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Effect of Heated Sunflower Oil and Dietary Supplements on the Composition, Oxidative Stability, and Sensory Quality of Dark Chicken Meat

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A factorial design was used to study the effect of dietary oxidized sunflower oils (fresh, heated at low temperatures, and heated at high temperatures), dl-α-tocopheryl acetate (0 or 100 mg/kg), and Zn supplementation (0 or 600 mg/kg) on the composition, oxidative stability, and sensory quality of dark chicken meat with skin from animals fed with a Se supplement (Se-enriched yeast, 0.6 mg of Se/kg). The positional and geometrical isomers of linoleic acid were increased in raw meat from chickens fed oils oxidized at high temperatures. In addition, supplementation with α-tocopheryl acetate increased the α-tocopherol content, whereas 2-thiobarbituric acid (TBA) values and lipid hydroperoxide content were reduced. Likewise, TBA values, rancid aroma, and rancid flavor also decreased in cooked dark meat. However, none of the dietary factors studied affected consumer acceptability scores of cooked meat. Furthermore, Zn supplementation increased the Se content in raw meat.

KEYWORDS: Oxidized sunflower oil; α-tocopherol supplementation; Zn supplementation; Se supplementation; fatty acid isomers

INTRODUCTION

Oils rich in polyunsaturated fatty acids are prone to lipid oxidation, which can occur at low or high temperatures, such as during long-term storage at room temperature or during frying. Nevertheless, a considerable number of distinct oxidation products are produced depending on the oxidative conditions. At low temperatures, fatty acid hydroperoxides are formed mainly in the initial stages of oxidation. They reach a plateau and then decompose into secondary oxidation products (*1*), whereas at high temperatures, these hydroperoxides are immediately decomposed into secondary oxidation products (*2*). Several of these oxidation products have a range of biological effects, which are mainly detrimental and related to a number of chronic and degenerative diseases (*3*−*5*). In addition, lipid oxidation products, at high doses, affect animal growth and metabolism (*6*−*8*). Nevertheless, the consumption of used frying fats and oils with polar compounds ≤25% is not detrimental to experimental animals even in long-term feeding trials (*7*−*9*).

However, few studies have addressed the effects of moderate levels of dietary supplements with distinct types of oxidized oils on the composition, oxidative stability, acceptability, and rancidity of meat. The consumption of oxidized oil leads to reduced oxidative stability of meat, which can be attributed to oil tocopherol loss in these oils (*10*, *11*). Moreover, decreased oxidative stability of meat from chickens fed oxidized oils is reversed by α-tocopheryl acetate supplementation (α-TA) (*11*−*13*), which is related to an improved sensory quality (*14*−*16*).

In addition, Se and tocopherol are interrelated in the in vivo antioxidant system in which the former, through glutathione peroxidase (GPx), contributes to the decomposition of lipid hydroperoxides into non-pro-oxidant species, whereas tocopherol acts as a chain-breaking antioxidant (*17*, *18*). Furthermore, the highest susceptibility to oxidation has been observed in animals fed diets deficient in Se and tocopherol (*19*−*21*), which demonstrates that both are crucial in the antioxidant system. Although inorganic and organic sources of Se restore GPx activity, supplementation with the latter is preferred in poultry farming because it is associated with lower meat drip loss, higher increase in meat Se content, and better productive characteristics (*22*, *23*).

Zn is also related to the antioxidant system through superoxide dismutase. Supplements of this mineral have no effect on the Zn content, oxidative stability, or sensory quality of chicken meat; however, they do lead to an increased Se content in mixed dark and white chicken meat (*24*).

Here we studied the effect of various dietary factors (oxidation degree of the fat source, α-TA supplements, and Zn supplements) on animal performance, fatty acid composition, crude fat, α-tocopherol, Zn, Se, Fe, and Cu contents, consumer

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Table 1. Oxidation Variables and α-Tocopherol Content of the Sunflower Oils Added to the Basal Diet

sunflower oil	PV[a]	AV[b]	polymers[c] (%)	α-tocopherol[d] (mg/L)
fresh	2.1	6.4	0.7	683
peroxidized	91.9	8.5	0.7	480
oxidized	7.0	83.1	4.9	655
very oxidized	17.2	153.0	9.4	619

[a] Peroxide value in milliequiv of peroxide/kg (*Official Method Cd 8-53*; American Oil Chemists' Society: Champaign, IL, 1998). [b] *p*-Anisidine value (*Official Method Cd 8-90*; American Oil Chemists' Society: Champaign, IL, 1998). [c] Expressed as percentage of polymerized triacylglycerols (*IUPAC Standard Method 2.508*; International Union of Pure and Applied Chemistry; Blackwell: Oxford, U.K., 1987). [d] Tocopherol content analysis was determined as described under Material and Methods section.

acceptability and rancidity scores, and TBA and ferrous oxidation−xylenol orange (FOX) values of raw and cooked dark chicken meat.

MATERIALS AND METHODS

Preparation of Oxidized Oils. Two thermal treatments were performed. The first consisted of heating 40 L of refined fresh sunflower oil (FSO) in a heat exchanger at 55−60 °C under agitation for 12 days. This procedure produced a peroxide value of 90 mequiv of O_2/kg of oil, that is to say, peroxidized sunflower oil (PSO). The second treatment involved heating 90 L of FSO in a direct heating fryer at 190−195 °C under agitation for 28 h until the oil had reached a *p*-anisidine value of 150 and 9.4% of polymerized triacylglycerols, that is, very oxidized sunflower oil (VOSO). By mixing 50% of FSO with 50% of VOSO, we obtained oxidized sunflower oil (OSO). Various oxidation variables and the α-tocopherol content of these sunflower oils are shown in **Table 1**. Oils were frozen at −20 °C until feed preparation.

Animals and Housing. Three hundred and thirty-six female broiler chicks (Ross 308, 1 day old) were randomly assigned to 48 floor pens (7 birds per pen) corresponding to 16 triplicated dietary treatments. Further housing parameters are as described elsewhere (*23*). Feed and water were provided ad libitum for 42 days. The animals were reared and slaughtered in compliance with national regulations, and the experiment received prior approval by the Animal Care and Use Committee of Copaga Soc. Coop (Lleida, Spain).

Diets and Experimental Design. A factorial design was used to study the effect of various dietary factors on animal performance, α-tocopherol, crude fat, Zn, Se, Fe, and Cu contents, fatty acid composition, consumer acceptability and rancidity scores, and TBA and FOX values of raw and cooked meats. Up to 10 days of age, a typical basal diet containing 6% of animal fat was supplied to the chickens (**Table 2**). From 11 to 42 days, chickens were given 1 of 16 dietary treatments, all supplemented with Se-enriched yeast at 0.6 mg of Se/kg. The treatments were prepared from a basal diet (**Table 2**) by combination of the dietary factors studied (**Table 3**). As oil thermal treatments caused α-tocopherol loss, diets with added PSO, OSO, and VOSO were adjusted by adding α-TA to contain the same amount of α-tocopherol as those feeds prepared with FSO. Thereafter, each α-tocopherol rectified feed was supplemented with Zn (0 or 600 mg/kg) and α-TA (0 or 100 mg/kg). These 16 dietary treatments were prepared every 10 days and supplied to chickens from 11 to 21 days, from 22 to 32 days, and from 33 to 42 days of age. For feed analyses, ∼200 g of each dietary feed was immediately vacuum packed in high-barrier multilayer bags (Cryovac BB-4L; Cryovac Europe, St. Boi de Llobregat, Spain) and kept at −20 °C.

Zinc sulfate and dl-α-TA were purchased from Andrés Pintaluba, S.A. (Reus, Spain). The organic source of Se came from Se-enriched yeast (Sel-Plex) and was supplied by Probasa (Sta. Perpetua de la Moguda, Spain).

Preparation, Cooking, and Storage of Samples. The chickens were slaughtered and were then stored for 24 h at 4 °C. Two random legs plus their skin were then chosen from each pen to study the composition and oxidative stability of raw meat. These samples were hand-deboned, mixed, ground, and weighed (∼30 g per bag), placed in high-barrier multilayer bags (Cryovac BB-4L; permeability to $O_2 = 30$ cm^3/m^2, 24 h, 1 bar, ASTMD-3985), and then vacuum packed and immediately stored at −20 °C until analysis of α-tocopherol, mineral content, fat content, and fatty acid composition. The remaining legs (with skin) from each pen were hand-deboned and vacuum packed in high-barrier multilayer bags (Cryovac CN-300; permeability to $O_2 = 15$ cm^3/m^2, 24 h, 1 bar, ASTMD-3985). At 35 h after slaughter, these samples were cooked in an oven at 85 °C (99% relative humidity) to an internal temperature of 78 °C. They were then cooled and stored at −20 °C until the consumer acceptability and rancidity scores and TBA values of cooked meat were determined.

Reagents and Standards. The reagents and standards used in element analysis were as described in Bou et al. (*26*), whereas those used in the other analyses were described in Bou et al. (*24*). Tocopherol analogues (α, *β*, *γ*, and *δ*) were quantified using a kit from Calbiochem (San Diego, CA).

Determination of Fatty Acid Composition. Lipid extraction was carried out with chloroform/methanol (2:1, v/v) in raw meat and in milled feed. Then fatty acid methyl esters were prepared from this fraction and determined by gas chromatography, as described elsewhere (*25*). The resulting 48 (16 treatments × 3) raw dark meat samples and the 16 feeds prepared for chickens from 22 to 32 days of age were analyzed.

Determination of Crude Fat Content. The fat content of the resulting 48 raw meat samples was measured according to AOAC Official Method 991.36 (*27*).

Determination of Tocopherols. α-Tocopherol was measured in raw meat and in milled feed following the method described elsewhere (*24*). In addition, using the same method, *β*-, *γ*-, and *δ*-tocopherol analogues were also measured. The chromatographic conditions did not separate *β*- and *γ*-tocopherol. The resulting 48 raw meat samples were analyzed. For feed analysis, the 16 feeds prepared for each period (11−21, 22−32, and 33−42 days of age) were analyzed.

Determination of Zn, Se, Fe, and Cu. The method used to measure element content was as described in Bou et al. (*26*). The resulting 48 raw meat samples and the 16 feeds prepared for chickens from 33 to 42 days of age were analyzed.

Determination of TBA and FOX Values. The TBA values of raw meat samples were measured through third-derivative spectrophotometry after acid aqueous extraction (*28*). Susceptibility to oxidation was determined by using the FOX method, as described by Grau et al. (*29*), after 130 h of incubation at room temperature. After incubation, absorbance was measured at 560 nm and lipid hydroperoxide content was quantified as described by Navas et al. (*30*).

Samples of cooked chicken legs were thawed, as for sensory analyses, by heating in a water bath at 35 °C for 20 min and were then ground before TBA measurement (*28*). TBA values of the blind control and the maximum and minimum references used for the sensory analysis were also determined.

Sensory Analysis. A consumer acceptability panel test was done on cooked meat after 4 months of storage at −20 °C. Thirty-two experienced panelists were selected from our department. Criteria for panel selection and sample preparation were as described elsewhere (*24*). Samples were presented to the panelists in a balanced incomplete block design (*31*): 16 blocks, 6 samples per block, and 6 replicates for each sample. This design was duplicated. In addition, each panelist evaluated the acceptability of a blind control (total samples presented to each panelist = 7), which was a vacuum-packed freshly cooked commercial chicken sample that had been stored for 1 day at −20 °C. Panelists were asked to rank the overall acceptability of the product using a 9-point scale (1 = very bad; 9 = very good).

In addition, a sensory analysis of the rancid aroma and flavor of the meat was carried out after 30 months of storage at −20 °C. Sample preparation was as follows: bags containing frozen samples were opened and kept at 4−5 °C for 9 days. Then, as for the consumer acceptability test, 20-g chicken pieces, with a similar amount of skin, were placed in screw-capped flasks. They were then heated at 75 °C for 20 min in a conventional air oven and served to trained panelists.

Twenty-four volunteers were trained to evaluate rancid aroma and flavor using cooked meat after refrigeration for a range of periods.

Table 2. Ingredients and Compositions of Basal Diets

diet up to 10 days	percentage	diet from 11 to 42 days[a]	percentage
ingredient		ingredient	
barley	45.42	wheat	56.61
soybean meal, 48% protein	23.37	soybean meal, 48% protein	24.69
sorghum	10.00	sunflower oil[b]	6.00
meat meal, 50% protein	6.00	sunflower meal	5.03
animal fat	6.00	soy grass	3.14
tapioca, 62% starch	3.03	calcium carbonate	1.69
soy grass	1.89	monocalcium phosphate	1.23
sepiolite	1.49	trace mineral–vitamin mix[c]	0.50
trace mineral–vitamin mix[d]	1.00	L-lysine	0.38
L-lysine	0.44	DL-methionine	0.26
DL-methionine	0.34	salt	0.20
salt	0.31	sodium bicarbonate	0.14
monocalcium phosphate	0.27	Avizyme 1300	0.12
calcium carbonate	0.26	choline chloride	0.03
choline chloride	0.12		
DL-threonine	0.05		
phytase[e]	0.04		
calculated composition		calculated composition	
dry matter	89.4	dry matter	90.1
crude protein	21.5	crude protein	20.9
crude fat	8.6	crude fat	8.2
crude fiber	2.5	crude fiber	3.6
ash	6.6	ash	6.1
metabolizable energy (cal/g)	3025	metabolizable energy (cal/g)	3000

[a] Supplemented with 0.6 mg of Se/kg from Se-enriched yeast. [b] Sunflower oils with distinct degrees of oxidation were added depending on the dietary treatments. [c] Supplies the following per kilogram of complete feed: 10000 IU of vitamin A, 2000 IU of vitamin D_3, 30 mg of vitamin E (dl-α-tocopheryl acetate), 5 mg of vitamin B_2, 2 mg of vitamin B_6, 2 mg of vitamin K_3, 1 mg of vitamin B_1, 10 μg of vitamin B_{12}, 30 mg of nicotinic acid, 48 μg of biotin, 10 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous carbonate), 100 mg of Mn (manganese oxide), 100 μg of Se (sodium selenite), 80 mg of Zn (zinc oxide), 10 mg of Cu (copper sulfate), 2 mg of I (potassium iodide), and 200 μg of Co (cobalt sulfate). [d] Supplies the following per kilogram of complete feed: 10000 IU of vitamin A, 2000 IU of vitamin D_3, 30 mg of dl-α-tocopheryl acetate, 20 μg of vitamin B_{12}, 4 mg of vitamin B_6, 5 mg of vitamin K_3, 5 mg of vitamin B_2, 2 mg of vitamin B_1, 66 mg of nicotinic acid, 200 μg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 μg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 μg of Co (cobalt sulfate), and 4 mg of butylated hydroxytoluene. [e] (EC 3.1.3.8), which releases 1000 FTU/g.

Table 3. Dietary Treatments Supplied from 11 Days of Age

sunflower oil[a] (6%)	α-tocopheryl acetate supplement (mg/kg)	Zn supplement (mg/kg)
fresh	0	0
fresh	100	0
fresh	0	600
fresh	100	600
peroxidized	0	0
peroxidized	100	0
peroxidized	0	600
peroxidized	100	600
oxidized	0	0
oxidized	100	0
oxidized	0	600
oxidized	100	600
very oxidized	0	0
very oxidized	100	0
very oxidized	0	600
very oxidized	100	600

[a] Sunflower oil diets were adjusted, by adding α-tocopheryl acetate, to contain the same amount of α-tocopherol as fresh sunflower oil diets. All dietary treatments were supplemented with Se-enriched yeast at 0.6 mg of Se/kg.

Afterward, eight preselected panelists received samples of this meat. After this test, five panelists, which showed correlation coefficients between TBA values and either descriptor of ≥0.5, were selected.

Samples were presented to each selected panelist in a Latin square design (*31*): Panelists received all samples in four working sessions in which four random samples were presented. They were asked to score rancid aroma and flavor, each on a 15 cm unstructured line scale anchored on the left side with the term "weak" and on the right side with "strong". Vertical lines drawn by the panelists through the horizontal line scales were converted into percentage scores. In addition to the samples to be evaluated, the panelists also received two reference samples corresponding to the minimum and the maximum of the scale. As a minimum sample, we chose meat from animals fed FSO and supplemented with α-TA that had been thawed overnight. As a maximum sample, we used meat that had been stored for 10 days at 4−5 °C from animals fed PSO and not supplemented with α-TA.

Statistical Analysis. Multifactor ANOVA was used to determine significant effects produced by the dietary factors on productive parameters, fatty acid composition, crude fat, tocopherol, Zn, Se, Fe, and Cu contents, and oxidative stability (FOX and TBA values) of the raw meat. Likewise, multifactor ANOVA was used to determine significant effects on consumer acceptability, rancidity scores, and TBA values of cooked meat stored under various conditions. Interactions between more than two factors were ignored. One-way ANOVA was used to determine significant differences between dietary treatments and the blind control used in the consumer acceptability test. Least-squares means for the main factors with a significant effect were separated using the Duncan test. In all cases, $P \leq 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Animal Performance. No differences were recorded in final body weight, feed conversion, or mortality after 42 days of breeding. Although feed intake was significantly lower in animals on Zn supplements (3720 versus 3801 g/bird), this reduced intake did not result in a significant decrease in body weight (1898 versus 1906 g) or feed conversion ratio (1.963 versus 1.996). The lack of effect of Zn supplements on body weight and feed conversion ratio is consistent with previous results (*23*).

Fatty Acid Composition and Total Fat Content. The fatty acid composition of feed was affected by the addition of

Table 4. Fatty Acid Composition (Expressed as Area Normalization in Percent) of the Experimental Feeds and the Effect of Dietary Factors on the Fatty Acid Composition of Raw Dark Chicken Meat[a]

	feeds with added sunflower oils				raw dark chicken meat with skin							
					sunflower oil added				tocopherol added[b]		Zn added[c]	
fatty acid	FSO	PSO	OSO	VOSO	FSO	PSO	OSO	VOSO	0 mg/kg	100 mg/kg	0 mg/kg	600 mg/kg
14:0	0.114	0.118	0.111	0.114	0.370	0.373	0.369	0.364	0.368	0.370	0.370	0.368
16:0	9.114a	9.309bc	9.204ab	9.379c	16.135	16.259	16.446	16.273	16.282	16.274	16.406	16.150
18:0	3.920a	3.961b	3.952ab	4.019c	5.729	5.625	5.710	5.598	5.650	5.681	5.644	5.687
20:0[d]	0.287a	0.291ab	0.292b	0.297c	0.206ab	0.201a	0.224b	0.226b	0.214	0.215	0.211	0.218
22:0	0.564a	0.567a	0.576b	0.585c								
24:0	0.197b	0.186a	0.200b	0.200b								
total SFA	14.196a	14.431b	14.334b	14.593c	22.441	22.457	22.749	22.460	22.407	22.433	22.528	22.312
16:1n–9	0.036a	0.039b	0.039b	0.040b	0.413	0.429	0.400	0.402	0.409	0.413	0.415	0.407
16:1n–7	0.154	0.158	0.149	0.153	2.371	2.527	2.586	2.713	2.563	2.535	2.597	2.502
18:1n–9[d]	24.276a	24.470bc	24.395ab	24.596c	29.930	30.477	30.581	30.794	30.605	30.286	30.648	30.244
18:1n–7	0.748	0.758	0.786	0.785	1.110	1.124	1.088	1.124	1.106	1.118	1.087	1.136
20:1n–9[d,e]	0.237	0.245	0.239	0.241	0.259	0.263	0.259	0.262	0.264a	0.258b	0.262	0.260
total MUFA	25.452a	25.670b	25.607b	25.815c	34.084	34.820	34.914	35.295	34.947	34.610	35.008	34.548
18:2n–6	57.254a	56.777b	56.838[b]	56.244c	39.294	38.613	38.292	38.011	38.458	38.647	38.243	38.862
18:3n–6					0.109	0.109	0.109	0.106	0.108	0.108	0.108	0.108
20:2n–6					0.315	0.309	0.295	0.303	0.304	0.307	0.304	0.307
20:3n–6					0.238	0.247	0.242	0.256	0.240a	0.252b	0.245	0.247
20:4n–6	0.028	0.030	0.028	0.031	0.889	0.882	0.820	0.891	0.845	0.895	0.861	0.880
22:4n–6					0.257	0.249	0.227	0.244	0.235a	0.254b	0.241	0.247
22:5n–6					0.063	0.063	0.058	0.065	0.060	0.065	0.061	0.063
total n–6 PUFA	57.282a	56.807b	56.866b	56.275c	41.165	40.469	40.043	39.878	40.356	40.641	40.173	40.824
18:3n–3[e]	2.660	2.693	2.679	2.740	1.784	1.753	1.768	1.787	1.768	1.778	1.758	1.788
20:5n–3					0.035	0.031	0.035	0.037	0.033	0.036	0.035	0.034
22:5n–3					0.096	0.093	0.084	0.093	0.087a	0.096b	0.091	0.092
22:6n–3	0.022	0.024	0.019	0.020	0.067	0.062	0.051	0.060	0.057	0.063	0.062	0.058
total n–3 PUFA	2.683	2.716	2.698	2.760	1.983	1.937	1.938	1.976	1.945	1.973	1.946	1.972
total PUFA	60.025a	59.523b	59.564b	59.035c	43.148	42.407	41.981	41.854	42.301	42.614	42.119	42.795
trans-18:1[f]	0.011a	0.011a	0.042b	0.066c	0.116	0.114	0.122	0.127	0.118	0.122	0.110a	0.129b
di-*trans*-LA	0.021a	0.021a	0.048b	0.071c	0.046a	0.045a	0.051a	0.066b	0.052	0.052	0.053	0.051
mono-*trans*-LA	0.404ab	0.382a	0.428b	0.471c	0.187a	0.182a	0.201b	0.207b	0.193	0.196	0.196	0.193
total LA isomers	0.425a	0.404a	0.476b	0.543c	0.233ab	0.227a	0.253b	0.272c	0.245	0.243	0.244	0.244
9*c*,11*t*-CLA	0.025a	0.032a	0.036b	0.041c	0.050	0.045	0.048	0.051	0.049	0.048	0.048	0.049
10*t*,12*c*-CLA	0.019	0.022	0.026	0.028	0.010	0.007	0.006	0.008	0.007	0.009	0.009	0.006
di-*trans*-CLA	0.062a	0.061a	0.102b	0.144c	0.034a	0.036a	0.047b	0.060c	0.045	0.037	0.044	0.044
total CLA	0.102a	0.108a	0.134b	0.164c	0.094a	0.086a	0.101a	0.119b	0.100	0.101	0.102	0.100

[a] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LA, linoleic acid; CLA, conjugated linoleic acid; FSO, fresh sunflower oil; PSO, peroxidized sunflower oil; OSO, oxidized sunflower oil; VOSO, very oxidized sunflower oil. Values given in this table for feed samples correspond to means ($n = 4$), whereas those for meat samples correspond to least-squares means obtained from multifactor ANOVA ($n = 48$). Means and least-squares means with different letters differ significantly ($P \leq 0.05$). [b] Supplementation with 0 or 100 mg/kg of α-tocopheryl acetate. [c] Supplementation with 0 or 600 mg/kg of Zn as zinc sulfate. [d] Interaction of dietary sunflower oil source × α-tocopheryl acetate supplementation significant at $P \leq 0.05$. [e] Interaction of α-tocopheryl acetate supplementation × Zn supplementation significant at $P \leq 0.05$. [f] Interaction of dietary sunflower oil source × Zn supplementation significant at $P \leq 0.05$.

sunflower oil (**Table 4**). The thermal treatment of these oils led to a significant decrease in linoleic acid (LA) and total polyunsaturated fatty acids (PUFA) and, consequently, provoked a relative increase in monounsaturated and saturated fatty acids, as discussed in Bou et al. (*25*). Fats and oils heated at 190 °C or above suffer geometric isomerization (*25*, *32*−*34*). This explains the differences recorded in feeds containing OSO and VOSO for a mixture of *trans*-octadecenoic acids (*trans*-18:1), *trans*-9,*trans*-12-octadecadienoic (di-*trans*-LA), *trans*-9,*cis*-12- and *cis*-9,*trans*-12-octadecadienoic acid group (mono-*trans*-LA), *cis*-9,*trans*-11-octadecadienoic acid (9*c*,11*t*-CLA), and a mixture of di-*trans*-conjugated linoleic acids (di-*trans*-CLA). The identification, formation, and deposition of these geometrical and positional isomers in dark meat have been discussed elsewhere (*25*).

Dietary sunflower oil produced significant differences in the fatty acid composition of dark meat (**Table 4**). These differences reflected, to a certain extent, the differences in feeds and explained the increase in 20:0 in meat from animals fed OSO and VOSO diets. Moreover, the total LA isomer group, which is the sum of mono-*trans*-LA and di-*trans*-LA, was higher in animals on VOSO, followed by animals on OSO. This result shows the effect of the fatty acid composition of the feed on meat. Likewise, di-*trans*-CLA content showed significant differences between the OSO and VOSO groups because of heating treatments, whereas the di-*trans*-CLA content of meat from animals on FSO and PSO did not differ. Thus, di-*trans*-CLA and total LA isomer content in meat reflected the differences observed in feeds. However, taking into the account total CLA isomers in dark meat, this group was different only in animals fed VOSO.

In contrast, 9*c*,11*t*-CLA and 10*t*,12*c*-CLA did not differ between treatments. Nonetheless, the former was more easily incorporated than the latter. In addition, LA, total n−6 PUFA,

Table 5. Fat, α-Tocopherol, Zn, Se, Fe, and Cu Contents in Raw Dark Chicken Meat Expressed in 100 g of Edible Portion[a]

studied factor	fat (g)	α-tocopherol (mg)	β- + γ-tocopherol (mg)	Zn (mg)	Se (μg)	Fe (mg)	Cu (mg)
sunflower oil							
fresh	14.7	2.58	0.123	1.38	32.6	0.71	35
peroxidized	15.2	2.17	0.116	1.36	32.4	0.77	36
oxidized	15.7	2.44	0.119	1.39	33.1	0.73	34
very oxidized	15.4	2.26	0.117	1.40	31.6	0.75	33
SEM[b]	0.47	0.11	0.0063	0.025	0.54	0.024	1.4
α-tocopheryl acetate supplementation							
0 mg/kg	15.5	1.68a	0.116	1.39	32.5	0.75	35
100 mg/kg	15.0	3.04b	0.121	1.38	32.3	0.73	35
SEM	0.33	0.080	0.0044	0.018	0.38	0.017	1.0
Zn supplementation							
0 mg/kg	15.5	2.33	0.114	1.36	31.9a	0.73	35
600 mg/kg	15.0	2.39	0.123	1.41	33.0b	0.76	34
SEM	0.33	0.080	0.0044	0.018	0.38	0.017	1.0

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 48$). Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$). [b] Standard error of the least-squares means.

and total PUFA did not show significant differences between meats from animals fed different sunflower oils, whereas significant differences were recorded in those feeds.

Therefore, these results seemed to indicate that feed fatty acid composition but also some metabolic pathways influence both isomerized and nonisomerized fatty acid content of chicken meat.

Significant increases in 20:3n−6, 22:4n−6, and 22:5n−3 were recorded in meat from α-TA-supplemented treatments (**Table 4**), which is consistent with other findings (*35*, *36*). In this regard, several authors propose that tissue PUFA are protected from oxidation by tocopherol supplementation or that tocopherols enhance the synthesis of a number of PUFA through the Δ^6 desaturase pathway (*36*, *37*). Conversely, α-TA supplementation has no effect on the fatty acid composition of meat from animals on hydrogenated soybean oil (*38*), olive oil or tallow (*15*), or fish oil when comparing animals fed 1.25 or 2.5% of this oil α-TA supplemented at either 70 or 140 mg/kg (*24*).

In addition, the increase in the content of some PUFA may cause a relative decrease in 20:1n−9, which was recorded when animals received α-TA supplements. Nevertheless, the interaction between sunflower oil source and α-TA supplements affected the 18:1n−9, 20:0, and 20:1n−9 content. The animals on FSO had more 18:1n−9 and 20:1n−9 when supplemented with α-TA, whereas those on the PSO and VOSO treatments had higher 18:1n−9 and 20:1n−9 contents in the absence of α-TA supplements. Indeed, Lauridsen et al. (*39*) also reported a significant effect, without finding a pattern, of the interaction between dietary oil source (tallow and olive oil) and α-TA supplementation (20 and 200 mg/kg) on the occurrence of fatty acids in chicken muscle membranal fractions. In relation to our findings, the distinct amounts of PUFA provided by the feeds and the effect of α-tocopherol on oxidative stability, and probably on Δ^6 desaturase, could partially explain this interaction.

Zn supplementation at 600 mg/kg caused a significant increase in *trans*-18:1 content. No explanation can be given for this effect, and further studies are required to confirm this result and also the effect of interactions observed between sunflower oil source and Zn supplementation on *trans*-18:1 and between α-TA and Zn supplements on LA and 20:1n−9.

However, Zn supplementation had no effect on any other fatty acid. Our results are consistent with a previous study on Zn supplementation (0, 300, and 600 mg/kg) that reported no effect on the fatty acid composition of meat from chickens fed various nonheated fat sources (*25*). Furthermore, dietary sunflower oil source and Zn and α-TA supplements did not affect the crude fat content of meat (**Table 5**).

Tocopherol Content. The average contents of α-tocopherol and other tocopherols supplied by the feeds are shown in **Table 6**. As tocopherol losses from sunflower oil caused by the thermal treatments were rectified (see Materials and Methods), there were no significant differences in feed α-tocopherol content. No differences were observed for other tocopherols between treatments. Nevertheless, as expected, dietary supplements with α-TA provided distinct feed α-tocopherol supplies.

Chickens on feeds rectified with α-TA did not show significant differences in the α-tocopherol content of raw meat (**Table 5**). However, nonrectified diets in which added oxidized oils were heated at temperatures below 80 °C, reaching peroxide values >150 mequiv of O_2/kg, led to lower α-tocopherol content in chicken meat (*10*, *11*, *40*, *41*). Therefore, these results may be attributed to either the decreased oil tocopherol content, the destruction of α-tocopherol in the feeds by oxidation products, or the use of some of the α-tocopherol in protecting tissue lipids from oxidized oil-induced free radical attack (*10*, *11*, *40*).

Similar results have been reported when animals received diets rectified with α-tocopherol, in which the oxidized oils added were previously heated at temperatures >120 °C (*12*, *13*). Therefore, diets containing oxidized oils are rich in secondary oxidation products and are comparable to our OSO and VOSO treatments.

Sheehy et al. (*12*) rectified with α-TA oxidized sunflower and linseed oils (heated at 140 °C for 24 h). Dietary treatments using these oxidized oils, containing 50 mg/kg of α-tocopherol, produced a lower α-tocopherol content in chicken plasma and breast and thigh tissues than diets including fresh oils.

Likewise, these authors compared the effect of feed supplements with fresh sunflower oil, containing 30 mg of α-tocopherol/kg of feed, with the addition of heated sunflower oil (11 h at 120 °C), containing 25 mg of α-tocopherol/kg of feed, on the α-tocopherol content of several chicken tissues (*13*). In that study, chicken plasma, liver, thigh, breast, lung, pancreas, and spleen had reduced α-tocopherol contents, whereas the brain was not affected by the addition of oxidized sunflower oil. The lowered tissue α-tocopherol content was attributed to impaired intestine hydrolysis of α-TA by lipid oxidation products, the destruction of α-tocopherol in the gastrointestinal tract by free radical attack, and the effect of certain oxidation products of

Table 6. Tocopherol, Zn, Se, Fe, and Cu Contents in Feeds[a]

studied factor	α-tocopherol (mg/kg)	β- + γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	Zn (mg/kg)	Se (μg/kg)	Fe (mg/kg)	Cu (mg/kg)
sunflower oil[b]							
fresh	110	22.2	2.8	417	633	173	25
peroxidized	105	21.3	2.7	336	617	167	22
oxidized	111	22.4	2.7	386	623	178	22
very oxidized	103	21.0	2.7	370	626	174	18
SEM[c]	3.7	0.10	0.38	28	11	2.3	3.2
α-tocopheryl acetate supplementation							
0 mg/kg	70a	22.0	2.7	364	614	172	21
100 mg/kg	144b	21.5	2.7	390	633	174	23
SEM	2.6	0.39	0.06	20	8.1	1.6	2.3
Zn supplementation							
0 mg/kg	104	21.3	2.7	107a	631	176	21
600 mg/kg	110	22.2	2.8	647b	619	170	22
SEM	2.6	0.39	0.06	20	8.1	1.6	2.3

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 48$ and $n = 16$ for tocopherol analogues and element content, respectively). Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$). [b] α-Tocopherol losses due to heating in peroxidized, oxidized, and very oxidized oil diets were adjusted, by adding α-tocopheryl acetate, to contain the same amount of α-tocopherol as fresh sunflower oil diets. All dietary treatments were supplemented with Se-enriched yeast at 0.6 mg of Se/kg. [c] Standard error of the least-squares means.

the heated oils, which may be absorbed and may destroy α-tocopherol in animal tissues.

Although the diets used in the above-mentioned studies were rectified by adding α-TA, they provided a much lower α-tocopherol content than those tested in our study (nonsupplemented diets provided 70 mg of α-tocopherol/kg). Therefore, in diets containing oxidized oils, only those diets providing a low and/or different content in α-tocopherol cause a decreased α-tocopherol content in chicken meat. Nevertheless, our results are consistent with the trend reported by these authors.

In contrast, a significant increase in α-tocopherol was observed in broilers fed α-TA supplements (**Table 5**). This effect has been widely reported in raw chicken meat (*37*, *39*, *42*, *43*).

Zn supplementation had no influence on chicken meat α-tocopherol content (**Table 5**), which is in agreement with previous studies (*23*, *24*).

In relation to tocopherol analogues, δ-tocopherol was not detected in meat; therefore, we report only the sum of β- and γ-tocopherols, which were not affected by any of the dietary factors studied (**Table 5**).

Element Content. Feed element composition is shown in **Table 6**. As expected, Zn supplementation led to significant increase in this element in feeds.

The contents of Zn, Se, Fe, and Cu in raw meat were not affected by the sunflower oil source (**Table 5**). Meat from chickens fed 1.25% animal fat, linseed oil, or fish oil 1 week before slaughter did not show significant differences in the content of these elements (*23*).

α-TA supplementation did not have a significant effect on the element content of raw meat (**Table 5**). However, when poultry are reared under environmental stress, plasma and tissue concentrations of minerals such as Fe and Zn are reduced (*44*, *45*). In this situation, in Japanese quail, these decreases are counteracted by increasing both α-TA and Se supplementation, which causes increased Fe and Zn serum concentrations, although a decrease in Cu also occurs (*46*).

The Se content of mixed dark and white raw meat is increased by organic Se supplementation (*23*). In that study, the organic Se supplementation (0.2 mg of Se/kg) came from Se-enriched yeasts. These are rich in selenomethionine, which is incorporated nonspecifically into distinct proteins (*22*, *23*). In addition, Bou et al. (*23*) also reported an increased Se content as a result of Zn supplementation (0, 300, or 600 mg/kg).

In the present study it is confirmed that Zn supplementation (600 mg/kg) provokes an increase of Se content in dark meat when animals receive a high dose of organic Se supplementation (0.6 mg of Se/kg) (**Table 5**). Nevertheless, the Se content reported here was greater and the differences were smaller than those reported by Bou et al. (*23*) in mixed dark and white raw meat. This finding can be explained by the higher dose of organic Se added to the feeds and by the higher GPx activity in thigh than in breast meat (*47*). In addition, it has been hypothesized that metallothionein, induced by dietary Zn, can interact with some Se forms (*23*). Therefore, because several Se forms are present in different amounts in either thigh or breast meat (*47*), this could explain both the higher Se content and the smaller increase found in dark chicken meat as a result of Zn supplementation.

However, Zn supplementation did not affect the Zn, Fe, or Cu content of dark meat (**Table 5**), which is also consistent with previous reports (*23*, *24*). However, Japanese quails reared under heat stress (34 °C), fed Zn (30 or 60 mg/kg) supplements, showed increased serum Zn, ascorbic acid, and α-tocopherol concentrations as dietary Zn supplementation increased (*45*). Moreover, laying hens, reared at low temperature (6.8 °C) and fed a Zn supplement (30 mg/kg), have increased serum α-tocopherol, ascorbic acid, Fe, Zn, Mn, and Cr concentrations (*44*). Therefore, although some vitamin and element serum concentrations are increased through Zn supplements in animals reared under environmental stress, in our conditions this supplementation did not increase the Fe, Zn, or Cu content of dark meat. In contrast, supplements with Zn did increase the content of Se.

Oxidative Stability of Raw Dark Chicken Meat (TBA and FOX Values). The oxidative stability of dark meat was studied using the TBA and FOX methods. The former measures the malondialdehyde content, a secondary oxidation product, whereas the latter measures lipid hydroperoxide formation.

Dietary sunflower oil source did not increase TBA values (**Table 7**). These results are in accordance with those reported by Grau et al. (*43*), who described that the addition of 6% of oxidized sunflower (160 °C, 12 h) did not increase TBA values in raw and cooked dark chicken meat compared with animals

Table 7. TBA and FOX Values of Raw Dark Chicken Meat[a]

studied factor	TBA (μg of malondialdehyde/kg)	FOX (μmol of cumen hydroperoxide equiv/kg)
sunflower oil		
fresh	18	713
peroxidized	22	965
oxidized	20	762
very oxidized	22	699
SEM[b]	1.6	148
α-tocopheryl acetate supplementation		
0 mg/kg	22a	1222a
100 mg/kg	19b	348b
SEM	1.1	105
Zn supplementation		
0 mg/kg	20	660
600 mg/kg	21	909
SEM	1.1	105

[a] Values correspond to least-squares means obtained from multifactor ANOVA ($n = 48$). Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$). [b] Standard error of the least-squares means.

fed 6% beef tallow, sunflower oil, or linseed oil. Similarly, Sheehy et al. (*13*) reported that the plasma TBA values of animals fed 4% FSO did not differ from those fed a heated sunflower oil (120 °C, 11 h) when this oil contained the same amount of α-tocopherol as FSO. Nevertheless, these authors reported higher TBA values for animals fed the same heated sunflower oil without rectifying the tocopherol losses caused by oil heating. In accordance with these findings, the authors proposed that the increased TBA values in plasma of chicks fed oxidized sunflower oil without rectified α-tocopherol losses resulted from peroxidation of plasma lipids rather than from the absorption of malondialdehyde and other TBA-reactive substances from the gastrointestinal tract (*13*).

Galvin et al. (*11*) compared the TBA values of dark and white chicken meat on several diets containing 6% FSO or 6% of an oxidized sunflower oil. The TBA values from animals fed FSO plus 30 mg/kg of α-TA did not differ from those on oxidized sunflower oil plus 200 mg/kg of α-TA. However, the TBA values from the oxidized oil or oxidized oil plus 30 mg of α-TA/kg treatments were higher than in the FSO plus 30 mg of α-TA/kg group. Nevertheless, it should be taken into the account that Galvin et al. (*11*) did not rectify the tocopherol losses caused by oil heating. Thus, these authors hypothesized that the lower tocopherol and higher oxidation product contents in treatments with oxidized sunflower oil lead to higher TBA values in dark and white meats. In our study, because the α-tocopherol content of the oxidized oils was rectified and the supply of tocopherol was high (nonsupplemented diets contained 70 mg of α-tocopherol/kg), no differences for TBA values were detected between treatments. However, the same trend for these values was observed in animals on PSO and VOSO compared with those on FSO (**Table 7**).

Similarly to TBA values, lipid hydroperoxide formation (FOX values) did not show differences between sunflower oil sources (**Table 7**). This result is consistent with findings on raw chicken meat from oxidized sunflower oil and FSO treatments (*43*). Therefore, the consumption of oils rich in secondary or primary oxidation products did not provoke a higher oxidative status (TBA values) or susceptibility to oxidation (FOX values).

Furthermore, animals on α-TA supplements showed decreased TBA and FOX values (**Table 7**). These results are in agreement with the values reported in dark chicken meat from animals on α-TA (0 versus 225 mg/kg) supplements (*43*). Similar results have also been described in meat from chickens on oxidized sunflower oil supplemented with α-TA (*10*, *11*). Thus, the meat from animals on α-TA supplements contained lower secondary oxidation products and was also less susceptible to oxidation.

Therefore, the α-tocopherol content in feeds and their further incorporation into tissues protect meat against oxidation. Moreover, the high α-tocopherol content supplied by the α-tocopherol-rectified feeds assayed (**Table 6**) accounted for the absence of significant differences between animals fed oxidized oils. Thus, feed producers and stockbreeders using fat sources oxidized or very susceptible to oxidation should increase

Table 8. Effect of Dietary Factors on Acceptability, Rancid Aroma, Rancid Flavor, and TBA Values of Cooked Dark Chicken Meat Stored for a Range of Periods under Different Conditions[a]

studied factor	acceptability[b]	TBA, 4 months[c]	TBA, 30 months[d]	rancid aroma[e]	rancid flavor[e]	TBA, 30 months + 9 days[f]
sunflower oil						
fresh	5.3	115	147	56	48	779
peroxidized	5.2	119	194	57	49	768
oxidized	4.8	104	179	40	33	850
very oxidized	5.1	102	212	48	34	670
SEM[g]	0.29	11	19	6.7	5.7	57
α-tocopheryl acetate supplementation						
0 mg/kg	5.1	130a	210a	59a	50a	841a
100 mg/kg	5.1	90b	156b	41b	33b	692b
SEM	0.20	8.1	13	4.8	4.0	41
Zn supplementation						
0 mg/kg	5.1	108	177	52	43	719
600 mg/kg	5.1	112	189	48	39	814
SEM	0.20	8.1	13	4.8	4.0	41

[a] Values correspond to least-squares means obtained from multifactor ANOVA. Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$). [b] Overall acceptability of samples stored for 4 months at –20 °C ($n = 192$) was ranked using a 9-point scale (1 = very bad; 9 = very good). Treatments did not differ from the blind control. [c] TBA values (μg of malondialdehyde/kg, $n = 32$) were measured in samples stored for 4 months at –20 °C. Blind control averages 123 μg of malondialdehyde/kg. [d] TBA values (μg of malondialdehyde/kg, $n = 32$) were measured in samples stored for 30 months at –20 °C. [e] Rancid aroma and flavor scores of samples stored for 30 months at –20 °C and then for 9 days at 4–5 °C ($n = 80$). Scores for rancid aroma and flavor ranged from 0 (minimum reference) to 100 (maximum reference). [f] TBA values (μg of malondialdehyde/kg, $n = 32$) were measured in samples stored for 30 months at –20 °C and then for 9 days at 4 °C. Among the samples evaluated for rancidity, the minimum and maximum references showed the lowest and highest TBA values, respectively. [g] Standard error of the least-squares means.

the level of α-tocopherol in the trace mineral−vitamin mix added to the feed because this will improve the oxidative stability and nutritional value of meat.

Zn supplementation did not affect TBA or FOX values (**Table 7**). However, lower serum and liver TBA values have been reported in poultry, reared under environmental stress conditions of low and high temperature, on Zn supplements in comparison with untreated animals (*44*, *45*).

Finally, it should be taken into account that dietary Se, especially in combination with α-tocopherol, lowers TBA values in chicken tissues and increases GPx activity in several tissues (*20*, *46*, *48*) and also in chicken muscles (*19*, *49*).

Sensory Analysis and TBA Values of Cooked Dark Chicken Meat. Two sensory analyses were carried out. First, a consumer panel performed an acceptability test of cooked meat after 4 months of frozen storage. Second, the rancidity of the meat was evaluated by a trained panel.

Dietary sunflower oil did not have a significant effect on overall consumer acceptability, although the greatest differences were observed within this dietary factor (**Table 8**), which is consistent with the results from a previous study (*50*).

Likewise, α-TA supplementation did not affect consumer acceptability scores (**Table 8**). However, this dietary treatment (225 mg/kg) has been reported to increase the consumer acceptability of cooked dark meat with skin from chickens fed a range of fats (beef tallow, sunflower oil, oxidized sunflower oil, linseed oil) after storage for 13 months at −20 °C (*50*). However, animals fed fats with a low degree of unsaturation and/or receiving enough α-TA supplementation provide acceptable meat even after long-term frozen storage (*23*, *24*, *50*). In addition, Zn supplementation did not have a significant effect on consumer acceptability, as previously reported (*23*, *24*).

In relation to TBA values, samples stored for 4 months at −20 °C were affected only by α-TA supplementation (**Table 8**). Therefore, α-tocopherol, which is increased after this supplementation (**Table 5**), led to more stable meat because it delays the oxidative deterioration of meat (*39*, *51*, *52*). Thus, α-tocopherol reduces TBA values and undesirable flavors and odors in chicken meat, both of which are highly correlated (*15*, *16*, *50*, *53*, *54*). However, the TBA values and their differences between dietary treatments (**Table 8**) are too low to detect significant differences in acceptability scores (*50*).

The TBA values of cooked samples were analyzed again after 30 months of frozen storage (**Table 8**). Neither dietary sunflower oils nor Zn supplementation affected these values. However, α-TA supplementation led to a decrease in TBA values, indicating its protective effect (**Table 8**). Again, the TBA values and their differences between dietary treatments were not high enough to detect sensory differences between samples (*50*). Consequently, samples were further stored under refrigeration to induce oxidation. Thus, after 30 months, the bags containing frozen samples were opened and stored for 9 days at 4−5 °C before rancidity evaluation.

Neither rancid aroma nor flavor scores were affected by dietary sunflower oils or Zn supplementation after further storage of the cooked meat for 9 days under refrigeration (**Table 8**). However, α-TA supplementation led to a decrease in these rancid scores. In addition, α-tocopherol supplementation significantly affected the TBA values of meat stored for 30 months at −20 °C and then for 9 days at 4−5 °C. As commented above, this effect was also significant after 4 and 30 months of frozen storage. Therefore, TBA determination is a much more sensitive method to assess lipid oxidation in chicken meat than the sensory evaluation of rancidity. Moreover, from these results, differences in meat rancidity can be set by the trained panel at >700 μg of malondialdehyde/kg of meat, which is in agreement with the results reported by Gray and Pearson (*55*).

ABBREVIATIONS USED

α-TA, α-tocopheryl acetate; GPx, glutathione peroxidase; FSO, fresh sunflower oil; PSO, peroxidized sunflower oil; OSO, oxidized sunflower oil; VOSO, very oxidized sunflower oil; LA, linoleic acid; CLA, conjugated linoleic acid; FOX, ferrous oxidation−xylenol orange; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; TBA, 2-thiobarbituric acid.

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