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Effect of Supplementation of Hen Diet with Pennyroyal Extract (*Mentha pulegium*) on Performance, Egg Quality and Yolk TBARS Values

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ABSTRACT

The aim of this study was to investigate the effects of pennyroyal extract supplementation at different levels (0, 32.5, 65 and 130 mg/kg) and 50 mg/kg BHA (Butylated hidroxyanisole) into diets of laying hens on performance, egg quality traits, thiobarbituric acid reactive substans (TBARS) of yolk, the contents of malondialdehyde (MDA), superoxide dismütase (SOD) and glutation peroxidas (GSHPx) in serum. Sixty Lohman LSL white layers, 40 weeks of age, kept in individual cages were assigned randomly to five treatment groups, each group included 12 hens. The hens received one of five diets with 0, 32.5, 65 or 130 mg/kg pennyroyal extract and 50 mg/kg BHA, respectively. Experiment lasted for 60 days. At the end of the experiment, the supplementation of pennyroyal extract did not affect feed intake, rates of albumen, yolk and shell of egg, shell tickness, spesific gravity, Haugh unit and some serum parameters. It however, significantly improved feed conversion rate and egg production. Also, egg weights of groups fed on diets including 65 and 130 mg/kg of pennyroyal extract increased in present study. It was found that supplementation of 130 mg/kg pennyroyal extract significantly improved shell strength. TBARS was reduced in eggs stored during 42 days (P<0.05). In conclusion, pennyroyal extract ameliorated performance and lipid oxidation of eggs.

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Authors' Contribution
SCB conceived the idea and supervised the study. AA executed the experiments. SCB and AA performed analytical examinations. SCB wrot the manuscript.

Key words
Laying hen, Pennyroyal extract, Performance, Egg quality, TBARS

INTRODUCTION

The prohibition of growth stimulating antibiotic feed additives reduced the profitability in industrial animal production, therefore the use of alternative feed additives has become widespread. One of the most interesting alternative feed additives is plant extracts. Herbs and herbal extracts have been used since many years in many countries for medical purposes. Some researchers reported that plant extracts can be successfully used as feed additives in poultry (Hertrampf, 2001; Alçiçek *et al*., 2004; Williams and Losa, 2001; Bölükbaşı *et al*., 2009; Rahman *et al*., 2018).

It is considered that plants and plant extracts have a number of beneficial effects on poultry. They increase feed consumption and improve the immune system since they have antibacterial, anticoxidical, antihelminthic, antiviral and antioxidant properties.

Mentha belongs to the genus of Labiatae family, and it is estimated that there are 20 species of mentha spread all around the world. It is known that some species of mentha have been used by various traditional medical practices (Jager *et al*., 2006; Stafford *et al*., 2008; Vitalini *et al*., 2009). Furthermore, it is a commonly served garnish as herbal tea or spice in the Mediterranean diet (Conforti *et al*., 2008).

Mentha pulegium L. is a native species of Asia and the near East (Chalchat *et al*., 2000). It has been reported that *M. pulegium* L. has been used in traditional medicine for flu, respiratory illnesses, gastrointestinal disorders etc. Some researchers reported that *M. pulegium* L. have exhibited antimicrobial and antioxidant properties *in vitro* and *in vivo* (Sivropoulou *et al*., 1995; Mahboubi and Haghi, 2008).

There is no enough research which investigated the antioxidant activities of *M. pulegium* L. in laying hens. This study was to determine the effects of dietary supplementation of *M. pulegium* L. extract on performance, the quality of egg, some blood parameters and glutathione peroxidase (GPx), malondialdehyde (MDA) and superoxide dismutase (SOD) in serum of laying hens.

MATERIALS AND METHODS

The experiment was performed with 60, 40 weeks old laying hens (Lohman LSL). They were fed ad libitum with feed and water. The control group was fed on basal

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diet, and the experiment group was given 32.5 mg/kg MPE (half of activity of BHA), 65 mg/kg MPE (equivalent to BHA activity) and 130 mg/kg MPE (twice the activity of BHA) and 50 mg/kg BHA supplementation.

Table I. The composition of the basal diet (g/kg).

Item	Composition (g/kg)
Corn	371.21
Wheat	208.63
Soybean meal	96.03
Sunflower meal	95.00
Poultry meal	40.00
Bonkalite	75.00
Vegetable oil	5.00
Salt	1.76
Lysine-HCL	0.85
Limestone	100.98
Vitamin mineral mixture[1]	2.00
$NaHNO_3$	1.07
DCP	2.47
Total	1000
Calculated composition (%)	
Crude protein	16.70
Crude fat	3.43
Crude cellulose	4.13
Crude ash	13.40
ME (kcal/kg)	2724
Analysed composition (%)	
Crude protein	16.90
Crude fat	3.27
Crude cellulose	4.59
Crude ash	13.6
ME (kcal/kg)	2700

[1]Per kg diet added: 12000 IU vitamin A, 2500 IU vitamin D_3, 30 IU vitamin E, 3.4 mg vitamin K_3, 3 mg vitamin B_1, 6 mg vitamin B_2, 30 mg niacin, 10 mg calcium D-pantothenate, 5 mg vitamin B_6, 0.015 mg vitamin B_{12}, 1 mg folic acid, 0.050 mg D-biotin, 50 mg vitamin C, 125 mg choline chloride, 80 mg manganase, 60 mg iron, 60 mg zinc, 5 mg copper, 0.5 mg cobalt, 0.2 mg iodine, 0.15 mg selenium.

M. pulegium L. plant was harvested from its natural habitat, Erzurum, Turkey, in early July. The plant material was cleaned and then dried at room temperature. Then, *M. pulegium* was extruted according to Kordali *et al*. (2009). The antioxidant activity of *M. pulegium* extract was determined by DPPH method according to Gülçin (2005). The antioxidant activity of the extract is 65% based on BHA. The antioxidant activity of the extract is equivalent to 65% of BHA.

Feed intake and egg production were measured every two weeks and accordingy, feed conversion ratio was calculated. The criteria of egg quality such as Hough units, egg weight, shell breaking strength, shell thickness, yellowness, shape index and ratio of albumen, yolk and shell were also determined every two week.

The blood samples collected from six birds in each group at the end of the experiment were centrifuged at 4000×g for 10 minutes at +20 ºC. Then, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), glucose, serum cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured using biochemical automatic analysis. Serum superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) values have been determined by commercial kits (SOD Assay kit, No:706002, Cayman, USA; Glutathione Assay kit, No: 10009055, Cayman, USA; Glutathione Assay kit, N0: 703002, Cayman, USA).

Egg samples from each group at the end of the experiment were stored at + 4 ° C to determine the value of thiobarbituric acid reactive substance (TBARS), a marker of beta-oxidation of lipids. Malondialdehyde (MDA) levels were determined according to Tarladgis *et al*. (1960) at 0, 21 and 42 d.

Statistical analyses

The data obtained from this experiment were analyzed using one-way ANOVA and Duncan's multiple range test (SPSS, 1999). Least Significant Difference Test ($P<0.05$) was applied to the treatment means.

RESULTS

The measured values of feed intake, egg weight, egg production and feed conversion ratio are presented in Table II. Dietary supplementation of *M. pulegium* L . extract (MPE) had no effect on feed intake. Feed conversion ratio and egg production was significantly different between the control and experimental groups ($P < 0.05$). The dietary supplementation of MPE improved the feed conversion ratio and increased egg weight.

The impact of MPE on some egg quality criteria were listed in the Table III. The difference among the groups was not significant in terms of shell thickness, shape index, Hough units and ratio of albumen, yolk and egg shell. However, shell breaking strength increased in MPE supplemented groups ($P<0.05$), and the highest shell breaking strength value was observed in 130 mg/kg

Table II. Effects of MPE on performance of the laying hens.

Groups	Feed intake (g)	Egg weight (g)	Egg production (%)	Feed conversion ratio (g:g)
Control	128.3± 5.1	59.7±3.2b	76.2±3.2b	2.82±0.2a
MPE 32.5 mg/kg	126.6±3.9	66.3±1.3a	89.7±1.3a	2.13±0.1b
MPE 65 mg/kg	135.9±4.5	65.3±1.8a	88.2±1.8a	2.36±0.1b
MPE 130 mg/kg	123.5±6.0	59.5±2.9b	85.0±2.9a	2.44±0.1b
BHA 50mg/kg	122.1±4.1	63.3±3.8b	72.6±3.8b	2.65±0.9ab
P	NS	0.000**	0.000**	0.05*

a, b, The column average is significantly different; SE, standard error ; *, p<0.05; **, p<0.01; NS, Not significant.

Table III. Effects of MPE and vitamin E supplements on egg quality of the laying hens.

Groups	Albumen (%)	Yolk (%)	Egg shell (%)	Shape index	Shell breaking strength (kg cm2)	Yellowness	Shell thickness (µm)	Hough units
Control	62.2±0.6	27.7±0.5	10.1±02	76.2±054	2.12±0.34b	12.3±0.4a	442.1±11.3	74.5±4.1
MPE 32.5 mg/kg	61.6±0.6	28.5±0.5	9.91±0.2	76.3±0.94	2.20±0.49b	12.3±0.5a	445.0±27.7	77.7±3.6
MPE 65 mg/kg	61.9±0.5	28.2±0.5	9.90±0.2	77.0±0.75	2.02±0.23b	11.0±0.3ab	447.9±24.4	76.7±3.0
MPE 130 mg/kg	60.7±0.6	28.9±0.6	10.5±0.2	77.4±0.66	2.52±0.31a	12.4±0.4a	465.8±23.8	73.7±4.1
BHA50 mg/kg	63.1±0.6	27.3±0.5	9.57±0.3	76.2±0.85	2.61±0.34a	10.4±0.8b	494.2±31.2	85.8±2.6
P	NS	NS	NS	NS	0.05*	0.020*	NS	NS

a, b, The column average is significantly different; SE, standard error; *, p<0.05; **, p<0.01; NS, Not significant.

Table IV. Effects of MPE supplements on blood serum biochemical parameters and some antioxidant enzymes of the laying hens.

Groups	Control	MPE 32.5 mg/kg	MPE 65mg/kg	MPE 130 mg/kg	BHA 50 mg/kg	P
MDA	5.30 ± 0.3a	6.90 ± 0.3a	6.25±0.2a	3.43 ± 0.4b	5.70 ± 0.2a	0.04*
GSHPx	51.8± 1.9a	45.6± 3.0a	53.9 ±2.0a	48.1 ± 3.9a	37.2 ±2.2b	0.05*
SOD	0.86 ± 0.1b	0.88 ±0.0b	1.04 ± 0.2a	1.08±0.1a	0.64±0.0b	0.037*
AST	209.3 ± 9.1	247.0± 23.3	171.3±9.5	209.3 ± 21.8	205.0± 12.1	NS
ALT	6.0±0.4	8.0±0.4	10.0±0.2	2.0±0.5	2.6±0.8	NS
TG	537±11.7	651±17.0	842±43.0	442±27.0	990±69.0	NS
Cholesterol	66.0±6.0	164±18.0	129±8.5	108±22.0	120±13.0	NS
HDL-C	30.0±5.5	36.3±7.4	42.0±5.6	45.0±4.1	34.0±1.0	NS
LDL-C	50.0±9.3	38.0±4.9	44.0±0.6	36.6±3.7	40.64±3.8	NS
Glucose	240±10.7	165±38.4	158±5.1	209±25.5	210±34.0	NS

a, b, The raw average is significantly different; SE, standard error; *, p<0.05; NS, Not significant.

MPE and 50 mg/kg BHA supplemented groups. In the BHA group, the value of yellowness was found to be significantly lower than the other groups.

There is no statistically significant difference (P> 0.05) between the groups in terms of AST, ALT, triglyceride (TG), total cholesterol, HDL-cholesterol, LDL-cholesterol and glucose in Table IV. Blood serum MDA, GSHPx and SOD values were significantly (P <0.05) different between the groups. MDA ration, which is an end product of lipid peroxidation and used to indicate the level of oxidative damage, was found to be significantly lower (P <0.05) in the group supplemented with 130 mg / kg of the extract than the other groups. GSHPx was found to be significantly lower in the BHA group as a synthetic antioxidant than in the other groups. SOD value was significantly higher in groups supplemented with 65 and 130 mg / kg of yarn

extract.

MDA value decreased by supplementation with the addition of 65 mg /kg MPE at 1d. On the 21st and 42nd days of storage yolk MDA level was significantly decreased in MPE and BHA groups compared with control group. Group x Day interaction was found to be insignificant.

Table V. Effects of MPE supplements on TBARS (mg MDA/kg) values in egg yolk of the laying hens

Groups	1 d	21 d	42 d
Control	0.148±0.002a	0.160±0.026a	0.220±0.027a
MPE 32.5 mg/kg	0.146±0.003a	0.148±0.007b	0.198±0.022b
MPE 65mg/kg	0.111±0.003b	0.141±0.004b	0.154±0.019b
MPE 130 mg/kg	0.148±0.001a	0.149±0.007b	0.160±0.017b
BHA 50 mg/kg	0.110±0.015b	0.139±0.009b	0.155±0.011b
P	0.043*	0.045*	0.050*
Groups	0.015 *		
Days	0.003**		
Group × Days	NS		

a, b, The column average is significantly different; SE, standard error; *, $p<0.05$; **, $p<0.01$; NS, Not significant.

DISCUSSION

We have determined that dietary supplementation of MPE did not lead to any change on feed intake. Similarly, Arjomandi *et al*. (2011) and Nobakth *et al*. (2011) discovered that pennyroyal supplement had no effect on the feed intake of laying hens. The supplementation of 32.5 and 65 mg/kg MPE in the diet increased egg weight. However, Paymard *et al*. (2013) noted that 0.3% pennyroyal extract supplementation decreased the feed intake and egg weight of laying hens.

The addition of MPE in the diet increased the egg production, and the feed conversion ratio was improved in all the dietary groups compared to the control group. Contrary to these results, Arjomandi *et al*. (2011), Nobakth *et al*.(2011a) and Paymard *et al*. (2013) reported that egg production and feed conversion ratio of laying hens decreased after the pennyroyal supplementation. However, Nobakht ve Mehmannavaz (2010) revealed that dietary supplementation of pennyroyal improved the performance and egg quality of laying hens. Furthermore, some researchers discovered the positive effects of dietary pennyroyal supplementation on performance of broilers (Erhan *et al*., 2012; Nobakht *et al*., 2011b; Hardai *et al*., 2010; Modiry *et al*., 2010; Geran *et al*., 2010).

The results of this experiment are inconsistent with the results of previous studies. The results obtained from the present study and the previous studies are contraversial, which may be related to practical differences such as the amount of supplementation of pennyroyal, the selection of animal breeds or using pennyroyal extract instead of pennyroyal plant.

Yellowness, which is an internal quality criterion which is gaining importance especially for marketing and consumer, is formed by color materials known as xanthophylls. It was determined that the BHA supplement significantly reduced the yellowness value. There was no relationship observed between shell thickness, shape index, Hough Unit and ratio of albumen, yolk and egg shell and dietary MPE and BHA supplementation. However, breaking strength significantly increased in the experimental group with MPE and BHA supplementation. Contrary to the findings of this study, Nobakth *et al*. (2011) reported that adding pennyroyal to the diet increased shell thickness in layers. Paymard *et al*. (2013) reported that dietary supplementation of pennyroyal extract in the diets of laying hens did not change egg shell weight, egg shell thickness, shell breaking strength and yellowness whereas the Hough Unit of egg increased. Similarly, Nobakth *et al*. (2011) concluded that pennyroyal did not cause any change in shell weight, yellowness and Hough Unit.

AST and ALT are important enzymes indicating the degree of damage in the liver. In this study, it was determined that no blood parameters including ALT and AST was affected by MPE and BHA. Similar our results, Bölükbaşı *et al*. (2018) reported that 50 and 100 mg/kg *M. pulegium* extract did not effect on ALT, AST, glucose, TG, cholesterol, HDL and LDL in blood serum of layers.

MDA, which is a marker of the oxidation level in the body, was reduced by addition of MPE in serum. Serum MDA level significantly decreased in 130 mg/kg MPE group compared to the other groups. Many researchers have found that some aromatic plants have antioxidant properties (Lado *et al*., 2004; Bölükbaşı *et al*., 2006; Chikhi *et al*., 2012). A series of studies conducted to elucidate the antioxidant properties of pennyroyal support the findings of this study (Kamkar *et al*., 2010; Çöteli *et al*., 2013). Ruberto and Baratta (2000) reported that menthole and pulegone have antioxidant properties. For this reason, it is estimated that the reduction in MDA level is due to the main components of MPE, menthone and pulegone.

GSH-Px and SOD are endogenously synthesized antioxidant enzymes. SOD neutralizes superoxide radicals and protects cells from harmful effects of superoxide radicals. According to our findings, BHA significantly reduced the GSHPx value, but the MPE had no effect on the GSHPx value. SOD activity significantly increased 65 and 130 mg/kg MPE groups. It is estimated that pennyroyal may have a positive effect on the SOD activity by lowering the MDA in the blood serum in 130 mg/kg MPE group.

Some previous studies have obtained similar data (Lin *et al*., 2005; Jiang *et al*., 2013).

It was reported that 40 g/L *Mentha spicata* tea significantly decreased activity of SOD and GSH-Px in the rats kidney (Akdoğan *et al*., 2003). Similarly, it was reported supplementation thymol and carvacrol in broiler diet increased GSH-PX and SOD and reduced the MDA level in the muscles of broilers (Hashemipour *et al*., 2013). However, Kostadinoviç *et al*. (2010) found that 200 g/kg of *Mentha piperita* improved GSH-Px in blood of the broilers.

Thiobarbituric acid reactive substances (TBARS), secondary products of lipid peroxidation, are an important parameter used in the determination of lipid peroxidation. In our study, we found that egg yolk MDA values were significantly reduced by the addition of the MPE. It has been reported that *M. pulegium* belonging to the Labiatae family has antioxidant properties. The antioxidant effect of phenolic compounds is due to their ability to purify free radicals, to form compounds with metal ions, and to inhibit or reduce the formation of singlet oxygen. Erhan *et al*. (2015) reported that the addition of 0.50% *M. pulegium* powder to broiler chicks diets increased oxidative stability in the muscles.

CONCLUSION

As a result, it was found that the addition of MPE to the ration improved egg weight, egg production, feed conversion ratio, and egg shell strength. The supplementation of 130 mg/ kg MPE to layer hen rations had positive effects on SOD enzyme activity, which is responsible for the enzymatic defense of antioxidant metabolism, and also reduced serum MDA levels. In general, it has been concluded that it may be beneficial for MPE to participate as feed additives due to these positive effects on laying hens.

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Statement of conflict of interset

The declare there is no conflict of interset.

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