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Full Length Research Paper

The effects of using different levels of lemon balm (*Melissa officinalis L.)* medicinal plant on performance, egg traits, blood biochemical parameters and Immunity cells of laying hens

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This experiment was conducted to evaluate the effects of different levels of Lemon balm *(Melissa officinalis L.)* medicinal plant dried aerial part powder on performance, egg traits, blood biochemical and Immunity cells of laying hens. 180 laying hens (W36 strain) from 46 until 58 weeks of age were used in a completely randomized design in five treatments (0, 0.5, 1, 1.5 and 2%) and three replicates (12 birds per replicate). Using different levels of lemon balm significantly affected the performance, egg traits and blood biochemical parameters of laying hens ($p < 0.05$). The lowest feed conversion ratio (3.1), the highest egg production (56.85%) and egg mass (35.51 g/hen/day) were observed in Treatment 2 (0.5% lemon balm); the best yolk index (5.17) was obtained in Treatment 4 by using 1.5% of lemon balm medicinal plant whereas the highest Haugh unit (87.91) obtained in control group. The lowest (188.22 mg/dl) and the highest (280.22 mg/dl) amounts of blood glucose were observed in Groups 1 and 2. The results showed that there are no significant differences between treatments on blood immunity cells levels, but numerically the highest percentage of lymphocytes (86.67%) and the lowest percentage of hetrophiles (11%) and the lowest ratio of H/L (0.132) were observed in Treatment 2 by using 0.5% of lemon balm. The overall results showed that the use of 0.5% of lemon balm medicinal plant in the diets of laying hens have positive effects on their performance, egg traits, blood biochemical parameters and Immunity cells.

Key words: Blood metabolites, egg traits, laying hens, *Melissa officinalis L.,* performance.

INTRODUCTION

Antibiotics and other chemical feed additives have been used for more than 50 years to enhance growth performance and to prevent disease in livestock feeding environments. However, the current trend is to look for alternatives to antibiotic feed additives because of public concern about antibiotic residues in animal products and the potential evolving of antibiotic resistant bacteria. As a consequence, new commercial additives of plant origin, considered to be natural products that the consumer would accept, have been proposed to animal producers. The chemical components of most essential oils from plants are generally recognized as safe, and are used commonly in the food industry (Craig, 1999; Varel, 2002).

Adding 2% of Nettle *(Urtica dioica L.)* dried areal parts powder into laying hens diets significantly increased egg production percentage, egg mass and eggshell weight, whereas the highest Haugh unit was recorded by using 2% of Savory *(Satureja hortensis L.)* in this experiment, different levels of Nettle, Savory and Ziziphora could not significantly affected blood biochemical parameters and immunity cells (Jaderi et al., 2011). In another study by using different levels of Thymyus *valgaris, Lamiaceae menthapiperita* and *Oreganum valgare* the highest egg

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production percent, egg mass and the best feed conversion, the highest Haugh unit and egg yolk color index were resulted by using 2% of *O. valgare*, whereas the highest amount of feed intake and the lowest level of blood Triglyceride were observed by adding 2% of *L. menthapiperita.*

Lemon balm *(Melissa officinalis L.)* is one of the oldest and still most popular medicinal plants. It is a representative of the Lamiaceae family that is known for many aromatic and medicinal plants commonly used in Europe's traditional medicine and gastronomy (Toth et al., 2002). The essential oil contains geraniol, citronellol, cintronellal, linalool, eugenol acetate and nerol (Abbaszadeh et al., 2009). The most commonly known therapeutic properties of lemon balm are sedative, carminative, antispasmodic, antibacterial, antiviral, anti-inflammatory and antioxidative (Yamasaki et al., 1998). Lemon balm has traditionally been employed against catarrh, flatulence (Weizman et al., 1993) headaches (Wake et al., 1999) and fever (Kabala-Dzik et al., 2004). Polyphenolic compounds are commonly found in medicinal plants and they have been reported to have multiple biological effects, including antioxidant activity (Wojdylo et al., 2007). Oil yield of lemon balm in 17 regions of Europe was reported from 0.06 to 0.16% (Mrlianova et al., 2001). Aerial parts of lemon balm cultivated in three states of Iran were used for isolation of oil and reported oil yield 0.14 to 0.26%. Major components of oil were citronellal and β- caryophyllene. Carvacrol was dominant in Tehran sample (Askari and Sefiedkon, 2004). 17 components isolated from the extracted oil of the dried aerial parts ob Limon balm and identified (Sadraei et al., 2003). Geranial (35.3%) and neral (24.5%) were the major components. 14 constituents found in leaf oil of lemon balm which citral (48.0%) and citronellal (40.0%) was the majors (Carnat et al., 1998). The composition and quantity of essential oil from a particular species could be markedly affected by harvesting season (Atti-Santos et al., 2004), geographical environment and other agronomical factors (Naghdi et al., 2004; Jordan et al., 2006). This study focuses on evaluation effects of using different levels of lemon balm *(M. officinalis L.)* medicinal plant dried aerial part powder on performance, egg traits, biochemical and Immunity parameters of blood in laying hens.

MATERIALS AND METHODS

Animals and dietary treatments

180 laying hens (W36 strain) from 46 until 58 weeks of age were used in a completely randomized design in five treatments and three replicates (12 birds per replicate). The treatment groups consisted of a control group (1) with no lemon balm supplementation, and experimental groups 2, 3, 4 and 5 receiving in 0.5, 1, 1.5 and 2% of lemon. The diets were formulated to meet the requirements of laying hen as established by the NRC (1994).

Dried aerial part of lemon balm was supplied from local market and the compositions of it were determined according to AOAC (1994) (Table 2); after fine milling, mixed with other ingredients. The diets and water was provided *ad libitum.* The lighting program during the experimental period consisted of a period of 16 h light and 8 h of darkness. Average of environmental temperature was 15℃. The composition of experimental diets of layin g hens is given in Table 1.

Performance parameters and egg traits

Birds were individually weighted at the beginning and at the end of the study and body weight gain was calculated. feed intake, Feed Conversion Ratio (FCR), egg production (%), egg mass (g/hen/day) and egg weight (g) 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 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 eggs specific gravity was done by floated 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 four 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, egg albumin index, Haugh units were determined (Card and Nesheim, 1972).

Blood biochemical parameters and immunity cells

At end of this experiment two hens from each replicate were randomly chosen for blood collection and approximate 5 ml blood samples were collected from the brachial vein. Collected (1 ml) blood was transferred to tubes with EDTA for determination of heterophil and lymphocyte 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 4 ml of blood was centrifuged to obtain serum for determination the blood biochemical parameters include: glucose, cholesterol and Triglyceridet 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 Institute (2005). Means were compared using the Duncan multiple range test. Statements of statistical significance are based on $P < 0.05$ (Table 1).

RESULTS AND DISCUSSION

Performance parameters

The effects of different levels of Lemon balm in Diets on performance of laying hens are summarized in Table 3.

Table 1. Composition of experimental diets.

Feeds ingredient	Control group	0.5% lemon balm	1% lemon balm	1.5% lemon balm	2% lemon balm
Corn	53.12	52.38	51.64	50.90	50.16
Wheat	20	20	20	20	20
Soybean meal-44%	16.56	16.61	16.65	16.70	16.75
Vegetable oil	0.3	0.49	0.68	0.88	1.07
Lemon balm	0	0.5	1	1.5	2
Oyster shell	1.09	1.09	1.09	1.09	1.09
Dicalcium phosphate	8.15	8.15	8.15	8.15	8.15
Salt	0.28	0.28	0.28	0.28	0.28
Vitamin premix1	0.25	0.25	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25	0.25	0.25
Calculated composition					
Metabolisable energy (Kcal/kg)	2800	2800	2800	2800	2800
Crude protein (%)	14	14	14	14	14
Ca (%)	3.4	3.4	3.4	3.4	3.4
Available phosphor (%)	0.31	0.31	0.31	0.31	0.31
Sodium (%)	0.15	0.15	0.15	0.15	0.15
Lysine (%)	0.64	0.64	0.64	0.64	0.64
Methionine + Cysteine (%)	0.55	0.55	0.55	0.55	0.55
Tryptophan (%)	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 k3, 2200 mg; thiamine, 1477 mg; riboflavin, 4000 mg; panthothenic acid, 7840 mg; pyridoxine, 7840 mg; cyanocobalamin, 10 mg; folic acid, 110 mg; 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; Se (NaSeO3, 45.56% Se), 200 mg.

Table 2. Composition of lemon balm (%).

Dry matter	Crude protein	Crude fiber	Ca	P
91	8.2	32	0.39	0.28

Table 3. Effects of using different levels of lemon balm on performance of laying hens.

Lemon balm (%)	Egg weight (g)	Egg production (%)	Egg mass (g/hen.d)	Feed intake (g)	Feed conversion ratio
0	61.97	44.65^{b}	27.63^{b}	106.94	3.89^{a}
0.5	62.66	56.85^{a}	35.51^{a}	109.07	3.1^{b}
1	62.42	51.44^{a}	32.09^{ab}	107.14	3.41^{ab}
1.5	62.70	48.78^{ab}	30.62^{ab}	106.65	3.52^{ab}
2	62.66	52.05^{ab}	32.56^{ab}	106.85	3.40^{ab}
SEM	0.54	2.47	1.64	0.89	0.21
P Value	0.54	2.47	1.64	0.89	0.21

Values in the same row not sharing a common superscript differ significantly (P<0.05). SEM = Standard error of mean.

Using different levels of lemon balm had significant effects on feed conversion ratio, egg production and egg mass of laying hens ($p < 0.05$). The best feed conversion ratio (3.1), the highest percentage of egg production (56.85%) and egg mass (35.51 g/hen/day) were observed in Treatment 2 by using 0.5% of lemon balm. Also the highest amount of feed intake was resulted in Group 2. Using more than 0.5% of lemon balm did not have any significant effects on feed conversion, egg production and egg mass. Improving of laying hens performance is in agreement of (Nobakht and Mehmannavaz, 2010; Jaderi et al., 2011) findings. Most of the spices stimulate the function of pancreatic enzymes (lipases, amylases and proteases); some also

Table 4. Effects of using different levels of lemon balm on egg traits of laying hens.

Lemon balm (%)	Specific gravity (mg/cm^3)	Yolk index (%)	Color yolk index	shell weight (g)	Albumin weight (g)	Yolk weight (g)	Haugh unit	Shell thickness (mm)
0	1.07	41.55	2b	6.37	38.72	19.60	87.91^{a}	0.332
0.5	1.082	42.05	4.84^{a}	6.2	40.09	19.74	73.51^{b}	0.345
1	1.078	41.78	5^{a}	6.47	39.74	18.55	69.64^{b}	0.353
1.5	1.077	42.26	5.17^{a}	5.68	44.37	18.74	73.85^{b}	0.354
2	1.067	43.83	5^{a}	6.12	41.32	2.24	78.06^{ab}	0.336
SEM	0.005	1.22	0.51	0.33	1.5	0.51	3.38	0.01
P Value	0.21	0.71	0.006	0.52	0.81	0.18	0.03	0.83

Values in the same -not sharing a common superscript differ significantly (P < 0.05).

Table 5. Effects of using different levels of lemon balm on biochemical parameters and immunity cells of laying hens.

Lemon balm (%)	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Hetrophile (%)	Lymphocyte (%)	H/L
0	188.22^{b}	132.52	3087.2	16.5	83	0.199
0.5	280.22^{a}	201.77	3223.5	11	87.67	0.126
1	279.50^{b}	197.32	3149.2	12.67	86.67	0.146
1.5	258.59^{ab}	128.48	2182.2	13.34	85	0.157
2	236.57^{b}	204.98	2231.4	13.5	85.5	0.158
SEM	29.01	26.12	439.19	3.40	3.58	0.05
P Value	0.03	0.75	0.97	0.33	0.67	0.27

Values in the same - not sharing a common superscript differ significantly (P < 0.05).

increase the activity of digestive enzymes of gastric mucosa (Srinivasan, 2005). Besides the effect on bile synthesis and enzyme activity, extracts from herbs and spices accelerate the digestion and shorten the time of feed/food passage through the digestive tract (Platel and Srinivasan, 2004; Suresh and Srinivasan, 2007). So, increase the availability of nutrients and shorten the time of feed/food passage through the digestive tract due to increase the activity of digestive enzymes of gastric mucosa and bile synthesis are may be the main cause of improvement in feed conversion, egg production and egg mass. Also, there are positive correlation between feed conversion ratio with egg mass and egg mass with egg production. So, perhaps increase of egg mass in Group 2 lead to increasing egg production and improving feed conversion ratio in this group.

Egg traits

The effects of different levels of lemon balm in feeds on egg traits of laying hens are summarized in Table 4. Using different levels of lemon balm had significant effects on Haugh unit and color index of egg yolk in laying hens ($p < 0.05$). Adding lemon balm into laying hens diets in contrast with control group significantly improved the yolk color index; however there were no significant difference between experimental treatments in this respect. So, the best color index of the yolk (5.17) was observed in Group 4 by using 1.5% of lemon balm whereas the highest Haugh unit (87.91) was observed in control group. The colorants for increasing yolk color in laying hens or skin color in broilers in intensive production can be of natural (carotenoids) in spices and medicine plants or synthetic origin (Nobakht and Mehmannavaz, 2010). So, exist of pigments and carotenoids lead to increasing of color index of yolk in groups contained Limon balm especially by using 1.5% of lemon balm. Many of studies showed that there are positive correlation between Haugh unit and quality of egg inside components. Egg albumin height and egg weight are indexes for evaluation of Haugh unit. So, perhaps increase albumin height in control group may be the main cause of improvement in Haugh unit.

Blood biochemical and immunity parameters

The effects of different levels of Lemon balm on blood biochemical parameters and immunity cells of laying hens are presented in Table 5. The effects of different levels of Lemon balm had significant effects on blood glucose ($p < 0.05$). The lowest (188.22 mg/dl) and the highest (280.22 mg/dl) amounts of glucose were observed

in Groups 1 and 2. Concentration of blood glucose is relative to concentration of carbohydrates in blood. These carbohydrates are energy sources that using for synthesis of glycogen and fatty acids. So, exist species of carbohydrates in lemon balm and increase absorption of carbohydrates are may be the main cause of increase concentration of glucose blood in Group 2 by using 0.5% of lemon balm medicinal plant. The effects of different levels of lemon balm had not significant effects on immunity parameters of blood ($p < 0.05$). But in numerically the highest percentage of lymphocyte (86.67%) and the lowest percentage of hetrophile (11%) and the lowest ration of H/L (0.132) were observed in Group 2 by using 0.5% of lemon balm. The immune system generally benefits from the herbs and spices rich in flavonoids, vitamin C and carotenoids. These compositions increase immunity of body of laying hens by decrease their stress (Frankič et al., 2009). So, decrease of stress by exit composition of herbs and spices lead to improving immunity parameters of blood.

Conclusion

From the obtained data can be concluding that Lemon balm *(M. officinalis L.)* medicinal plant at level 0.5% of laying hens diet increased egg production percentage, egg mass, improve feed conversion ratio, and increased yolk color index, so had positive effects on blood biochemical parameters and immunity cells and increased the concentration of blood glucose during 46 to 58 weeks of hens age.

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