Effect of Dietary Fat Sources and Zinc and Selenium Supplements on the Composition and Consumer Acceptability of Chicken Meat

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ABSTRACT A factorial design was used to study the effect of changes in broiler feed on the composition and consumer acceptability of chicken meat. One week before slaughter, 1.25% dietary fish oil was removed from the feed and replaced by other fat sources (animal fat or linseed oil) or we continued with fish oil, and diets were supplemented with Zn (0, 300, or 600 mg/kg), and Se (0 or 1.2 mg/kg as sodium selenite or 0.2 mg/kg as Se-enriched yeast). The changes in dietary fat led to distinct fatty acid compositions of mixed raw dark and white chicken meat with skin. The fish oil diet produced meat with the highest eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) content, whereas the linseed oil diet led to meat with the highest content in total n-3 polyunsaturated acids (PUFA), especially linolenic acid. However, meat from animals on the animal fat diet was still rich in very long-chain n-3 PUFA. Se content was affected by Se and Zn supplements. Se content increased with Zn supplementation. However, only Se from the organic source led to a significant increase in this mineral in meat compared with the control. Consumer acceptability scores and TBA values of cooked dark chicken meat after 74 d or after 18 mo of frozen storage were not affected by any of the dietary factors studied.

(*Key words*: fat source, zinc supplementation, selenium supplementation, chicken meat composition, consumer acceptability)

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INTRODUCTION

One of the goals of food scientists is to increase the nutritional value of foods without compromising sensory quality. In this regard, n-3 polyunsaturated fatty acids (PUFA), especially eicosapentanoic (EPA) and docosahexanoic acid (DHA), have beneficial effects on human health (Food and Nutrition Board, 2002a). Many studies have explored enrichment of chicken meat with EPA and DHA through addition of dietary fish oil (FO; Hargis and Van Elswyk, 1993; Scaife et al., 1994; Wood and Enser, 1997; Surai and Sparks, 2000). However, these acids are prone to oxidation, and, consequently, their use in meat enrichment may produce off-tastes and off-odors, thereby reducing consumer acceptability.

Several strategies have been studied to enrich poultry meat in n-3 PUFA while maintaining optimal sensory quality (López-Ferrer et al., 1999a, 2001b; González-Esquerra and Leeson, 2000, 2001; Bou et al., 2004b). These are based on combining distinct amounts of dietary tocopherol with a range of doses of dietary FO, blends of FO with vegetable seeds or oils rich in n-3 PUFA, or replacement of FO by other fat sources prior to slaughter. Although these strategies reduce off-flavors, they also decrease EPA and DHA contents in comparison with high FO doses (Hargis and Van Elswyk, 1993). Therefore, it is essential to establish the best strategy leading to an enriched meat in these fatty acids (FA) and ensure that consumer acceptability is not lowered.

Furthermore, α-tocopheryl acetate (α-TA) supplements increase the α-tocopherol content in chicken tissues (Cherian et al., 1996; Morrissey et al., 1997; Galvin et al., 1998; Surai and Sparks, 2000). The effects of α-tocopherol on health have also been described (Food and Nutrition Board, 2000). In addition, α-tocopherol prevents lipid oxidation in chicken meat (Lin et al., 1989, Sheehy et al., 1993; Jensen et al., 1995; Grau et al., 2001a,b) thereby increasing the sensory quality (De Winne and Dirinck, 1996; Bou et al., 2001; Mielnik et al., 2002), even when animals are fed mainly on saturated (SFA) or monounsaturated FA (Lin et al., 1989; O'Neill et al., 1998).

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Abbreviation Key: AF = animal fat; DHA = docosahexanoic acid; EPA = eicosapentanoic acid; FA = fatty acid; FO = fish oil; GPx = glutathione peroxidase; LO = linseed oil; MT = metallothionein; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; α-TA = α-tocopheryl acetate.

Meat products are one of the main sources of Fe, Cu, Se, and Zn (Pennington and Young, 1991; Buss and Rose, 1992; Foster and Sumar, 1995; Subar et al., 1998) showing a high bioavailability (Fairweather-Tait, 1992). In addition, these elements are involved in a wide range of biochemical functions. Besides, several communities do not achieve the recommended daily intakes of some elements, even in developed countries (Pennington and Young, 1991; Bou et al., 2004b). This is the case for Zn and Se intakes in elderly people (Girodon et al., 1999; De Jong et al., 2001; Savarino et al., 2001). Therefore, meat products enriched in selected FA, tocopherol, and elements such as Zn and Se can be of great nutritional benefit.

Here we studied the effect of various dietary factors (supplementation with distinct levels of n-3 PUFA, Zn and Se, and a fixed amount of α-TA) on α-tocopherol, Zn, Se, Fe, and Cu contents and FA composition in raw chicken meat. Furthermore, we analyzed the oxidative stability and the consumer acceptability of cooked dark chicken meat.

MATERIALS AND METHODS

Birds and Housing

Three hundred twenty-four female broiler chicks (Ross 308, 1 d old) were assigned to 54 floor pens (6 birds per pen) corresponding to 27 replicated dietary treatments. Assignment of the replicated dietary treatments to the pens was made to provide a completely randomized design. Pens (0.8 m^2 with wire walls) contained wood shavings. Environmental temperature was set at 33°C on d 1 and was lowered stepwise to between 21 and 22°C. For the first 10 d, lights were on 24 h/d, and then lighting was lowered stepwise to 21 h/d. Relative humidity and ventilation were under standard conditions. Feed and water were provided ad libitum. Birds were reared and slaughtered in compliance with national regulations, and the experiment received prior approval by Copaga Soc. Cooperativa (Lleida, Spain) Animal Care and Use Committee.

Diets and Experimental Design

Treatments were prepared from 2 basal meal diets (Table 1). Diets were formulated according to requirements recommended by the NRC (1994) and supplemented with dl-α-TA at 100 mg/kg. Nine treatments containing 6% of animal fat (AF) were fed until 19 d of age (Table 2); treatments were 3 doses of Zn supplementation (0, 300, or 600 mg/kg) and 3 levels of Se supplement (0 or 1.2 mg Se/kg from sodium selenite or 0.2 mg Se/kg from Se-enriched yeast, an organic source of Se). From 20 to 39 d, the second basal diet was used, and the above treatments were maintained, although total added fat was 1.25% FO plus 5.81% of AF (Table 2). From 40 to 45 d of age, 27 dietary treatments resulted from combining the distinct levels of Zn and Se supplementation and 3 types of fat source at 1.25% AF,[2] linseed oil[3] (LO), or FO[4] plus 5.81 % of AF (Table 2).

Zinc sulfate, sodium selenite and α-TA were purchased from Andrés Pintaluba, SA.[5] The organic source of Se came from Se-enriched yeast (Sel-Plex) and was supplied by Probasa.[6]

Preparation, Cooking, and Storage of Samples

The chickens were slaughtered according to commercial procedures and were stored for 4 h at 4°C. Carcasses from each pen were then longitudinally cut and divided into 2 groups (left and right sides). Two random right sides (legs and breasts with skin) from each pen were used to study the composition and nutritional value of the meat. These samples were hand-deboned, mixed, ground, and weighed (approximately 30 g/bag) into high-barrier multilayer bags (Cryovac[7] BB-4L; permeability to O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985), vacuum-packed, and immediately stored at –20°C until determination of FA composition, and α-tocopherol, elements, and crude fat contents. Because of increased susceptibility of legs to oxidation due to higher Fe and fat content, only this part was used to study consumer acceptability. Thus, the remaining legs with skin from each pen were hand-deboned and stored at 4°C. Ten hours postslaughter, samples were 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) and 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 consumer acceptability and TBA values were determined.

Reagents and Standards

Reagents and standards used in element analyses were as described in Bou et al. (2004a), whereas those used in the other analyses were as described by Bou et al. (2004b).

Determination of FA Composition

The FA composition in mixed dark and white raw meat plus skin (hereafter referred to as raw meat) and in milled feed was determined by gas chromatography, as described by Bou et al. (2004b). The resulting 54 (27 × 2) meat samples were analyzed. For feed analysis, one sample for each dietary treatment was examined. Thus, 9 samples were taken from treatments used from 0 to 19 d and from 20 to 39 d, whereas 27 samples were taken from the diets supplied from 40 to 45 d.

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[4]FF of Denmark, Skagen, Denmark.
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[6]Probasa, Sta. Perpetua de la Moguda, Spain.
[7]Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain.

TABLE 1. Ingredients and composition of the basal diets[1]

Diet up to 19 d	Percentage	Diets from 20 to 45 d	Percentage
Ingredient		Ingredient	
Barley	25.00	Wheat	31.42
Soybean meal, 48% CP	22.57	Soybean meal, 48% CP	16.31
Wheat	16.72	Sorghum	15.00
Sorghum	10.00	Barley	15.00
Full-fat soy	6.82	Added fat[6]	7.06
Meat meal, 50% protein	6.00	Rapeseed	4.00
Animal fat[2]	6.00	Poultry by-product meal	4.00
Tapioca, 62% starch	3.00	Meat meal, 50% protein	3.66
Sepiolite[3]	1.50	Sepiolite[3]	1.50
Trace mineral-vitamin mix[4]	1.00	Trace mineral-vitamin mix[7]	1.00
Sodium chloride	0.32	L-Lysine	0.50
DL-Methionine	0.32	DL-Methionine	0.24
L-Lysine	0.25	Sodium chloride	0.20
Calcium carbonate	0.22	Calcium carbonate	0.09
Calcium phosphate	0.13	Phytase[5]	0.06
Choline chloride	0.10	Choline chloride	0.01
Phytase[5]	0.06		
Calculated composition		Calculated composition	
Dry matter	90.06	Dry matter	89.35
Crude protein	22.60	Crude protein	20.64
Crude fat	9.54	Crude fat	9.54
Crude fiber	3.32	Crude fiber	3.12
Ash	6.78	Ash	5.41

[1]Both basal diets were supplemented with 100 mg/kg of dl-α-tocopheryl acetate.

[2]Animal fat was 80% lard and 20% beef tallow.

[3]Hydrated magnesium silicate.

[4]Supplied the following per kilogram of complete feed: 10,000 IU of vitamin A, 2,000 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.

[5]EC 3.1.3.8, which liberated 1,000 phytase units per gram.

[6]From d 20 to 39, added fat contained 1.25% fish oil and 5.81% animal fat (80% lard and 20% tallow). Diets from d 40 to 45 added fat contained 5.81% animal fat (containing 80% lard and 20% tallow) and 1.25% additional animal fat, linseed oil, or fish oil.

[7]Supplied the following per kilogram of complete feed: 7,500 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of dl-α-tocopheryl acetate, 15 μg of vitamin B_{12}, 5 mg of vitamin K_3, 5 mg of vitamin B_2, 40 mg 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.

Determination of α-Tocopherol

Determination of α-tocopherol in raw meat and in milled feed was achieved by liquid chromatography, as described by Bou et al. (2004b). The resulting 54 (27 × 2) meat samples were analyzed. For feed analysis, we examined the 27 dietary treatments from 40 to 45 d and the 9 treatments from 0 to 19 d and from 20 to 39 d.

Determination of Zn, Se, Fe, and Cu

Element determination in raw meat and in milled feed was performed as described by Bou et al. (2004a). In this method, after mineralization of samples, Zn and Fe were determined by means of inductively coupled plasma atomic emission spectrometry, Cu was measured by inductively coupled plasma mass spectrometry, and Se determined with hydride generation inductively coupled plasma mass spectrometry. The resulting 54 (27 × 2) chicken meat samples were analyzed. For feed analysis, only the 27 dietary treatments given from 40 to 45 d were examined.

Sensory Analysis

Two consumer acceptability panel tests were performed on cooked dark chicken meat with skin stored at –20°C for 74 d and 18 mo. Thirty-one and thirty-three experienced consumer panelists were used in each test, respectively. Because of the high number of dietary treatments assayed (27), consumer acceptability of samples supplemented with 300 mg/kg of Zn was not evaluated, thereby reducing the samples for evaluation to 18. Criteria for panelist selection, sample preparation, and presentation were as described by Bou et al. (2004b).

Samples were presented to each panelist in a completely randomized design in 3 working sessions. In each session, 6 randomized samples and a blind control were presented. The blind control was a vacuum-packed freshly cooked commercial chicken meat sample stored

TABLE 2. Dietary treatments

Up to 19 d[1]		From 20 to 45 d		
Zn supplement (mg/kg)	Se supplement[2]	Fat source[3]	Zn supplement (mg/kg)	Se supplement[2]
0	0	FO or LO or AF	0	0
0	Selenite	FO or LO or AF	0	Selenite
0	Se yeast	FO or LO or AF	0	Se yeast
300	0	FO or LO or AF	300	0
300	Selenite	FO or LO or AF	300	Selenite
300	Se yeast	FO or LO or AF	300	Se yeast
600	0	FO or LO or AF	600	0
600	Selenite	FO or LO or AF	600	Selenite
600	Se yeast	FO or LO or AF	600	Se yeast

[1]Up to 19 d of age 6% of animal fat was added to the feed.

[2]Selenite provided 1.2 mg Se/kg of feed, and Se-enriched yeast provided 0.2 mg of Se/kg of feed.

[3]From 20 to 39 d of age added fat contained 1.25% fish oil (FO) and 5.81% animal fat (AF). From 40 to 45 d of age added fat was 5.81% AF and 1.25% additional FO, linseed oil (LO), or AF.

for 1 d at –20°C. Panelists were asked to rank the overall acceptability using a 9-point scale (where 1 = very bad and 9 = very good). A comment section was also available on the score sheet.

Determination of TBA Values

Vacuum-packed cooked chicken legs with skin were thawed, as for sensory analysis, by heating in a water bath at 35°C for 20 min and were then ground. Two grams of ground cooked dark meat was then weighed for analysis. TBA values were measured through a third derivative spectrophotometry method after acid aqueous extraction (Grau et al., 2000). As in the sensory analysis, only samples from 18 treatments and the blind control were analyzed.

Determination of Crude Fat Content

Fat content of the raw meat from all the experimental treatments was measured by the AOAC Official Method 991.36 (AOAC, 2000). Thus, the resulting 54 (27×2) samples were analyzed.

Statistical Analysis

Multifactor ANOVA was used to determine whether any significant effects were produced by the factors studied on animal performance parameters, FA composition, and α-tocopherol, Zn, Se, Fe, Cu, and crude fat contents of the raw meat as well as on consumer acceptability and TBA values of cooked dark meat with skin. Interactions between factors higher than an order of 2 were discarded. One-way ANOVA was used to determine significant differences in consumer acceptability scores between dietary treatments and the blind control used in the sensory test. Multifactor ANOVA was used to determine significant differences caused by the factors studied in α-tocopherol, Zn, Se, Fe, and Cu content of feeds. In all cases, least squares means for the main factors that had a significant effect were separated using Duncan test. In all cases, $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Bird Performance

Final BW, feed intake, feed conversion, and mortality were not affected by dietary treatments. Averages for BW and feed conversion after 44 d were 2,318 g and 1.41 g/g, respectively. The lack of effect of Zn supplementation on BW agrees with previous results for chickens fed 0 or 200 mg/kg of Zn (Bou et al., 2004b). However, chickens fed 600 mg/kg had a slightly lower BW (2,250 g), which was almost significant ($P = 0.052$) compared with those given 0 or 300 mg/kg of Zn (2.344 and 2.359 g, respectively). Nevertheless, this BW decrease, which has been reported to be significant in chickens fed higher Zn supplements (Sandoval et al., 1998; Williams et al., 1989), did not significantly affect carcass weight in our experiment (carcass weights for 0, 300, and 600 mg Zn/kg were 1.722, 1.715, and 1.687 g, respectively). Based on our BW results and those reported by Sandoval et al. (1998) the upper tolerable Zn supplementation for broiler production purposes should be set between 600 and 1,000 mg/kg. Therefore, it could be assumed that mixed dark and white raw chicken meat plus skin as well as cooked dark chicken meat with skin samples were similar and comparable among treatments.

FA Composition and Total Fat Content

Feed and raw meat FA compositions are shown in Table 3. In relation to feed FA composition, when FO was added to the feed from 20 to 39 d of age, EPA and DHA increased compared with levels in feed administered up to 19 d of age. Distinct feeds from 40 to 45 d of age also showed differences for n-3 PUFA. The LO diets showed a higher linolenic acid content, whereas FO diets had higher EPA and DHA contents. Furthermore, feeds including AF had the lowest amount of n-3 PUFA and total PUFA and the highest percentage of total monounsaturated FA and SFA.

The FA composition of meat was affected by dietary fat source (Table 3). The effect of the FA composition of feed on chicken meat composition has been widely

TABLE 3. Fatty acid composition (expressed as area normalization in %) of the experimental feeds and the effect of the dietary fat source on chicken meat[1]

	Feeds[2]					Mixed, raw, dark, and white chicken meats with skin[3]			
			From 40 to 45 d			Fat source			
Fatty acid	Up to 19 d	From 20 to 39 d	FO	LO	AF	FO	LO	AF	SEM
C14:0	1.51	2.56	2.43	1.49	1.81	1.66[a]	1.59[b]	1.59[b]	0.014
C16:0	21.12	23.60	21.86	19.98	22.71	22.81	22.61	22.47	0.121
C18:0	10.68	13.71	11.09	11.14	13.03	8.30	8.06	8.05	0.100
C20:0	0.25	0.25	0.24	0.22	0.22	0.09	0.08	0.09	0.002
Total SFA	33.56	40.12	35.62	32.82	37.76	32.87[a]	32.35[b]	32.21[b]	0.171
C14:1 n-9	0.18	0.22	0.18	0.17	0.20	0.27	0.26	0.27	0.004
C16:1 n-9	0.26	0.30	0.29	0.25	0.31	0.58[ab]	0.56[a]	0.60[b]	0.010
C16:1 n-7	2.41	3.17	2.91	2.19	2.59	4.77	4.79	4.86	0.066
C18:1 n-9	32.10	33.21	33.24	33.58	36.01	40.69[a]	40.82[a]	41.51[b]	0.140
C18:1 n-7	1.81	1.98	2.10	1.82	2.03	2.07	2.06	2.03	0.021
C20:1 n-9	0.48	1.25	1.50	0.53	0.59	0.77[a]	0.71[b]	0.71[b]	0.005
C22:1 n-9	0.03	0.13	0.14	0.06	0.04	0.64[ab]	0.60[b]	0.66[a]	0.016
Total MUFA	37.26	40.26	40.36	38.60	41.77	49.79[a]	49.77[a]	50.68[b]	0.179
C18:2 n-6	25.41	14.54	18.04	18.96	17.90	12.88	13.04	13.02	0.114
C18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.12	0.12	0.12	0.003
C20:2 n-6	0.18	0.21	0.24	0.21	0.24	0.23	0.23	0.23	0.003
C20:3 n-6	0.21	0.16	0.17	0.16	0.16	0.17[a]	0.17[a]	0.18[b]	0.004
C20:4 n-6	0.06	0.07	0.08	0.07	0.22	0.30[a]	0.25[b]	0.24[b]	0.004
C22:4 n-6	0.05	0.07	0.07	0.06	0.07	0.10	0.10	0.10	0.002
C22:5 n-6	0.00	0.05	0.06	0.01	0.01	0.03[a]	0.02[b]	0.02[b]	0.001
Total n-6 PUFA	25.91	15.05	18.53	19.46	18.44	13.83	13.93	13.94	0.121
C18:3 n-3	2.98	1.69	1.84	8.80	1.72	1.50[a]	2.29[b]	1.51[a]	0.037
C18:4 n-3	0.01	0.39	0.48	0.02	0.01	0.21[a]	0.18[b]	0.18[b]	0.005
C20:4 n-3	0.01	0.11	0.13	0.03	0.02	0.06[a]	0.06[a]	0.05[b]	0.001
C20:5 n-3	0.07	0.90	1.13	0.06	0.07	0.50[a]	0.40[b]	0.38[b]	0.008
C22:5 n-3	0.07	0.18	0.20	0.08	0.09	0.39[a]	0.35[b]	0.35[b]	0.007
C22:6 n-3	0.12	1.25	1.58	0.11	0.11	0.86[a]	0.67[b]	0.71[b]	0.014
Total n-3 PUFA	3.27	4.52	5.38	9.10	2.03	3.51[a]	3.94[b]	3.18[c]	0.043
Total PUFA	29.18	19.57	23.97	28.57	20.46	17.34[a]	17.87[b]	17.11[a]	0.151

[a–c]Least squares means for fatty acids of mixed raw dark and white chicken meat with skin with different letters differ significantly ($P \leq 0.05$). Statistical results for feeds are not stated.

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; FO = fish oil; LO = linseed oil; AF = animal fat.

[2]Data in these columns correspond to means (n = 9).

[3]Data in these columns correspond to least squares means (n = 18) obtained from multifactor ANOVA (n = 54).

reported (Hargis and Van Elswyk, 1993; Cherian et al., 1996; Wood and Enser, 1997; Surai and Sparks, 2000). Therefore, these differences in chicken meat FA composition reflected differences in diets, which had been given to the chickens for only 5 d before slaughter. The effect of 8.2% dietary FO replacement, 1 or 2 wk before slaughter, on the FA composition of thigh and breast meats has been previously reported (López-Ferrer et al., 1999a). These authors found that replacement of dietary FO by LO just 1 or 2 wk before slaughter produces smaller amounts of EPA and DHA and higher amounts of linolenic acid. In our study, the removal of FO and replacement by LO led to a slight decrease in 18:4 n-3, 22:5 n-3, EPA, and DHA (Table 3). In addition, the LO diet led to the highest increase in total n-3 PUFA content because of the great increase in linolenic acid. On the contrary, the lowest total n-3 PUFA content was observed in meat from AF treatments. However, AF diets still produced high concentrations of EPA, DHA, and total n-3 PUFA compared with diets using nonmarine fat sources throughout the feeding period (López-Ferrer et al., 1999a,b, 2001a; Grau et al., 2001b). Furthermore, total monounsaturated FA content was higher in AF treatments, whereas total SFA was greater in birds on FO diets. The former observation could be explained by the amount of these FA in the feed. However, the differences observed in meat were not as marked as those observed in feeds given from 40 to 45 d. This result could be explained by the short period of fat replacement, although some metabolic pathways or homeostatic mechanism might also be involved (Asghar et al., 1990; Wood and Enser, 1997). These factors could explain why the highest total SFA content was detected in meat from the FO diet, whereas no differences in total SFA content were observed between that from the LO or AF treatments.

The FA composition of feed also explained the significant differences for 14:0, 16:1 n-9, 18:1 n-9, and 20:1 n-9 in meats. However, 22:1 n-9 content was higher in meat from AF treatments but very low in feeds containing AF. The 20:4 n-6 content of meat increased when FO was added to feed. This finding is in disagreement with other authors' results when comparing increasing amounts of FO (Phetteplace and Watkins, 1990; López-Ferrer et al., 2001b). In our study, the formation of this metabolite

TABLE 4. Crude fat, α-tocopherol, Zn, Se, Fe, and Cu contents in mixed raw dark and white chicken meat with skin (expressed per 100 g of edible portion)[1]

Factor studied	Crude fat[2] (%)	α-Tocopherol (mg)	Zn content (mg)	Se content (μg)	Fe content[3] (mg)	Cu content (μg)
Fat source						
Fish oil	10.5	2.18	0.855	15.5	0.472	27
Linseed oil	11.1	2.08	0.851	15.5	0.500	24
Animal fat	10.5	2.01	0.870	14.7	0.508	27
Zn supplementation						
0 mg/kg	10.9	2.13	0.864	11.5[a]	0.487	25
300 mg/kg	10.5	2.00	0.854	12.9[b]	0.506	26
600 mg/kg	10.7	2.13	0.864	21.3[c]	0.487	27
Se supplementation						
0	10.6	2.19	0.867	14.8[a]	0.487	23
Selenite, 1.2 mg/kg	10.7	2.04	0.862	15.1[ab]	0.494	29
Se yeast, 0.2 mg/kg	10.9	2.03	0.847	15.8[b]	0.493	29
Pooled SEM	0.12	0.041	0.0058	0.17	0.0056	1.4

[a–c]Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

[1]Values given in this table correspond to least squares means (n = 18) obtained from multifactor ANOVA (n = 54).

[2]Significant interactions between fat source × Zn supplementation and between Zn × Se supplementation.

[3] Significant interactions between fat source × Se supplementation.

might not have been inhibited through Δ6 desaturase by the amount of n-3 FA provided at 1.25% FO or LO, and enhanced deposition of this FA might have occurred in these 2 dietary groups.

Furthermore, the FA composition (Table 3) and crude fat content (Table 4) of raw meat were not affected by Zn or Se supplementation. The lack of effect of Zn supplementation on raw meat FA composition and crude fat content has been previously reported in a similar study with chickens fed 200 mg of Zn/kg (Bou et al., 2004b).

Additionally in our study, dietary fat sources had no effect on crude fat content of raw meat (Table 4). Therefore, the enrichment expressed as area normalization (%) in some very long-chain n-3 PUFA was comparable between treatments. The lack of effect of fat source on crude fat is consistent with the results on dark and white meat with and without skin from chickens fed different fat sources (Ajuyah et al., 1992; Scaife et al., 1994; Crespo and Esteve-Garcia, 2001).

Tocopherol Content

All diets were supplemented with 100 mg/kg of α-TA, a dose that ensures good consumer acceptability for chickens fed 1.25% FO (Bou et al., 2004b). The averages for α-tocopherol content in diets up to 19 d and from 20 to 39 d of age were 131 and 129 mg/kg of feed, respectively. In diets from 40 to 45 d, the average α-tocopherol content is shown in Table 5. As there were no significant differences between fat sources for α-tocopherol, it can be assumed that the fat sources assayed did not modify the dietary α-tocopherol supply.

The α-tocopherol content of raw meat was not affected by any of the factors studied (Table 4). Thus, the replacement of 1.25% FO with 1.25% AF or LO 1 wk before slaughter did not alter α-tocopherol content. These results are consistent with those reported in studies of chicken meat from birds fed on different fat sources (saturated and unsaturated) and different α-TA supplements (Lin et al., 1989; Ruiz et al., 1999; Surai and Sparks, 2000; Grau et al., 2001b; Bou et al., 2004b).

Conversely, the α-tocopherol contents of dark and white meats have been described to increase in chickens fed olive oil compared with those fed tallow supplemented at 30 or 200 mg/kg of α-TA (O'Neill et al., 1998). Nevertheless, this effect could be explained by the endogenous content of α-tocopherol present in these feeds, which was slightly higher in diets containing olive oil.

Several factors, such as feed endogenous α-tocopherol content, could also influence the levels of this antioxidant. We did not address this factor because dietary amounts of α-tocopherol were similar in all treatments (Table 5). However, some studies that have provided similar levels of α-tocopherol reported a decreased α-tocopherol content in various tissues of chickens fed highly unsaturated diets. This decrease was attributed to a higher oxidation susceptibility of these tissues (Maraschiello et al., 1999; Surai and Sparks, 2000). In addition, inefficient absorption of tocopherol, other antioxidants in feed, or stressful periods of early and rapid growth can explain some differences reported in α-tocopherol levels of chicken meat, eggs, and other tissues (Galobart et al., 2002; Cherian and Sim, 2003; Zanini et al., 2003).

Furthermore, Zn may act as an antioxidant (Bray and Bettger, 1990; Oteiza et al., 1995; Powell, 2000; Zago and Oteiza, 2001), and deficiencies in animals are alleviated by tocopherols and other antioxidants (Kraus et al., 1997). In addition, laying hens reared at low temperature (6.8°C) and given supplemental Zn (30 mg/kg) have higher serum tocopherol contents than those not receiving supplements (Onderci et al., 2003). Likewise, Japanese quails supplemented with Zn (0, 30, or 60 mg/kg) and reared under high temperature (34°C) have higher serum tocopherol contents (Sahin and Kucuk, 2003). However, these authors did not find differences in α-tocopherol content between birds on distinct levels of Zn supple-

ments and reared at 22°C. Thus, these former results agreed with our study in which the meat of chickens on Zn supplements (300 or 600 mg/kg) did not have higher α-tocopherol content in raw meat (Table 4). This finding was consistent with a previous study (Bou et al., 2004b).

Deficiencies in Se combined with low dietary tocopherol supply have been related to various chicken diseases, which are exacerbated by an excess of dietary PUFA and can be minimized by Se or tocopherol supplements (NRC, 1983). This result can be explained by the fact that both take part in the antioxidant system. In fact, Se supplements can increase glutathione peroxidase (GPx), which converts hydroperoxides into non-prooxidant molecules (Diplock et al., 1998; Surai, 2002a). Therefore, those reported increases in α-tocopherol levels in different animal tissues as a result of Se supplementation could be related with the role of Se in the antioxidant system (Surai, 2002b).

This explanation is in agreement with the increased liver and serum tocopherol content reported for Japanese quails fed Se supplements (0.1 vs. 0.2 mg/kg) and reared under high temperature (34°C) (Sahin et al., 2002). However, in our study the levels of Se supplementation did not affect α-tocopherol content (Table 4). Similarly, hamsters fed FO do not show differences in tocopherol content of heart and liver tissues when fed adequate amounts of tocopherol (27 mg/kg α-TA) and a Se supplement (3.4 mg/kg as sodium selenite; Poirier et al., 2002). Therefore, under nonstressing conditions, α-tocopherol levels did not appear to be affected by Se supplements.

Element Content

The element composition of feed is shown in Table 5. Differences in Zn and Se content reflect the supplements of these elements. In addition, the fat source led to significant differences for Cu content. Feeds containing FO had a higher Cu content, whereas those with LO had a lower content of this metal, although the AF treatment did not differ from the FO or LO treatments.

Contents of each element in chicken meat are listed by dietary factor in Table 4. Dietary fat source did not affect raw meat content of elements, although Fe content was affected by an interaction between Se supplement and dietary fat source (P = 0.027). Supplemental Zn had no effect on meat Zn content, which is consistent with a previous study in which animals were supplemented at 200 mg/kg of Zn (Bou et al. 2004b). Chicken muscle Zn content decreases mainly with age (Mohanna and Nys, 1998; Sandoval et al., 1998). In this regard, body Zn concentrations are lower and stable from 21 to 50 d of age compared with earlier periods (Mohanna and Nys 1998). However, increasing dietary Zn supplementation results in higher concentrations in distinct tissues, although at 3 wk of age a poor linear regression is observed between muscle Zn concentration and dietary Zn supplementation (Sandoval et al., 1998). These results support the Zn homeostatic control metabolism (Cousins, 1996; Food and Nutrition Board, 2002b).

Furthermore, we observed that Zn supplementation led to a significant increase in Se content in meat (Table 4). Greater Zn supplementation led to higher Se content in raw chicken meat. Yin et al. (1991) also reported that rats supplemented with Zn showed higher Se concentrations in plasma, erythrocytes, and muscle, heart, and liver tissues.

This increase in chicken meat Se content induced by Zn supplementation is difficult to explain because several factors may be involved. Se, like As, is excreted through biomethylation (Foster and Sumar, 1997; Schrauzer, 2000; Gailer, 2002; Jiang et al., 2003), and many forms of Se have been reported in chicken meat (Daun et al., 2004). Furthermore, metallothionein (MT) is a cysteine-rich protein that controls the Zn pool (Cousins, 1996, Coyle et al., 2002), and its synthesis is induced by Zn and other cations (Nordberg, 1998). This protein, through thiol groups, acts as a chelating agent for divalent cations (Nordberg, 1998; Maret et al., 1999; Coyle et al., 2002), as a reductant of biological oxidants (Klotz et al., 2003; Maret, 2003) and reacts with various forms of Se (Jacob et al., 1999; Chen and Maret, 2001), reduced glutathione (Maret, 2000), and methylated species of As (Jiang et al., 2003). Thus, MT acts in detoxifying and antioxidant systems (Schwarz et

TABLE 5. α-Tocopherol and Zn, Se, Fe, and Cu contents in feeds given from 40 to 45 d[1]

Factor studied	α-Tocopherol (mg/kg)	Zn content (mg/kg)	Se content (μg/kg)	Fe content (mg/kg)	Cu content (mg/kg)
Fat source					
Fish oil	122	460	581	465	17[a]
Linseed oil	114	425	507	400	13[b]
Animal fat	119	378	493	399	15[ab]
Zn supplementation					
0 mg/kg	115	131[a]	560	406	16
300 mg/kg	120	419[b]	504	430	15
600 mg/kg	120	712[c]	517	428	13
Se supplementation					
0	117	388	136[a]	435	14
Selenite, 1.2 mg/kg	120	457	1069[b]	404	15
Se yeast, 0.2 mg/kg	118	412	376[c]	425	15
Pooled SEM	1.4	11	20	21	1.7

[a–c]Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

[1]Values given in this table correspond to least squares means (n = 9) obtained from multifactor ANOVA (n = 27).

al., 1994; Maret, 2000, 2003; Coyle et al., 2002; Klotz et al., 2003)

On the other hand, the equimolar binding of Hg and Se with selenoprotein P has been described in plasma as a detoxification mechanism (Yoneda and Suzuki, 1997). Therefore, this and the MT detoxification mechanism could explain the intertwined toxicity of Se, As, and Hg (Yoneda and Suzuki, 1997; Goyer, 1997; NRC, 1999; Gailer, 2002)

Given these relationships, some forms of Se present in chicken meat (Daun et al., 2004), which does not depend on the type of dietary Se source, may be bound to MT or other selenoproteins. Thus, this MT or other Se forms could present an altered metabolism under high doses of Zn supplementation leading to an increased Se content. However, further studies are required to determine the Se content and the different Se forms present in different chicken tissues under Zn supplementation. Conversely to this effect, the contents of Zn, Fe, and Cu in chicken meat were not affected by Zn supplementation.

Organic Se supplementation increased meat Se content in comparison with chickens that received no Se addition (Table 4). However, when an inorganic Se source (sodium selenite) was added to feeds at a relatively high dose (1.2 mg/kg feed), the content of this element did not differ between treatments with an organic Se supplement (0.2 mg/kg feed) and those without supplement.

The organic form of Se used in this study was obtained from Se-enriched yeast. This yeast mainly contains this element in the form of selenomethionine, which cannot be synthesized by mammals or poultry (Schrauzer, 2000). Furthermore, selenomethionine is actively absorbed in the intestine and follows the same pathways as methionine, whereas selenite is absorbed passively (Thomson, 1998; British Nutrition Foundation, 2001; Surai, 2002b). Selenomethionine is incorporated nonspecifically into distinct proteins such as those in muscle (Thomson, 1998; Surai, 2002b). In contrast, selenite and other forms of Se appear to be under homeostatic regulation and incorporated specifically as selenocysteine into functional selenoproteins (Thomson, 1998; British Nutrition Foundation, 2001).

In addition, selenomethionine and other organic Se forms are also converted into selenocysteine, which can be used in functional selenoproteins (Foster and Sumar, 1997; Schrauzer, 2000), explaining the maintained activities of GPx after Se depletion in animals on selenomethionine supplements (Surai, 2000). These observations indicate that selenomethionine can act as a Se reserve (Schrauzer, 2000; Surai, 2002b).

Thus, when birds were fed the inorganic Se supplement, if there was an increase in muscle Se content, it would be expected to be mainly due to the increase in functional selenoproteins such as GPx. In fact, after inorganic Se supplementation, increased GPx activity has been reported for chicken meat compared with that for animals on a basal diet containing 0.09 mg Se/kg (De Vore et al., 1983) and also in chicken erythrocytes coming from Se-supplemented chickens compared with those coming from animals receiving 0.03 mg Se/kg (Aydemir et al., 2000). Nevertheless, liver GPx activity of chickens fed a basal diet containing 0.12 mg Se/kg was not increased compared with those of animals on inorganic and organic Se supplements (Holovská et al., 2003). Indeed, GPx activities can be used as indicators for estimating the requirements of Se. In relation to this, Holovská et al. (2003) reported that a diet containing 0.1 mg Se/kg provided the Se requirement for the synthesis of GPx in chicken liver. The same Se requirement was reported to maintain serum GPx activity in pigs (Mahan and Parret, 1996).

Moreover, Mahan and Parret (1996) found that Se content in pig loins from animals on Se-enriched yeast was higher than in pigs on inorganic Se or no supplementation. These observations are consistent with our results in chicken meat, indicating that inorganic Se supplementation did not affect Se content, whereas chickens on organic Se supplement had higher Se content in meat, probably in the form of selenomethionine (Table 4).

Supplementation with Se did not affect Fe, Zn, or Cu contents of raw meat (Table 4). These results are consistent with those reported by Sahin et al. (2002) for Fe, Zn, and Cu serum concentrations of Japanese quails on diets supplemented with inorganic Se (0.1 and 0.2 mg of Se/kg).

Sensory Analysis and TBA Values

Two consumer tests were conducted to assess the consumer acceptability of cooked leg samples. The first was carried out after 74 d of frozen storage, and the second was carried out after 18 mo of frozen storage. After 74 d, consumer acceptability and TBA values did not show significant differences for the factors studied (Table 6). In addition, the one-way ANOVA performed for the sample factor did not show significant differences in acceptability between the blind control and the dietary treatments. Nevertheless, the former showed lower consumer acceptability than the latter. This observation could be explained by the higher TBA values in the blind control. These values are inversely correlated with sensory scores (Ang and Lyon, 1990; Mielche, 1995; Bou et al., 2001).

Consumer acceptability of cooked samples was evaluated again after 18 mo of frozen storage. Similarly to at 74 d of frozen storage, none of the dietary factors showed significant differences in consumer acceptability and TBA values. In addition, the one-way ANOVA for the sample factor showed no differences in acceptability between the blind control and the dietary treatments. Nevertheless, in this second sensory analysis, the differences in consumer acceptability scores between samples and the blind control were smaller, which could be also explained because TBA values were quite similar between experimental samples and the blind control.

Although TBA values increased after 18 mo of frozen storage (Table 6), these values were too low to find significant differences in acceptability scores (Bou et al., 2001). In addition, these low TBA values could be ex-

TABLE 6. Effect of the dietary factors on consumer acceptability[1] scores and TBA values (μg of malondialdehyde/kg) of cooked dark chicken meat after 74 d and 18 mo of storage at –20°C[2]

	After 74 d of frozen storage		After 18 mo of frozen storage	
Factor studied	Acceptability	TBA	Acceptability	TBA[3]
Fat source[4]				
Fish oil	4.9	86	5.1	137
Linseed oil	5.0	84	5.1	139
Animal fat	5.1	74	5.3	139
Zn supplementation[5]				
0 mg/kg	4.9	93	5.1	137
600 mg/kg	5.1	71	5.2	140
Se supplementation[4]				
0	5.0	91	5.3	141
Selenite, 1.2 mg/kg	5.2	73	5.1	135
Se yeast, 0.2 mg/kg	4.8	83	5.0	139
Pooled SEM	0.11	4.4	0.10	1.2
Blind control[6]				
Mean	4.2	267	4.9	202
SE	0.26	2.7	0.22	1.4

[1]Overall acceptability was ranked using a 9-point scale (where 1 = very bad and 9 = very good).

[2]Values given in this table correspond to least squares means obtained from multifactor ANOVA, except for mean values of the blind control.

[3]Significant interaction between Zn × Se supplements.

[4]n = 186 for acceptability after 74 d, 198 for acceptability after 18 mo, and 6 for TBA values.

[5]n = 279 for acceptability after 74 d, 297 for acceptability after 18 mo, and 9 for TBA values.

[6]n = 93 for acceptability after 74 d, 99 for acceptability after 18 mo, and 3 for TBA values.

plained by the protective effect of α-tocopherol (100 mg/kg α-TA supplementation) against oxidation, which has been reported for cooked chicken meat stored at 4 and –20°C for various periods (Ajuyah et al., 1993; Jensen et al., 1995; Galvin et al., 1998, Grau et al., 2001a).

Dietary FO replacement by other fat sources rich in PUFA before slaughter might lead to a decrease in very long-chain n-3 PUFA, depending on several factors, and improve sensory quality (Hargis and Van Elswyk, 1993; López-Ferrer et al., 1999a). López-Ferrer et al. (1999a) studied the effect of 8.2% FO fed to birds for 5 wk on consumer acceptability of dark and white chicken meat and compared acceptability scores of these meats with those from chickens fed diets in which FO was replaced by linseed or rapeseed oil 1 or 2 wk before slaughter. Through a triangular test, these authors showed a decrease in acceptability scores of dark meat from birds on FO diets in comparison with those fed on diets with rapeseed or LO 2 wk before slaughter. However, the FO dose used in that study was much higher than that applied in our work (1.25%), and diets were not supplemented with α-TA.

In another study with more comparable FO doses, López-Ferrer et al. (2001b) compared thighs from chickens fed 8% tallow for 5 wk with those fed first with a high FO dose (4% FO + 4% tallow) and then with a low FO dose (1% FO + 3% LO + 4% tallow) for 1 or 2 wk before slaughter. A triangular test showed no significant differences between dietary treatments using a 5-point acceptability scale.

Our results, therefore, are consistent with these studies because our commercial blind control (probably similar to meat from chickens fed 8% tallow from the former work) did not differ from the FO treatment (similar to the replacement with 1% FO 1 wk before slaughter from the former work). In addition, it is noteworthy that treatments assayed by López-Ferrer et al. (2001b) were not supplemented with α-TA.

In our study, Zn supplementation at 600 mg/kg did not affect the sensory quality or TBA values, which was consistent with another study using 200 mg/kg of Zn supplement (Bou et al., 2004b). Various authors (De Vore et al., 1983; Surai, 2002b) have reported a crucial role of organic and inorganic Se supplements in enhancing oxidative meat stability in combination with tocopherol during storage. This increase in stability could be explained through the antioxidant effect of tocopherol combined with increased GPx activity, which is related to a decrease in TBA values (De Vore et al., 1983). However, in our study, Se supplementation did not affect the sensory scores or TBA values of cooked meat, probably because of the protective effect of α-TA supplementation at an efficient dose (100 mg/kg) in all treatments.

TABLE 7. Nutrients provided by 100 g of edible portion (mix of raw dark and white chicken meat with skin) depending on different dietary factors and comparison with the human recommended daily dietary intakes (mg/d)

	Linolenic acid			EPA plus DHA[1]			Vitamin E	Selenium					
	Fat source			Fat source				Zn supplements (mg/kg)			Se supplements[2]		
	FO	LO	AF	FO	LO	AF	All treatments	0	300	600	0	Selenite	Se yeast
100-g edible portion[2]	186	889	174	137	108	110	2.00–2.19	0.0115	0.0129	0.0213	0.0148	0.0151	0.0158
Recommended		1,600[4]			650[5]		15[6]				0.055[6]		
Recommendation[7] (%)	11.6	55.6	10.9	21.1	16.6	16.9	13.3–14.6	20.9	23.4	38.7	26.9	27.5	28.7

[1]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; FO = fish oil; LO = linseed oil; AF = animal fat.

[2]Selenite provided 1.2 mg Se/kg of feed, whereas Se yeast provided 0.2 mg of Se/kg of feed.

[3]Results for fatty acids are calculated by taking the fat content average (10.7%).

[4]Adequate intake (Food and Nutrition Board, 2002a).

[5]Simopoulos et al. (2000).

[6]Recommended dietary allowances (Food and Nutrition Board, 2000).

[7]Values are the percentages of the recommended daily dietary intakes provided by 100 g of edible portion.

In summary, the removal of dietary FO and its replacement by LO or AF 1 wk before slaughter led to a distinct FA composition of raw meat. FO produced meat with a higher EPA and DHA content, whereas LO led to meat with a higher content in total n-3 PUFA, especially linolenic acid. Nevertheless, meat from AF treatments was still rich in EPA, DHA, and total n-3 PUFA, thereby providing a good source to cover the human recommended daily dietary intakes for these FA (Table 7). In addition, chicken meat can be Se-enriched by Zn and organic Se supplementation (Table 7). Consumer acceptability scores and TBA values of cooked dark chicken meat from birds fed 100 mg of supplemental α-TA/kg were not affected by any of the dietary factors studied after 74 d and 18 mo of frozen storage. We concluded that, under our conditions, this α-TA supplementation dose brought about oxidative stability and provided a source of vitamin E (Table 7).

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