω6	0.12	0.13	NS	0.15[a]	0.03[b]	0.10[a]	0.20[a]	***	0.025
C22:6 ω3	2.13	2.13	NS	0.48[d]	2.07[c]	2.60[b]	3.37[a]	***	0.082
PUFA:SFA	1.21	1.21	NS	0.38[d]	0.98[c]	1.33[b]	2.13[a]	***	0.039

[a–d]Values in the same row with no common superscripts are significantly different.

[1]Values given in this table correspond to least-squares means obtained from ANOVA (n = 48) and their pooled SE. *P* values for the effect of dietary α-TA supplementation and interactions between different factors (cooking process, dietary polyunsaturation, and α-TA supplementation) in all cases were not significant.

[2]FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[3]PU15 = 15 g of PUFA/kg of feed; PU34 = 34 g of PUFA/kg of feed; PU45 = 45 g of PUFA/kg of feed; PU61 = 61 g of PUFA/kg of feed.

[4]C18:1 ω9 includes sum of *cis* and *trans* forms.

*$P \leq 0.05$; *** $P \leq 0.001$; † $P \leq 0.10$.

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