Effect of multiple levels of propolis added to the diets on productive and physiological status of aged ISA Brown chickens

Qais Al-Saada

Department of Animal Production, Agriculture College, Tikrit University, Tikrit, Iraq
Correspondence email: qais.m.abdulrahman@tu.edu.iq

Abstract

The study aimed to determine the effect of multiple levels of propolis (0, 250, 500 mg/kg) on the productive and physiological characteristics of ISA Brown laying hens. The results showed a significant improvement ($P<0.05$) in egg mass and feed conversion ratio for the treatments with the addition of 250 and 500 mg/kg, and an improvement in egg production for the treatment with the addition of 500 mg/kg compared to the first control treatment (T1). Regarding blood plasma protein parameters, a significant increase in total protein (T.Protein) and globulin in both the 250 mg and 500 mg supplementation treatments, compared to T1. For blood lipids, a significant decrease in total cholesterol(TC) and LDL concentration was observed in T2, and in triglyceride (TG) and VLDL in both T2 and T3, compared to T1. Liver enzyme concentrations decreased significantly in both the 250 mg and 500 mg supplementation treatments compared to T1; no significant differences were observed in glucose and uric acid concentrations between the experimental treatments. Propolis supplementation affected the histological condition of the intestines. The addition of propolis at a concentration of 250 mg significantly improved villus width and area, as well as the villus length-to-width ratio (v/c), while the addition of propolis at a concentration of 500 mg significantly improved villus length compared to the control treatment.

Key words: propolis, physiological state, ISA Brown, productive, histology

Introduction

In recent years, the demand for functional foods for human consumption has increased due to their diverse and beneficial nutritional content. Consequently, those involved in the agricultural sector are increasingly interested in using raw materials that provide natural nutrients such as minerals and vitamins, rather than synthetically produced compounds [1]. Plant-derived substances are of great importance due to their biological activity as anti-disease and anti-inflammatory agents. The potential side effects of using chemical preservatives and antibiotics are a source of concern for consumers, particularly with the emergence of resistance to growth-enhancing antibiotics in Europe, such as avoparcin, whose use has led to increased resistance to vancomycin. Consequently, many research bodies, institutions, and consumers have criticized the use of these substances, calling for their prohibition to ensure that animal products are free of any potentially harmful residues [2]. But banning antibiotics in many countries could lead to the spread of infectious diseases, causing significant losses for poultry farmers [3], which leads to an

ISSN 2072-3857

increase in harmful bacteria in the digestive tract, causing an imbalance in the gut flora. This negatively impacts feed conversion efficiency, thus increasing breeding costs and consequently higher feed consumption [4]. Therefore, poultry industry professionals have turned to searching for safe, naturally derived alternatives to replace antibiotics.

Propolis, once considered a useless or wasteful substance, has gained significant attention in recent years due to its health benefits for both animals and humans [5]. It is a complex natural resinous compound containing over 200-300 active components, primarily phenolic compounds, vitamins, amino acids, phenols, urea, and minerals. It is considered anti-inflammatory, antibacterial, antiseptic, antioxidant, and immune-boosting. Studies have also shown it to have antimutagenic and hepatoprotective effects. Bees collect propolis from the buds, leaf margins, and stems of various plants, then mix it with wax, enzymes, and pollen. The main components of propolis are plant resin, beeswax, and essential oils in proportions of 50%, 30%, and 10%, respectively, and 5% organic matter and 5% pollen [6] . Bees collect it to repair

Materials and Methods

Experimen Design

The experiment was conducted in the animal production fields of the College of Agriculture at Tikrit University, Iraq. 72 ISA Brown hybrid laying hens were used in the experiment, divided into three groups. Each group was further divided into three subgroups (8 hens per subgroup). Propolis was added to T2 and T3 at concentrations of 250 and 500 mg/kg feed, respectively. The first treatment (T1) served as the control for comparison and received no propolis supplementation. The hens were fed a locally produced ration with an energy level of 2810.88kcal/kg and a protein level of 15.59 %, according to the ISA Brown 2018 Guide. The lighting program was 16 hours of light and 8 hours of darkness.

damaged cells [7-8]. In addition to the above, numerous studies have demonstrated the many benefits of adding propolis to laying hen diets, n a study conducted by [9], in which green propolis was added to the diets of Hy-Line Brown hybrid laying hens at a rate of 10, 20 and 30 g/kg of diet, it was noted that the addition of propolis led to an improvement in egg quality by reducing fat peroxidation in fresh and stored eggs, and also significantly reduced microbial contamination on the eggshell. Similarly. [10] conducted a study to determine the effect of adding propolis at rates of 250, 500, and 1000 mg/kg to the diets of Lohmann hybrid laying hens. They observed that propolis significantly increased egg production and improved blood biochemical characteristics.

Most previous studies were conducted on young laying flocks. As is known, the ageing of the flock leads to several issues, including a decline in immunity and health, reduced production, and a deterioration in egg quality. Therefore, this study aimed to determine the effect of using propolis on the productive, physiological, and health status of older laying flocks.

ISSN 2072-3857

Table (1) Composition of diets used in the experiment (%).

Ingredients (%)	Diet
yellow corn	13
wheat	64.4
soybean meal	7
Premix 5%*	5
DICAL	1
Caco3	9
Salt	0.6
Calculate values	100%
Chemical composition**	
M.E (Kcal/kg)	2810.88
Crude protein %	15.59
Crude fiber %	2,70
Methionine	0.27
Lysine	0.42
Met+cysteine	0.51
Calcium	3.73
Available phosphor	0.35

* The premix used in the diet is produced by the Dutch company WAFI.
**The chemical composition of the diet was calculated according [11].

Productive Parameters

The eggs were collected once daily at 11:30 AM, and the average egg weight, egg production, feed conversion ratio, and egg mass were calculated during the

study period.

Blood sample

For physiological parameters, three hens were selected from each treatment group (one bird per replicate). 5 mL of blood was withdrawn from the wing vein and 4 mL placed into anticoagulant-free tubes. The blood was allowed to coagulate, and then the tubes were centrifuged at 3000 rpm to get serum. The serum was frozen until analysis for blood lipids, blood proteins, glucose, uric acid, and liver enzymes. The remaining blood (1 mL) was placed in anticoagulant tubes for complete blood count (CBC).

Histology Samples

For histological parameters, a 2 cm section of the small intestine of a chicken was excised from the region between the scar and the duodenum. The specimen was prepared according to the method of [12]. The length of the villi and the depth of the crypts were then measured using an Am Scope microscope.

Statistical Analysis

The data were analyzed using the general linear model in the statistical analysis program [13], and to compare the means of the experimental treatments, Duncan's multiple test was used [14].

ISSN 2072-3857

Result and Discussion

The effects of propolis addition are clearly shown in the data presented in Table 2, where egg mass and feed conversion ratio (FCR) improved significantly ($P<0.05$) in both the T2 and T3, as did egg production in T3 compared to the control treatment(T1). Researchers believe that the antioxidant and immune-boosting properties of propolis, along with its high content of phenolic acids, flavonoids, vitamins and minerals, have positively impacted the bird's health and thus improved its productive performance [15], are the reason for the improved production performance of hens fed propolis compared to the control group. The results were agreement with those of [16] who observed that adding propolis to laying hen diets improved egg production, weight, and feed conversion ratio. Also, the results of this study were consistent with those of researchers [17] who observed that adding propolis improved egg production, egg weight, and egg mass. They were also consistent with the findings of researchers [10] who observed that adding propolis to laying hen diets improved the overall productive performance of the flock, particularly egg production and feed conversion ratio.

Table (2) effect of propolis levels on productive performance of laying hens

Treats*	T1	T2	T3
Egg mass (gm)	36.58±0.07b	37.93±0.36a	38.64±0.37a
FCR (g/g)	3.31±0.01a	3.18±0.03b	3.14±0.02b
Egg production(HD)	56.79±0.29b	57.82±0.32b	60.65±0.29a
Egg weight (gm)	64.48±0.28ab	65.54±0.33a	63.77±0.38b

* T1 , T2 and T3 received 0 , 250 and 500 mg/kg of propolis respectively.

Results in Table 3 showed a significant improvement ($P\leq 0.05$) in T.protein and globulin in T2 and T3, and a significant decrease in albumin for T3 compared to the control treatment(T1). The significant improvement in blood protein levels can be attributed to the positive effect of propolis on liver tissue, protecting it from toxins and harmful substances [18]. Or perhaps the reason is the improvement in the functions of immune organs and tissues as a result of the positive effect of propolis on the tissues of the bird's immune system, which increases the production of globulins, and thus raises the concentration of total proteins and globulins in the blood serum, as shown in Table 2 of this conclusion [19] Propolis also affects the increased differentiation and proliferation of lymphatic system cells, activating lymphatic tissues, which positively impacts the activity of the immune system [20]. These results are consistent with those of [21] who found that adding propolis at a low dose of 3 g/kg stimulated the production of immunoglobulins IgG and IgM in laying hens, thus enhancing the bird's immune status, which is reflected in its productive performance and health

ISSN 2072-3857

Table (3) Total protein parameters of laying hens fed multi levels of propolis.

Traits*	**T1**	**T2**	**T3**
T.protein (g/dl)	5.77±0.48b	7.64±0.48a	7.32±0.42a
Albumin (g/dl)	1.89±0.04a	1.66±0.15ab	1.39±0.04b
Globulin (g/dl)	3.88±0.53b	5.98±0.38a	5.93±0.45a

* T1 , T2 and T3 received 0 , 250 and 500 mg/kg of propolis respectively.

The results shown in Table 4 indicate a significant decrease in total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in T2, as well as a significant decrease in triglyceride concentration and very low-density lipoprotein (VLDL) in both T2 and T3 compared to the control treatment, The reduction in TC, TG, LDL and VLDL and levels may be attributed to the effects of certain propolis components, such as flavonoids, which have been scientifically proven to lower cholesterol. Flavonoids have been found to influence metabolic products by activating triglyceride metabolism and utilizing it as a primary energy source [22]. These results are agreement with [23] who found that adding propolis at a concentration of 300 mg/kg to chicken feed for 35 days reduced triglyceride and cholesterol levels in blood plasma, The results also agreed with [24] who found that adding propolis at levels of 200 and 400 mg/kg in broiler diets led to a reduction in TC, TG, and LDL concentrations.

Table (4) lipids profile parameters of laying hens fed multi levels of propolis.

Traits*	**T1**	**T2**	**T3**
TC (mg/dl)	236.22±15.29a	174.37±1.93b	259.22±8.09a
TG (mg/dl)	114.00±1.15a	81.00±0.57b	83.00±2.64b
HDL (mg/dl)	55.00±0.57a	44.33±0.88b	60.33±3.17a
LDL (mg/dl)	158.42±15.68a	113.83±2.85b	182.28±7.98a
VLDL (mg/dl)	22.80±0.23a	16.20±0.11b	16.60±0.52b

* T1 , T2 and T3 received 0 , 250 and 500 mg/kg of propolis respectively.

Table 5 shows the effect of propolis supplementation at various levels on liver enzymes, blood glucose, and uric acid. A significant decrease in the concentration of ALT and AST enzymes was observed in treatments T2 and T3 compared to the control treatment, while no significant differences were found in glucose and uric acid concentrations between the supplementation treatments and the non-supplementation control treatment, The reduced concentration of liver enzymes in propolis treatments may be due to its ability to protect liver tissue from damaging agents, thus preserving optimal liver function [10], The results were agreement with those of [25] and [16] who observed that adding propolis to feed led to a reduction ALT and AST concentrations.

ISSN 2072-3857

Table (5) response of some biochemical parameters in laying hens to multiple levels of propolis.

Traits*	**T1**	**T2**	**T3**
AST (IU/L)	207.00±2.08a	183.66±4.05c	194.66±2.90b
ALT (IU/L)	49.66±0.66a	39.33±1.45b	39.33±1.20b
Uric acid (g/dl)	6.27±1.15	7.33±1.03	8.70±1.39
Glucose (mg/dl)	257.27±31.44	294.68±6.41	275.05±12.62

* T1 , T2 and T3 received 0 , 250 and 500 mg/kg of propolis respectively.

The health of the intestine and its ability to absorb food are measured by the length of the villi and the depth of the crypts; the greater these ratios, the better the digestion and absorption of nutrients [26]. Table 6 shows the effect of propolis on the histological condition of the intestines of laying hens. The T2 showed significant improvement in the villus length-to-crypt depth ratio, villus width, and villus area; T3 showed superiority in villus length and area compared to the control treatment. These results are agreement with those of [27] who observed a significant improvement in the histological condition of the intestines in terms of villus length, crypt depth, and the ratio between them, as well as a significant improvement in the absorption surface area of chickens fed a diet supplemented with propolis or a mixture of propolis and pollen. The improvement in intestinal histological condition may be due to propolis's effect in stimulating the growth and proliferation of beneficial bacteria. These bacteria protect the intestines and maintain their health by protecting the intestinal mucosa, thus increasing intestinal efficiency and improving digestion and absorption. [28].

Table (6) Effect of propolis on intestinal histology of ISA Brown hens.

Traits*	T1(µm)	T2 (µm)	T3(µm)
Villi length	633.30±66.34b	733.47±20.93b	825.19±15.37a
crypst	124.00±4.71a	93.27±5.77b	137.56±3.75a
v/c	5.10±0.51b	7.86±0.44a	5.99±0.06b
Villi wedth	104.43±3.36b	125.76±2.00a	101.33±2.27b
Villi area	77.12±5.01b	106.01±2.77a	98.46±4.162.57a

* T1 , T2 and T3 received 0 , 250 and 500 mg/kg of propolis respectively.

Conclusions

There was no significant difference in the effect of propolis at the 250 mg/kg and 500 mg/kg levels, although both significantly outperformed the control treatment in terms of egg mass and feed conversion efficiency. The 250 mg/kg supplementation treatment significantly outperformed both the control and 500 mg/kg treatments in terms of significant improvements in total protein and globulin concentrations in the blood, as well as in reducing total cholesterol, LDL cholesterol, and triglyceride levels, and increasing HDL cholesterol levels. No significant difference was observed in the positive effect of the 250 mg/kg and 500 mg/kg supplementation treatments on the levels of the liver enzymes GOT and GPT. Histological

ISSN 2072-3857

measurements of the intestines showed that the second treatment was superior in terms of the ratio of villus length to crypt depth and villus width, and was not significantly different from the third treatment in terms of villus area. From the above results, we conclude that the optimal supplementation rate of propolis in this study, in terms of its effect on productive, physiological, and histological performance, was the ratio 250 mg/kg diets.

References

[1] Panaite, T. D., Nour, V., Saracila, M., Turcu, R. P., Untea, A. E., & Vlaicu, P. A. (2021). Effects of linseed meal and carotenoids from different sources on egg characteristics, yolk fatty acid and carotenoid profile and lipid peroxidation. Foods, 10(6), 1246.

[2] Houlihan, A. J., & Russell, J. B. (2003). The susceptibility of ionophore-resistant Clostridium aminophilum F to other antibiotics. Journal of Antimicrobial Chemotherapy, 52(4), 623-628.

[3] Cetin, N., Güçlü, B. K., & Cetin, E. B. R. U. (2005). The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. Journal of Veterinary Medicine Series A, 52(6), 263-267.

[4] Stanley, D., Denman, S. E., Hughes, R. J., Geier, M. S., Crowley, T. M., Chen, H., ... & Moore, R. J. (2012). Intestinal microbiota associated with differential feed conversion efficiency in chickens. Applied microbiology and biotechnology, 96(5), 1361-1369.

[5] Apata, D. F. (2009). Antibiotic resistance in poultry. International Journal of Poultry Science, 8(4), 404-408.

[6] Abd El-Ghany, W. A. (2024). Propolis (bee glue): a promising natural feed additive for poultry and rabbits–a review. Annals of Animal Science, 24(4), 1051-1064.

[7] Al-Nayef, H. H. N., & Al-Nuaimi, M. M. H. A. (2015). Effect of addition of Bee Propolis (bee gum) and kinder (Olibanum) and their blend to the diet in the productive traits of broilers. Anbar Journal of Agricultural Sciences, 13, 88-100.

[8] Omar, N. A. A., Allithy, A. N. E. A., Baghdadi, H., Zolaly, M., Abdel-haleem, M., Helmy, M. M., ... & El Sayed, S. M. (2016). Hepatoprotective effects exerted by propolis against doxorubicin-induced rat liver toxicity: a biochemical and histopathological study. Am. J. Cancer Prev, 4, 36-40.

[9] Casagrande, A. C., Machado, G. C., Brunetto, A. L., Galli, G. M., Rosa, G. D., Araujo, D. N., ... & Silva, A. S. D. (2021). The addition of green propolis to laying hens had positive effects on egg quality: lower bacteria counts in the shell and lipid peroxidation in the yolk. Anais da Academia Brasileira de Ciências, 93(suppl 4), e20210315.

[10] Abdel- Kareem, A. A. A., & El-Sheikh, T. M. (2017). Impact of supplementing diets with propolis on productive performance, egg quality traits and some haematological variables

ISSN 2072-3857

of laying hens. Journal of Animal Physiology and Animal Nutrition, 101(3), 441-448.

[11] National ResearchCouncil (N.R.C). 1994. Nutrient requireents of poultry, 9th revisited. National Academy Press, Washington. DC. USA.

[12] Kim, J. S., Hosseindoust, A., Lee, S. H., Choi, Y. H., Kim, M. J., Lee, J. H., ... & Chae, B. J. (2017). Bacteriophage cocktail and multi-strain probiotics in the feed for weanling pigs: effects on intestine morphology and targeted intestinal coliforms and Clostridium. Animal, 11(1), 45-53.

[13] SAS Version Statistical Analysis System 2005 Statistical Analysis System (USA: Institute Inc Cary) NC 27512-8000.

[14] Duncan DB 1955 Multiple Range Multiple F-test-Biometeics 11 pp 1–42.

[15] Wang, B. J., Lien, Y. H., & Yu, Z. R. (2004). Supercritical fluid extractive fractionation–study of the antioxidant activities of propolis. Food Chemistry, 86(2), 237-243.

[16] Galal, A., Abd El-Motaal, A. M., Ahmed, A. M. H., & Zaki, T. G. (2008). Productive performance and immune response of laying hens as affected by dietary propolis supplementation. International Journal of Poultry Science, 7(3), 272-278.

[17] El-Neney, B. A. and Awadien, N. B.(2016) The use of propolis as a source of natural additives to improve the productive performance and 40 immune system of chickens local, 2 – Effect on growing Dokki4, 9th International Poultry Conference-Proceedings, organized by Animal Production Research Institute (APRI), Ain Sukhna, Egypt, 62–78, 7–10 November, https://www.researchgate.net/publication /320258260 (last access: 12 March 2023).

[18] Bhadauria, M., & Nirala, S. K. (2009). Reversal of acetaminophen induced subchronic hepatorenal injury by propolis extract in rats. Environmental Toxicology and Pharmacology, 27(1), 17-25.

[19] Babaei, S., Rahimi, S., Torshizi, M. A. K., Tahmasebi, G., & Miran, S. N. K. (2016, March). Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. In Veterinary Research Forum (Vol. 7, No. 1, p. 13).

[20] Tayeb, I. T., & Sulaiman, B. F. (2014). Effect of propolis supplementation on productive performance in local quail.

[21] Cetin, E. B. R. U., Silici, S. İ. B. E. L., Çetin, N., & Güçlü, B. K. (2010). Effects of diets containing different concentrations of propolis on hematological and immunological variables in laying hens. *Poultry science*, *89*(8), 1703-1708.

[22] Matsui, T., Ebuchi, S., Fujise, T., Abesundara, K. J., Doi, S., Yamada, H., & Matsumoto, K. (2004). Strong antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3, 4, 5-tri-O-caffeoylquinic acid. Biological and Pharmaceutical Bulletin, 27(11), 1797-1803.

[23] Attia, Y. A., Abd Al-Hamid, A. E., Ibrahim, M. S., Al-Harthi, M. A.,

ISSN 2072-3857

Bovera, F., & Elnaggar, A. S. (2014). Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. Livestock Science, 164, 87-95.

[24] Rabie, A. H., El-Kaiaty, A. M., Hassan, M. S., & Stino, F. R. (2018). Influence of some honey bee products and a growth promoter supplementation on productive and physiological performance of broiler chickens. Egyptian Poultry Science Journal, 38(2), 513-531.

[25] El-Hanoun, A. M., Kamel, K. I., El Sebaei, M. S., & Gad, H. A. M. (2007, March). Effect of Egyptian propolis supplementation on productive, reproductive performance and some hematobiochemical parameters and steroid hormones of female rabbits during winter and summer seasons. In Proc.: 4th World Poultry Conference (pp. 27-30).

[26] Chegini, S., Kiani, A., & Rokni, H. (2018). Alleviation of thermal and overcrowding stress in finishing broilers by dietary propolis supplementation. Italian Journal of Animal Science, 17(2), 377-385.

[27] Prakatur, I., Miskulin, M., Pavic, M., Marjanovic, K., Blazicevic, V., Miskulin, I., & Domacinovic, M. (2019). Intestinal Morphology in Broiler Chickens Supplemented with Propolis and Bee Pollen. Animals, 9(6), 301. https://doi.org/10.3390/ani9060301

[28] Hosseini, S., Afshar, M., Ahani, S., & Vakili Azghandi, M. (2015). Heat shock protein 70 mRNA expression and immune response of heat-stressed finishing broilers fed propolis (bee glue) supplementation. Archives Animal Breeding, 58(2), 407-413.

ISSN 2072-3857