J. Agr. Sci. Tech. (2016) Vol. 18: 347-359

Omega-3 Enrichment of Quail Eggs: Age, Fish Oil, and Savory Essential Oil

M. Kamely[1], M. A. Karimi Torshizi[1]*, and H. Khosravinia[2]

ABSTRACT

The aim of this study was to develop a diet suitable for obtaining quail eggs enriched with omega-3 fatty acids with minimum disadvantages on egg quality. This 12 weeks study was performed to investigate the effects of Fish Oil (FO) and Savory (*Satureja khuzestanica*) Essential Oil (EO) supplementation in diets of laying quails at different ages, on their performance, egg quality, fatty acid composition, and oxidation of egg yolk. One hundred and ninety-two Japanese quails were allocated to 8 groups (24 birds in each) with four replicates (having 6 birds in each) in a factorial arrangement with 3 variables: Age (31 and 12 weeks), FO (0 and 15 g kg^{-1}), and EO (0 and 500 mg kg^{-1}). The results showed that FO supplementation partially improved feed conversion ratio, hen-day egg production, egg weight and egg mass. There were no significant differences in albumen and shell weight percentage, but yolk percentage was significantly lower in FO groups. Savory essential oil significantly decreased shell thickness ($P< 0.05$). Percentage of yolk eicosapentaenoic Acid (EPA) and docosahexaenoic Acid (DHA) increased but Arachidonic Acid (AA) percentage and the ratio of *n*-6/*n*-3 fatty acids decreased in the eggs of the birds fed on diets supplemented with FO. Also EO supplementation decreased omega-3 enriched egg yolk lipids oxidation during refrigeration and room temperature preservation. Thus, it is possible to produce quail's *n*-3 enriched eggs, which can improve public health and be used for marketing purposes without any loss in eggs quality through dietary administration of FO and EO.

Keywords: Dietary administration, Herbal antioxidant, Quail *n*-3 enriched eggs, Performance, Yolk oxidative stability.

INTRODUCTION

High intakes of long-chain *n*-3 polyunsaturated fatty acids are associated with a decreased risk of cardiovascular disease (Rymer *et al*., 2010). Long–chain polyunsaturated fatty acids, Docosahexaenoic Acid (22:6 n-3, DHA) is important for biological membranes, the retina, the cerebral cortex, nervous tissues, the testicles, blood platelets, and EPA for its effect on arteries (antithrombotic and anti-inflammatory) resulting from the metabolism of eicosanoids (biological molecules that act as signals and messengers) (Arantes da Silva *et al*., 2009; Shimizu *et al*., 2001).

The proportional concentration of these fatty acids in poultry egg is affected by many factors including age, genetics, ration, and season. Research revealed that the content of myristic, palmitic, palmitoleic, stearic, and linoleic acids of yolk decreased with increasing age of layer hen (Yilmaz-Dikmen and Sahan, 2009). Latour *et al*. (1998) demonstrated that palmitic and stearic acid contents were greater in the yolks of eggs from hens at 51 and 64 weeks than in eggs from hens at 36 week.

Consumer awareness of the health benefits of *n*-3 fatty acids is growing and is driving

[1] Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[2] Department of Animal Science, Lorestan University, Khoramabad, Islamic Republic of Iran.
* Corresponding author; e-mail: karimitm@modares.ac.ir

consumer demand for enriched food products (Zuidhof *et al*., 2009). All vegetable fat sources seem to be less effective than marine fats when meat is enriched with polyunsaturated Fatty Acids (PUFA, particularly with *n*-3 long-chain fatty acids (C≥ 20). This effect results from the content of *n*-3 fatty acids, because marine oils contain EPA and DHA, whereas vegetable oils contain greater levels of linolenic acids (LNA), whose conversion to longer-chain derivatives and deposition in peripheral tissues is not sufficient to give nutritionally valuable modified products (Lopez-Ferrer *et al*., 2001). However, Petrovic *et al.* (2012) reported the increase of C18:3 *n*-3 and C22:6 *n*-3 in eggs by supplementation of linseed oil (1-4%) to hens' feed.

In regular eggs, the *n*-6/*n*-3 ratio varies from 1:15 to 1:20, which is far from human nutritionist's recommendations of 4:1 to 6:1 (Kaźmierska *et al*., 2007). It is feasible to modify this ratio easily by feeding strategies such as providing hens with *n*-3 fatty acids enriched diets. Also, Kaźmierska *et al*. (2005) reported that layer quails were more efficient in production of *n*-3 enriched eggs than layer chicken.

Although fish is considered to be the primary source of *n*-3 PUFA, it may not serve as a primary source for people (Lewis *et al*., 2000b). It has been proved that *n*-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery diseases, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer (Lewis *et al*., 2000a; Simopoulos, 1991; Simopoulos *et al*., 1991). The *n*-6 and *n*-3 fatty acids are the parent fatty acids for the production of eicosanoids, e.g., prostaglandins, thromboxanes, and leukotrienes. Also, eicosanoids derived from *n*-6 fatty acids have opposite metabolic properties to those derived from *n*-3 fatty acids (Simopoulos, 2000).

Lipid oxidation during food processing and storage is of major importance. Oxidation of polyunsaturated lipids results in hydroperoxides which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones, and other oxygenated compounds that may adversely affect flavor, taste, nutritional value, and overall quality of foods (Vercellotti *et al*., 1992). Since most egg lipids are located in the yolk, they are susceptible to oxidation and thus require quality control (Aghdam Shahryar *et al*., 2010). Also, oxidation is influenced by dietary factors such as fat composition and storage times (Rahimi *et al*., 2011).

Therefore, adding antioxidants into poultry diets seems to be an efficient means to improve oxidative stability of eggs as reflected by decreased egg yolk malondialdehyde (MDA) levels. There is an inverse relationship between malondialdehyde concentration and dietary antioxidant level in poultry products (Akdemir *et al*., 2009). The most commonly used synthetic antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, have been restricted recently, mainly because of their potential carcinogenicity causing liver swelling and changing liver enzyme activities (Rajani *et al*., 2011).

Also, Orhan and Eren (2011) reported that addition of herbal mixtures to FO layer diets instead of synthetic antioxidants can be a natural method to prevent egg yolk from oxidation.

This experiment was carried out to study the relationship between age, Fish Oil (FO), and Essential Oil (EO) on production parameters, egg quality, yolk fatty acid profiles, and oxidative stability of egg yolk in Japanese quail eggs.

MATERIALS AND METHODS

One hundred and ninety-two Japanese quail *(Coturnix coturnix japonica*) were randomly divided into 8 groups (24 birds in each) with four replicates (having 6 birds in each) in a factorial arrangement, with 3 variables: age (31 and 12 weeks), FO (0 and

15 g per kg diet) and EO (0 and 500 mg per kg diet). Experimental diets were fed *ad libitum* for 12 weeks. Productive traits consisting of feed intake, egg weight, hen-day egg production, egg mass and feed conversion ratio were recorded weekly. The live weights of birds were recorded at the beginning and the end of study. Egg production was recorded daily and feed consumption and egg weight were recorded weekly. Feed conversion ratio was calculated by dividing the feed intake by egg mass production. The diets were formulated according to the recommendations of the National Research Council (NRC, 1994).

Egg Quality Parameters

At the end of each period (6^{th} and 12^{th} weeks), five eggs from each replicate were collected to determine interior and exterior egg quality. Egg shell thickness was determined by the mean of three measurements taken from three different sides of the shell by ultrasonic thickness gauge Karl deutsch D-56 (Wuppertal Echometer 1061). Shell strength was determined by eggshell breaking strength force gauge (eggshell force gauge, model-II, Robotmachine Co., LTD, Tokyo Japan). Yolk, albumen and shell weight were assessed manually using digital scale at 0.1 g precision.

Determination of Egg Yolk Lipid Oxidation

The extent of lipid peroxides in egg yolk samples was assessed by measuring Thiobarbituric Acid Reactive Substances (TBARS) as secondary oxidation product, according to the method described by Botsoglou *et al*. (1994). Tetraethoxy propane (1, 1, 3, 3-Tetraethoxy propane, T9889, 97%, Sigma, USA.) was used as a MDA precursor in the standard curve. To assess the effect of treatments on the oxidative stability of egg yolk lipids, 5 yolks from each replicate were pooled and divided into two sample sets. The first set of samples were assayed immediately for lipid oxidation while the second set of samples were assayed after 7 days refrigerated preservation at 4°C. The effect of 3 weeks storage under room temperature condition (25±2°C and 65±5% relative humidity) on shell eggs was also assayed.

Fatty Acid Analysis

Nine eggs from each pen were collected to determine Fatty Acid (FA) profiles. The FA composition of feed and eggs was determined after extraction by Folch method (Folch *et al*., 1957) and gas chromatography (Metcalfe *et al*., 1966) using a gas chromatograph (Unicam 4600, UK) equipped with a BPX70 fused silica capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness; SGE, USA) and a flame ionization detector. The operating conditions of the gas chromatograph were as follows: the initial temperature was 140°C, increasing by the rate of 20°C min^{-1} to 180°C; after 9 minutes, the temperature was increased at the rate of 20°C min^{-1} to 200°C. The temperature of the injector was 250°C and the detector (FID) remained stable at 300°C. The column head pressure of the carrier gas (Helium) was 20 psi and sample volume was 0.2 μL. Tricosanoic acid (Sigma, St. Louis, USA) was used as internal standard. Run time was 40 minutes for each sample. Fatty acids were identified by matching their retention times with those of their respective standards.

The composition and calculated nutrients contents of the experimental diets are presented in Table 1. Fatty acid profile of FO is shown in Table 2. Savory essential oil components are shown in Table 3.

Statistical Analysis

Data were subjected to analysis of variance using the GLM procedure of SAS software (SAS, 2004) for a factorial

Table 1. Ingredients and chemical composition of the diet.

Ingredients	Dietary levels of FO (g kg^{-1})	
	0	15
Yellow corn	588	589
Soybean meal (44% CP)	329	328
Soy oil	15	0
Fish oil	0	15
Limestone	53	53
Dicalcium phosphate[a]	10.6	10.6
Sodium chloride	3	3
DL-Methionine	zzzz1.4	1.4
Vitamin premix[b]	0.25	0.25
Mineral premix[b]	0.25	0.25
Calculated analysis		
ME (MJ kg^{-1})	11.89	11.87
Crude protein (g kg^{-1})	195.5	195.2
Calcium (g kg^{-1})	24.7	24.7
Available phosphorus (g kg^{-1})	3.5	3.5
Methionine (g kg^{-1})	4.5	4.5
Methionine+Cystine (g kg^{-1})	7.7	7.7
Lysine (g kg^{-1})	10.4	10.4

[a] Contained 230 g kg^{-1} Ca and 200 g kg^{-1} P. [b] Supplied the following per kilogram of diet: Retinyl acetate, 9,000 IU; Cholecalciferol, 2,000 IU; dl-α-Tocopheryl acetate, 12.5 IU; Menadione sodium bisulfite, 1.76 mg; Biotin, 0.12 mg; Thiamine, 1.2 mg; Riboflavin, 3.2 mg; Calcium d-pantothenate, 6.4 mg; Pyridoxine, 1.97 mg; Nicotinic acid, 28 mg; Cyanocobalamine, 0.01 mg; Choline chloride, 320 mg; Folic acid, 0.38 mg; $MnSO_4 \cdot H_2O$, 60 mg; $FeSO_4 \cdot 7H_2O$, 80 mg; ZnO, 51.74 mg; $CuSO_4 \cdot 5H_2O$, 8 mg; Iodized NaCl, 0.8 mg; Na_2SeO_3, 0.2 mg.

Table 2. Fatty acids profile of FO and soy oil.

	Fatty acids (g kg^{-1})										
	C16:0	C16:1	C18:0	C18:1	C18:2	C20:4	EPA	DHA	*n*-3	*n*-6	*n*-6/*n*-3
FO	291	84	47	348	16	01	59	150	210	17	0.08
Soy oil	157	ND	4	238	563	ND	ND	ND	ND	563	-

Table. 3. Savory essential oil components. [a]

Components	g kg^{-1}	Components	g kg^{-1}
α-Thujene	2.4 ± 1.4	*p*-Cymene	12.6 ± 8.6
α-Pinene	1.5 ± 0.5	Limonene	1.3 ± 0.4
α-Terpinene	2.4 ± 1.2	(Z)-*β*-Oeimene	5.4 ± 0.8
Carvacrol	921.6 ± 4.6	Trans-sabinene hydrate	1.7 ± 0.23
Myreene	2.6 ± 1.9	γ-Terpenene	7.4 ± 0.2
Thymol	Very low	*β*-Caryophyllence	1.6 ± 0.1

[a] Khosravinia *et al*. (2013).

experiment (2×2×2). Test of significance for the difference between means of different levels within each classification was done by Duncan's multiple range tests (Kaps and Lamberson, 2004). Statements of statistical significance were based on $P< 0.05$.

RESULTS

Productive Performance

Effects of treatments on daily feed intake, Feed Conversion Ratio (FCR), hen-day egg production, egg weight, egg mass, and birds body at the first 6 weeks of the trial and the second 6 wks of trial are shown in Tables 4 and 5, respectively.

Daily feed intake, egg weight, and body weight of the hens were not affected by diets during 12 weeks of the trial, while FCR, hen-day egg production, and egg mass were significantly improved in young birds (P< 0.05). Supplementation of FO numerically improved FCR, hen-day egg production, egg weight and egg mass in whole period of trial. Also, feed consumption did not change significantly (P> 0.05) in due to FO supplementation.

Egg Quality Parameters

These results showed that supplementation of FO decreased yolk percentage and shell strength (P< 0.05). However, albumen percentage was significantly more in FO fed birds, but there were no significant differences on shell percentage and shell thickness due to FO supplementation and ages (Table 6). The greater shell thickness was observed in birds that had received diets supplemented with EO (P< 0.05).

Yolk Lipid Oxidation

The results of oxidation, measured as the concentration of MDA in egg yolks in fresh eggs, after 7 days remaining in refrigerator at 4°C (as separated yolk) and after 3 weeks of storage at room temperature (as shell egg), are presented in Table 7. Results

Table 4. Effects of treatments on daily feed intake, Feed Conversion Ratio (FCR), hen-day egg production, egg weight, egg mass and hens body weight on the experimental birds at the first 6 weeks of trial. [a]

Treatment	Daily feed intake (g hen^{-1} day^{-1})	Feed conversion ratio (g g^{-1})	Hen-day egg production (%)	Egg weight (g)	Egg mass (g day^{-1})	Hens body weight (g)
Age						
Young	32.5	3.03 [b]	86 [a]	12.47	10.86 [a]	287.1
Old	33.5	3.36 [a]	82 [b]	12.33	10.12 [b]	290.3
EO (mg kg^{-1})						
0	32.68	3.16	84	12.30	10.45	289.1
500	33.35	3.22	84	12.50	10.52	288.3
FO (g kg^{-1})						
15	33.07	3.13	85	12.42	10.62	282.3
0	32.96	3.25	83	12.38	10.35	295.1
SEM	0.28	0.06	1	0.08	0.18	3.8
P-value						
Age	0.05	0.0006	0.04	0.32	0.04	0.75
EO	0.20	0.42	0.72	0.20	0.83	0.92
FO	0.84	0.17	0.39	0.80	0.44	0.21
Age×EO	0.03	0.06	0.73	0.51	0.62	0.036
Age×FO	0.86	0.37	0.18	0.39	0.41	0.91
EO×FO	0.14	0.26	0.53	0.01	0.13	0.85
Age×EO×FO	0.23	0.19	0.47	0.01	0.13	0.84

[a] Means with different letters into the column are statistically different (P< 0.05). EO= Savory Essential Oil, FO= Fish Oil.

Table 5. Effects of treatments on daily feed intake, Feed Conversion Ratio (FCR), hen-day egg production, egg weight, egg mass and hen body weight on the experimental birds at the second 6 weeks of trial. [a]

Treatment	Daily feed intake (g hen^{-1} day^{-1})	Feed conversion ratio (g g-^{1})	Hen-day egg production (%)	Egg weight (g)	Egg mass (g day^{-1})	Hens body weight (g)
Age						
Young	36.4 [b]	3.43 [b]	84 [a]	12.51	10.56 [a]	298.4
Old	39.6 [a]	4.49 [a]	74 [b]	12.35	9.29 [b]	290.7
EO (mg kg^{-1})						
0	37.98	3.97	79	12.43	9.95	293.3
500	38.15	3.95	79	12.42	9.90	295.8
FO (g kg^{-1})						
15	37.86	3.86	80	12.33	9.96	296.5
0	38.27	4.06	78	12.53	9.88	292.6
SEM	0.47	0.13	1	0.12	0.23	3.6
P-value						
Age	0.0005	<0.0001	0.001	0.50	0.005	0.56
EO	0.83	0.94	0.97	0.96	0.91	0.85
FO	0.60	0.33	0.40	0.41	0.85	0.77
Age×EO	0.38	0.48	0.40	0.03	0.09	0.11
Age×FO	0.64	0.89	0.82	0.08	0.25	0.27
EO×FO	0.69	0.30	0.11	0.43	0.41	0.29
Age×EO×FO	0.10	0.42	0.24	0.78	0.50	0.57

[a] Means with different letters into the column are statistically different (P< 0.05). EO= Savory Essential Oil, FO= Fish Oil.

Table 6. Effects of treatments on percent of shell, albumen, yolk, shell thickness and shell strength of laying quail. [a]

Treatment	Albumen (%)	Yolk (%)	Shell (%)	Shell thickness (mm)	Shell strength (N)
Age					
Young	60	29	9	0.229	1.33
Old	59	30	10	0.225	1.08
EO (mg kg^{-1})					
0	60	29 [b]	10 [a]	0.223 [b]	1.26
500	59	30 [a]	9 [b]	0.231 [a]	1.15
FO (g kg^{-1})					
15	60 [a]	29 [b]	10	0.229	1.05 [b]
0	59 [b]	30 [a]	9	0.225	1.36 [a]
SEM	0.3	0.3	0.1	0.137	0.07
P-value					
Age	0.07	0.18	0.28	0.15	0.05
EO	0.79	0.03	0.008	0.004	0.39
FO	0.02	0.002	0.28	0.17	0.01
Age×EO	0.003	0.023	0.68	0.25	0.23
Age×FO	0.01	0.17	0.05	0.41	0.06
EO×FO	0.05	0.38	0.05	0.03	0.18
Age×EO×FO	0.45	0.11	0.26	0.84	0.37

[a] Means with different letters into the column are statistically different (P< 0.05). EO= Savory Essential Oil, FO= Fish Oil.

Table 7. Influence of Japanese quail's age and diet on yolk MDA concentration (Optical density). [a]

Treatments	MDA of fresh egg yolk	MDA of yolk after 7 days at 4°C	MDA of yolk after 3 weeks in room temperature
Age			
Young	0.059	0.265	1.41
Old	0.045	0.259	1.38
EO (mg kg^{-1})			
0	0.046	0.275 [a]	1.47 [a]
500	0.058	0.249 [b]	1.32 [b]
FO (g kg^{-1})			
15	0.062 [a]	0.267 [a]	1.42 [a]
0	0.046 [b]	0.257 [b]	1.37 [b]
SEM	0.004	0.003	0.018
P-value			
Age	0.05	0.05	0.05
EO	0.10	< 0.0001	< 0.0001
FO	0.009	0.008	0.008
Age×EO	0.008	0.01	0.01
Age×FO	0.96	0.88	0.9881
EO×FO	0.45	0.0001	0.0001
Age×EO×FO	0.01	0.05	0.05

[a] Means with different letters into the column are statistically different (P< 0.05). EO= Savory Essential Oil, FO= Fish Oil.

showed that, after 21 days of storage, the MDA development was significantly reduced by dietary supplementation of EO (P< 0.01). Age of birds had no significant effects on egg yolk lipids oxidation (P> 0.05). Fish oils containing groups had higher egg yolk lipids oxidation rate in fresh eggs and eggs stored at room temperature and at 4°C (P< 0.05).

Fatty Acid Content

The FA composition of the experimental diets and egg yolk are presented in Table 8. Supplementation of FO increased percentage of EPA and DHA and decreased AA percentage in egg yolk (P< 0.05). Supplementation of FO to quails diet increased *n*-3 fatty acids and decreased the *n*-6 percentage in yolk (P< 0.01) and decreased the *n*-6/*n*-3 ratios from 30.63 to 5.25. Oleic acid and DHA percentage increased (P< 0.05) and palmitic acid, AA, and EPA decreased in the birds receiving EO. Nevertheless, there were some interactions among EO, age, and FO supplementation in FA profiles (Table 8).

DISCUSSION

Inclusion of FO in the diet significantly improved *n*-6/*n*-3 fatty acids ratio with no adverse effects on productive performance indicators. Irrespective of the birds age, FO diets supplementation with EO improved stability of yolk lipids during refrigeration or at room temperature preservation. Al-Daraji *et al*. (2010) reported that dietary FO at the inclusion level of 30 g kg^{-1} enhanced egg weight, hen-day egg production, egg mass, cumulative egg production, and feed conversion ratio in quail hens. Also, FO and flax oil at levels of 30 g kg^{-1} in Japanese quail's diet during the laying period showed higher economic efficiency with no adverse effects on productive and reproductive performance.

Table 8. Fatty acid composition [a] (%) of the egg yolk of quails in different diets. [b]

Treatments	C16:0	C16:1	C18:0	C18:1	C18:2	C20:4	EPA	DHA	*n*-3	*n*-6	*n*-6/*n*-3
Age											
Young	29.2 [b]	8.6	7.9	41 [a]	10.6	0.9 [b]	0.02	1.1 [a]	1.1	11	18.6 [a]
Old	30.0 [a]	8.8	7.9	40 [b]	10.5	1.2 [a]	0.11	1.0 [b]	1.2	11	17.1 [b]
EO (mg kg^{-1})											
0	29.9 [a]	8.7	7.9	40 [b]	10.6	1.1 [a]	0.12 [a]	1.0 [b]	1.2	11	17.77
500	29.4 [b]	8.7	7.9	41 [a]	10.5	1.0 [b]	0.01 [b]	1.1 [a]	1.1	11	18.11
FO (g kg^{-1})											
15	30.4 [a]	8.7	7.8 [b]	41	9.4 [b]	0.5 [b]	0.14 [a]	1.7 [a]	1.9 [a]	9.9 [b]	5.25 [b]
0	28.9 [b]	8.7	8.0 [a]	40	11.8 [a]	1.6 [a]	0.00 [b]	0.4 [b]	0.4 [b]	13.4 [a]	30.63 [a]
SEM	0.22	0.20	0.04	0.35	0.25	0.10	0.03	0.12	0.1	0.3	2.30
P-value											
Age	0.002	0.56	0.91	0.003	0.07	< .0001	0.06	< 0.0001	0.54	0.79	0.0007
EO	0.045	0.98	0.81	0.03	0.15	0.04	0.02	< 0.0001	0.64	0.06	0.51
FO	< 0.0001	0.94	0.04	0.06	< 0.0001	<0.0001	0.007	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Age×EO	0.22	0.72	0.12	0.02	0.03	0.45	0.02	< 0.0001	< 0.0001	0.03	0.08
Age×FO	0.004	0.31	0.07	0.03	0.02	0.0002	0.06	< 0.0001	0.50	0.001	0.01
EO×FO	0.007	0.009	0.002	< 0.0001	< 0.0001	0.009	0.02	< 0.0001	0.54	< 0.0001	0.04
Age×EO×FO	0.15	0.29	0.63	0.009	0.24	0.003	0.02	< 0.0001	0.0003	0.84	0.78

[a] C16:0= Palmitic; C16:1= Palmitoleic; C18:0= Stearic; C18:1= Oleic; C18:2= Linoleic; C20:4= Arachidonic; C20:5= Eicosapentaenoic, C22:6= Docosahexaenoic acid. *N*-3 PUFA was calculated as 20:5 *n*-3+22:6 *n*-3. *N*-6 PUFA was calculated as 18:2 *n*-6 + 20:4 *n*-6. [b] Means with different letters into the column are statistically different (P< 0.05). EO= Savory Essential Oil, FO= Fish Oil.

Bozkurt *et al*. (2008) demonstrated that FO supplementation to broiler breeder diet significantly decreased settable egg weight, possibly due to increased dietary *n*-3 fatty acid content, which in turn might cause a reduction in circulating triglycerides of the birds and limited availability of lipids for yolk formation. Gonzalez-Esquerra and Leeson (2000) reported that *n*-3 fatty acids could affect circulating estradiol. This hypothesis could partially explain the slight lower egg weight found in birds fed FO in this study in the second period of the experiment.

Body weight gain of 12-week birds was not affected by the dietary treatments (P> 0.05), which was in accordance with the results of Gonzalez-Esquerra and Leeson (2000) study.

Radwan *et al*. (2008) investigated that addition of 1 g kg^{-1} (thyme, rosemary or oregano) or 5 g kg^{-1} *Curcuma longa* as natural antioxidants to laying hens diets increased egg mass, egg production, and improved feed conversion, but, in the present study, similar to Yesilbag *et al*. (2013) and Botsoglou *et al*. (2005), EO exhibited no significant effects on the same parameters (P> 0.05). Also, Malekizadeh *et al*. (2012) indicated that the inclusion of Ginger rhizome powder into the diets increased egg production percent, egg mass, and feed intake.

Dalton (2000) reported that feeding 50 g kg^{-1} menhaden dietary FO to Japanese quail decreased egg production, egg quality, and specific gravity of eggs. These results are in harmony with the finding of the present study. Similar to Güçlü *et al*. (2008) findings, there were no significant differences in shell quality between groups that were fed FO. Demanding further research, the effect of herbal essential oils on egg shell attributes of various birds has not been fully investigated yet. Essential oils are rich sources of several compounds with well known antioxidant activity. These bioactive molecules could be transferred to poultry products (egg and meat). In general, in agreement with Radwan *et al*. (2008), supplementation of 0.5 g kg^{-1} EO in the quail feed decreased MDA formation in egg yolk and had positive effect on oxidative stability of shell eggs storage.

Botsoglou *et al*. (1997) showed that thyme reduced oxidation of lipids in liquid yolk but the extent of lipid oxidation, as measured by MDA formation did not change with storage time. They suggested that the MDA found in the yolk of fresh eggs might be due to either the consumption or subsequent deposition of MDA that was already present in the diets or the *in vivo* production of MDA by the hens fed the diets. The former possibility appears unlikely because in that case the levels of MDA should have been equal among the treatments; however, our results in this study did not prove that hypothesis. Also, Kassis *et al.* (2012) reported that high level of *n*-3 PUFAs as EPA and DHA in eggs make them more prone to oxidation than others.

Yilmaz-Dikmen and Sahan (2009) reported that the content of myristic, palmitic, palmitoleic, stearic, and linoleic acids of yolk decreased with increasing the age of layer hen. In contrast, Latour *et al*. (1998) showed that the palmitic and stearic acid contents were higher in the egg yolks from aged hens compared to young hens. Similarly, in this study, palmitic acid was significantly higher in old birds (P< 0.05). Similar to the results of Shimizu *et al*. (2001), Alvarez *et al*. (2004), Cachaldora *et al*. (2005), and Cachaldora *et al*. (2006) studies, feeding FO increased percentage of EPA and DHA and decreased AA percentage in egg yolk (P< 0.05). Furthermore, Huang *et al*. (1990) showed that *n*-3 polyunsaturated fatty acids (EPA and DHA) in the egg yolk can be increased without causing a fishy flavor by feeding up to 30 g kg^{-1} FO, stabilized with 1 g kg^{-1} ethoxyquin to prevent rancidity. In addition, Lawlor *et al*. (2010) showed that the total content of the *n*-3 fatty acids increased from 141 to 299 mg egg^{-1} as the FO increased from 0 to 60 g kg^{-1} diet. In contrast with the current study, Dalton (2000) reported that feeding menhaden FO increased yolk *n*-3 fatty acid concentrations and feeding FO and

soybean oil increased the total monounsaturated fatty acids and polyunsaturated fatty acids in yolk. In agreement to the current study, Güçlü *et al*. (2008) reported that *n*-3 percentage and *n*-3/*n*-6 ratio increased and *n*-6 percentage decreased significantly in yolks of birds fed on diets supplemented with FO ($P< 0.001$). Moreover, Laca *et al*. (2009) showed that diet containing 15 g kg^{-1} cantabrian blue FO and 50 g kg^{-1} linseed as sources of *n*-3 fatty acids allowed obtaining commercially acceptable levels of EPA and DHA in the egg.

CONCLUSIONS

Inclusion of FO in the diet had no adverse effects on productive performance indicators. Savory essential oils supplementation (500 mg kg^{-1}) in quails' diets decreased omega-3 enriched egg yolk lipids oxidation during refrigeration and room temperature preservation. The *n*-6/*n*-3 ratio decreased from 30.63 to 5.25 by 15 g kg^{-1} FO supplementation to the quails' diet, which is close to the value recommended for humans. It is possible to produce quail egg enriched with omega-3 fatty acids, which can improve public health and be used for marketing purposes without any losses in eggs quality with FO addition along with EO supplementation.

REFERENCES

1. Aghdam Shahryar, H., Salamatdoust, R., Chekani-Azar, S., Ahadi, F. and Vahdatpoor, T. 2010. Lipid Oxidation in Fresh and Stored Eggs Enriched with Dietary ω3 and ω6 Polyunsaturated Fatty Acids and Vitamin E and A Dosages. *Afr. J. Biotechnol*., **9:** 1827-1832.
2. Akdemir, F. and Sahin, K. 2009. Genistein Supplementation to the Quail: Effects on Egg Production and Egg Yolk Genistein, Daidzein, and Lipid Peroxidation Levels. *Poult. Sci*., **88:** 2125-2131.
3. Al-Daraji, H. J., Al-Mashadani, H. A., Al-Hayani, W. K., Mirza, H. A. and Al-Hassani, A. S. 2010. Effect of Dietary Supplementation with Different Oils on Productive and Reproductive Performance of Quail. *Int. J. Poult. Sci*., **9:** 429-435.
4. Alvarez, C., Cachaldora, P., Méndez, J., GarcÍa-Rebollar, P. and De Blas, J. C. 2004. Effects of Dietary Conjugated Linoleic Acid and Fish Oil Supplementation on Performance and Egg Quality in Laying Hens. *Bri. Poult. Sci.,* **45:** 524-529.
5. Arantes da Silva, W., Naiverti Elias, A. H., Aparecida Aricetti, J., Izumi Sakamoto, M., Eiko Murakami, A., Marques Gomes, S. T., Vergı´lio Visentainer, J., Evela´zio de Souza, N. and Matsushita, M. 2009. Quail Egg Yolk (*Coturnix coturnix* Japonica) Enriched with Omega-3 Fatty Acids. *LWT-Food Sci. Tech.,* **42:** 660-663.
6. Artemis, P. and Simopoulos, M. D. 2008. The Omega-6/Omega-3 Fatty Acid Ratio, Genetic Variation, and Cardiovascular Disease. *Asia Pac. J. Clin. Nutr.,* **17:** 131-134.
7. Botsoglou, N. A., Yannakopoulos, A. L. and Fletouris, D. J. 1997. Effect of Dietary Thyme on the Oxidative Stability of Egg Yolk. *J. Agri. Food Chem*., **45:** 3711-3716.
8. Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J. and Trackatellis, A. G. 1994. Rapid, Sensitive, and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animal Tissue, Food and Feedstuff Sample. *J. Agri. Food Chem*., **42:** 1931-1937.
9. Botsoglou, N., Florou-Paneri, P., Botsoglou, E., Dotas, V., Giannenas, I., Koidis, A. and Mitrakos, P. 2005. The Effect of Feeding Rosemary, Oregano, Saffron and α-Tocopheryl Acetate on Hen Performance and Oxidative Stability of Eggs. *South African J. Anim. Sci*., **35(3):** 143-151.
10. Bozkurt, M., Cabuk, M. and Alcicek, A. 2008. Effect of Dietary Fat Type on Broiler Breeder Performance and Hatching Egg Characteristics. *J. Appl. Poult. Res*., **17:** 47-53.
11. Cachaldora, P., García-Rebollar, P., Álvarez, C., Méndez, J. and de Blas, J. C. 2005. Effect of Conjugated Linoleic Acid, High-Oleic Sunflower Oil and FO Dietary Supplementation on Laying Hen Egg Quality. *Span. J. Agric. Res.* **3**: 74-82.

12. Cachaldora, P., García-Rebollar, P., Alvarez, C., De Blas, J. C. and 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. *British Poult. Sci*., **47**: 43-49.
13. Dalton, M. N. 2000. Effects of Dietary Fats on Reproductive Performance, Egg Quality, Fatty Acid Composition of Tissue and Yolk and Prostaglandin Levels of Embryonic Tissues in Japanese Quail (*Coturnix coturnix* Japonica). Master's Thesis, Virginia Polytechnic Institute and State University, USA.
14. Folch, J., Lees, M. and Sloane-Stanley, G. H. 1957. A Simple Method for Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem*., **226**: 497–509.
15. Gonzalez-Esquerral, R. and Leeson, S. 2000. Effect of Feeding Hens Regular or Deodorized Menhaden Oil on Production Parameters, Yolk Fatty Acid Profile, and Sensory Quality of Eggs. *Poult. Sci*., **79**: 1597-1602.
16. Güçlü, B. K., Uyanık, F. and İşcan, K. M. 2008. Effects of Dietary Oil Sources on Egg Quality, Fatty Acid Composition of Eggs and Blood Lipids in Laying Quail. *South African J. Anim. Sci*., **38**: 91-100.
17. Huang, Z., Leibovitz, H., Lee, C. M. and Millar, R. 1990. Effect of Dietary Fish Oil on *n*-3 Fatty Acid Levels in Chicken Eggs and Thigh Flesh. *J. Agri. Food Chem*., **38**: 743-747.
18. Kaps, M. and Lamberson, W. R. 2004. *Biostatistics for Animal Science*. CABI Publishing, London.
19. Kassis, N. M., Gigliotti, J. C., Beamer, S. K., Tou, J. C. and Jaczynski, J. 2012. Characterization of Lipids and Antioxidant Capacity of Novel Nutraceutical Egg Products Developed with Omega-3-Rich Oils. *J. Sci. Food Agric.* **92**:66-73.
20. Kaźmierska, M., Jarosz, B., Korzeniowska, M., Trziszka, T. and Dobrzański, Z. 2005. Comparative Analysis of Fatty Acid Profile and Cholesterol Content of Egg Yolks of Different Bird Species. *Polish J. Food Nutr. Sci*., **14/55**: 69-73.
21. Kaźmierska, M., Korzeniowska, M., Trziszka, T. and Jarosz, B. 2007. Effect of Fodder Enrichment with PUFAS on Quail Eggs. *Polish J. Food Nutr. Sci*., **57**: 281-284.
22. Khosravinia, H., Karimi Torshizi, M. A., Alireza, M., Shahsevari, R. and Ghasemi, S. 2013. Effect oF *Satureja khuzistanica* Essential Oils in The Drinking Water On *ω*-6 and ω-3 Fatty Acids Ratio, Cholesterol Content and Lipid Stability of Breast Muscle in Broiler Chicken. *Iran. J. Anim. Sci.,* **44**: 71-81.
23. Laca, A., Paredes, B. and Díaz, M. 2009. Quality Characteristics of *n*-3 Polyunsaturated Fatty Acid-enriched Eggs. *J. Anim. Feed Sci*., **18**: 101-112.
24. Latour, M. A., Peebles, E. D., Doyle, S. M., Pansky, T., Smith, T. W. and Boyle, C. R. 1998. Broiler Breeder Age and Dietary Fat Influence The Yolk Fatty Acid Profiles of Fresh Eggs and Newly Hatched Chicks. *Poult. Sci*., **77**: 47-53.
25. Lawlor, J. B., Gaudette, N., Dickson, T. and House, J. D. 2010. Fatty Acid Profile and Sensory Characteristics of Table Eggs from Laying Hens Fed Diets Containing Microencapsulated Fish Oil. *Anim. Feed Sci. Tech*., **156**: 97-103.
26. Lewis, N. M., Schalch, K. and Scheideler, S. E. 2000a. Serum Lipid Response to *n*-3 Fatty Acid Enriched Eggs in Persons with Hypercholesterolemia. *J. Am. Diet Assoc.,* **100**: 365-367.
27. Lewis, N. M., Seburg, S. and Flanagan, N. L. 2000b. Enriched Eggs as a Source of *n*-3 Polyunsaturated Fatty Acids for Humans. *Poult. Sci*., **79**: 971-974.
28. Lopez-Ferrer, S., Baucells, M. D., Barroeta, A. C. and Grashorn, M. A. 2001. N-3 Enrichment of Chicken Meat. 1. Use of Very Long-chain Fatty Acids in Chicken Diets and Their Influence on Meat Quality: Fish Oil. *Poult. Sci*., **80**: 741-752.
29. Malekizadeh, M., Moeini, M. M. and Ghazi, Sh. 2012. The Effects of Different Levels of Ginger (*Zingiber officinale* Rosc) and Turmeric (*Curcuma longa* Linn) Rhizomes Powder on Some Blood Metabolites and Production Performance Characteristics of Laying Hens*. J. Agr. Sci. Tech.,* **14**: 127-134.
30. Metcalfe, L. D., Schmitz, A. A. and Pelka, J. R. 1966. Rapid Preparation of Fatty Acid Esters from Lipid for Gas Chromatographic Analysis. *Analytical Chem*., **38**: 514-515.
31. NRC (National Research Council). 1994. *Nutrient Requirements of Poultry*. 9th Revised Edition, National Academy Press, Washington, DC.
32. Orhan, F. and Eren, M. 2011. Effect of Herbal Mixture Supplementation to Fish

Oiled Layer Diets on Lipid Oxidation of Egg Yolk, Hen Performance and Egg Quality. *Ankara Üniv Vet Fak Derg*., **58**: 33-39.
33. Petrovic, M., Gacic, M., Karacic, V., Gottsteinc, Z., Mazijac, H. and Medic, H. 2012. Enrichment of Eggs in *n*-3 Polyunsaturated Fatty Acids by Feeding Hens with Different amount of Linseed Oil in Diet. *Food Chem.*, **135**: 1563-1568
34. Radwan, N. L., Hassan, R. A., Qota, E. M. and Fayek, H. M. 2008. Effect of Natural Antioxidant on Oxidative Stability of Eggs and Productive and Reproductive Performance OF Laying Hens. *Int. J. Poult. Sci*., **7**: 134-150.
35. Rajani, J., Karimi Torshizi, M. A. and Rahimi S. 2011. Control of Ascites Mortality and Improved Performance and Meat Shelf-life in Broilers Using Feed Adjuncts with Presumed Antioxidant Activity. *Anim. Feed Sci. Tech*., **170**: 239-245.
36. Rahimi, S., Kamran Azad, S. and Karimi Torshizi, M. A. 2011. Omega-3 Enrichment of Broiler Meat by Using Two Oil Seeds. *J. Agr. Sci. Tech*., **13**: 353-365
37. Rymer, C., Gibbs, R. A. and Givens, D. I. 2010. Comparison of Algal and Fish Sources on the Oxidative Stability of Poultry Meat and Its Enrichment with Omega-3 Polyunsaturated Fatty Acids. *Poult. Sci.,* **89**: 150-159.
38. SAS Institute. 2004. *SAS System for Windows*, *Version 9.1.2.* SAS Institute Inc., Cary, NC.
39. Shimizu, Y., Arai, K., Ise, S. and Shimasaki, H. 2001. Dietary Fish Oil for Hens Affects the Fatty Acid Composition of Egg Yolk Phospholipids and Gives a Valuable Food with an Ideal Balance of *n*-6 and *n*-3 Essential Fatty Acids for Human Nutrition. *J. Oleo Sci*., **50:** 797-803.
40. Simopoulos, A. P. 2000. Requirement for *n*-3 Polyunsaturated Fatty Acids. *Poult. Sci*., **79**: 961-970.
41. Simopoulos, A. P. 1991. Omega-3 Fatty Acids in Health and Disease and in Growth and Development. *Am. J. Clin. Nutr.,* **54**: 438-463.
42. Simopoulos, A. P., Kifer, R. R., Martin, R. E. and Barlow, S. M. 1991. Health Effects of ω3 Polyunsaturated Fatty Acids in Seafoods. *World Rev. Nutr. Diet.,* **66**: 1-592.
43. Vercellotti, J. R., Angelo, A. J. St. and Spanier, A. M. 1992. *Lipid Oxidation in Foods*. American Chemical Society, Washington, DC.
44. Yesilbag, D., Gezen, S. S., Biricik, H. and Meral, Y. 2013. Effects of Dietary Rosemary and Oregano Volatile Oil Mixture on Quail Performance, Egg Traits and Egg Oxidative Stability. *British Poult. Sci*., **54**: 231-237.
45. Yilmaz-Dikmen, B. and Sahan, U. 2009. The Relationship among Age, Yolk Fatty Acids Content, and Incubation Results of Broiler Breeders. *Poult. Sci.,* **88**: 185-190.
46. Zuidhof, M. J., Betti, M., Korver, D. R., Hernandez, F. I. L., Schneider, B. L., Carney, V. L. and Renema, R. A. 2009. Omega-3-Enriched Broiler Meat. 1. Optimization of a Production System. *Poult. Sci.*, **88**: 1108-1120.

غنی‌سازی تخم بلدرچین از نظر اسیدهای چرب امگا-۳: سن، روغن ماهی، اسانس مرزه

م. کاملی، م. ا. کریمی ترشیزی و ح. خسروی نیا

چکیده

هدف از انجام این آزمایش تولید تخم بلدرچین غنی شده با اسیدهای چرب امگا – ۳ با حداقل تاثیرات منفی بر خصوصیات کیفی بود. این آزمایش به مدت ۱۲ هفته جهت بررسی اثر روغن ماهی و

اسانس مرزه (*Satureja khuzestanica*) در جیره بلدرچین نخمگذار ژاپنی با دو سن متفاوت، بر عملکرد، کیفیت تخم، ترکیب اسیدهای چرب و اکسیداسیون زرده انجام شد. ۱۹۲ بلدرچین ژاپنی به ۸ گروه (۲۴ پرنده در هر گروه) با ۴ تکرار در قالب طرح فاکتوریل با ۳ عامل: سن پرنده (در دو سن ۳۱ و ۱۲ هفتگی)، روغن ماهی (صفر و ۱۵ میلی‌گرم در کیلوگرم) و اسانس گیاه مرزه (۰ و ۵۰۰ میلی‌گرم در کیلوگرم) در جیره اختصاص یافتند. نتایج نشان داد که افزودن روغن ماهی سبب بهبود جزئی ضریب تبدیل غذایی، تولید تخم، وزن تخم و توده وزنی تخم تولیدی شد. تفاوت معنی‌داری در مقدار آلبومن و درصد پوسته تخم مشاهده نشد ($P>0/05$) درحالی که درصد زرده تخم در گروه تغذیه شده با روغن ماهی کاهش یافت ($P<0/05$). اسانس مرزه سبب کاهش معنی‌دار پوسته تخم بلدرچین شد ($P<0/05$). افزودن روغن ماهی سبب افزایش درصد اسیدهای چرب ایکوزاپنتونوئیک اسید و دیکوزاهگزانوئیک اسید و کاهش آراشیدونیک اسید و بهبود نسبت اسیدهای چرب امگا -۳ و امگا – ۶ شد. افزودن اسانس مرزه سبب کاهش فساد اکسیداتیو زرده تخم بلدرچین نگهداری شده در دمای اتاق و یخچال شد($P<0/05$). با افزودن ۱۵ میلی‌گرم در کیلوگرم روغن ماهی و مکمل سازی با اسانس مرزه می‌توان تخم بلدرچین غنی شده با اسیدهای چرب امگا – ۳ بدون اثرات نامطلوب تولید نمود که می‌تواند برای سلامت انسان مفید باشد.