Study of the effect of Bee Propolis on some biochemical, immunological traits and intestinal microflora of broiler chickens (Ross 308)

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Abstract

This study was conducted to know the effect of adding Bee Propolis to a diet in some biochemical, immunological traits and intestinal microflora of broiler chickens, where 225 unsexed broiler chicks were used. The chicks were randomly divided into five treatments, each treatment was 45 birds, and each treatment was divided into three replicates, each replicate of 15 birds, the Bee Propolis material was added with the diet and at levels (0, 2, 2.5, 3 & 3.5 g Bee Propolis/kg feed) and for treatments T1, T2, T3, T4, T5 respectively. The chicks were raised for 35 days, and during the study period, we reached the following results: a significant difference (P<0.05) for the treatment T1 in glucose, uric acid, AST (Aspartate amino transferase) and height significant (P<0.01) in cholesterol concentration compared to the rest of the studied treatments, and a significant excelled (P<0.05) for the T2 treatment in the ALT (Alanine amino transferase) concentration compared with the rest of the treatments studied. The results also indicated that there was significant (P<0.05) increased for T3 and T5 in the protein level, a significant increased (P<0.05) for the T3 in IgG (immunoglobulin Gama) and IgM (immunoglobulin Mua) and T5 in IgA (immunoglobulin Alpha), a significant superior (P<0.05) for T1 and T2 in E.coli bacteria in jejunum and superior T5 in lactobacilli in jejunum and ileum meanwhile height significant (P<0.01) for T1 and T2 in E.coli bacteria in ileum this is at 14 days for age, while in 35 days for age significant superior for T1 and T2 in E.coli, T4 and T5 in lactobacilli in jejunum and ileum.

Keywords: Bee Propolis, broilers, biochemical, Immunological, intestinal microflora.

1.Introduction

Bee Propolis or bee gum is a natural product with a complex resinous structure that is collected by honey bees workers from growing parts of trees and leaf buds for use in restoring beehives, which prevents pathogens and rodents from entering the cells. propolis with natural antimicrobial substances [1]. Thus, it has a considerable antibacterial and antifungal effect on bacteria, fungi, and yeasts [2]. Due to these anti-microbial. The studies have proven that the Bee Propolis contains more than 300 active substances, the most important of which are Flavonoids, Flavones, uremic acids, phenolic compounds, amino and fatty acids, vitamins and minerals, these compounds are responsible for the biological activity of the Bee Propolis. [3,4], [5] mentioned that the most important activities of biological Bee Propolis are its action as antibacterial, viral, antifungal, antioxidant, and anti-inflammatory. [6] indicated Lower dosages of propolis in chicken nutrition significantly (P<0.05) reduced the count of Enterobacteriaceae family isolates in chicken’s crops, while the number of beneficial lactic acid bacteria in chicken’s crops with presence of propolis was increased, all so [7] noted that dietary propolis supplementation did not affect cecal concentration of Escherichia coli, total coliforms, Enterococcus and Lactobacilli meanwhile it has reduced uric acid in serum. [8] indicated that use of bee Propolis at different levels in the broilers diet achieved a significant increase (P <0.05) in albumin/globulin ratio, AST, triglyceride, VLDL, HDL concentration but there was no difference significant in total protein, albumin, globulin, and ALT concentration, this study aims to know the effect of adding different levels of bee Propolis to the diet in some biochemical parameters, immunological and intestinal microflora of broiler chickens Ross 308.

2.Materials and methods

This study was conducted in one of the poultry breeding farms in Babylon province, for the period from 2/8/2019 to 7/9/2019, Where 225 unsexed broiler chicks Ross 308 the Turkish origin were used It was raised for 35 days in (pins) cages of 1 x 1.5 dimensions. The birds were randomly distributed into five treatments. Each treatment was divided into three replicates, each replicate of 15 birds. Obtained the Chinese propolis substance in powder form and the experiment treatments were as follows (T1: Without adding, 2, 2.5, 3 and 3.5 g Bee Propolis/kg feed for treatments T2, T3, T4, T5 respectively.
The chicks were feed on the starter diet from the one day age of until the third week of the bird's age (Table 1), containing a protein of 23% and a representative energy of 3027 kcal/kg for feed, then they were replaced by final diet until the end of the fifth week, a container containing a protein of 20% and a representative energy of 3195.3 kcal/kg. The feed and water were provided free of charge (ad libitum) and the feed used is as shown in the table below.

| Feeding Materials          | % starter diet | % Final diet |
|---------------------------|---------------|--------------|
| yellow corn               | 30            | 40           |
| wheat                     | 28.25         | 24           |
| Soybean meal (48% protein)| 31.75         | 24.8         |
| protein concentrate *     | 5             | 5            |
| Sunflower oil             | 2.9           | 4.4          |
| limestone                 | 0.9           | 0.6          |
| DCP Calcium Diphosphate   | 0.7           | 0.9          |
| salt                      | 0.3           | 0.1          |
| Mix vitamins and minerals | 0.2           | 0.2          |
| Total                     | 100           | 100          |
| General protein (%)       | 23            | 20           |
| Calculated energy (kilocalories/kg feed) | 3027 | 3195.3 |
| Lysine (%)                | 1.2           | 1.1          |
| Methionine (%)            | 0.49          | 0.46         |
| Cystine (%)               | 0.36          | 0.32         |
| Methionine + cysteine (%) | 0.85          | 0.76         |
| Available phosphorus (%)  | 0.45          | 0.49         |
| C / P%                    | 131.61        | 159.77       |

* BROCON-5 SPECIAL W protein concentrate *: Chinese origin, each kg contains: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% phosphorus available, 3.25% lysine, 3.90% methionine + cysteine 2.2% sodium, 2100 kcal / kg energy represented, 20,000 IU vitamin A, 40000 IU vitamin D3, 50 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6, 300 B12 mg, 10 mg folic acid, 100 mcg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 6 mg cobalt, 900 mg antioxidant (BHT).

** According to the chemical analysis of the diet according to [9].

### 2.1 Biochemical treats

The concentration of glucose, cholesterol, and ALT, AST enzymes in the blood serum of birds was estimated at the age of 35 days, where blood samples were taken from the birds immediately after the slaughter and collected in tubes that do not contain anticoagulant, after which the serum was separated from the blood using a centrifuge at a speed of 3000 c/m for 15-minute course and then the qualities mentioned were measured in the Life Sciences Laboratory at the Faculty of Science/University of Babylon, where the concentration of glucose was estimated using a measurement (Kit) from Roche of Germany and by method of [10], and the concentration of cholesterol was estimated using several measurements (Kit) from The German company Roche and by method of [11]. The concentration of ALT and AST enzymes was estimated using a kit from Roche of Germany and by method of [12]. The concentration of uric acid was measured using a kit produced by the Spanish company Spinreact and by a method of [13].

### 2.2 Immunological treats

To measure antibodies in the serum, the indirect method used to test the ELISA, this test depending on the susceptibility of many antigens to the association with polystyrene, this method includes several steps as mentioned by [14].

### 2.3 Counting the numbers of microflora in the Jejunum and ileum

At the age of 14 and 35 days, two birds were taken from each replicate and after slaughtering the birds, they were dissected, the small intestine was extracted and The contents of the Jejunum and ileum area were then collected separately, where approximately 2 g was placed in a 5 mm tube for each replicates, which was kept at 20 °C in the refrigerator and microorganism numbers were then estimated as following:
2.3.1 Counting the numbers of Lactobacilli bacteria in the Jejunum and ileum

One gram of the Jejunum contents was taken, for all the replicates and in sterile conditions and a Decimal dilutions to dilute (10) -10 was made from it using sterilized Peptone water by Micropipette, the numbers of lactobacilli bacteria were then estimated using Pour - plate method according to [15]. By transferring 1 mL of each decimal diluent to two empty and sterile Petri dishes and directly added to each dish 15 mL of agricultural media (MRS Agar) prepared instantaneously and preserved in a water bath at 46 °C and after hardening gar, it is add in agar and put a candle with it to consume oxygen and after covering it, it was placed in incubator at a temperature of 37 °C for 48 hours and the numbers of growing colonies were then calculated by multiplying the number of colonies × inverting the dilution.

2.3.2 Counting the numbers of coliform bacteria in the jejunum and ileum

The numbers of coliform bacteria were calculated as in the case of the numbers of lactobacilli, but when transferring 1 mL of each decimal diluent to two of the sterile Petri dishes directly, 15 mL of sterile MacConkey Agar was added to each dish. After hardening the agricultural media in the dishes, it was kept inverted at 37 °C for 48 hours, the growing colonies in the dishes were then calculated as mentioned in No. (1).

2.4 statistical analysis

The Statistical Analysis System –[16] was used in data analysis to study the effect of different treatments on the traits studied according to a Completely Randomized Design (C.R.D), and the mean differences between the averages were compared to the [17] polynomial test.

\[ Y_{ij} = \mu + Ti + e_{ij} \]

where

- \( Y_{ij} \): the value of viewing j to treatment i.
- \( \mu \): general average for the trait.
- \( Ti \): effect of treatment i (the study included the effect of five treatments).
- \( e_{ij} \): a random error that is normally distributed with an average of zero and a variation of \( \sigma^2e \)

3. Results and discussion

3.1 Biochemical traits.

Table 2. showed that the effect of adding different levels to the diet of Bee Propolis on some biochemical parameters in 35 days of bird's age. In glucose concentration, there was a significant difference (P <0.05) for T1 treatment compared to rest treatments and excelled on the treatments T2, T3 on treatment T4 and T5. In the uric acid, however, (P <0.05) significantly increased in the T1 treatment compared to the T3, T4, and T5 treatments. Also, the T3 and T5 treatments significantly increased compared T4 treatment, as well as no significant difference, occurred between the T2 and T1, T3 and T5 treatments, and the T1 treatment was excelled significantly (P <0.01) in cholesterol concentration compared to the rest of the experimental treatments and The T2 and T3 treatments was excelled compared to the T4 and T5 treatments and there was no significant difference between the T2 and T3 all so T4 and T5 treatments, meanwhile in AST enzyme concentration a significant increased (P <0.05) of the T1 treatment compared to all treatments, The T2 and T5 treatments has excelled on treatments T3 and T4 treatments, The table did not show significant differences between T2 and T5 treatments, all so T3 and T4 but in the ALT concentration T2 treatment have significantly increased (P <0.05) compared to the rest of the treatments and T1 treatment was excelled to T3, T4, and T5 treatments, and T3 and T4 treatments were excelled to T5 and there was no significant difference between T3 and T4 treatment.

A significant decrease in the concentration of glucose, uric acid, cholesterol, AST and ALT enzymes may be due to the role of adding propolis in the preservation of protein and not using it as an energy source for its antioxidant effect [5], and considering it as a protector of the liver cells [18]. If uric acid is the product of the final metabolism of protein metabolism in birds and its increased concentration reflects the amount of damage to proteins caused by bird stress and the use of protein as an energy source, then there is a positive correlation between the concentration of uric acid and the effectiveness of AST and ALT enzymes because those enzymes are responsible about the transfer of the amino acid group for the purpose of its introduction through the liver [19], where we note through the table (2) the low concentration of uric acid and low levels of AST and ALT enzymes in the treatment of the addition of propolis (Especially the T3, T4 and T5 treatment) may be due to the containment of propolis on compounds containing antioxidants that protect unsaturated fatty acids in cellular membranes of oxidative processes and retaining these membranes as optional permeability and keeping the cell contents and not sipping them out of the cell as propolis shows a similar effectiveness For vitamin C to protect the unsaturated fatty acids in the cell membrane of oxidation [20], and that propolis plays a role in preventing liver injury and protecting it from damage by maintaining the activities of enzymes within the liver and not coming out into the blood stream [21] where the activity of enzymes indicates Low that propolis is able to reduce tissue damage and prevent the leakage of enzymes through cellular membranes due to its antioxidant function, and that the increased of these concentrations in the treatment of control may be
due to damage to liver cells, as well as may be due to the low concentration of uric acid in the blood of birds propolis treatment to propolis activity in and its work to inhibit the action and production of xanthin due to the containment of propolis on some bioactive compounds such as chrysin, galangin, caffeic acid phenethl ester, p-coumaric acid and artepillin which inhibits the production of oxide Exanthinthus thus reduces the production of uric acid [22], and a significant decrease in cholesterol concentration may be due to the role of propolis in increasing unsaturated fatty acids compared to Saturated fatty acids [8], which lowers cholesterol, where propolis contains essential fatty acids that inhibit the reductive activity of hepatic enzyme 3-Hydroxy-3-Methyglutaryl Coenzyme A (HMG-CO A), a primary regulatory enzyme in the process of synthesis of cholesterol [23], meanwhile that the low level of Glucose in the blood may be due to the role of propolis to enhance the role of antioxidants in the cell and reduce the effect of oxidative stress, which activates the action of body cells, including pancreatic beta cells, and thus activates the secretion of insulin, which lowers the level of blood glucose.

### Table 2. Study of the effect of Bee Propolis biochemical traits of broiler chickens (Ross 308)

| Treatments | Glucose mg/100 ml | Uric acid mg/100 ml | Cholesterol mg/100 ml | ATS U/L | ALT U/L |
|------------|------------------|---------------------|----------------------|--------|--------|
| T1         | 210.02 ± 2.10a   | 9.13 ± 2.71a        | 193.00 ± 2.38a       | 114.80 ± 2.22a | 5.80 ± 2.35b |
| T2         | 182.69 ± 2.40b   | 8.75 ± 3.79ab       | 182.14 ± 2.14b       | 109.65 ± 3.11b | 6.67 ± 2.53a |
| T3         | 180.13 ± 3.32b   | 8.35 ± 2.34b        | 185.26 ± 3.15b       | 94.39 ± 2.74c | 5.12 ± 3.41c |
| T4         | 171.50 ± 3.90c   | 7.50 ± 3.40c        | 170.30 ± 3.50c       | 84.55 ± 2.00d | 5.10 ± 3.00c |
| T5         | 179.40 ± 2.82c   | 8.20 ± 2.00b        | 174.95 ± 2.25c       | 102.13 ± 2.38b | 4.23 ± 3.02d |

Level of significance: * * * * *

Averages with different letters indicate a significant difference in probability level * (P <0.05), **(P <0.01). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 2.25, 3.35 g of Bee Propolis / kg feed, respectively.

#### 3.2 Immunological traits

Table 3. showed that the effect of adding different levels to the diet of Bee Propolis on some immunological traits in 35 days of bird’s age. In protein concentration, there was a significant difference increased (P <0.05) for T3 and T5 treatment compared to T1 and T4 treatments and there was no significant difference between T2 and T1, T3, T4, and T5 treatments. In the immunoglobulin IgG, however, (P <0.05) significantly increased in the T3 treatment compared to the T1, T2, and T4 treatments. Also, the T2 and T4 treatments significantly increased compared T1 treatment, as well as no significant difference, occurred between the T5 and T2, T3 and T4 treatments, and the T5 treatment was excelled significantly (P <0.05) in IgA level compared to the T1, T2, and T3 treatments and The T1 treatment was improvement compared to the T2 and T3 treatments and The T2 and T3 treatments was increased compared to the T4 treatment meanwhile there was no significant difference between the T4 and T1 and T5 treatment, meanwhile in IgM concentration a significant increased (P <0.05) of the T3 treatment compared to all treatments, The T2 treatment has excelled on treatments T1, T4 and T5, The table did not show significant differences between T4 and T1, T3 treatments.

Stimulation of the immune system and immunoglobulins by natural products has already been reported [24,25]. Not only in broilers but also rodents these effects of propolis have been confirmed [26]. The effect of natural products such as propolis on the immune system of different species is interesting and complicated. The direct effect might be related to stimulating the lymphatic tissue in the digestive system, and an indirect effect via changing the microbial population of the lumen. Propolis is a natural product which in numerous experiments, has revealed different actions on the immune system. For example, increasing the macrophage activity [27], increasing the IL1 [28,29], IL2 [30] and IL4 [31]. In this relation increasing the humoral response in broilers might be related to a combination of these responses. Because it is very obvious that in immune system B lymphocytes are stimulated by these cytokines, and then they are changed to plasma cells which would be able to produce antibodies. On the other hand, propolis has anti-oxidant [32] and anti-inflammatory [33] effects and these are related to inhibition of prostaglandin synthesis[34]as an anti-immune substance and resulting better humoral response, This explains the high level of immunoglobulins in bird blood treated with propolis compared to the treatment of control. The high concentration of total protein in T3 and T5 treatments is also due to the role of propolis as an antioxidant within the body and the non-use of protein as an energy source within cells.
Table 3. Study of the effect of Bee Propolis on immunological traits of broiler chickens (Ross 308)

| Treatments | Average ± standard error |
|------------|-------------------------|
|            | Total protein g/100 ml   | IgG mg/ml | IgA mg/ml | IgM mg/ml |
| T1         | 4.90 ± 0.11 b            | 2.08 ± 0.60 c | 3.10 ± 0.25 b | 1.60 ± 0.32 c |
| T2         | 5.00 ± 0.39 ab           | 2.54 ± 0.75 b | 2.64 ± 0.21 c | 3.09 ± 0.28 b |
| T3         | 5.10 ± 0.27 a            | 3.32 ± 0.50 a | 2.27 ± 0.50 c | 3.45 ± 1.10 a  |
| T4         | 4.98 ± 0.50 b            | 2.69 ± 0.23 b | 3.57 ± 1.00 ab| 1.98 ± 0.50 cd |
| T5         | 5.22 ± 0.25 a            | 3.17 ± 0.37 ab| 3.80 ± 0.45 a | 2.25 ± 0.30c   |

Level of significance

* Averages with different letters indicate a significant difference in probability level * (P <0.05). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 2,2.5,3,3.5 g of Bee Propolis/kg feed, respectively.

3.3 Intestinal microflora

3.3.1 Study of the effect of Bee Propolis on the Jejunum and ileum microflora at the age of 14 days (log/ g) for broiler chickens (Ross 308).

Table 4. shows the effect of adding different levels to the diet of Bee Propolis on the numbers of bacteria in Jejunum and ileum at the age of 14 days from birds age. It was noticed in the Jejunum that there was a significant superiority (P<0.05) for the treatments T1, T2 in E.coli bacteria compared to the rest of the treatments as well as the treatments T3 have excelled on the T4 and T5 treatments and it did not show significant differences between treatments T1, T2 all so the treatments T4 and T5. As for the number of beneficial bacteria, the treatment T5 gave the highest number from it, The T3 treatment has excelled in the treatments T1 and T2 meanwhile there was no significant difference between the treatments T4, T5, T3. As for the number of harmful bacteria in ileum, the T1 and T2 treatments was significantly excelled (T <0.01) on the rest of the treatments as well as the T3 and T4 treatments was significantly excelled on the treatment T5 and statistical analysis did not show significant differences between the two treatments T1 and T2 all so T3 and T4 treatments. The superiority of the T5 treatment continued to ileum in the number of beneficial bacteria, where It was significantly (P<0.05) excelled on the rest of the treatments, the T3 and T4 treatments also excelled on the treatments T2, and there were no significant differences between the T1, T2, T3, and T4 treatments.

Table 4. Study of the effect of Bee Propolis on the numbers of bacteria at the age of 14 days (log/ g) for broiler chickens (Ross 308).

| Treatments | Average ± standard error |
|------------|-------------------------|
|            | Jejunum | Ileum |
|            | E.coli  | Lactobacilli | E.coli  | Lactobacilli |
| T1         | 4.30 ± 3.11 a | 6.18 ± 1.60 c | 3.80 ± 1.25 a | 7.58 ± 2.25 bc |
| T2         | 4.20 ± 2.39 a | 6.25 ± 1.75 c | 3.74 ± 1.21 a | 7.14 ± 2.00 c  |
| T3         | 3.80 ± 1.27 b | 7.13 ± 2.50 b | 2.19 ± 1.00 b | 8.29 ± 3.20 b  |
| T4         | 3.41 ± 1.50 c | 7.47 ± 2.23 ab| 2.35 ± 1.10 b | 8.39 ± 3.75 b  |
| T5         | 3.45 ± 1.25 c | 7.78 ± 2.37 a | 1.90 ± 0.75 c | 8.91 ± 3.00 a  |

Level of significance

* Averages with different letters indicate a significant difference in probability level * (P <0.05). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 2,2.5,3,3.5 g of Bee Propolis/kg feed, respectively.
3.3.2 Study of the effect of Bee Propolis on the Jejunum and ileum microflora at the age of 35 days (log/ g) for broiler chickens (Ross 308).

Table 5. shows the effect of adding different levels to the diet of Bee Propolis on the numbers of bacteria in Jejunum and ileum at the age of 35 days from birds age. In the Jejunum, there was a significant superiority (P<0.05) for the treatments T1, T2 in harmful E.coli bacteria compared to the rest of the treatments as well as the treatments T3 and T5 has excelled on the T4 treatment and it did not show significant differences between treatments T1, T2 all so the treatments T3 and T5. As for the number of beneficial bacteria lactobacilli, the treatments T4 and T5 were the highest numbers from it, The T1 and T2 treatments have excelled on the treatment T3 as well as there was no significant difference between the treatments T1 and T2 all so T4 and T5 treatments. As for the number of harmful bacteria in ileum, the T1 treatment was significantly excelled (T <0.05) on the rest of the treatments as well as the T2 treatment was significantly excelled on the treatment T3, T4 and T5 meanwhile T4 and T5 treatments increased the compared the T3 treatment and there were no significant differences between the T1 and T2 all so T4 and T5 treatments, In the number of beneficial bacteria, where It was height significant (P<0.01) excelled for T4 and T5 treatment on the rest of the treatments, the T2 and T3 treatments also excelled on the treatment T1, and there were no significant differences between the T1 and T2 all so T4 and T5 treatments.

According to [35] the decline of harmful bacteria family counts in chicken's crops may be associated with the antibacterial activity of propolis. In comparison with our results, [36] found a comparable count of lactic acid bacteria on average (6.55 log CFU. g-1) in the jejunum of broiler chickens after propolis addition. The higher increase of lactic acid bacteria count in jejunum and ileum of T4 and T5 was significant because of the addition of propolis and its work as a prebiotic or stimulant for the growth of these beneficial bacteria. [37] revealed that plant extract additives increased the numbers of lactic acid bacteria in the ileum and cecum of broilers while significantly reducing the numbers of total anaerobic, coliform and C.perfringes bacteria. Jejunum and ileum of broiler chickens without nutrition with propolis had not a significantly higher count of any observed beneficial bacteria (lactobacilli) but there was a higher significantly in harmful bacteria (E. coli).

| Treatments | Average ± standard error |
|------------|--------------------------|
|            | Jejunum | Ileum |
|            | E. coli | Lactobacilli | E. coli | Lactobacilli |
| T1         | 4.19 ± 1.75 a | 7.31 ± 2.15 c | 3.82 ± 0.50 a | 7.64 ± 1.18 c |
| T2         | 4.13 ± 1.20 a | 7.74 ± 2.60 b | 3.40 ± 0.91 b | 8.48 ± 1.65 b |
| T3         | 3.60 ± 1.00 b | 7.20 ± 2.30 c | 1.98 ± 0.25 d | 8.55 ± 1.80 b |
| T4         | 2.59 ± 1.23 c | 8.38 ± 3.10 a | 2.33 ± 0.55 c | 9.17 ± 2.10 a |
| T5         | 3.60 ± 1.00 b | 8.40 ± 3.22 a | 2.31 ± 0.35 c | 9.12 ± 2.25 a |

Level of significance: * * * * *

Averages with different letters indicate a significant difference in probability level * (P <0.05). The treatment T1, T2, T3, T4, T5 are control treatments without addition, adding 2,2.5,3,3.5 g of Bee Propolis/kg feed, respectively.

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