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INFLUENCE OF ADDING PROPOLIS AND / OR WHEY ON PRODUCTIVE AND PHYSIOLOGICAL PERFORMANCE IN BROILER CHICKS

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ABSTRACT: Due to the prohibition of antibiotic use, attributed to their adverse health effects on both poultry and humans, researchers and specialists in poultry nutrition and management have been prompted to explore natural alternatives aimed at enhancing broiler chicken performance. The purpose of the current study was to ascertain how broiler chickens responded to propolis and/or liquid whey as natural supplements. 180 one-day-old unsexed broiler chicks (Ross 308) were divided into 5 groups at random (3 replicates each, 12 birds / replicate): The first treatment was fed basal diet without any additives and served as a control. The second treatment was fed the basal diet supplemented with propolis powder 5 g per kg of diet. The third treatment was fed the basal diet and their water was supplemented with propolis extract (10 ml/L). The forth treatment was fed the basal diet and their water was supplemented with liquid whey (10 ml/L). The fifth treatment was fed the basal diet and their water was supplemented with propolis extract + liquid whey (10 ml/L in drinking water from each additive). The findings showed that adding propolis and whey in individual forms enhanced the feed conversion ratio (FCR), feed intake (FI), final body weight (FBW) and body weight gain (BWG) of broilers. There were no discernible variations in carcass characteristics across all experimental treatments ($P>0.05$). For the blood analysis, the inclusion of additives significantly ($P<0.05$) increased phosphorus, total protein, calcium, albumin and Vitamin C and decreased total lipids, glucose, cholesterol and Heterophils/Lymphocytes H/L ratio. Moreover, improved tibiotarsal strength was observed in propolis and whey treatments in comparison with the control group ($P<0.05$). Therefore, it can be concluded that propolis and liquid whey could be successfully used in separate forms as natural supplementations in reinforcing broilers' performance without any negative effects on birds' carcass traits.

Key words: Broilers; performance; propolis; liquid whey; natural additives.

1. INTRODUCTION

Nowadays, there is an inclination to diminish the use of synthetic growth promoters (such as antibiotics) because of their negative effects on humans (issues with antibiotic resistance and leftovers in animal products), and this has led to a general restriction on using them in poultry production worldwide (AL-Kahtani *et al.,* 2022). Poultry nutritionists strive to discover natural feed additives that are capable of enhancing feed utilization, immune system, growth performance and meat quality of broiler chickens (Phillips *et al.,* 2023). Bee products, including propolis, royal jelly and bee pollen are being used with great emphasis on broilers' nutrition. (Hascik *et al.,* 2019; Petricevic *et al.,* 2022).

Propolis is a resinous, balsamic and impermeable essence produced by bees (Vieira *et al.,* 2020). It contains various components, including polyphenols, steroids and terpenoids. According to a study by Kacaniova *et al.* (2012), propolis has the potential to increase poultry productivity and physiological indicators by altering the gut microbiome, which increases the number of helpful bacteria and decreases the number of harmful microorganisms. Furthermore, in the words of AL-Kahtani *et al*. (2022), incorporating propolis into broiler diets strengthened the hens' antioxidant defenses and immunological systems.

Whey is a worthy by-product of the cheese-making process; it contains a great amount of protein (35% crude protein Sugiharto *et al.,* 2023). Antioxidant properties have been reported for peptides generated from lactoglobulin and lactalbumin, as well as free amino acids including Trp, Phe, and Cys present in whey (Corrochano *et al.,* 2018). Recent studies summarized that oral supplementation or adding a dried form of whey to broiler chickens diet had a valuable effect in improving growth performance (Kumar *et al.,* 2022), antioxidant status (Afkhami *et al.,* 2020), antimicrobial properties (Pineda-Guiroga *et al.,* 2018) and meat quality (Ashour *et al.,* 2019). Notwithstanding the aforementioned essential impacts of whey, Tsiouris *et al.* (2020) stated that high dietary whey consumption (more than 2%) had a negative effect on the performance and tibiotarsus of broilers.

The current study was conducted to ascertain the effects of adding propolis (either as an extract or as a powder) and whey, either separately or in combination, on the physiological and productive performances of broiler chickens. It was inspired by the aforementioned prestigious literature.

2. MATERIALS AND METHODS

The Animal Research Ethics Committee of the Faculty of Agriculture at Assiut University in Egypt authorized all research methods, including the care and husbandry of the animals (Reference No: RECFAAU-23-04).

2.1. Growth performance, carcass characteristics and relative organ weight of broilers.

A reputable commercial hatchery provided 180 one-day-old, un-sexed broiler chicks (Ross 308) with an average initial body weight of 42.5 ± 0.2 g/chick. All chicks were randomly distributed into 15 littered pens (1×1 m, 5 cm depth) with five treatments (3 replicates each, 12 birds/ replicate). The experimental treatments were: T1control group, T2: propolis powder (PP; 5 g /kg diet), T3: propolis extract (PE; 10 ml/L), T4: liquid whey (LW) (10 ml/L drinking water), T5: propolis extract + liquid whey (10 ml/L from each additive). Commercial basal diets were fed to all birds during the starter (0 to 14 d), grower (15 to 28 d), and finisher (29 to 35 d) phases (Table 1). The chicks were raised for 35 days at

34°C of room temperature and then progressively cooled to 24°C (with 60% humidity). Feed and water were provided *ad libitum*. The birds were exposed to 24 hours of light on the first day of the experiment, and after that, the amount of light was reduced by 1 hour each day until a total of 18 hours of light/ day. Individual body weights were recorded each week. Feed intake / bird was determined weekly. The feed conversion ratio (FCR, g feed/gain) was calculated weekly by dividing feed intake (FI, g) by body weight gain (BWG, g). Two birds from each replication, totaling six birds / treatment, were randomly chosen at 35 days of age after fasting for 4 hours prior to slaughter. Each bird was then weighed, slaughtered, then subjected to bleeding, de-feathering, and evisceration. Carcass yield, organs (liver, heart, spleen, and gizzard), cut portions (breast, thighs, wings, and neck), and Abdominal fat were weighed and computed as percentages of the birds' pre-slaughter live body weight.

2.2. Blood Profile Analyses

Blood samples from each treatment were obtained using vacuum blood collection tubes 35 days following euthanasia. The samples were then frozen at -25^{o}C until analysis and centrifuged at 3,000 rpm for 15 minutes to extract serum. The colorimetric kits used to measure total protein, albumin, Phosphorus, calcium, blood glucose; total cholesterol, total lipids, and vitamin C were supplied by the Egyptian Company for Biotechnology (S.A.E.). The Haemagglutination inhibition test was used to evaluate antibody titers against the novel castle vaccine (Al-Kassie *et al.*, 2012); the H/L ratio was calculated by calculating the proportion of heterophils (H) and lymphocytes (L).

2.3. Tibiotarsal strength

After the left and right tibia were boiled for eight minutes, all tissues were removed, dried for nineteen hours at 100°C, and then placed in a desiccator, and their tibiotarsal strength was assessed. An Instron device with a flexure fixture head was used to measure the tibia's strength.

2.4. Statistical analysis

Statistical analysis was performed using the general linear model (G.L.M.) from SAS, (2013) programe. The Duncan Multiple Range Test was used to see if there were significant variations between the treatment means (Steel and Torrie, 1980). All percentages were subjected to the arcsine transformation to approximate a normal distribution before analysis. The data are presented as means and SEM. A significance level of $P < 0.05$ was applied to probability values. A one-way analysis of variance (ANOVA) was employed to assess the impact of various treatments on various measured parameters. The data were analyzed according to the following model:

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

Y_{ij}= Average effect observed, μ = Total average, T_i = Effect of treatments, e_{ij} = Effect of errors.

3. RESULTS

3.1. Growth performance

Throughout the trial period no mortality was recorder. Table 2 displays the impact of several dietary interventions on broiler growth metrics. The addition of both types of propolis or whey considerably ($P<0.05$) raised the broiler's average gain (AVG) and final body weight (FBW) according to the results. The combination group and the control therapy did not significantly differ

(P>0.05). We observed that, propolis treatments significantly reduced feed intake (FI) and enhanced feed conversion ratio (FCR) compared to other dietary groups.

3.2. Carcass characteristics

Table 3 shows the carcass proportions of broilers in different experimental treatments. The statistical analysis did not show any differences (P>0.05) among all groups with respect to carcass yield (%) and relative carcass cuts and organs.

3.3. Blood analysis

Regarding the parameters set for blood analysis, Table 4 revealed that, compared to the control group, birds supplemented with natural additives showed a significant (P< 0.05) increase in serum levels of phosphorus, calcium, total protein, albumin and vitamin C, as well as a significant (P< 0.05) decrease in glucose, cholesterol and total lipids. We found that the H/L ratio significantly (P<0.05) decreased after applying our evaluated additives.

3.4. Tibiotarsal strength

The impact of propolis and/or whey supplementation on tibia-breaking strength (TBS) is presented in Table 5. The accomplished results elucidated that TBS for birds in supplementary treatments significantly (P<0.05) increased in compared to those in the control group. Birds supplemented with either whey in individual forms or in combination with propolis reflected the highest TBS (25.03 and 24.96, respectively).

3. DISCUSSION

The improved growth performance indices in this study were in line with Al-Kahtani *et al.* (2022), who indicated that dietary inclusion of propolis at a level of 0.1% improved FBW and BWG of broiler chickens. Also, Kumar *et al.* (2022) observed that supplementation of whey (10%) in the drinking water enhanced broilers growth performance. Because propolis and whey are additional sources of lipids, protein, carbohydrates, minerals, and vitamins, their combined effects may have improved the performance indices of broilers fed them (Patel, 2015; Al-Kahtani *et al*., 2022). This could explain the improved performance indices of broilers treated with propolis and/or whey. Conversely, Dosoky *et al.* (2022) found no discernible impact on growth performance metrics at 42 days of age when propolis (100– 400 mg/kg diet) was added to broiler diets. According to a related study by Sugiharto *et al.* (2023), broiler diets supplemented with whey protein (1%) had no discernible impact on growth performance metrics. Consistent with the results of Hascík *et al*. (2019), Pourakbari *et al.* (2016), Vieira *et al.* (2021), and Ma'rifah *et al.* (2023), the carcass features of broiler chickens fed diets supplemented with either whey or propolis showed no discernible change. The decline that was observed in cholesterol, glucose and total lipids values that were parallel with adding propolis and/or whey may be due to lipid absorption reduction through the small intestine by ligating bile acids, which led to an increase in cholesterol elimination (Ashour *et al.*, 2019). In addition, propolis contains components like essential fatty acids that restrain the hepatic 3-hydroxy-3-methlglutaryl coenzyme A reductase activity, which is a key regulatory enzyme in cholesterol synthesis (Babińska *et al.*, 2013). The impact on the H/L ratio due to adding propolis and/or whey may be explained by the ability of whey and propolis to stimulate the immune system, increasing the function of lymphatic tissues and macrophage activity (Mona *et al*., 2021). Comparable to our findings, Szczurek *et al.* (2013) indicated that adding whey protein concentrate to broiler diets (32 g/kg diet) decreased the H/L

ratio. Moreover, Hosseini *et al.* (2016) noticed a decrease in the H/L ratio as a result of the supplementation of propolis (3 g/kg diet) into broiler chickens reared under heat stress. The influence of dietary additives suggested on birds' tibia-breaking strength may be due to the enhancement of calcium and phosphorus metabolism in birds, as well as the fact that propolis and whey contain high levels of Vit. D (Koutoulis *et al.,* 2009; Pineda-Quiroga *et al.,* 2018; El-Faham *et al.,* 2019).

4. CONCLUSION

The application of nutritional strategies for natural additives in broilers' production is considered a promising tool for enhancing their productive and physiological performances. The novelty of our study is that propolis extract and liquid whey (1%) in individual forms improved the growth performance, tibia-breaking strength and blood parameters of broiler chickens with no significant effect on carcass characteristics. While, the combination of both boosted blood parameters and tibia-breaking strength only. Therefore, we recommend using either propolis or whey in separate forms for broiler producers as alternatives to synthetic growth promoters.

Table (1): Components and computed percentage of nutrients in baseline diets:

Ingredient (%)	**Starter (0-14 d)**	**Grower (15-28 d)**	**Finisher (29-35 d)**
Yellow Corn (….% CP)	52.26	59.07	63.23
Soybean meal (44%CP)	34.02	26.68	22.48
Corn gluten (60% CP)	6.00	7.00	6.30
Sunflower Oil	3.00	3.00	4.00
Di-Calcium phosphate	1.84	1.69	1.58
Limestone	1.42	1.20	1.11
L- Lysine Hcl	0.33	0.30	0.27
DL -Methionine	0.26	0.20	0.16
Sodium Chloride	0.25	0.23	0.24
Sodium bicarbonate	0.22	0.23	0.23
Vitamins Premix *	0.10	0.10	0.10
Minerals Premix**	0.30	0.30	0.30
Calculated nutrient composition %			
Crude Protein	23.17	21.25	19.04
Metabolizable Energy (Kcal/Kg)	3100	3110	3207
Crude fiber	3.80	3.45	3.22
Crude fat	5.55	5.75	5.85
Calcium	1.04	0.90	0.84
Av. Phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54
Methionine+Cyctine	1.06	0.95	0.86

* Vitamin A, 11000 IU; Vitamin D_3, 5000 IU; Vitamin E, 50 mg; Vitamin K_3, 3 mg; Vitamin B_1, 2 mg; Vitamin B_2, 6 mg; B_6, 3 mg; Vitamin B_{12}, 14 mcg; Nicotinic acid, 60 mg; Folic acid, 1.75 mg; Pantothenic acid, 13 mg; and Biotin, 120 mcg are provided/ Kg diet.

** 600 mg of choline, 16 mg of copper, 40 mg of iron, 120 mg of manganese, 100 mg of zinc, 0.20 mg of selenium, and 1.25 mg of iodine are provided/ Kg diet.

Table (2): Growth performance of broiler chickens in the experimental treatments.

Treatment / Items	Control	PP. (5g /kg diet)	PE (10 ml/ L)	LW (10 ml/L)	Combination PE+LW (10 ml/ L)	SEM	*P* Value
Initial g/bird	42.58	42.44	42.47	42.47	42.49	0.08	0.827
FBW g/bird	2195.29^{c}	2290.76^{b}	2341.80^{a}	2270.29^{b}	2158.89^{c}	16.72	<.0001
AVG g/bird	2152.71^{c}	2248.32^{b}	2299.33^{a}	2227.82^{b}	2116.40^{c}	36.06	<.0001
FI g/bird	3873.62^{a}	3623.28^{c}	3653.02^{c}	3815.87^{b}	3767.69^{b}	16.75	<.0001
FCR	1.80^{a}	1.61^{b}	1.59^{b}	1.71^{a}	1.78^{a}	0.03	<.001

$^{a-c}$ Means with different superscripts, within time, differ significantly (P < 0.05). PP= propolis powder group, PE= propolis extract group, LW= liquid whey. FBW= final body weight, AVG= average gain, FI= feed intake, FCR= feed conversion ratio.

Table (3): The carcass properties of the experimentally treated broiler chicks.

Treatment Items (%)	Control	PP. (5g /kg diet)	PE. (10 ml/ L)	LW. (10 ml/ L)	Combination PE+LW (10 ml/ L)	SEM	*P* value
Carcass yield	67.82	70.83	68.99	69.37	68.72	0.68	0.0830
Carcass cuts:							
Breast	25.82	26.00	25.63	25.73	25.66	0.28	0.8895
Thigh	24.41	24.42	24.29	24.15	24.21	0.21	0.8659
Wing	5.31	5.37	5.69	5.66	5.57	0.13	0.3340
Neck	2.39	2.45	2.36	2.37	2.42	0.06	0.8279
Organs:							
Liver	2.92	2.87	2.79	2.82	2.75	0.06	0.3151
Heart	0.39	0.40	0.39	0.40	0.40	0.01	0.7835
Gizzard	0.75	0.77	0.78	0.77	0.76	0.02	0.7608
Abdominal fat	1.55	1.54	1.50	1.49	1.51	0.03	0.7215

*Means with different superscripts, within time, differ significantly (P < 0.05). PP= propolis powder group, PE= propolis extract group, LW= liquid whey.

Table (4): Blood parameters of broiler chickens in the experimental treatments.

Treatment [1] / Items	Control	PP. (5g /kg diet)	PE (10 ml/ L)	LW (10 ml/ L)	Combination PE+LW (10 ml/ L)	SEM	*P* value
Protein(g/dl)	1.96^{b}	2.0^{b}	2.30^{b}	3.05^{a}	2.85^{a}	0.15	0.010
Albumin(g/dl)	0.97^{b}	1.09^{b}	1.17^{b}	2.02^{a}	1.20^{b}	0.07	<.0001
Phosphorus(mg/dl)	8.38^{b}	8.90^{b}	9.12^{ab}	10.4^{a}	9.5^{ab}	0.35	0.0495
Calcium(mg/dl)	9.22^{b}	9.58^{b}	9.49^{b}	14.86^{a}	13.28^{a}	1.19	0.0034
Cholesterol(mg/dl)	126.6^{a}	112.7^{b}	111.3^{b}	86.77^{d}	103.3^{c}	6.01	<.0001
Total lipids(mg/dl)	909.9^{a}	770.25^{b}	747.7^{b}	738.4^{b}	777.1^{b}	30.3	0.0266
Glucose(mg/dl)	124.2^{a}	48.20^{c}	88.2^{b}	105.5^{ab}	123.9^{a}	6.06	<.0001
Immune indicator							
H/L Ratio	0.82^{a}	0.59^{bc}	0.50^{c}	0.67^{b}	0.65^{b}	0.04	0.0007
AbNDV	4.77^{b}	5.13^{a}	5.18^{a}	5.29^{a}	5.35^{a}	0.049	0.049

$^{a-c}$ Means with different superscripts, within time, differ significantly (P < 0.05). [1]PP= propolis powder group, PE= propolis extract group, LW= liquid whey. [2]H/L= Heterophils/Lymphocytes.

Table (5): Tibia breaking strength of broiler chickens in the experimental treatments.

Treatment1 Items	Control	PP. (5g /kg diet)	PE. (10 ml/ L)	LW. (10 ml/ L)	Combination PE+LW (10 ml/ L)	SEM	P value
Tibia breaking Strength (Newton)	24.54c	24.78b	24.75b	25.03a	24.96a	0.12	<.0001

[a-c] Means with different superscripts, within time, differ significantly (P < 0.05). [1]PP= propolis powder group, PE= propolis extract group, LW= liquid whey. Newton (symbol: N) is the International System of Units (SI) derived unit of force.

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الملخص العربى

تأثير إضافة البروبوليس و/أو شرش اللبن على الأداء الإنتاجي والفسيولوجي في دجاج التسمين

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نتيجة حظر استخدام المضادات الحيوية لما لها من تاثيرات غير صحية سواء على الطائر او الانسان مما اضطر الباحثين والمهتمين برعاية وتغذية الدواجن إلى البحث عن بدائل طبيعية وذلك لتحسين أداء دجاج التسمين. تهدف هذه الدراسة الحالية إلى معرفة استجابة دجاج التسمين للمكملات الطبيعية والتي تشتمل علي البروبوليس و/أو شرش اللبن. تم توزيع عدد 180 كتكوت تسمين (Ross 308) عشوائيًا عمر يوم على 5 مجاميع وكل مجموعة تم تقسمها الى 3 مكررات كل مكرر يحتوى على 12 كتكوت وتم معاملة هذه المجاميع كما يلى:

- المعاملة الاولى: وهى الكنترول (Control) وفيها لا تحتوى العليقة على اى اضافات.
- المعاملة الثانية: وهى كنترول + مسحوق بودر البروبوليس بمعدل (5 جم / كجم علف)
- المعاملة الثالثة: تم اضافة مستخلص البروبوليس فى ماء الشرب بمعدل (10 مل/لتر ماء شرب).
- المعاملة الرابعة: تم اضافة شرش اللبن فى ماء الشرب بمعدل (10 مل/لتر ماء شرب)
- المعاملة الخامسة: تم اضافة مستخلص البروبوليس + شرش اللبن فى ماء الشرب بمعدل (10 مل/لتر من كل إضافة)

تم تقييم الأداء الإنتاجي للمعاملات خلال فترة التجربة، كما تم تقدير صفات الذبيحة. أظهرت النتائج أن إضافة البروبوليس والشرش كلا على حدى قد حسّنت من معدل التحويل الغذائي، واستهلاك العلف، والوزن النهائي، ومعدل الزيادة الوزنية. لم تُلاحظ فروق معنوية في صفات الذبيحة بين جميع المعاملات التجريبية ($P>0.05$). أما من الناحية الفسيولوجية، فقد أدى استخدام الإضافات إلى ارتفاع معنوي ($P>0.05$) في مستويات الفوسفور، والبروتين الكلي، والكالسيوم، والألبومين، وفيتامين C، مع انخفاض معنوي في الدهون الكلية، والجلوكوز، والكوليسترول، Heterophils/Lymphocytes H/L ratio بالإضافة إلى ذلك، لوحظ تحسن في قوة صلابة عظم الساق في معاملات البروبوليس والشرش مقارنة بالكنترول ($P>0.05$). وعليه، يمكن الاستنتاج بأن استخدام البروبوليس وشرش اللبن كلا على حدى كمكملات طبيعية يُعد وسيلة فعّالة لتحسين أداء دجاج التسمين دون أي تأثيرات سلبية على صفات الذبيحة.