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RESEARCH ARTICLE

Effects of Dietary Inclusion of Plant Extract Mixture and Copper into Layer Diets on Egg Yield and Quality, Yolk Cholesterol and Fatty Acid Composition [1]

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Summary

This study was conducted to determine the effects of plant extract mixture (PEM) inclusion at different levels and copper into diets of hens on performance, egg quality traits, yolk and serum cholesterol content and yolk fatty acid composition. A total of 192 Lohmann white layers, 38 wks of age, were used in this study. The experiment was carried out on a control and 7 treatments groups (basal diet plus 200 mg/kg copper ($CuSO_4.5H_2O$), 500 mg/kg PEM, 500 mg/kg PEM + 200 mg/kg copper, 750 mg/kg PEM, 750 mg/kg PEM + 200 mg/kg copper, 1000 mg/kg PEM + 200 mg/kg copper). PEM, copper and PEM plus copper combinations did not affect performance parameters. Increasing level of PEM increased quadratically shell stiffness and linearly shell thickness, but did not affect other egg traits. Treatments had no a significant effect on cholesterol and trigliserid concentrations of egg and serum. While supplemented PEM and copper did not affect fatty acid level of yolk, PEM plus copper combination increased oleic acid and total MUFA levels. Results obtained from present study showed that supplementation of a mixed herbal product containing *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil to diets of laying hens can be beneficial to improve egg quality traits especially such as shell stiffness and thickness.

Keywords: *Plant extract mixture, Copper, Performance, Egg quality, Cholesterol, Fatty acid composition of yolk*

Yumurtacı Tavuk Rasyonlarına Farklı Seviyelerde Bitki Ekstraktı ve Bakır İlavesinin Yumurta Verimi ve Kalitesi, Yumurta Sarısı Kolesterolu ve Yağ Asidi Kompozisyonuna Etkileri

Özet

Bu çalışma, yumurtacı tavuk rasyonlarına ilave edilen farklı seviyelerde bitki ekstrakt karışımının ve bakırın performans, yumurta kalitesi, yumurta sarısı ve serum kolesterol içeriği ile yumurta sarısı yağ asidi kompozisyonu üzerine etkisini belirlemek amacıyla yürütülmüştür. Araştırmada; 38 haftalık yaşta 192 adet Lohmann beyaz yumurtacı tavuk kullanılmıştır. Deneme, bir kontrol ve 7 muamele (200 mg/kg bakır, 500 mg/kg bitki ekstrakt karışımı, 500 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır, 750 mg/kg bitki ekstrakt karışımı, 750 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır, 1000 mg/kg bitki ekstrakt karışımı, 1000 mg/kg bitki ekstrakt karışımı + 200 mg/kg bakır) grubundan oluşmuştur. Bitki ekstrakt karışımı, bakır ve bakır ile bitki ekstrakt karışımının performans parametrelerini etkilemediği tespit edilmiştir. Bitki ekstrakt karışımının artan seviyesi kırılma mukavemetini ve kabuk kalınlığını artırmasına rağmen diğer yumurta kalite kriterlerin de önemli bir etkiye sahip olmadığı saptanmıştır. Muamele grupları arasında yumurta sarısı kolesterol ve trigliserid oranı ile serum kolesterol ve trigliserid oranları bakımından önemli bir farklılığın olmadığı tespit edilmiştir. Bitki ekstrakt karışımı ile bakır kombinasyonu yumurta sarısında oleik asit ve toplam MUFA seviyesini artırırken diğer uygulamaların yumurta sarısı yağ asidi oranı üzerine etkisinin olmadığı belirlenmiştir. Sonuç olarak; yumurtacı tavuk rasyonlarına kekik, kekik otu, kekik yağı, sarımsak yağı, anason ve rezene yağından oluşan bitki ekstrakt karışımı ilavesinin yumurta kalite kriterlerini özellikle kabuk kalınlığı ve kırılma mukavemetini artırmada faydalı olabileceği söylenebilir.

Anahtar sözcükler: *Bitki ekstrakt karışımı, Bakır, Performans, Yumurta kalitesi, Kolesterol, Yumurta sarısı yağ asit kompozisyonu*

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INTRODUCTION

It is known that a raised concentration of serum LDL-cholesterol increases the risk of coronary heart disease [1]. It is also known that the intake of egg cholesterol contributes to an elevation of the level of serum is considered relevant to find ways to produce eggs low cholesterol [2,3]. Nutritionist have been able to alter the diet of laying hens so that their eggs contain less cholesterol than the standard eggs. Results obtained from different studies have successfully demonstrated that natural products [4] and copper [5] could offer solutions to the health problem and cholesterol reduction. Natural products, essential extracts or oil derived from herbs and spices, exhibit hypocholesterolemic effect [6]. The hypocholesterolemic effect of essential oils has been reported for chickens [6-9]. It was reported that the pure components of essential oils inhibit hepatic 3-,hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis [10].

Copper (Cu) is an essential mineral which serves as a cofactor in many enzyme system [11]. Research evidences demonstrated that pharmacological levels of Cu (>250 mg/kg diet) caused changes in 17 beta-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in mature laying hens and suggested that copper supplements can affect reproductive physiology and lipid metabolism [12-14]. Kim et al.[15] indicated that reduced glutathione by addition of copper may play a major role in cholesterol homeostasis. Also, Iduwu et al.[16] reported that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentrations which is known to regulate cholesterol biosynthesis through the stimulation of the enzyme 3- hydroxy-3-methyl glutaryl coenzyme A (HMG-Co A) reductase, a key regulatory enzyme in cholesterol synthesis. Pesti and Bakalli [14] obtained a reduction in egg yolk cholesterol concentration and an increase in egg production when 125 and 250 mg/kg dietary levels of Cu were fed on White Leghorn hens. Skrivan et al.[17] suggested that copper supplementation at 200 mg /kg level in broiler diet had beneficial effects on growth performance. The maximum dietary tolerable level of copper for poultry was set at 300 mg kg^{-1} according to NRC [18]. Chiou et al.[19] reported that Layer diets containing 200 mg Cu per kg gave a positive response on the performance, liver function and enzyme activities but high dietary copper dosage (400, 600 or 800 mg kg^{-1}) gived a progressively negative response.

The objective of this study was to examine the dietary effect of the single or combination feeding of plant extracts mixture including *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil at different levels and copper on laying performance, egg quality, yolk fatty acid composition, yolk and serum cholesterol and trigliserid content.

MATERIAL and METHODS

This experiment was conducted by the researchers based on protocols by Atatürk University Ethical Commission Report (No: 24.04.2009/5/54).

A total of 192 Lohmann white layers, 38 weeks of age, were randomly allocated eight groups, each formed 6 replicate cages as subgroups, comprising of four hens. Experimental diets were (1) a standard commercial layer diet (Control), (2) a diet including 200 mg/kg copper ($CuSO_4.5H_2O$), (3) a diet including 500 mg/kg plant extracts mixture, (4) a diet including 500 mg/kg plant extracts mixture + 200 mg/kg copper, (5) a diet including 750 mg/kg plant extracts, (6) a diet including 750 mg/kg plant extracts mixture + 200 mg/kg copper, (7) a diet including 1.000 mg/kg plant extracts and (8) a diet including 1.000 mg/kg plant extracts mixture + 200 mg/kg copper for 14 weeks including adaptation period of 2 weeks. Diets were formulated according to NRC [18] and chemically analyzed by the AOAC [20] *(Table 1)*. A commercial blend of PEM Fitococci ® contained 463 g/kg active ingredients *, basically including *Origanum vulgare* (dried leaf), *Thymus vulgaris* (dried leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil. PEM and Cupric sulfate ($CuSO_4.5H_2O$) as a copper source were provided from Farmavet International Inc., Kocaeli, 41400, Turkey. During the experimental period, hens were fed ad libitum once daily at 08:30 h and water was available all the times. Hens were subjected to a 16L:7D cycle.

Feed intake and egg production were recorded daily, egg weight was measured bi-weekly, and body weight was measured at the end of experimental period. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. To evaluate egg quality parameters 12 egg samples were randomly picked up from each experimental group every month. Before determination of egg quality parameters such as shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit, samples were stored for 24 h in room temperature. They were assessed according to the method of Kaya et al.[21].

At the end of the experiment period, blood and egg samples were taken from the each replicate in order to determine the ratio of triglyceride and cholesterol. Triglyceride and cholesterol rations of blood and egg samples were assessed according to the method of Kaya and Macit [11].

Sample of 5 eggs was randomly collected from each

** Active ingridients given by Farmavet International Inc: 1,8-Cineole (0.24%), Allicine (0.24%), Alliine (0.12%), Alpha-Pinene (0.12%), Alpha Terpineol (0.70%), Borneol (0.18%), Caffeic-Acid (2.28%), Camphene (0.08%), Carvacrol (4.48%), Eugenol (0.12%), Geraniol (1.04%), Limonene (0.56%), Linalool (0.96%), Myrcene (0.18%), P-Cymene (2.38%), Phenol (0.86%), Polyphenol (6.00%), Tannin (12.9%), Rosmarinic-Acid (7.60%), Terpinen-4-Ol (0.06%), Ursolic Acid (1.92%), Tymol (3.26%)*

experimental group for fatty acid (FA) analysis at the end of the trial. The FA profile was determined with gas chromatograph (HP Agilent Technologies 6890N, Inc. Headquarters Santa Clara, US). Method identified by Yüksel et al.[22] was used to determine FA levels of yolk samples.

The experimental data were sitatistically analysed to the general linear models procedure of SPSS [23]. Polynomial contrast was constructed to determine the effect of the feeding level of PEM in the diets. The effects of the dietary treatments on response variables were declared to be significant at P<.05.

Table 1. *Ingredients and chemical composition of the experimental basal diet*
Tablo 1. *Bazal rasyonun bileşimi ve kimyasal komposizyonu*

Ingredients	(%)	Chemical Composition (Analyzed on Dry Matter Basis)	
Corn	52.81	Dry matter (%)	89.47
Soybean meal	18.13	Crude protein (%)	16.50
Wheat	6.00	Crude fiber (%)	4.49
Full fat soybean oil	1.65	Crude ash (%)	11.70
Sunflower meal	7.50	Ether extract (%)	4.88
Corn gluten meal	2.04	Lysine (%)	0.7
Soybean oil	1.60	Methionine (%)	0.33
Ground limestone	6.82	Calcium (%)	3.4
Salt	0.30	Total phosphorus (%)	0.7
Di-calcium phosphate 18	2.65	ME* kcal/kg	2720
DL-Methionine 99%	0.15		
L-Lysine	0.10		
Vit-Min	0.25		

Each two kilogram of vitamin- mineral premix contained 12.000 IU of retinyl palmitate, 2.500 IU of cholecalciferol, 30 IU of DL-α-tocopheryl acetate, 34 mg of phylloquinone, 3 mg of thiamin, 6 mg of riboflavin, 30 mg of nicotin amid, 10 mg of Ca-D-pantothenate, 5 mg of pyridoxine, 15 mg of cobalamin, 1 mg of folic acid, 50 mg of D-Biotin, 300 mg of choline chloride, 50 mg of ascorbic acid, 80 mg of manganese, 60 mg of iron, 60 mg of zinc, 5 mg of copper, 2 mg of iodine, 5 mg of cobalt, 150 mg of selenium
Metabolizable Energy Calculated

RESULTS

The effects of single feeding of PEM or Cu and combination of copper and PEM on the performance traits are presented in *Table 2*. Dietary additives did not have significant effect on final body weight and body weight change of hens. Similarly, feeding as the single or combination of plant extract mixture at different levels and copper did not affect the egg weight, egg production, feed intake, feed conversion ratio and broken egg rate.

The egg quality traits of hens fed a diet supplemented with PEM and Cu or combination of copper and PEM are shown in *Table 3*. Shape index, shell weight, yolk index, albumen index and haugh unit were not affected by adddition of PEM (P>.05). But PEM supplementation affected shell stiffnes and shell thickness (P<.05). Shell stiffnes was quadratically increased by increasing level of PEM. On the other hand, a significant linear increase was found in shell thickness when PEM at 500, 750 and 1.000 mg/kg levels was added to layer diets. The supplementation of copper had no significant effect on egg quality traits except for yolk color. Egg quality traits were not influenced by feeding of combination of PEM and copper.

The effect of inclusion of PEM at 500, 750 and 1.000 mg/kg levels into diet on ratios of cholesterol and triglyceride as a percentage of total lipids in serum and egg yolk is presented in *Table 4*. Cholesterol and trigliserid concentrations of yolk and serum were not affected by dietary treatments.

Table 5 shows the dietary effects of PEM, 200 ppm

Table 2. *Effects of plant extract mixture and copper on laying performance parameters*
Tablo 2. *Bitki ekstrakt karışımı ve bakırın performans parametreleri üzerine etkisi*

PEM mg/kg	Cu ppm	IBW (g)	FBW (g)	BWC (g)	EW (g)	EP (%)	FI (g/d)	FCR[1]	DER (%)
0	0	1627.27	1760.00	132.83	67.54	89.02	131.85	2.20	0.03
	200	1560.83	1705.83	145.00	66.72	85.68	123.35	2.17	0.27
500	0	1525.63	1650.63	125.00	65.96	90.29	128.20	2.16	0.13
	200	1593.96	1668.96	75.00	65.89	90.03	130.12	2.20	0.17
750	0	1622.08	1723.33	101.25	67.64	87.54	128.44	2.19	0.20
	200	1576.04	1681.67	105.63	64.56	90.39	128.40	2.18	0.23
1000	0	1597.92	1698.13	100.21	66.22	90.78	127.87	2.14	0.07
	200	1587.71	1670.00	82.29	64.12	85.75	125.24	2.32	0.40
SEM		29.95	30.55	21.93	1.13	2.27	2.41	0.24	0.11
PEM		0.55	0.12	0.16	0.39	0.65	0.73	0.54	0.83
Cu		0.53	0.23	0.41	0.06	0.37	0.18	0.25	0.06
PEM*Cu		0.14	0.66	0.50	0.56	0.33	0.17	0.59	0.47

***IBW**= Initial body weight, **FBW**= Final body weight, **BWC**= Body weight change, **EW**= Egg weight, **EP**= Egg production rate, **FI**= Feed intake, **FCR**= Feed conversion ratio (kg feed:kg egg), **DER**= Damage egg rate, **PEM**= plant extracts mixture, **SEM**= Standart error of mean, **Cu**= Copper*

Table3. *Effects of plant extract mixture and copper on egg quality parameters*
Tablo 3. *Bitki ekstrakt karışımı ve bakırın yumurta kalite parametreleri üzerine etkisi*

PEM mg/kg	**0**		**500**		**750**		**1000**		**SEM**	**PEM / Polinomial**	**Cu**	**PEM*Cu**
Cu mg/kg	**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
SI (%)	74.22	74.44	73.89	73.83	73.42	73.61	74.17	74.94	0.65	0.38	0.54	0.93
SS (kg/cm^2)	0.84	1.03	1.14	1.20	0.95	0.91	0.90	0.84	0.07	0.01/**(Q)**	0.44	0.26
ST (mm×10^{-2})	0.39	0.40	0.39	0.40	0.39	0.40	0.43	0.42	0.01	0.00 /**(L)**	0.49	0.58
SW (g)	8.55	8.37	8.32	8.80	8.22	8.22	8.37	8.17	0.17	0.18	0.84	0.20
YC	8.83	8.39	8.44	8.11	8.33	7.50	8.17	7.78	0.23	2.02	0.00	0.70
YI (%)	42.71	42.44	43.09	43.74	43.15	43.58	43.20	42.50	0.54	0.35	0.95	0.58
AI (%)	8.32	8.44	8.44	8.90	8.85	7.93	8.20	8.86	0.39	0.87	0.76	0.20
HU	82.01	82.65	83.03	84.52	84.20	80.17	80.83	85.04	1.66	0.77	0.62	0.11

SI*= Shape index,* ***SS****= Shell strenght,* ***ST****= Shell thickness,* ***SW****= Shell weight,* ***YC****= Yolk color,* ***YI****= Yolk index,* ***AI****= Albumen index,* ***HU****= Haugh unit,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper,* ***L****= Linear effect of PEM supplementation;* ***Q****= Quadratic effect of PEM supplementation*

Table 4. *Effects of plant extract mixture on cholesterol and trigliserid level of egg and serum (%)*
Tablo 4. *Bitki ekstrakt karışımı ve bakırın yumurta sarısı ve serum kolesterol ile trigliserid oranları üzerine etkisi (%)*

PEM mg/kg		**0**		**500**		**750**		**1000**		**SEM**	**PEM**	**Cu**	**PEM*Cu**
Cu mg/kg		**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
Egg yolk	Chol	18.84	18.19	18.25	20.99	17.77	18.95	17.45	16.22	1.26	0.19	0.57	0.39
	Trig	60.54	56.00	56.33	60.35	58.52	61.33	61.29	59.03	1.72	0.57	1.0	0.06
Serum	Chol	24.35	23.52	23.33	22.83	23.33	23.12	23.34	23.00	0.65	0.54	0.32	0.97
	Trig	43.87	45.17	45.36	45.26	42.63	44.12	44.15	43.09	0.92	0.15	0.53	0.47

Chol*= Cholesterol,* ***Trig****= Trigliserid,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper*

copper and combination of PEM and copper on fatty acid composotion of yolk. While supplemented PEM and copper did not affect fatty acid level in yolk, PEM with copper combination increased oleic acid and total monounsaturated fatty acid (MUFA) level (P<.05).

DISCUSSION

Present study, significant differences were observed among the groups with respect to performance parameters at the end of the 14-week experimental period. A similar observation was reported by Bozkurt et al.[9] who determined that body weight was not affected by addition of an essential oil combination to the layer diets. Arpasova et al.[24] concluded that body weight was not influenced diet including 0.25 ml/kg thyme essential oil in the laying hens. But, Çabuk et al.[25] found that essential oils had the positive effects on body weight of laying hens. In accordance with the present findings, Orhan and Eren [26] reported that the addition of essential oil mixture including *Origanum vulgare* (leaf), *Thymus vulgaris* (leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil into laying hen diets had no significant effect on egg weight, egg production, feed conversion ratio, feed consumption. In agreement with the present study, Aksu and Bozkurt [27] found that body weight, feed intake, and feed efficiency of broilers were not affected by the adding of essential oils at the level of 1.000 mg/kg. Similarly, Köksal and Küçükersan [28] reported that supplementation of essential oil into basal diet at level of 0.75 g/kg did not have significant effect on performance including body weight, body weight gain, feed intake and feed conversion ratio in broilers. Specific effects of the essential oils on laying performance have not received much attention because poultry may not acutely respond to flavor [29]. In contrast to our results, a study has observed that the addition of essential oil mixture into a layer diet has positive effect on egg weight and egg production rate [30]. Chandra et al.[31] reported that the supplementation of dietary copper at different levels (50, 75, 100, 125, 150 and 175 mg Cu per kg diet) into layer diets had no significant influence on performance parameters. Kaya and Macit [11] reported that egg weight, egg production and feed consumption decreased with 200 mg/kg Cu sulfate supplementation but feed conversion ratio and cracked egg were not affected. Pekel and Alp [32] postulated that supplementation with 250 mg/kg of Cu sulfate improved egg production but decreased egg weight and feed intake.

Except for shell stiffness and shell thickness, none of the egg quality traits were affected by PEM. In contrast to our study, other researchers have observed that the addition of essential oil mixture to layer diet did not have significant effect on shell strenght [9,26,30]. Similarly Nasiroleslami and Torki [33] reported that adding 300 mg/kg fennel essential oil to laying hen diet improved shell thickness. In contrast

Table 5. *Effects of plant extract mixture and Cu on fatty acid composition of egg yolk (%)*
Tablo 5. *Yumurta tavuk rasyonlarına bitki ekstrakt karışımı ve bakır ilavesinin yumurta sarısı yağ asidi komposizyonu üzerine etkisi (%)*

PEM mg/kg	**0**		**500**		**750**		**1000**		**SEM**	**PEM**	**Cu**	**PEM*Cu**
Cu mg/kg	**0**	**200**	**0**	**200**	**0**	**200**	**0**	**200**				
C14:0	0.28	0.28	0.27	0.32	0.27	0.27	0.23	0.26	0.02	0.08	0.17	0.49
C14:1	0.01	0.01	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.28	0.22	0.89
C15:0	0.03	0.04	0.03	0.04	0.02	0.02	0.02	0.02	0.01	0.12	0.90	0.38
C16:0	25.93	25.78	25.45	26.58	26.10	26.04	25.19	25.93	0.45	0.66	0.20	0.41
C16:1	2.16	2.34	2.33	2.72	2.44	2.63	2.28	2.35	0.20	0.37	0.15	0.88
C17:0	0.18	0.17	0.18	0.17	0.16	0.15	0.18	0.16	0.01	0.367	0.10	0.98
C17:1	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.01	0.68	0.58	0.56
C18:0	9.63	9.57	9.33	9.11	8.95	8.80	9.16	9.58	0.30	0.13	0.99	0.71
C18:1t (n-9)	0.06	0.06	0.06	0.07	0.06	0.02	0.06	0.05	0.02	0.38	0.36	0.43
C18:1c (n-9)	37.95^{b}	38.27^{ab}	37.98^{b}	36.02^{c}	37.68^{b}	39.05^{a}	37.95^{b}	38.46^{ab}	0.55	0.07	0.88	**0.03**
C18:2t (n-6)	1.50	1.59	1.60	1.60	1.62	1.76	1.60	1.59	0.06	0.16	0.18	0.57
C18:2c (n-6)	16.97	16.57	17.21	17.79	17.25	16.02	17.48	16.54	0.60	0.50	0.25	0.46
C18.3 (n-6)	0.12	0.11	0.12	0.11	0.10	0.07	0.09	0.10	0.02	0.13	0.34	0.51
C18.3 (n-3)	0.40	0.42	0.44	0.48	0.45	0.39	0.45	0.40	0.03	0.45	0.60	0.33
C20:1	0.19	0.22	0.20	0.19	0.22	0.21	0.20	0.21	0.01	0.50	0.51	0.47
C20:2	0.19	0.20	0.21	0.21	0.21	0.19	0.19	0.20	0.01	0.61	0.87	0.65
C20:3 (n-6)	0.18	0.19	0.19	0.19	0.18	0.17	0.14	0.17	0.02	0.14	0.40	0.56
C20:4	1.89	1.90	1.90	1.84	1.80	1.79	1.94	1.85	0.05	0.13	0.28	0.74
C24:1	0.71	0.73	0.69	0.65	0.71	0.69	0.76	0.68	0.02	0.18	0.13	0.17
∑ SFA	36.05	35.83	35.26	36.21	35.51	35.26	34.78	35.96	0.47	0.56	0.22	0.29
∑ UFA	62.43	62.69	63.03	62.01	62.79	63.08	63.19	62.69	0.45	0.67	0.45	0.41
∑ MUFA	41.17^{b}	41.71^{ab}	41.36^{b}	39.77^{c}	41.19^{b}	42.70^{a}	41.30^{b}	41.83^{ab}	0.50	0.06	0.49	**0.03**
∑ PUFA	21.27	20.99	21.66	22.24	21.60	20.38	21.89	20.86	0.66	0.48	0.30	0.51
∑ (ω-6)	20.67	20.36	21.01	21.54	20.94	19.79	21.25	20.26	0.62	0.49	0.28	0.52
∑ (ω-3)	0.41	0.42	0.44	0.49	0.45	0.39	0.45	0.40	0.03	0.37	0.62	0.29

C14:0*= Miristic Acid,* ***C14:1****= Miristioleic Acid,* ***C16:0****= Palmitic Acid,* ***C16:1****= Palmitoleic Acid,* ***C17:0****= Heptadecanoic Acid,* ***C17:1****= Heptadecenoic Acid,* ***C18:0****= Stearic Acid,* ***C18:1t (n-9)****= Elaidik Acid,* ***C18:1c (n-9)****= Oleic Acid,* ***C18:2t (n-6)****= Linoleic Acid,* ***C18:2c(n-6)****= Linoleic Acid,* ***C18.3(n-6)****= γ-Linolenic Acid,* ***C18.3(n-3)****= α-Linolenic Acid,* ***C20:1****= Gadoleic Acid,* ***C20:2****= Eikosadienoic Acid,,* ***C20:3 (n-6)****= Eikosatrienoic Acid,* ***C20:4****= Arashidonic Acid,* ***C24:1****= Nervonic Acid,* ***∑ SFA****= Total saturated fatty acid,* ***∑ UFA****= Total unsaturated fatty acid,* ***∑ MUFA****= Total Monounsaturated Fatty Acid,* ***∑ PUFA****= Total unsaturated fatty acid,* ***∑ (ω-6)****= Total omega-6,* ***∑ (ω-3)****= Total Omega-3,* ***PEM****= Plant extract mixture,* ***SEM****= Standart error mean,* ***Cu****= Copper*

to present study, there have been some researches who reported the unbeneficial effects of essential oil mixture on shell thickness in laying hens [9,30,34]. Information acquired from literature showed that herbal extracts used in poultry nutrition caused feed intake and feed efficiency improvement [35,36] because of increased production of digestive enzymes [37]. Increase in shell thickness may lead to higher nutrient retention and nutrient availability through the intestines during shell formation in this study. Yolk color was decreased by suplementation of 200 mg/kg copper to laying hen diets. In contrast to our results, Kaya and Macit [11] observed that yolk color was not affected by addition of 200 mg/kg copper to layer diets.

The effects of supplemental treatments on cholesterol and triglyceride ratios of yolk and serum were not significant in the present study. In accordance with the present findings, some researchers who reported that cholesterol and triglyceride concentrations of serum were not affected by addition of 24 mg/kg essential oil mixture [9] or 300 mg essential oils of fennel per kg diet [33]. A different observation was reported by Bölükbaşı et al.[38] who determined that the addition of 200 ppm thyme essential oil to the layer diet resulted in remarkable reduction in serum cholesterol and trigliserid ratios. Bölükbaşı et al.[39] showed that adding essential oils of thyme (200 mg/kg) into layer diet decreased triglyceride rations of yolk but not effected cholesterol ratios of yolk. The results of cholesterol and triglyceride ratios of egg and serum were supported by Kaya and Macit [11] who founded that the supplementation of 200 ppm copper did not have a significant effect on cholesterol and triglyceride ratios of egg and serum. On the contrary to the result of this experiment, in previous studies, egg yolk cholesterol in laying hens was decreased

by dietary Cu supplementation [5,14,40,41]. Also Abaza et al.[42] stated that Cu supplementation at 0 to 100 and 200 mg/kg levels in the laying quail diets decreased cholesterol and triglyceride concentrations of serum.

Inclusion of PEM with copper combination into the diets of laying hens increased oleic acid and total mono-unsaturated fatty acid (MUFA) level. The increase in MUFA observed in the eggs from layer fed the combination of PEM and copper diets was mainly contributed by oleic acid (C18:1c [n-9]). Recently, MUFA have been a subject of interest due to their possible hypolipidemic and anti-thrombotic effects [43]. Ansari et al.[44] reported that fatty acid level of yolk in layer was not effected by 250 ppm copper. Maurice and Lightsey [45] postulated that adding of 250 ppm copper into layer diets did not effect fatty acid level of yolk except for arashidonic acid level.

The differences between present study and other experiments may be due to differences in the laying period, age and genotype of hen and the combination, and kind of used extract and levels of Cu and extract in the diets of laying hens. In previous years, effects of supplementation of PEM together with Cu as combination in laying hens diets have not been studied. Therefore there have not been yet any literature to compare the results related to performance and some egg quality traits containing cholesterol and fatty acid levels obtained from present study with findings reported by other researchers.

Results from present study showed that addition of plant extract mixture containing leaves of *Origanum vulgare* (leaf), *Thymus vulgaris* (leaf), thyme oil, origanum oil, garlic oil, anise oil and fennel oil into the laying hen diets can be beneficial to improve the shell stiffness and thickness from egg quality traits. In addition, feeding of laying hens with diet containing combination of plant extract mixture and copper increased MUFA level of yolk, but did not decrease the cholesterol level of egg yolk in laying hens.

In conclusion, combination of 750 mg/kg PEM and Cu may be used in the diets of 38 wks of age laying hens for 3-month experimantal period, but more experiment on the supplementation of extracts and Cu combinations into diets of laying hens should be conducted by researchers related to this subject.

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