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The Effects of Licorice (Glycyrrhriza glabra) Root on Performance, Some Serum Parameters and Antioxidant Capacity of Laying Hens

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ABSTRACT

The current study was conducted to determine the effects of the licorice root *(Glycyrrhriza glabra)* in laying hens diets on performance, egg cholesterol, some plasma parameters and antioxidant capacity. One hundred, 40-week old laying hens were divided into four groups, each group consisted of 25 hens and were placed in individual cages. The mean of the initial body weight of laying hens was 1829.18±9.595 g. Commercial laying hen diet was supplemented with 0, 0.5, 1.0 and 2.0% levels of licorice root powder and four different dietary groups were formed. From the experimental findings, it was ascertained that the licorice root supplementation had no significant effect on egg weight and feed conversion ratio (p>0.05), but feed consumption decreased with increasing licorice root (p<0.05).Egg yield was recorded as 88.94%, 89.56%, 86.82% and 85.02% in the groups of 0, 0.5, 1.0 and 2.0, respectively (p<0.05).Plasma low density lipoprotein (LDL) and egg yolk cholesterol level decreased with the addition of licorice root, while plasma high density lipoprotein (HDL) level was increased with licorice root addition (p<0.05). Licorice root addition had a positive effect on total antioxidant capacity (TAS) of plasma. It was determined that the total antioxidant capacity was increased by increasing amount of licorice root.

From the overall findings, it can be concluded that licorice root could be used as a feed additive without any adverse effect on performance. It has been demonstrated that the licorice root enables the production of functional eggs.

INTRODUCTION

Over the centuries, due to having various pharmacological effects, many natural medicinal and aromatic plants are being used as medicine and spices. Enriched with several bioactive compounds, these plants are also an abundant source of various functional properties (Kohlert *et al.*, 2000). Plants and their extracts are consumed by humans and animals regarded as health-friendly and safe additives in terms of chemical structure. However, from the study of literatures regarding the application of these plants in animal nutrition up to 2006, it can be found that, the implementation of these plants as food additives has increased after the prohibition of antibiotics.

Licorice root (*Glycyrrhriza glabra*) is among the oldest and most widely known medicinal plants throughout the world. Although licorice plant has been used for many years in traditional herbal medicine, compounds and pharmacological effects have been revealed in recent scientific research conducted over the last 25-30 years (Asl & Hosseinzadeh, 2008). Huge amounts of pharmacologically active compounds have been isolated from licorice plant and have been identified and verified by modern analytical techniques. The majority of these bioactive

compounds are form triterpene saponins (4-20%) and various types of phenolic compounds (Fiore *et al.,* 2008; Tan *et al*., 2010).

It has been reported that glabridin has the strong antioxidant activity from the main isoflavonoid compounds of the plant (Shibata, 2000). Ju (1989) has shown that the antioxidant effect of licorice flavonoids is 100-fold greater than that of E vitamin. Glycyrrhizin and glycyrrhizinic acid, the main compounds of the triterpen saponins, are mainly antioxidant (Ju 1989; Vaya *et al*., 1997; Doğan, 2004), anti-inflammatory (Yokota *et al*., 1998) anti-ulcer (Aly *et al*., 2005), anti-viral (Utsunomiya *et al*., 1997; Fiore *et al*., 2008), anti-allergic, anticarcinogenic and immunomodulatory effects have been demonstrated as a result of clinical and experimental studies (Shibata, 2000; Asl & Hosseinzadeh, 2008).Other important effects of glycyrrhizin and glycyrrhizinic acid have been reported to have cardioprotective, hepatoprotective and plasma lipid-lowering effects (Fuhrman *et al*., 2002, Nakagawa *et al*., 2004, Visavadiya & Narasimhacharya, 2006).

Studies on the effects of triterpenoid saponins and flavonoid compounds possessed by licorice root on human and experimental animals have been intensified in recent years. Visavadiya & Narasimhacharya (2006) stated that significant decreases in plasma total hepatic lipid, cholesterol, triglyceride, and plasma LDL and VLDL in rabbits supplemented with 5 and 10% *Glycyrrhiza glabra* root in their diets were observed, while they reported an increase in HDL-cholesterol content. In the same study, licorice root was reported to improve hepatic HMG-CoA reductase activity, prevent lipid peroxidation by increasing the amount of superoxide dismutase (SOD) and catalase enzyme.

Nitalikar *et al*. (2010) investigated the antimicrobial activity of licorice root extracts and reported that it has a significant effect on gram positive (Bacillus subtili and Stapphylococcus aureus) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. Sen *et al*. (2011) reported that glycyrrhizin, one of the key compounds of the licorice root normalized Superoxide dismutase (SOD) and catalased activity and Malondialdehyde (MDA) levels which are oxidative stress parameters in diabetic rats. Karami *et al*. (2013) reported that the licorice root was also effective on gram positive and gram negative bacteria such as Salmonella enteritidis (gr-), Escherichia coli (gr-), Bacillus cereus (gr+) and Staphylococcus aureus (gr+). The studies conducted using licorice root was carried out especially with broiler chickens, but limited number of studies were found for the effect of it on laying hens.

For this purpose, in the present study, the effects of bioactive compounds contained in the licorice plant were investigated on the performance and on some plasma lipid profile of lipid metabolism in terms HDL, LDL, total cholesterol and total triglyceride levels of laying hens. In addition, the effects of the plant on antioxidant status of the hens were revealed with total antioxidant and oxidant status (TAS and TOS) parameters. At the same time, the effect of licorice to the cholesterol content of the eggs was also determined.

MATERIAL AND METHODS

Animal and Feed Material

A total of 100 Lohman Brown laying hens at 40 week of age with an initial body weight range between 1828.50 to 1830.20 g were obtained from research and application farm poultry unit, Faculty of Agriculture, Çukurova University, Turkey (latitude is 36.976 and longitude is 35.4535).The hens were weighed individually and divided into four groups of 25 hens each and housed individually in 55cmx50cmx45cm battery cages equipped with nipple drinkers. Corn and soybean based commercial diet containing 2700 kcal/kg ME and 16% crude protein was used as feed material for the laying hens. The basic ingredient and chemical composition of commercial diet is shown in Table 1. Commercial laying hen diet was supplemented with 0, 0.5, 1.0 and 2.0% levels of licorice root powder and four different dietary groups were formed. Licorice root was obtained from Hatay province which is situated in southern Turkey, on the eastern Mediterranean coast. The latitude of Hatay is 36.3524, and the longitude is 36.2935.

The experiment lasted 8 weeks. During the experiment, all hens were housed in an environment with 16 hours of light and 8 hours of darkness, temperature of 21°C, ambient humidity of 50 - 60% and feed-water were given *ad-libitum.* All protocols for the experiment were reviewed and approved by the local animal care and use committee of Çukurova University (30.10.2014/7)

Productive Performance

During the study, feeds were given daily to hens in individual cages and weekly feed consumption was calculated by taking advantage of daily feed consumption. The individual weekly feed consumption was divided by the individual weekly egg weight, and the individual feed conversion ratio was calculated.

Table 1 – Ingredient and chemical composition of the diet.

Ingredient	%	Calculated Nutrient	Composition
Maize	65.27	ME, kcal/kg	2700
Soybean meal	16.95	Crude protein, %	16.00
Sunflower meal	3.70	Crude fiber, %	3.90
Fish meal	3.00	Crude fat, %	4.67
Dicalcium phosphate	1.2	Calcium, %	4.00
Marble powder	8.98	Available phosphorus, %	0.52
Salt	0.20	Methionine, %	0.30
Vitamin premix*	0.10	Methionine & Cysteine, %	0.61
Mineral premix**	0.10	Lysine, %	0.68
DL-Methionine	0.50	Sodium, %	0.16
100.00			

*Vitamin premix in per kg of diet: 15,000 I.U. Vitamin A, 5,000 I.U. Vitamin D, 100 I.U. Vitamin E, 5 I.U. Vitamin K, 4 I.U. Vitamin B_1, 10 I.U. Vitamin B_2, 5 I.U. Vitamin B_6, 0.03 I.U. Vitamin B_{12}, 50 mg Vitamin C, 60 mg Niacin, 18 mg Calcium D-Pantothenic acid, 2 mg Folic Acid, 0.25 mg Biotin

**Mineral premix in per kg of diet: 100 mg Manganese, 80 mg Iron, 100 mg Zinc, 10 mg Copper, 0.2 mg Cobalt, 1.5 mg Iodine, 0.2 mg Selenium.

Feed Conversion Ratio= Individual feed consumption (g/week) /Individual egg weight (g/week)

During the experiment, eggs were collected daily from the cage and weighed with a scale of 0.01 g. Egg yields were calculated by recording the number of eggs collected daily.

Egg yield (hen day) % = (number of eggs (week) / number of chickens) *100

Egg Yolk Cholesterol

At the 4th and 8th weeks of the experiment, egg yolk cholesterol levels were analyzed by collecting 15 eggs from each experimental group. For this purpose, the egg samples were boiled until the egg yolk solidified, then were transferred to the tubes by taking samples from the boiled egg yolk and stored at -80 ˚C until analysis. Fat extraction from samples was performed according to the method developed by Boselli *et al*. (2010). According to this method egg yolk samples were added in a 2: 1 ratio of 30/70 ethanol / chloroform solution and stirred for 30 min at 21 °C. The solution obtained at the end of the period was filtered clean tube by vacuum filtration method. Egg yolk cholesterol levels were estimated by spectrophotometric method using the Boehringer Manheim GmbhBiochemica (1989).

Biochemical Analysis

To determine the effect of licorice root on some plasma parameters and antioxidant capacity, eight randomly selected animals from each group were chosen at the end of the experiment and blood samples were taken from the brachial wing vein. Blood samples were placedin EDTA anticoagulant tubes and centrifuged for 10 minutes at 3000 rpm to separate plasma from whole blood. Plasma samples were kept at -80 ° C until analyzed. Plasma glucose levels and plasma lipid profiles (HDL, LDL, total cholesterol, total triglyceride) of the samples were tested spectrophotometrically according to the protocols of commercial kits (Erba Mannheim, CZ).

Total Antioxidant and Oxidant Status, Oxidative Stress Index

At the end of the study, total antioxidant and oxidant status (TAS and TOS) parameters were detected in plasma samples taken from eight randomly selected animals from each group. TAS and TOS levels were determined by spectrophotometer following the methodology of commercial kits (Rel Assay, Diagnostics).

The effect of licorice root on oxidant stress and the antioxidant system were determined by oxidative stress index by measuring the total antioxidant and oxidant status (TAS and TOS) parameters. The oxidative stress index is equal to the percentage of total oxidant status to total antioxidant status. The TAS value obtained in the study as mmol / L was converted to μmol/L and the oxidative stress index was calculated according to the following formula (Kosecik *et al*., 2005).

OSI = [TOS, μmol H_2O_2 equiv./L]/[TAS, μmol Trolox equivalent/L] × 100

Statistical Analysis

Data obtained in the study were analyzed using the One-way ANOVA procedure of SPSS (SPSS for Windows release 18.0) with Duncan's Multiple Range Test to identify the significant differences between the means.

RESULTS AND DISCUSSION

The effects of licorice root supplement on performance of laying hens are shown in Table 2. The body weights at the beginning of the experiment were set to be similar to each other and body weights varied between 1828.50 and 1830.20 g in the diets groups. However, body weights at the end of the study were reduced with increasing level of licorice root supplement to diets. At the end of the experiment, body weights were determined as 1748.63, 1680.30, 1648.90 and 1635.78 g in groups containing 0, 0.5, 1.0 and 2.0% licorice root, respectively. The differences in body weights between the groups were statistically significant (p=0.086)at the end of the experiment. The feed consumption in the groups also decreased with

the addition of licorice root powder at increasing levels in diets (p=0.001). The highest feed consumption was obtained as to be 105.28 g in the control group and the lowest feed consumption was obtained as 97.47 g in 2.0 % supplemented group. The decrease in feed consumption with the addition of licorice root between the hens may be due to the aromatic odor of the plant. Indeed, Fiore *et al*. (2008) have indicated that licorice has a characteristic odor and sweet taste. Rezaei *et al*. (2014) reported similar results to those obtained in the present study. Researchers investigated the effects of *Thymus vulgaris* L., *Glycyrrhiza glabra,* and their mixture and enzyme supplement on broiler performance and they stated that feed consumption and body weight gain were_significantly lower in the *Glycyrrhiza glabra* supplemented group than in the other groups. Similarly, Amen & Muhammad (2016) obtained results that showed that the highest body weight was in 1 g/kg licorice root extract supplemented group for broiler at the 6th week. They stated that the other licorice root extract on supplemented groups (1.25 and 1.5 g/kg) had lower body weight than the control groups. Moradi *et al*. (2014) reported that body weights of broiler at the end of the experiment (42 days) were 2667.8, 2604.5, 2629.6, 2611.6 g in 0, 0.1, 0.2 and 0.3 mg/lit licorice root extract supplemented to drinking water. They stated that there was no difference between the groups in terms of feed consumption. In this study, final body weight of the licorice root addition group was found to be lower than the control group. Some studies have shown that licorice flavonoids have suppressed body weight by reducing the fat content of the body (Nakagawa *et al*., 2004; Tominaga *et al*., 2006). Researchers suggested that the possible mechanism for the reduction of visceral fat and low body weight gain; increase of fatty acid oxidation and reduction of fatty acid biosynthesis.

Unlike the current experiment, Safari & Zahedi (2016) reported that supplementation of licorice root extract to quail's diets and Salary *et al*. (2014) reported that the addition of licorice extract to drinking water increased the feed consumption and body weight. Salary *et al*. (2014) reported that increased consumption of feed could be attributed to a change in the taste of the feed and that this stimulated appetite. Sedgi *et al*. (2010) stated that *Glycyrrhiza glabra* added to feeds at a level of 0.5, 1, or 2 g / kg had no significant effect on body weight and feed consumption.

In present study, the best feed conversion ratio was obtained from the group of 0.5% licorice root supplementation and the worst feed conversion ratio from the control group (p=0.745). Safari & Zahedi (2016) found that the addition of licorice root extract to quail diets, Salary *et al*. (2014) reported that the addition of licorice extract to drinking water had no effect on the feed conversion ratio. The findings of these researchers are consistent with the results of the present study. Awadein *et al*. (2010) used 0.1 and 0.5% licorice root as a source of phytoestrogens in the layer's diets and stated that feed consumption level is lower in 0.1 and 0.5% licorice root supplemented groups than in the control group. They reported that feed conversion ratio was worse in the control group than in the supplemented groups. Moradi *et al*. (2014) reported that feed conversion ratio was better in the control group than in the current study (p>0.05).

There was no difference between the groups in terms of egg weight (Table 2) (p=0.200).The highest egg yield was obtained from 0 and 0.5% licorice root supplemented groups (p=0.021. Similar Results to the present findings also reported by Awadein *et al*. (2010). Researchers have reported that egg yields were higher in 0.1 and 0.5% licorice root supplemented group as a source of phytoestrogen than in the control group. In the present study, the highest egg yield was obtained 0.5% licorice root supplemented group and followed by the control group.

Effects of licorice root on some plasma biochemical parameters and egg yolk cholesterol

The effects of licorice root supplement on plasma parameters is shown in Table 3. Plasma cholesterol

Table 2 – The effects of licorice root supplementon performance of laying hens*

Parameters	Licorice Root Supplementation, (%)				p
	0	0.5	1.0	2.0	
Initial body weight (g)	1828.50±18.774	1829.05±19.915	1829.00±18.527	1830.20±20.946	1.000
Final body weight (g)	1748.63±32.978^{a}	1680.30±40.943^{ab}	1648.90±27.329^{b}	1635.78±28.633^{b}	0.086
Feed Consumption (g day hen)	105.28±1.464^{a}	103.86±1.504^{a}	101.25±1.329^{ab}	97.47±1.554^{b}	0.001
Feed conversion ratio (kg feed/kg egg)	1.98±0.029	1.93±0.032	1.96±0.036	1.94±0.037	0.745
Egg weight (g)	60.42±0.311	60.77±0.327	61.16±0.366	60.26±0.269	0.200
Egg yield (%)	88.94±1.066^{a}	89.56±1.041^{a}	86.82±1.180^{ab}	85.02±1.245^{b}	0.021

*The difference between the averages shown in different letters on the same line is significant (p<0.05)

level was 92.00 mg/dl in the control group, while it was 86.56, 89.43 and 86.35 mg/dl in 0.5, 1.0 and 2.0% in licorice root supplemented groups respectively. There was no statistically significant difference in plasma triglyceride levels among the experimental groups (p=0.780). Although the triglyceride levels of the groups are statistically insignificant, it tended to be lower in licorice added groups than in the control group. While the plasma LDL level decreased with the addition of licorice root (p=0.071), then an increase in HDL level was observed (p=0.001). In the current study, it was determined that the plasma glucose level increased with the addition of licorice root and this result was in correlation with the findings of Al-Daraji (2012a) and Al-Daraji (2012b). Al-Daraji (2012a) reported that the addition of 0, 150, 300 or 450 mg/l of licorice root extract to the drinking water in order to investigate the effects of the licorice root extract on broilers, increased plasma glucose level and the cholesterol level decreased (p<0.05). In the present study, it was observed that the plasma cholesterol level decreased with the addition of licorice root. In an earlier study of Al-Daraji (2012b), the effect of 0, 150, 300, 450 mg/kg licorice root extract supplement to broiler diets was investigated, and it was found that the blood glucose level was increased with increasing levels of licorice root extract. The researchers determined that the cholesterol levels were as 128.2, 134.6, 130.5 and 122.2 mg/dl in the supplemented groups, respectively and there was a statistically difference between the groups. Sedghi *et al*. (2010) found that blood glucose levels were higher in the control group in contrast to the results obtained in the current study. The researchers reported that the triglyceride levels were lower in the licorice root supplemented groups compared with the control group, and also stated that the HDL level increased and the LDL level decreased with the licorice root supplementation, but no statistically differences were destined between the groups (p>0.05). They also reported that cholesterol levels were significantly reduced by the addition of licorice root (p<0.05). The results obtained in the study are consistent with the findings of the researchers. Salary *et al*. (2014) stated that 0.2 and 0.4% licorice root extract in drinking water of broilers increased blood HDL level (p<0.05), and the cholesterol levels were 115.66, 123.66 and 106 mg/dl in the 0, 0.2 and 0.4% supplemented groups, respectively. They stated that triglyceride and LDL levels increased with the addition of licorice root, unlike the existing findings. Visavadiya & Narasimhacharya (2006) have reported a significant decrease in plasma hepatic lipid, cholesterol, triglycerides, LDL and VLDL levels while the increase in HDL-cholesterol content. Sharifi *et al*. (2013) reported that it may be due to the inhibition of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA), an enzyme active at plasma HDL levels. Sharifi *et al*. (2013) reported that the increase in plasma HDL levels may be due to the inhibition of the active enzyme hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA). Indeed, it is suggested that medicinal plants cause a decrease in HMG-COA enzyme synthesis, which is the key enzyme in cholesterol synthesis in the liver (Yu *et al*., 1998). Rezaei *et al*. (2014) reported that *Thymus vulgaris* L. and *Glycyrrhiza glabra* or a mixture thereof in broiler diets reduced the triglyceride levels in the blood. They also found that glucose levels were lower in *Glycyrrhiza glabra* supplemented groups.

It was determined that the egg yolk cholesterol level decreased with the addition of licorice root (p=0.005) as a result of the analyzed egg samples collected in the 4th and 8th weeks of the experiment. In the 4th and 8th weeks of the experiment, the control group had the highest egg yolk cholesterol content while the lowest egg yolk cholesterol level was determined in the 1.0% supplemented group. Awadein *et al*. (2010) investigated the effect of phytoestrogen source before pre-sexual maturity on performance of laying hens and indicated that egg cholesterol levels were significantly lower in 0.1% and 0.5% licorice supplemented groups than in the control group.

Table 3 – The effects of licorice root supplement on plasma biochemical parameters*

Parameters	Licorice Root Supplementation, (%)				p
	0	0.5	1.0	2.0	
Cholesterol (mg/dl)	92.00±6.667	86.56±8.956	89.43±2.677	86.35±2.871	0.892
Triglyceride (mg/dl)	398.12±21.203	376.17±23.797	394.22±20.177	373.14±17.891	0.780
LDL(mg/dl)	118.17±5.290^{a}	100.36±4.095^{b}	101.82±6.676^{ab}	114.23±4.703^{ab}	0.071
HDL (mg/dl)	25.40±2.731^{b}	34.80±1.624^{a}	35.20±1.529^{a}	41.40±2.502^{a}	0.001
Glucose (mg/dl)	132.00±3.674^{c}	191.60±8.096^{ab}	181.60±8.823^{b}	205.60±6.345^{a}	0.000
Egg yolk cholesterol 4. week (mg/g)	21.90±1.253^{a}	17.17±1.480^{b}	15.00±0.855^{b}	17.52±1.561^{b}	0.009
Egg yolk cholesterol 8. week (mg/g)	24.78±1.939^{a}	19.31±1.436^{b}	15.68±0.961^{b}	18.12±1.657^{b}	0.005

*The difference between the averages shown in different letters on the same line is significant (p<0.05).

Examined in Table 4, it was determined that the oxidant capacity was the best for the use of 0.5% level of the root and that the effect of increasing levels was not significant (p=0.001). Total antioxidant capacity was increased with the addition of licorice root, and it was determined as 0.893, 1.113, 1.239 and 1.107 µmol / L in the groups containing 0, 0.5, 1.0 and 2.0% licorice root, respectively (p=0.010).

The results found in the present studies smoothly correlate with earlier findings and it depicts that the licorice root has antioxidant capacity (Visavadiya & Narasimhacharya, 2006; Sen *et al*., 2011; Zhao *et al*., 2011; Habibi *et al*., 2014).

Habibi *et al*. (2014) reported that the addition of 0, 7.5 and 15 g/kg of licorice root increased the antioxidant enzyme activity and lowered the MDA level in which they investigated the effect on antioxidant capacity in broiler chickens. Visavadiya & Narasimhacharya (2006) reported that *Glycyrrhiza glabra* root increased hepatic HMG-CoA reductase activity in rats supplemented with 5 and 10 gm% levels of *Glycyrrhiza glabra* root in their diets and increased lipid peroxidation by increasing superoxide dismutase (SOD) and catalase enzyme activity. Sen *et al*. (2011) stated that glycyrrhizin, one of the important compounds of the licorice root, brought to normal levels oxidative stress parameters such as SOD, catalase and malondialdehyde (MDA) in diabetic mice. Zhao *et al*. (2011) found that 0, 5, 10, 15 and 20 g/kg ginger root supplemented to layer diet increased plasma and egg antioxidant content.

Table 4 – The effects of licorice root supplement on plasma total oxidant and antioxidant capacity in laying hens.*

Parameters	Licorice Root Supplementation, (%)				*p*
	0	0.5	1.0	2.0	
Total Oxidant Status (µmol/L)	0.531±0.041^{b}	0.377±0.041^{c}	0.629±0.383^{ab}	0.671±0.041^{a}	0.001
Total Antioxidant Status(mmol/L)	0.893±0.031^{b}	1.113±0.049^{a}	1.239±0.086^{a}	1.107±0.048^{a}	0.010
Oxidative Stress Index	0.059±0.004^{a}	0.034±0.004^{b}	0.051±0.002^{a}	0.061±0.005^{a}	0.002

*The difference between the averages shown in different letters on the same line is significant (p<0.05).

From the above mentioned findings of the current study, it was noted that the licorice root could be used in laying hens diets to lower the egg cholesterol content without negative effect on performance. In addition, the licorice root had a positive effect on plasma cholesterol, triglyceride, LDL, HDL levels in laying hens. The antioxidant capacity in laying hens could be increased by licorice root supplement. In this context, it has been concluded that the licorice root could have a potential role to promote the production of functional eggs from healthy hens.

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