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Performance and Egg Quality of Laying Hens Fed Diets Supplemented with Herbal Extracts and Flaxseed

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Abstract

In this experiment, the effects of fennel and thyme extracts with and without flaxseed were investigated on performance and egg quality of Leghorn-type laying hens (Hy-Line W-36). 200 laying hens from 26 to 38 weeks of age were assigned to five dietary treatments with five replications. The treatment groups were: 1) Control (a diet without any additives); 2) control diet plus fennel (40 mg/kg feed); 3) control diet plus thyme (40 mg/kg feed); 4) a diet containing flaxseed and fennel; and 5) a diet containing flaxseed plus thyme. There were significant differences in feed intake and egg weight between the treatments ($P < 0.05$). The egg yolk color index in hens that received thyme extract and flaxseed treatment was significantly higher than other treatments ($P < 0.05$). Hens fed control diet had lower Haugh unit compared to other treatments that contained herbal extracts. The eggshell strength was significantly higher in hens that received thyme extract and flaxseed treatments than control ($P < 0.05$). The eggshell weight in treatments containing flaxseed was significantly higher compared to the other treatments ($P < 0.05$). The lowest egg yolk cholesterol concentration was found in hens fed thyme and flaxseed treatment. The hens fed plant extracts and flaxseed diets had eggs with low palmitic and stearic acids and high α-linolenic acid. It is concluded that thyme and fennel extracts, as well as flaxseed, improved the performance and egg quality of laying hens. The use of flaxseed and thyme extract improved egg yolk omega-3 fatty acids and decreased yolk cholesterol content.

Introduction

Herbal plant species contain chemical substances that can be effective on growth and livability of animals. The positive properties of herbal extracts are variable and include antioxidant (Lee *et al*., 2003), hypocholesterolemic (Craig, 1999), digestion–stimulatant and enzymatic (Jamroz *et al*., 2003; Ramakrishna *et al*., 2003; Hernández *et al*., 2004; Jamroz *et al*., 2005). These substances also improve utilization of digestive products through enhanced liver functions (Hernández *et al*., 2004). The use of herbal essential oils in poultry diets improves performance traits and assists colonization of beneficial microbial population (Zaika, 1988; Jang *et al*., 2007) and inhibits the growth of pathogenic bacteria that may pollute meat and egg products (Bassett, 2000; Hertrampf, 2001).

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Among herbal extracts, thyme has received great attention due to its antioxidant and antibacterial properties (Varel, 2002). The major components of thyme essential oils are thymol and carvacrol which have been shown to possess antioxidant properties (Aeschbach *et al*., 1994). Fennel is recommended for the total digestive system (Simon *et al*., 1984). The aroma and flavor components of the essential oils for fennel seeds consist of anethole, limonene, fenchone, estragole, safrole, alpha-pinene, camphene, beta-pinene, sabeinene, beta-myrcene, phellandrene, cis-ocimene, para-cymene, gamma-terpinene, camphor and several other volatile component as well as affixed oil (Charles *et al*., 1993). Supplementation of diet with thyme improved egg weight, egg mass, and hen-day-egg production but had no effect on egg qualitative traits (Abdel-Wareth, 2013). The use of mixed herbal essential oils in poultry feeding as a supplement to improve production is of recent interest (Abdel-Wareth *et al*., 2012; Olgun and Yildiz, 2014). In contrast, flaxseed has been shown to enrich omega-3 fatty acid levels in eggs of laying hens (Klatt, 1986; Scheideler and Froning, 1996). Flaxseed also reduces the severity of ovarian disease in laying hen (Aeschbach *et al*., 1994). It seems that supplementation of a diet containing flaxseed with thyme and fennel essential oil can improve egg production and egg quality in hens. Meanwhile, there is no report for interaction between flaxseed and herbal extract on laying hens performance. In spite of the vast amount of research papers published on herbal extract supplementation to broiler diets in the past decade (Alcicek *et al*., 2004; Hernández *et al*., 2004; Jamroz *et al*, 2005; Bölükbaşı *et al*., 2006), there is relatively little published data on laying hens (Botsoglou *et al*., 2005; Ma *et al*., 2005; Cabuk *et al*., 2006).

Therefore, the objective of this experiment was to study the effects of thyme and fennel extracts on performance and egg quality parameters and also to investigate the possibility of improving cholesterol and fatty acid composition of egg yolks by the supplementation of herbal extracts and flaxseed in laying hen diets.

Materials and Methods

Experimental design and measurements

The trial was conducted in a windowless house at Kashmar Research Station according to the Animal Care Committee of Islamic Azad University (Kashmar Branch, Kashmar, Iran) from December 2011 to March 2012. Two hundred 26-weeks old commercial White Leghorn laying hens (W-36 Hy-Line strain) were used in a completely randomized design experiment. Hens were assigned to five dietary treatments with five replications. Each replication consisted of two cages (four hens per cage), with 40 hens in each treatment. The dietary treatments were: 1) a control diet without flaxseed and any growth promoters; 2) control diet plus 40 mg fennel extract/kg feed; 3) control diet plus 40 mg thyme extract/kg feed; 4) 100 g flaxseed and 40 mg fennel extract/kg feed, and 5) 100 g flaxseed and 40 mg thyme extract/kg feed. The ingredients and chemical composition of the dietary treatments are presented in Table 1. The diets were offered in mash form and birds had free access to feed and water throughout the experiment. Alcoholic extracts from thyme *(Thymus vulgaris* L.) and fennel (*Foeniculumvulgare)* were used (Barij Essence, Pharmaceutical Co., Tehran, Iran). Dry matter of alcoholic extract was determined by lyophilization of 5 mL of extract.

Body weight was measured by weighing all hens individually at the onset and end of the experiment. A daily photoperiod of 17:7 hrs lightness: darkness was used throughout the experiment. Egg production and broken-cracked eggs were recorded daily from 26 to 38 weeks of age. All eggs were weighed during the experiment by an electronic balance (Sartorius, accuracy 0.001). Feed intake was recorded weekly. Feed conversion ratio was calculated as the ratio of mass of feed consumed per egg mass produced. Egg mass production was determined based on the percent of hen-day-production according to the following formula. Egg production was calculated based on hen house as shown in the formula below:

$$\text{Average egg mass} = \%\text{hen} - \text{day production} \times \text{Average egg weight in gram}$$

$$\text{Hen house egg production} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of hens housed at the beginning of laying period}}$$

Table 1. Ingredients and nutrient composition of experimental diets (% as feed basis)

Ingredients	Control	Control + Fennel	Control + Thyme	Control + Flaxseed + Fennel	Control + Flaxseed +Thyme
Yellow Corn	60.70	60.70	60.70	54.95	54.95
Soybean Meal, 44% CP	16.10	16.10	16.10	14.80	14.80
Flaxseed	0.00	0.00	0.00	10.00	10.00
Wheat Bran	3.40	3.40	3.40	2.67	2.67
Barley	10.00	10.00	10.00	8.00	8.00
Dicalcium Phosphate	0.69	0.69	0.69	0.69	0.69
Oyster Shell	7.90	7.90	7.90	7.70	7.70
Salt	0.26	0.26	0.26	0.26	0.26
Vitamin Premix[1]	0.50	0.50	0.50	0.50	0.50
Mineral Premix[2]	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.11	0.11	0.11	0.09	0.09
Calculated Values					
ME (Kcal/Kg)	2700	2700	2700	2700	2700
Crude Protein (%)	14.70	14.70	14.70	14.70	14.70
Lysine (%)	0.65	0.65	0.65	0.68	0.68
Methionine (%)	0.34	0.34	0.34	0.34	0.34
Methionine + Cystine (%)	0.59	0.59	0.59	0.60	0.6
Threonine (%)	0.50	0.50	0.5	0.53	0.53
Calcuim (%)	3.20	3.20	3.20	3.20	3.20
Available Phosphorous (%)	0.28	0.28	0.28	0.28	0.28
Herbal Extracts Value:					
Thyme (*Thymus vulgaris* L.) (mg/Kg)	0	0	40	0	40
Fennel (*Foeniculum vulgare*) (mg/Kg)	0	40	0	40	0

[1] Vitamin premix supplied the following per kilogram of diet: Vitamin A, 440.000 IU; Vitamin D_3, 80.000 IU; Vitamin E, 96 mg; Vitamin K_3, 2000 mg; Vitamin B_1, 6120 mg; Vitamin B_2, 3000 mg; Vitamin B_6, 612 mg; Calcium Pantothenate, 8800 mg; Niacin, 50 mg; Biotin, 2 gr; Folic acid, 1.25 mg; Vitamin B_{12},640 mg (Telavang Co., Tehran,Iran).

[2] Mineral permix supplied the following per kilogram of diet: Cu ($CuSO4 \cdot 5H2O$, 25.45% Cu) 8 gr; Fe ($FeSO_4 \cdot 7H_2O$, 20.29% Fe) 100 gr; Mn ($MnSO_4 \cdot H_2O$,32.49% Mn) 64.52 gr; Zn (ZnO, 80.35% Zn) 33.8 gr; Se ($NaSeO_3$, 45.56% Se) 8 mg (Telavang Co., Tehran, Iran)

Egg quality traits

Egg quality traits were evaluated at 4, 8 and 12 weeks of the experiment. 30 eggs per treatment were randomly selected and sampled daily on two consecutive days in the mentioned weeks. Eggs and eggshells were weighed using an electronic balance. A tripod micrometer (B.C. Ames Co., Waltham, MA) was used to measure the height of the albumen midway between the yolk and the edge of the albumen. The haugh unit score (Haugh, 1937) was calculated using the following formula:

$$HU = 100 \log_{10}(H - 1.7W^{0.37} + 7.57)$$

where H = height of the albumen (mm) and W = weight of egg (g)

Yolk index (YI) was calculated by Roche yolk color fan (RYCF) scale (Roche Ltd., Basel Switzerland) based on 15 sample colors ranging from 1 (the lightest) to 15 (the darkest). Eggshell strength was evaluated using an eggshell strength tester (Fujihira industry Co., LTD, Tokyo Japan) to determine the force that causes cracking under longitudinal compression, and the shell maximum deformation was recorded (g/cm^2).

Serum and yolk cholesterol concentration, egg fatty acids composition

Blood (~5 ml) was collected from the brachial wing vein of two birds in each replication at the end of 4, 8 and 12 weeks of the experiment. All blood samples were collected in a glass tube (16 mm × 100 mm) and left to stand at room temperature to clot. Serum was then obtained by centrifugation at 4000 × g for 3 min. Cholesterol was determined in serum by auto-analyzer

(analyzer A-15 Biosystem, barcelona, Spain) and corresponded kit (Pars Azmoon, Iran).

Yolk cholesterol and fatty acid composition were measured in egg samples. Yolk cholesterol was determined by an enzymatic method using colorimetry according to Shen *et al*., (1982). A cholesterol reagent kit (Pars Azmoon Cholesterol assay) was used for enzymatic determination using cholesterol esterase and cholesterol oxidase. The color intensity was determined photometrically at 540 nm, using a spectrophotometer (Hitachi U-2000, Japan). The fatty acid composition of eggs was determined by gas chromatography (GC) using a Hewlett Packard 5890 series I gas chromatograph equipped with a flame ionization detector and integrator 3392A under Stibilj and Koman-Rajsp (1997). The samples were prepared by fat extraction from the yolk of two eggs. After hydrolysis and methylation of extracts, 0.5 µL of samples were injected into the GC apparatus. The quantities of fatty acids were determined by internal standard method (Park and Goins, 1994).

Statistical Analysis

Data analyzed using the Mixed procedure of SAS (1996) according to the following model:

$$Y_{ijkm} = \mu + T_i + W_j + TW_{ij} + \Phi_{k(i)} + E_{ijkm}$$

where Y_{ijkm} is the dependent variable, µ is the mean, T is the treatment effect, W_j is the period effect, TW_{ij} is the treatment and period interaction , $\Phi_{k(i)}$is random effect of animal nested in each treatment and E_{ijkm} is random residual error term. Significant differences were separated by Tukey's multiple range test at $P < 0.05$.

Results

Results showed that egg production and feed conversion ratio were not significantly affected by experimental treatments, but significant effects were observed in feed intake and egg weight ($P < 0.05$, Table 2).

Table 2. Effects of dietary treatments on the performance of laying hens

Variables	Treatments					*SEM*	*P*-values
	Control	C+F	C+T	C+F+Flax	C+T+Flax		
Egg production (%)	69.43	71.42	68.48	67.77	67.32	1.17	0.1044
Feed intake (g/hen/day)	89.28[a]	90.42[a]	88.71[a]	88.6[a]	85.79[b]	1.54	0.0197
Feed conversion ratio	2.31	2.21	2.28	2.29	2.24	0.05	0.6801
Egg weight (g)	56.76[b]	60.00[a]	59.54[a]	56.60[b]	57.65[b]	0.61	0.0276
Egg mass (g/hen)	3938.73[b]	4287.00[a]	4056.75[a]	3825.46[b]	3880.21[b]	178.13	0.045

[a, b] Means within a row lacking a common superscript differ significantly ($P < 0.05$).
C: Control, F: fennel, T: thyme, Flax: Flaxseed

Hens fed control diets with fennel or thyme extracts had significantly higher egg weight and egg mass than others ($P < 0.05$), and no significant differences were observed between other treatments. Birds fed the diet containing flaxseed with thyme extract had significantly lower feed intake than others ($P < 0.05$), but no significant differences were observed between other treatments.

Table 3. Effects of dietary treatments on egg yolk index from eggs of laying hens in different weeks (Roche unit)

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	6.0[b]	7.0	6.8[b]	6.6 [b]
C + F	6.6[b]	6.8	6.4[b]	6.6 [b]
C + T	6.8[b]	6.4	6.8[b]	6.7 [b]
C + F + Flax	7.0[a]	7.0	6.8[b]	6.9[b]
C + T + Flax	7.8[a]	7.2	7.8[a]	7.6[a]
SEM	0.12	0.18	0.11	0.17
P-values	0.013	0.15	0.037	0.022

[a,b]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

Results regarding the effects of dietary treatments on the egg yolk index are shown in Table 3. Egg yolk index was significantly higher in hens fed flaxseed with thyme extract than others at 30 and 38 weeks as well as during the total period ($P < 0.05$), but no significant

differences were observed between other treatments during these periods. There were also no significant differences in egg yolk index between treatments at 34 weeks. Hens that received thyme and fennel extracts produced eggs that had significantly higher Haugh unit compared to hens that received control diet at all weeks ($P < 0.05$, Table 4).

Table 4. Effects of dietary treatments on egg Haugh unit in different weeks

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	76.9[b]	90.4[b]	91.2[b]	86.1[b]
C+F	95.1[a]	96.0[a]	96.5[a]	95.9[a]
C+T	99.0[a]	96.3[a]	96.5[a]	97.2[a]
C+F+Flax	94.9[a]	97.5[a]	97.4[a]	96.6[a]
C+T+Flax	97.3[a]	97.7[a]	97.9[a]	97.6[a]
SEM	3.55	2.04	1.54	1.81
P-values	<0.0001	<0.0001	<0.0001	<0.0001

[a,b]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

No significant differences were observed between dietary treatment for eggshell strength at 30, 34, and 38 weeks (Table 5). However, throughout the experiment, the greatest eggshell strength was in eggs from hens fed the control diet with thyme extract ($P < 0.05$), followed by the control diet with fennel extract .

Table 5. Effects of dietary treatments on eggshell strength (kg/cm²) of laying hens in different weeks

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	0.32	0.26	0.28	0.28[b]
C + F	0.31	0.32	0.33	0.32[ab]
C + T	0.35	0.37	0.36	0.36[a]
C + F + Flax	0.30	0.30	0.31	0.31[b]
C + T + Flax	0.31	0.25	0.36	0.31[b]
SEM	0.05	0.06	0.03	0.02
P-values	0.54	0.24	0.27	0.047

[a,b]Means within a column lacking a common superscript differ ($P < 0.05$).

No significant differences were observed between treatments for eggshell weight at 30, 34 and 38 weeks (Table 6). The largest eggshell weight throughout the experiment was from hens fed flaxseed diet with thyme or fennel extracts which significantly differed with other treatments ($P < 0.05$).

Table 6. Effects of dietary treatments on eggshell weight (g) of laying hens in different weeks

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	7.0	6.3	6.9	6.7[b]
C + F	6.3	6.2	6.3	6.3[b]
C + T	6.5	6.9	6.8	6.7[b]
C + F + Flax	7.7	7.6	7.8	7.7[a]
C + T + Flax	7.7	7.8	7.7	7.8[a]
SEM	0.31	0.29	0.27	0.19
P-values	0.63	0.43	0.46	0.023

[a,b]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

Table 7. Effects of dietary treatments on egg yolk cholesterol (mg/egg yolk) of laying hens in different weeks

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	255.6	260.2	165.8	227.2[a]
C + F	230.9	155.4	223.4	203.2[a]
C + T	227.2	157.7	223.3	202.8[a]
C + F + Flax	220.5	216.1	190.9	209.1[a]
C + T + Flax	167.1	162.9	161.3	163.7[b]
SEM	22.6	24.3	23.7	11.4
P-values	0.53	0.50	0.45	0.012

[a,b]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

Evaluation of cholesterol content in egg yolk and serum indicates that use of thyme and flaxseed in the diet significantly decreased cholesterol in egg yolk compared to control in the total period of the experiment ($P < 0.05$), but the use of thyme alone showed no statistical difference than control diet (Table 7). Use of flaxseed or thyme significantly decreased cholesterol of serum in comparison to the control diet whereas fennel alone did not impact cholesterol of serum in the total period (Table 8).

Experimental diets had a significant effect on palmitic, stearic and linolenic acid content of egg yolk ($P < 0.05$), but no significant effect on oleic and linoleic acids (Table 9). The content of linolenic acid (as an omega-3 fatty acid) in eggs from hens with diets containing herbal extracts was significantly higher but saturated fatty acid content (palmitic and stearic fatty acids) was lower in comparison to the control treatment.

Table 8. Effects of dietary treatments on blood serum cholesterol of laying hens (mg/dL) in different weeks

Treatment	30 weeks	34 weeks	38 weeks	total period
Control	177.8	133.6	148.4	153.3[a]
C+F	169.8	162.2	162.2	165.9[a]
C+T	124.0	93.0	90.4	102.5[b]
C+F+Flax	117.6	125.2	127.2	123.3[b]
C+T+Flax	126.2	99.8	161.3	107.0[b]
SEM	16.7	18.3	17.9	11.2
P-values	0.27	0.15	0.17	0.03

[a,b]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

Table 9. Effects of dietary treatments on egg fatty acids composition of laying hens (mg/g)

Fatty acid	Treatments					*SEM*	*P*-value
	Control	C + F	C + T	C + F + Flax	C + T + Flax		
Palmitic	29.10[a]	20.70[d]	22.70[c]	24.20[b]	21.10[d]	0.54	0.0001
Stearic	11.45[a]	8.70[c]	8.40[c]	9.60[b]	8.00[d]	0.20	0.0001
Oleic	38.40	45.70	72.20	48.30	43.90	11.90	0.3377
Linoleic	10.30	13.60	12.80	13.70	15.50	45.10	0.1403
Linolenic	0.32[c]	0.48[b]	0.46[b]	0.61[a]	0.49[b]	0.02	0.0001

[a-d]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Discussion

In the present study, the effect of herbal extracts on feed conversion ratio and egg production are in accordance with previous studies (Vogt, 1990; Botsoglou *et al*., 2005). Hens fed a diet containing thyme or fennel extracts produced heavier eggs which may be related to the volatile compounds in these extract. Adding flaxseed in addition to herbal extracts reduced egg weight compared to herbal extract alone. This may be associated with lower true amino acid availability of glutamate, serine, valine, phenylalanine, isoleucine, leucine, and methionine in respect to soybean meal (Barbour and Sim, 1991) and presence of some toxic substances in flaxseed products (Ajuyah *et al*., 1991). Anethole is major substance in fennel essence that reduces and stops gastrointestinal spasms and improves gastrointestinal digestion, and subsequently increases nutrient intake. Also, carvacrol and thymol in thyme essence increase nutrient metabolism in hepatocytes and can also act as antioxidants (Liu, 2011).

Increasing nutrient metabolism in liver may provide more ovalbumin for egg formation as our result showed a greater Haugh unit for treatments containing herbal extract.

The yolk color index of eggs from hen diets supplemented with flaxseed and thyme had a significant difference from other treatments in 38 weeks and the total period of sampling. ($P < 0.05$). Carotenoid in thyme extract and flaxseed (Lutein and zeaxanthin) could be responsible for a greater color index in related treatments (fennel seed contains less carotenoid than thyme) (Danish food composition databank-ed.7.01[1], Technical University of Denmark). Haugh unit was affected by the treatments in all periods, reflecting egg quality. Egg quality is largely concerned with egg white strength or jelly structure. Therefore egg quality increases

[1]National food institute- technical university of Denmark

when white strength enhances. The protein ovomucin causes a jelly-like structure in egg whites (Leeson and Summers, 2001), so its high content increases egg white quality, thereby increasing Haugh unit. The nutritional effect on egg white quality has been investigated by limited studies (Leeson and Summers, 2001). There is a significant difference in Haugh unit between treatments containing herbal extracts and the control group. Compounds in thyme herbal extract, such as thymol, carvacrol, and anethole likely play a role in the stimulation of ovomucin protein synthesis (Abdel-Wareth, 2013). Shell strength in eggs from hens fed diets containing flaxseed and thyme extracts is higher than other treatments when calculated over the total period. Thymol has previously been shown to have an indirect effect on the beta-adrenergic receptors and can relax the smooth muscle of uterus (Wienkotter *et al*., 2007). This increases delay time of egg in the uterus and the secretion of calcium carbonate. Hens that received flaxseed produced eggs with higher shell weight. Flaxseed contains lignans that are considered phytoestrogens, which are plant chemicals that mimic the oestrogen. Brooks *et al*. (2004) investigated the effect of lignans on bone metabolism. Calcium storage in medullary bone develops as oestrogen levels increase. On the other hand, calcium binding protein (CBP) levels increase in uterine cells in response to oestrogen (Squires, 2010). Both mechanisms are effective in increasing the secretion of calcium carbonate in eggshell, subsequently producing a heavy eggshell.

In this study, use of flaxseed and thyme resulted in the decrease of egg and sera blood cholesterol. These findings agree with those of another study showing that lignans from flaxseed can decrease cholesterol and lipoproteins in serum blood of male rat (Cho *et al*., 2004). It is also reported by Sosin-Bzduca and Krawczyk (2012) that use of 10% flaxseed in the diet of hens can reduce cholesterol content in egg yolk. Case *et al.* (1995) showed a 9% reduction in serum cholesterol in leghorn chicks fed 15 ppm thyme essential oil. It seems that the reduction of cholesterol in blood seen by flaxseed diets is not related to lignans compounds, because they have no effect on gene expression in cholesterol biosynthesis (HMG-CoA reductase, cholesterol 7-hydroxylase,and acyl-CoA oxidase) but the effects of lignans on cholesterol metabolism are not rejected (Pellizzon, *et al*., 2007; Felmlee, *et al*., 2009). It was suggested that the enterolignans are metabolites of food lignans in the intestine and their weak estrogenic and biochemical properties suggest potential for reduction of cholesterol *via* biochemical methods (Peterson *et al*., 2010). Some future studies plan to identify the biochemical mechanism(s) through which flaxseed lignans exert their beneficial effects and to also identify the lignan form(s) responsible. (Felmlee *et al*., 2009). Therefore, there are two suggested mechanisms for hypolipidemic effects of flaxseed. Firstly, flaxseed contains about 7% soluble fiber that bind to cholesterol and make a cholesterol-fiber complex which is unavailable for absorption in the intestine (Bloedon and Szapary, 2004). Secondly, this plant included many phytoestrogenic compounds (lignans family) that are effective in cholesterol biosynthesis but their molecular mechanisms of cholesterol reduction are unknown (Felmlee *et al*., 2009).

Decreasing of cholesterol content in blood sera and egg yolk in hens that received thyme extract may be related to the active components (thyme and carvacrol). These components may reduce the liver enzyme activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which is a key enzyme in cholesterol synthesis (Abdulkarimi *et al*., 2011). While a 5% reduction in HMG-CoA reductase activity has been reported, there was also a 2% reduction in poultry serum cholesterol (Bloedon and Szapary, 2004). Similar results have been obtained for cholesterol in chickens fed thymol and carvacrol, but carvacrol significantly reduce triglycerides and total phospholipids in chicken blood (Abdulkarimi *et al*., 2011).

Hens fed diets containing thyme and fennel extracts or flaxseed produced eggs with higher concentration of linolenic acid. This finding is in agreement with other studies (Yalcin and Unal, 2010; Sosin-Bzducha and Krawczyk, 2012). Flaxseed is a very rich source of α-linolenic acid with a 55% concentration (Cunnane *et al*., 1993). Linolenic acid is a precursor of other n-3 fatty acids and can further elongate and desaturate to other n-3 fatty acids family by Δ-6 desaturase and elongase enzymes (Sosin-Bzducha and Krawczyk, 2012). The result of the current study also shows the low concentration of saturated fatty acids in egg yolks from hens with diets supplemented with herbal extract. Similar results reported by Antruejo *et al.* (2011) showed

that hens fed diets containing flaxseed, rapeseed or chia seed produced eggs with low levels of saturated fatty acids. Hence, the composition of fatty acids in layer diets is effective on the fatty acid profile of egg yolks which should be considered for enriching eggs.

Conclusion

According to the results of this study, thyme and fennel extracts are recommended to improve the performance of laying hens and fatty acid profiles in egg yolk. Also, thyme extracts in combination with flaxseed are useful for reducing egg cholesterol while the combination of flaxseed and fennel extract improves yolk omega-3 fatty acids and decrease cholesterol contents.

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