

Synergistic and Individual Effects of Licorice Root and Plantain Herb Extracts Supplemented Through Drinking Water on Performance and Egg Quality in Laying Hens

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Abstract

This study examined the effects of licorice root (*Glycyrrhiza glabra*) and plantain herb (*Plantago major*) extracts, individually or combined, in drinking water on laying performance and egg quality in laying hens. A total of 280 Lohmann Brown hens (30 weeks old) were assigned to four groups: (1) control (no extract), (2) licorice root extract (3 ml/l), (3) plantain herb extract (3 ml/l), and (4) licorice root + plantain herb extract (1.5 + 1.5 ml/l). Licorice root extract supplementation resulted in a significantly higher egg weight compared to the control group ($P < 0.05$). Egg production was also improved in both the licorice root and plantain herb groups relative to the other treatments. Similarly, egg mass and feed efficiency were significantly better in hens receiving licorice root and plantain herb extracts compared to the control ($P < 0.05$). Regarding egg quality, licorice root extract improved the Haugh unit, while plantain herb extracts enhanced shell quality, both separately and in combination. Overall, licorice root extract positively affected the egg production and quality, whereas plantain herb extract mainly improved shell strength. These findings suggest that supplementation of licorice root or plantain herb extract into drinking water may enhance the performance and egg quality of laying hens.

Introduction

Due to the European Union's ban in 2006 for usage of antibiotics as growth promoter in animal feeding, an increasing interest has been arisen about natural and reliable alternative feed additives in livestock production (Butaye *et al.*, 2000; O'Neill, 2016; Perera *et al.*, 2025). In this context, medicinal and aromatic plants have gained increasing importance due to their low toxicity and lack of residues. Regarding their natural structure, strong antimicrobial effects, and their ability to prevent the development of resistance, these plants and feed additives obtained from their extracts are emerging as an important alternative in animal nutrition, particularly in terms of improving performance (Selaleli *et al.*, 2020; Redoy *et al.*, 2021; Galib *et al.*, 2023). Besides, aromatic plants and their

extracts are widely used both in the treatment of various diseases and in extending the shelf life of foods (Çabuk *et al.*, 2003; Dalkılıç *et al.*, 2005; Işık *et al.*, 2025). Furthermore, due to their low cost and easy accessibility, they are widely used by small-scale producers (Gholami-Ahangaran *et al.*, 2022). Licorice root (*Glycyrrhiza glabra*), known as the "father of plants," has been widely used for medicinal purposes since 500 BC (Ody, 1993). This plant, which contains approximately 300 flavonoids, carries active components such as glycyrrhizin, licochalcone A, liquiritigenin, 18β-glycyrrhetic acid, and glabridin (Wang *et al.*, 2015). *Glycyrrhizin*, present at a rate of 4–20%, is 50 times sweeter than sucrose and exhibits anti-inflammatory, anti-viral, anti-allergic, and gastro-

protective effects (Yang *et al.*, 2013). The antioxidant power of the flavonoids contained in licorice root is reported to be 100 times greater than that of vitamin E (Visavadiya *et al.*, 2009). Furthermore, various studies have demonstrated that licorice root extract exhibits broad-spectrum antimicrobial effects (Gupta *et al.*, 2008; Alagawany *et al.*, 2019). Considering all these properties, the use of licorice root in animal nutrition has been investigated in recent years. A study by Dorhoi *et al.* (2006) found that adding licorice root extract, along with garlic, plantain herb, and sea buckthorn extracts, to the feed of 55-week-old laying hens (Rhode Island Red crossbreeds) strengthened their immune systems. Another study indicated that adding 6 g/kg of licorice root extract to the feed of Japanese quails reduced abdominal fat, but had no significant effect on other production parameters (Sedghi *et al.*, 2010a). The *Plantago lanceolata*, commonly known as ribwort plantain, is a narrow-leaved plant that grows easily around the world, particularly in temperate regions, and is widely found in pastures and green areas. It has approximately 275 subspecies. Based on its active components, it has been widely used in both traditional and modern medical practices from ancient times to the present day for its healing effects on skin wounds, respiratory and digestive organs, reproductive system, and blood circulation disorders (Beara *et al.*, 2009, 2012). Research has shown that the *Plantago* genus, to which plantain belongs, contains active compounds belonging to five different chemical classes: flavonoids, monoterpenoids, triterpenoids, iridoid glycosides, and phenolic compounds (Stewart, 1996; Moore *et al.*, 2006) and these active components have been shown to give plantain antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties (Samuelsen, 2000). This study was carried out to determine the synergistic and individual effects of licorice root and plantain herb by drinking water on laying performance and egg quality in laying hens. In the study, the supplementation of plant extracts was applied by drinking water, due to its high bioavailability and fast metabolism. Based on current literature, the supplementation of plantain herb in poultry nutrition, especially laying hens, has not studied regarding various factors during production period. Besides, this study, focused on usage of plant extract combination, could potentially provide beneficial results to understand of phytogenic synergy, which put forward the strategic use of hybridizing multiple plant extracts in poultry nutrition.

Materials and Methods

Animal Material

The study was conducted for 8 weeks (March–May 2025) on a commercial egg-laying poultry farm in Izmir province. A total of 280 Lohmann Brown laying hens, 30 weeks of age and with similar body weight, were used in the experiment. The hens were randomly

allocated to four experimental groups, each consisting of seven replicates (pens) with ten hens per replicate. Thus, each treatment group included 70 hens, and the total number of hens used in the study was 280.

Method

Daily egg production was monitored during a 2-week adaptation period prior to the start of the study. At the end of this period, the hens were randomly assigned to the experimental pens and started the experimental treatment (during 8 weeks). Four experimental groups were according to the supplementation different plant extracts in the drinking water:

- Group-1: Control group (no plant extract added)
- Group-2: Licorice root extract (*Glycyrrhiza glabra*)
- Group-3: Plantain herb extract (*Plantago lanceolata*)
- Group-4: Licorice root and plantain herb extract combination

The active ingredients of the plant extracts used in the experiment are shown in Table 1 (Greenext Global Organic Chemistry, Izmir, Turkey). The plant extract was added daily and regularly at a level of 3 ml/l to the drinking water in the tanks located in each pen. The combination of licorice root and plantain herb extract was added at a total of 3 ml/l, with 1.5 ml/l of each extract. The drinking water in the experimental groups was monitored and refreshed daily, and the supplementation of plant extract were applied on daily basis.

Commercial layer feed based on corn and soybean meal (16.8% crude protein and 2730 ME kcal/kg) was used for feeding the laying hens, and the feed ingredients and nutrient contents of the feed are given in Table 2. Feed and water were provided ad libitum throughout the experimental period. A lighting program of 8 hours of darkness/16 hours of light was applied, and both environmental and indoor temperature and humidity values were recorded daily (Figure 1).

Performance Parameters

At the beginning and end of the study (weeks 30 and 38), the body weights of the laying hens in the experimental groups were determined individually with a precision of ± 0.1 g. Throughout the experimental period, feed consumption (weekly), number of eggs (daily), and egg weight (daily) were determined for each pen. Using these data, the egg mass and feed efficiency (Zhai *et al.*, 2013) were calculated using the following formulas:

$$\text{Egg mass} = \text{Egg weight} \times \text{Egg yield}$$

$$\text{Feed conversion ratio} = \frac{\text{Amount of Feed Consumed}}{\text{Egg Mass}}$$

During the experiment, eggs were collected daily and at the same time each day. To monitor changes in egg weight, the collected eggs were weighed using a digital scale with ± 0.01 g accuracy on the same day of the week in two-week intervals.

Egg Quality Parameters

At the end of the trial (38 weeks of age), internal and external quality analysis were performed on 28 eggs from each trial group (4 eggs randomly selected from each pen). The eggs were weighed with an accuracy of ± 0.01 g and then measured for length and width using a digital caliper (± 0.01 mm accuracy, Mitutoyo, 300 mm, Neuss, Germany). These values were used in the formula provided by Reddy *et al.* (1981) to determine the egg shape index:

$$\text{Egg shape index (\%)} = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

Eggshell breaking strength was determined in kg/cm² using a special breakage resistance measuring device developed by Rauch (1958) (Akbaş *et al.*, 1996). After the eggs were broken on a flat surface, the height of the yolk and albumen was measured with a tripod micrometer with a sensitivity of ± 0.01 mm (Froning and Funk, 1958). The length and width of the albumen and the diameter of the yolk were measured using a digital caliper with a sensitivity of ± 0.01 mm. The pigmentation of the egg yolk was determined using the DSM egg yolk color chart (Roche Ltd., Switzerland), with the lightest yolk color scored as 1 and the darkest egg yolk color scored as 15 on this scale (Beardsworth and Hernandez, 2004). The egg yolk was then carefully separated from the albumen and weighed using a 0.01 g precision balance. The shells from each broken egg were carefully washed to remove membrane residue and then were numbered. The shells were then dried in an oven at 105°C for 24 hours and weighed at the end of this period using a scale with a sensitivity of ± 0.01 g. The shell thickness of the eggs was measured using a micrometer with a sensitivity of ± 0.01 mm on membrane-covered shell samples taken from the pointed, middle, and blunt ends (Ehtesham and Chowdhury, 2002).

The albumen weight was determined by subtracting the total yolk and shell weight from the egg weight specified at the beginning of the analysis. Subsequently, the yolk, white, and shell weight values were calculated as percentages relative to the egg weight.

The albumen index was calculated using the following formula based on the height, length, and width of the albumen (Wilhelm and Heiman, 1936). The yolk index was calculated using the following formula based on the height and diameter of the yolk (Sharp and Powell, 1930). The Haugh unit was calculated according to the formula given below (Haugh, 1937).

$$\text{Albumen Index (\%)} = \frac{\text{White height (mm)}}{\text{White length (mm)} + \text{White width (mm)}} \times 100$$

$$\text{Yolk Index (\%)} = \frac{\text{Yolk Height (mm)}}{\text{Yolk Diameter (mm)}} \times 100$$

$$\text{Haugh Unit} = 100 \times \text{Log}_{10} (\text{H} + 7.57 - 1.7\text{G}^{0.37})$$

The measurement of, pH in yolk and albumen samples from each experimental group were performed using a digital pH meter (Mettler Toledo FiveEasy Plus FP20 benchtop model).

Statistical Analyses

The study was conducted according to a completely randomized design. The replicates were considered as replicates in determining the differences in performance parameters between the experimental groups (n: 10 hens/pen, 7 pens/experimental group). For the egg quality analyses, egg samples representing each experimental group were taken randomly, and each egg was considered a replicate. Statistical analyses of the data obtained in the study were performed using one-way ANOVA with the Minitab 17 software package. The following statistical model was used to determine the effects of the addition of plant extracts on the parameters examined in the experiment:

$$Y_{ij} = \mu + \alpha_{ij} + e_{ij}$$

Here, μ = overall mean, α_{ij} = effect of extract applications, e_{ij} = random error, and Y_{ij} = dependent variable.

The percentage values obtained in the experiment were subjected to variance analysis after applying the arc-sin transformation. Statistical differences between the experimental groups were determined by comparing them using the Tukey test. Statistical analyses of the data were performed at a probability level of $P < 0.05$.

Results and Discussion

The effects of adding licorice root extract and plantain herb extract to the drinking water of laying hens on performance parameters are presented in Table 3. The highest mean value for egg weight was observed in the licorice root extract group (62.85 g), while the lowest value was observed in the control group (61.60 g) ($P < 0.05$). Egg weight in the group supplemented with plantain herb extract was 62.54 g, which was higher than both the control and licorice root+plantain herb groups. Similarly, egg production was found to be higher in the licorice root extract (94.11%) and plantain herb (93.47%) groups than in the other experimental groups ($P < 0.05$). These findings clearly demonstrate that the individual supplementation of licorice root and plantain herb extracts to drinking water has an egg weight-increasing effect. Better results were obtained in the licorice root extract (59.15 and 1.92, respectively) and plantain herb extract (58.46 and 1.93, respectively) groups in terms of egg mass and feed conversion ratio ($P < 0.05$). There were no statistically significant differences between the experimental groups in terms of feed consumption and water consumption ($P > 0.05$). The positive effects of licorice root extract and plantain herb extract on egg weight, production, mass, and feed

conversion ratio is similar to the results of previous studies. These beneficial effects of licorice root extract could be explained by the content of glycyrrhizin and glycyrrhetic acid in licorice root extract, has a promoting effect for steroid sex hormones including estrogen and prolong its action effect (Al-Daraji *et al.*, 2009). Besides, licorice improves digestive and utilisation of nutrients (Al-Daraji *et al.*, 2006), which could potentially increase egg production in this study. Indeed, Awadein *et al.* (2010) emphasized that adding 0.1% and 0.5% licorice root to the diet of Mandarrah chickens had a positive effect on both egg production and egg weight. Similar results were also reported by Dogan *et al.* (2018a) and Dosoky *et al.* (2021). On the other hand, Safari and Zahedi (2016) stated that adding licorice root to the diet of Japanese quails had no effect on egg weight. The number of studies investigating the effects of plantain herb on performance parameters in laying hens is quite limited. However, when the studies conducted are examined, it is emphasized that the use of plantain herb alone or in combination with other medicinal and aromatic plants has positive effects on performance parameters, and it is stated that these effects may originate from the active components contained in the plant (Temür and Uslu, 2019; Redoy *et al.*, 2021). However, in this study, it was observed that the combined use of licorice root and plantain herb extract did not have an enhancing effect on the performance parameters examined. This observation suggests that there may be an antagonistic relationship between licorice root and plantain herb. Furthermore, it should be considered that the differences observed between studies may be related to various factors such as the use of herbal extracts at different doses, differences in the active content of the extracts used, and the age and health status of the herd.

In our study, similar average values were determined between the experimental groups in terms of feed consumption and water consumption, and this finding is similar to the findings reported by Sedghi *et al.* (2010b). On the other hand, Dogan *et al.* (2018b) reported that adding licorice root powder to the feed of laying hens at increasing levels (0.5%, 1.0%, and 2.0%) resulted in a decrease in feed consumption, and that this effect may be related to the plant's distinctive aromatic odor. Contrary to these findings, there are also research results reporting that the addition of licorice root extract to feed or drinking water has an effect in increasing both feed consumption and live weight (Safari and Zahedi, 2016; Salary *et al.*, 2014).

The effects of adding licorice root and plantain herb extract to drinking water on egg composition in laying hens are presented in Table 4. In the quality assessment conducted in the final week of the experiment, the average weights and yolk weights (%) of the eggs used were found to be similar among the experimental groups. The egg albumen weight was found to be proportionally highest in the licorice root extract and licorice root + plantain herb experimental

groups, with average values of 64.10% and 64.32%, respectively ($P < 0.05$). The eggshell weight ratio was observed to be highest in the plantain herb extract group, with an average value of 10.44% ($P < 0.01$). When previous studies are examined, there are significant differences in the reported results regarding the effects of adding licorice root extract or plantain herb extract to the diet of laying hens on egg composition. In a study by Zeweil *et al.* (2019), it was suggested that adding licorice root to the diet of Japanese quails (500 mg/kg) resulted in a significant increase in egg weight and yolk weight. Aghdam *et al.* (2018) also found that yolk weight was higher in the groups supplemented with licorice root extract (0.5%, 1%, 1.5%, and 2%) compared to the control group, but there was no difference between the experimental groups in terms of albumen and shell weight ratios.

The effects of adding licorice root and plantain herb extract to drinking water in the diet of laying hens on egg internal quality characteristics are presented in Table 5. The shape index was found to be higher in the groups supplemented with plantain herb and licorice root extract compared to the control group ($P < 0.05$). The yolk color was found to be darker in the control group with a value of 13.0 ($P < 0.05$). The yolk and albumen indices were similar among the experimental groups ($P > 0.05$). In the evaluation based on Haugh units, the highest average values were observed in the licorice root extract (74.76%) and licorice root + plantain herb extract (74.37%) groups ($P < 0.05$). Zeweil *et al.* (2019) reported that in their study, in which Tylosin and licorice root were added to the diets of 180 nine-week-old Japanese quails, the yolk index in the group supplemented with 1000 mg/kg licorice root was 448.40. Dogan *et al.* (2018c) examined the effects of licorice root supplementation, different storage temperatures, and storage times on the quality of quail eggs. The study reported that licorice root supplementation at levels of 0, 0.5%, 1.0%, and 1.5% had no significant effect on the Haugh unit value, but the highest value was 86.84% in the group supplemented with 1.5% licorice root. The egg albumen weight was found to be highest at 4.14% in the group with 1.0% licorice root addition ($P < 0.05$). The egg albumen index was reported to be 9.62% in the 1.0% licorice root group, while the egg yolk index was reported to be 39.08% in the group with 0.5% licorice root addition ($P < 0.05$).

The study clearly showed that adding plant extracts to drinking water had no effect on egg yolk color in laying hens. This finding differs from the results reported by Ghasemi *et al.* (2010), Połtowicz and Wexyk (2001), and Aghdam *et al.* (2018). This difference may be related to the potential for licorice root extract added to feed to directly affect yolk color. On the other hand, the increase observed in Haugh units is similar to the results reported by Aghdam *et al.* (2018), Al-Daraji *et al.* (2009), and Zeweil *et al.* (2019). The effect of supplementing different plant extracts on eggshell quality is shown in Figure 2. Shell thickness and shell breaking strength

were significantly higher in the experimental groups supplemented with plantain herb extract (0.455 mm and 2.133 g/cm², respectively) and licorice root + plantain herb extract (0.433 mm and 1.968 g/cm², respectively) (P<0.01). These findings reveal that plantain herb potentially has a positive effect on eggshell quality. This may be related to the fact that plantain herb contains flavonoids, which have a function in regulating calcium metabolism (Bahadori *et al.*, 2020). On the other hand, the fact that the eggs in the group supplemented with licorice root extract had similar values to the control group eggs indicates that licorice root has no effect on shell quality. However, this finding differs from the findings of Hanafy *et al.* (2022), who reported that licorice root has a beneficial effect on shell quality. The differences observed in studies on egg quality may be related to the use of different amounts of plant extracts.

The effect of different plant extracts on egg pH is shown in Table 6. The egg yolk pH value was found to be highest in the licorice root extract (6.12) and licorice root + plantain herb (6.13) groups (P<0.05). On the other hand, the egg albumen pH value was found to be lower in the licorice root extract (8.81) and licorice root + plantain herb (8.89) groups than in the control (9.07) and plantain herb extract (9.08) groups (P<0.05). These groups showed a pH-lowering effect. Although there are not enough studies in the literature where pH values have been measured, Dogan *et al.* (2018c) stated in a similar study that the pH level of egg albumen decreased as the level of licorice root powder added to the ration increased, but there was no statistically significant difference.

CONCLUSION

When the findings obtained as a result of the study were examined, it was observed that licorice root extract could rank first among the experimental groups in terms of its potential effect on improving performance in laying hens. This was followed by plantain herb extract in terms of egg production and egg weight. In terms of egg quality, licorice root extract was found to be more effective on Haugh unit, while plantain herb extract was more effective on shell quality. However, the limited number of studies conducted specifically on plantain herb restricts the broader evaluation of the current findings. Therefore, new studies are needed that take into account more variables, particularly related to the plantain herb, in the nutrition of laying hens.

REFERENCES

- Aghdam, S. H., Ahmadzadeh, A., Nobakht, A. (2018). Effects of different levels of Licorice (*Glycyrrhiza glabra*) medicinal plant powder on performance, egg quality and some of serum biochemical parameters in laying hens.
- Akbaş, Y., Altan, Ö., Koçak, Ç. (1996). Effects of hen age on the internal and external quality characteristics of chicken eggs Turkish Veterinary Journal, 20, 445-460.
- Alagawany, M., Elnesr, S. S., Farag, M. R., Abd El-Hack, M. E., Khafaga, A. F., Taha, A. E., Dhama, K. (2019). Use of licorice (*Glycyrrhiza glabra*) herb as a feed additive in poultry: Current knowledge and prospects. *Animals*, 9(8), 536. <https://doi.org/10.3390/ani9080536>
- Al-Daraji, H. J., H. A. Al-Saraf (2006). Effect of using high levels of licorice extract from the first day of chicks' age on productive performance of broiler chickens. *Iraqi Poultry Science Journal*, 1(1), 38-52. <https://www.iasj.net/iasj/article/59541>
- Al-Daraji, H. J., W. K. Al-Hayani, H. A. Al-Mashadani., D. H. Al-Hassani (2009). The use of different concentrations of licorice extract (*Glycyrrhiza glabra*) for improving egg quality traits of Lohmann hens reared during different periods of the year. *Alfurat Journal of Agricultural Sciences*, 1(2): 71–80.
- Al-Sofee, K. H. M. (2018). Effect of different levels of licorice roots powder to diets on productive performance and some blood traits for quail. *Mesopotamia Journal of Agriculture*, 46(4), 135-144.
- Awadein, N. B., Eid, Y. Z., Abd El-Ghany, F. A. (2010). Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. *Poultry Science Journal*, 30(3), 829-846.
- Bahadori, M. B., Sarikurkcu, C., Kocak, M. S., Calapoglu, M., Uren, M. C., Ceylan, O. (2020). *Plantago lanceolata* as a source of health-beneficial phytochemicals: Phenolics profile and antioxidant capacity. *Food Bioscience*, 34, 100536. <https://doi.org/10.1016/j.fbio.2020.100536>
- Beara, I. N., Lesjak, M. M., Jovin, E. Đ., Balog, K. J., Anackov, G. T., Orcic, D. Z., & Mimica-Dukic, N. M. (2009). Plantain (*Plantago L.*) species as novel sources of flavonoid antioxidants. *Journal of Agricultural and Food Chemistry*, 57(19), 9268-9273.
- Beara, I.N., Lesjak, M.M., Orcic, D.Z., Simin, N.D., Cetojevic-Simin, D.D., Bozin, B.N., and amp; Mimica-Dukic, N.M. (2012). Comparative analysis of phenolic profile, antioxidant, anti-inflammatory and cytotoxic activity of two closely-related Plantain species: *Plantago altissima L.* and *Plantago lanceolata L.* *LWT-Food Science and Technology*, 47, 64-70. <https://doi.org/10.1016/j.lwt.2012.01.001>
- Beardsworth, P.M., Hernandez, J.M. (2004). Yolk colour-an important egg quality attribute. *International Poultry Production*. 12(5): 17-18.
- Butaye P, Van Damme K, Devriese LA, Van Damme L, Bael M, Lauwers S, Haesebrouck F. (2000). In vitro susceptibility of *Enterococcus faecium* isolated from food to growth-promoting and therapeutic antibiotics. *International Journal of Food Microbiology*, 54, 181–187. [https://doi.org/10.1016/S0168-1605\(99\)00198-1](https://doi.org/10.1016/S0168-1605(99)00198-1)
- Çabuk, M., Alçiçek, A., Bozkurt, M. ve İmre, E. (2003). Antimicrobial properties of essential oils obtained from aromatic plants and their potential use as alternative feed. II. Ulusal Hayvan Besleme Kongresi Bildiri Kitabı (ss. 184-187), Selçuk Üniversitesi Ziraat Fakültesi, Konya, Türkiye, 18–20 Eylül.
- Dalkılıç, B., Güler, T., Ertaş, O.N., Çiftci, M. (2005). The Effect Of Thyme And Anise Oils And Antibiotic on Total Cecum Coliform Bacteria III. Ulusal Hayvan Besleme Kongresi Bildirileri, Çukurova Üniversitesi Ziraat Fakültesi, Adana, 7–10 Eylül.

- Dogan, S. C., Erdoğan, Z., Şekeroğlu, A., Baylan, M., & Küçükgül, A. (2018a). The effects of licorice root powder (*Glycyrrhiza glabra*) on performance, serum parameters, egg yolk cholesterol and antioxidant capacity of laying Japanese quail. *Turkish Journal of Agriculture-Food Science and Technology*, 6(9), 1290-1296. DOI: <https://doi.org/10.24925/turjaf.v6i9.1290-1296.2124>
- Dogan, S. C., Baylan, M., Erdoğan, Z., Küçükgül, A., & Bulancak, A. (2018b). The effects of Licorice (*Glycyrrhiza glabra*) root on performance, some serum parameters and antioxidant capacity of laying hens. *Brazilian Journal of Poultry Science*, 20, 699-706. <https://doi.org/10.1590/1806-9061-2018-0767>
- Dogan, S. C., Baylan, M., Yaman, S., & Bulancak, A. (2018c). Effects of dietary licorice root (*Glycyrrhiza glabra*) supplementation, storage time and temperature on quality of quail eggs. *Progress in Nutrition*, 20(4), 665-671. DOI: 10.23751/pn.v20i4.7854
- Dorhoi, A., Dobrea, V., Zăhan, M., Virag, P. (2006). Modulatory effects of several herbal extracts on avian peripheral blood cell immune responses. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 20(5), 352-358. <https://doi.org/10.1002/ptr.1859>
- Dosoky, W. M., Zeweil, H. S., Ahmed, M. H., Zahran, S. M., Ali, A. M., Abdelsalam, N. R., & Naiel, M. A. (2021). The influences of Tylosine and licorice dietary supplementation in terms of the productive performance, serum parameters, egg yolk lipid profile, antioxidant and immunity status of laying Japanese quail under heat stress condition. *Journal of Thermal Biology*, 99, 103015. <https://doi.org/10.1016/j.jtherbio.2021.103015>
- Ehtesham, A., Chowdhury, S.D., (2002). Responses of laying hens to diets formulated by using different feeding standards. *Pakistan Journal of Nutrition*. 1, 127-131. DOI: <https://doi.org/10.3923/pjn.2002.127.131>
- Froning, G. W., Funk, E. M. (1958). Seasonal variation in quality of eggs laid by caged layers and their sisters on the floor. *Poultry Science*, 37(1), 215-223. <https://doi.org/10.3382/ps.0370215>
- Galib A.A.K., Hasan S.A.J., Nihad A.L.A., and Ihsan H.A.T. (2023). Protein replacers in poultry diets. *Revis Bionatura*, 8 (2), 78.
- Ghasemi, R., Zarei, M., Toriki, M. (2010). Adding medicinal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. *American Journal of Animal and Veterinary Sciences*, 5(2), 151-154.
- Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M, Derakhshan M. (2022). Thymol and carvacrol supplementation in poultry health and performance. *Veterinary Medicine and Science* 8(1): 267–288. <https://doi.org/10.1002/vms3.663>
- Gupta, V. K., Fatima, A., Faridi, U., Negi, A. S., Shanker, K., Kumar, J. K., Khanuja, S. P. (2008). Antimicrobial potential of *Glycyrrhiza glabra* roots. *Journal of Ethnopharmacology*, 116(2), 377-380. <https://doi.org/10.1016/j.jep.2007.11.037>
- Hanafy, M. M. (2022). The impact of stocking density and licorice powder supplementation on the productive state and some physiological traits of laying hens. *Egyptian Poultry Science Journal*, 42(1), 17-38. <https://doi.org/10.21608/EPSJ.2022.225211>
- Haugh, R. R., (1937). The Haugh unit for measuring egg quality, *US Poultry Magazine*, 43,552-555, 572-573. <https://doi.org/10.24925/turjaf.v6i9.1290-1296.2124>
- Işık, Y., Babacan, O., Taş, T. (2025). Use of medicinal and aromatic plants in poultry nutrition. *Düzce Üniversitesi Ziraat Fakültesi Dergisi*, 3(1), 54-66.
- Moore, G., Sanford, P., Wiley, T. (2006). *Perennial pastures for Western Australia* (Bulletin 4690). Perth: Department of Agriculture and Food.
- O'Neill J. (2016). Review on Antimicrobial Resistance: Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Available. Accessed date: 09.09.2024
- Ody, P., (1993). *The Complete Medicinal Herbal*. New York, Dorling Kindersley Limited, 70: 138-139.
- Perera, W. N. U., Ravindran, V. (2025). Role of feed additives in poultry nutrition: Historical, current and future perspectives. *Animal Feed Science and Technology*, 116371. <https://doi.org/10.1016/j.anifeedsci.2025.116371>
- Połowicz K., Wężyk S. (2001). Wpływ dodatku ziół w żywieniu kur niesnych na produktywność i jakość jaj. *Roczniki Naukowe Zootechniki*, 28, 2: 215-225.
- Rauch W. (1958). Vergleichende Untersuchungen Zur Qualitats Beurteilung Von Frischeiem. *Archives für Geflügelkunde*, 22: 74-104.
- Reddy, P. M., Reddy, V. R., Reddy, C. V., Rao, P. S. P. (1981). Egg weight, shape index and hatchability in Khaki Campbell duck eggs.
- Redoy, M.R.A., Rahman, M.A., Atikuzzaman, M., Shuvo, A.A.S., Hossain, E., Khan, M.J., and Al-Mamun, M. (2021). Dose titration of plantain herb (*Plantago lanceolata* L.) supplementation on growth performance, serum antioxidants status, liver enzymatic activity and meat quality in broiler chickens. *Italian Journal of Animal Science*. 20(1), 1244-1255. <https://doi.org/10.1080/1828051X.2021.1952114>
- Rezaei M, Kalantar Nasr J. (2014). Thymus vulgaris L., Glycyrrhiza glabra, and combo enzyme in corn or barley-basal diets in broiler chickens. *International Journal of Plant, Animal and Environmental Science*; 4(3):418-423.
- Safari A, Zahedi A. (2016). The effect of different levels supplementation of Glycyrrhiza glabra extract on growth performance in male quail. *International Journal of Advanced Biological and Biomedical Research*; 4(2):214-219.
- Salary J, Kalantar M, Sahebi ala MK, Ranjbar K, Hemati Matin HR. (2014). Drinking water supplementation of licorice and aloe vera extracts in broiler chickens. *Scientific Journal of Animal Science*; 3(2):41-48. doi: 10.14196/sjas.v3i2.1144
- Samuelson AB. (2000). The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. *Journal of Ethnopharmacology*. Jul;71(1-2):1-21.
- Sedghi M, Golian A, Kermanshahi H, Ahmadi H. (2010a). Effect of dietary supplementation of licorice extract and a prebiotic on performance and blood metabolites of broilers. *South African Journal of Animal Science*; 40(4):371-380. DOI:10.4314/sajas.v40i4.65259
- Sedghi, M., Golian, A.G.H. and Soleymani, P. (2010b). Effect of dietary supplementation of licorice extract on egg quality and performance of hens. *Veterinary Clinical Pathology* 4(15), 933-941.

- Selaledi, A.L., Hassan, M.Z., Manyelo, T.G., & Mabelebele, M. (2020). The current status of the alternative use to antibiotics in poultry production: an African perspective. *Antibiotics*, 9(9),594612. <https://doi.org/10.3390/antibiotics9090594>
- Sharp, P. F., and C. K. Powell, (1930). Decrease in internal quality of hen's eggs during storage as indicated by the yolk. *Industrial and Engineering Chemistry Research*. 22: 909-910.
- Stewart, A.V. (1996). Plantain (*Plantago lanceolata*) A potential pasture species. *Proceedings of the New Zealand Grassland Association*, 58, 77-86.
- Temür, C., Uslu, S. (2019). Effects of plantain (*Plantago lanceolata*) containing diets of quails on growth performance, carcass characteristic, some blood parameters and mast cell numbers. *Yuzuncu Yil University Journal of Agricultural Sciences*, 29(1), 114-120. <https://doi.org/10.29133/yyutbd.495271>
- Visavadiya, N. P., B. Soni., N. Dalwadi, (2009). Evaluation of antioxidant and anti-atherogenic properties of Glycyrrhiza glabra root using in vitro models. *International Journal Food Sciences and Nutrition*, 60 (Suppl.2): 135-149. <https://doi.org/10.1080/09637480902877998>
- Wang, L., R. Yang, B. Yuan, Y. Liu., C. Liu, (2015). The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb. *Acta Ppharmaceutica Sinica B*, 5 (4): 310-315. <https://doi.org/10.1016/j.apsb.2015.05.005>
- Wilhelm, L. A., and Victor Heiman, (1936). The constancy of the albumen index of eggs from individual hens. *U. S. Egg and Poultry Magazine*. 42:750.
- Yang, L., L. L. Li, T. T. Liu, Y. G. Zu, F. J. Yang, C. J. Zhao, L. Zhang, X. Q. Chen., Z. H. Zhang, (2013). Development of sample preparation method for isoliquiritigenin, liquiritin, and glycyrrhizic acid analysis in licorice by ionic liquids-ultrasound based extraction and high-performance liquid chromatography detection. *Food Chemistry*, 138 (1): 173-179. <https://doi.org/10.1016/j.foodchem.2012.10.059>
- Zeweil, H. S., Dosoky, W. M., Zahran, S. M., Ahmed, M. H., Mohamed, A. A. (2019). Effect of licorice as an alternative to antibiotics on productive, egg quality and yolk and blood lipid profile of laying japanese quail. *Journal of the Advances in Agricultural Researches*, 24(2), 164-177. DOI: 10.21608/jalexu.2019.163066
- Zhai, Q. H., Dong, X. F., Tong, J. M., Guo, Y. M., Bao, Y. E. (2013). Long-term effects of choline on productive performance and egg quality of brown-egg laying hens. *Poultry Science*, 92(7), 1824-1829. DOI: 10.3382/ps.2012-02854

Table 1. Active ingredients of licorice root (*Glycyrrhiza glabra*) and plantain herb (*Plantago lanceolata*) extracts

Extracts	Active substances*
Glycyrrhiza glabra	Triterpenoid saponin (5-13%) Glycyrrhizin (10%) Glycyrrhetic acid Isoliquiritin Isoflavones
Plantago lanceolata	Iridoid glycosides (aucubin) Flavonoids (rutin, baicalin, %1-2) Phenolic acids (caffeic acid) Mucilage (1-3%) Other Molecules: Allantoin (0.5-1%), iridoid glycosides (0.1-0.5%), aucubin (0.1-0.3%), catalpol (0.05-0.1%)

* The active ingredients have been reported by the manufacturer

Table 2. The composition of the feed used in the trial and its nutrient content

Raw materials	%	Nutrient content	%
Corn	58.00	Metabolic energy (ME, kcal/kg)	2742
Soybean meal (44%)	23.70	Crude protein	16.56
Corn gluten	4.80	Calcium	3.84
Vegetable oil	2.00	Available phosphorus	0.42
Limestone	5.20		
Marble dust	3.60		
Dicalcium phosphate	1.60		
NaCl	0.35		
L-lysine-HCL	0.05		
DL-methionine	0.20		
Premix*	0.50		

*Vitamin premix provided per kg of ration: Vitamin A: 2.40 mg; Vitamin D3: 75.00 µg; Vitamin E: 5.00 mg; Vitamin K3: 2.20 mg; Vitamin B1: 1.50 mg; Vitamin B2: 4.00 mg; Vitamin B3: 8.00 mg; Vitamin B5: 35.00 mg; Vitamin B6: 2.50 mg; Vitamin B9: 0.50 mg; Vitamin B12: 10.00 µg; Vitamin H2: 0.15 mg; Choline: 468.70 mg; Mn: 80.00 mg; Fe: 75.00 mg; Zn: 64.00 mg; Cu: 6.00 mg; Se: 0.30 mg

Table 3. Effect of different plant extract additions on performance parameters

Parameter	Test groups ¹				Standard error	Significance level
	Control	Licorice root extract	Plantain Herb extract	Licorice root + Plantain herb		
Egg weight (g)	61.60 ^c	62.85 ^a	62.54 ^{ab}	61.90 ^{abc}	0.31	0.004
Egg yield (%)	90.93 ^b	94.11 ^a	93.47 ^a	92.59 ^{ab}	0.74	0.004
Egg mass	56.01 ^c	59.15 ^a	58.46 ^a	57.31 ^b	0.46	<0.001
Feed consumption (g)	116.72	111.78	112.78	116.17	4.87	0.548
Water consumption (ml)	250.88	277.27	276.54	263.32	13.31	0.120
FCR	2.08 ^a	1.92 ^b	1.93 ^b	2.00 ^{ab}	0.05	0.027

¹Control: no plant extract added; Licorice root extract: 3 ml/l drinking water, Plantain herb extract: 3 ml/l drinking water; Licorice root + Plantain herb 1,5 + 1,5 ml/l drinking water; FCR: Feed conversion ratio

^{a-c} Differences between values indicated by different letters in the same row are significant (P<0.05).

Table 4. Effect of different plant extract additions on egg composition

Parameter	Test groups ¹				Standard error	Significance level
	Control	Licorice root extract	Plantain herb extract	Licorice root + Plantain herb		
Egg weight (g)	63.88	64.97	64.34	64.07	1.14	0.176
Egg yolk (%)	27.12	26.25	27.26	25.94	1.63	0.214
Egg albumen (%)	63.89 ^{ab}	64.10 ^{ab}	62.31 ^b	64.32 ^a	1.58	0.029
Eggshell (%)	8.99 ^b	9.65 ^{ab}	10.44 ^a	9.74 ^{ab}	0.77	0.002

¹Control: no plant extract added; Licorice root extract: 3 ml/l drinking water, Plantain herb extract: 3 ml/l drinking water; Licorice root + Plantain herb 1,5 + 1,5 ml/l drinking water;

^{a-c} Differences between values indicated by different letters in the same row are significant (P<0.05).

Table 5. Effect of different plant extract additions on egg internal quality characteristics

Parameter	Test groups ¹				Standard error	Significance level
	Control	Licorice root extract	Plantain herb extract	Licorice root + Plantain herb		
Shape index	77.30 ^b	79.40 ^a	79.60 ^a	79.10 ^{ab}	1.73	0.020
Egg yolk color	13.00 ^a	11.60 ^b	12.80 ^{ab}	12.20 ^{ab}	1.10	0.031
Egg yolk index	40.29	41.67	40.02	42.31	5.58	0.764
Egg albumen index	5.10	5.99	5.26	5.91	1.04	0.152
Haugh unit	70.81 ^b	74.76 ^a	70.24 ^b	74.37 ^a	2.69	<0.01

¹Control: no plant extract added; Licorice root extract: 3 ml/l drinking water, Plantain herb extract: 3 ml/l drinking water; Licorice root + Plantain herb 1,5 + 1,5 ml/l drinking water;

^{a-c} Differences between values indicated by different letters in the same row are significant (P<0.05)

Table 6. Effect of different plant extract additions on egg pH

Parameter	Test groups ¹				Standard error	Significance level
	Control	Licorice root extract	Plantain herb extract	Licorice root + Plantain herb		
Egg yolk pH	6.03 ^{bc}	6.12 ^{ab}	6.02 ^c	6.13 ^a	0.07	0.002
Egg albumen pH	9.07 ^a	8.81 ^b	9.08 ^a	8.89 ^b	0.10	<0.001

¹Control: no plant extract added; Licorice root extract: 3 ml/l drinking water, Plantain herb extract: 3 ml/l drinking water; Licorice root + Plantain herb 1,5 + 1,5 ml/l drinking water;

^{a-c} Differences between values indicated by different letters in the same row are significant (P<0.05).

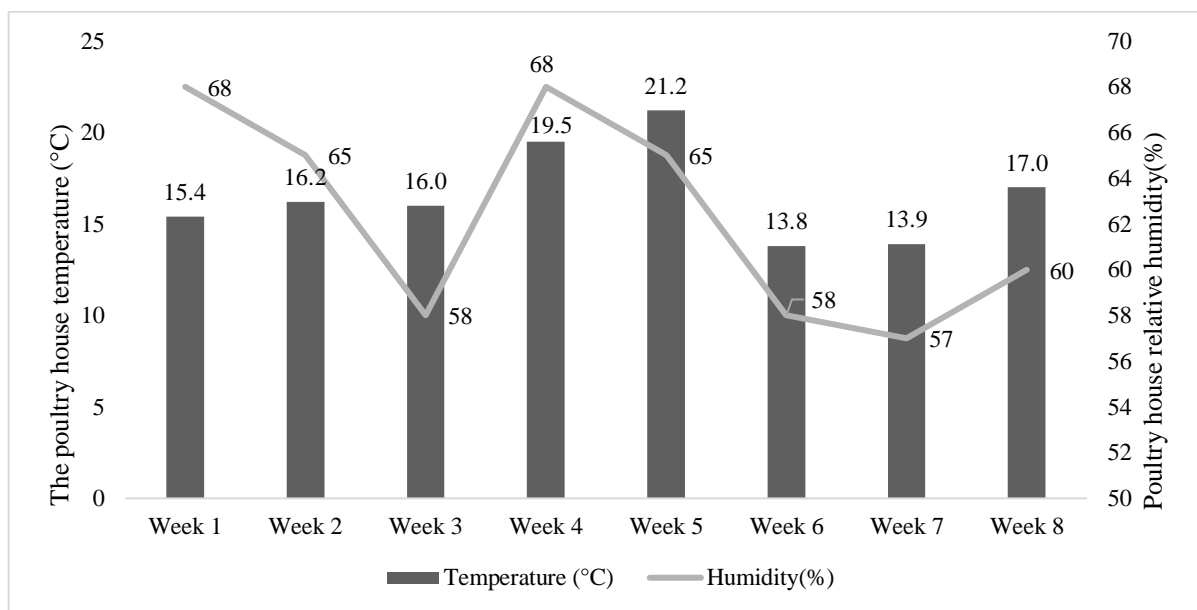


Figure 1. Indoor temperature and humidity values during the trial period (weekly)

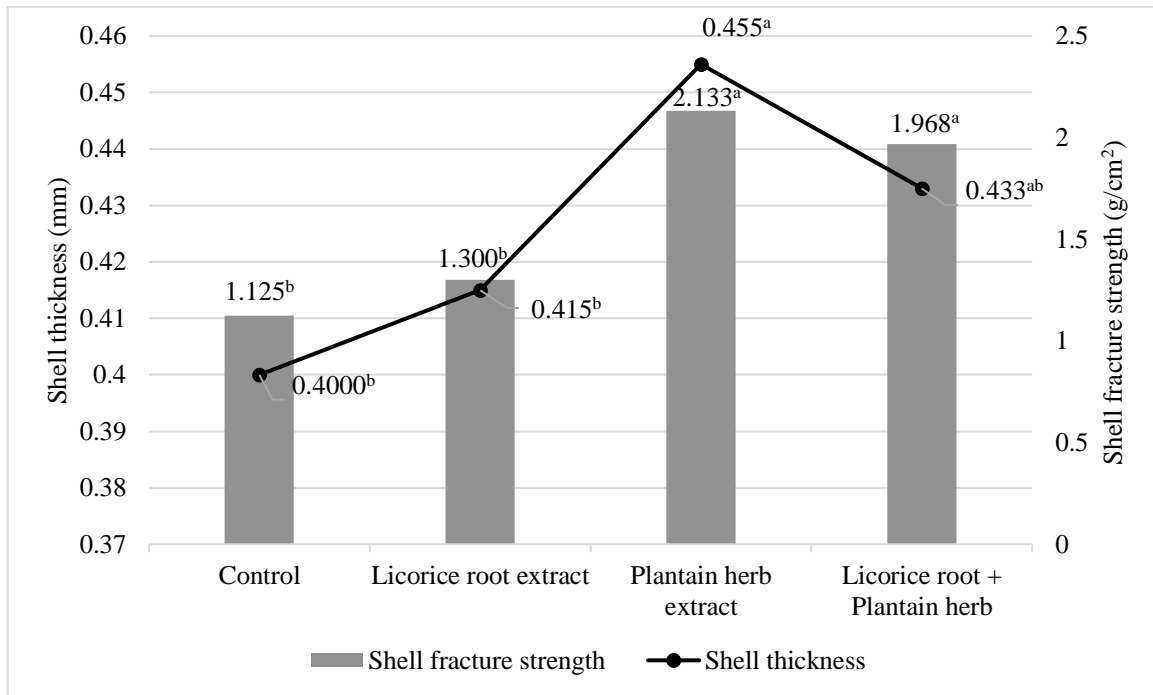


Figure 2. Effect of different plant extract additions on eggshell quality