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Effects of Different Levels of Licorice (*Glycyrrhiza glabra*) Medicinal Plant Powder on Performance, Egg Quality and some of Serum Biochemical Parameters in Laying Hens

Research Article

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ABSTRACT

An experiment was conducted to evaluate the effects of different levels of licorice powder (LP) in diet on performance, egg traits and some serum biochemical parameters of laying hens in the late laying period. Totally 180 Hy-Line W-36 layer hens aged 55-68 weeks with similar weight were selected and assigned to 5 treatments, 3 replicates and 12 hens in each replicate in a completely randomized design. Experimental groups included 1) basal diet (without LP), 2, 3, 4 and 5) basal diet supplemented with 0.5, 1.0, 1.5 and 2.0% of LP, respectively. The results showed that the use of different levels of LP significantly affected the performance and egg traits of laying hens ($P<0.01$). The highest performance for egg production (60.96%), egg weight (61.50 g), egg mass (37.60 g) and the best feed conversion ratio (2.93) was achieved with 2% LP treatment. The amount of daily feed intake was not affected by dietary treatments. The highest amounts of yolk weight (29.43%), egg shell thickness (0.366 mm), haugh unit (74.79) and yolk color index (4.78) were obtained using 2% LP diet. Licorice powder supplementation in the diets of hens did not significantly alter the serum blood parameters compared to the control ($P>0.05$). It can be concluded that in laying hens in late laying period, using 2.0% of Licorice powder, without having any significant effects on serum biochemical parameters, can improve their performance and egg traits.

KEY WORDS egg mass, egg yield, laying hens, licorice powder, serum metabolites.

INTRODUCTION

Medicinal plants and their extracts and essential oils have a wide range of effects, 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 can have an impact on other physiological functions, help to sustain good health and welfare and improve performance (Frankic *et al.* 2009). In recent years the use of various forms of medicinal plants in poultry has been increased.

Licorice (*Glycyrrhiza glabra*) is one of the most important medicinal plant that has been used for 4000 years for various applications in human and animals. Licorice is a perennial herb belonging to the legume family (pea family) and is native to Asia. So, it can easily grow in different regions such as south central Russia, Asia, including Iran. It contains different chemically active compounds such as glycyrrhizin acid mixed with potassium and calcium salts, glabridin and also glycyrrhetinic acid (enoxolone). However, the main active secondary substances of licorice is glycyrrhizin. Glycyrrhizin can be found in different parts of licorice, but the root contains the highest amount of this

compound. Glycyrrhizin which is a valuable chemical active substance is 50 times sweeter than sugar (Armanini *et al*. 2002). The chemical constituents of the roots include several bioactive compounds, such as glycyrrhizin (~16%), different sugars (up to 18%), flavonoids, saponoids, sterols, starches, amino acids, gums and essential oils. However, licorice is also known to exhibit many pharmacological actions, including antibacterial (Fukai *et al*. 2002), anti-atherosclerotic (Fuhrman *et al*. 2002), anti-infective (Nowakowska, 2006) and reduces blood cholesterol (Bensky and Gamble, 1993). Previous findings showed that feeding licorice to mice and humans reduces the weight and abdominal fat (Nakagawa *et al*. 2004; Tominaga *et al*. 2006; Aoki *et al*. 2007). In broilers, using licorice extract up to 0.3% in drinking water had no effects on their performance, but significantly reduced the serum levels of low-density lipoprotein (LDL) and total cholesterol, however it could not alter the amounts of triglyceride and high-density lipoprotein (HDL) (Khamisabadi *et al*. 2014). In laying hens, using licorice extract up to 0.6% in the diets increased egg production and egg shell thickness and reduced the level of blood cholesterol (Sedghi *et al*. 2010). However, the use of licorice powder (LP) in laying hens has not been reported yet.

According to the abundance of licorice and medicinal properties of this plant, Iran will probably use it as an additive in the feed of laying hens. So it is important that this additive is evaluated in this study. Therefore, the aim of this study was to evaluate the effect of licorice powder on egg production, egg yield performance, egg quality and serum biochemical parameters in laying hens at the end of production.

MATERIALS AND METHODS

One hundred eighty, 58- wk-old Hy-Line W-36 laying hens with similar weight were used in standard three-story high cages in this experiment. Birds were randomly assigned to 5 groups (n=12), each of which included 3 cages (50×45×45 cm) with four birds. The experiment was conducted as a completely randomized design (CRD). The temperature and relative humidity of the hen house were 20 + 2 ˚C and 65%, respectively. Hens were maintained on a 14:10 hr light: dark cycle during the experiment. The treatments consisted of diets containing 0, 0.5, 1.0, 1.5 and 2.0% licorice powder. Composition of the experimental diets is presented in Table 1. The diets were isoenergetic and isonitrogenous. Before starting the experiment, two weeks was conducted of pre- tests to ensure that all egg laying hens were in the same production condition. The experiment lasted for 12 weeks. During the experimental period, hens were free to access feed and water. Egg production, feed intake, feed conversion rate, egg weight and egg mass were recorded daily from each cage. As well as, this characteristics of the estimated in the end of period.

Egg quality

For analyzing egg quality, 6 eggs were collected per replicate during 3 consecutive days at the end of the experiment. These eggs were transported to the lab, and were weighed by means of a precision scale (0.01 g). Percentage of yolk, albumen and shell, shell thickness and Haugh unit values were measured biweekly using 8 eggs from each dietary treatment.

A Vernier caliper was used to measure eggshell thickness at three different eggshell locations and the related values were recorded. Then their average was considered as final thickness of eggshell for each experimental unit. A micrometer was used to determine the height of the thick albumen (egg white). Haugh unit was calculated by the following formula (Stadelman and Cotterill, 1986):

HU= 100 log (H+7.57-1.7 W 0.37)

Where:
H: stands for the albumen height (mm).
W: refers to egg weight (g).
7.57: considered to be the correction factor for albumen height.
1.7: regarded as the correction factor for egg weight.

Color index of the yolk was measured using Roche color index (Leeson and Caston, 2004).

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.

Four ml blood was centrifuged to obtain serum for determination of blood biochemical parameters including: glucose, total cholesterol, triglyceride, albumin, total protein and uric acid. Kit packages (Pars Azmoon Company; Tehran, Iran) were used for the determination of the blood bio-chemical parameters using the Anision-300 auto-analyzer system.

The remaining one ml of collected blood was transferred to tubes with ethylenediaminetetraacetic acid (EDTA) for determination of heterophile, lymphocyte, hematocrit, hemoglobin, RBC and leukocyte of blood cells counts.

One hundred leukocytes per sample were counted by heterophile to lymphocyte separation under an optical microscope. The heterophile to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983).

Table 1 Composition and calculated analysis of experimental diets

Ingredients (%)	Control	0.5% licorice	1.0% licorice	1.5% licorice	2.0% licorice
Corn	46.00	45.75	45.60	45.30	45.00
Wheat	15.00	15.00	15.00	15.00	15.00
Soybean meal	17.00	17.00	17.00	17.00	17.00
Wheat bran	7.50	7.30	7.10	7.00	6.90
Licorice	-	0.50	1.00	1.50	2.00
Soybean oil	4.45	4.40	4.25	4.15	4.05
Oyster meal	8.10	8.10	8.10	8.20	8.10
Dicalcium phosphate	1.10	1.10	1.10	1.10	1.15
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.05	0.05	0.05	0.05	0.05
Salt	0.20	0.20	0.20	0.20	0.20
Calculated chemical composition	100	100	100	100	100
Metabolizable energy (kcal/kg)	2860	2859	2856	2856	2856
Crude protein (%)	14.21	14.21	14.21	14.21	14.21
Crude fiber (%)	3.44	3.68	3.92	4.14	4.42
Ca (%)	3.40	3.41	3.40	3.41	3.40
Available P (%)	0.32	0.32	0.32	0.32	0.32
Lys (%)	0.69	0.68	0.68	0.68	0.69
Met (%)	0.33	0.33	0.33	0.33	0.33
Met + Cys (%)	0.55	0.55	0.56	0.57	0.56

[1] Vitamin premix per kg of diet: vitamin A (retinol): 3600000 IU; vitamin D_3 (cholecalciferol): 80000 IU; vitamin E (tocopheryl acetate): 7200 IU; vitamin K_3: 700 mg; Thiamine: 2640 mg; Riboflavin: 3920 mg; Pantothenic acid: 1176 mg; Pyridoxine: 6 mg; Cyanocobalamin: 400 mg; Folic acid: 400 mg; Biotin: 40 mg; Niacin: 11880 mg and Choline chloride: 1176 mg.
[2] Mineral premix per kg of diet: Fe ($FesO_4.7H_2O$, 20.09% Fe): 20000 mg; Mn ($MnsO_4.H_2O$, 32.49% Mn): 3968 mg; Zn (ZnO, 80.35% Zn): 33880 mg; Cu ($CusO_4.5H_2O$): 4000 mg; I (KI, 58% I): 396 mg; Se ($NaseO_3$, 45.56% Se): 200 mg.

Differences between groups were analyzed with analysis of variance (ANOVA) using the statistical software SAS (2004). Significant means were subjected to a multiple comparison test (Turkey's test) at $P < 0.05$ level.

RESULTS AND DISCUSSION

Performance parameters

The effects of different levels of licorice powder (LP) on performance of laying hens are shown in Table 2. LP supplementation significantly affect the performance of laying hens ($P<0.01$). The highest values of egg production (60.96%), egg weight (61.50 g), egg mass (37.60 g) and the best feed conversion ratio (2.93) were obtained in group supplemented with 2% LP. There was no significant difference among the experimental groups in daily feed intake. Improving the performance of laying hens by using different levels of licorice powder can be related to the effective secondary substances which are found in the licorice powder (Sedghi *et al.* 2010).

Glycyrrhizin is the main compound that is found in large amount in the root of licorice (Nowakowska, 2006). Glycyrrhizin is a phytobiotic compound with antiviral and antibacterial properties (Brenes and Roura, 2010). So, use of it in hen diets may be improving their performance via reducing the amount of harmful digestive tract microorganisms (Khamisabadi *et al.* 2014).

In another study it was shown that the phytobiotic compounds such as glycyrrhizin are able to activate the mechanisms of sensory peripheral in the cavities of the mouth and nose, the gut to receive feed and also stimulate gastrointestinal motility and secretions of the stomach for best digestion of feed ingredients (Vaya *et al.* 1997). In agreement with the present study result about the amount of feed intake, using licorice extract in laying hens (Sedghi *et al.* 2010) and broilers (Khamisabadi *et al.* 2014) should not change the amount of feed intake.

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). Therefore, 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 licorice medicine plant to accelerate the performance. Previous studies with other species of animals have shown that licorice flavonoids suppress body weight (BW) by reducing body fat mass (Armanini *et al.* 2002; Nakagawa *et al.* 2004; Tominaga *et al.* 2006; Aoki *et al.* 2007). 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 increased egg production in group 5 by using 2% powder licorice medicine plant.

Table 2 The effect of different levels of licorice powder on performance of laying hens in the late stage

Treatments	Feed intake (g/day)	Egg production (%)	Egg weight (g)	Egg mass (g)	Feed conversion rate (FCR)
Control	110.52	52.45^{c}	60.59^{c}	31.78^{c}	3.48^{a}
0.5% licorice powder	110.19	53.53^{c}	60.95^{b}	32.63^{c}	3.38^{a}
1% licorice powder	110.45	54.96^{bc}	60.86^{bc}	33.45^{bc}	3.30^{ab}
1.5% licorice powder	110.33	57.14^{b}	61.09^{b}	34.91^{b}	3.17^{b}
2% licorice powder	110.35	60.96^{a}	61.50^{a}	37.60^{a}	2.93^{c}
P-value	0.3513	0.0008	0.0005	0.0006	0.0008
SEM	0.112	0.981	0.092	0.642	0.061

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

Table 3 The effects of different levels of licorice powder on egg quality traits of laying hens in the late laying period

Treatments	Yolk weight (%)	Albumen weight (%)	Shell weight (%)	Shell thickness (mm)	Haugh unit	Yolk color
Control	28.56^{b}	**58.48**	**12.96**	0.357^{a}	73.30^{c}	2.11^{d}
0.5% licorice powder	29.22^{a}	59.04	11.74	0.337^{b}	73.90^{b}	3.00^{c}
1% licorice powder	29.41^{a}	58.49	11.26	0.363^{a}	74.12^{b}	3.78^{b}
1.5% licorice powder	29.49^{a}	58.19	12.32	0.339^{b}	74.69^{a}	4.55^{a}
2% licorice powder	29.43^{a}	59.33	12.28	0.366^{a}	74.79^{a}	4.78^{a}
P-value	0.0473	0.7649	0.5869	0.0002	0.0001	0.0001
SEM	0.250	1.235	0.508	0.003	0.087	0.132

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

The increase of percent yolk and albumen (Table 3) could probably lead to an increase of egg weight in the control group. Several studies indicate that egg weight and egg production were indices for evaluation of egg mass. It is possible that the increase of egg weight due to the increase in percent of albumin and yolk might be the main cause of improvement in egg mass in the experimental group fed 2% licorice powder.

The results showed that the improvement of the feed conversion ratio was related to the increase of egg mass.

Egg quality

The effects of different levels of licorice powder in experimental diets on egg quality of laying hens are shown in Table 3. Adding different levels of licorice powder in laying diets significantly effects on yolk weight (P<0.05), shell thickness, haugh unit and yolk color in laying hens (P<0.01). So, adding different licorice powder in compared with control group could be significantly improving the egg quality.

Linear increases in yolk weight (P<0.05), Haugh unit and yolk color (P<0.01) were found with increasing dietary licorice powder. This is consistent with data from Ghasemi *et al.* (2010) who reported that yolk color responded linearly with increasing levels of medicinal herbs. Poltowicz and Wezyk (2001) had also showed that the herbs used significantly increased yolk color intensity in the experimental groups. In contrast with the results of the present study, Sedghi *et al.* (2010) reported that using licorice could not change the egg traits except egg shell thickness that is inconsistent with our experiment.

These differences in the results may be related to the amount and form of licorice products used in the experiments. An increase in egg shell thickness (P<0.01) was found with increasing dietary licorice, whereas egg shell percentage was not affected by the dietary treatments.

Blood biochemical parameters

The feeding of licorice powder did not have a significant effect on the amounts of serum biochemical parameters (P>0.05) (Table 4).

Results also revealed that the addition of licorice powder to the diet of hen layer resulted did not have significant (P>0.05) decrease in serum cholesterol compared with control group (Table 4). Furthermore, birds in experimental group fed 2% licorice powder the lowest value concerning this trait in comparison with other treatments used in the present study. This result may be explained by the hypocholesterolemia property of licorice (Fuhrman *et al.* 2002). High level licorice powder has been shown to reduce low density lipoprotein and cholesterol.

The active components of licorice inhibit the formation of lipid peroxides and protect low density lipoprotein associated carotenoids (Belinky *et al.* 1998). Also, this could be due to actions of licorice such as protecting of LDL cholesterol from oxidation, inhibiting cyclooxygenase and lipoxygenase enzymes and inhibiting lipid peroxidation (Craig, 1999). In the present experiment, none of hematological parameters between the groups was not significant (Table 5). Huang *et al.* (2008) found that licorice plant contains phytoestrogens that increases the erythrocyte sedimentation rate and reduce the number of them.

Table 4 Effect of different levels of licorice powder on serum biochemical parameters of laying hens in the late laying period

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Albumin (mg/dL)	Total protein (g/dL)
Control	135.10	167.00	1222.20	12.48	98.07	2.67	6.33
0.5% licorice powder	159.81	151.36	830.60	12.78	75.73	2.76	6.00
1% licorice powder	146.56	154.08	968.00	13.84	68.40	2.71	6.80
1.5% licorice powder	152.33	147.61	840.50	15.67	75.57	2.69	9.36
2% licorice powder	162.61	99.650	1108.61	15.33	56.41	2.58	5.42
P-value	0.0634	0.8070	0.7878	0.7047	.2172	0.9628	0.3950
SEM	0.359	41.00	261.78	1.798	14.352	0.175	0.481

HDL: high-density lipoprotein and LDL: low-density lipoprotein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of different levels of licorice powder on blood hematology of laying hens in the late laying period

Treatments	Hematocrit (%)	Hemoglobin (%)	RBC (mm^3)	WBC (mm^3)	Hetrophile (%)	Lymphocyte (%)	H/L (%)
Control	30.67	9.47	2.74	26500	21.00	76.67	0.275
0.5% licorice powder	33.00	11.17	2.92	25167	16.00	83.67	0.194
1% licorice powder	43.34	11.17	2.66	26833	12.67	86.34	0.148
1.5% licorice powder	31.67	9.77	2.70	27200	12.34	87.66	0.142
2% licorice powder	29.67	10.34	2.74	27333	12.67	83.33	0.190
P-value	0.4335	0.1316	0.3242	0.5910	0.3591	0.2182	0.3507
SEM	1.81	0.517	0.088	1015.55	3.16	3.22	0.058

RBC: red blood cell; WBC: whit blood cell and H/L: hetrophile to lymphocyte ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Ibrucker and Burdock (2006) reported injected licorice extract, stimulates cell activity and cell cycle was in human lymphocytes.

CONCLUSION

It can be concluded that in laying hens in late laying period, using 2% of licorice powder, without having any significant effects on serum biochemical parameters, can improve their performance and egg traits. According to the growing conditions the licorice in Iran, economically, its use in poultry diets affordable.

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