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Impact of Adding Different Levels of Licorice Root Powder to Japanese Quail Diet on Egg Production and Quality

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Abstract: The present study aimed to evaluate the influence of dietary supplementation of licorice root powder (LRP) Glycyrrhiza glabra on the productive performance of Quail and some qualitative egg traits. A total of 128 female quails aged 49 days were randomly distributed into four groups, each containing four replicates with eight females per replicate. The groups were as follows: G1 (control) was fed a standard diet without supplementation, while G2, G3 and G4 were supplemented with licorice root powder 0.5%, 1.0% and 1.5%, respectively, added to the same standard diet. The results of the study showed significant rise ($p \leq 0.05$) in the total number of eggs and percentage rate of egg production (%H.D.) in the third group (G3) when compared to the control group. In addition, no differences were noted in average egg weight, total egg mass, total feed intake, efficiency of feed conversion, body weight change, or overall death rate. The present work also showed no differences in shell thickness and weight, albumen weight, yolk weight, or shape index of the egg. In conclusion, this study suggests using licorice root powder as an ecologically safe and sustainable dietary supplement in layer quail feeding at 1% to boost egg production rate.

Keywords: licorice, Quail, production performance, egg traits.

Introduction

Poultry products, such as eggs and meat, are an essential source of high-quality protein due to their nutritional value and the content of essential amino acids needed for human growth and health [1]. In addition, they are affordable compared to other animal protein sources [2]. Recently, there has been increased demand for the breeding and production of quails due to high demand for their products. It is a dual-purpose bird due to its rapid growth rate and abundant egg production with a high metabolism rate. Quails are easy to raise because they are small birds and therefore require limited space. They also possess a high tolerance to various environmental conditions and excellent disease resistance [3].

The most daunting challenge that faces the poultry industry is provision of adequate quality feed for enhancing productive and health condition of poultry, while reducing or not incorporating feed additives such as antibiotics and other chemicals, especially given that their utilization in animal food is not permitted in the majority of countries, including European Union [4], given concerns of their adverse effects on the well-being of consumers. Therefore, research has increasingly emphasized the use of medicinal plants, some herbs and spices are used as feed additives to enhance poultry productivity and support health as alternatives to antibiotics and traditional growth promoters. Among the alternatives, licorice was chosen, since it is a possible alternative due to its active constituents. It is one of the oldest of drug plants and used worldwide extensively as a result of its very helpful therapeutic effects upon different diseases [5]. It goes by the names "Eiraq Alsuws" in Arabic and "Licorice" or "Liquorice" in English, though its botanical title is Glycyrrhiza glabra [6]. The licorice plant belongs to the family Leguminosae. It is a perennial herbaceous plant, which is prevalent in Europe and Asia, and it grows naturally in the Mediterranean, Middle East, and East Asia. It is also cultivated commercially in some countries, particularly countries that specialize in their pharmaceutical industries, including China, Japan, and Europe. Licorice in Iraq is a shrub that grows on farms, orchards and riversides [7].

The most active part of licorice plant is roots since it has active components, plant power source, found in its roots. Of major significance are such active ingredients such as Glycoside that consist of carbohydrate and non-carbohydrate constituents. The most important of these is Saponin, which is also called Glycoside Saponin and Glycyrrhizin, and is hydrolyzed in an acidic medium to form Glycyrrhetinic Acid [7]. It has been discovered in studies that upon hydrolysis of Glycyrrhizin in the body, its composition is similar to adrenal cortex hormones, which might be the main reason for its anti-inflammatory activity. Glycyrrhizin also produces similar effect to ACTH in adrenal cortex, which is enhancing secretion of glucocorticoids and mineralocorticoids. Glycyrrhetinic acid resembles cortisone in anti-inflammatory and anti-stress effect because their structural makeup is same as their molecular structure is similar at the 3 and 11 bond position. The cause of the effectiveness of Saponin is its structure which is like adrenal steroid hormones [8]. There

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are a number of flavonoids found in licorice roots such as clabridin, chalcones, clabrin, isoliquirtin, liquiritin, and glabroin [9]. Flavonoids and glycyrrhizin have been shown to be anti-inflammatory and antitumor agents. They also exhibit antioxidant activities [10]. As indicated by Haleem et al. [11], flavonoids are effective antioxidants by virtue of their capacity to protect many vital molecules such as low-density lipoprotein and red blood cells against all components of oxidative stress by inhibiting cell membrane lipid peroxidation [2]. Abdul-Majeed [9] also illustrated that flavonoids have an important biological role against certain diseases, may prolong effectiveness of vitamin C, and inhibit platelet aggregation. Volatile fatty acids such as propionic acid and butyric acid also exist in roots of licorice [12]. Roots of licorice are rich in vitamins (including vitamin E and B vitamins) and minerals that are critical in forming body tissues like calcium, phosphorus, iron, cobalt, tin, zinc, and manganese [13].

In this study, researchers sought to determine the optimal ratios of adding licorice root powder (LRP) to Japanese quail feed and its impact on production performance and egg quality.

Materials and Methods

Housing and Bird Management

This experiment was conducted in the poultry house of the College of Agriculture and Forestry, University of Mosul. A total of 128 female quails aged 49 days were used and distributed into four groups, with each group containing four replicates of eight females each. All birds were raised in a dedicated quail rearing hall equipped with wire cages measuring 50×50×50 cm (length, width and height). The birds were maintained under the same managerial and environmental conditions, with poultry house temperature ranging between 18-21°C and relative humidity between 55-65%. A lighting program of 16 hours of light and 8 hours of darkness per day was implemented.

Feed Ingredients and Feeding Method

Diet was prepared in the form of a mixed and homogeneous mash, formulated according to the recommendations established by the National Research Council (N.R.C) [14]. Table (1) illustrates the composition of diet used in the study. LRP was added to diet of each group according to the specified percentages before being provided to the birds. The powder was thoroughly and uniformly mixed with the other diet components. Feed and water were provided ad libitum throughout the experimental period. The study lasted for six weeks (42 days). The experimental groups were as follows:

G1: (Control group) Fed a standard diet without any additives.

G2: Supplemented with 0.5% LRP added to the standard diet.

G3: Supplemented with 1% LRP added to the standard diet.

G4: Supplemented with 1.5% LRP added to the standard diet.

Performance Standards

Productive performance indicators of laying quails were studied over a period of six weeks. Eggs were collected daily for each replicate, and the average number of eggs produced per female was calculated by dividing the total number of eggs produced per replicate by the number of females in that replicate. Additionally, the egg production rate (%H.D) was calculated using the following formula:

$$\%\mathrm{H.D} = \frac{\text{Number of eggs produced on daily basis}}{\text{Number of birds available in the flock on that day}} \times 100$$

Eggs produced were weighed daily for each replicate using a sensitive electronic scale to determine average egg weight per group. This was done by dividing total weight of eggs produced per replicate by number of eggs produced in the same replicate. Egg mass was calculated using the following formula:

$$\text{Egg mass (g/bird)} = \text{Average total number of eggs} \times \text{Average egg weight during the same period}$$

Feed intake for each replicate was calculated by weighing remaining feed at the end of the week and then subtracting it from feed intake at the beginning of the week for each replicate. Feed conversion ratio (FCR) was calculated based on the amount of feed consumed (g of feed) required to produce one gram of egg. To determine the change in average body weight, live weight of the birds was recorded at the beginning and end of the experiment to monitor changes in body weight during the experimental period. The change in body weight was calculated using the following formula:

$$\text{Change in body weight (g/bird)} = \text{Final weight (g/bird)} - \text{Initial weight (g/bird)}$$

The number of dead birds in each replicate was recorded, and death rate was calculated for each treatment using the following formula:

$$\%\ \text{Death} = \frac{\text{Number of dead birds}}{\text{Total number of birds}} \times 100$$

Table (1): Components of the laying quail feed used in this study.

Feed material	%
Soybean meal	29
Yellow corn	47
Wheat	12
Protein concentrate*	5
Sunflower oil	1.5
calcium carbonate	5
NaCl	0.5
total	100
Estimated Chemical Composition **	
Crude protein	20.45
The representative energy	2819
Crude fiber	3.6
Lysine	1.09
Methionine	0.35
Methionine + cysteine	0.72
Calcium	2.33
Available phosphorus	0.28

* Protein Concentrate: Supplied by WAFI (a Dutch company), containing: 40% crude protein, 2100 kcal/kg metabolizable energy, 5% crude fat, 2% crude fiber, 3.85% lysine, 3.70% methionine, 4% methionine + cystine, 6.5% calcium, 2.6% phosphorus, 2.3% sodium,1600 mg/kg manganese,1200 mg/kg zinc,1000 mg/kg iron, 20 mg/kg iodine, 3 mg/kg cobalt, 5 mg/kg selenium,1000 mg/kg selenomycin, 600 mg/kg vitamin E, 220 IU/kg vitamin A, 60 IU/kg vitamin D3, 60 mg/kg vitamin B1, 80 mg/kg vitamin B6, 20 mg/kg folic acid, 400 µg/kg vitamin B12, 800 mg/kg niacin, vitamin K_3 50 mg/kg, B_2 140 mg/kg, choline 5000 mg/kg, and copper 200 mg/kg.

** Chemical composition calculated based on analysis of feed materials reported in NRC (1994).

Egg Quality Standards

At the end of the study, six eggs were collected from each replicate (totaling 24 eggs per group) required qualitative examinations. The length and width of the eggs were measured, and shell thickness was determined using a Dogmatic Caliper. The egg shape index was calculated using the following formula:

$$\text{Egg shape index} = \frac{\text{egg width mm}}{\text{egg length mm}} \times 100$$

A sensitive balance (0.01 g precision) was used to weigh the whole egg, including the shell, albumen and yolk. The percentage weights of these components were calculated using the following formula:

$$\text{Shell weight \%} = \frac{\text{shell weight}}{\text{egg weight}} \times 100$$

In the same way, the relative weight of yolk and albumin was measured.

Statistics

The experiment was conducted using a completely randomized design (CRD), and the study data were analyzed using one way ANOVA with the ready-made statistical analysis software (SAS) [15]. The Duncan multiple range test was also applied to determine the significant differences among the means [16].

Results and Discussion

Table 2 shows the effects of adding different levels of LRP to the diet of quail on average total number of eggs (eggs/bird). The results showed a notable improvement ($p \leq 0.05$) in the third group (1%) as opposed to the control group. Still, there were no apparent differences between the control group and each of the second and fourth groups. Total number of eggs was (30.33, 30.93, 34.08, and 31.30 eggs/bird), respectively. Furthermore, the results showed a notable improvement ($p \leq 0.05$) in egg production rate (%H.D) for the third group in comparison with the control, whereas no significant differences were obtained for the second and fourth groups compared to the control. Rates of egg production were (72.20, 73.69, 81.13, and 74.52 %), respectively.

The findings of this study coincide with Li et al. [17] who noted a significant enhancement ($p \leq 0.05$) in egg production rate of Chinese yellow quails after supplementation of licorice at 600 mg with 100 mg of rutin per kg of feed. In a later study by Hanafy et al. [18] an improvement was reported ($p \leq 0.01$) for egg production when licorice powder was supplemented in the diet of local hens (Inshas) compared to the control group during summer months. On the other hand, the results of our study do not agree with the results of Sabzipoor et al. [19] who found no enhancement on egg production when licorice extract was included in the diet of laying hens. Zeweil et al. [20] also did not observe any remarkable increase in egg production (%) of quails fed with diets containing licorice roots. Furthermore, Doğan et al. [3] also reported a remarkable decrease in egg production (%) in quails when LRP was included in diets at 0.5 and 1% levels.

The improvement in egg count and egg production rate (%HD) may be due to the presence of glycyrrhizin and glycyrrhetinic acid in licorice root powder, which promotes the production of steroid sex hormones such as estrogen and prolongs the action of its effect [21]. Also, licorice promotes digestion and helps utilization of amino acids, fatty acids, and glucose by increasing blood flow rates in mucous membranes lining the digestive tract and enhancing the use of essential nutrients. Anabolic effects of steroid hormones include promotion of protein synthesis and inhibition of protein degradation, enhance basal metabolic rates and promote the body's capacity to retain calcium, which is essential in egg formation [22]. In addition, the release of estrogen from the hen's ovary into the bloodstream increases the levels of fats, vitamins, and calcium in the blood, which are essential for egg formation. Additionally, estrogen stimulates the liver to produce various yolk components, which are then carried in the blood to growing follicles and deposited via receptor-specific mechanisms. Estrogen functions to promote the growth of the oviduct and also stimulates the secretion of the tubular gland, which is involved in the synthesis of egg-specific formation proteins [21]. This effect may also be attributed to licorice's flavonoids. Flavonoids have also been found to increase the lifespan of vitamin C in the body, which plays a significant role in enhancing birds' ability to resist and neutralize stress agents. It also facilitates amino acid metabolism and reduces protein breakdown [9], thereby ensuring sufficient protein utilization for egg synthesis. Licorice also contains a vast array of necessary vitamins and minerals that take part in the synthesis of many hormones and enzymes needed in vital body processes [13], thus leading to egg formation.

The results reported in Table 2 also show no effect of feeding laying quails a diet with LRP on the mean egg weight (g/egg) and the mean total egg mass (g egg/female). These findings are similar to those of Zeweil et al. [20] who observed no significant difference in the mean weight of eggs and egg mass in quails when licorice was supplemented in the feed at levels of 250, 500 and 1000 mg/kg. Similarly, the same was reiterated by Sabzipoor et al. [19] when no increase in egg weight and egg mass was observed after the addition of licorice extract into the diet of laying hens. However, these results were not in parallel with Hanafy et al. [23] since they obtained a highly significant increase ($p \leq 0.01$) in the mean egg weight and egg mass per day in the treatment provided with 0.5 and 1% licorice powder supplementation in the diet of laying hens compared with the control treatment lacking licorice.

Table (2): Effect of adding different levels of LRP to quail diet on average total egg number, egg production (%H.D), average egg weight, and average total egg mass (means ± standard error).

Groups		Average total number of eggs	Egg production %H.D	Average egg weight (g/egg)	Average total egg mass (g egg/bird)
G1	Control	30.33±0.67 ^{b}	72.20±1.60 ^{b}	11.93±0.13 ^{a}	361.81±10.68 ^{a}
G2	LRP % 0.5	30.95±1.39 ^{ab}	73.69±3.31 ^{ab}	11.99±0.17 ^{a}	370.58±14.52 ^{a}
G3	LRP % 1.0	34.08±1.20 ^{a}	81.13±2.85 ^{a}	11.67±0.27 ^{a}	398.21±21.61 ^{a}
G4	LRP % 1.5	31.30±0.76 ^{ab}	74.52±1.81 ^{ab}	11.73±0.19 ^{a}	366.86±6.01 ^{a}
Significant		*	*	N.S	N.S

* Different letters within a column indicate significant differences at ($p \leq 0.05$).

Table 3 shows the effect of adding different levels of LRP to the diet of quail on feed intake. The results indicated that there were no significant differences in the average total feed intake (g feed/bird) among all the supplemented groups and the control. In addition, there was no significant variation in the total feed conversion ratio (g feed: g egg) among all groups with a minor and insignificant improvement in favor of the supplement group compared to the control group, with values of 3.61, 3.41, 3.31, and 3.45 g feed: g egg, respectively. Moreover, the outcomes of statistical analysis revealed that the rate of live body weight change (g/bird) was not affected by the addition of LRP to all groups compared with the control group. The addition of LRP did not affect the death (%) of laying quails either. This indicates that these traits are not affected by the addition of any level of LRP into the diet. This result conforms to Zeweil et al. [20] whose report indicated that there was no variation in feed intake level, feed conversion ratio, or quail live body weight change when licorice root was added at levels of 250, 500, and 1000 mg/kg diet. It is also consistent with the results of Doğan et al. [4] when LRP was added to laying quail diets because they found no significant difference in feed intake or feed conversion ratio. In addition, Hanafy et al. [18] also demonstrated that there was no variation in daily feed intake, whereas a significant improvement ($p \leq 0.01$) was observed in the feed conversion ratio while supplementing licorice powder at levels of 0.5, 1.0 and 1.5% in the local laying hens' (Inshas) feed relative to the control during summer, and there was a great superiority in the final live weight during supplementing licorice at a level of 0.5% relative to the control.

Table (3): Effect of adding different levels of LRP to quail diet on average total feed intake, feed conversion ratio, average change in live body weight, and death rate (means ± standard error).

Groups		Average total feed intake (g feed/bird)	Feed conversion ratio (g feed: g egg)	Average of change in live body weight (g/bird)	% Death
G1	Control	1304.93±28.03 ^{a}	3.61±0.08 ^{a}	11.75±11.37 ^{a}	0.25±0.25 ^{a}
G2	LRP % 0.5	1258.30±11.70 ^{a}	3.41±0.13 ^{a}	18.25±5.60 ^{a}	0.00±0.00 ^{a}
G3	LRP % 1.0	1309.10±28.99 ^{a}	3.31±0.19 ^{a}	26.25±4.07 ^{a}	0.25±0.25 ^{a}
G4	LRP % 1.5	1264.78±24.27 ^{a}	3.45±0.10 ^{a}	11.25±10.17 ^{a}	0.00±0.00 ^{a}
Significant		N.S	N.S	N.S	N.S

* Different letters within a column indicate significant differences at ($p \leq 0.05$).

Table 4 shows the effects of incorporating different levels of LRP on egg quality traits of Japanese quails. No significant differences were found between groups for any of the studied traits ($p \leq 0.05$). These results are similar to those of Zeweil et al. [20], who evaluated the impact of incorporating licorice at 250, 500, and 1000 mg/kg feed on quail eggs and concluded that it did not markedly change egg shell thickness or the relative weights of the shell, albumin, and yolk. Similarly, Sabzipoor et al. [19] reported that the addition of licorice extract to hens' feed at 5 to 10 g/kg showed no improvement in the egg shell thickness and weight. In regard to this, Hanafy et al. [18] reported that adding LRP to the diet of laying hens in summer did not produce any significant changes in the egg shape index or the relative weights of yolk, albumin, and shell, while a highly significant increase ($p \leq 0.01$) in eggshell thickness was observed in the licorice-supplemented treatments compared to the control. Nevertheless, the introduction of LRP into the diet of laying quails was shown by Doğan et al. [24] to improve significantly ($p \leq 0.05$) the thickness of the eggshells.

Table (4): Effect of adding different levels of LRP to quail diet on average shell thickness and relative weights of shell, albumin, and yolk as well as egg shape index (means ± standard error).

Groups		Shell thickness (mm)	Shell relative weight (%)	Albumen relative weight (%)	Yolk relative weight (%)	Egg shape guide
G1	Control	0.22 ± 0.01 ^{a}	9.38 ± 0.43 ^{a}	58.22 ± 1.37 ^{a}	32.42 ± 1.08 ^{a}	78.43 ± 1.25 ^{a}
G2	LRP % 0.5	0.23 ± 0.02 ^{a}	8.93 ± 0.32 ^{a}	58.69 ± 0.99 ^{a}	32.37 ± 1.02 ^{a}	78.64 ± 1.74 ^{a}
G3	LRP % 1.0	0.21 ± 0.07 ^{a}	9.10 ± 0.44 ^{a}	59.10 ± 1.23 ^{a}	31.80 ± 1.07 ^{a}	80.44 ± 1.64 ^{a}
G4	LRP % 1.5	0.22 ± 0.03 ^{a}	9.45 ± 0.31 ^{a}	58.15 ± 1.66 ^{a}	32.39 ± 1.26 ^{a}	77.72 ± 3.67 ^{a}
Significant		N.S	N.S	N.S	N.S	N.S

* Different letters within a column indicate significant differences at ($p \leq 0.05$).

Conclusion

This study revealed the potential of licorice root powder as an effective nutritional supplement that enhances egg production when added to the feed of laying quails at a rate of 1% of the feed components. The results of this study also showed that the qualitative characteristics of the eggs were not affected by this addition. Based on this, licorice can be considered a safe and sustainable feed supplement, characterized by its potential for local production at a low cost, making it an effective alternative to growth promoters and antibiotics in poultry nutrition. Its use contributes to reducing reliance on traditional additives, promoting the production of organic, drug-free food, thereby minimizing potential health risks for consumers and providing greater protection for human health. We also recommend conducting future studies using the extract resulting from the roots of the licorice plant and adding it to the poultry diet or using it through drinking water, and studying its effect on different types of poultry.

Disclosure statement

- **Ethics approval and consent to participate:** The study was conducted in accordance with the guidelines issued by the Scientific Ethics Committee of the College of Veterinary Medicine, University of Mosul, Iraq. according to its document No. UM.VET.2023.072 dated August 1,2023.
- **Consent for publication:** No applicable.
- **Availability of data and materials:** Data were collected and documented throughout the study, then analyzed to extract the results presented in the manuscript.
- **Author's contribution:** The authors contributed to preparing this study as follows:
- **Study design:** Mustafa, K.H.; Saaduldeen, FS. Practical implementation of the study: Mustafa, K.H.; Saaduldeen, FS; Al-Tamee, NG. Collection of scientific references: Abdel-Wareth, A. Manuscript preparation and writing: Mustafa, K.H. Manuscript review: Abdel-Wareth, A. Making modifications to the manuscript: Mustafa, K.H.; Saaduldeen, FS. All authors participated in reviewing the results and approving the final version of the manuscript.
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