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Full Length Research Paper

Lipid oxidation in fresh and stored eggs enriched with dietary ω3 and ω6 polyunsaturated fatty acids and vitamin E and A dosages

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Two experiments were planned to study the influence of dietary fat sources (fish oil (FO) or sunflower oil (SO)) and dietary doses of α-tocopheryl acetate (α-TA) (0, 60 and 120 mg/kg of feed) and vitamin A (0 IU, 10000 IU and 20000 IU) on lipid oxidation of stored eggs in three stages of 0 or fresh, 1 and 2 months of storage time. In the first experiment, 96 hen layers in six treatments including two oil sources (FO and SO) and two dietary [0, 60 and 120 mg/kg doses of α -tocopheryl acetate (α-TA)] were fed for 75 days. In the second experiment, 96 hen layers in six treatments including two sources of ω3 and ω6 (FO and SO) and three doses 0, 10000 and 20000 IU of vitamin A were fed for 75 days. The results showed that using α-TA supplementation, lipid stability of enriched eggs increased and was very effective throughout the stored period of the eggs. Yolk TBA value was higher in fish oil than sunflower oil groups ($p < 0.01$). The treatments that contained 120 mg/kg of α-TA in diets, showed lower lipid peroxidation than other groups in stages of 2 and 3 storage time (30 and 60 days). The degree of lipid oxidation in fresh, 1 and 2 months of storage eggs was measured by the lipid TBA values. The results showed that TBA value in fresh and stored eggs was higher in groups containing fish oil than other groups ($p < 0.01$). The MDA value in stage 1 was higher in fish oil group and in 2 and 3 stages was lower in FO + A1. Therefore, addition of Vitamin E and A as natural antioxidants in diets containing oil source for the prevention of lipid oxidation is recommended.

Key words: Fish oil, sunflower oil, α-tocopheryl acetate, vitamin A, TBA value, egg.

INTRODUCTION

Nowadays, it is widely accepted that dietary manipulation, especially dietary lipid modifications, can alter lipid composition of different animal products (Hargis et al., 1991). Eggs, one of the cheapest sources of animal protein are easily available all over the world (Leskanich and Noble, 1997). Furthermore, eggs are nutritious favorites providing complete nutritional proteins, lipids, vitamins, minerals and some key nutrients such as omega - 3 fatty acid, amino-acids, vitamins A, D, B_1, B_2 and E, selenium and iron. These are considered particularly important in human nutrition and body health (Surai et al., 2000). Since most egg lipids are located in the yolk, they are susceptible to oxidation and thus requires quality control (Yang and Chen, 2001; Martins et al., 2002). Yolk lipids generally include triglycerides, phospholipids, cholesterol and other trace compounds. These and other different components of yolk lipids may result in adverse effects on human health. Omega - 3 (ω3) and omega - 6 (ω6) fatty acids gotten from natural sources such as fish and sunflower oils are highly unsaturated and are susceptible to peroxidation when there is excessive level of its consumption without sufficient amount of antioxidants (Chen and Hsu, 2003, 2004).

Dietary supplementation with α-tocopheryl acetate has been demonstrated to beneficially affect enhancement of lipid stability in foods from animal origin, such as avian

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Table 1. Ingredients and composition of the basal diet.

Ingredients	Percentage
Corn	57.27
Soybean meal (44%CP)	18.97
Wheat	5.00
Added oil[1]	3.50
Oyster shell	9.66
Fish mail	2.00
Bone meal	0.94
DL-methionine	0.06
Salt	0.10
Vitamin premix[2]	0.25
Trace-Mineral premix[3]	0.25
Sand	2.00
Composition	
Metabolizable energy (Kcal/kg[4)]	2850
Crude protein	15
Crude fiber	2.72
Calcium[3]	3.06
Available Phosphorus[4]	0.37
Lysine[4]	0.76
Methionine+cystine[4]	0.57

[1]Fish oil or sunflower oil; [2]Supplied per kilogram of diet: 3520000 IU vit. A, 1000000 IU vit. D_3, 4400 IU vit. E, 880 mg vit. K_3, 738.5 mg vit. B_1, 1600 mg vit. B_2, 3136 mg vit. B_3, 13860 mg vit. B_5, 984.8 mg vit. B_6, 192 mg vit. B_9, 4 mg vit. B_{12}, 60 mg biotin, 80000 mg choline chloride, 400 mg anti oxidant; [3]Supplied per kg of diet: 25870 mg Zn, 30000 mg Fe, 29760 mg Mn, 2400 mg Cu, 346.8 mg I, 80 mg Se. [4]Calculated value.

eggs and meat. However, it is well known that oxidative deterioration, quality characteristics, nutritional value of eggs, consumer acceptability and deleterious biological effects are important nutritional factors that researchers do not test for without antioxidant substances (Florou-Paneri et al., 2005). The effect of dietary α - tocopheryl acetate supplementation on enhancing lipid stability in egg yolk has been repeatedly reported (Chen and Hsu, 2006; Bourre and Galea, 2006; Aghdam Shahriar et al., 2007).

Carotenoids are antioxidants and immunomodulants that cannot be synthesize *de novo* in bird and must be obtained in the diet (Moller et al., 2000). Maternally derived carotenoids in egg yolk are thought to enhance egg quality (Blount et al., 2002a, b). Supplemental feeding experiments have shown that carotenoid availability can limit antioxidant and immune defences in adult birds (Blount et al., 2002b, 2003).

In order to prevent the oxidation (formation of thiobarbituric acid reactive substances; TBARS) of yolk lipids and improve the oxidative stability of enriched products such as omega- 3 fatty acids in eggs, two experiments were planned to study the influence of dietary fat sources (fish oil or sunflower oil) and dietary doses of vitamin A (0,10000 and 20000 IU) and α- tocopheryl acetate (α-TA) (0, 60 and 120 mg/kg of feed) on lipid oxidation of fresh, one and two months-stored eggs.

MATERIALS AND METHODS

Two 2 × 3 factorial experiments were planned and replicated four times in order to study the influence of 6 dietary treatments for each trial on lipid oxidation in fresh, one and two months-stored eggs. In these experiments the Ninety six hen of Hey-line laying for each experiment were randomly distributed into the 6 dietary treatments. These were fed and provided water *ad libitum*. The lighting regimen was 15 h of continuous light per day. Diets (Table 1) were formulated to meet the requirements for nutrient and energy for laying hens on the base of nutrients recommended by (NRC, 1994). It resulted in the supplementation of a basal diet containing 3.5% fish oil (FO) or sunflower oil (SO) in the first trial with dietary doses of vitamin A (0, 10000 and 20000 IU retinyl acetate). In the second trial, 3.5% fish oil (FO) or sunflower oil (SO) and dietary doses of α-tocopheryl acetate (α-TA) (0, 60 and 120 mg/kg) were used.

To investigate the effect of enriched diet on lipid oxidation of shell eggs during refrigerated storage, 4 freshly collected eggs from each subgroup total 16 eggs from each dietary treatment were stored pending further handling and were placed in a refrigerated cabinet at 4°C to be analyzed for malondialdehyde (MDA) levels in yolk of 3 stages stored eggs at 0, 30 and 60 days after storage.

Thiobarbituric acid (TBA) determination

The TBA values were determined for the Malonaldehyde (MDA) formed in fresh eggs and those that were refrigerated. This secondary oxidation product (MDA) was measured according to the TBA method described by Botsoglou et al. (1994) using third derivative spectrophotometry with some modifications. Yolk samples were homogenized (Polytron homogenizer, PCU, Switzerland) in the presence of 8 ml 5% aqueous trichloroacetic acid (TCA). 5 ml 0.8% butyrate hydroxytoluene in hexane was immediately added and the mixture centrifuged. The top layer was discarded and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of 0.8% aqueous 2 -thiobarbituric acid, and was further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, Japan) in the range of 400 - 650 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of MDA in analyzed samples (ng/ g yolk) was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of a standard calibration curve prepared using 1, 1, 3, 3 tetraethoxypropane, the precursor of MDA.

Statistical analysis

Data were subjected to a one-way analysis of variance using the General Linear Models (GLM), procedure of SAS User's guide (2001). When significant difference among means was found, means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

The effect of dietary treatments without vitamin supplementation on lipid oxidation of fresh eggs and those that were refrigerated and stored for 30 and 60 days are

Table 2. The effect of oil source and vitamin E levels on MDA in different times of storage.

Treatments	Storage time		
	0	30 d	60 d
Oil source (p value)	**	**	**
Fish oil (3.5%)	3.29	3.37	6.28
Sunflower oil (3.5%)	2.88	2.74	5.49
SEM	0.36	0.44	0.49
Vitamin E Levels (mg/kg) (p value)	**	**	**
0	3.10	3.35	7.03
60	2.43	2.82	5.69
120	2.57	2.64	4.32
SEM	0.15	0.28	0.26
Oil × Vitamin (p value)	**	**	**

Table 3. The effect of oil source and vitamin A levels on MDA in different times of storage.

Treatments	Storage time		
	0	30 d	60 d
Oil source (p value)	**	**	**
Fish oil (3.5%)	3.44	3.16	6.74
Sunflower oil (3.5%)	2.74	4.26	6.04
SEM	0.26	0.39	0.47
Vitamin A Levels (IU) (p value)	**	**	**
0	3.1	3.98	7.04
10000	2.35	3.35	6.11
20000	2.25	3.39	4.71
SEM	0.23	0.51	0.89
Oil × Vitamin (p value)	**	**	**

shown in Tables 2 and 3. The TBA value in fresh and stored eggs was higher in fish oil when compared to sunflower oil groups ($p < 0.01$). The extent of MDA in enriched eggs by fish and sunflower oils rich in omega - 3 and omega - 6 without vitamin A and E supplementation were clearly increased only in the second stage of storage time (60 days).

In the first experiment, dietary supplementation of diets with 0, 60 or 120 mg of α-TA/ kg decreased the oxidative stability of PUFA-enriched eggs refrigerated and stored for 30 and 60 days. Inclusion of vitamin E, resulted in a significant ($p < 0.01$) reduction in the TBARS values of eggs at 0, 1 and 2 months storage time in layer hens (Table 2). Although, the MDA values were increased in eggs during storage time, significant reduction in yolk of ω3 and ω6 containing eggs, occurred with increasing levels of vitamin E when compared to those not supplemented with α-TA.

In the second experiment, addition of vitamin A at 0, 10000 and 20000 IU doses to diet containing fish oil decreased the extent of lipid oxidation, as measured by MDA formation, when the storage time was increased. These changes were lightly observed in FO + 20000 IU vitamin A than fish oil diet without vitamin A supplementation. The same results were observed in SO diets supplemented with higher levels of vitamin A dosage. Blount et al. (2002a) showed in wild gulls that maternally derived carotenoids could increase the level of antioxidant activity and reduce susceptibility to peroxidation in egg yolk. Very low density lipoprotein (VLDL) has a highly unsaturated lipid profile (Speake et al., 1998) and consequently is vulnerable to peroxidation (Blount et al., 2000). Therefore, carotenoid supplementation could have increased the production and/or antioxidant protection of circulating VLDL, giving rise to an increased capacity to produce eggs.

During the storage time, eggs supplemented with vitamin A and E treatments had lower TBA values than those not supplemented (Figure 1). Although, the MDA values in fish oil treatments were higher when compared to sunflower groups, the stability of omega- 3 eggs to lipid oxidation was increased due to α-TA antioxidant supple-

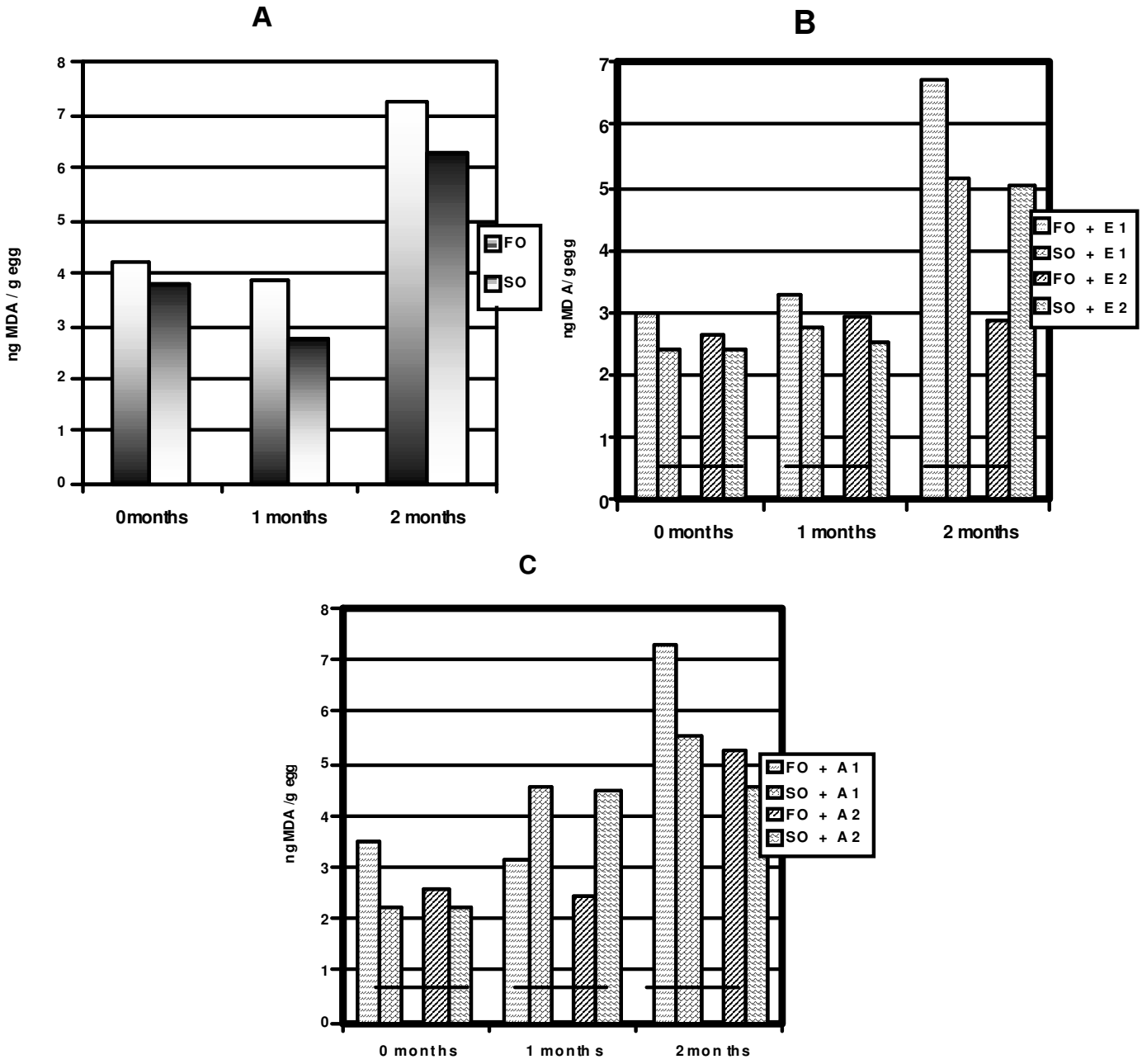

Figure 1. Influence of dietary oils without vitamin supplementation (A) dietary oils and *α*-tocopheryl acetate supplementation (B) dietary oils and vitamin A supplementation (C) on TBA values (expressed as ng malondialdehyde/g egg) in spray dried eggs during 12 months storage. FO = diet with 3/5% fish oil; SO = diet with 3/5% sunflower oil; E1 and E2 = 60 and 120 mg *α*-tocopheryl acetate/kg of feed, respectively; A1 and A2 = 10000 and 20000 IU vitamin A supplementation, respectively; MDA = malondialdehyde.

mentation. These results are in agreement with previous reports that showed that fat types enriched with ω3 and ω6 polyunsaturated fatty acids (PUFA) obtained in eggs are necessary for the improvement of oxidative stability (Marshall et al., 1994; Cherian et al., 1996; Galobart et al., 1999). The extent of MDA was higher in omega-3 eggs than omega-6 enriched eggs, whereas oxidative stability in both enriched eggs of α-TA supplemented treatments were increased and were very effective in storage time with increasing levels of vitamin E.

Grashorn and Steinhilber (1999) used different dietary oils to obtain different n- 6/ n- 3 ratios and reported that TBA values were higher in fresh eggs with a higher content of n- 3 FA than in those rich in n- 6 FA. However, the major susceptibility to oxidation in FO eggs when compared to those from SO diets could be explained by

the higher content of total and long chain n- 3 PUFA (Galobart et al., 2001). The results of the present study are in agreement with those published by Cherian et al.(1996) using fish, flax, palm and sun flower oils to enrich eggs with different FA and found that TBA values from fresh eggs rich in omega-3 FA (fish and flax oils) were higher than those from eggs rich in omega- 6 FA (SO).

α-Tocopherol was very effective in preventing lipid oxidation at 30 and 60 days of enriched eggs. It could still be effective for 6 months of storage accordance to reports of Wahle et al. (1993). According to Galobart et al. (2001), α-tocopherol was very effective in preventing lipid oxidation for 6 and 12 months of storage after spray-drying. Differences obtained in this study when compared to other reports could be attributed in part to the evolution of LHP and TBA values that may depend on unsaturation degree as well as processing and storage conditions. Therefore, determination of several oxidation parameters to assess lipid oxidation is necessary. Florou-Paneri et al. (2005) reported that the MDA existed in yolks of fresh eggs. This was attributed to either the consumption and subsequent deposition of MDA that was already present in the diets or to the *in vivo* production of MDA by the hens during the feeding trial. The former possibility appeared unlikely because in that case, the level of MDA would have been equal among all treatments. Possible transfer of the antioxidant constituents of the oils rich in omega- 3 and omega- 6 or α-tocopherol into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk.

Consistently with these results, other workers (Marshall et al., 1994; Aymond and Van Elswyk, 1995) found that MDA levels could not be attributed to lipid oxidation during shell egg storage. The higher MDA concentrations (180ng/g) found by these workers most likely reflect differences in the methods applied to determine lipid oxidation in eggs, as the widely used distillation method (Tarladgis et al., 1964) may cause oxidation of lipids during the distillation stage even in the presence of added antioxidants (Raharjo et al., 1993).

Conclusion

Marked oxidation and accumulation of oxidation products (including cholesterol oxides) may take place when eggs are processed under pre-oxidative conditions (Lai et al., 1995), which is particularly important when processed eggs are stored prior to consumption. In these cases, it is essential to prevent oxidation and therefore it may be necessary to customize the vitamin E concentration in egg yolk by dietary intervention. It may be concluded from the results of this study that dietary supplementation with 60 or 120 mg of *α*-TA/kg and 10000, 20000 IU of vitamin, increased the oxidative stability of PUFA-enriched eggs refrigerated and stored for 30 and 60 days. On the other hand, eggs enriched with omega -3FA appeared to be more susceptible to lipid oxidation than those enriched with omega - 6FA.

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