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The Effects of Different Levels of Dried Aerial Parts Powder and Extract of Pennyroyal (*Mentha pulegium*) Medicinal Plant on Performance, Egg Quality, Blood Biochemical and Immunity Parameters of Laying Hens

Research Article

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ABSTRACT

This experiment was conducted to evaluate the effects of different levels of dried aerial parts powder and extract of pennyroyal (*Mentha pulegium*) medicinal plant on performance, egg traits, blood biochemical and immunity parameters of laying hens. Two-hundred-fifty-two Hi-line (W36 strain) laying hens from 46 until 58 weeks of age were used in a completely randomized design in 7 treatments and 3 replicates (12 birds per replicate). The treatment groups consisted of a control group (1) with no dried aerial parts powder or extract of pennyroyal supplementation, and experimental groups 2, 3 and 4 contained 0.5%, 1% and 1.5% of pennyroyal powder respectively, whereas experimental groups 5, 6 and 7 contained 0.1%, 0.2% and 0.3% of pennyroyal extract, respectively. Using different levels and forms of pennyroyal significantly affected the performance, egg traits, blood biochemical and immunity parameters of laying hens ($P<0.05$). By increasing the level of pennyroyal extract to 0.2% and 0.3% of rations, the performance of laying hens significantly decreased, whereas pennyroyal powder improved the performance of laying hens, consequently the best feed conversion ratio (2.46) was achieved by using 1.5% pennyroyal powder. The highest specific gravity (1.092) and Haugh unit (89.22) were resulted in groups 5 and 6, respectively. The lowest amount of blood triglyceride (270.4 mg/dL) and the highest amounts of albumin (3.05 mg/dL), total protein (5.40 mg/dL) and uric acid (5.39 mg/dL) were observed in group 7. The lowest percent of hetrophil (9.67%) and the highest percent of lymphocyte (90%) were observed in group 2 and the highest amount of white blood cells (266601/mm^3) was resulted in group 5. The overall results showed that the use until 1.5% of dried aerial parts powder and 0.1% of pennyroyal (*Mentha pulegium*) extract compared with control group had positive effects on performance of laying hens. Using different levels of pennyroyal powder and extract could change the egg traits, blood biochemical parameters and immunity levels of laying hens.

KEY WORDS blood metabolism, egg traits, laying hens, *Mentha pulegium*.

INTRODUCTION

Medicinal plants, their extracts and essential oils have a wide range of activities, including inhibitory action on pathogens, effects on physio-pathologies and activity in different body systems, e.g. endocrine and immune system (Francois, 2006). In poultry, herbs and spices are not just appetite and digestion stimulants, but they can impact on other physiological functions, help to sustain good health and welfare and also improve their performance (Frankic *et al*. 2009). In recent years, the use of various forms of medicinal plants in poultry has a growing trend. They reported

that nettle powder had positive effects on carcass traits of broilers (Nasiri *et al.* 2011), whereas 1 g/kg nettle extract did not have any significant effects on growth performance of broilers (Khosravi *et al.* 2008). Mixture of three medicinal plants (0.5% *Malva silvestris*, 1% *Alhaji maurorum*, 0.5% *Mentha spicata*) improved the carcass percent and decreased the blood glucose, however did not have significant effects on immune cells of broilers (Nobakht and shahryar, 2010).

It was shown that adding 0.5% pennyroyal aerial parts powder significantly improved the performance of broilers (Nobakht *et al.* 2011).

Adding 2% of nettle nettle (*Urtica dioica*) dried areal parts powder in diets of laying hens significantly increased egg production percent, egg mass and eggshell weight.

The highest Haugh unit was recorded by using 2% of savory (*Satureja hortensis*), however, different levels of nettle, savory and ziziphora could not significantly affected the blood biochemical parameters and immunity cells (Jaderi *et al.* 2011) in this experiment. In another study by using different levels of *Thymus valgaris*, *Lamiaceae menthapiperita* and *Oreganum valgare*, the highest egg production percent, egg mass and the best feed conversion ratio, the highest Haugh unit and egg yolk color index were resulted by using 2% of *Oreganum valgare*, whereas the highest amount of feed intake and the lowest level of blood triglyceride were observed by adding 2% of *Lamiaceae menthapiperita* (Nobakht and Mehmannavaz, 2010). According to the results of other study, 2% blend of *Melissa officinalis*, *Tamacetum balsamila* and *Ziziphora clinopodioides* herbs could remarkably boost the performance and immunity of laying hens (Nobakht *et al.* 2012). Using 2% blends of ziziphora, menta, peppermint and nettle improved egg mass, feed conversion and egg Haugh unit in laying hens (Sayedpiran *et al.* 2011).

Mentha pulegium is one of the *Mentha* species commonly known as pennyroyal. It is native species of Europe, North Africa and in Asia Minor and near East.

The flowering aerial parts of *Mentha pulegium* has been traditionally used for its antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis, and also as anti-flatulent, carminative, expectorant, diuretic, antitussive, menstruate (Mahboubi and Haghi, 2008).

The ingredients of *Mentha pulegium* oil have been subjected to a number of studies which have shown a difference in its constituents depending on the region of cultivation and there have been some variations in the constituents from different countries. These studies showed three chemo-types of *Mentha pulegium* with the following major oil components (1) pulegone, (2) piperitenone and / or piperitone and (3) isomenthone / neoisomenthol (Mahboubi and Haghi, 2008).

This study focused on evaluation the effects of using different levels of dried aerial parts powder and extract of pennyroyal (*Mentha pulegium*) medicinal plant on performance, egg traits, blood biochemical and immunity parameters of laying hens.

MATERIALS AND METHODS

Animals and dietary treatments

Two-hundred-fifty-two of Hy-line laying hens (W36 strain) from 46 until 58 weeks of age were used in a completely randomized design in 7 treatments and 3 replicates (12 birds per replicate). The treatment groups consisted of a control group (1) with no levels of powder or extract of pennyroyal supplementation, and experimental groups 2, 3 and 4 contained 0.5%, 1% and 1.5% of dried aerial parts powder pennyroyal supplementation respectively, and experimental groups 5, 6 and 7 received 0.1%, 0.2% and 0.3% of pennyroyal extract respectively. The diets were formulated to meet the requirements of laying hen as established by the NRC (1994) (Table 1). Dried aerial parts of pennyroyal were supplied from local market and the compositions of it were determined according to AOAC (1994). Pennyroyal extraction was done by Shadnosh Company. Pennyroyal powder and extract fine mixed with other ingredients. The diets and water were provided ad libitum. The lighting program during the experimental period consisted of a period of 16 hours light and 8 hour of darkness. An average of environmental temperature was 15 ˚C.

Performance parameters and egg traits

Feed intake, feed conversion ratio, egg production percent, egg mass and egg weight were determined weekly on bird bases. Mortality was recorded if it occurred. The collected eggs were classified as normal or damaged; the latter included the following: fully cracks eggs (an egg with broken shell and destroyed membrane), hair cracks eggs (an egg with broken shell but intact membrane), the eggs without shell (an egg without shell but with intact membrane). For measuring of egg traits at the end of experiment period 3 egg sampled collected from each replicate. Determination of egg specific gravity was done by floating eggs in salty water. Content of egg shells were cleaned and shells were maintained in environmental temperature for 48 h until dried, then weighed with a digital scale in an accuracy of 0.01 (g). The thickness of egg shell was measured by micrometer with accuracy of 0.001 (mm) in the middle of egg and in three spots on three eggs. Then their average was considered as final thickness of egg shell for each experimental unit. Color index of the yolk (Roche color index), yolk index, albumen index, Haugh units were determined (Card and Nesheim, 1972).

Table 1 Composition of experimental diets

Feeds Ingredients	Control Group	0.5% Pennyroyal powder	1% Pennyroyal powder	1.5% Pennyroyal powder	0.1 % Pennnyroyal extract	0.2 % Pennnyroyal extract	0.3 % oyalPennnyr extract
Corn	53.12	52.38	51.64	50.90	52.58	52.70	52.90
Wheat	20	20	20	20	20	20	20
Soybean meal-44%	16.56	16.61	16.65	16.70	16.81	16.85	16.90
Vegetable oil	0.3	0.49	0.68	0.88	0.49	0.68	0.88
Pennyroyal	0	0.5	1	1.5	0.1	0.2	0.3
Oyster shell	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Dicalcium phosphate	8.15	8.15	8.15	8.15	8.15	8.15	8.15
Salt	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated composition							
Metabolisable energy (kcal/kg)	2800	2800	2800	2800	2800	2800	2800
Crude protein (%)	14	14	14	14	14	14	14
Ca (%)	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Available phosphor (%)	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine (%)	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Methionine + Cysteine (%)	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Tryptophan (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[1] Vitamin premix per kg of diet: vitamin A (retinol): 8500000 IU; vitamin D3 (cholecalciferol): 2500000 IU; vitamin E (tocopheryl acetate): 11000 IU; vitamin k_3: 2200 mg; Thiamine: 1477 mg; Riboflavin; 4000 mg; Panthothenic acid; 7840 mg; Pyridoxine: 7840 mg; Cyanocobalamin: 10 mg; Folic acid, 110 mg and Choline chloride: 400000 mg.
[2] Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 75000 mg; Mn (MnSO4.H2O, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu (CuSO4.5H2O): 6000 mg; I (KI, 58% I); 867 mg and Se (NaSeO3, 45.56% Se): 200 mg.

Blood biochemical parameters and immunity cells

At the end of experiment, two hens from each replicate were randomly chosen for blood collection and approximate 5 mL blood samples were collected from the brachial vein.

One mL of collected blood was transferred to tubes with EDTA for determination of heterophil, lymphocyte, hematocrit, hemoglobin, RBC and lukocyte blood cells counts. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope the heterophil to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983). The remaining 4ml blood was centrifuged to obtain serum for determination the blood biochemical parameters include: glucose, cholesterol include: glucose, cholesterol, triglyceride, albumin, total protein and uric acid. Kit package (Pars Azmoon Company; Tehran, Iran) were used for determination the blood biochemical parameters using Anision-300 auto-analyzer system.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005).

Means were compared using the Duncan multiple range test. Statements of statistical significance were based on $P<0.05$.

RESULTS AND DISCUSSION

Performance parameters

The effects of different levels of dried aerial parts powder and extract of pennyroyal in feeds on performance of laying hens are summarized in Table 2. Treatments included different levels of powder and extract of pennyroyal showed significant effects on performance of laying hens ($P<0.05$). Pennyroyal extract decreased the egg weight as well as the lowest amount of egg weight (56.71 g) was resulted by adding 0.3% pennyroyal extract into diet in group 7, meanwhile the heaviest eggs (59.58 g) were seen in control and 2 groups. The highest percent of egg production (57.85%), highest amount of egg mass (34.46 g), and the best feed conversion ratio (2.46) obtained in group 4 by adding 1.5% pennyroyal. Pennyroyal extract levels especially upper levels (0.3%) in compared with its dried aerial parts had adverse effects on egg production parameters and significantly decreased the amount of feed intake and increased feed conversion ratio. Indeed, the lowest percent of egg production, and the lowest amounts of egg weight, egg mass production, daily feed intake and the highest feed conversion were observed in group 7 by using 0.3% Pennyroyal extract. Most of the spices stimulate the function of pancreatic enzymes (lipases, amylases and proteases), some also increase the activity of digestive enzymes of gastric mucosa (Srinivasan, 2005).

Table 2 Effects of using different levels of pennyroyal powder and extract on performance of laying hens

Treatments	Egg weight (g)	Egg production (%)	Egg mass (g)	Feed intake (g)	Feed conversion ratio
1	59.58^{a}	56.22^{a}	33.50^{a}	84.50^{ab}	2.54^{bc}
2	59.58^{a}	57.43^{a}	34.23^{a}	86^{ab}	2.52^{bc}
3	59.31^{a}	52.68^{a}	31.25^{b}	84.50^{ab}	2.88^{b}
4	59.54^{a}	57.85^{a}	34.46^{a}	84.32^{ab}	2.46^{c}
5	58.98^{a}	54.98^{a}	32.44^{a}	82.32^{ab}	2.55^{bc}
6	58.21^{a}	47.67^{b}	27.68^{b}	76.28^{c}	2.60^{bc}
7	56.71^{b}	28.22^{c}	16.04^{c}	65.81^{d}	4.1^{a}
SEM	0.44	1.62	1.06	1.99	0.12
P-value	0.004	0.0001	0.0001	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Besides the effect on bile synthesis and enzyme activity, extracts from herbs and spices accelerated the digestion and shorten the time of feed / food passage through the digestive tract (Platel and Srinivasan, 2005; Suresh and Srinivasan, 2007).

Spices and herbs with growth promoting activity increased the stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganism's growth (Windisch *et al.* 2008). So, it might be possible that the increase of digestion and absorption of essential nutrients due to increasing the enzyme activity and / or inhibition of pathogenic microorganism's growth could be the main reason of pennyroyal medicine plant to accelerate the performance.

Indeed, increase of digestion and absorption of essential nutrients and increasing the availability and utilization of feed ingredients' energy and exist of sterols plant might lead to increasing egg production in group 4 by using 1.5% powder pennyroyal medicine plant.

There were positive correlation between egg weight with percent or weight of albumen and egg weight with percent or weight of yolk. So, increase of percents yolk and albumen could probably lead to increasing of egg weight in control group. Several studies indicated that egg weight and egg production were indices for evaluation of egg mass.

Hence, it could be possible that the increasing of egg weight due to increase percents of albumin and yolk might be the main cause of improvement in egg mass in control group. Most of findings showed that improve of feed conversion ratio were related to increase amount of egg mass. Consequently, increase of egg mass might lead to improve feed conversion ratio in control group. Our results about pennyroyal aerial parts powder were in agreement with reports of (Nobakht *et al.* 2011; Sayedpiran *et al.* 2011). The higher concentration of active ingredients such as antimicrobial agents in pennyroyal extract might cause a wide range loss in beneficial microorganisms of digestive tract, therefore due to biological imbalance of digestive tract, digestion and absorption was impaired.

Regarding, a bitter smell of pennyroyal extract, which is an adverse factor, the amount of feed intake, and laying hens performance were reduced. Considering the higher density of adverse effects of pennyroyal by increasing the levels of extract, the lowest performance was resulted in group 7, which included 0.3% pennyroyal extract.

Egg traits

The effects of different levels of pennyroyal dried aerial parts powder and extract in feeds on egg traits of laying hens are shown in Table 3.

Different levels of pennyroyal powder and extract had significant effects on specific gravity and Haugh unit in laying hens (P<0.05).

However, adding different pennyroyal forms in compared with control group could not significantly improve the egg traits.

Use of more than 0.1% pennyroyal extract significantly decreased the amount of eggshell specific gravity, whereas the highest specific gravity (1.092) was resulted in groups 5. Significantly, reduction in egg specific gravity in 6 and 7 groups might be associated with the amount of feed intake, since by increasing the amount of pennyroyal extract, the amount of feed intake decreased; therefore, much less amount of calcium deposited in shell and decreased egg specific gravity.

Most of studies indicated that increase availability and utilization of energy of feeds ingredients was major reason that led to increasing yolk weight in laying hens. Indeed, several findings showed that increasing of yolk weight was main reason for increasing of albumin weight. Furthermore, other studies indicated that there were positive correlation between Haugh unit and quality of egg internal components (yolk and albumin). Egg albumen height and egg weight were indices for evaluation of Haugh unit. So, increase percents of yolk and albumen due to higher availability and utilization of energy of feeds ingredients and existence of sterols plant in group 6 by using 0.2% of pennyroyal might probably be the main cause of improvement in Haugh unit.

Table 3 Effects of using different levels of pennyroyal powder and extract on egg traits of laying hens

Treatments	Specific gravity (mg/cm^3)	Yolk index (%)	Shell strength (g/cm^2)	Yolk color	Shell weight (%)	Albumen weight (%)	Yolk weight (%)	Haugh unit	Shell thickness (mm)
1	1.083^{ab}	40.21	25.64	6	8.97	62.04	28.94	82.74^{ab}	0.404
2	1.085^{ab}	41.07	24.30	6	9.25	62.1	28.68	74.08^{b}	0.078
3	1.081^{ab}	40.41	27.52	6.33	8.72	66.39	26.55	86.82^{ab}	0.073
4	1.082^{ab}	40.67	25.86	6.67	9.24	63.86	26.79	85.17^{ab}	0.078
5	1.092^{a}	41.12	28.77	5.44	9.60	63.58	26.67	83.51^{ab}	0.082
6	1.076^{b}	40.96	25.52	5.78	8.87	62.36	31.01	89.22^{a}	0.073
7	1.075^{b}	39.06	26.29	5.89	8.70	62.56	28.78	82.83^{ab}	0.073
SEM	0.004	0.82	3.63	0.37	0.32	1.5	1.71	3.98	0.002
P-value	0.09	0.60	0.98	0.4	0.41	0.43	0.51	0.03	0.08

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Improving of Haugh unit by using medicinal plants was in agreement with previous findings (Nobakht and Mehmannavaz, 2010; Sayedpiran *et al.* 2011), while they did not report any significantly differences in egg specific gravity.

Blood biochemical and immunity parameters

The effects of different levels of dried aerial parts powder and extract of pennyroyal in feeds on blood biochemical parameters and immunity cells of laying hens are presented in Tables 4 and 5.

The effects of different levels of pennyroyal powder and extract had significant effects on blood triglyceride, albumen, total protein and uric acid (P<0.05).

The lowest amount of blood triglyceride (270.4 mg/dL), the highest amounts of albumen (3.05 mg/dL), total protein (5.40 mg/dL) and uric acid (5.39 mg/dL) were observed in group 7 by using 0.3% of pennyroyal extract. Since, 0.3% pennyroyal extract significantly reduced the performance of laying hens; the highest amounts of nutrients remained in body and increased the amounts of blood biochemical parameters. Pennyroyal powder significantly increased the amount of blood triglyceride in compared with pennyroyal extract and control group. The highest amount of blood triglyceride (1703.6 mg/dL) was observed in group 2 by using 0.5% pennyroyal powder. Most of spices and herbs enhance the synthesis and excretion of bile acids in the liver.

Table 4 Effects of using different levels of pennyroyal powder and extract on biochemical parameters of laying hens

Treatments	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Albumin (mg/dL)	Total protein (mg/dL)	Uric acid (mg/dL)	HDL (mg/dL)
1	785.9^{bc}	91.97	2.24^{bc}	3.93^{c}	2.55^{bc}	9.39
2	1703.6^{a}	115.26	2.63^{ab}	4.75^{abc}	1.71^{ab}	6.51
3	1040.2^{ab}	107.53	2.26^{bc}	4.35^{bc}	2.25^{bc}	7.79
4	1043.6^{ab}	119.56	2.31^{bc}	4.49^{abc}	2.27^{ab}	12.24
5	519.3^{bc}	159.91	2.40^{bc}	5.11^{ab}	3.50^{bc}	29.44
6	422.9^{c}	74.55	2.11^{c}	4.75^{abc}	2.37^{abc}	15.03
7	270.4^{c}	115.49	3.05^{a}	5.40^{a}	5.39^{a}	24.96
SEM	210.51	29.09	0.15	0.28	0.5	8.98
P-value	0.005	0.57	0.01	0.04	0.003	0.48

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Table 5 Effects of using different levels of pennyroyal powder and extract on immunity cells of laying hens

Treatments	Hematocrit (%)	Hemoglobin (%)	RBC (mm^3)	WBC (mm^3)	Hetrophile (%)	Lymphocyte (%)	H/L (%)
1	2.84	9.64	2.76	233300^{ab}	18.34^{ab}	80^{b}	0.24
2	28.34	9.30	3	196601^{b}	9.67^{b}	90^{a}	0.11
3	28	9.37	2.65	233300^{ab}	14^{ab}	85.67^{ab}	0.26
4	29.67	9.77	2.72	253300^{a}	20.34^{a}	79.67^{b}	0.26
5	29.34	9.60	2.83	266601^{a}	14^{a}	84.67^{ab}	0.17
6	26.50	9.54	2.62	211601^{ab}	17.67^{ab}	80.67^{ab}	0.22
7	28	9.37	2.82	241601^{ab}	16^{ab}	84^{ab}	0.19
SEM	1.36	0.48	0.31	16401	3.07	2.92	0.04
P-value	0.61	0.64	0.98	0.12	0.31	0.21	0.33

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

As bile acids had beneficially effects on lipids' digestion and absorption, it could improve the lipids' digestion and absorption, which led to increase the level of blood triglyceride (Srinivasan, 2005). Change in blood biochemical parameters in the present experiment did not supported by other researchers (Nobakht and Mehmannavaz, 2010; Sayedpiran *et al.* 2011). The effects of different levels of pennyroyal powder and extract had significant effects on white blood cells, hetrophil and lymphocyte ($P<0.05$). The lowest amount of hetrophil (9.67%) and white blood cells (196601/mm^3) and the highest amount of lymphocyte (90%) were observed in group 2 by using 0.5% of powder of pennyroyal, whereas the highest amounts of white blood cells (266601 mm^3) was in group 5 by using 0.1% of pennyroyal extract. The immune system generally benefits from the herbs and spices rich in flavonoids, vitamin C and carotenoids. These compositions increase immunity of laying hens by decreasing stress (Frankič *et al.* 2009). Decrease of immunity status by using 1.5% of pennyroyal powder might be associated with the production levels of laying hens. Regarding, the increase of performance, as well as, transfer of highest amounts of nutrients such as flavonoids, vitamin C and carotenoids into eggs, lower levels would remain in body, which could decrease the immunity levels of laying hens.

CONCLUSION

The overall results showed that the use up to 1.5% of dried aerial parts powder and 0.1% of pennyroyal (*Mentha pulegium*) extract had positive effects on performance of laying hens compared with control group. Indeed, different levels of pennyroyal powder and extract could change the egg traits, blood biochemical parameters and immunity levels of laying hens.

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