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Effect of herbal mixture supplementation to fish oiled layer diets on lipid oxidation of egg yolk, hen performance and egg quality*

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Summary: In this study, the effects of a specific herbal mixture as a feed additive in layer diets which are enriched with omega-3 fatty acids by fish oil on egg yolk lipid oxidation, hen performance and egg quality were investigated. A total of 140 Lohmann white 34 week old hens were divided into four groups and the study was carried out 16 weeks. Negative control diet was not included supplemental oil, herbal mixture and synthetic antioxidant (NC); fish oiled control diet (FOC) was involved fish oil (1.5 %) and soy oil (1.5 %) but herbal mixture and synthetic antioxidants free; herbal mixture diet was formulated as of FOC diet + herbal mixture [Origanum Vulgare (dried leaf), Thymus Vulgaris (dried leaf), Thyme Oil, Origanum Oil, Garlic Oil, Anise Oil and Fennel Oil] (FOHM) and synthetic antioxidant diet (FOSA) was prepared with FOC diet + synthetic antioxidants (ethoxyquine, butylhydroxitoluene, butylhydroxianisole and citric acid). Egg production, feed conversion ratio, feed consumption egg weight, eggshell breaking strength and haugh unit were not affected by the dietary treatments. There were no significant differences between egg yolk MDA values of NC and FOC until 42th storage day (+4^{o}C) but MDA values in egg yolk of FOC was higher($p<0.05$) than NC's after 42th storage day. Addition of herbal mixture and synthetic antioxidant separately to FOC' diet reduced egg yolk MDA values during 56 days storage period even if it is in the first day ($p<0.05$). According to these data, addition of herbal mixtures to fish oiled layer diets instead of synthetic antioxidants can be a natural method to prevent egg yolk from oxidation.

Key words: Antioxidant, egg yolk, fish oil, herbal product, oxidation.

Balık yağı içeren yumurta tavuğu rasyonlarına bitkisel karışım katkısının yumurta sarısı oksidasyonu ve yumurta verimi üzerine etkileri

Özet: Bu çalışmada, balık yağı ile omega-3 yağ asitlerince zenginleştirilmiş yumurta tavuğu yemlerinde yem katkı maddesi olarak kullanılan bir bitkisel karışımın yumurta sarısı lipit oksidasyonu, yumurta verimi ve yumurta kalitesi üzerine etkileri incelendi. Deneme süresi 16 hafta olan çalışmada, 34 haftalık yaşta 140 adet beyaz Lohmann yumurtacı tavuk 4 gruba ayrıldı. Negatif kontrol (NC) rasyonuna ilave yağ, bitkisel karışım ve sentetik antioksidan katılmadı. Kontrol rasyonuna (FOC) ise balık yağı (%1.5) ve soya yağı (%1.5) katkısı yapıldı. Deneme rasyonlarından biri (FOHM) balık yağlı kontrol grubunun (FOC) rasyonuna %0.5 bitkisel karışım (kurutulmuş Origanum vulgare ve Thymus vulgaris, kekik yağı, oregano yağı, sarımsak yağı, anason yağı ve rezene yağı), diğeri (FOSA) ise FOC rasyonuna %0.5 sentetik antioksidan karışımı (ethoxyquine, butylhydroxitoluene, butylhydroxianisole ve sitrik asit) ilave edilerek hazırlandı. Yumurta verimi, yumurta ağırlığı, yemden yararlanma, yem tüketimi, kabuk kırılma direnci ve haugh birimi yeme katılan bitkisel karışım ile sentetik antioksidandan etkilenmedi ($p>0.05$). Balık yağlı (FOC) ve yağsız (NC) kontrol gruplarının yumurta sarısı MDA değerleri arasında depolamanın (+4^{o}C) 42. gününe kadar önemli farklılık bulunmadı, ancak depolamanın 56. gününde FOC' un yumurta sarısı MDA değeri NC' un değerinden yüksekti ($p<0.05$). Balık yağlı kontrol grubu yemine sentetik antioksidan ve bitkisel karışımın ayrı ayrı katılması depolamanın 1. gününden 56. gününe kadar yumurta sarısı MDA değerlerini düşürdü ($p<0.05$). Bu verilere göre, balık yağlı yumurta tavuğu rasyonlarına sentetik antioksidan yerine bitkisel karışım katkısı, yumurta sarısını depolamada oluşacak oksidasyondan korumak için daha doğal bir yöntem olabilir.

Anahtar sözcükler: Antioksidan, balık yağı, bitkisel ürün, oksidasyon, yumurta sarısı.

Introduction

The egg is the most complete food in nutritional point of view. Consumers refrain from egg consumption because of the relatively high cholesterol content in eggs, which is supposed as a reason of the heart diseases and atheriosclerosis (26). The growing role of diet in prevention from heart diseases caused both consumer and governmental attention on the composition and the quality of foods (12). Soy oil commonly used for providing energy and optimum egg weight in layer diets. But soy oil has high level of omega-6 fatty acids (6). Whereas fish oil include omega-3 (n-3) polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic (EPA) and docosahexanoic (DHA) acids, which have positive

* This paper is summarized from Fatih ORHAN' s PhD thesis

effects for preventing from coronary heart diseases, hypertension, inflammation and autoimmune disorders (30). Feeding strategies are being incorporated to increase the n-3 fatty acid (FA) composition of chicken eggs (11). The increasing level of fish oil in the layer diets leads to an increase in omega-3 fatty acids of egg yolk (10). Nevertheless, PUFA are sensitive to oxidation (4). For this reason, the use of antioxidants take an important place in PUFA rich poultry diets. Some synthetic antioxidants (BHA; BHT and ethoxyquine) have been commonly used in foods and feeds. However, the detrimental effects of butylhydroxianisole (BHA), butylhydroxitoluene (BHT) and ethoxyquine have been reported as a reason of cancer (22). The effect of dietary tocopherol acetate supplementation to improve lipid stability in egg yolk has been repeatedly reported (14, 16). Recently, antioxidative ingredients (flavanoids, cineol, etc.) of aromatic herbal products (leaf, plant extract, essential oil, etc.) have been documented though their antimicrobial properties are well known for a long time (24). But the antioxidative effects of aromatic herbal products (leaf, plant extract, essential oil, etc.) on egg yolk have not been examined enough yet.

This study was conducted to determine efficiency of a specific herbal mixture on the lipid oxidation of egg yolk, hen performance and egg quality when it is supplemented to feeds which are enriched with n-3 fatty acids by using fish oil.

Materials and Methods

Hens Management and Production Parameters : A total of 140 Lohmann white laying hens, 34 week old, were used in the experiment. They were randomly distributed into 28 cages(50x50x50) at four dietary treatments replicated seven times with five hens per cage under conventional conditions with access to feed and water adlibitum and photoperiod of 16 hours was maintained. Experimental study was carried for 16 weeks. Feed consumption was recorded in each two weeks period and feed conversion ratio was calculated biweekly as feed consumption (kg) / egg production (kg) of each replication. Hen - day egg production was recorded daily but mean of data were statically analyzed weekly.

Experimental Diets and Treatments **:** Experimental diets (Table 1) were prepared weekly, to avoid oxidation, in powder form by grinding and mixing by a 500 kg capacity mixer. Diets were formulated by using Moonstar® computer program. In the experiment, both synthetic antioxidants and herbal mixture were supplemented to the diets as follows:

Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: Control diet + herbal mixture (0,5 g/kg), Group FOSA: Control diet + synthetic antioxidant preparation (0,5 g/kg)

Fish oil was supplemented to diet at value of 1.5% for preventing fish taste in egg yolk (17, 21) Unrefined fish oil (antioxidant free) used in experiment was provided by a anchovy processing fish factory in Black Sea Region. Unrefined soy oil (antioxidant free) was provided by a factory in Marmara Region. Fatty acid composition of fish and soybean oil are presented in Table 2. The soy oil and fish oil were stored at +4º during the experiment. The experimental diets were formulated according to N.R.C (27). The diets were chemically analyzed according to the A.O.A.C. (2) methods and metabolizable energy was calculated by the equation of Hartel (19) as follows:

ME (kcal/kg) = 239 x ((Ether extract, % x 0.3431) + (Crude Protein, % x 0.1551) + (Saccharose, % x 0.1301) + (Starch, % x 0.1669)) (Table 1).

Table 1. Experimental diets
Tablo 1. Deneme Rasyonları

Experimental Diets Feed Ingredient (%)	NC[4]	FOC[4]	FOHM[4]	FOSA[4]
Corn	63.28	58.16	58.11	58.11
Soybean Meal	25.57	24.86	24.86	24.86
Sun Flower Meal	0.12	3.00	3.00	3.00
Fish Oil	-	1.50	1.50	1.50
Soy Oil	-	1.50	1.50	1.50
Limestone	8.12	8.12	8.12	8.12
Dicalcium Phosphate	2.09	2.06	2.06	2.06
DL-Methiyonin	0.17	0.15	0.15	0.15
Salt	0.40	0.40	0.40	0.40
Vit-Min Premix[1]	0.25	0.25	0.25	0.25
Synthetic Antioxidant[2]	-	-	-	0.05
Herbal Mixture[3]	-	-	0.05	-
Total	100	100	100	100
Analyzed Chemical Composition				
Dry Matter(%)	90.09	89.23	89.23	89.23
Crude Protein(%)	16.54	16.65	16.65	16.65
Ether Extract(%)	2.67	5.68	5.68	5.68
Ash(%)	12.67	12.89	12.89	12.89
ME(kcal/kg)(calculated)	2702	2745	2745	2745
Ca(%)	3.54	3.54	3.54	3.54
P(%)	0.68	0.68	0.68	0.68

[1] Premix: 10.000.000 IU Vit. A, 2.500.000 IU Vit. D_3, 20.000 mg Vit. E, 2.500 mg Vit.K_3, 2.000 mg Vit.B_1, 5.000 mg Vit.B_2, 3.500 mg. Vit.B_6,15 mg.Vit.B_{12}, 30.000 mg Niacin, 8.000 mg. Cal-D-Pan, 1000 mg Folic acid, 25 mg D-Biotin, 160.000 mg Colin Clorid, 50.000 mg Vit. C, 1.000 mg Carofil red, 80.000 mg Mn, 40.000 mg Fe, 60.000 mg Zn, 5.000 mg Cu, 2.000 mg I, 500 mg Co, 150 mg Se.

[2] (0.95 % ethoxyquine, 4.74 %, butylhydroxitoluene, 0.95 % butylhydroxianisole and 0.48 % citric acid)

[3] [Origanum Vulgare (dried leaf), Thymus Vulgaris (dried leaf), Thyme Oil, Origanum Oil, Garlic Oil, Anise Oil and Fennel Oil]

[4] Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: FOC diet + herbal mixture (0.5 g/kg), Group FOSA: FOC diet + synthetic antioxidant preparation (0.5 g/kg)

Table 2. Fatty acid composition of oils used in diets
Tablo 2. Yemlerde kullanılan yağların yağ asidi bileşenleri

Fatty Acid (%)	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	22:0	20:5	22:6	20:4
Fish Oil	6.89	0.12	18.69	7.08	0.68	3.55	14.92	1.96	1.42	0.6	0.29	14.33	13.39	8.59
Soy Oil	0.07	-	10.63	0.09	-	4.83	23.18	52.1	7.71	0.39	0.4	0.21	-	-

Fatty Acid (%)	Total SFA	Total MUFA	Total PUFA	Total n-3	Total n-6	n-6/ n-3
Fish Oil	30.02	22	39.69	29.14	10.55	0.36
Soy Oil	16.32	23.27	60.02	7.92	52.1	6.58

Experimental Additives:

Herbal mixture (powder form): Origanum Vulgare (dried leaf), Thymus Vulgaris (dried leaf), Thyme Oil, Origanum Oil, Garlic Oil, Anise Oil and Fennel Oil. Active compounds of herbal mixture are shown in Table 3.

Synthetic Antioxidant Preparation (powder form): 0.95 % ethoxyquine, 4.74 %, butylhydroxitoluene, 0.95 % butylhydroxianisole and 0.48 % citric acid (Table 3).

Table 3. Active compounds of herbal mixture
Tablo 3. Bitkisel karışımın aktif bileşenleri

Active compounds[1]	Ppm
1,8-CINEOLE	2498
ALLYL DERIVATES	3520
ALPHA-PINENE	1150
ALPHA-TERPINEOL	7010
BORNEOL	1784
CAFFEIC-ACID	22796
CAMPHENE	754
CARVACROL	44828
EUGENOL	1236
GERANIOL	10340
LIMONENE	5540
LINALOOL	9576
MYRCENE	1836
P-CYMENE	23800
PHENOL	8520
POLYPHENOL	59960
TANNIS	129000
ROSMARINIC-ACID	76000
TERPINEN-4-OL	692
URSOLIC AC	19200
THYMOL	32616

[1] Active ingredients were notified by producer.

Egg Sampling Protocol and Egg Quality Parameters **:** In the experiment, all eggs were visually checked for cracks and breakage under artificial lighting and cracked egg ratio was calculated weekly. Egg quality characteristics (egg weight, eggshell strength, cracked egg, and haugh units) were measured weekly using mean of 14 eggs from each dietary treatment (2 eggs from each replication). At 105th day of trial, six eggs were collected from control (fish oiled – Group FOC) and negative control (oil free-Group NC) groups for fatty acid composition of egg yolks. At the end of the trial 30 shell eggs from each treatment were randomly collected for thiobarbituric acid (TBA) analysis. Egg samples were stored at 4 C° for 56 days. Egg samples were stored for 24 hours at room temperature and weighted. Eggshell breaking strength was measured as Newton (N) by using a cantilever system applying increased pressure by force gauge (Imada®) to the broad pole of the shell (7). Egg albumen height was measured by a tripod micrometer and haugh unit was calculated by equation as follows:

Haugh unit = $100 \times \text{Log}(h + 7.51 - 1.7G^{0,37})$

[h = albumen height (mm) , G = Egg weight (g)]

Fatty Acids of Experimental Oils and Egg Yolk **:** Eggs were boiled and egg yolks separated. Egg yolks were treated with ethyl ether (Merck) by Soxhaleth Fat Extractor. Both egg yolk extracts and experimental oils were esterified. For esterification 0.2 g sample was taken from oils then 4cc %2 Metanolic NaOH (Merck) added and boiled until saponification. After saponification 5cc %14 BF3-Methanol (Merck) was added in fat extract balloon and boiled for 5 minutes. 2cc n-heptane (Merck) was added and boiled for 1 minute after that 4cc NaCl (Merck) was added and mixed well. Solution was waited for 5-10 minutes for separation of phases. Colored supernatant was taken away to storage bottle. After esterification, samples were injected into Supercovacs - 10 silica capillary column of Gas Choromatograph (Hewlett Packard Agilent Tech. 6890N Network GS) and fatty acids profiles were determined by using A.O.A.C. (3) protocols.

Thiobarbituric Acid (TBA) Analyses **:** Thiobarbituric acid (TBA) analyses were used as described by Salih *et al.* (28) with some modifications for determination malondialdehyde (MDA) level of egg yolks. Fresh collected six shell eggs from each treatment for each group were placed in refrigerator at 4 C° to be analyzed for TBA levels at 1, 14, 28, 42 and 56 days of storage. Egg yolk samples (2g) were weighted into 50 ml test tubes and 18 ml of 3,86 % perchloric acid (Merck) added. The samples were homogenized and 0,1 ml butylhydroxytoluene (Merck) was added during homogenization to control lipid oxidation. The homogenated sample was filtered through Whatman 1 Filter paper. Filtrate (2ml) was mixed with 2 ml of 20

mM TBA (Merck) in distilled water, and incubated in a boiling water bath for 30 minutes. Absorbance was determined at 531 nm. The TBA levels were expressed as milligrams of malondialdehyde (MDA) per kilogram of yolk.

Statistical Analyses: The data from the entire experimental period were pooled and analyzed by analysis of variance. Differences between means were tested according to Tukey's test and t test (31,33). All analyses were performed using SPSS® 12.00 (SPSS Inc., Chicago, USA, 1999) computer software. Differences were considered significant when P values were less than 0.05.

Results

The values of egg production, feed consumption, feed conversion ratio (FCR), egg weight, eggshell breaking strength and cracked egg ratio were not significantly different between treatments (Table 4-5). Although haugh units were not significantly different when compared between experimental groups but haugh units were increased significantly (Table 7) in all groups by storage time (1^{st}, 14^{th}, 28^{th}, 42^{nd}, 56^{th} day). Mean value of omega-3 FA in egg yolk (Table 6) at FOC group was higher than NC group ($p<0.05$). Whereas, there was no significantly difference in total n-6 FA value between FOC group and oil NC group (Table 6). Herewith, n6/n3 FA ratio of FOC group was higher ($p<0.05$) than NC group (Table 6). Egg yolk MDA values of Group NC, FOC, FOHM and FOSA were increased significantly at 42^{nd}, 28^{th}, 28^{th} and 14^{th} storage days respectively. Malondialdehyde values of herbal mixture and synthetic antioxidant free groups (Group NC and FOC) were higher ($p<0.05$) than both herbal mixture and synthetic antioxidants supplemented groups (Group FOHM and FOSA) in the first and 14^{th} days of storage (Table 8). Egg yolk MDA values of Group FOHM and FOSA were lower ($p<0.05$) than Group FOC but the values were not significantly different with Group NC at the storage days 28^{th} and 42^{nd} . However, at the 56^{th} storage day egg yolk MDA values of fish oiled-Group FOC was higher than the other groups ($p<0.05$).

Discussion and Conclusion

We are reporting that neither herbal mixture nor synthetic antioxidant feeding could influence ($p>0.05$) egg production, feed consumption, feed conversion ratio (FCR), egg weight, eggshell breaking strength and cracked egg ratio (Table 4-5). As far as the effect of dietary supplementation with herbal products or synthetic antioxidants on layers performance is concerned, there were no clear evidence in pertinent studies (6, 8,14,15). Haugh units was not effected significantly by antioxidant type and oil addition (Table 7). However, haugh unit was started to decrease at first storage period and continue decreasing significantly for each storage period in all groups ($p<0.05$). It is known that, increasing storage time leads to decrease in haugh unit (29, 32).

Table 4. Production parameters
Tablo 4. Performans parametreleri

Groups	Egg Production Hen-day (%) n=16		Feed Intake (g/day) n=8		Feed Conversion Ratio, n=8	
	Mean	SEM	Mean	SEM	Mean	SEM
NC	88.98	0.772	108.66	0.557	2.07	0.023
FOC	89.43	0.533	108.50	0.846	2.04	0.027
FOHM	89.79	0.513	107.83	1.013	2.09	0.018
FOSA	90.57	0.394	107.16	0.307	2.05	0.016

Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: Control diet + herbal mixture (0.5 g/kg), Group FOSA: Control diet + synthetic antioxidant preparation (0.5 g/kg)

Table 5. Egg quality parameters
Tablo 5. Yumurta kalite parametreleri

Groups	Cracked Egg (%) n=16		Eggshell Strength, (N) n=16		Egg Weight (g) n=16	
	Mean	SEM	Mean	SEM	Mean	SEM
NC	1.42	0.127	35.08	1.724	61.51	1.133
FOC	1.36	0.084	36.32	1.257	61.11	1.122
FOHM	1.56	0.134	35.84	1.233	60.37	1.122
FOSA	1.30	0.081	37.33	1.288	62.51	1.122

Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: Control diet + herbal mixture (0.5 g/kg), Group FOSA: Control diet + synthetic antioxidant preparation (0.5 g/kg)
(N): Newton

Table 6. Fatty acid composition of egg yolk, (% of total fatty acids)
Tablo 6. Yumurta sarısı yağ asidi bileşimi, (toplam yağ asitlerine oranı,%)

Fatty Acid	NC		FOC		Fatty Acid	NC		FOC	
	mean	SEM	mean	SEM		mean	SEM	mean	SEM
14:0	0.44	0.01	0.41	0.04	20:5 n-3	0.27 ^{b}	0.02	0.65 ^{a}	0.03
16:0	26.57^{a}	0.89	24.26 ^{b}	0.30	22:6 n-3	-		3.64	0.01
16:1n-7	3.42	0.30	2.96	0.23	20:4n-6	-		0.11	0.02
18:0	10.42^{a}	0.09	7.62 ^{b}	0.48	Total SFA	37.51^{a}	0.92	32.32^{b}	0.54
18:1n-9	41.90	0.73	40.93	0.73	Total MUFA	45.33	0.82	43.89	0.87
18:2n-6	13.41	0.42	13.32	0.21	Total PUFA	13.97 ^{b}	0.43	18.64^{a}	0.25
18:3 n-3	0.29^{b}	0.01	1.42^{a}	0.08	Total n-3	0.55 ^{b}	0.02	5.71^{a}	0.06
20:0	0.033	0.01	-		Total n-6	13.41	0.42	13.32	0.21
22:0	0.043^{a}	0.01	0.016^{b}	0.02	n-6 : n-3	24.02 ^{a}	0.49	2.51^{b}	0.12

$^{a-c}$ Within a row values with no common superscripts indicate significantly different (P<0,05)
Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil)

Table 7. Haugh unit
Tablo 7. Haugh unit

Groups		1stday	14th day	28th day	42nd day	56th day	P^{1}
		n=6	n=6	n=6	n=6	n=6	
NC	Mean	94.02 ^{A}	86.14 ^{B}	73.29 ^{C}	61.24 ^{D}	51.32 ^{E}	*
	SEM	1.606	4.013	4.002	3.837	4.267	
FOC	Mean	94.35 ^{A}	84.54 ^{B}	73.46 ^{C}	64.24 ^{D}	49.11 ^{E}	*
	SEM	1.824	3.688	4.288	3.387	4.122	
FOHM	Mean	94.87 ^{A}	85.84 ^{B}	74.77 ^{C}	63.87 ^{D}	50.82 ^{E}	*
	SEM	1.824	3.688	4.288	3.387	4.122	
FOSA	Mean	95.24 ^{A}	85.25 ^{B}	72.56 ^{C}	62.84 ^{D}	49.71 ^{E}	*
	SEM	1.824	3.688	4.288	3.387	4.122	
P^{2}		N.S	N.S	N.S	N.S	N.S	

P^{1} : $^{A-E}$ Mean values within a row with different superscripts are significantly different (P<0,05)
P^{2} : N.S: Non-significant within a column. Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: Control diet + herbal mixture (0.5 g/kg), Group FOSA: Control diet + synthetic antioxidant preparation (0.5 g/kg)

Table 8. MDA values of Egg Yolks in storage periods (mg/kg)
Tablo 8. Depolama dönemlerine göre yumurta sarısı MDA değerleri (mg/kg)

Groups		1stday	14th day	28th day	42nd day	56th day
		n=6	n=6	n=6	n=6	n=6
NC	Mean	0.31$^{b\,A}$	0.34$^{b\,A}$	0.34^{abA}	0.38^{abAB}	0.39$^{a\,B}$
	SEM	0.020	0.031	0.026	0.033	0.019
FOC	Mean	0.31 ^{bA}	0.35 ^{bAB}	0.38 ^{bBC}	0.41 ^{bBC}	0.44 ^{bC}
	SEM	0.016	0.008	0.007	0.011	0.016
FOHM	Mean	0.24 ^{aA}	0.29 ^{aAB}	0.32 ^{aBC}	0.36 ^{aBC}	0.37 ^{aC}
	SEM	0.011	0.013	0.008	0.007	0.009
FOSA	Mean	0.23 ^{aA}	0.30 ^{aB}	0.32 ^{aB}	0.35 ^{aB}	0.36 ^{aB}
	SEM	0.009	0.010	0.008	0.014	0.007

$^{A-C}$ Mean values within a row with different superscripts are significantly different (P<0,05)
$^{a-c}$ Mean values within a column with different superscripts are significantly different (P<0,05) Group NC: Negative control (supplemental oil, antioxidant and herbal mixture free), Group FOC: Control (1.5 % Fish oil + 1.5 % Soy oil), Group FOHM: Control diet + herbal mixture (0.5 g/kg), Group FOSA: Control diet + synthetic antioxidant preparation (0.5 g/kg)

Addition of fish oil to experimental diets enriched n-3 FA in egg yolk but there were no change in n-6 FA (Table 6). Herewith, n6/n3 FA ratio decreased by addition of fish oil to the diets (Table 6). It is known that the type of dietary supplemented oil effects fatty acid composition of egg yolk (1, 11, 13,18). Fish oil in layer diets increases n-3 FA in egg yolk because of its high n-3 FA content (6, 9, 13, 20). High n-6 content of corn (23, 34) which used in all experimental diets may cause similar n-6 values in egg yolks of groups.

Malondialdehyde values of egg yolks were started to increase in different storage period (in Group NC, FOC, FOHM and FOSA at 42nd, 28th, 28th and 14th storage days respectively) during 56 days of storage (Table 8). Different data were documented about MDA values of egg yolk during storage time (14, 15). It is reported that this discrepancy is most likely to reflect a difference in the methods applied to detect lipid oxidation (14).

Addition of both synthetic antioxidant and herbal mixture to fish oiled diet caused a decrease in egg yolk MDA values until the end of the storage time (Table 8). This decreased value was even lower than the value of fish oil free diet at 14th storage day (Group NC). This decrease may be explained with possible transfer of the antioxidant constituents of herbal mixture (1,8- Cineole, Limonene, Eugenol, Carvacrol, Rosmarinic acid, etc.) or synthetic antioxidant (ethoxyquine, butylhydroxitoluene, butylhydroxianisole and citric acid) into hen organism through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreased oxidation products transferred into the yolk (5, 14-16, 25). From 28th to 42nd day of storage, egg yolk MDA values of fish oil and antioxidant free Group NC was similar with MDA values of other three groups. It is interesting that the MDA values of fish oil free Group NC and fish oiled antioxidant free Group FOC were similar until 42nd storage day. However, at the 56th storage day egg yolk MDA values of fish oiled-Group FOC was higher than the other groups ($P<0.05$). Oxidation of n-3 FA and n-6 FA from fish oil, corn oil and soy oil passed to egg yolk is concerned, they were similarly affected by refrigerated storage until 42nd day but oxidation of n-3 FA was higher than n-6 FA's at 56th day of storage. Also, the present study gives evidence that not only synthetic antioxidants but antioxidant constituents of herbal products could pass into egg yolk and efficiently prevent lipid oxidation.

Consequently, these data indicate that supplementation of synthetic antioxidant and herbal mixture to fish oiled layer diets retard oxidation in egg yolks until 56th day of storage. Also, according to these data using herbal mixtures, which involves antioxidant active ingredients, as a feed antioxidant instead of synthetic antioxidants in fish oiled layer diets can be a natural method to prevent egg yolk from oxidation during storage.

References

1. **Aida H, Hamamdzic M, Gagic A, Mihaljevic M, Krinic J, Vegara M, Baltic M, Trajkovic S, Kadric M, Pasic JE** (2005): *Egg yolk lipid modifications by fat supplemented diets of laying hens*. Acta Vet, **55**, 41-51.
2. **Association of Offical Analytical Chemists** (1984): *Official Methods of Analysis of the Association of Official Analytical Chemist*. 14th ed, Arlington, Virginia.
3. **Association of Offical Analytical Chemists** (2003): *Official Methods of Analysis of the Association of Official Analytical Chemist.* 17th ed., Gaithersburg, Maryland.
4. **Astawan M, Wahyuni M, Tadokoro T, Maekawa A** (1995): *Defatting and desalting treatment of indonesian dried-salted fish: dietary effects on alpha-tocopherol and peroxide levels in the serum and liver of rats.* Biosci-Biotechnol-Biochem, **59**, 1450-4.
5. **Bailey CA, Srınıvasan LJ, Mc Geachın RB** (1996): *The effect of ethoxyquin on tissue peroxidation and ımmune status of single comb white leghorn cockerels.* Poult Sci **75**, 1109–1112.
6. **Balevi T, Coşkun B** (2000): *Effects of some dietary oils on performance and fatty acid composition of eggs in layers.* Revue Med Vet, **151**, 847-854.
7. **Balnave D, Muherreza SK** (1997): *Improving eggshell quality at high temperatures with dietary sodium bicarbonate*. Poult Sci, **76**, 588-593.
8. **Basmacıoğlu H, Çabuk M, Ünal K, Özkan K, Akkan S, Yalçın H** (2003): *Effects of dietary fish oil and flaxseed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens*. S Afr J Anim Sci, **33,** 266-273.
9. **Baucells MD, Crespo N, Barroete AC, Lopez-Ferrer S, Grashorn MA.** (2000): *Incorparation of different polyunsaturated fatty acids into eggs.* Poult Sci, **79,** 51-59.
10. **Ceylan N, Çiftçi İ, Mizrak C, Kahraman Z, Efil H** (2002): *Yumurta Tavuğu Yemlerine Balık, Keten ve Kanola Yağı Katılmasının Yumurta Yağ Asitleri ve Kolesterol Düzeyi Üzerine Etkisi*. Proje sonuç raporu, proje kod no. TAGEM-GY-00-11-03-029, Tarım ve Köyişleri Bakanlığı, TAGEM.
11. **Cherian G, Wolfe FW, Sim JS** (1996): *Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids and oxidative stability*. Poult Sci, **75,** 423-431.
12. **Elswyk ME** (1997): *Comparison of n-3 fatty acid sources in laying hens rations for improvement of whole egg nutritional quality: review*. Br J Nutr, **78,** 61-69.
13. **Filardi SR, Junqueiera OM, Laurentiz AC, Casartelli EM, Rodrigues EA, Araujo LF** (2005): *Influence of different fat sources on the performance, egg quality and lipid profile of egg yolks of commercial layers in the second laying cycle.* J Appl Poult Res, **14**, 258-264.
14. **Florou-Paneri P, Dotas D, Mitsopoulos I, Dotas V, Botsoglou E, Nikolakakis I, Botsoglou N** (2006): *Effect of feeding rosemary and α- tocopherol acetate on hen performance and egg quality.* Poult Sci**, 43**, 143-149.

15. **Florou-Paneri P, Nikolakakis I, Giannenas I, Koidis A, Botsoglou E, Dot As V, Mitsopoulos I** (2005): *Hen performance and egg quality as affected by dietary oregano essential oils and tocopherol acetate supplementation,* Int J of Poult Sci, **4,** 449-454.
16. **Galobart J, Barroeta AC, Baucells MD, Codony R, Ternes W** (2001): *Effect of dietary supplementation with rosemary extract and α-tocopheryl acetate on lipid oxidation in eggs enriched with ω-3 fatty acids*. Poult Sci, **80**, 460-467.
17. **Gonzalez-Esquerral R, 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.
18. **Grobas S, Mendez J, Lazaro R, Blas C, Mateos GG** (2001): *Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens*, Poult Sci**, 80,** 1171-1179.
19. **Hartel H** (1977): *Relations between N-corrected metabolisable energy and nutrient conten of feeds for chickens.* Archiv für Geflügel, **41,** 152-182.
20. **Herber-Mcneill** SM, Van Elswyk ME (1998): *Dietary marine algae maintains egg consumer acceptability while enhancing yolk color*. Poult Sci, **77**, 493-496.
21. **Huang Z, Leibovit, H, Lee CM, Millar R** (1990): *Effect of dietary fish oil on omega-3 fatty acid levels in chicken eggs and thigh flesh.* American Chem Soc, **38**, 743-747.
22. **Ito N, Fukushima S, Tsuda** H.(1985): *Carcinogenicity and modification of the carcinogenic response by BHA, BHT and other antioxidants*. Crit Rev Toxic, **15,** 109-150.
23. **Perıago JL, Suarez MD, Pıta ML** (1990)**:** *Effect of dietary olive oil, corn oil and medium-chain triglycerides on the lipid composition of rat red blood cell membranes*. J. Nutrition**, 120,** 986-994.
24. **Kalemba D, Kunicka A** (2003): *Antibacterial and antifungal properties of essential oils.* Current Med Chemist, **10**, 813-829.
25. **Fellenberg1 MA, Speısky H. (**2006) *Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability.* World's Poult Sci J **62,** 53-70
26. **McNamara DJ** (2000): *Dietary cholesterol and atherosclerosis,* Biochimica et Biophysic Acta **1529,** 310-320.
27. **National Research Council** (1994): *Nutrient Requirements of Poultry*. 9th review. National Academy Press, Washington, DC
28. **Salih AM, Smith DM, Price JF, Dawson LE** (1987): *Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry*. Poult Sci, **66**,1483-1488.
29. **Samlı HE, Agma A, Senkoylu N** (2005): *Effect of storage time and temperture on egg quality in old laying hens.* App Poult Res, **14,** 548-553.
30. **Simopoulos AP** (2000): *Human requirement for n-3 polyunsaturated fatty acids*. Poult Sci, **79,** 961-970.
31. **Snedecor GW, Cochran WG** (1980): *Statistical Methods*. 8th Edn., Iowa State Uni. Press, Ames, Iowa.
32. **Tilki M, Saatci M** (2004): *Effect of storage time on external and internal characteristics in partridge* (Alectoris gracea) eggs, Revue Med Vet, **155**, 561-564.
33. **Tukey J.W.** (1949): *Comparing individual means in the analysis of variance.* Biometrics, **36**, 99-114.
34. **Collıns VP, Cantor AH, Pescatore AJ, Straw ML, Ford MJ** (1997): *Pearl Millet in Layer Diets Enhances Egg Yolk n-3 Fatty Acids,* Poult Sci **76**:326–330.

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