Influence of the Dietary Polyunsaturation Level on Chicken Meat Quality: Lipid Oxidation

L. Cortinas,* A. Barroeta,*[,1] C. Villaverde,* J. Galobart,* F. Guardiola,† and M. D. Baucells*

*Department of Animal and Food Science, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain; †Department of Nutrition and Food Science-CeRTA, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII, E-08028 Barcelona, Spain

ABSTRACT The present study was carried out to evaluate the influence of increasing amounts of dietary polyunsaturated fatty acids (PUFA) and α-tocopheryl acetate (α-TA) supplementation on lipid oxidation of raw and cooked thigh meat stored under refrigeration. One hundred ninety-two female, 1-d-old, broiler chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary PUFA (15, 34, 45, and 61 g/kg) and 4 levels of supplementation with α-TA (0, 100, 200, and 400 mg/kg). Thiobarbituric acid reactive substance (TBARS) values in cooked meat and cooked refrigerated meat were 12- and 24-fold higher, respectively, than in raw meat. Dietary polyunsaturation and α-TA supplementation affected lipid oxidation more markedly in cooked meat and cooked refrigerated meat than in raw meat and raw refrigerated meat. Lipid oxidation in cooked meat showed a significant linear increase as the concentration of PUFA in raw meat increased. The oxidative stability of meat was not affected by an increase in the dietary α-TA level from 200 to 400 mg/kg. Nonlinear relationship between TBARS values in cooked meat and α-tocopherol content of raw meat showed saturation in the antioxidant effect of α-Toc. The equation $y = x (11.88 + 63.38e^{-0.007z})$ was calculated to predict the minimum inclusion of α-tocopherol to diets (z) of chickens with certain dietary PUFA content (x) to assure a certain TBARS value (y).

(*Key words*: lipid oxidation, polyunsaturation, α-tocopherol, thigh and breast meat, cooked meat and meat storage)

INTRODUCTION

There is interest in foods containing higher levels of polyunsaturated fatty acids (PUFA) because of their beneficial effects on human health, mainly in the prevention of cardiovascular disease (Krauss et al., 2001). For this reason, there are several studies concerning the enrichment of chicken meat with PUFA by the addition of polyunsaturated fats to the diet (Lin et al., 1989; Ajuyah et al., 1993; López-Ferrer et al., 1999, 2001). However, chicken meat enriched with PUFA contains longer fatty acids (FA) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life. One such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among all the methods proposed for assessing MDA, the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. In practice, meat is stored and cooked for consumption. These processes of cooking and storage of meat promote degradation of its lipid fraction (Pikul et al., 1984; Lin et al., 1989; Jensen et al., 1997; Ruiz et al., 1999; Grau et al., 2001a,b).

The negative consequences of lipid oxidation can be overcome by the use of antioxidants in the diet, such as α-tocopherol (α-Toc). α-Toc supplementation prevents lipid oxidation and, therefore, increases the shelf life of meat (Lin et al., 1989; Ahn et al., 1995; De Winne and Dirinck, 1996; Bou et al., 2001; Grau et al., 2001a,b). Thus, it is of great commercial interest to assess the protective effect of α-Toc during storage and cooking processes of poultry meat (Ahn et al., 1995; King et al., 1995; Ruiz et al., 1999; Grau et al., 2001a,b). Second, dietary supplementation with α-Toc permits the enrichment of chicken meat in this antioxidant vitamin (Miller and Huang, 1993; O'Neill et al., 1998; Grau et al., 2001a,b; Bou et al., 2004).

Abbreviation key: C = cooked; CF = cooked refrigerated; MDA = malondialdehyde; PUFA = polyunsaturated fatty acids; R = raw; RF = refrigerated raw; α-TA = α-tocopheryl acetate; TBARS = thiobarbituric acid reactive substances; α-Toc = α-tocopherol.

TABLE 1. Ingredients and composition of the diets

Ingredient	%	Chemical analysis[1]	%
Wheat	39.30	Dry matter	90.78
Soybean meal 48% CP	34.09	Crude protein	22.98
Barley	13.39	Crude fat	10.17
Added fat	9.00	Crude fiber	3.47
Dicalcium phosphate	2.17	Ash content	6.08
Calcium carbonate	0.98	Crude energy (kcal/kg)	4481
Salt	0.45	Metabolizable energy (kcal/kg)[3]	3100
Vitamin-mineral mix[2]	0.40		
DL-Methionine	0.28		
L-Lysine	0.04		

[1]Values given in this table are means of 16 dietary treatments, results 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; thiamin, 2.2 mg; riboflavin, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 11 μg; folic acid, 1.5 mg; biotin, 150 μg; calcium pantotenate, 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.

Some authors point out that the protective effect of α-Toc against lipid oxidation in chicken meat depends on the lipid profile and the α-Toc content of the meat and, hence, of the diet (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). However, most works have studied different fat sources, but few studies are specifically designed to study the effect of an increasing dietary polyunsaturation degree on lipid oxidation by regression analysis.

The objective of the present study was to determine the influence of diets containing increasing amounts of PUFA, at different levels of α-tocopheryl acetate (α-TA), on the development of the lipid oxidation in raw and cooked thigh meat stored under refrigeration for different periods.

MATERIALS AND METHODS

Birds and Diets

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

One hundred ninety-two Ross female broilers at 1 d of age were randomly distributed into 16 dietary treatments with 3 replicates each. The animals were housed in groups of 4 in 48 cages under standard conditions of temperature, humidity, and ventilation. The diet was formulated to meet or exceed recommendations of the NRC (1994) (Table 1). The experimental treatments resulted from a combination of 4 levels of dietary PUFA: 15 (PU15), 34 (PU34), 45 (PU45), and 61 (PU61) g/kg of feed and of 4 levels of supplementation with α-TA:[2] 0 (E0), 100 (E1), 200 (E2),

[2]Rovimix E-50 Adsorbate. F. Hoffmann- La Roche Ltd., Basel, Switzerland.
[3]Tallow and linseed oil were provided by Cailá-Parés S.A., Barcelona, Spain.
[4]Fish oil was provided by Agrupación de Fabricantes de Aceites Marinos, S.A., Vigo, Spain.
[5]Cryovac, Sant Boi de Llobregat, Spain.
[6]J. P. Selecta, S.A., Abrera, Spain.
[7]Sigma, St. Louis, MO.

and 400 (E4) mg/kg of feed. The dietary PUFA levels were achieved by replacing 9% tallow with different proportions of a mixture of linseed[3] and fish oils[4] (ratio of 4:1).

Feed and water were provided ad libitum during the 44 d on study. Feed samples were taken 3 times during the experiment (0, 21, and 44 d) for fatty acid and α-Toc content analysis.

Sample Collection

At the end of the experimental period, 2 birds per cage were randomly selected and processed in a commercial slaughterhouse. The edible portions of both thighs were removed and weighed individually and then deboned, ground with the skin, and then packed in Cryovac CN300[5] bags. Four samples from each thigh were obtained. One was kept as raw (R), another was refrigerated raw (RF) for 3 d (0 to 4°C), and the last 2 were cooked in a water bath under agitation (Unitronic 320 OR[6]) for 30 min to an internal temperature of 80°C. One of the cooked (C) samples was used for quality analyses, and the other was refrigerated (CF) for 2 mo (0 to 4°C). R, RF, C, and CF samples were divided in 3 subsamples. One was stored at –80°C until TBA analysis, and the other 2 were stored at –20°C until FA and α-Toc analysis.

Determination of Thiobarbituric Acid Reactiive Substances

The extent of lipid oxidation in R, RF, C, and CF thighs was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Grau et al. (2000), using third derivative spectrophotometry. The height of the third-order derivative peak that appeared at approximately 521.5 nm was used for calculation of the MDA concentration in the samples. Tetraethoxypropane[7] was used as an MDA precursor in the standard curve. TBARS was expressed as micrograms of MDA per kilogram of sample.

Fatty Acid Content

Fatty acid content of the feeds was determined by gas chromatography following the method described by Sukhija and Palmquist (1988). Thigh samples were analyzed as described previously by Carrapiso et al. (2000). Nonadecanoic acid[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 capillary column[9] (60 m × 0.25 mm i.d.) with a 0.25 μm film thickness of stationary phase. Helium was used as carrier gas. Oven temperature was programmed as follows: increasing 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; and sample volume injected, 1 μL. FA were identified by matching their retention times with those of their relative standards, as well as by mass spectrometry (HP5973[10]) of each peak.

α-Toc Analysis

Tocopherol content in feeds and thighs were extracted as previously described by Jensen et al. (1999) starting from 2 g of feed and 100 mg of freeze-dried thigh meat. Chromatographic separation was achieved on a HS-5-Silica column[11] (125 × 4 mm). Heptane modified with 2-propanol (99.5:0.5 vol/vol) and degassed with helium constituted the mobile phase. HPLC determination was performed according to the conditions described by Jensen et al. (1999). Fluorescence detection was performed with an excitation wavelength of 290 nm and an emission wavelength of 327 nm.

Statistics

Multifactorial ANOVA with repeated measures (n = 384) was performed to determine whether processing, dietary PUFA, and α-TA supplementation affected TBARS values in thigh meat. Data were treated using the proc mixed procedure of SAS software (SAS Institute, 2000). Differences among treatment means were tested using Tukey's test correction for multiple comparisons. The relationship between TBARS values in cooked thigh meat and PUFA content of raw meat was fitted by linear regression analysis. The comparative response of TBARS values depending on the variation in the level of supplementation with α-TA was assessed with the GLM procedure of SAS software (n = 96). The relationship between TBARS values in cooked thigh meat and α-Toc content of raw meat was fitted by an exponential equation of type y = a + be$^{(-cx)}$, where a and a + b are the minimum and the maximum levels that could be reached, respectively,

---

TABLE 2. Fatty acid composition of the experimental diets (expressed as g/kg)[1]

Fatty acid[3]	Polyunsaturation level[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
Total MUFA	41.30	32.55	28.32	20.31
Total PUFA	15.40	33.77	45.03	60.84
C 18:2 ω6	13.16	16.23	17.98	20.17
C 18:3 ω3	1.55	16.45	24.62	36.27
C 20:5 ω3	ND	0.81	1.77	3.35
C 22:6 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]PU15 = 15 g of polyunsaturated fatty acids per kilogram of feed; PU34 = 34 g of polyunsaturated fatty acids per kilogram of feed; PU45 = 45 g of polyunsaturated fatty acids per kilogram of feed; PU61 = 61 g of polyunsaturated fatty acids per kilogram of feed.

[3]FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ND = not detected.

---

and c is the fractional rate of reduction of the TBARS value. The fit was performed by using nonlinear regression by means of NLIN procedure of SAS software. The comparative response of TBARS values in thigh depending on the dietary PUFA content was assessed by the likelihood ratio test (n = 96). The evolution of TBARS values (n = 96) in cooked thigh due to variation in dietary α-Toc and PUFA was fitted by using nonlinear regression by means of the NLIN procedure of SAS software. This data analysis was represented using SigmaPlot 8.02 (2002). In all cases, $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

The fatty acid content of the experimental diets is shown in Table 2. Fatty acid content of diets and thigh meat were discussed in Cortinas et al. (2004). Supplementation with 0, 100, 200 and 400 mg α-TA/kg of feed resulted in dietary α-Toc content of 6 ± 0.6 g/kg, 136 ± 1.5 g/kg, 236 ± 14.5 g/kg, and 451 ± 18.1 g/kg for E0, E1, E2, and E4 treatments, respectively.

Lipid Oxidation in Thigh Meat

The TBARS values in R, RF, C, and CF thigh meat were expressed as micrograms of MDA per kilogram of solids and were compared (Table 3). TBARS values in R meat were low, but during cooking and refrigeration they significantly increased. Thus, lipid oxidation in C and CF thigh meat was 12- and 24-fold higher, respectively, than in R thigh meat. An increase of the lipid oxidation in cooked thigh meat (Ang, 1988; Sheehy et al., 1993; Galvin et al., 1997; Jensen et al., 1997; Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b) and cooked thigh meat stored in various conditions for different periods (Pikul et al., 1984; Lin et al., 1989; Ajuyah et al., 1993; Galvin et al., 1997; Jensen et al., 1997; Ruiz et al., 1999) has

---

[8]Agilent, D-76337 Waldbronn, Germany.
[9]HP19091-136 Hewlett Packard, Newtown, PA.
[10]Agilent, D-76337 Waldbronn, Germany.
[11]Perkin Elmer, D-88662 Überlingen, Germany.

TABLE 3. Effect of dietary polyunsaturation, α-tocopheryl acetate (α-TA) supplementation and processing on thiobarbituric acid reactive substances values in thigh meat (μg of malondialdehyde/kg on a dry-matter basis)[1]

Process[2]	Global Means	Dietary polyunsaturation level[3]			
		PU15	PU34	PU45	PU61
R	338[C]	88[b]	159[c]	287[c]	816[c]
RF	946[C]	146[b]	339[c]	775[c]	2526[c]
C	3870[B]	1141[bz]	3274[byz]	4644[byz]	6421[by]
CF	7939[A]	4366[az]	8466[ay]	10126[ay]	8798[ay]
Global Means		1435[C]	3059[B]	3958[AB]	4640[A]

	Supplementation with α-TA[4]			
	E0	E1	E2	E4
R	901[c]	164[c]	144.0[b]	142[b]
RF	2654[by]	682[cyz]	251.2[byz]	199[bz]
C	7860[ax]	3884[by]	2195.9[byz]	1540[bz]
CF	9258[ay]	9433[ay]	6688.4[az]	6377[az]
PU15	2448[c]	1438[b]	1081	774
PU34	4419[bcy]	3980[abyz]	1760[z]	2078[yz]
PU45	5653[aby]	3736[abyz]	3322[yz]	3123[bz]
PU61	8153[ax]	5009[ay]	3116[yz]	2282[z]
Global Means	5168[A]	3540[B]	2320[C]	2064[C]
SE	501			

P values

Process	***	Process × PUFA	***
α-TA level	***	Process × α-TA level	***
PUFA	***	PUFA × α-TA level	**

[A–C]Grand means within the same column or row with different superscripts are significantly different.

[a–c]Different superscripts indicate significant differences within the same column.

[x–z]Different superscripts indicate significant differences within the same row.

**P ≤ 0.01; ***P ≤ 0.001.

[1]Values given in this table correspondence to least squares means obtained from multifactor ANOVA (n = 384) and their pooled SE.

[2]R = raw thigh meat; RF = raw refrigerated thigh meat; C = cooked thigh meat; CF = cooked refrigerated thigh meat.

[3]PU15 = 15 g of polyunsaturated fatty acids per kilogram of feed; PU34 = 34 g of polyunsaturated fatty acids per kilogram of feed; PU45 = 45 g of polyunsaturated fatty acids per kilogram of feed; PU61 = 61 g of polyunsaturated fatty acids per kilogram of feed.

[1]E0 = without supplementation with α-tocopheryl acetate; E1, E2 and E4 = supplemented with 100, 200, or 400 mg/kg α-tocopheryl acetate, respectively.

FIGURE 1. Influence of processing on thiobarbituric acid reactive substance (TBARS) values in thigh meat [μg of malondialdehyde (MDA)/kg on a dry matter basis] from the dietary treatment with 61 g of polyunsaturated fatty acids (PUFA)/kg of feed and nonsupplemented with α-tocopheryl acetate. R = raw thigh meat; RF = raw refrigerated thigh meat; C = cooked thigh meat; CF = cooked refrigerated thigh meat. [a–c]Different letters indicate significant differences.

have suggested that reduction in TBARS values observed as a function of storage time is probably associated with increased concentrations of highly polar products, probably resulting from polymerization of secondary oxidation products. It has been reported that MDA reacts with a wide range of compounds or can form dimers or trimers of MDA, which decreases the amount of MDA available to react with TBA and, as a result, decreases the TBARS values (Gutteridge, 1975; Esterbauer et al., 1991; Aubourg, 1993). The present data indicate that TBARS numbers may not be a good method for determining the oxidative stability of meat during extended storage. In this sense, some authors who observed a decrease in TBARS values did not find a reduction in total volatile compounds (Ajuyah et al., 1993) and lipid hydroperoxides (Grau et al., 2001a).

The design of the present study permitted the observation that TBARS values in thigh meat depended on dietary polyunsaturation ($P \leq 0.001$). An interaction between dietary polyunsaturation level and processing of meat showed that dietary polyunsaturation significantly affected TBARS values in C and CF thigh meat (Table 3). Hence, TBARS values in C thighs from PU45 and PU61 treatments were 4.1 and 5.6% higher, respectively, than in those from PU15 treatments ($P \leq 0.001$). A similar interaction showing higher TBARS values for cooked and stored thighs of chickens fed polyunsaturated fat sources has been reported (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b). At the same time, α-TA supplementation resulted in a significant decrease of oxidation for RF, C, and CF thigh meat (Table 3). An interaction between dietary α-TA supplementation and processing of meat indicated that the antioxidant effect of α-Toc increased as the oxidative tendency increased, i.e., cooking and refrigeration. Reduction of TBARS values as a consequence of dietary tocopherols has been reported by several authors and has been attributed to the accumulation of the α-Toc in thigh meat (Lin et al., 1989; Sheehy et al., 1993; Ahn et al., 1995; King et al., 1995; De Winne and Dirinck, 1996; Wen et al., 1996; Galvin et al., 1997;

been reported. However, the magnitude of the increase in the TBARS values after cooking and storage of thigh meat differs among reports. Generally, it is difficult to make comparisons of TBARS values between studies because differences in the magnitude of TBARS variation could be attributed to different factors such as the analytical method used, cooking and storage conditions (time, temperature and packaging), vitamin E content, and fatty acid profile of the meat.

Although CF thigh meat showed TBARS values higher than C thigh meat (Table 3), this was not observed in thigh meat from the more polyunsaturated treatment nonsupplemented with α-TA (PU61+E0) (Figure 1). A decrease in TBARS values has been previously observed in thigh meat after prolonged refrigeration (King et al., 1995; Wen et al., 1996; Grau et al., 2001a). Several authors
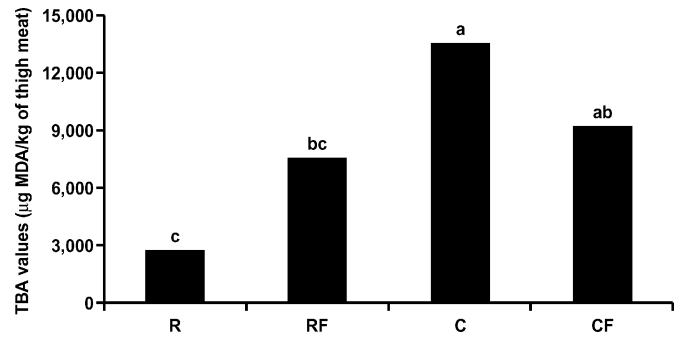
TABLE 4. Equations: y = thiobarbituric acid values (μg/kg of malondialdehyde on a wet-weight basis) of cooked thigh meat, x = PUFA content of raw thigh meat (mg/kg), and z = α-tocopherol content (mg/kg) of raw thigh meat

Independent variable (mg/kg)	Dietary treatments[1,2]	Equation	r^2	P
PUFA content of raw thigh meat	E0	y = 69.67x	0.92	***
	E1	y = 35.62x	0.82	***
	E2, E4	y = 18.13x	0.73	***
α-Tocopherol content of raw thigh meat	PU15	$y = 170.7 + 1{,}133.5e^{-0.106z}$	0.75	***
	PU34	$y = 434.5 + 2{,}285.1e^{-0.106z}$		
	PU45	$y = 1138.7 + 1{,}616.5e^{-0.106z}$		
	PU61	$y = 580.5 + 4{,}419.7e^{-0.106z}$		

[1]E0 = without supplementation with α-tocopheryl acetate; E1, E2, and E4 = supplemented with 100, 200, or 400 mg/kg α-tocopheryl acetate, respectively.

[2]PU15 = 15 g of polyunsaturated fatty acids per kilogram of feed; PU34 = 34 g of polyunsaturated fatty acids per kilogram of feed; PU45 = 45 g polyunsaturated fatty acids per kilogram of feed; PU61 = 61 of polyunsaturated fatty acids per kilogram of feed.

***$P \leq 0.001$.

Lauridsen et al., 1997; O'Neill et al., 1998; Ruiz et al., 1999; Grau et al., 2001a,b).

The lower TBARS values in cooked meat for treatments supplemented with α-Toc indicated that this antioxidant remained active after processing at high temperatures (80°C). Hence, α-Toc is an effective antioxidant for preventing lipid oxidation in meat subjected to heat processes. Obviously, the protective effect of α-Toc against lipid oxidation depended on the level of dietary polyunsaturation. Thigh meat from chickens fed the more polyunsaturated diets and, therefore, containing more PUFA was more protected by α-TA supplementation. Thus, α-Toc protected thigh meat against lipid oxidation in diets containing more PUFA, whereas this effect was not significant in thighs from more saturated treatments (PU15). This finding could be attributed to the higher PUFA content of meat from the more polyunsaturated treatments, which involved greater vulnerability of its lipid fraction to free radical attack. Other studies have also reported an interaction between dietary fat source and α-TA supplementation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a,b).

It is well established that PUFA contents of thigh meat increase with their levels in diets (Lin et al., 1989; Ajuyah et al., 1993; López-Ferrer et al., 1999, 2001). Table 4 shows linear regressions between PUFA content of raw thigh meat and TBARS values in cooked meat for different levels of supplementation with α-TA. Lipid oxidation in cooked thigh meat increased linearly when the concentration of PUFA in raw thigh increased, but this increase was lower as the dietary α-TA supplementation increased. However, the response was similar for the treatments supplemented with 200 and 400 mg of α-TA/kg. These results indicated that α-TA inclusion at levels higher than 200 mg/kg did not further improve lipid stability of thigh meat in terms of TBARS values. These results are in agreement with those of Jensen et al. (1995), who found that the reduction in TBARS values of thigh meat was similar for dietary α-TA levels of 100 and 500 mg/kg. Bou et al. (2004) showed that cooked dark meat in chickens fed α-TA levels of 70 and 140 mg/kg of feed did not have significant differences in TBARS values. Similarly, Ahn et al. (1998) did not find significant differences between TBARS of aerobically packaged cooked thighs from turkeys fed 200, 400, or 600 mg α-TA/kg. Sheldon et al. (1997) showed that breast meat of turkeys fed α-TA at 120 and 300 mg/kg of feed did not have significant differences in TBARS values.

In relation to α-Toc content of raw thigh meat, an increase of 10 mg of α-Toc/kg in the broiler diets caused an increase from 0.71 to 1.14 mg of α-Toc/kg of thigh meat depending on dietary polyunsaturation level (data not shown). The relationship between TBARS values of cooked thighs and α-Toc content of raw thighs for the different polyunsaturation levels showed a nonlinear relation. We established equations of the type $y = a + be^{(-cx)}$, where y is TBARS values in cooked thigh, and x is α-Toc content in raw thigh. As an example, the correlation between TBARS and α-Toc content in thigh meat from PU61 treatment is represented in Figure 2. It can be observed that within the range of α-TA doses used, there was saturation in the antioxidant effect of α-Toc. That is, an increase in α-Toc content of thigh meat did not always imply a reduction in lipid oxidation. Thus, at low α-Toc content in thigh, a marginal increase of α-Toc sharply reduced lipid oxidation, whereas at high α-Toc content in thigh, a large α-Toc increase in thigh only caused slight or no improvement of oxidative stability. Except for TBARS values from PU45 treatments that presented higher variability, in general, maximum and minimum TBARS values obtained through α-TA supplementation increased as dietary polyunsaturation increased. For all levels of dietary PUFA, supplementation with α-TA prevented 84 to 88% of the maximum lipid oxidation in terms of TBARS values. The fractional rate of reduction of the TBARS values was 10.6% in all cases. Despite the fact that α-Toc antioxidant effectiveness was the same for all dietary polyunsaturation levels, in the most polyunsaturated treatments the effectiveness was much more evident because maximum TBARS values were 4-fold higher than

those in the least saturated treatments. Other authors have reported different relationships between α-Toc concentration and TBARS values in tissues: inverse linear (Bartov and Bornstein, 1978; Bartov and Frigg, 1992), inverse logarithmic (Mercier et al., 1998), potential (Ruiz et al., 1999), and binomial (Yamauchi et al., 1982). Contrary to our results, the prediction equations from all of these authors did not find saturation in the antioxidant effect of α-Toc. However, these authors worked with lower concentrations of α-Toc in thighs of poultry than those used in our study.

Considering the different factors which affect lipid oxidation, the dietary supplementation with α-TA should be adjusted depending on dietary polyunsaturation level and on the processing and storage conditions of thigh meat, as well as the objective of this supplementation: either to prevent lipid oxidation or to enrich poultry meat with vitamin E. To predict the minimal dietary supplementation with α-TA in meat enriched with PUFA to minimize lipid oxidation, we performed a regression analysis of the evolution of TBARS values in cooked thigh in response to variation in dietary PUFA and α-Toc content. Therefore, the following equation was obtained (Figure 3): $y = x(11.88 + 63.38e^{-0.007z})$ ($P \leq 0.001$), where y is TBARS value in cooked thigh (μg of MDA/kg), x is dietary PUFA (g/kg), and z is dietary α-Toc (mg/kg). A TBARS value ≥ 800 μg of MDA/kg of meat has been considered as threshold for warmed-over flavor detection in cooked dark chicken meat (O'Neill et al., 1998; Bou et al., 2001). Thus, when the polyunsaturation level of PUFA is low in a diet (15 g of PUFA/kg) only 60 mg of dietary α-Toc per kilogram of feed is necessary to assure TBARS values below 800 μg of MDA/kg, whereas at high level of dietary PUFA (30 g of PUFA/kg), 200 mg of dietary α-Toc per kilogram of feed are necessary. Moreover, supplementation with α-Toc greater than 200 mg/kg did not

improve lipid stability (see Table 4). Therefore, dietary PUFA level should not be greater than 30 g PUFA/kg of feed to avoid appearance of warmed-over flavor in cooked chicken meat.

In conclusion, dietary polyunsaturation and α-tocopheryl acetate supplementation affected lipid oxidation more markedly in cooked meat and cooked refrigerated meat, with higher TBARS values, than in raw meat and raw refrigerated meat. The experimental design used permitted the study of lipid oxidation pattern in function of polyunsaturated fatty acids and α-Toc content in the diet and thigh. Lipid oxidation increased linearly as the concentration of polyunsaturated fatty acids in raw meat increased, but this increase was lower with greater dietary α-TA supplementation. In fact, the nonlinear relationship between TBARS values in cooked meat and α-Toc content of raw meat showed saturation in the antioxidant effect of α-tocopherol. These results indicate that α-TA inclusion at levels higher than 200 mg/kg did not further improve lipid stability of thigh meat in terms of TBARS values, and, therefore, it was necessary to limit dietary polyunsaturated fatty acids content to minimize lipid oxidation. The equation calculated in the present work allowed prediction of the minimal dietary supplementation with α-TA to minimize lipid oxidation of cooked thigh meat in response to variation in dietary PUFA.
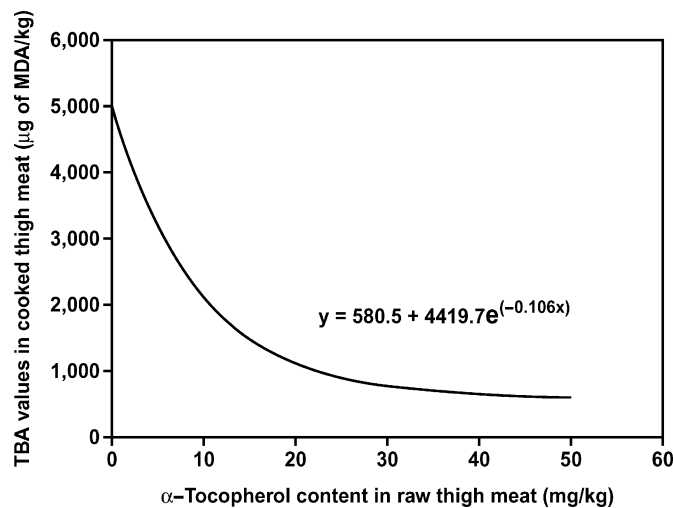
ACKNOWLEDGMENTS

FIGURE 2. Relationship between α-tocopherol content (mg/kg) in raw thigh and thiobarbituric acid reactive substance (TBARS) values [μg of malondialdehyde (MDA)/kg on a wet weight basis] in cooked thigh meat from chickens fed diets containing 61 g of polyunsaturated fatty acids per kilogram of feed.
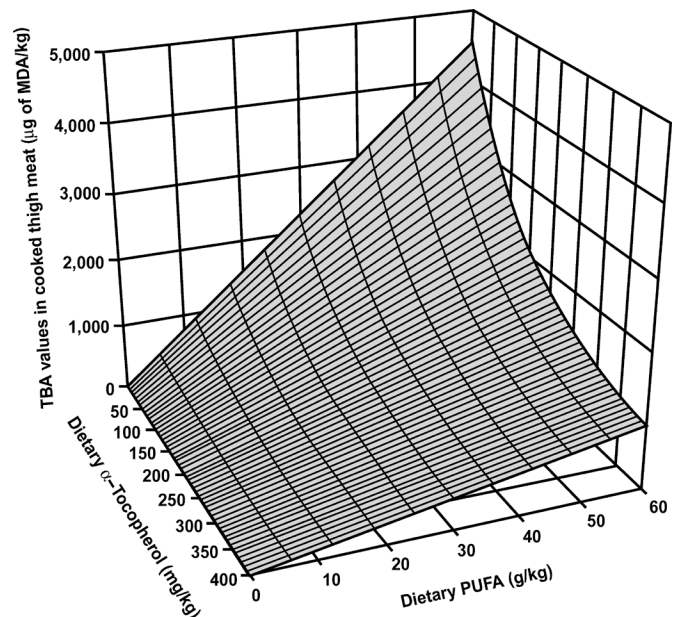
$$y = 580.5 + 4419.7e^{(-0.106x)}$$

FIGURE 3. Estimated response surface for thiobarbituric acid reactive substance (TBARS) values [μg of malondialdehyde (MDA)/kg on a wet weight basis] in cooked thigh to variation in dietary content of α-tocopherol (mg/kg) and polyunsaturated fatty acids (PUFA; g/kg).

REFERENCES

Ahn, D. U., J. L. Sell, C. Jo, X. Chen, C. Wu, and J. I. Lee. 1998. Effects of dietary vitamin E supplementation on lipid oxidation and volatiles content of irradiated, cooked turkey meat patties with different packaging. Poult. Sci. 77:912–920.

Ahn, D. U., F. H. Wolfe, and J. S. Sim. 1995. Dietary α-linolenic acid and mixed tocopherols, and packaging influences on lipid stability in broiler chicken breast and leg muscle. J. Food Sci. 5:1013–1018.

Ajuyah, A. O., R. T. Hardin, and J. S. Sim. 1993. Dietary antioxidant and storage affect chemical characteristics of ω-3 fatty acid enriched broiler chicken meats. J. Food Sci. 58:43–46.

Ang, C. Y. W. 1988. Comparisons of broiler tissues for oxidative changes after cooking and refrigerated storage. J. Food Sci. 53:1072–1075.

Aubourg, S. P. 1993. Review: Interaction of malondialdehyde with biological molecules- new trends about reactivity and significance. Int. J. Food. Sci. Tecnol. 28:323–335.

Bartov, I., and S. Bornstein. 1978. Stability of abdominal fat and meat of broilers: Effect of duration of feeding antioxidants. Br. Poult. Sci. 19:129–135.

Bartov, I., and M. Frigg. 1992. Effect of high concentrations of dietary vitamin E during various age periods on performance, plasma vitamin E and meat stability of broiler chicks at 7 weeks of age. Br. Poult. Sci. 33:393–402.

Bou, R., F. Guardiola, A. Grau, S. Grimpa, A. Manich, A. Barroeta, and R. Codony. 2001. Influence of dietary fat source, α-tocopherol, and ascorbic acid supplementation on sensory quality of dark chicken meat. Poult. Sci. 80:1–8.

Bou, R., F. Guardiola, A. Tres, A. C. Barroeta, and R. Codony. 2004. Effect of dietary fish oil, and α-tocopheryl acetate and zinc supplementation on the composition and consumer acceptability of chicken meat. Poult. Sci. 83:282–292.

Carrapiso, A. I., M. L. Timón, M. J. Petrón, J. F. Tejeda, and C. García. 2000. In situ transesterification of fatty acids from Iberian pig subcutaneous adipose tissue. Meat Sci. 56:159–164.

Cortinas, L., C. Villaverde, J. Galobart, M. D. Baucells, R. Codony, and A. C. Barroeta. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poult. Sci. 83:1155–1164.

De Winne, A., and P. Dirinck. 1996. Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. J. Agric. Food Chem. 44:1691–1696.

Esterbauer, H., R. F. Schaur, and H. Zollner. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11:81–128.

Galvin, K., P. A. Morrisey, and D. J. Buckley. 1997. Influence of dietary vitamin E and oxidised sunflower oil on the storage stability of cooked chicken muscle. Br. Poult. Sci. 38:499–504.

Grau, A., R. Codony, S. Grimpa, M. D. Baucells, and F. Guardiola. 2001b. Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and α-tocopherol and ascorbic acid supplementation. Meat Sci. 57:197–208.

Grau, A., F. Guardiola, J. Boatella, A. C. Barroeta, and R. Codony. 2000. Measurement of 2-thiobarbituric acid values in dark chicken meat through derivative spectrophotometry: Influence of various parameters. J. Agric. Food Chem. 48:1155–1159.

Grau, A., F. Guardiola, S. Grimpa, A. C. Barroeta, and R. Codony. 2001a. Oxidative stability of dark chicken meat through frozen storage: Influence of dietary fat and α-tocopherol and ascorbic acid supplementation. Poult. Sci. 80:1630–1642.

Gutteridge, J. M. C. 1975. The use of standards for malonyldialdehyde. Anal. Biochem. 69:518–526.

Jensen, C., R. Enberg, K. Jakobsen, L. H. Skibsted, and G. Bertelsen. 1997. Influence of the oxidative quality of dietary oil on broiler meat storage stability. Meat Sci. 47:211–222.

Jensen, C., L. H. Skibsted, K. Jakobsen, and G. Bertelsen. 1995. Supplementation with all-rac-α- or a mixture of natural source RRR-α-,γ-,δ-tocopheryl acetate. 2. Effect on the oxidative stability of raw and precooked broiler meat products. Poult. Sci. 74:2048–2056.

Jensen, S. K., R. M. Engberg, and M. S. Hedermann. 1999. All-rac-α-tocopherol acetate is a better vitamin E source than all-rac-α-tocopherol succinate for broilers. J. Nutr. 129:1355–1360.

King, A. J., T. J. Uijttenboogaart, and A. W. Vries. 1995. α-Tocopherol, β-carotene and ascorbic acid as antioxidant in stored poultry muscle. J. Food Sci. 60:1009–1012.

Krauss, R. M., R. H. Eckel, B. Howard, L. J. Appel, S. R. Daniels, R. J. Deckelbaum, J. W. Erdman, P. Kris-Etherton, I. J. Goldberg, T. A. Kotchen, A. H. Lichtenstein, W. E. Mitch, R. Mullis, K. Robinson, J. Wylie-Rossett, S. S. Jeor, J. Suttie, D. L. Tribble, and T. L. Bazzare. 2001. Revision 2000: Statement for healthcare professionals from the nutrition committee of the American Heart Association. J. Nutr. 131:132–146.

Lauridsen, C., D. J. Buckley, and P. A. Morrisey. 1997. Influence of dietary fat and vitamin E supplementation on α-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. Meat Sci. 46:9–22.

Lin, C. F., J. I. Gray, A. Ashgar, D. J. Buckley, A. M. Booren, and C. J. Flegal. 1989. Effects of dietary oils and α-tocopherol supplementation on lipid composition and stability of broiler meat. J. Food Sci. 54:1457–1460.

López-Ferrer, S., M. D. Baucells, A. C. Barroeta, and M. A. Grashorn. 1999. N-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. Poult. Sci. 78:356–365.

López-Ferrer, S., M. D. Baucells, A. C. Barroeta, and M. A. Grashorn. 2001. N-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: Fish oil. Poult. Sci. 80:741–752.

Maraschiello, C., C. Sárraga, and J. A. García Regueiro. 1999. Glutathione peroxidase activity, TBARs, and α-tocopherol in meat from chicken fed different diets. J. Agric. Food Chem. 47:867–872.

Mercier, Y., P. Gatellier, M. Viau, H. Remington, and M. Renerre. 1998. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. Meat Sci. 48:301–318.

Miller, E. L., and Y. X. Huang. 1993. Improving the nutritional value of broiler meat through increased n-3 fatty acid and vitamin E content. Pages 404–411 in Proceedings of the 11th European Symposium on the Quality of Poultry Meat, Tours, France.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

O'Neill, L. M., K. Galvin, P. A. Morrissey, and D. J. Buckley. 1998. Comparison of effects of dietary olive oil, tallow and vitamin E on the quality of broiler meat and meat products. Br. Poult. Sci. 39:365–371.

Pikul, J., D. E. Leszczynski, P. J. Betchel, and F. A. Kummerow. 1984. Effects of frozen storage and cooking on lipid oxidation in chicken meat. J. Food Sci. 49:838–843.

Ruiz, J. A., A. M. Pérez-Vendrell, and E. Esteve-García. 1999. Effect of β-carotene and vitamin E on peroxidative stability in leg meat of broilers fed different supplemental fats. J. Agric. Food Chem. 47:448–454.

SAS Institute. 2000. SAS 8.01. SAS Institute Inc., Cary, NC.

Sheehy, P. J. A., P. A. Morrissey, and A. Flynn. 1993. Influence of heated vegetable oils and α-tocopheryl acetate supplementation on α-tocopherol, fatty acids and lipid peroxidation in chicken muscle. Br. Poult. Sci. 34:367–381.

Sheldon, B. W., P. A. Curtis, P. L. Dawson, and P. R. Ferket. 1997. Effect of dietary vitamin E on the oxidative stability, flavor, color, and volatile profiles of refrigerated and frozen turkey breast meat. Poult. Sci. 76:634–641.

SigmaPlot. 2002. SigmaPlot 2002 for Windows, Version 8.02. SPSS Inc., Chicago, IL.

Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method of determination of total fatty acid content and composition of feedstuffs and faeces. J. Agric. Food Chem. 36:1202–1206.

Wen, J., P. A. Morrisey, D. J. Buckley, and P. J. A. Sheehy. 1996. Oxidative stability and α-tocopherol retention in turkey burgers during refrigerated and frozen storage as influenced by dietary α-tocopheryl acetate. Br. Poult. Sci. 37:787–795.

Yamauchi, K., Y. Nagai, and T. Ohashi. 1982. Quantitative relationship between alpha-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in chicken skeletal muscle. Agric. Biol. Chem. 46:2719–2724.