Dose and Duration Effect of α-Tocopheryl Acetate Supplementation on Chicken Meat Fatty Acid Composition, Tocopherol Content, and Oxidative Status

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The effects of dietary α-tocopheryl acetate (α-TA) doses (75, 150, and 225 mg/kg) and the duration of this supplementation (0, 10, 21, 32, and 43 days prior to slaughter) on fatty acid composition, α-tocopherol content, and oxidative status were studied either in raw or in cooked dark chicken meat with its skin. With regard to fatty acid composition, raw meat was affected by both dietary factors. Various polyunsaturated fatty acids decreased as a result of higher α-TA doses, whereas these fatty acids increased with longer supplementation periods. Cooked meat showed similar trends for the duration of α-TA supplementation. On the other hand, α-tocopherol content in raw and cooked meat increased as a result of the dose and duration of α-TA supplementation. Formation of lipid hydroperoxides and thiobarbituric acid values of these meats were also influenced by these two dietary factors, and the dietary combination of 150 mg/kg of α-TA during the last 32 days was optimal in terms of supplementation costs and meat oxidative stability.

KEYWORDS: Tocopheryl acetate supplementation; chicken meat; fatty acid composition; tocopherol content; meat oxidative status

INTRODUCTION

Because consumers have become more concerned about food quality and safety there is an interest in increasing the content of α-tocopherol, the most abundant naturally occurring lipid-soluble antioxidant in skeletal muscle, in meat (*1*). α-Tocopherol, present in membranes and lipoproteins, blocks the chain reaction of lipid peroxidation by scavenging peroxyl radicals (*2*). If these radicals are not scavenged, they can oxidize fatty acids and damage DNA and membrane proteins, so they not only impair cell functions but also generate oxidation products that can be detrimental to health (*2, 3*).

In addition, α-tocopherol has been related in humans with positive effects on preventing cardiovascular disease, cancer, and inflammatory and age-related diseases, although several large-scale epidemiological studies and intervention trials were not conclusive (*4−6*).

Nevertheless, tocopherols cannot be synthesized by animals, and so they are dependent on dietary sources (*7*). Esters of tocopherols are resistant to oxidation and, in this form, display no antioxidant activity, although they are hydrolyzed in the gut, releasing the native tocopherols that show antioxidant activity

(*7*). Therefore, increasing the dietary supplementation of α-tocopheryl acetate (α-TA) results in an increase in the accumulation of α-tocopherol in chicken plasma (*8*), skeletal muscle (*9−11*), and other tissues (*10, 11*).

Moreover, in relation to its antioxidant activity, many works have reported that dietary α-TA supplementation led to meat with higher oxidative stability (*7, 9, 12, 13*). Dietary supplementation is preferred to post-mortem addition because it is more effective, as α-tocopherol is incorporated into the membrane where lipid oxidation is initiated (*7*).

Apart from its effect on lipid oxidation, α-TA supplementation prevents off-flavor and off-odor formation in chicken meat (*14, 15*). In addition, several authors have reported that chickens fed α-TA supplements or mixed tocopherols provided meat with increased content of some PUFA (*11, 16*).

Given these beneficial effects on meat quality, it is important to find the appropriate duration and dosage of α-TA supplementation. This subject is of nutritional and economic importance because animals fed an α-TA supplement a few days prior to slaughter may provide meat with an oxidative stability comparable to that obtained by feeding throughout the growth period.

The aim of this work was thus to study the effect of α-TA supplementation on animal performance, meat fatty acid composition, and α-tocopherol content as well as to find the appropriate duration and dosage of α-TA supplementation that

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Table 1. Ingredients and Composition of the Basal Diet

ingredient	percentage
corn	54.0
soybean meal, 48% CP	33.7
full fat extruded soybeans	4.7
lard	3.4
dicalcium phosphate	2.2
calcium carbonate	1.0
salt	0.5
DL-methionine	0.2
trace mineral–vitamin mix[a]	0.4
calculated composition[b]	
dry matter	89.1
crude protein	21.8
crude fat	7.0
crude fiber	3.2
ash	6.4

[a] Includes *all-rac*-α-tocopheryl acetate (20 mg/kg of feed). [b] Metabolizable energy of the basal diet is 3100 cal/g.

would lead to meat with an optimal oxidative stability. The effect of this α-tocopherol supplementation on meat sensory quality has been reported elsewhere (*14*).

MATERIALS AND METHODS

Birds and Diets. A 3 × 5 factorial design was planned and conducted in triplicate to study the influence of α-TA supplements (75, 150, and 225 mg/kg of feed) and periods of α-TA supplementation (0, 10, 21, 32, and 43 days prior to slaughter) on several parameters [fatty acid composition, α-tocopherol content, thiobarbituric acid (TBA) values, and lipid hydroperoxide (LHP) content] in raw and cooked dark chicken meat.

Two hundred and twenty-five female broiler chicks (Hubbard, 1-day-old) were randomly assigned to 45 cages (5 chickens per cage) corresponding to the 15 dietary treatments replicated 3 times. Females were chosen because of their higher meat fat content and, therefore, influence on meat oxidative stability.

Diets were formulated according to requirements recommended by the NRC (*17*) and were prepared from a commercial basal diet (**Table 1**). The analyzed fatty acid composition of this diet was as follows: 24.80% saturated fatty acids (SFA), 34.75% monounsaturated fatty acids (MUFA), 2.53% n-3 polyunsaturated fatty acids (PUFA), and 37.96% n-6 PUFA.

Birds were fed ad libitum and reared in compliance with national regulations. This study received prior approval by the Universitat Autònoma de Barcelona Animal Care and Use Committee.

Sample Preparation. Briefly, broiler chickens (44 days old) were slaughtered according to commercial procedures at Copaga Soc. Cooperativa slaughterhouse. Because of their increased susceptibility to oxidation, legs with skin from each cage were hand-deboned and divided into three groups. One group of three legs was ground and vacuum-packed in 13.5 × 16 cm polyamide/polyethylene bags and stored at −20 °C. The remaining seven legs were divided into two groups (three and four legs), and each group of legs was vacuum-packed in 20 × 40 cm polyamide/polyethylene bags and cooked in an oven (90 °C and 95% relative humidity) until the center of the leg reached 80 °C. The cooked bag containing three legs was then opened, and the legs plus their exudates were ground and vacuum-packed in 13.5 × 16 cm polyamide/polyethylene bags and stored at −20 °C. The other group of cooked legs was stored at −20 °C until sensory analysis (*14*). Permeability to oxygen of all bags used was 50 cm^3 × m^{-2} × bar × 24 h at 23 °C (DIN 53,380).

Reagents and Standards. Butylated hydroxytoluene (BHT), pyrogallol, and *all-rac*-α-tocopherol were obtained from Sigma (St. Louis, MO). The methanol and ethanol (96%) used in α-tocopherol analysis were of HPLC grade. Extraction reagents for fatty acid determination were of ACS grade.

Fatty Acid Composition. One and a half grams of a ground sample (leg with skin) or 3 g of a milled feed was weighed in 32 × 210 mm tubes, with 1.5 mL of 0.1% aqueous EDTA that was immediately added. Subsequently, 20 mL of chloroform/methanol (2:1, v/v) was added, and the mixture was homogenized for 40 s at 19800 rpm using a Polytron PT 2000. Extracts were filtered through Whatman no. 1 filter paper into 50 mL screw-capped tubes, and the residues were re-extracted twice with the same solvent: first with 7 mL (30 s at 19800 rpm) and then with 5 mL (10 s at 19800 rpm). Ten milliliters of water was then added to these tubes, and they were stoppered and shaken for 30 s before being centrifuged for 20 min at 500*g*. The chloroform phase was filtered through anhydrous sodium sulfate (using a Whatman no. 1 filter paper), which was then washed twice with 5 mL of chloroform. The lipid extract obtained was concentrated to 1 mL in a vacuum rotatory evaporator at 35 °C, and the rest of the solvent was removed in a light nitrogen stream, with the flask then stored in a vacuum desiccator (10 mmHg overnight). Fatty acid methyl esters were prepared from the extracted lipid fraction and determined as described by Guardiola et al. (*18*).

Determination of α-Tocopherol. Two grams of a ground sample (leg with skin) was homogenized using a polytron PT 2000 (Kinematica, Lucerne, Switzerland) for 30 s at 19800 rpm with 5 mL of absolute ethanol containing 1% pyrogallol (w/v) and 0.012% BHT (w/v). Ten milliliters of 1.6 N methanolic KOH was then added, and saponification was carried out at 70 °C for 30 min. Nonsaponifiables were then extracted with petroleum ether and filtered through a 0.45 μm Teflon membrane. After solvent evaporation under a nitrogen stream at 30 °C, the residue was redissolved in 96% ethanol. Chromatographic separation of this solution was performed using a Hewlett-Packard series 1100 liquid chromatograph (Waldbronn, Germany) equipped with a Rheodyne 7725i model injector (Cotati, CA) featuring a loop volume of 20 μL, a column (25 × 0.46 cm) packed with 5 μm, 80 Å Extrasil ODS2, and a precolumn (1 × 0.4 cm) packed with 5 μm, 100 Å Kromasil ODS (Teknokroma, St. Cugat del Vallès, Spain). Sample compounds were isocratically eluted with methanol and detected through a Hewlett-Packard 1046A fluorescence detector (Waldbronn, Germany) in which the excitation and emission wavelengths were set at 288 and 330 nm, respectively. Raw and cooked meat samples spiked with different amounts of *all-rac*-α-tocopherol were used to determine the recoveries (70.85 and 85.21%, respectively). α-Tocopherol content was determined by means of the recovery applied and an experimental calibration curve, using *all-rac*-α-tocopherol as an external standard.

Determination of TBA Values. As described by Grau et al. (*19*), an acid aqueous extraction of meat samples protected with EDTA and BHT was made. Then this extract reacted with added TBA after 30 min at 70 °C. This reaction mixture was submitted to third-derivative spectrophotometry to measure the peak height at 512.5 nm in the third-derivate spectrum.

Determination of Lipid Hydroperoxides. LHP were measured according to the ferrous oxidation−xylenol orange method described by Grau et al. (*20*). Briefly, meat methanol extracts were added to an acid ferrous medium containing xylenol orange. Reaction mixtures containing 140 μL of sample extract were incubated at room temperature for 80 h to induce LHP formation.

Statistical Analysis. Multifactor ANOVA was used to determine whether the dietary factors (dose and duration of dietary α-TA supplementation) affected feed intake, body weight, carcass weight, fatty acid composition, α-tocopherol content, TBA values, and LHP content in either raw or cooked meat. The interaction between factors was included in the statistical model except for fatty acid composition. Least-squares means for main factors having a significant influence were separated by means of Scheffé's test. In addition, a multiple linear regression analysis was used to study whether meat α-tocopherol deposition is related to dietary α-TA supplements.

RESULTS

Bird Performance. Feed intake from 11 to 21 days, from 22 to 32 days, and from 33 to 43 days was not affected by the dose or the days of α-TA supplementation (**Table 2**). Likewise, body weight at 21 days, final body weight, and carcass weight

Table 2. Feed Intake and Body and Carcass Weight of Chickens Fed Different Doses of α-Tocopheryl Acetate Supplements for Different Numbers of Days[a]

		dose (mg/kg)			days of supplementation					
	period of supplementation	75	150	225	0	10	21	32	43	SE[b]
feed intake (g)	up to 10 days	229	224	216	236	215	216	233	215	3.1
feed intake (g)	from 11 to 21 days	768	802	772	786	799	753	788	776	8.1
feed intake (g)	from 22 to 32 days	1322	1325	1313	1321	1360	1332	1319	1269	11
feed intake (g)	from 33 to 43 days	1372	1379	1354	1372	1415	1355	1359	1342	12
body weight (g)	21 days	768	790	771	785	785	781	763	765	6.9
body weight (g)	43 days	2140	2142	2136	2146	2146	2149	2124	2133	11
carcass wt[c] (g)	43 days	1742	1752	1747	1748	1754	1757	1737	1739	10

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 45$). [b] Standard error of the global least-squares means. [c] Birds had been plucked, eviscerated, and weighed without neck and head.

Table 3. Effect of the Dose and Duration of α-Tocopheryl Acetate Supplementation on Raw Dark Meat Fatty Acid Composition (Expressed as Area Normalization in Percent)[a]

	dose (mg/kg)			days of supplementation					
fatty acid[b]	75	150	225	0	10	21	32	43	SE[c]
12:0	0.08	0.07	0.07	0.06	0.08	0.07	0.08	0.07	0.004
14:0	0.85	0.83	0.89	0.85	0.86	0.86	0.86	0.86	0.009
16:0	21.75a	22.25ab	23.12b	23.08	22.69	22.22	21.94	21.93	0.163
18:0	6.93	6.60	6.66	6.75	6.79	6.58	6.62	6.91	0.087
20:0	0.08	0.08	0.09	0.09	0.09	0.09	0.07	0.08	0.004
22:0	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.001
total SFA	29.70	29.87	30.85	30.86	30.52	29.85	29.59	29.87	0.198
14:1 n-9	0.13a	0.14ab	0.16b	0.15	0.15	0.15	0.14	0.13	0.003
16:1 n-9	0.61	0.61	0.58	0.58	0.60	0.59	0.62	0.60	0.011
16:1 n-7	3.88	4.25	4.52	4.27	4.46	4.32	4.20	3.84	0.093
18:1 n-9	35.70	37.00	36.16	36.33	36.96	36.17	35.85	36.10	0.226
18:1 n-7	2.14	2.22	2.22	2.17	2.21	2.15	2.23	2.21	0.022
20:1 n-9	0.39	0.40	0.38	0.38	0.39	0.38	0.39	0.40	0.006
22:1 n-9	0.04a	0.03b	0.03b	0.03	0.03	0.03	0.04	0.03	0.001
total MUFA	42.89	44.64	44.03	43.90	44.81	43.77	43.47	43.32	0.285
18:3 n-3	1.41	1.33	1.32	1.33	1.29	1.38	1.38	1.38	0.016
18:4 n-3	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.002
20:5 n-3	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.001
22:5 n-3	0.13a	0.12b	0.11b	0.11a	0.11a	0.12ab	0.13b	0.13b	0.002
22:6 n-3	0.11a	0.09ab	0.08b	0.08	0.09	0.09	0.11	0.11	0.003
total PUFA n-3	1.73	1.62	1.60	1.60	1.56	1.68	1.70	1.71	0.020
18:2 n-6	23.53	21.97	21.68	21.87	21.33	22.70	23.14	22.94	0.297
18:3 n-6	0.24	0.24	0.25	0.25	0.25	0.25	0.23	0.25	0.005
20:2 n-6	0.29	0.27	0.27	0.26	0.25	0.28	0.29	0.30	0.004
20:3 n-6	0.23	0.22	0.22	0.21	0.22	0.23	0.22	0.24	0.003
20:4 n-6	1.03a	0.87b	0.82b	0.79a	0.80a	0.92ab	1.00b	1.02b	0.015
22:4 n-6	0.27a	0.23b	0.21c	0.21a	0.20a	0.25b	0.27b	0.28b	0.003
22:5 n-6	0.09a	0.07b	0.07b	0.06a	0.07ab	0.08ab	0.10b	0.09ab	0.003
total PUFA n-6	25.68	23.87	23.52	23.64	23.11	24.70	25.24	25.10	0.318
PUFA/SFA	0.93a	0.85ab	0.82b	0.82	0.81	0.89	0.91	0.90	0.015

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 15$). Least-squares means corresponding to a dietary factor (dose or days of supplementation) with different letters differ significantly ($P \leq 0.05$). [b] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [c] Standard error of the global least-squares means.

were not affected by dose or duration of α-TA supplementation. Therefore, under our conditions, neither the dose nor the period of α-TA supplementation improved animal performance, which agrees with other studies supplementing diets above the α-tocopherol physiological requirements (8, 21).

Fatty Acid Composition. Fatty acid composition in raw dark chicken meat was affected by the dose and the duration of α-TA supplementation (**Table 3**). Meat content of 16:0 and 14:1 n-9 increased with higher α-TA supplements, whereas the contents

of 22:5 n-3, 22:6 n-3, 20:4 n-6, 22:4 n-6, and 22:5 n-6 followed the opposite trend.

As for the duration of α-TA supplementation, the content of 22:5 n-3, 20:4 n-6, 22:4 n-6, and 22:5 n-6 in raw meat increased with the duration of α-TA supplementation.

In cooked dark chicken meat, fatty acid composition was also influenced by the dose and duration of α-TA supplementation (**Table 4**), although, in comparison to raw meat, fewer fatty acids were affected by α-TA supplements. In fact, only the 20:1

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Table 4. Effect of the Dose and the Duration of α-Tocopheryl Acetate Supplementation on Cooked Dark Meat Fatty Acid Composition (Expressed as Area Normalization in Percent)[a]

fatty acid[b]	dose (mg/kg)			days of supplementation					SE[c]
	75	150	225	0	10	21	32	43	
12:0	0.08	0.07	0.07	0.08	0.07	0.08	0.06	0.06	0.004
14:0	0.84	0.79	0.83	0.84	0.83	0.84	0.80	0.77	0.013
16:0	21.58	21.87	22.49	22.62	22.44	21.78	21.68	21.37	0.174
18:0	7.03	6.94	7.05	6.82	7.08	6.87	7.04	7.22	0.058
20:0	0.10	0.12	0.10	0.08	0.12	0.12	0.10	0.12	0.011
22:0	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.001
total SFA	29.68	29.84	30.58	30.50	30.59	29.75	29.74	29.58	0.172
14:1 n-9	0.13	0.15	0.17	0.15	0.17	0.14	0.14	0.13	0.006
16:1 n-9	0.66	0.63	0.63	0.66	0.63	0.66	0.65	0.60	0.021
16:1 n-7	3.78	4.10	4.19	4.08	4.28	4.10	3.99	3.66	0.081
18:1 n-9	36.14	37.17	36.30	37.01	36.77	36.47	35.97	36.42	0.189
18:1 n-7	2.18	2.26	2.12	2.10	2.23	2.25	2.17	2.18	0.024
20:1 n-9	0.43ab	0.46b	0.42a	0.41	0.43	0.44	0.45	0.45	0.005
22:1 n-9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.001
total MUFA	43.32	44.77	43.81	44.40	44.53	44.07	43.38	43.45	0.221
18:3 n-3	1.33	1.30	1.30	1.26	1.27	1.31	1.35	1.37	0.013
18:4 n-3	0.06	0.06	0.08	0.07	0.08	0.07	0.06	0.06	0.004
20:5 n-3	0.05	0.06	0.08	0.05	0.06	0.06	0.06	0.06	0.002
22:5 n-3	0.15	0.13	0.16	0.12	0.13	0.18	0.16	0.14	0.008
22:6 n-3	0.09	0.12	0.18	0.13	0.14	0.11	0.13	0.15	0.016
total PUFA n-3	1.68	1.67	1.80	1.62	1.68	1.72	1.76	1.78	0.028
18:2 n-6	23.36	21.69	21.75	21.79	21.27	22.49	22.88	22.91	0.258
18:3 n-6	0.23	0.23	0.23	0.22	0.23	0.23	0.25	0.23	0.007
20:2 n-6	0.28	0.28	0.27	0.25a	0.26a	0.28ab	0.30b	0.30b	0.003
20:3 n-6	0.23	0.25	0.24	0.21	0.24	0.25	0.25	0.25	0.005
20:4 n-6	0.87	0.91	0.98	0.73	0.88	0.85	1.05	1.08	0.030
22:4 n-6	0.26	0.26	0.24	0.19	0.24	0.27	0.29	0.29	0.010
22:5 n-6	0.10	0.11	0.10	0.07	0.09	0.10	0.11	0.13	0.007
total PUFA n-6	25.33	23.73	23.81	23.47	23.21	24.46	25.12	25.18	0.262
PUFA/SFA	0.91	0.85	0.84	0.83	0.81	0.88	0.90	0.91	0.012

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 15$). Least-squares means corresponding to a dietary factor (dose or days of supplementation) with different letters differ significantly ($P \leq 0.05$). [b] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [c] Standard error of the global least-squares means.

n-9 meat content was affected by the dose of α-TA, being higher when animals were fed 150 mg/kg of α-TA.

The period of α-TA supplementation affected the content of only 20:2 n-6, which is higher in those meats from animals fed with α-TA supplementation over longer periods.

α-Tocopherol Content. As a result of higher α-TA dietary amounts, α-tocopherol content in raw dark chicken meat increased (**Table 5**). However, this increase was significant only when the animal received the highest dose of α-TA.

In addition, the period of α-TA supplementation also influenced α-tocopherol content in raw dark chicken meat (**Table 5**). Raw meat α-tocopherol content increased as a result of the days of α-TA supplementation, although meat α-tocopherol content from animals fed α-TA supplements for 10 days was not different from that of animals not receiving supplements. Similarly, meat α-tocopherol content from animals that received α-TA supplements for 32 days was not different from those of animals fed for either 21 or 43 days of supplementation.

Cooked meat α-tocopherol content was also influenced by the dose and duration of α-TA supplementation (**Table 5**). Greater α-TA doses led to significantly higher α-tocopherol content. Similarly, animals that received α-TA supplements for more days increased α-tocopherol content except for those animals fed α-TA supplementation for 43 days, which were not different from those fed for 32 days.

Table 5. Effect of α-Tocopheryl Acetate Supplementation on α-Tocopherol, Oxidative Status, and Susceptibility to Oxidation in Raw and Cooked Meat[a]

|	dose (mg/kg)			days of supplementation					SE[b]
	75	150	225	0	10	21	32	43	
	Raw Meat								
tocopherol[c]	17.6a	22.2a	29.1b	7.9a	14.9a	24.6b	30.9bc	36.5c	0.77
TBA[d]	35	34	28	53a	31b	29b	23b	26b	1.2
LHP[e]	314a	186b	112c	400a	291b	155c	133c	41d	8.5
	Cooked Meat								
tocopherol[c]	14.6a	20.5b	27.3c	6.9a	13.5b	22.4c	30.2d	31.0d	0.58
TBA[d]	651a	445b	321b	941a	506b	461bc	267cd	188d	23
LHP[e]	389a	215b	167b	440a	374a	192b	147b	132b	10

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 45$). Least-squares means corresponding to a dietary factor (dose or days of supplementation) with different letters differ significantly ($P \leq 0.05$). [b] Standard error of the global least-squares means. [c] Meat α-tocopherol content expressed in mg/kg. [d] TBA, thiobarbituric acid values (μg of malondialdehyde/kg). [e] LHP, induced lipid hydroperoxides (mg of cumene hydroperoxide equiv/kg).

In addition, there is an interaction between dietary factors (dose and duration of dietary α-TA supplementation) in α-tocopherol content in raw ($P = 0.020$) and cooked ($P = 0.001$) meat (**Figure 1**). The multiple linear regression analysis gave the following fitted model: $y = -2.03 + 6.77 \times$ days of α-TA
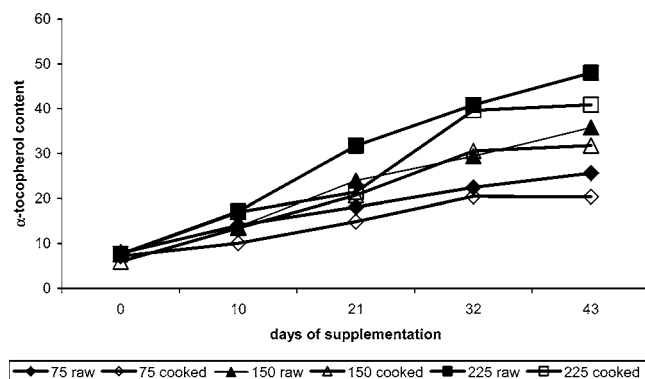
Figure 1. Influence of the dose (mg/kg) and duration of α-tocopheryl acetate supplementation on raw (filled symbols) and cooked (unfilled symbols) chicken meat α-tocopherol content (mg/kg).
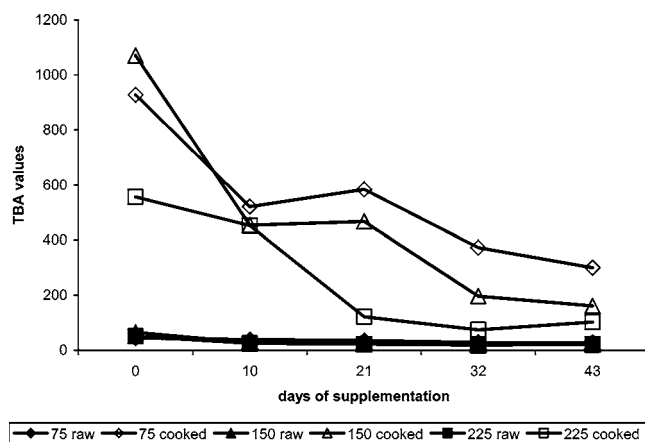
Figure 2. Influence of the dose (mg/kg) and duration of α-tocopheryl acetate supplementation on raw (filled symbols) and cooked (unfilled symbols) chicken meat thiobarbituric acid (TBA) values (μg of malondi-aldehyde/kg).

supplementation + 0.07518 × dose of α-TA supplementation ($P \leq 0.001$), where y is the α-tocopherol content in raw meat expressed in mg/kg. The adjusted R^2 statistic is 0.795, and the standard error of the estimate shows the standard deviation of the residuals to be 5.793.

Oxidative Status and Susceptibility to Oxidation. The oxidative status of raw meat, measured by means of TBA values, was influenced by the duration of α-TA supplementation. Animals that did not receive the α-TA supplementation produced raw meat with higher TBA values than meat from animals fed α-TA supplements. On the other hand, the dose of α-TA supplementation showed no significant effect on the oxidative status of raw meat (**Table 5**). In addition, TBA values in raw meat were lower than in cooked meat (**Figure 2**).

However, raw meat susceptibility to oxidation, measured by means of the ferrous oxidation−xylenol orange method that measures the induced LHP content, was affected by both the dose and duration of α-TA supplementation (**Table 5**). In addition, there is an interaction between dietary factors for the LHP content in raw meat ($P = 0.001$) (**Figure 3**).

In relation to cooked meat, TBA values and LHP content were influenced by either the dose or the duration of α-TA supplementation (**Table 5**). LHP content in cooked meat showed an interaction between dietary factors ($P = 0.002$) (**Figure 3**).

DISCUSSION

The dose and duration of α-TA supplementation led to small differences in some fatty acids. As for raw meat, there were
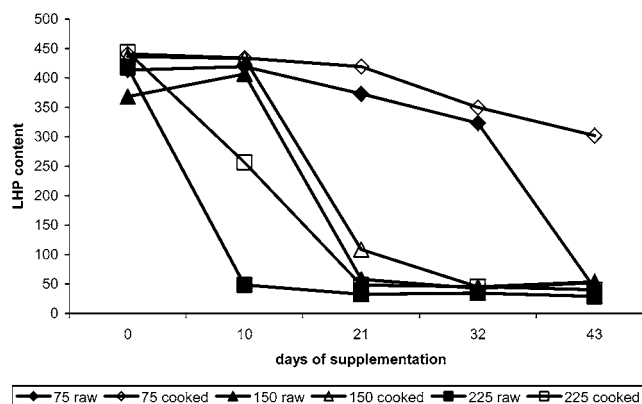
Figure 3. Influence of the dose (mg/kg) and duration of α-tocopheryl acetate supplementation on raw (filled symbols) and cooked (unfilled symbols) chicken meat lipid hydroperoxide (LHP) content (mg of cumene hydroperoxide equiv/kg).

small increases in various PUFA as a result of longer periods of α-TA supplementation (**Table 3**), whereas TBA values were significantly higher in meat from animals that did not receive the supplement (**Table 5**). Therefore, α-TA supplements increased meat α-tocopherol, and this protected PUFA from oxidation. In fact, Surai and Sparks (*11*), who studied the effect of 160 mg/kg α-TA supplementation in cockerels fed 3% fish oil, recorded an increased α-tocopherol content and PUFA increases in the phospholipid fraction of several cockerel tissues (testes, cerebellum, and heart muscle), although this latter effect was not recorded in the triacylglycerol fraction (*11*). Moreover, in laying hens fed 3.5% fish oil, both dark and white meat from those animals receiving a mixed tocopherols supplement (40 vs 367 mg of tocopherol analogues/kg) recorded lower TBA values, whereas these supplemented animals recorded some PUFA increases in only the white meat lipid fraction (*16*).

However, PUFA increases have not been reported in raw dark and white meats from animals fed tocopherol-supplemented diets with a lower degree of unsaturation such as hydrogenated soybean oil (*22*), palm oil, or sunflower oil (*16*). Furthermore, when animals that received two levels (70 and 140 mg/kg) of α-TA supplementation were compared, one supplement or the other was added to feeds containing 1.25 and 2.5% of fish oil, and these α-TA supplements led to meat with no differences in their fatty acid composition (*23*). Therefore, meats with low levels of α-tocopherol and relatively prone to oxidation, such as that situation at 0 days of α-TA supplementation (see Birds and Diets and **Table 5**), might lead to meat with a lower PUFA content as a consequence of lipid oxidation.

However, when the dose of α-TA supplementation was considered, the opposite trend in PUFA content was observed. These PUFA decreases, as a result of increased α-TA amounts, cannot be attributed to lipid oxidation because TBA values were lower in those meats from animals fed higher α-TA amounts (**Table 5**). Despite the fact that further studies are necessary to confirm this tocopherol dose-dependent effect on fatty acid composition, the effect on PUFA/SFA ratio is mainly explained by a higher content in 16:0 and a lower content in 20:4 n-6. Thus, the fact that tocopherol has been reported to alter the enzymatic activities of chain elongation−desaturation (*11, 24*) and, in mammalian cells, phospholipase A_2 presented a toco-pherol dose-dependent 20:4 n-6 release related with the synthesis of prostanoids (*25, 26*) may suggest these results are affected by some tocopherol-regulated enzymes.

In relation to cooked-in-bag dark chicken meat, the dose of α-TA supplementation led to trends in total SFA, total n-6

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PUFA, and PUFA/SFA ratio similar to those observed in raw meat, whereas for total n-3 PUFA the opposite trend was observed (**Tables 3** and **4**). On the other hand, the effect of the duration of α-TA supplementation on fatty acid composition in cooked meat showed trends that were very similar to those observed in raw meat (**Tables 3** and **4**).

Furthermore, despite the fact that Ajuyah et al. (*27*) reported no differences in fatty acids in the total lipid fraction of cooked dark meat, the authors reported higher contents of several PUFA in triglyceride and phosphatidyl fractions of cooked dark meat from broilers fed 15% full-fat flaxseed diet supplemented with mixed tocopherols at 200 mg/kg. These latter results could be explained by the antioxidant activity of tocopherols. In fact, a clear increase in α-tocopherol content was observed in the cooked meats from animals fed longer periods of α-TA supplementation (**Figure 1**), which clearly protected against lipid oxidation (**Table 5**).

In relation to α-tocopherol content in chicken meat, by means of a multiple linear regression analysis, a fitted model, taking into account the dose and duration of α-TA, was described. The influence of these two dietary factors on raw and cooked dark chicken meat α-tocopherol content was also observed in **Table 5**. In fact, α-tocopherol content has been reported to be increased in dark and white chicken meats by the dose (ranging from 20 to 600 mg/kg) and duration of α-TA supplementation (*7, 10*).

Nevertheless, by considering each α-TA dose, the longest period of α-TA supplementation (43 days prior to slaughter) led to raw and cooked dark chicken meat with α-tocopherol contents not statistically different from those of meats from animals fed α-TA supplements for 32 days (**Table 5**). Thus, in our experimental conditions, this is the optimum period for α-TA supplementation in relation to α-tocopherol content in meat.

Figure 1 shows how both dietary factors (dose and duration of α-TA supplementation) affected both raw and cooked meat in terms of α-tocopherol content. Thus, α-tocopherol in meat increased with higher periods or doses of α-TA supplementation, and the low levels found for 0 days may explain the interactions recorded in raw and cooked meat. However, cooked meat recorded a lower level of α-tocopherol (**Table 5** and **Figure 1**), so heating, which promotes oxidation, will explain this (*6, 7, 28*).

In relation to the oxidative status, TBA values in raw and especially cooked meat showed a trend that was opposite to the α-tocopherol content in meat (**Table 5** and **Figure 2**), indicating its protective effect. TBA values in raw meat decreased with increased periods of α-TA supplementation, whereas in cooked meat they decreased with increased doses and periods of α-TA supplementation. Nevertheless, TBA values in cooked meat from animals fed 225 mg/kg of α-TA for 21, 32, and 43 days were very similar and, moreover, were also similar to values for animals fed 150 mg/kg of α-TA for 32 and 43 days (**Figure 2**). This effect was observed in cooked meat because TBA values are increased as a result of cooking (**Figure 2**) and can be attributed to several factors: denaturalization of proteins, which means that antioxidant enzymes are inactivated; iron and other transition metals are released; and membrane disruption, which favors the contact between antioxidants and pro-oxidants (*7, 13, 28−31*). In addition, heat decomposes hydroperoxides into radicals (*32, 33*), which provokes an increase of the content of secondary oxidation products such as TBA values in cooked meat.

In relation to the susceptibility to oxidation, the induced LHP content tended to decrease with the duration of α-TA supple-

mentation. Therefore, animals fed longer periods of α-TA supplementation would result in meat with higher shelf life. However, different trends were observed in **Figure 3**, which will explain the interactions reported in both raw and cooked chicken meat. As can be observed, meat from animals that had received 225 mg/kg of α-TA had a lower LHP content in both raw and cooked meat, which indicates the effect of α-tocopherol on oxidation. Nevertheless, meat from animals fed 150 mg of α-TA/kg for 21 days reached a LHP content similar to meat from animals fed 225 mg of α-TA/kg for 43 days. Moreover, raw meat from animals fed 75 mg of α-TA/kg showed a lower LHP content only after 43 days of supplementation, whereas the LHP content in cooked meat did not decrease even when this supplement was fed throughout the animals' life.

Therefore, with regard to TBA values and LHP content in both raw and cooked dark chicken meat, meat from animals fed 225 mg/kg of α-TA for 21, 32, and 43 days showed an oxidative stability similar to that of meat from animals fed 150 mg/kg of α-TA for 32 or 43 days. However, this meant using different dietary α-TA amounts (525, 561, 617, 776, or 816 mg of α-TA) depending on the feeding regimen (150 mg/kg for 32 days, 150 mg/kg for 43 days, 225 mg/kg for 21 days, 225 mg/kg for 32 days, or 225 mg/kg for 43 days, respectively), so, taking into account economic criteria, the optimum feeding regimen can be set at 150 mg/kg of α-TA for 32 days. In addition, as can be observed in **Figures 1−3**, this dietary combination led to meat with a certain α-tocopherol content that seemed to be the minimum content providing an optimal meat oxidative stability.

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