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Research Article

EFFECT OF FEEDING *MENTHA PULEGIUM* L. POWDER ON EGG QUALITY CHARACTERISTICS AND CHOLESTEROL VALUE OF LAYING JAPANESE QUAILS

Tahereh Mohammadabadi [1]*, Fanos Zarei [2]

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ABSTRACT: The aim of this experiment was to evaluate the effect of feeding *Mentha pulegium* L. leaf powder on egg quality indexes and cholesterol content of laying quails. 150 one-day-old Japanese quail were used with 5 treatments, 3 replicates and 10 quail per replicate in a completely randomized design, then fed with experimental diets for 12 weeks. The diets were consisted of control diet and diets containing 0.5, 1, 1.5 and 2 % *Mentha pulegium* powder. In the end of trial, the egg quality parameters and cholesterol content were measured. The results revealed that diets containing *Mentha pulegium* caused to improvement haugh unit, yolk index, white weight, egg shell thickness and resistance ($p<0.05$), but the egg weight, egg shape index, yolk index, white weight percentage, yolk weight, yolk weight percentage and percentage of shell weight were not influenced by experimental diets ($p>0.05$). The highest haugh unit (89.63) was for diet containing 2 % *Mentha pulegium.* The using of *Mentha pulegium* in laying quail diet resulted in decrease egg cholesterol in compared with the control diet ($p<0.05$). It is concluded that the inclusion *Mentha pulegium* to 2 % in laying quail diet had beneficial effects on some egg indexes and decreasing of cholesterol, therefore it is recommended that *Mentha pulegium* to be used at 2 % level in nutrition of laying quail.

Key words: *Mentha pulegium*, Egg quality, Quail, Cholesterol.

INTRODUCTION

Currently, because of antibiotic resistant, the animal feed industry reduces the use of antibiotic as growth promoters in poultry diet, that can have substituted by herbal medicine. *Mentha pulegium* L., one of the species of peppermint and belongs to *Lamiaceae* family is commonly known as pennyroyal that is widespread in the nature (Chalchat *et al*. 2000). This plant grows in the humid plains and the margins of water currents (even in the water), most in the central, southern and western parts of Europe, southwest Asia, northern Africa, Habsche and the Canary Islands. Also it is distributed in the northern parts of Iran; Gilan, Rasht, sandy beaches of Bandar Anzali, Shah Darvishan near the Lagoon, wetlands of Lahijan, Gorgan and Minoodasht (Shahverdi *et al.* 2004).

Chemical compositions of different parts of this plant contain tannin, resin and pectics materials, glucose and essential oils. Its essential oils contain pulegeone, alcohol, linalool, limonene, dipentene and azulen. The species of labiate family, like thyme and *Mentha pulegium*, show the antioxidant and anti-microbial properties due to the high amounts of monoterpenes, thymol and carvacrol. In addition to high anti-microbial and antioxidant properties, *Mentha pulegium* also is antifungal and stimulating appetite, and increases digestibility of nutrients and improves digestive system environment (Nobakht and Shahryar 2008). Aerial parts of *Mentha pulegium* flowers are commonly used as a substance in the treatment of colds, sinusitis, cholera, food poisoning, inflammation of the pancakes, and also in the treatment of tuberculosis, and has the property anti-bloating, spatula and anti-cough and anti-menstrual bleeding (Parlat *et al*. 2005). The dominant component of the essential oil of *Mentha pulegium*, is pulegeone. The remaining components of this essential oil are α-pinene 0.4%, sabinene 0.15%, betapinene 0.48%, mirsen 0.3%, limonene 0.4%, 1,8 cineole 0.1 % and 0.45% germacron (Babakhanlou *et al.* 1998).

Cholesterol is an indicator of animal metabolism and many researchers have shown that there is a relationship between increased serum lipids and cardiovascular disease in humans, and cholesterol is a major contributor

[1]Associate Professor, [2] M. Sc. Graduated student, Department of Animal Science, Faculty of Animal Science and Food Technology, Khuzestan Agricultural Sciences and Natural Resources University, Ahvaz, Iran.
** Corresponding author. e-mail: mohammadabadi@ramin.ac.ir*

to these diseases (McDonald *et al*. 2002). Since cholesterol of egg has not been successfully reduced by genetic methods (Malekian *et al.* 2000), as well as the lack of acceptance of low cholesterol eggs produced by chemical and synthetic supplements (Sutton *et al.* 1984), therefore need to use natural compounds in the diet of birds for reducing of egg cholesterol is quite evident. Medicinal plants exhibit anti-oxidant, anti-microbial and immune-stimulating effects, gastrointestinal stimulant, lipid lowering, lowering blood cholesterol, and ultimately growth stimulants due to presence of active compounds in their tissues (Jafarnia *et al.* 2007).

It has been shown that the antioxidant compounds found in medicinal herbs can be transmitted to the egg via the circulatory system when used in laying hens (Jafarnia *et al.* 2007). In a report, adding ginger root to the diet of laying hens significantly reduced egg yolk cholesterol (Akbarian *et al.* 2010). But other researchers showed adding of aniseed oil to laying quail had no statistical effect in terms of egg cholesterol (Bayram *et al.* 2007). Therefore, aim of this trial was study of using *Mentha pulegium* on egg quality indexes and egg cholesterol content in laying quails.

MATERIALS AND METHODS

Animals and diets

At the beginning of the experiment, 150 Japanese quails fed until the age of 12 weeks with experimental diets. The chicks were divided into 15 experimental units including 5 treatments, 3 replicates, and 10 replicates in each replicate (cage). Diets were arranged according to the requirements of the poultry diet (NRC 1994). All birds were placed on floor pens in a temperature-controlled house and had free access to feed and water daily. The lighting program consisted of a period of 16 h light and 8 h darkness. The ambient temperature in experimental house was maintained at 34-35 ^{0}C on the first day and gradually decreased to 23 ^{0}C upto 35 days and then fixed on 21-25 ^{0}C to end of laying period. The chemical composition of *Mentha pulegium* powder was given in Table 1. The ingredients of the experimental diets are shown in Table 2.

The wheat bran was replaced by *Mentha pulegium* powder, as a percentage of the diet. The experimental diets during laying was including: control diet (without *Mentha plegium* powder), diet containing 0.5% *Mentha pulegium* powder, diet containing 1% *Mentha plegium* powder, diet containing 1.5% *Mentha pulegium* powder, and diet containing 2% *Mentha plegium* powder. Egg production recorded daily. Egg quality parameters and egg cholesterol levels were also evaluated in this experiment.

Cholesterol measuring

After separating the yolk from the whites, the amount of egg yolk cholesterol was measured by enzymatic method (Pasin *et al.* 1998). First, one g yolk was added to 9 ml of 2% salt solution. Then was vigorously shaken in a container with a tight lid, at room temperature for 2 hours. Then, 10 µl of the sample with 100 µl of 2% salt solution and one µl of the reagent (commercial kit of Pars Azmon) was injected in the micro tube. To prepare the standard, 10 µl of the standard of Pars Azmon (at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg) added to 100 µl of salt solution and all the samples and standard put in the incubator at 37°C for 15 minutes. To prepare blank, we used from 10 µl distilled water, 100 µl of salt solution and 1 ml reagent. At the end, put 200 µl of each sample, as well as standard, and blank in microplate and read at 546 nm. Then, based on the dilution factors, the primary weight of sample, the standard concentration and the optical absorption results; the cholesterol concentration was calculated as mg/g of egg yolk.

Statistical analysis

All data were analyzed using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC, USA, 2005) for a completely randomized design based on the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$. where Y_{ij} is the observation, μ is the general mean, T_i is the effect of *Mentha pulegium* powder and e_{ij} is the SE of term. The Duncan's multiple range test was used to compare the mean difference at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of different levels of *Mentha pulegium* powder on weight and qualitative characteristics of yolk and white of quail egg at 12 weeks is shown in Table 3. *Mentha pulegium* powder significantly improved the

Table 1. Chemical composition of *Mentha plegium* powder.

Gross energy (Kcal/Kg)	DM(%)	CP(%)	Ash(%)	CF (%)	EE(mg)	Ca(%)	P(%)	Urea(%)
4903.6	97.40	14.96	8.86	9.38	8.99	2.46	0.04	0.00

Table 2. Ingredients and chemical composition of experimental diets containing *Mentha plegium* in laying quails (%).

Ingredients	The level of *Mentha plegium* (%)				
	0	**0.5**	**1**	**1.5**	**2**
Corn	55.45	55.45	55.45	55.45	55.45
Soybean meal	31.44	31.44	31.44	31.44	31.44
Wheat bran	3.00	2.5	2.00	1.5	1.00
Mentha plegium powder	0.00	0.5	1.00	1.5	2.00
Vegetable oil	2.30	2.30	2.30	2.30	2.30
Oyster meal	5.72	5.72	5.72	5.72	5.72
Dicalcium phosphate	1.03	1.03	1.03	1.03	1.03
NaCl	0.16	0.16	0.16	0.16	0.16
DL-methionine	0.16	0.16	0.16	0.16	0.16
$NaHCO_3$	0.22	0.22	0.22	0.22	0.22
Mineral permix[1]	0.25	0.25	0.25	0.25	0.25
Vitamin permix[2]	0.25	0.25	0.25	0.25	0.25
Metabolizable energy (Kcal/Kg)	2781.69	2781.69	2781.69	2781.69	2781.69
Protein	19.18	19.18	19.18	19.18	19.18
E/P	145.00	145.00	145.00	145.00	145.00
Ca	2.50	2.50	2.50	2.50	2.50
P	0.35	0.35	0.35	0.35	0.35
Lysine	1.00	1.00	1.00	1.00	1.00
Methionine	0.45	0.45	0.45	0.45	0.45
Na	0.15	0.15	0.15	0.15	0.15
Cl	0.14	0.14	0.14	0.14	0.14

[1] Each 2.5 kg of mineral premix is containing: Mn 66000 mg; Fe 33000 mg; Cu 880 mg; Zn 66000 mg; I 900 mg; Se 300 mg.
[2] Each 2.5 kg of vitamin premix is containing: vitamin A 7700000 IU; vitamin D_3 300000 IU; vitamin E 6600 IU; vitamin K3 300000 mg; thiamin 1500 mg, riboflavin 4400 mg; nicotinic acid 5500 mg; pantothenic acid 22000 mg; vitamin B_{12} 8.8 mg; pyridoxine 3000; folic acid 110 mg.

haugh unit, yolk color index and white weight ($p < 0.05$). According to the obtained data, 2% *Mentha pulegium* powder had the highest haugh unit ($p < 0.05$). Also, in agreement of this result, Chehrei *et al.* (2010) reported the use of levels 0.05, 0.1 and 0.15 of herbal supplement (containing 0.05, 0.1 and 0.15 of thyme and garlic essential oil) in laying hen had a significant effect on increasing the haugh unit in compared to the control. Florou Paneri *et al.* (2006) concluded the supplementation of rosemary herb in diets significantly increased Haugh unit value.

In contrast to the current trial, the researchers reported levels of 0.5, 1, 1.5 and 2 % of *Mentha pulegium* in laying hen had no significant effect on the haugh unit (Nobakht and Shahryar 2008). Also, Bayram *et al.* (2007) reported that the addition of anise oil to the quail diet did not change the haugh unit. The other researchers reported addition of peppermint (Cetingul *et al.* 2008) to diet of laying quails and different plant extracts (Bozkurt 2009) had not any significant influence on haugh unit.

On base of the result, all levels of *Mentha pulegium* powder increased the yolk color index and diet containing 1.5% had the highest yolk color index ($p<0.05$). The increase of the yolk index in group containing herbal medicine was due to the transfer of pigments such as the xanthophyll from plant to the eggs and their sedimentation in the yolk that lead to change in yolk color (Jaderi *et al*. 2011). The addition of peppermint to diet of laying quails positively influence yolk color index that could be due to carotenoids in peppermint (Cetingul *et al.* 2008). In

Table 3. Effect of experimental diets containing *Mentha plegium* on egg yolk and white quality of laying quails.

	The level of *Mentha plegium* (%)						
Parameters	**0**	**0.5**	**1**	**1.5**	**2**	**SEM**	**p value**
Egg weight	9.11	10.26	9.68	10.21	10.19	0.35	0.16
Haugh unit	87.42^{ab}	85.62^{b}	85.51^{b}	84.00^{b}	89.62^{a}	0.96	0.02*
Egg shape index	77.02	75.00	77.31	76.71	81.52	2.45	0.51
Yolk index	46.02	44.46	42.63	44.46	45.19	1.42	0.55
Yolk color index	3.83^{b}	5.00^{a}	5.66^{a}	6.00^{a}	5.66^{a}	0.33	0.003**
White weight	4.58^{b}	4.93^{ab}	4.85^{b}	5.50^{a}	5.16^{ab}	1.63	0.03*
White weight percentage	50.42	50.74	50.22	53.91	50.77	151	o.68
Yolk weight	3.25	3.48	3.45	3.21	3.64	0.18	0.56
Yolk weight percentage	35.51	36.05	35.58	31.45	35.66	1.41	0.42

Values bearing different superscripts differ significantly ($p > 0.05$). SEM = standard error of mean. (** = $p < 0.01$; * = $p < 0.05$).

addition, fructo-oligosaccharides in medicinal herbs have stimulating effects on digestive enzymes of the stomach, pancreas and mucous membranes of the intestine, which improve digestibility and absorption of ileum and increase the absorption of these pigments. Increasing the color of egg yolk by using natural colors in diet of laying hens has been confirmed by Aydin *et al.* (2008). Also, Seyed Piran *et al.* (2011) reported that the use of probiotic, organic acid and mixed herb medicines had a significant effect on egg yolk color index.

The treatments containing 1.5% *Mentha plegium* had the highest white weight ($p<0.05$). The results of the present experiment were in agreement with the results of Taghinejad (2010) that reported that using 1% *Mentha pulegium* in laying hens increased white weight.

The result indicated the egg weight, egg shape index, yolk index, white weight percentage, yolk weight, and yolk weight percentage was not affected by treatments containing *Mentha pulegium.* The other researchers also reported adding of anise oil as medicinal herb in laying quail were not influenced egg quality parameters (Bayram *et al.* 2007). Also Cetingul *et al*. (2008) reported that the addition of peppermint to diet of laying quails had not any significant influence on egg shape index.

Florou Paneri *et al*. (2006) found there is no significant difference between groups receiving rosemary herb in terms of egg shape index and yolk index. But Cetingul *et al*. (2008) reported supplementation of peppermint to diets decreased the yolk index value. The lowest yolk index value was determined in high content of peppermint, while highest value was seen in 10 ppm peppermint.

The results of Table 4 showed, *Mentha pulegium* powder significantly improved the egg shell thickness and resistance ($p <0.05$) but shell weight percentage was not different between diets containing various levels of *Mentha pulegium* ($p >0.05$). The shell thickness in the treatment containing 1.5% *Mentha pulegium* had the highest values ($p <0.05$). Increase in shell thickness can be due to the beneficial effects of the active matters in this plant on improving the digestive system and increasing its function in absorbing various nutrients, including minerals, that resulting in increased calcium deposits in the shell, and greater weight of shells of eggs, so that the improvement of digestive system function to absorb these materials especially calcium, that caused to increasing of the shell thickness.

The effect of experimental treatments on shell thickness was reported in a study on the effects of cumin on the performance of laying hens that in addition to the yolk weight and egg components, the percentage of production and shell thickness were improved by adding 3% cumin to the diet. Akhtar *et al.* (2003) also reported that the addition of *Nigella sativa* at 0.5, 1, and 1.5%, significantly improved the egg shell thickness. Contrary to the current study, Arshami *et al.* (2010) reported levels of 0.5, 1.5 and 2.5 % of cumin was not affected shell thickness in laying hens.

The diet containing 1.5% *Mentha pulegium* had the highest amount of shell weight ($p <0.05$). Significant increase in egg shell weight in experimental groups containing medicinal herbs could be related to the reduction of egg size, which caused to the ratio of shell weight to egg weight increased (Jaderi *et al.* 2011).

In contrast to the present experiment, Jaderi *et al.* (2011) explained using Ziziphora plant in diet of laying

Table 4. Effect of experimental diets containing *Mentha plegium* on egg shell indexes of laying quails.

	The level of *Mentha plegium* (%)						
	0	**0.5**	**1**	**1.5**	**2**	**SEM**	**p value**
Shell weight	1.27^{b}	1.27^{b}	1.37^{ab}	1.49^{a}	1.38^{ab}	0.55	0.04*
Shell weight percentage	14.05	13.19	14.18	14.63	13.55	0.54	0.57
Shell thickness	23.66^{ab}	19.66^{b}	25.00^{a}	27.00^{a}	22.40^{ab}	1.42	0.04*
Shell resistance	0.21^{b}	0.14^{b}	0.12^{b}	0.47^{a}	0.16^{b}	0.07	0.03*

Values bearing different superscripts differ significantly (p > 0.05). SEM = standard error of mean. (** = p <0.01; * = p <0.05).

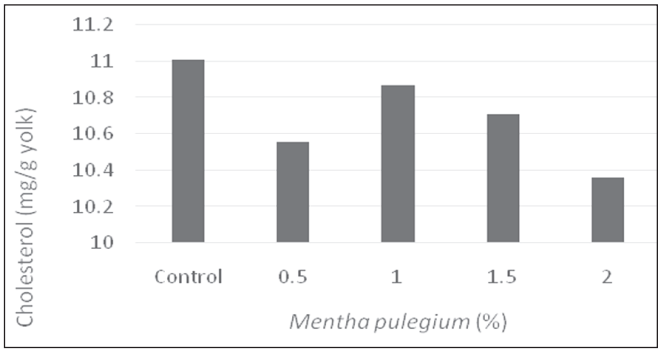

Fig. 1. Effect of experimental diets containing *Mentha plegium* on egg cholesterol of laying quails (mg/g yolk).

hens significantly decreased the egg weight. Aami Azghadi *et al.* (2010) also reported the effect of different levels of thyme on the weight of egg shell and other quality traits of egg was not significant.

The shell resistance was highest at level of 1.5% *Mentha pulegium* (p <0.05). It seems that by increasing the shell thickness, eggs resistance increases. Aydin *et al.* (2008) reported that adding 3% *Nigella sativa* extract to diet of laying hens increased the shell thickness and resistance (kg/cm$_2$) that were consistent with the present study.

The results of the experimental treatments on the cholesterol amount in quail eggs shown in Fig. 1, that yolk cholesterol levels were significantly affected by the treatments (p <0.05). The treatments containing *Mentha pulegium* had lower cholesterol than control treatment. According to current results, Nobakht and Shahryar (2008) explained the using of 2% *Mentha pulegium* significantly reduced the level of blood cholesterol in laying hens due to the type and level of the used plant and the production stage of hens. Also Bayram *et al.* (2007) reported there were no statistical differences in terms of egg cholesterol among the groups fed with anise oil. The recent study confirmed by the result of Cetingul *et al.* (2007) who have studied the effect addition *Thymus vulgaris* leaf to the laying quail diet on egg cholesterol as well.

Aydin *et al.* (2008) reported, adding *Nigella sativa* to the diet of laying hens reduced triglycerides and total serum cholesterol, as well as increased serum HDL. The mechanism that herbs reduces yolk cholesterol is still unknown. Cholesterol biosynthesizes in the liver of laying hens and enters the vitiliginin and VLDL particles that enter the bloodstream and then are absorbed by endocytosis by the growing egg. Therefore, it has been suggested that the reduction of egg cholesterol due to decrease in the synthesis of cholesterol in the liver (Aidin *et al.* 2008). Also, the pure components of essential oils prevent the activity of the β-hydroxy- β-methyl glutaryl coenzyme reductase (HMG-CO), an enzyme that regulates cholesterol production in the liver, which is the reason of the essential oils to be expected to reduce cholesterol (Ali *et al.* 2007). Also, the effect of *Nigella sativa* on reduce of egg cholesterol has been reported by Akhtar *et al.* (2003). But Cetingul *et al.* (2008) concluded cholesterol values of quail eggs were significantly different between the groups, and the highest was for 10 and 20 ppm peppermint.

CONCLUSION

The adding of *Mentha pulegium* herb significantly improved the Haugh unit, yolk color index, white weight, shell weight, egg shell thickness and shell resistance, but the egg weight, egg shape index, yolk index, white weight percentage, yolk weight, yolk weight percentage and the percentage of shell weight was not affected. Also according to the results it is revealed that all levels of *Mentha pulegium* powder reduce the cholesterol of egg yolk. Therefore, it is concluded that high levels of *Mentha pulegium* powder (1.5% and 2%) in diets of laying quail, had a positive effect on improving quality traits of egg, that using of *Mentha pulegium* herb as a benefit supplement in laying quails diet, is recommended.

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