International Scholars Journals

Advances in Food Science and Technology ISSN: 6732-4215 Vol. 4 (4), pp. 001-008, April, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effects of adding vitamin E to diets supplemented with sardine oil

***Alfonso N. Slim, Alejandro V. I and Lupita F. Rivera**

Department of Microbiology, Faculty of Medicine, Universidad Veracruzana, Xalapa, Mexico.

Accepted 16 November, 2015

The aim of this study was to know the effect of adding vitamin E (VE) (100 and 200 mg/kg) to diets supplemented with sardine oil (SO) on egg fatty-acids (FA) composition. 240 Bovans hens were grouped into four treatments: T1- basal diet (BD), T2-BD+2.5% SO, T3-BD+2.5% SO+100 mg/kg VE, and T4-BD+2.5% SO+200 mg/kg VE. After four weeks, eggs were collected from each treatment, and analyzed FA content using gas chromatography. Results: a) 14:0, 16:0, 16:1, 20:5n3, 22:6n3 and 22:6n3 content were higher in eggs from treatments with SO (T2) and n6:n3 ratio was better (11:1 vs 2:1) than the control group (T1) (P<0.05); b) T3 had a reduction of 16:0, 16:1, 18:2n6 and 18:3n3 in eggs (P>0.05); c) T4 had a reduction on the concentration of saturated and polyunsaturated FA in eggs (P<0.05). The productive parameters were no affected, only egg yolk color increased when SO was added to the diets (P<0.05). It is concluded that adding SO to laying hens diet increases n3 FA in eggs, and that adding large quantities (100 or 200 mg/kg) of VE to diets supplemented with SO reduce the concentration of the polyunsaturated FA.

Key words: Vitamin E, sardine oil, egg fatty acid, laying hen, egg composition, egg lipids.

INTRODUCTION

Omega-3 fatty acids (n3FA), mainly eicosapentaenoic acid (C20:5 n3 EPA) and docosahexaenoic acid (C22:6 n3 DHA), are essential for normal growth and development. Also, they have an important role in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders and some types of cancer, and in neuronal development (Simopoulos, 2000; Kris-Etherton et al., 2002). Recent dietary fat studies have centered on the manipulation of specific fatty acids. In an attempt to increase n3 FA content in eggs, the utilization of fish and flaxseed as feed ingredients has been a common practice (Baucells et al., 2000; González- Esquerra and Leeson, 2001; Castillo-Badillo et al., 2005).

In Mexico, as in other western countries, there is a low consumption rate of fish due to common eating habits and cost, among other factors (CONAPESCA, 2010; Kris-Etherton et al, 2002). For this reason, one alternative has been the incorporation of fishing industry byproducts with high n3 PUFAs content, such as fish oil, into the diet of hens, with the goal of concentrating n3 PUFAs in the egg yolk.

Some researchers (González-Esquerra and Leeson 2001; Simopoulos 2000; Leskanich and Noble, 1997) believe that eggs are excellent vehicles to achieve this objective for multiple reasons, including their low calorie content (75 kcal/egg), protein quality, culinary versatility and low cost. In Mexico, the consumption rate of fresh eggs is the highest in the world (21.6 kg/per capita annually) (UNA, 2010). A fresh egg is understood as an

*Corresponding author. E-mail: alfonso.slim12@uv.mx

egg that has not been submitted to any preservation processes, whose physical characteristics and chemical and microbiological properties are maintained at an optimal level of edible quality, and whose age since laying is no greater than 15 days; included in this classification are products stored in refrigerators for periods no longer than 10 days (NOM-159-SSA1-1996; NOM-FF-079-SCFI-2004).

Many studies have been conducted (Marshall and Van Elswyk 1994; Van Elswyk et al., 1995, 1997; Castillo-Badillo et al., 2005) in which laying hen diet were supplemented with fish oil in order to increase the n3 PUFAs content of their eggs (1.02% ALA, 15.54% EPA and 10.70% DHA / percent of total fatty acids). Although the results have been satisfactory, one drawback has been that n3 PUFAs fast oxidize due to the long-chain hydrocarbons they contain, causing rancidity in the product and, as a result, reducing their shelf life.

Consequently, it has been considered appropriate to add antioxidants to fish oil-supplemented diet hens, with the goal of reducing the risk of lipid peroxidation. The most commonly used antioxidant is vitamin E (VE) (Chen et al., 1998; Cherian et al., 1996).

Due to the fact that these FAs are highly prone to oxidation, VE reserves are rapidly exhausted when dietary EPA and DHA quantities are increased (Surai and Spark, 2000; Surai, 2003). It has been suggested that increasing the quantity of vitamin E in rations supplemented with fish oil (FO) might help to prevent or reduce undesirable effects in the hens and to avoid the loss of long-chain fatty acids by reducing the risk of lipid peroxidation (Meluzzi et al., 2000; Surai, 2003).

However, an association among dietary FO and reduced sensory quality of eggs has been reported (Van Elswyk et al., 1995; Marshall and Van Elswyk, 1994; Van Elswyk, 1997; González-Esquerra and Leeson, 2000 Castillo-Badillo et al., 2005). These modifications, off-flavors and, their lipid inestability, have been associated to the inclusion of FO in the formulation of the diet hens. Lipids are not the only substances responsible for fishy flavors; it might also be a non-lipid fraction that, interacting with some lipid compounds, produces those fishy flavors. For example, amines can impart a fishy taint (González-Esquerra and Leeson, 2001; Honkatukia et al., 2005). Also, oxidation products could be responsible in part for fishy odors found in eggs, but the use of stabilized n3 FA sources in poultry diets could in theory diminish this problem (Van Elswyk et al., 1995; Cherian et al., 1996; Leskanich and Noble, 1997).

The aim of this study was to know the effect of adding high levels of VE (100 and 200 mg/kg) to diets supplemented with sardine oil (SO) on the egg FA profile.

MATERIALS AND METHODS

Sources of sardine oil and vitamin E

Sardine oil (SO) used was obtained from Guaymas, Sonora, Mexico. The SO was not refined, bleached, and deodorized, with a Butylated Hydroxytoluene (BHT) as an antioxidant. The VE was feed-grade and supplied by BASF Mexicana (Lutavit E 50%).

Diet formulation and preparation

Four diets were prepared: T1- basal diet (BD), T2-BD+2.5% SO, T3-BD+2.5% SO+100 mg/kg VE, and T4-BD+2.5% SO+200 mg/kg VE. Diets were formulated using Nutrition Windows™ (version 5.0 pro) software to contain 15% crude protein and 2800 kcal of metabolizable energy and other nutrients to meet the nutrient requirements established by the National Research Council (1994) for laying hens (Table 1). Diets were prepared weekly. The fatty acid compositions of the soy and sardine oils and the diets used in this study were measured using gas chromatography following AOAC method 969.33 (AOAC, 2000).

Experimental design

240 Bovans White hens were randomly assigned to four treatments with five replicates of 12 birds each. Each replicate represented one experimental unit. The experimental diets were randomly assigned to the groups of hens. After one week of adaptation, the trial was carried out for four weeks. Throughout the period, the productive parameters were measured: feed intake, egg production, egg weight, egg mass, and feed conversion ratio. To measure the yolk color, 50 eggs per treatment (10 per replicate) were randomly collected at end of the four weeks. The color was measured by Roche color fan. The study was conducted in accordance with the policies established by the Ethics Committee for Animal Care of the Faculty of Veterinary Medicine and Animal husbandry, National Autonomous University of Mexico. After four weeks, 50 eggs per treatment (10 per replicate) were randomly collected. The yolks from each replicate were mixed to form a pooled sample. Each pooled sample was analyzed by duplicate. The lipids were extracted using chloroform: ethanol (1:1) (method 923.07). The lipid extract was methylated with boron trifluoride in a methanolic solution (method 969.33) (AOAC, 2000). The samples were injected in a Varian gas chromatograph (model 3380 CX) equipped with a CP8400 autosampler, flame ionization detector, and a 30 m DB23 column with an internal diameter of 0.25 mm. The miristoleic acid was used as internal standard. The nitrogen was used as the carrier gas with a flow rate of 30 ml/min. The temperatures used were: column, 230°C; injector, 150°C; and detector, 300°C. To calculate FA concentration, a mix of FA standards with known concentrations (Supelco™ 37 FAME Mix SIGMA) was used as reference. The results were analyzed using Star Chromatography Workstation v. 6.3 software from Varian Associates, Inc., and are reported as percentages of total FA (%TFA).

Organoleptic test

At the end of the 4th week, 30 untrained panelists, who were usual egg consumers participated in organoleptic test. This test was performed in single booths and under white light, in the Organoleptic Evaluation Laboratory. A hedonic acceptance test was used (Watts et al., 1992). Four samples of fried eggs, one for each treatment, labeled with random numbers, were provided to the panelists. Salt was not added. They received a questionnaire to indicate the degree of acceptance of each sample choosing from the following options: “like very much”, “like moderately”, “neither like nor dislike”, “dislike moderately” and “dislike very much”. They also received bread and water to be consumed before testing each sample in order to eliminate any residual flavor brown color, and translucent. It was stabilized with 200 ppm of Butylated

Table 1. Compositions of experimental diets (%).

Ingredient	T1
Sorghum grain	62.67
Soybean meal	20.71
Calcium carbonate	9.85
Sardine oil	0.00
Soy oil	4.50
Orthophosphate *	1.39
Salt	0.40
Vitamin premix [I]	0.10
Vitamin E [+]	0.00
Avelut [§]	0.10
DL-Methionine	0.07
Choline chloride 60	0.05
Mineral premix [¶]	0.05
Avired **	0.05
Antioxidant	0.04
Antimicotic	0.02
L-Threonine	0.00
Total	100.00

*Monobasic phosphate. P 21% min, Ca 18% min, F 0.21% max, moisture 5% max. [†] Vitamins (per kg): A, 10 000 000 IU; D_3, 3 000 000 IU; E, 20 000 IU; K_3, 2.500 g; Thiamin, 2.500 g; Riboflavin, 5 g; Niacin, 35 g; Pantotenic acid, 10 g; Pyridoxine, 4 g; Folic acid, 1 g; Cyanocobalamin, 10 mg; Biotin, 200 mg; excipient, q.s. 1 000. [‡]Lutavit 50 (BASF Mexicana SA de CV). [§] Source of natural yellow xanthophylls (marygold flower), 15 g/kg. [¶] Minerals (mg/kg in diet): Mn 120; Zn 100; Fe 120; Cu 12; I 0.7; Se 0.4; Co 0.2; excipient, c.b.p. 1 000. **Red vegetal pigment: Lucantin Red as a source of canthaxanthin, 5 g total carotenoids/kg.

Hydroxytoluene (BHT) as an antioxidant. The VE was feed-grade and supplied by BASF Mexicana (Lutavit E 50%).

Statistical analysis

The productive parameters variables and FA concentrations were subjected to an analysis of variance with a fully randomized design using PROC ANOVA in the SAS software package. Tukey's test was used to compare means ($P<0.05$) (SAS v.6.12).

RESULTS

Eggs fatty-acid composition

The sardine oil used in this study had a greater content of EPA (14%) than DHA (10%) (Table 2). As a consequence, the FA compositions of diets that included SO showed similar proportions: more EPA (8%) than DHA (6%). Moreover, the addition of SO to laying hen diets also resulted in higher saturated FA (SFA) content than in control diet (Table 3). However, more DHA than EPA was deposited in the eggs (Table 4). Productive parameters were not affected by supplementation with 2.5% SO ($P>0.05$) (Table 5). No difference ($P>0.05$) were found in the egg flavor, among the four treatments.

DISCUSSION

Fatty-acid composition of eggs

The results of fatty acid composition of oils used in diet formulation, agrees with the data obtained by Cachaldora et al. (2006), who indicated that the proportion of these two fatty acids in fish oil varies by species; in anchovy oil, the EPA concentration is greater than the DHA concentration (approximately 185 vs. 85 g/kg), while the opposite relationship exists in tuna oil (approximately 80 g/kg EPA vs. 220 g/kg DHA).

The behavior of the fatty acid composition of the diet and content in eggs, may be related to the fact that in hens, as in mammals, EPA is converted to DHA and vice-versa through lipid metabolism as a result of elongation and desaturation processes, with a preference for DHA deposition (González-Esquerra and Leeson, 2001; Cachaldora et al., 2006). These results agree with the observations of other authors using menhaden oil (Huang et al., 1990; Van Elswyk et al., 1995; González-Esquerra and Leeson, 2000) and tuna oil (Castillo-Badillo et al., 2005).

The fatty-acid composition of the eggs obtained from each treatment is shown in Table 4. In all treatments, saturated fatty acids with least of 13 carbons, and

Table 2. Fatty-acid composition of oils used in diet formulation.

Fatty acid (Percentage FAME)	Soy oil	Sardine oil
Myristic (C14:0)	0.11	6.09
Palmitic (C16:0)	10.77	18.54
Palmitelaidic (C16:1)	0.00	0.13
Palmitoleic (C16:1)	0.18	7.47
Heptadecanoic (C17:0)	0.10	0.56
Cis-10-heptadecanoic (C17:1)	0.07	0.99
Stearic (C18:0)	4.22	3.82
Oleic (C18:1 n9)	21.91	12.20
Cis-vaccenico	0.83	3.06
Linoleic (C18:2 n6 LA)	53.23	13.70
Gamma linolenic (C18:3 n6 GLA)	0.06	0.31
Alpha linolenic (C18:3 n3 ALA)	6.95	0.99
CLA c9,t11 y c11, t9 (C18:2)	0.00	2.55
CLA t10, c12 (C18:2)	0.00	0.29
Arachidic (C20:0)	0.31	0.29
Eicosenoic (C20:1)	0.22	2.17
Cis-11,14-eicosenoic (C20:2)	0.05	0.25
Cis-11,14,17-eicosenoic (C20:3)	0.10	0.18
Arachidonic (C20:4 n6 AA)	0.00	0.75
Eicosapentaenoic (C20:5 n3 EPA)	0.40	14.20
Docosapentaenoic (C22:5 n3 DPA)	0.00	1.57
Docosahexaenoic (C22:6 n3 DHA)	0.00	10.26
Total	99.51	100.37

FAME – Fatty acids methyl esters.

elaidic, behenic, erucic, lignoceric and nervonic acids were no delected. Trace amounts ranging from 0.05 to 1 g/100 g FAME of 14:0, 14:1, 16:1, 17:0, 17:1, 18:2 (CLA and linolelaidico), 18:3 (gama linolenico), 20:0, 20:2, 20:3,20:5, 22:0, 22:5 and 23:0, were present in treatments containing SO.

Adding SO to the diet resulted in a significant increase ($P<0.05$) in the myristic acid (C14:0), palmitic acid (C16:0), palmitoleic (C16:1), eicosapentaenoic acid (C20:5 n3EPA), docosapentaenoic acid (C22:5 n3 DPA), and docosahexaenoic acid (C22:6 n3 DHA) content in eggs ($P<0.05$); while that the concentration of oleic (C18:1 n9), linoleic (C18:2 n6 LA), alpha-linolenic (C18:3n3) and arachidonic (C20:4 n6 AA) acids were reduced.

The total SFA content in the eggs increased when diets were supplemented with SO, while that the monounsaturated fatty acids (MUFA) content and polyunsaturated acids (PUFA) did decrease significantly ($P<0.05$). The total quantity of n3FA increased, whereas the total quantity of n6FA significantly decreased ($P<0.05$). The calculation of the ratio n6:n3 was performed as follows:

Σn6/Σn3:Σn3/Σn3

Eggs treated with SO maintained an n6:n3 ratio of 2:1 vs. 11:1 for the control group ($P<0.05$). These observations agree with the findings of Hargis et al. (1991), who supplemented hen diets with 3% menhaden oil. These authors found a significant increase in n3 and a decrease in n6, resulting in an n6:n3 ratio of 3:1. They also observed that MUFA content did decline, mainly oleic acid content, but in their study SFA content was not influenced by the diet.

The increase of n3FA in eggs accompanied by a noticeable decrease in n6 concentration when diets are supplemented with 2.5% SO occurs because Δ6–desaturase delta-6 is more active in the n3 pathway than in the n-6 pathway (Meluzzi et al., 2000; Castillo-Badillo et al., 2005). However, maintaining an appropriate n6:n3 ratio in the diet, and in the birds, is more important than the total concentrations of these fatty-acid types because the n6:n3 ratio determines membrane composition and, consequently, the types and quantities of eicosanoids that are produced. For example, high n6:n3 ratios are pro-inflammatory, and low ratios are anti-inflammatory (Klasing, 1998).

With regard to the benefits on n3FA when VE is add to diets supplemented with SO, we observed only a higher content of EPA and DPA in eggs when 100 mg/kg VE

Table 3. Fatty-acid compositions of diets (Percentage fatty acids methyl esters).

Fatty acid	T1	T2	T3	T4
Myristic (C14:0)	0.24	3.46	3.60	3.68
Palmitic (C16:0)	13.03	17.01	16.80	17.38
Palmitelaidic (C16:1)	0.08	0.10	0.09	0.08
Palmitoleic (C16:1)	0.44	4.11	4.12	4.13
Heptadecanoic (C17:0)	0.12	0.35	0.36	0.37
cis10-heptadecanoic (C17:1)	0.07	0.29	0.35	0.54
Stearic (C18:0)	3.86	3.27	3.09	3.09
Oleic (C18:1)	27.78	20.41	20.35	20.54
cis-vaccenic (C18:1)	0.72	2.23	2.22	2.21
Linoleic (LA) (C18.2)	43.71	20.36	21.89	21.92
Gama-linolenic (C18:3)	0.00	0.14	0.15	0.13
Alpha-linolenic (ALA) (C18:3 n3)	4.54	1.60	1.57	1.61
CLA c9, t11 and c11, t9 (C18:2)	0.00	1.33	1.36	1.35
CLA t10, c12 (C18:2)	0.00	0.09	0.12	0.10
Arachidic (C20:0)	0.26	0.22	0.22	0.23
Eicosenoic (C20:1)	0.28	1.32	1.15	1.44
cis-11,14-eicosadienoic (C20:2)	0.04	0.11	0.10	0.10
cis-11,14,17eicosatrienoic (C20:3)	0.04	0.06	0.04	0.00
Arachidonic (AA) (C20:4)	0.19	0.45	0.41	0.41
Eicosapentaenoic (EPA) (C20:5 n3)	0.15	8.05	8.07	7.93
Docosapentaenoic (C22:5 DPA n6)	0.00	0.16	0.16	0.15
Docosapentaenoic (C22:5 DPA n3)	0.00	1.08	1.07	1.07
Docosahexaenoic (DHA) (C22:6 n3)	0.07	6.11	6.05	5.91
Other fatty acids	4.46	7.69	6.66	5.71
Total	100.00	100.00	100.00	100.00

T1- basal diet (BD), T2- BD+2.5% SO, T3- BD+2.5% SO+100 mg/kg vitamin E, T4- BD+2.5% SO+200 mg/kg vitamin E.

was added to diets supplemented with SO (T3 vs. T2; P>0.05), but DHA no change (Table 4). These results agree with the findings of Meluzzi et al. (2000), who found no effect on the FA composition of eggs when adding 50 or 100 mg/kg VE to diets supplemented with 3% FO. Some authors attributed the lack of an effect to the fact that eggs are highly resistant to lipid oxidation because they are closed systems containing natural antioxidants, such as VE, ovotransferrin, phosvitin, and carotenoids (Pike and Peng, 1985; Guérin-Dubiard et al., 2007).

The addition of 200 mg/kg VE to diets supplemented with 2.5% SO cause to noticeably decrease of palmitic acid (C16:0), palmitoleic (C16:1), n6FA (C18:2 and C20:4) and n3FA (C18:3, C20:5, C22:5, C22:6) in eggs, compared with the treatment containing 100 mg/kg VE (P<0.05). These results agree with those of Meluzzi et al. (2000), who found that the n3 content of eggs decreased when 200 mg/kg VE was added to diets supplemented with 3% FO.

However, Qi and Sim (1998), found no effect on the FA content of eggs when high concentrations of VE (200, 400, and 800 mg/kg) were added to diets supplemented with 15% linseed oil + 0.5% FO. Similarly, Cherian et al. (1996) have reported that diets containing 3.5% FO and 367 to 423 µg/g VE do not affect FA composition.

Some studies have shown that under certain situations, VE can act as a prooxidant rather than as an antioxidant. Chen et al. (1998) have proposed that VE acts as a prooxidant in yolks when its concentration reaches 120 ppm or more in the diet. Franchini et al. (2002) have indicated that high VE concentrations (100 to 200 mg/kg) in diets increase the content of malonaldehyde in eggs. A possible mechanism for this process is described subsequently.

The oxidative process consists of three stages: initiation, propagation, and finalization. During initiation, high concentrations of free radicals are generated, and the generation of peroxides remains low. The free radical is in the carbon atom close to the double bond, creating an alkene radical. This reaction is generated in molecules with numerous unsaturated carbons. Thus, susceptibility to oxidation declines from high to low for DHA, DPA, EPA, arachidonic (AA), ALA, LA, and oleic acids. Antioxidants such as tocopherols act during the initiation stage, slowing the generation of free radicals (Wong, 1989).

In the case of VE, its antioxidant effect begins when the

Table 4. Fatty-acid content in eggs from hens fed diets including fish oil and different levels of vitamin E.

Fatty acids (Percentage TFA)	T1	T2	T3	T4
Myristic (C14:0)	0.34 ± 0.02 b	0.48 ± 0.02 a	0.48 ± 0.01 a	0.47 ±
Palmitic (C16:0)	24.62 ± 0.20 b	26.90 ± 0.24 a	25.78 ± 0.14 b	25.22 ±
Palmitoleic (C16:1)	2.33 ± 0.05 b	3.61 ± 0.11 a	3.24 ± 0.03 b	2.85 ±
Palmitelaidic (C16:1)	0.54 ± 0.01 a	0.54 ± 0.01 a	0.56 ± 0.05 a	0.56 ±
Stearic (C18:0)	8.57 ± 0.09 a	8.45 ± 0.40 a	8.29 ± 0.04 a	8.45 ±
Oleic (C18:1 n9)	40.90 ± 0.41 a	37.34 ± 0.71 b	37.44 ± 0.42 b	37.84 ±
Cis-vaccenic (C18:1)	0.75 ± 0.12 b	1.73 ± 0.18 a	1.69 ± 0.17 a	1.62 ±
Linoleic (C18:2 n6)	15.95 ± 0.13 a	9.46 ± 0.11 b	8.95 ± 0.13 c	7.83 ±
Alpha-linolenic (C18:3 n3)	0.65 ± 0.01 a	0.37 ± 0.01 b	0.33 ± 0.01 c	0.28 ±
Arachidonic (C20:0 n6)	1.82 ± 0.07 a	0.65 ± 0.03 b	0.69 ± 0.05 b	0.56 ±
Eicosapentaenoic (C20:5 n3)	0.02 ± 0.00 b	0.41 ± 0.02 b	0.50 ± 0.02 a	0.23 ±
Docosapentaenoic (C22:5 n3)	0.11 ± 0.01 b	0.35 ± 0.01 b	0.43 ± 0.03 a	0.22 ±
Docosahexaenoic (C22:6 n3)	0.84 ± 0.09 c	3.12 ± 0.33 a	3.17 ± 0.16 a	2.47 ±
Saturated fatty acids	33.53 ± 0.31 b	35.44 ± 0.52 a	34.56 ± 0.19 b	34.11 ±
Monounsaturated fatty acids	44.52 ± 0.60 a	43.12 ± 1.01 b	42.92 ± 0.66 b	42.67 ±
Polyunsaturated fatty acids	19.40 ± 0.13 a	14.37 ± 0.30 b	14.06 ± 0.22 c	11.59 ±
Total n6	17.77 ± 0.10 a	10.11 ± 0.09 b	9.63 ± 0.15 c	8.39 ±
Total n3	1.62 ± 0.09 c	4.26 ± 0.35 a	4.43 ± 0.16 a	3.20 ±
n6:n3	10.98 ± 0.63 a	2.40 ± 0.24 bc	2.18 ± 0.09 c	2.63 ±

Different letters within rows indicated statistical differences (P<0.05). T1 = basal diet (BD), T2 = BD + 2.5% SO, T3= BD + 2.5 mg/kg vitamin E, T4= BD + 2.5% SO + 200 mg/kg vitamin E.

Table 5. Productive parameters for hens fed diets including sardine oil and different levels of vitamin E.

Parameter	Feed intake g/hen/day	Egg production (%)	Egg weight (g)	Egg mass (g)	Feed conversio
BD	114.95 ± 6.88	83.70 ± 8.03	71.95 ± 2.20	56.45 ± 6.33	2.07 ± 0.2
BD + 2.5% SO	117.90 ± 5.91	81.45 ± 8.36	70.45 ± 2.58	56.95 ± 6.10	2.10 ± 0.2
BD + 2.5% SO + 100 mg/kg VE	118.50 ± 4.26	80.95 ± 7.74	70.75 ± 2.38	57.65 ± 5.58	2.02 ± 0.2
BD + 2.5% SO + 200 mg/kg VE	115.70 ± 5.35	80.45 ± 6.64	71.80 ± 2.48	57.20 ± 4.97	2.01 ± 0.1

a,b In each column, different letter indicate statistical difference (P<0.05).

hydrogen of the aromatic ring of VE is released, generating two free radicals, hydrogen, and a α-tocopherol radical. The tocopherol radical can react with one of the peroxyl radicals that come from the fatty acid and can form radical products derived from quinone, α-

tocopherolquinone, and quinone epoxide, which do not have the do not have the antioxidant activity of VE. Therefore, the activities of other antioxidants, such as vitamin C, are important to restore or regenerate VE from the tocopherol radical so that it can recover its antioxidant function (Wong, 1989). Regeneration of the tocopherol radical occurs in the presence of any antioxidant that releases hydrogen radicals without turning into a reactive oxygen species in the process. For example, vitamin C is a powerful antioxidant that turns into dehydroascorbic acid when donating hydrogen (Li and Min, 2006). However, this possibility remains to be assessed in laying hens, which metabolize vitamin C but do not transfer it to their eggs (Pardue and Thaxton, 1986). Therefore, we should look for another antioxidant that, like ascorbic acid, would help VE to regenerate and recover its antioxidant activity to protect the n3FA present in the eggs. Further studies are needed to test this hypothesis.

Productive parameters

These results agree with those of Cornejo et al. (2008) and Cherian et al. (2006), who detected no changes in productive parameters when incorporating 6% FO or 0.25 and 0.5% FO, respectively. Likewise, Cachaldora et al. (2006) no found effect on egg weight when hen diets were supplemented with various types and levels of FO (1.5, 3, 4.5, and 6%).

Although egg weight was not affected (P>0.05), a numerical decrease was observed when SO and VE were added in laying hen diets. Whitehead (1995) reported that egg weight was affected by fat type and amount. High level of FO gave a decrease in egg weight with regard to the control group. That decrease caused by fish oil was caused by a decrease in yolk weight. These decreases by FO were mediated through a common mechanism involving oestradiol. Oestradiol regulate hepatic synthesis of triglyceride and very low density lipoprotein apoliprotein as well as albumen synthesis in the oviduct. Fatty acids with a chain length of 18 carbon atoms with moderate degree of unsaturation would appear to be most effective in enhancing oestrogen metabolism, but the presence of some other compound in the dietary fats also affect oestrogen metabolism cannot be omitted.

Thus, the oestrogen has a great influence on egg weight as dietary. Similarly, adding VE to diets supplemented with SO had no effect on production variables in our study, this is consistent with the observations of Meluzzi et al. (2000) who observed no effect on productive parameters in hens supplied with diets containing 3% FO and three levels of VE (50, 100, and 200 mg/kg).

The egg yolk color increased when diets were supplemented with SO. The SO employed in this study was a by-product of the fish industry. The increase of the yolk color could be due the pigments present in the SO.

Organoleptic test

The results suggest that SO could be used in the laying hen rations until 2.5%, because the panelists did not detect fish flavor in eggs.

Conclusion

Under the conditions of this study, (1) adding 2.5% SO to the diet of laying hens is an alternative method to enrich eggs with n3FA without affecting productive parameters; (2) adding large quantities (100 or 200 mg/kg) of VE to diets supplemented with SO did not protect the PUFAs.

REFERENCES

AOAC (2000). Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists. Washington D.C.

Baucells M, Crespo N, Barroeta A, López -Ferrer S, Grashorn M (2000). Incorporation of different polyunsaturated fatty acids into eggs. Poult Sci., 79: 51-59.

Cachaldora P, García-Rebollar P, Alvárez C, De Blas J, Méndez J (2006). Effect of type and level of fish oil supplementation on yolk fat composition and n-3 fatty acids retention efficiency in laying hens. Br. Poult. Sci., 47: 43-49.

Castillo-Badillo C, Vázquez-Valladolid J, González-Alcorta M, Morales-Barrera E, Castillo-Domínguez R, Carrillo-Domínguez S (2005). The tuna oil as w-3 fatty acids source for egg of laying hens. Grasas y Aceites. 56: 153-159.

Chen J, Latshaw I, Lee H, Min D (1998). Alpha-tocopherol content and oxidative stability of egg yolk as related to dietary alpha-tocopherol. J. Food Sci., 63: 919-922.

Cherian G, Wolfe F, Sim J (1996). Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poult. Sci., 75: 423-431.

Cornejo S, Hidalgo H, Araya J, Pokniak J (2008). Supplementation of commercial layer diets with different refined fish oils. Effects on layer performance and sensory egg quality. Arch. Med. Vet. 40: 45-50.

FAO (2003) Nutrition Country Profiles. "Organization of the United Nations Food and Agriculture ", Rome, Italy.

Franchini A, Sirri F, Tallarico N, Minelli G, Iaffaldano N, Meluzzi A (2002). Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamin E and C. Poult. Sci., 81: 1744-1750.

González-Esquerra R, Leeson S (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory evaluation of eggs. Poult. Sci. 79: 1597-1602. González-Esquerra R, Leeson S (2001). Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. Can. J. Anim. Sci., 81: 295-305.

Guérin-Dubiard C, Castellani O, Anton M (2007). Egg compounds with antioxidant and mineral binding properties. In: Huopalahti R, López-Fandiño R, Anton M, Schade R (eds.), Bioactive Egg Compound, Springer-Verlag, Berlin Heidelberg, pp. 223-228.

Hargis P, Van Elswyk M, Hargis B (1991). Dietary modification of yolk lipid with menhaden oil. Poult. Sci., 70: 874-883.

Honkatukia M, Reese K, Presinger R, Tuiskula-Haavisto M, Weigend S, Roito J, Maki-Tanila A, Vilkki J (2005). Fishy taint in chicken eggs is associated with a substitution within a conserved motif of the *FMO3* gene. Genomics 86: 225-232.

Huang Z, Leibovitz H, Lee C, Miller R (1990). Effect of dietary fish oil on w-3 fatty acid levels and general performance of commercial broilers fed practical levels of redfish meal. Poult. Sci., 68: 153-162.

Klasing K (1998). *Comparative Avian Nutrition*, CAB International, Cambridge, U.K.

Kris-Etherton M, Williams D, Harris S, Lawrence A (2002). Fish consumption, fish oil, omega 3 fatty acids, and cardiovascular disease. Circulation. 106: 2747-2757.

Leskanich O, Noble C (1997). Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. J. Food Sci., 54: 1457-1460.

LiJ, Min D (2006). Nutraceuticals aging and food oxidation. In: Akoh C (ed.), Handbook of Functional Lipids Boca Raton, USA: CRC Press, pp. 325-350.

Marshall C, Van Elswyk E (1994). Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil. J. Food Sci., 59: 261-263.

Meluzzi A, Sirri F, Manfreda G, Tallarico N, Franchini A (2000). Effects of Dietary vitamin E on the quality of table eggs enriched with n3 long-chain fatty acids. Poult. Sci., 79: 539-545.

National Research Council (1994). Nutrient Requirement of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

NMX-FF-079-SCFI-2004. Mexican standards for poultry products. Fresh egg hen. Specifications and Test Methods. p. 23.

NOM-159-SSA1-1996. Official Mexican Standard. Goods and Services. Egg and derivatives. And products Requirements sanitary specifications. Mexico.

Qi G, Sim J (1998). Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. J. Agric. Food Chem., 46: 1920-1926.

Pardue S, Thaxton P (1986). Ascorbic acid in poultry: A Review. World´s Poult. Sci. J., 42: 107-123.

Pike O, Peng I (1985). Stability of shell egg and liquid yolk to lipid oxidation. Poult. Sci., 64: 1470-1475.

SAS Institute (2008). SAS/STAT. User's Guide. SAS Institute Inc., Cary, NC.

Simopoulos P (2000). Human requirement for n-3 polyunsaturated fatty acids. Poult. Sci., 79: 961-970.

Surai P, Sparks N (2000). Tissue-Specific and alpha-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci., 79: 1132-1142.

Surai P (2003). Natural antioxidants in avian nutrition and reproduction. Nottingham University Press, England.

UNA (2010). Digest of Poultry Sector Economic Indicators "National Poultry Union, Mexico City. p. 105.

Van Elswyk M, Dawson P, Sams A (1995). Dietary menhaden oil influences sensory characteristics and headspace volatiles of shell eggs. J. Food Sci., 60: 85-89.

Van Elswyk M (1997). Comparision of n-3 fatty acids sources in laying hen rations for improvement of whole egg nutritional quality: a review. Br. J. Nutr., 78(Suppl 1): S61-S69.

Watts BG, Ylimaki L, Jeffery Y, Elías L (1992). Basic methods for the evaluation of foods. International Center for Development Research, Ottawa, Canada. p.171.

Whitehead C (1995) Plasma oestrogen and the regulation of egg weight in laying hens by dietary fats. Anim. Feed Sci. Technol., 53: 91-98.

Wong DW (1989). Food Chemisty Acribia, Zaragoza, Spain.