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Original article

Effects of dietary licorice root (*Glycyrrhriza glabra*) supplementation, storage time and temperature on quality of quail eggs

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Summary. This study was conducted to investigate the effect of licorice root (*Glycyrrhriza glabra*) supplementation, different storage temperature and storage time on egg quality in quails. Two hundred and forty 10-wk-old laying Japanese quails, randomly divided into four equal groups for dietary treatments, were fed using the commercial diet as a control group and the commercial diet supplemented with 0.5, 1.0 and 1.5% licorice root powder. The forty eggs were sampled and weighed for each one of storage time groups (i.e. 15, 30 and 45 days), and were stored at +4°C and +25°C. Egg internal and external quality characteristics were determined at the end of each storage period for each temperature. The egg albumen pH level was increased in a parallel of the escalation of storage time and temperature, whereas it was decreased as the licorice level was increased. Haugh Unit (HU) value was decreased with an increase of storage time and temperature, but it was increased in a parallel of accelerating level of licorice root. Though there were no significant differences in egg weight loss, yolk index and egg shell weight between the treatments, they were significant for the albumen index and egg shell thickness ($P<0.05$). Moreover, albumen index and egg shell thickness were increased with an enlarged licorice root supplementation. It was concluded that the use of licorice root in laying quail diet may be effective for the expansion of egg shelf life.

Key words: Egg quality, licorice root, Quail, storage temperature, storage time.

Introduction

The egg is an excellent and inexpensive food, and it contains high quality protein and many crucial nutrients. A medium-sized egg contains up to 6 grams of protein, and the essential amino acids exist in ideal proportions therefore, egg proteins are used as a standard of the protein quality for other foods. Egg protein has a digestibility of 98% and a biological value of 94%, which is a good source of protein. About 65% of the fatty acids of egg yolk are unsaturated fatty acids and 34% are saturated fatty acids (1). However, as soon as the egg is laid, its internal quality starts to decrease. Eggs deterioration is enhanced by high temperature and storage time. HU value which is the indicator of albumin quality decreases and pH increases with an increase in storage time and temperature (2,3).

Eggs have high levels of unsaturated fatty acids. These are more susceptible to lipid oxidation (4) which is one of primary mechanisms of quality deterioration in eggs (5). Lipid oxidations during storage may produce toxic compounds. Oxidative products in eggs can reduce its nutritive value (6). It is necessary to prevent or reduce lipid oxidation in order to avoid the decrease

in the nutritional value of the egg (2). For this purpose, studies have been carried out with medicinal and aromatic plants and extracts used in laying hens feeds so as to prevent the decline of egg quality and shelf life (3,7,8). To avoid the egg quality losses during storage time, the use of herbal products could be a beneficial tool in the industry. Much focus have been given to the use of herbs and herbal products to improve performance and to some extent on the quality in freshly laid eggs but limited research data are available for the impact of herbs on the storage quality of eggs (9).

Due to the various pharmacological effects of many medicinal and aromatic plants found in nature, they have been used extensively for medicinal purposes and spices for centuries. These plants have various functional properties due to the bioactive components they contain (10). When the plants and their extracts are consumed by humans and animals, they are considered to be safe additives in terms of their chemical structure. In food industry, plants or their extracts having antioxidative properties are frequently used to improve quality and shelf life of meat products (11) and egg (12).

Licorice (Glycyrrhriza glabra) is one of the oldest and most widely known medicinal plants in the world. A large number of pharmacological active compounds were isolated from the licorice plant and were identified and verified by modern analytical techniques. Triterpene saponins (4–20%) and various types of phenolic compounds are the major bioactive compounds (13). It has been reported that isoliquiritigenin, one of the major flavonoid compounds of the plant, has estrogen-like activity (14), whereas glabridin isoflavonoid compounds show strong antioxidant activity (15). Ju (16) found that the antioxidant effect of licorice flavonoids was 100 times higher than vitamin E. Therefore, due to its antioxidant activity in animal (8,17) licorice root powder may improve the egg quality in different storage time and temperature. Limited studies are available on the use of licorice root powder and its extract in laying quails diet concerning egg quality traits during storage conditions. Therefore, the objective of this study was to investigate the potential use of dietary licorice root powder in the quality of quail eggs during different storage time and temperature.

Table 1. Ingredient and chemical composition of the diet

Ingredient	g/kg	Calculated Nutrient	Composition
Maize	48.55	ME, kcal/kg	2900
Soybean meal	33.95	Crude protein, %	19.78
Meat-bone meal	3.00	Crude fiber, %	2.98
Vegetable oil	5.00	Available phosphorus, %	0.60
Lime stone	7.60	Calcium, %	3.20
Di calcium phosphate	1.2	Methionine, %	0.40
Salt	0.25	Methionine & Cysteine, %	0.65
Vitamin premix*	0.10		
Mineral premix**	0.10		
DL-Methionine	0.20		
Lysine, %	0.05		
	100.00		

** Each kg vitamin premix includes : 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, and 0.25 mg Biotin ** Each kg mineral premix includes: 100 mg Manganese, 80 mg Iron, 100 mg Zinc, 10 mg Copper, 0.2 mg Cobalt, 1.5 mg Iodine, and 0.2 mg Selenium.*

Material and Methods

Two hundred and forty 10-wk-old laying Japanese quails were randomly divided into four equal numbers of dietary treatment groups and consisted of 4 replicates with 15 birds each. Quails were fed a commercial diet as a control group and other three groups were fed commercial diet supplemented with 0.5, 1.0 and 1.5 % licorice root powder (*Glycyrrhriza glabra*). Ingredient composition of feed and its calculated nutrient compositions are given in Table 1. The birds were kept in 50 x 50 x 20 cm (length, width, height) cages. During the experiment, a 16 h light and 8 h dark lightining program was applied to laying quails. Water and diets were *ad- libitum*. Licorice root was obtained from Hatay province which is located on the eastern Mediterranean coast of the Southern Turkey.

A total of 560 eggs were collected from quails fed with 0, 0.5, 1.0 and 1.5 % licorice root powder supplemented diets during the 5th and 6th week of the experiment. Total of 80 eggs (20 eggs each group) were analyzed

within 2 hours after being laid (0 day storage). Remaining 480 eggs were numbered and weighed, and half of eggs was stored at +4 ˚C in the refrigerator and the remaining part was kept in at +25 ˚C for 15, 30 and 45 days and at 60-65% humidity. For each storage time, 40 eggs from each group were taken and half of eggs stored at + 4 ˚C and the other half eggs was kept on + 25 ˚C.

At the end of each storage period, 40 eggs (i.e. 20 eggs in the refrigerator and 20 eggs in +25 ˚C) were taken from each group and weighed to measure egg weight loss (EWL). After storage, EWL was calculated by subtracting the final egg weight from the initial one. Egg internal and external quality characteristics were ascertained at the end of the storage period. The egg external characteristics were weight (g), length and width (mm), shell weight (g) and shell thickness (mm), whereas the internal quality parameters were albumen height (mm), long diameter of albumen, short diameter of albumen (mm), yolk height (mm), yolk diameter (mm) and Haugh unit. Other parameters measured included egg shape index, albumen pH and yolk color. For this purpose, the egg width and height were measured using a digital compass, and the egg shape index was determined. The eggs were then broken on a flat surface and albumen and yolk height were measured with tripod micrometer, albumen length and width and yellow diameter were measured using a digital compass. Using these measurements, the yolk index, the albumen index and the HU value were calculated using the following formulas (18).

The Haugh unit (HU) values were calculated by the formula

Haugh Unit = 100 log (h +7.57- 1.7$W^{0.37}$)

Where h=height of albumen (mm) and W=weight of egg (gr).

The egg albumen was separated from the egg yolk by using egg yolk separator. After the egg albumen had homogenized, the pH value was measured. The egg inner shell was cleaned, weighed and then the egg shell thickness was gauged using a point micrometer by taking the egg shell from three different areas of the egg. A calibrated micrometer was used for measuring the thickness of the eggshell, which is reported as the mean of three different sides, in millimeters.

Data were analyzed by using the SPSS 18.0 (Statistical Package for Social Sciences) program. The diets, storage time and temperature were factors in using the One-way ANOVA. Duncan Multiple Range Test was used to compare means. The interactions were examined with the use of general linear model (univariate).

Results and Discussion

The effects of licorice root powder supplementation, storage time and temperature on egg external quality are presented in Table 2. Licorice root powder supplementation did not have significant effect on egg weight after storage, egg weight loss during storage and egg shell weight ($P>0.05$). Egg shell thickness was remarkably increased with the addition of licorice root ($P<0.05$). It can be attributed to calcium content of the licorice root. Isbrucker and Burdock (19) and Fiore et al. (20) reported that a mixture of potassium and calcium salts of glycyrrhizin constituted the majority of the triterpenes saponins present at the ratio of 4-20% in licorice root. In contrast to our finding Awadein et al. (21) used 0.1 and 0.5% licorice root as a source of phytoestrogens in the Mandarah hens diets and stated that 0.5% licorice reduced shell thickness. Similar results for shell weight were reported by these researchers; Sedghi et al. (22) indicated that *Glycyrrhiza glabra*, added to feeds at the level of 0, 2, 4 and 6 g/kg, had significant effect on shell thickness in laying hens. They described that hens fed with 4 g/kg of licorice extract produced thicker shell significantly ($P<0.05$) than those supplemented with 6 g/kg. In present experiment, egg shell weight was not affected with the additional licorice root, and similar results were also reported by Sedghi et al. (22).

Egg weight after storage, egg weight loss during storage and egg shell thickness were increased parallel to increase of storage temperature ($P<0.05$). Egg weight loss during storage was significantly less in eggs stored at 4˚C than those kept on 25 ˚C . This may be due to the less loss of solvents (water plus other gaseous products) from egg contents than those in room temperature (2). Moreover, egg weight after storage was decreased, but egg weight loss during storage was increased with an escalation of storage time of the eggs ($P<0.05$). These results are in the agreement with

those of Eke et al. (23); Akter et al. (2). The weight loss is mainly associated with the evaporation of water and loss of carbon dioxide from the albumen through the porous shells (24). Shape index was not affected significantly by licorice root supplementation, storage time and storage temperature (Table 2). Ahmad et al. (7) who reported that shape index value remained unchanged during the experiment. These results are also supported by Akter et al. (2) who reported similarly.

No differences were detected for albumen height, albumen pH and yolk index among licorice root supplemented groups (P>0.05). However, Haugh Unit and albumen index value were increased with additional licorice root supplementation. Haugh unit value which is the indicator of albumin quality was increased with a supplemented licorice root in comparison with the control group (Table 3). The higher Haugh Unit value in supplemented group can be attributed to the antioxidant property of the bioactive compounds that the plant possesses. This may be preserved the egg shell life. Interestingly, Ju (16) showed that the antioxidant effect of licorice root flavonoids is 100 times higher than that of vitamins E. Natural antioxidants such as tocopherols, vitamin C, flavonoids and synthetic antioxidants, are generally used to slow down or stop lipid peroxidation and preserve product freshness (25). Akter et al. (2) explained that yolk lipid oxidation was increased significantly with storage time and tempera-

Table 2. The effects of licorice root, storage time and temperature on egg external quality

	Egg weight before storage (g)	Egg weight after storage (g)	Egg weight loss during storage, %	Egg shell weight (g)	Egg shell thickness (mm)	Shape index
Licorice Root, %						
0	12.2±0.07	11.7±0.09	4.40±0.290	1.32±0.014	0.204±0.002^{b}	79.71±0.250
0.5	12.1±0.06	11.6±0.07	4.31±0.277	1.31±0.010	0.215±0.001^{a}	80.40±0.238
1.0	12.2±0.07	11.6±0.07	4.89±0.302	1.32±0.013	0.215±0.001^{a}	80.47±0.215
1.5	12.2±0.08	11.6±0.09	4.49±0.270	1.31±0.013	0.216±0.001^{a}	79.91±0.240
SEM[1]	0.037	0.042	0.142	0.006	0.000	0.118
P value[2]	0.739	0.957	0.505	0.854	0.000	0.065
Storage Temperature (°C)						
4 °C	12.1±0.05	10.4±0.05^{b}	2.56±0.126^{b}	1.29±0.010	0.210±0.001^{b}	80.09±0.193
25 °C	12.2±0.05	11.8±0.06^{a}	6.49±0.195^{a}	1.31±0.009	0.215±0.001^{a}	80.06±0.183
SEM[1]	0.041	0.043	0.142	0.006	0.000	0.133
P value[2]	0.177	0.000	0.000	0.230	0.013	0.906
Storage Time (day)						
0	12.5±0.07^{a}	---	---	1.37±0.017^{a}	0.212±0.001^{b}	80.41±0.256
15	12.1±0.06^{b}	11.8±0.06^{a}	2.83±0.159^{c}	1.32±0.010^{b}	0.220±0.001^{a}	80.46±0.197
30	12.1±0.07^{b}	11.5±0.07^{b}	4.93±0.202^{b}	1.24±0.012^{c}	0.212±0.001^{b}	79.95±0.217
45	12.2±0.07^{b}	11.4±0.08^{c}	5.81±0.304^{a}	1.36±0.011^{a}	0.205±0.001^{c}	79.79±0.267
SEM[1]	0.037	0.043	0.142	0.006	0.000	0.118
P value[2]	0.007	0.005	0.000	0.000	0.000	0.110
Main effects						
LRxSTE	0.312	0.501	0.015	0.014	0.000	0.861
LRxST	0.995	0.872	0.000	0.758	0.000	0.028
STExST	0.218	0.000	0.000	0.000	0.002	0.002
LRxSTExST	0.079	0.358	0.050	0.163	0.000	0.000

^{abc}: The difference between the averages represented by different letters in the same column for each parameter which is significant (P<0.05). LR: licorice root; STE: Storage temperature; ST: Storage time [1]SEM: Standard error of the mean, [2] Level of significance was set at P<0.05

ture, because egg storage for a longer period at different temperatures may reduce the antioxidant activity.

The similar results were demonstrated by Awadein et al. (22), who explained that Haugh unit value was significantly increased with an increase of licorice root supplementation. Al-Harthi, (25), who investigated the effects of natural and synthetic antioxidants on egg quality of laying hens, reported that Vitamin E significantly increased Haugh unit by 4.6%.

It has been determined that all internal egg quality characteristics was deteriorated with an acceleration of storage time and temperature (Table 3). These results in agreement with Olobatoke and Mulugeta (26), who explained that HU and albumen height were decreased and albumen pH was increased with an increase of storage temperature in laying hens. Albumen height, HU, albumen index and yolk index were all decreased with an extended storage time (P<0.05). The results suggest that these changes were faster in higher temperature (25°C) than that of lower temperature (4°C). These results are in harmony with those of Tayeb, (27) and Eke et al. (23), who stated that HU and yolk index were significantly decreased with an increased storage time and temperature.

The results of current study were also supported by Akter el al. (2), who observed HU of fresh eggs was significantly declined (P<0.05) with an increase of storage time and temperature. Researchers explained that HU reduction occurred due to the decrease in thick albumen height, because during storage the ovomucin-lysozyme complex breaks down, which contributes to increase the pH level. The modifications in

Table 3. The effects of licorice root, storage time and temperature on egg internal quality parameters

	Albumen height	Haugh Unit	Albumen pH	Albumen index	Yolk index
Licorice Root, %					
0	3.89±0.090	85.05±0.564	9.08±0.016	8.75±0.282	38.92±0.957
0.5	4.04±0.091	85.95±0.556	9.05±0.014	9.11±0.285	39.08±0.917
1.0	4.14±0.086	86.53±0.530	9.05±0.014	9.62±0.279	38.53±0.805
1.5	4.12±0.079	86.84±0.486	9.06±0.014	9.57±0.250	39.04±0.809
SEM[1]	0.043	0.268	0.007	0.137	0.436
P value[2]	0.111	0.092	0.383	0.080	0.970
Storage Temperature (°C)					
4 °C	4.37±0.032[a]	88.36±0.188[a]	9.02±0.008[b]	10.70±0.130[a]	46.30±0.279[a]
25 °C	3.12±0.055[b]	80.23±0.377[b]	9.21±0.005[a]	5.87±0.132[b]	27.09±0.425[b]
SEM[1]	0.041	0.269	0.006	0.132	0.482
P value[2]	0.000	0.000	0.000	0.000	0.000
Storage Time (day)					
0	5.49±0.056[a]	94.070.288[a]	8.78±0.007[d]	13.63±0.189[a]	47.33±0.566[a]
15	4.35±0.048[b]	88.21±0.273[b]	9.01±0.010[c]	9.80±0.182[b]	40.38±0.576[b]
30	3.63±0.063[c]	83.74±0.420[c]	9.13±0.009[b]	8.05±0.205[c]	37.40±0.801[c]
45	3.30±0.077[d]	81.34±0.522[d]	9.21±0.009[a]	7.20±0.252[d]	33.47±0.993[d]
SEM[1]	0.043	0.268	0.007	0.137	0.436
P value[2]	0.000	0.000	0.000	0.000	0.000
Main Effects					
LR x STE	0.532	0.651	0.794	0.477	0.003
LR x ST	0.423	0.402	0.110	0.185	0.162
STE x ST	0.000	0.000	0.005	0.000	0.000
LR x STE x ST	0.028	0.030	0.218	0.106	0.024

[abcd]: The difference between the averages represented by different letters in the same column for each parameter which is significant (P<0.05)
LR: licorice root; STE: Storage Temperature; ST: Storage Time, [1]SEM:Standard error of the mean, [2] Level of significance was set at P<0.05

egg weight and in dens albumen consistency lead to a gradual decrease in HU values (28).

Dudusola (29) reported that HU and yolk index were decreased, egg weight loss was increased with an increase in storage time and temperature. Similar reduction in yolk index value has also been reported by Akter et al. (2). Akter et al. (2) explained that after eggs are laid, water moves from the albumen to the yolk due to differences in osmotic pressure, and this may change the yolk index and may cause the weakening of the vitelline membrane.

Significant increases in albumen pH were observed with the increased storage time and temperature ($P<0.05$). Similar results were demonstrated by Caner and Cansız (30). The increase in egg albumen pH level is caused by the loss of carbon dioxide after egg laying (28). Thus, Decuypere et al. (31) reported that at oviposition the egg contains a high concentration of carbon dioxide which starts to escape after laying and during storage. Excessive carbon dioxide loss causes the albumen to have an extremely high pH level. If the loss of carbon dioxide was too low, the albumen pH level would be low resulting in eggs which are too fresh. CO_2 loss from the egg also varies depending on the temperature (32). In the present study, the pH content of the albumin was increased with an acceleration of storage temperature.

The current study proved that dietary supplementation of licorice root powder in laying quails had significant effect on egg quality during different storage temperature and time. It was concluded that licorice root supplementation has the potential to extend the shelf life of quail eggs.

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