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The influence of dietary fish oil and vitamin E on the fatty acid profile and oxidative stability of frozen stored chicken breast meat*

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ABSTRACT

The experiment was conducted on 640 Cobb chickens allocated to 16 groups, 5 replications of 4 males and 4 females each. Chickens from 22 to 42 days of age were fed diets containing 50, 47, 45 and 42 $g \cdot kg^{-1}$ of rapeseed oil and 0, 3, 5 and 8 $g \cdot kg^{-1}$ fish oil, respectively. The diets were supplemented with 0, 40, 150 and 300 $mg \cdot kg^{-1}$ of α-tocopheryl acetate. At the end of the experiment, the chickens were killed and samples of breast meat were prepared, frozen (-20°C) and stored for 6 months. Samples were analysed for fatty acid composition, thiobarbituric acid reactive substances (TBA-RS), and α-tocopherol content.

Fish oil positively affected the performance of chickens, elevated the content of stearic ($C_{18:0}$) and eicosapentaenoic ($C_{20:5,n-3}$) acids, and lowered the polyunsaturated fatty acid (PUFA) n-6:n-3 ratio in stored breast meat. Fish oil at 8 $g \cdot kg^{-1}$ in the diet decreased linolenic acid ($C_{18:3,\ n-3}$), unsaturated fatty acid, and n-6 PUFA contents, increased saturated fatty acid (SFA) content in lipids and lowered the PUFA:SFA ratio. The addition of 150 or 300 $mg \cdot kg^{-1}$ α-tocopheryl acetate to the diets increased the level of vitamin E in stored breast meat and lowered the TBA-RS value. The levels of fish oil and vitamin E used had no effect on the sensory properties of meat.

KEY WORDS: broiler chicken, breast meat, frozen storage, fatty acids, fish oil, rapeseed oil, α-tocopherol, TBA-RS

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INTRODUCTION

Fish oil added to chicken diets can provide polyunsaturated n-3 fatty acids, increase the degree of unsaturation, and increase the susceptibility to oxidation of meat lipids (Lopez-Ferrer et al., 1999). A high concentration of polyunsaturated fatty acids (PUFA) in poultry meat makes it particularly sensitive to oxidative changes affecting smell and taste. Dietary α-tocopheryl acetate supplementation affects the vitamin E content of meat and, because of this vitamin's antioxidant properties, may inhibit oxidation (De Winne and Dirinck, 1996). The quality of stored frozen meat may deteriorate as the result of lipid oxidation, especially in cooked, refrigerated meat (Cortinas et al., 2005).

The aim of this study was to evaluate the effect of moderate levels of fish oil and α-tocopheryl acetate incorporated to diets fed during the last 3 weeks of life on chicken performance, fatty acid composition, thiobarbituric acid reacting substances (TBA-RS), and vitamin E content in frozen stored breast meat.

MATERIAL AND METHODS

Standardized H465 fish oil stabilized with ethoxyquin (250 mg · kg $^{-1}$) from Lysi h.f., Reykjavik, and DL-α-tocoferyl acetate (Lutavit E 50 from BASF) were used in experiment. The fatty acid composition of the fish oil (in percent) declared by the producers was: 6.7 myristic acid ($C_{14:0}$), 13.4 palmitic acid ($C_{16:0}$), 1.3 stearic acid ($C_{18:0}$), 14.1 oleic acid ($C_{18:1}$), 1.3 linoleic acid ($C_{18:2,\ n-6}$), 0.8 α-linolenic acid ($C_{18:3,\ n-3}$), 7.9 eicosapentaenoic acid EPA ($C_{20:5,\ n-3}$), 0.9 docosapentaenoic acid DPA ($C_{22:5,\ n-3}$) and 8 docosaheksaenoic acid DHA ($C_{22:6,\ n-3}$).

The control diet contained 50 g · kg^{-1} of rapeseed oil (Table 1). The experimental diets were supplemented with 40, 150 or 300 mg α-tocopheryl acetate and with 3, 5 or 8 g · kg^{-1} of fish oil. Fish oil replaced rapeseed oil w/w.

This design resulted in 16 treatments. The experiment was performed on 640, 21-day-old sexed Cobb chickens, fed a standard diet during the first 3 weeks of life. The chickens were weighed and randomly allocated to 16 groups, each in 5 replications of 8 birds (4 males and 4 females) kept in wire-floor cages. Experimental diets were fed *ad libitum* in mash form to chickens from 22 to 42 days of age. Feed intake and body weight were measured, weight gain (BWG) and feed conversion (FC) were calculated.

On day 42, 4 males and 4 females from each group were slaughtered and 6 samples (3 males + 3 females) of breast muscle (*M. pectoralis maior and minor*) were chosen on the basis of similar weight. The right breast muscle (6 samples) without skin and outer fat was excised, packed in plastic bags and frozen at -20^{o}C.

Table 1. Composition of basal diet, g or MJ · kg^{-1}

Item	Content
Maize	582.5
Soyabean meal	325
Rapeseed oil	50
Limestone	10
Dicalcium phosphate	19.5
NaCl	3
DL-methionine (99%)	2.2
L-lysine HCl (78%)	2.8
Vitamin-mineral premix[1]	5
Metabolizable energy[2]	12.7
Crude protein	198
Crude fat	76.5

[1] contained no antioxidants and vitamin E, supplied to 1 kg of diet: IU: vit. A 12 000; vit. D_3 3250; mg: vit. K_3 2.25; vit. B_1 2; vit. B_2 7.25; vit. B_6 4.25; vit. B_{12} 0.03; biotin 0.1; Ca-pantothenate 12; niacin 40; folic acid 1.0; choline-Cl 450; Mn 100; Zn 65; Fe 65; Cu 15; J 0.8; Se 0.25 and Co 0.4

[2] according to European Tables of Energy Values for Poultry Feedstuffs (1989) as a sum of ME content of feed components calculated on the base of nutrients content

After 6 months the caudal part of the muscle was taken for chemical analyses and in selected groups (I, IV, V, VIII, IX, XII, XIII, XVI) the cranial part of the muscle was used for sensory testing. After 20 min of boiling in slightly salted water the meat was evaluated by a 6-member panel. The panelists ranked smell and taste, juiciness and tenderness on a 4-point scale for degree of acceptability (2 - flavour and taste and smell unacceptable, 3 - acceptable, 4 - good, 5 - very good).

Samples (5 g) of breast meat were extracted as described by Folch et al. (1957), evaporated under nitrogen, saponified with NaOH, converted to methyl esters according to Morrison and Smith (1964), extracted with hexane, and separated. The fatty acid composition was determined with a GC Varian 3400 gas chromatograph equipped with a CP-Wax 58, 25 m x 0.53 mm, 1.0 μm column, He as the carrier gas, 6 ml per min. Peak areas were measured with Star Chromatography Workstation software (Varian Star 4.5).

The content of α-tocopherol was determined with a Merck-Hitachi HPLC equipped with a LiChroCART 250-4 Superspher 100 RP-18, 4 μm column and FL, Ex. 295 nm, Em. 350 nm detector according to Manz and Philipp (1981). As a measure of oxidative stability of meat lipids, thiobarbituric acid reactive substances (TBA-RS) were determined according to Salih et al. (1987) with the modifications of Pikul et al. (1989).

Data were subjected to two-way analysis of variance and differences were examined by Duncan's multiple range test (Statistica ver. 5.0 PL software). As the

sensory properties of meat were determined only in selected groups, these data were subjected to one-way analysis of variance.

RESULTS

The lipid fraction of diets enriched with fish oil contained more EPA and DHA than the control diet (Table 2).

Adding fish oil to the basal diet improved ($P \leq 0.05$) feed intake and body weight gain (BWG) in chickens (Table 3). Supplementing vitamin E resulted in a significant reduction ($P \leq 0.05$) of feed intake and feed conversion per kg of BWG. An interaction was found between added fish oil and vitamin E for BWG. Average mortality in the experiment was 0.5% and feeding had no effect on this parameter.

As a consequence of including fish oil in the diet, after an extended period of storage (6 months) the fatty acid profile of lipids from breast meat was significantly changed in relation to some fatty acids (Table 3). The SFA content was elevated, whereas that of linolenic and arachidonic acids ($C_{20:4}$), decreased at 5 and 8 g fish oil $\cdot kg^{-1}$ diet. The contribution of long-chain EPA was increased ($P<0.05$), but the increase in the DHA content in total fatty acids was only numerical and

Table 2. Fatty acid composition of lipids in used oils and in experimental diets, % of total fatty acids

Item	Oils		Diets			
	rapeseed	fish	rapeseed oil 50 g $\cdot kg^{-1}$ control	rapeseed oil 47 g $\cdot kg^{-1}$ + fish oil 3 g $\cdot kg^{-1}$	rapeseed oil 45 g $\cdot kg^{-1}$ + fish oil 5 g $\cdot kg^{-1}$	rapeseed oil 42 g $\cdot kg^{-1}$ + fish oil 8 g $\cdot kg^{-1}$
$C_{14:0}$	0.0	8.95	0.09	0.26	0.65	0.77
$C_{16:0}$	4.24	18.14	7.42	6.74	8.76	8.28
$C_{16:1}$	0.21	6.97	0.19	0.29	0.49	0.58
$C_{18:0}$	1.10	1.26	1.58	0.98	1.47	1.34
$C_{18:1}$	52.2	15.7	42.0	39.1	37.7	36.5
$C_{18:2\ n-6}$	29.0	27.6	39.8	42.7	41.8	43.0
$C_{18:3\ n-3}$	11.3	1.38	6.34	6.89	6.54	6.55
$C_{22:0}$	0.0	0.0	0.45	0.34	0.24	0.0
$C_{22:1}$	0.41	17.30	0.59	0.59	0.39	0.37
$C_{20:5\ n-3}$ (EPA)	nd	6.98	nd	0.17	0.22	0.40
$C_{22:6\ n-3}$ (DHA)	nd	6.09	nd	0.10	0.17	0.35
SFA	5.63	28.5	9.95	8.77	11.45	10.72
MUFA	52.8	40.0	42.7	40.0	38.5	37.4
PUFA	41.6	31.6	47.3	51.2	50.0	51.8
n-6/n-3 PUFA	2.56	0.24	6.20	5.97	6.04	5.89

nd - not detected

Table 3. Effects of dietary fish oil and vitamin E supplementation on performance, major fatty acids (in % of total fatty acids), vitamin E and TBA-RS levels in chicken breast meat stored for 6 months

Item	Fish oil, g per kg diet				Vitamin E, mg per kg diet				Significance			
	0	3	5	8	0	40	150	300	RMSE[1]	fish oil	vit. E	interaction
Performance												
Feed intake, kg	2.95	3.10	2.99	3.05	3.09	3.03	2.99	2.99	0.01	***	*	ns
Body weight gain, kg	1.80	1.88	1.81	1.86	1.82	1.85	1.84	1.83	0.01	***	Ns	**
FCR, kg feed/kg BWG	1.66	1.65	1.65	1.64	1.70	1.64	1.63	1.63	0.01	ns	**	ns
Major fatty acids in breast meat, % of total fatty acids												
$C_{16:0}$	15.7	15.5	15.2	16.1	15.5	15.8	15.9	15.4	0.21	ns	ns	ns
$C_{18:0}$	7.50	7.53	7.84	8.51	7.76	7.88	7.96	7.79	0.11	**	ns	ns
$C_{18:1}$	33.6	34.4	34.9	34.1	34.3	33.8	34.1	34.9	0.20	ns	ns	ns
$C_{18:2\ n-6}$	28.9	28.2	28.5	27.9	28.0	28.5	28.7	28.2	0.21	ns	ns	ns
$C_{18:3\ n-3}$	3.26	3.15	2.87	2.75	2.94	2.98	3.03	3.07	0.06	**	ns	ns
$C_{20:4\ n-6}$	5.28	4.62	4.39	4.31	4.65	4.69	4.66	4.60	0.13	*	ns	ns
$C_{20:5\ n-3\ (EPA)}$	0.49	0.58	0.62	0.70	0.62	0.58	0.62	0.57	0.02	**	ns	ns
$C_{22:6\ n-3\ (DHA)}$	2.47	3.08	2.86	3.02	3.10	2.88	2.55	2.88	0.09	ns	ns	ns
SFA	23.5	23.5	23.7	25.0	23.8	24.2	24.2	23.5	0.24	*	ns	ns
UFA	76.5	76.5	76.3	75.0	76.2	75.8	75.8	76.5	0.24	*	ns	ns
UFA/SFA	3.26	3.26	3.22	3.00	3.20	3.14	3.13	3.25	0.04	*	ns	*
PUFA	41.3	40.5	40.1	39.4	40.8	40.2	40.4	40.1	0.29	ns	ns	*
PUFA n-6	34.4	33.1	33.0	32.2	33.2	33.2	33.4	32.9	0.22	**	ns	ns
PUFAn-3	6.28	6.63	6.27	6.40	6.50	6.37	6.20	6.50	0.09	ns	ns	ns
PUFA/SFA	1.77	1.73	1.70	1.57	1.72	1.67	1.67	1.71	0.03	*	ns	**
n-6/n-3 PUFA	5.53	5.04	5.32	5.08	5.21	5.24	5.41	5.12	0.06	**	ns	ns
Vitamin E, mcg/g breast meat	6.37	6.76	7.19	6.20	2.39	3.65	8.13	12.35	0.47	ns	***	ns
TBA-RS, mg/kg breast meat	0.524	0.461	0.387	0.379	0.514	0.436	0.418	0.382	0.01	***	***	***

[1] root of mean square error; TBA-RS - tiobarbituric acid reacting substances; * P<0.05; ** P<0.01; *** P<0.001; ns - no significant

statistically not confirmed. At the highest 8 g·kg^{-1} dietary level of fish oil, an increase of SFA and decrease of the unsaturated fatty acid (UFA) contents in meat lipids were observed. The n-6 PUFA content in total fatty acids was also lowered. The UFA:SFA and PUFA:SFA ratios in meat lipids were lowered and the ratio of n-6 : n-3 PUFA, decreased.

Different levels of α-tocopheryl acetate added to the control diet with an α-tocopherol content of 8.1 mg·kg^{-1} did not affect the fatty acid composition of breast meat lipids (Table 3). Similarly, dietary fish oil levels had no effect on the content of vitamin E in stored meat. The vitamin E content in meat increased in a dose-dependent manner (Table 3). The analytically confirmed vitamin E content in α-tocopheryl acetate-supplemented diets was 36, 126 and 234 mg· kg^{-1}, respectively.

Increasing dietary fish oil levels significantly influenced TBA-RS values ($P \leq 0.05$). A lower TBA-RS content (0.38 mg malondialdehyde·kg^{-1} of meat) was found in chickens receiving higher levels of fish oil (5-8 g·kg^{-1} diet) as compared with a TBA-RS content of 0.52 mg·kg^{-1} when fish oil was not added to the diet (Table 3). Incorporation of vitamin E into the diet affected TBA-RS and its values declined from 0.51 in the unsupplemented diet to 0.42-0.38 mg malondialdehyde·kg^{-1} of meat in chickens fed 150-300 mg α-tocoferyl acetate. Diet supplementation with fish oil and α-tocopheryl acetate did not affect smell, taste or other sensory indices in boiled breast meat (Table 4).

Table 4. Sensory properties of stored breast meats, points (from 2-unaccepted to 5-very good)

Group	Fish oil, g·kg^{-1} diet	Vitamin E addition mg·kg^{-1} diet	Smell	Taste	Juiciness	Tenderness
I	0	0	4.42	4.45	4.55	4.50
IV	0	300	4.42	4.40	4.48	4.42
V	3	0	4.57	4.48	4.60	4.58
VIII	3	300	4.57	4.50	4.50	4.50
IX	5	0	4.52	4.32	4.48	4.38
XII	5	300	4.58	4.43	4.45	4.42
XIII	8	0	4.57	4.47	4.50	4.43
XVI	8	300	4.58	4.42	4.52	4.48
SEM			0.023	0.024	0.024	0.025

DISCUSSION

Fish oil contains no natural antioxidants and its unsaturated fatty acids are particularly sensitive to oxidation. For this reason stabilized fish oil has uses as a component of feed mixtures. The levels of EPA and DHA in the fish used

in this experiment were relatively high and were a rich source of long-chain polyunsaturated fatty acids in the diet. For comparison, the EPA and DHA contents and PUFA n-6:n-3 ratio in oil from fish coagulates were 3.98, 5.88 and 1.07%, respectively (Dobrzański et al., 2002a).

The reduced MUFA percentage and PUFA n-6:n-3 ratio and increased PUFA and SFA contents in lipids of fish oil-enriched diets as compared with the control diet were a result of the higher oleic and linolenic acid contents in rapeseed oil. Rapeseed oil was substituted on a weight-to-weight basis with increasing amounts of fish oil. The levels of fish oil incorporated into the diet in the experiment were limited in view of a possible negative effect on the taste and smell of meat and on the costs of the feed mixture.

Fish oil added to the diets increased feed intake and improved BWG. It is quite likely that the combination of rapeseed and fish oils had a positive effect on feed palatability. Zolltisch et al. (1997) reported, however, that feed consumption was not improved by a higher percentage of PUFA from soyabean oil and rapeseed oil in the diet. Lopez-Ferrer et al. (1999) found higher feed consumption in chickens fed a vegetable oil-enriched diet than a fish oil one. On the other hand, the gain-to-feed ratio linearly increased in broilers fed higher dietary PUFA levels (Zollitsch et al., 1997) and BW and FC were better in chickens fed 3% fish oil replacing rapeseed oil (Dobrzański et al., 2002b).

A significant reduction of feed intake and improved feed conversion were observed when diets were supplemented with a higher level of α-tocopheryl acetate (150 and 300 $mg \cdot kg^{-1}$) as compared with the non-supplemented control diet with a vitamin E content of 8.1 $mg \cdot kg^{-1}$ or to this diet supplemented with 40 $mg \cdot kg^{-1}$ of α-tocopheryl acetate. The diets used in this experiment contained rapeseed oil supplemented with fish oil, so the dietary content of PUFA from both sources was generally considerable. Taking into account the protective effect of vitamin E in lipid peroxidation processes and that PUFA may increase the degradation of vitamin E in the gastrointestinal tract (Villaverde et al., 2004), it seems likely that also the levels of vitamin E used in this experiment could reduce fat oxidation processes in the diet and protect a pool of non-oxidized fat for absorption. The results reported by Guo et al. (2003) showed that addition of vitamin E (100 $mg \cdot kg^{-1}$) to diets improved growth and feed conversion in chickens during 0-3 weeks of age. Soto-Salanova and Sell (1996) reported that addition of 80 or 150 IU $\cdot$ kg^{-1} α-tocopheryl acetate did not influence the performance of poults during the first 21 days of life. Higher dietary PUFA levels reduced the hepatic vitamin E concentration in chickens (Villaverde et al., 2004), suggesting greater systemic use of this vitamin.

In the presented experiment, significant interaction was revealed between dietary fat and vitamin E for BWG. The kind of fat (rapeseed oil + fish oil) significantly influenced BWG and feed intake in chickens, but adding vitamin

E to the diet improved FC. This result may suggest a positive effect of rapeseed oil enriched with a small amount of fish oil as a fat component of the diet and supports the efficacy of adding vitamin E to diets for broiler chickens.

Inclusion of 0.5-0.8% fish oil in the diet with rapeseed oil changed the fatty acid profile in dietary lipids and breast meat. The increased dietary PUFA and SFA content modified the chicken meat lipid composition: the contribution of stearic acid and EPA was greater, whereas the contents of linolenic and arachidonic acids and of UFA decreased. The UFA:SFA, PUFA:SFA, and n-6:n-3 PUFA ratios in stored frozen meat were also reduced. The range of meat lipid modification was much smaller in this experiment than in other studies in which higher amounts (8.2%) of fish oil were added to the diet (Lopez-Ferrer et al., 2001) and did not confirm in principle the increase of the PUFA content in tissues (Cortinas et al., 2004). The fatty acid levels in breast meat lipids in groups fed 5 or 7.8% of rapeseed oil reported by Mieczkowska and Smulikowska (2005) and in the control group in this experiment were generally similar.

Supplementation of diets with increased levels of α-tocopheryl acetate did not change the fatty acid profile of lipids in breast meat and this result agrees with the data reported by Bou et al. (2004) in mixed white and dark meat stored for 5 months at -20°C. The vitamin E content in meat increased with α-tocopheryl acetate level in diets, which confirms the data of other authors (Surai et al., 2000). Dietary fish oil levels had no effect on the vitamin E content in stored breast meat. This finding confirms the data reported by Bou et al. (2004), who used diets with 1.25 or 2.3% fish oil and 70 or 140 $mg \cdot kg^{-1}$ added vitamin E.

For the PUFA content in meat lipids and the UFA:SFA, PUFA:SFA ratios, a significant interaction was revealed between the kind of dietary fat and level of vitamin E. Only the kind of dietary fat (rapeseed oil+fish oil) supplementation, however, but not vitamin E, significantly influenced the fatty acid composition of lipids in stored chicken breast meat.

Dietary fish oil and vitamin E levels influenced the lipid oxidation rate assessed by measuring TBA-RS. The higher dose of dietary fish oil predominantly correspond to higher TBA-RS values in meat (Bou et al., 2004). In contrast, in our experiment, in chickens fed the control diet, the TBA-RS content in breast meat was higher than for diets with fish oil. In diets supplemented with fish oil, total PUFA levels in stored meat lipids were similar to control levels or even lower. This is because the rapeseed oil used in the control diet was rich in unsaturated fatty acids. This could also result from the use of fish oil stabilized with ethoxyquin in our experiment. It is likely that ethoxyquin may also have an antioxidative effect in meat. Bartov and Bornstein (1981) reported increased stability of meat lipids when a combination of ethoxyquin and α-tocopheryl acetate were used in the diet. They found that in chickens fed diets simultaneously supplemented with

an antioxidant and vitamin E, the decrease of the TBA-RS content in thigh meat was beyond the effect of each of these additives fed alone.

Adding vitamin E to the diets (150-300 mg·kg^{-1}) lowered TBA-RS values in stored breast meat and this effect can be attributed to accumulation of vitamin E in tissues. This result is in line with the values found in dark meat, which is less susceptible to oxidation (Grau et al., 2001), and in chicken breast meat with a higher content of vitamin E (Giannenas et al., 2005).

Under the conditions of this experiment, a significant interaction was revealed between dietary fat and vitamin E for TBA-RS in stored breast meat. The results suggest that addition of stabilized fish oil to diets supplemented with rapeseed oil and vitamin E lowers the TBA-RS values in stored breast meat.

The results of sensory evaluation of boiled breast meat showed that the smell, taste and other indices in the meat of control and experimental chickens fed fish oil were not different. These results are in agreement with an earlier experiment (Koreleski et al., 1997) where no effect on consumer meat acceptability was observed when similar low levels of fish fat were introduced into the diet for chickens.

CONCLUSIONS

When feeding chickens with a rapeseed-oil-containing diet, a supplement of 8 g of stabilized fish oil and 40-300 mg·kg^{-1} vitamin E 3 weeks before slaughter may be advisable. Fish oil and vitamin E raised the level of EPA in the lipid fraction, positively affected the TBA-RS and vitamin E contents in stored breast meat and did not deteriorate the sensory quality of stored meat.

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