



## Original Research Article

# Effect of supplementing layer hen diet with phytogetic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents



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## ABSTRACT

This study was conducted to evaluate the effects of supplementing laying hen diet with phytogetic additives on laying performance, egg quality, blood constituents and egg lipid peroxidation. Two hundred Lohmann Brown Lite laying hens were randomly allotted to 4 dietary treatments: control (without phytogetic additive), fennel seeds (5 g/kg), black cumin seeds (5 g/kg) and hot red pepper (5 g/kg). Each of the 4 diets was fed to 5 replicates of 10 hens for 8 weeks. No significant differences were observed in body weight or feed intake between the groups. Dietary inclusion of fennel followed by red pepper improved ( $P < 0.05$ ) egg weight, egg production, egg mass and feed conversion ratio compared with control. Higher yolk shape index, shell and albumen weight percentages and Haugh unit ( $P < 0.05$ ) were recorded in the fennel supplemented group compared with control. The egg yolk color score increased by the addition of fennel or hot red pepper in laying hen diets compared with control. The inclusion of black cumin or hot red pepper decreased serum and egg yolk cholesterol and malondialdehyde concentrations ( $P < 0.05$ ) compared with control. Serum aspartate aminotransferase concentration was lower in black cumin group ( $P < 0.05$ ) than in other treatments. In conclusion, the best laying performance and egg quality were obtained by dietary inclusion of fennel, followed by hot red pepper and black cumin. Dietary supplementation of black cumin or red pepper may lead to the development of low-cholesterol concentration and better antioxidant capacity of eggs.

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## 1. Introduction

Natural medicinal products derived from herbs and spices used in animal and poultry nutrition to enhance performance have been called “phytogetic feed additives” (Windisch et al., 2008). The use of phytogetic feed additives or herbal plants has recently received

much greater attention as alternatives to traditional antibiotics, probiotics and prebiotics. These phytogetic plants are considered as natural products, and thus consumers may willingly accept them to be included in poultry feeds. Comprehensive investigations of phytogetic plants have indicated their growth promoting, antimicrobial, antioxidant and anti-inflammatory functions (Windisch et al., 2008; Gheisar and Kim, 2017). Additionally, they possess a stimulatory effect on the digestive system through increasing the production of digestive enzymes and improving feed utilization efficiency by enhancing liver functions (Hernandez et al., 2004; Prakash and Srinivasan, 2010; Abou-Elkhair et al., 2014). However, our knowledge about their applications in poultry nutrition is still rather limited.

Fennel (*Foeniculum vulgare* Mill.) belongs to the Apiaceae family. Fennel seeds contain about 2% to 6% essential oil, with trans-anethole as the dominant constituent (Najdoska et al., 2010).

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Research on fennel leaves and fruits revealed that its essential oil possesses antioxidant, antimicrobial, and hepatoprotective functions (Özbek et al., 2003; Gende et al., 2009; Shahat et al., 2011). Dietary inclusion of fennel in laying hen diets was shown to alleviate the negative effects of heat stress on egg quality parameters (Gharaghani et al., 2015).

Another example of natural phytochemical product is black cumin seeds (*Nigella sativa* L.). Black cumin seeds are good sources of protein (23%), energy and unsaturated fatty acids; linoleic and oleic acids. It has been reported that black cumin seeds have biological activities such as antioxidant (Guler et al., 2007), digestive and appetite stimulant (Gilani et al., 2004), and hepatoprotective activity (Mahmood et al., 2003).

Red pepper (*Capsicum annuum* L.) is a rich source of carotenoids such as vitamin C, E and provitamin A, with well-known antioxidant functions (Krinsky, 2001). The active compounds found in red pepper have been reported to have chemopreventive and chemotherapeutic effects (Jancso et al., 1997). Efficient red pepper active compounds are capsaicin, capsinin and capsantine. Red pepper fruits are used by broiler and layer producers for increasing chicken appetite (Özer et al., 2005), darkening the yolk color and improving laying performance (Özer et al., 2006). Al-Kassie et al. (2012) observed that dietary inclusion of hot red pepper decreased the heterophil/lymphocytes (H/L) ratio, indicating its role in the immune system of birds.

All these observations encourage the hypothesis that these additives may positively affect laying performance and egg quality, but the number of *in vivo* studies in laying hens is still limited. Researches on the effects of supplementing layer hen diet with fennel, black cumin or hot red pepper on laying performance, egg quality, blood biochemical parameters and egg lipid peroxidation are limited. Therefore, the current study was designed to evaluate the effect of fennel, black cumin and hot red pepper supplementation to laying hen diets on laying performance, egg quality, hepatoprotective activity and egg lipid peroxidation.

## 2. Materials and methods

### 2.1. Birds, housing and experimental diets

All the procedures used in the current study have been approved by Animal Care and Use Committee of Menoufia University, Egypt. In total, 200 commercial Lohmann Brown Lite laying hens at 32 weeks old with uniform body weight were assigned to 4 equal groups replicated 5 times with 10 hens per replicate. Laying hens in the control group were given a corn-soybean-basal diet in mash form without phytochemical additives. The remaining three groups were given the same basal diet supplemented with an additional 0.5% (5 kg/t) of fennel seeds in powder form (T1), black cumin seeds in powder form (T2) or hot red pepper (T3). Table 1 shows the ingredients, the nutrient concentration and chemical composition of the basal diet (AOAC, 2005). The experimental diets were formulated to meet the nutrient requirements for layer hens according to the recommendations of the breeder (Lohmann Brown Lite layers, Commercial Management Guide, Lohmann Tierzucht, Cuxhaven, Germany). Table 2 presents the proximate chemical composition of the tested fennel seeds, black cumin seeds and hot red pepper, including moisture, crude protein, ether extract, crude fiber and ash (AOAC, 2005). Feed and water were provided *ad libitum* during the experiment. The experiment was carried out between 32 and 40 weeks of age (8 weeks experimental period). Hens were housed in battery cages (100 cm long, 45 cm wide, and 40 cm high) equipped with trough feeders and nipple drinkers. The lighting regimen was 16 h of continuous light per day from 06:00 to 22:00. The laying hens were kept in optimal and standard

**Table 1**

Ingredients and nutrients composition of the basal diet (air-dry basis).

Item	Amount
Ingredients, %	
Yellow corn	64.04
Soybean meal (44%)	25.28
Vegetable oil	1.15
Salt	0.30
Dicalcium phosphate <sup>1</sup>	1.07
Premix <sup>2</sup>	0.30
Limestone	7.80
DL-methionine <sup>3</sup>	0.06
Calculated energy and nutrient content, g/kg	
ME, MJ/kg	11.43
Crude protein	170.2
Crude fiber	40.1
Ether extract	37.2
Calcium	32.8
Available phosphorus	5.40
Methionine	3.40
Lysine	9.00
Chemical analysis, g/kg	
DM	903.7
Crude protein	169.6
Crude fiber	38.4
Ether extract	30.3
Ash	95.4

<sup>1</sup> Dicalcium phosphate, 18% granular phosphate and 23% calcium.

<sup>2</sup> Supplied per kg of diet: Vitamin A 12,000 IU, vitamin D<sub>3</sub> 3,000 IU, vitamin E 40 mg, vitamin K<sub>3</sub> 3 mg, vitamin B<sub>1</sub> 2 mg, vitamin B<sub>2</sub> 6 mg, vitamin B<sub>6</sub> 5 mg, vitamin B<sub>12</sub> 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg.

<sup>3</sup> DL-methionine, Met AMINO (DL-2-amino-4-(methyl-thio)-butane acid, DL-methionine,  $\alpha$ -amino- $\gamma$ -methyl-oily acid) by Feed Grade 99% (EU).

**Table 2**

Proximate chemical composition (g/100 g) of the tested fennel seeds, black cumin seeds and hot red pepper.<sup>1</sup>

Item	Fennel seeds	Black cumin seeds	Hot red pepper
Moisture	6.50	5.53	4.83
Crude protein	19.20	29.30	7.70
Ether extract	7.50	8.13	2.60
Crude fiber	17.50	9.50	4.00
Ash	8.02	6.05	5.62

<sup>1</sup> Proximate chemical composition of the tested phytochemical feed additives according to AOAC (2005).

bio-climatic and welfare conditions. In this work, the national guidelines for care and precautions for animal use were followed and all procedures were approved by local ethic committee.

### 2.2. Collection and analyses

All hens weighed individually at 32nd and 40th week of age. Hen per day egg production (%) was measured. The feed consumption and feed conversion ratio were recorded in 7-d intervals. The feed conversion ratio (FCR) was expressed as grams of feed consumed per grams of egg produced. Egg mass was calculated by multiplying egg weight by egg production. All production variables were determined on a replicate basis.

An additional sample of 20 eggs was randomly collected from each experiment (4 eggs per replicate) at 36 and 40 weeks of age to assess interior and exterior egg quality parameters. Eggshell thickness (without inner and outer shell membranes) was

measured at 3 different points (top, middle, and bottom) using an ultrasonic micrometer. Albumen height, albumen index, yolk index, Haugh unit, weight percentages of albumen and yolk were measured as internal egg quality parameters. The weight of albumen and yolk were divided into whole egg weight and then multiplied by 100 to determine the weight percentage. Egg shape index (%), egg yolk index (%) and egg yolk albumen index (%) were recorded according to Romanoff and Romanoff (1949). Egg yolk visual color was measured by matching the yolk with one of the 15 bands of the Roche yolk color fan. Haugh units, a measure of the height of the albumen of eggs broken out on a flat surface were measured using a tripod micrometer.

At the end of the study, an additional sample of 4 eggs per replicate (20 eggs per experimental group) was collected for analyzing egg cholesterol and malondialdehyde (MDA) concentrations. The cholesterol (mg of cholesterol/g of egg yolk) and MDA (ng of MDA/g of egg yolk) concentrations in the egg yolk were measured by an ultraviolet spectrophotometer UV4802 (Unico Co., Dayton, USA) using commercial kits (Biosystem S.A, Costa Brava, 30, Barcelona, Spain) according to the methods described by Kaya et al. (2001) for cholesterol, and Botsoglou et al. (1994) and Galobart et al. (2001) for MDA.

Blood samples were taken from wing vein of 10 hens per experimental group at the end of the experiment (40 weeks of age). The serum was isolated and stored at  $-20^{\circ}\text{C}$ . Individual samples were analyzed for serum cholesterol and MDA concentrations alanine, and aminotransferase (ALT) and aspartate aminotransferase (AST) activities by an ultraviolet spectrophotometer UV4802 (Unico Co., Dayton, USA) (Botsoglou et al., 1994) using commercial kits (Biosystem S.A, Costa Brava, 30, Barcelona, Spain) according to manufacturer's instructions.

### 2.3. Statistical analysis

Experimental data were subjected to One-way ANOVA using IBM SPSS Statistics 22 statistical package (SPSS Inc., Chicago, IL, USA) as a completely randomized design. Significant differences among the treatments were determined using Tukey's test at  $P < 0.05$ .

## 3. Results

### 3.1. Phytogetic feed additives and growth performance

Based on the obtained results, the addition of fennel (T1), black cumin (T2) and hot red pepper (T3) in the diet of laying hens had no significant differences in body weight (Table 3). At the end of the experiment (40 weeks of age), laying hens fed a diet supplemented with fennel, black cumin or hot red pepper tended to have a higher body weight gain ( $P = 0.08$ ) than those fed the control diet. There was no significant difference in feed intake ( $P > 0.05$ ) between the treatment groups during 32 to 36, 36 to 40 and 32 to 40 weeks of age.

### 3.2. Phytogetic feed additives and laying performance

From the results in Table 4, dietary inclusion of phytogetic feed additives (fennel, black cumin or hot red pepper) led to significant ( $P < 0.05$ ) differences in egg number per hen, egg production, egg weight, egg mass and feed conversion ratio compared with the non-supplemented control group during the whole experimental period (32 to 40 weeks of age). During 32 to 40 weeks of age, addition of fennel, followed by hot red pepper and black cumin seeds to the layer diet had the highest egg number per hen, egg production, egg mass and better feed conversion ratio compared

**Table 3**

Effect of fennel, black cumin and red pepper supplementation on the productive performance of laying hens.

Parameters	Treatments <sup>1</sup>				SEM	P-value
	CON	T1	T2	T3		
BW, g						
Initial BW	1,589.0	1,588.9	1,588.2	1,589.9	3.48	0.96
Final BW	1,786.2	1,804.7	1,808.3	1,814.2	11.87	0.18
BW gain	197.17	215.73	220.07	224.33	9.234	0.08
Feed intake, g/hen						
Week 32–36	112.99	110.39	111.97	111.61	1.987	0.64
Week 36–40	126.99	125.01	126.15	125.99	1.123	0.42
Week 32–40	119.99	117.70	119.06	118.80	0.999	0.22

SEM = standard error of the mean.

<sup>1</sup> CON: control, the basal diet; T1: the basal diet + 0.5% fennel seeds; the basal diet + T2: 0.5% black cumin seeds; T3: the basal diet + 0.5% hot red pepper.

**Table 4**

Effect of fennel, black cumin and red pepper supplementation on the laying performance during the experimental period.

Parameters	Treatments <sup>1</sup>				SEM	P-value
	CON	T1	T2	T3		
Egg number, /hen						
Week 32–36	11.28 <sup>b</sup>	12.29 <sup>a</sup>	11.99 <sup>b</sup>	12.18 <sup>a</sup>	0.146	0.001
Week 36–40	11.31 <sup>b</sup>	12.45 <sup>a</sup>	11.71 <sup>ab</sup>	12.10 <sup>a</sup>	0.233	0.006
Week 32–40	11.25 <sup>c</sup>	12.52 <sup>a</sup>	11.80 <sup>b</sup>	12.09 <sup>ab</sup>	0.165	<0.001
Egg production, %						
Week 32–36	80.55 <sup>b</sup>	87.78 <sup>a</sup>	85.26 <sup>a</sup>	86.98 <sup>a</sup>	1.044	0.001
Week 36–40	80.17 <sup>c</sup>	91.15 <sup>a</sup>	83.32 <sup>bc</sup>	85.77 <sup>b</sup>	1.521	0.001
Week 32–40	80.36 <sup>c</sup>	89.46 <sup>a</sup>	84.29 <sup>b</sup>	86.38 <sup>ab</sup>	1.175	0.001
Egg weight, g						
Week 32–36	50.96	51.65	50.53	51.51	0.395	0.07
Week 36–40	53.52 <sup>c</sup>	56.46 <sup>a</sup>	54.19 <sup>b</sup>	54.46 <sup>b</sup>	0.151	<0.001
Week 32–40	52.24 <sup>b</sup>	54.05 <sup>a</sup>	52.36 <sup>b</sup>	52.99 <sup>b</sup>	0.201	<0.001
Egg mass, g/(hen·d)						
Week 32–36	40.51 <sup>c</sup>	44.09 <sup>a</sup>	41.97 <sup>b</sup>	43.85 <sup>a</sup>	0.239	<0.001
Week 36–40	40.51 <sup>d</sup>	50.47 <sup>a</sup>	44.10 <sup>c</sup>	45.95 <sup>b</sup>	0.480	<0.001
Week 32–40	40.51 <sup>d</sup>	47.28 <sup>a</sup>	43.03 <sup>c</sup>	44.90 <sup>b</sup>	0.285	<0.001
Feed conversion ratio						
Week 32–36	2.85 <sup>a</sup>	2.54 <sup>b</sup>	2.62 <sup>ab</sup>	2.58 <sup>ab</sup>	0.090	0.04
Week 36–40	3.19 <sup>a</sup>	2.44 <sup>c</sup>	2.86 <sup>b</sup>	2.74 <sup>b</sup>	0.067	<0.001
Week 32–40	3.05 <sup>a</sup>	2.47 <sup>c</sup>	2.76 <sup>b</sup>	2.64 <sup>bc</sup>	0.072	<0.001

SEM = standard error of the mean.

<sup>a–c</sup> Within a row, means with different letters are significantly different at  $P < 0.05$ .

<sup>1</sup> CON: control, the basal diet; T1: the basal diet + 0.5% fennel seeds; the basal diet + T2: 0.5% black cumin seeds; T3: the basal diet + 0.5% hot red pepper.

with control non-supplemented group. Egg weight was heavier ( $P < 0.05$ ) in the fennel and hot red pepper supplemented groups than that in the control and black cumin groups.

### 3.3. Phytogetic feed additives and egg quality

Egg quality traits were assessed 2 times in the current study, at weeks 4 and 8 of the experiment (Table 5). The egg shape index was significantly increased by including fennel in laying hen diets at weeks 4 and 8 of the experiment ( $P < 0.05$ ). Supplementation of the diet with 0.5% fennel significantly increased shell and albumen weight percentages ( $P < 0.05$ ) at 40 weeks of age. No significant effect on yolk weight percentage was observed with the addition of hot red pepper or black cumin to the diets of laying hens. Lower yolk weight percentage ( $P < 0.05$ ) and higher yolk shape index ( $P < 0.05$ ) were recorded in laying hens fed a diet supplemented with fennel compared with the control group at week 8 of the experiment. The egg yolk color score was significantly increased by the addition of fennel and hot red pepper in laying hen diets at

**Table 5**  
Effect of fennel, black cumin and red pepper supplementation on egg quality traits at 36 and 40 weeks of age.

Parameters	Treatments <sup>1</sup>				SEM	P-value
	CON	T1	T2	T3		
At the end of 36 weeks of age						
Egg shape index	77.32 <sup>b</sup>	81.38 <sup>a</sup>	79.09 <sup>ab</sup>	79.70 <sup>ab</sup>	1.169	0.04
Shell weight, %	13.91	14.61	13.77	13.61	0.375	0.11
Albumen weight, %	57.01	61.74	59.25	59.69	1.551	0.08
Albumen index, %	10.80 <sup>b</sup>	11.09 <sup>b</sup>	11.65 <sup>a</sup>	11.20 <sup>b</sup>	0.135	0.002
Yolk weight, %	29.08 <sup>a</sup>	23.65 <sup>b</sup>	26.97 <sup>ab</sup>	26.95 <sup>ab</sup>	1.641	0.05
Yolk shape index, %	67.57	70.31	70.13	69.39	1.811	0.45
Yolk color score	5.44 <sup>b</sup>	6.78 <sup>a</sup>	6.33 <sup>ab</sup>	7.00 <sup>a</sup>	0.351	0.01
Haugh units	81.31 <sup>b</sup>	85.28 <sup>a</sup>	81.97 <sup>b</sup>	82.52 <sup>b</sup>	0.589	0.001
Shell thickness, mm	0.344	0.357	0.360	0.352	0.008	0.26
At the end of 40 weeks of age						
Egg shape index	75.82 <sup>c</sup>	79.31 <sup>a</sup>	74.88 <sup>c</sup>	76.88 <sup>b</sup>	0.294	0.001
Shell weight, %	13.30 <sup>b</sup>	14.76 <sup>a</sup>	13.43 <sup>b</sup>	13.86 <sup>ab</sup>	0.337	0.01
Albumen weight, %	58.71 <sup>b</sup>	62.29 <sup>a</sup>	59.67 <sup>ab</sup>	60.04 <sup>ab</sup>	0.849	0.02
Albumen index, %	9.98	10.54	10.64	10.97	0.312	0.07
Yolk weight, %	27.99 <sup>a</sup>	22.95 <sup>b</sup>	26.89 <sup>a</sup>	26.09 <sup>a</sup>	0.939	0.004
Yolk shape index, %	65.73 <sup>c</sup>	69.11 <sup>a</sup>	65.57 <sup>c</sup>	67.54 <sup>b</sup>	0.489	0.001
Yolk color score	5.22 <sup>b</sup>	6.89 <sup>a</sup>	6.11 <sup>ab</sup>	7.06 <sup>a</sup>	0.336	0.002
Haugh units	79.39 <sup>c</sup>	83.07 <sup>a</sup>	79.72 <sup>c</sup>	81.43 <sup>b</sup>	0.377	0.001
Shell thickness, mm	0.353	0.370	0.382	0.365	0.011	0.16

SEM = standard error of the mean.

<sup>a-c</sup> Within a row, means with different letters are significantly different at  $P < 0.05$ .

<sup>1</sup> CON: control, the basal diet; T1: the basal diet + 0.5% fennel seeds; the basal diet + T2: 0.5% black cumin seeds; T3: the basal diet + 0.5% hot red pepper.

weeks 4 ( $P < 0.05$ ) and 8 ( $P < 0.05$ ) of the experiment compared with control. Using 0.5% fennel seeds as feed additive in laying hen diet had the highest Haugh unit percentage ( $P < 0.05$ ) during the first and the second experimental period, compared with other treatment groups. There was no significant difference in shell thickness among the experimental groups.

#### 3.4. Phytogetic feed additives, egg yolk and serum biochemical variables

Data of egg yolk and serum biochemical parameters at the end of the experiment (40 weeks of age) are presented in Table 6. The addition of black cumin or hot red pepper significantly decreased serum ( $P < 0.05$ ) and egg yolk ( $P < 0.05$ ) cholesterol concentrations compared with control group. However, there was no significant difference in serum and egg yolk cholesterol concentrations between control and fennel supplemented group. Serum ALT activity was lower in black cumin supplemented group ( $P < 0.05$ ) than in other groups. Dietary supplementation of different phytogetic feed additives (fennel seeds, black cumin seeds or hot red pepper) had no significant effect on serum ALT activity at the end of the experiment. Supplementation of layer hen diet with either

black cumin or hot red pepper significantly decreased both serum ( $P < 0.05$ ) and egg yolk ( $P < 0.05$ ) MDA concentrations compared with the control non-supplemented group. However, no differences in serum or egg yolk MDA concentrations were observed between fennel supplemented and control laying hens.

#### 4. Discussion

Different phytogetic plants have been investigated during the last 2 decades as natural feed additives. The growth promoting effects, antioxidant, antimicrobial and anti-inflammatory activities of herbal products have been reported in very recent studies (Gheisar and Kim, 2017). Herein, we focus on studying the effect of 3 different phytogetic feed additives in the diet of laying hens, in terms of their beneficial impact on growth efficacy, laying performance, egg quality, hepatoprotective functions and lipid peroxidation.

Data presented in this study showed that performance parameters, such as body weight, body weight gain and feed intake, experienced little influence of fennel, black cumin or hot red pepper consumption by laying hens. A tendency toward higher body weight gain was recorded in the red pepper group, followed by black cumin and fennel supplemented groups compared with control. Lokaewmanee et al. (2013), Khan et al. (2013) and Gharaghani et al. (2015) reported that the production performance of laying hens was not changed with dietary inclusion of red pepper, black cumin or fennel, respectively. Conversely, El Bagir et al. (2006) reported that dietary black cumin at the level of 1% or 3% increased final body weight of laying hens.

The beneficial impact of these phytogetic additives on improved body weight gain of laying hens could be clarified due to the bio-active ingredients in hot red pepper, black cumin seeds and fennel seeds. Capsaicin, the active component of hot red pepper, is efficient in augmenting nutrients and energy metabolism through enhancing the activities of glucose-6-phosphate dehydrogenase, lipoprotein lipase in adipose tissue, and pancreatic and intestinal enzymes (Reddy and Lokesh, 1992; Platel and Srinivasan, 2004). The active components of black cumin, particularly thymoquinone, and other constituents (e.g. anethole, carvacrol and 4-terpinol), have been reported to have antibacterial and antioxidant, as well as, stimulants of digestive enzymes (Gilani et al., 2004; Guler et al., 2007; Khan et al., 2012). The essential oil of fennel seeds was shown to have appetite stimulating effect, intestinal enzymes and bile acid's secretions enhancing activities, as well as, antioxidant and antimicrobial properties (Platel and Srinivasan, 2001; Gende et al., 2009; Shahat et al., 2011). Therefore, these above-mentioned bio-active components could be included in improving nutrient absorption and utilization, and modulating beneficial microbiota in gastrointestinal tract, which eventually

**Table 6**  
Effect of fennel, black cumin and red pepper supplementation on serum biochemical constituents and egg yolk parameters of laying hens at the end of the experiment (40 weeks of age).

Treatments <sup>1</sup>	In serum				In egg yolk	
	Cholesterol, mg/dL	AST, U/L	ALT, U/L	MDA, nmol/mL	Cholesterol, mg/g	MDA, ng/g
CON	129.00 <sup>a</sup>	24.44 <sup>a</sup>	12.30	12.82 <sup>a</sup>	12.70 <sup>a</sup>	28.20 <sup>a</sup>
T1	125.00 <sup>ab</sup>	24.59 <sup>a</sup>	12.13	11.69 <sup>ab</sup>	11.55 <sup>ab</sup>	27.34 <sup>ab</sup>
T2	110.50 <sup>c</sup>	23.14 <sup>b</sup>	12.09	9.64 <sup>c</sup>	11.08 <sup>b</sup>	24.10 <sup>b</sup>
T3	122.50 <sup>b</sup>	24.49 <sup>a</sup>	12.28	10.43 <sup>bc</sup>	11.13 <sup>b</sup>	24.50 <sup>b</sup>
SEM	1.306	0.084	0.282	0.506	0.408	0.995
P-value	<0.001	<0.001	0.46	0.001	0.01	0.02

AST = aspartate aminotransferase; ALT = alanine aminotransferase; MDA = malondialdehyde; SEM = standard error of the mean.

<sup>a-c</sup> Within a row, means with different letters are significantly different at  $P < 0.05$ .

<sup>1</sup> CON: control, the basal diet; T1: the basal diet + 0.5% fennel seeds; the basal diet + T2: 0.5% black cumin seeds; T3: the basal diet + 0.5% hot red pepper.



lead to improved growth performance of laying hens. Nevertheless, the mode of action of these phytogetic feed additives is still not fully elucidated.

Overall, the egg production, egg mass and FCR were increased with the dietary inclusion of fennel, followed by red pepper and black cumin in laying hen diets. The findings in the present study regarding the percentage of egg production, egg mass and FCR in the black cumin group agreed with those observed by Akhtar et al. (2003), Khan et al. (2013) and Boka et al. (2014). A beneficial effect of dietary inclusion of fennel or red pepper on egg production, egg mass or FCR have not been previously reported (Lokaewmanee et al., 2013; Hilmi et al., 2015; Vakili and Majidzadeh Heravi, 2016). Recently, Gharaghani et al. (2015) observed that the reduction in egg production during heat stress was smaller in laying hens receiving fennel in their diets. The observed increase in egg production, egg mass and FCR of laying hens in the current study was probably due to the presence of essential nutrients such as crude protein, essential fatty acids, minerals and carbohydrates in these phytogetic additives that have resulted in better laying performance. It is also possible that these phytogetic feed additives might have stimulated the hepatic secretion of egg yolk precursors through protecting hepatocytes from oxidative damage with subsequent enhancement of yolk formation and ovulation (Bollenger-Lee et al., 1998).

Our data showed that the egg weight was not affected by dietary inclusion of black cumin seeds or hot red pepper (5 g/kg of diet) during the whole experimental period. Boka et al. (2014) showed that supplementation of black cumin up to 30 g/kg of laying hen diets had no effect on egg weight. However, Aydin et al. (2008), Yalçin et al. (2009) and Khan et al. (2013) reported that dietary inclusion of black cumin seed at the levels of 10 to 15, 30 and 30 to 50 g/kg, respectively, increased egg weights. Furthermore, Melo et al. (2016) reported no significant differences in egg weight between control and black pepper supplemented groups. Conversely, inclusion of fennel in laying hens diet in the current study increased egg weights. Gharaghani et al. (2015) and Vakili and Majidzadeh Heravi (2016) showed that dietary inclusion of fennel in laying hen diets increased egg weight either under heat stress or normal ambient temperature, respectively. The significant impact of phytogetic feed additives on laying performance could be also attributed to the positive effects of these phytogetic additives in modulating gut microbiota, enhancing nutrient digestibility and absorption, and improving ovarian characteristics resulted in better health status and subsequent laying performance (Boka et al., 2014; Saki et al., 2014).

Haugh unit is a measure of the egg quality inside the shell. Haugh unit value obtained from the relationship between height of albumen and egg weight (albumen quality). Inclusion of fennel in laying hens diet had the highest Haugh unit percentage compared with control and other treatment groups. Gharaghani et al. (2015) observed that fennel supplementation in laying hen diets under heat stress increased Haugh unit. However, Saki et al. (2014) observed no significant effect on Haugh unit from fennel supplementation. In contrast to our results regarding black cumin seeds, Akhtar et al. (2003) and Khan et al. (2013) showed that the addition of black cumin seeds to layer diets increased Haugh unit. Hilmi et al. (2015) reported that supplementation of piperine in quail diets at a level of 30 mg/kg BW increased Haugh unit. In line with the current findings, Lokaewmanee et al. (2013) found that Haugh unit was not influenced by dietary supplementation of 0.5% red pepper in laying hen diets. The use of phytogetic additives with antibacterial and antioxidant properties, such as fennel seeds, may improve albumen quality, as previously reported by Bozkurt et al. (2012), who added a mixture of phytogetic essential oils, including fennel oil in the mixture, to laying hen diets. In addition, the bio-active ingredients

of herbal plants were shown to protect magnum and uterus, and encourage the albumen secretion in laying birds (Nadia et al., 2008).

The egg yolk color score was significantly increased by the addition of fennel and hot red pepper in laying hen diets compared with the control and black cumin groups. The egg yolk color was stated to be influenced by the consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids (Hammershøj et al., 2010). Santos-Bocanegra et al. (2004) and Lokaewmanee et al. (2013) reported that capsanthin improved egg yolk color and was responsible for the deep red color of the egg yolk. In contrast, Vakili and Majidzadeh Heravi (2016) observed that the inclusion of 40 mg fennel extract/kg feed did not influence egg yolk color index. The positive effect of fennel supplementation in laying hen diets on egg yolk color was not reported previously, there is relatively little published data on laying hens. The higher egg yolk color score in fennel and red pepper supplemented groups could be attributed to the presence of carotenoids pigment in fennel and red pepper.

Data showed that black cumin seeds and hot red pepper consumption reduced cholesterol and MDA concentrations in serum and egg yolk of laying hens. However, there was no difference in serum and egg yolk concentrations of cholesterol and MDA between fennel and control groups. The findings regarding black cumin were in line with the results of Akhtar et al. (2003), Aydin et al. (2008) and Boka et al. (2014). In addition, Puvača et al. (2015) reported that hot red pepper supplementation decreased blood total cholesterol concentration in broilers. It has been suggested that lower level of blood cholesterol could be related to the inhibitory effects of phytogetic bio-active components on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (a critical enzyme in cholesterol biosynthesis), thereby reducing cholesterol synthesis (Crowell, 1999) and to the reduction in intestinal cholesterol resorption (Brunton, 1999). In this regard, Badadry et al. (2000) demonstrated that thymoquinone components in black cumin decrease either *de novo* synthesis of cholesterol or enhance bile acid excretion. Moreover, dietary capsaicin was shown to enhance the conversion of cholesterol to bile acids via a stimulation of hepatic cholesterol-7-hydroxylase activity, which is a key pathway for cholesterol removal from the body (Srinivasan and Sambaiah, 1991). All together, these effects would lead to the reduction in serum and egg yolk cholesterol concentrations (Srinivasan and Sambaiah, 1991; Badadry et al., 2000). Accordingly, the current findings suggest that black cumin and hot red pepper could potentially improve the lipid profile of laying hens.

The dietary inclusion of black cumin or red pepper reduced serum and egg yolk MDA concentrations, suggesting the efficacy of their antioxidant action through reducing lipid peroxidation and the release of free radicals. The positive effects of these phytogetic additives on serum and egg yolk concentrations of MDA could be due to their main active components, thymoquinone, carvacrol, anethole, and 4-terpinol of black cumin (Guler et al., 2007) and to capsaicin in hot red pepper (Kentaro et al., 2002; Luqman and Razvi, 2006). Dietary inclusion of black cumin seeds at levels of 0.5% and 1% decreased the concentration of erythrocyte MDA and the production of lipid peroxidases in chickens (Tülüce et al., 2009). These authors suggested that black cumin seeds exhibit antioxidant properties through inhibiting free radical production by increasing the activity of antioxidant enzymes such as glutathione peroxidase (Tülüce et al., 2009). Additionally, capsaicin was reported to persuade antioxidant properties in terms of preventing lipid peroxidation via improvement the activities of antioxidant enzymes and decrease MDA concentration and protein carbonyl group content (Luqman and Razvi, 2006; Hassan et al., 2012). Consequently, all together, these effects would lead to the reduction of MDA

concentration in both serum and egg yolk of laying hens fed diets supplemented with either black cumin or hot red pepper.

It was observed in the present study that there were no significant differences among groups in the serum activity of ALT, but black cumin supplemented group had lower activity of AST compared with other groups. Al-Homidan et al. (2002) and Yalçın et al. (2009) failed to show any significant effects on serum AST and ALT activities of broiler chicks or laying hens fed diets containing black cumin seeds. The significant decrease in serum AST activity within the normal range in black cumin group suggested better hepatoprotective activity. The hepatoprotective activity of black seeds (lower serum AST activity) may be owing to the free radical scavenging (antioxidant) properties of its components, particularly thymoquinone, carvacrol, p-cymene, m-cymene and  $\alpha$ -thujene (Abdel-Wahhab and Aly, 2005; Hassan et al., 2012). In conclusion, these phytochemical additives did not have adverse effects on liver functions (AST and ALT).

## 5. Conclusions

Data presented in the current study indicate that fennel seeds, black cumin seeds and hot red pepper can be included in the diets of laying hens at a level of 0.5% without any deleterious effects on their laying performance, egg quality and serum biochemical constituents. According to the results of this study, fennel seeds and hot red pepper are recommended to improve the performance of laying hens and egg quality traits. Black cumin and hot red pepper are useful for reducing serum and egg cholesterol and MDA concentrations, and their dietary supplementation may lead to the development of low-cholesterol and better antioxidant capacity of eggs as demanded by health-conscious consumers. Moreover, black cumin seed supplementation enhanced the antioxidant capacity and provided health benefits to laying hens.

## Conflict of interest

The authors have no conflict of interest to declare.

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