Effect of propolis in diet supplementation on the immune response against Newcastle disease and hematological picture in broiler chicks

I. M. Shihab[*] and B. H. Ali[**]
[*]College of Agriculture\ University of Baghdad
[**]College of Veterinary Medicine\ University of Baghdad

Abstract

The study was conducted to evaluate the effect of dietary supplementation with propolis on immune response against Newcastle disease virus and hematological picture at 21 and 42 day old chicks. One hundred and eighty Ross broiler chicks were reared from 1 day to 47 day old they were divided randomly to six groups (30 birds/group), feed and drinking water offered ad libitum and experimental were groups treated as follows: Group 1(G1) received propolis (0.5 g/kg feed), while group 2 (G2) received propolis (1 g/kg feed), Group 3 (G3) received propolis (2 g/kg feed), group 4 (G4) received propolis (3 g/kg feed) and group 5 (G5) was control positive received (basal diet without propolis but vaccinated). Group 6 (G6) control negative received (basal diet without propolis and non vaccinated). The obtained results referred to high Abs titer ($P \leq 0.01$) in G4, G3 and G2 against Newcastle disease virus at the end of the experiment as compared with G1, G5 and G6. Although increase in blood picture was observed in G4, G3, G2 and G1 ($P \leq 0.01$) compared with G5 and G6 had no significant increase ($P \geq 0.01$).

تأثير إضافة البروبولس في العليقة على الاستجابة المناعية ضد مرض النيوكاسل والصورة الدمية في دجاج اللحم

إحسان محمد شهاب[*] وبلقيس حسن علي[**]
[*]كلية الزراعة/ جامعة بغداد
[**]كلية الطب البيطري/ جامعة بغداد

الخلاصة

أجريت هذه الدراسة لتقييم تأثير مادة البروبولس على الاستجابة المناعية ضد فايروس مرض النيوكاسل والصورة الدمية عند 21 و42 يوم من عمر الأفراخ. استخدمت 180 طير فروج لحم نوع روز من (1–47) يوم، وزعت عشوائيا لستة مجاميع كل مجموعة تحتوي على (30) طير وكالأتي: المجموعة الأولى: أضيف بروبولس بمعدل 0.5 غم/ كغم إلى العليقة وعدت (G1). المجموعة الثانية: أضيف بروبولس بمعدل 1 غم/ كغم إلى العليقة وعدت (G2). المجموعة الثالثة: أضيف بروبولس بمعدل 2 غم/ كغم إلى العليقة وعدت (G3). المجموعة الرابعة: أضيف بروبولس بمعدل 3 غم/ كغم إلى العليقة وعدت (G4). المجموعة الخامسة: مجموعة سيطرة موجبة أعطيت عليقه أساسية بدون إضافة البروبولس (ملقحة) وعدت (G5). المجموعة السادسة: مجموعة سيطرة سالبة أعطيت عليقه أساسية بدون إضافة البروبولس (غير ملقحة) وعدت (G6). أظهرت النتائج وجود معدل عالي ($P<0.01$) للأجسام المضادة المناعية في مصل الأفراخ ضد فيروس مرض النيوكاسل عند نهاية التجربة في المجموعة الرابعة والثالثة والثانية مقارنة بالمجموعة الأولى ومجموعة السيطرة الموجبة ومجموعة السيطرة السالبة. بالإضافة إلى ذلك فقد وجد ان هناك زيادة في الصورة الدموية للمجاميع الرابعة والثالثة والثانية والأولى ($P \leq 0.01$) مقارنة مع مجاميع السيطرة ($P \geq 0.01$).

Introduction

Propolis or bee glue, as it is commonly named, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds and exudates (1). This resin is masticated, salivary enzymes are added, and then it is mixed with beeswax and probably with other compounds of bee metabolism (2). Propolis, a resinous substance produced by honey bees from exudates collected from different parts of plants(3), presents a several biological activities (4, 5, 6), even though many of its action mechanisms are unknown. Propolis pharmacological activity against several viral infections has been evaluated in studies with influenza virus (7), adenovirus (8), and herpes simplex viruses (9, 10). The wide spectrum of propolis biological activities together with the need for new virucidal substances, renew the interest for this bee product regarding its antimicrobial potential (10). Within the several last year's using the prebiotics, probiotics and natural products is going to substitute for antibiotics in order to improve immune system and fight against pathogens in human and animal life. In contrast to antibiotics these products do not have side effects and are very useful in food chain (11).

Materials and Methods

- **Experimental Animals:** The chicks (Rose, Syria Origin), were brought from Hatchery Association of wade Al-Rafidian - Bagdad\ Abu grab. The 180 one day old chicks were divided randomly into 6 groups) (all biosecurity protocols were applied). Blood samples were taken from 20 chicks randomly for assessment of maternal immunity against Newcastle disease (ND), 5 blood samples from each group were aspirated from wing vein to determine blood picture and antibody titers against ND at days (21, 42) using ELISA for (ND). The chicks in G1, G2, G3, G4 and G5 were vaccinated with ND (La Sota) via drinking water at day 15 followed by booster dose of Newcastle virus vaccine (La Sota) at day 25 and with IBD Gumbo L strain (Ceva-Hungary) at day (19) and G6 left without any vaccine as control negative.
- **Preparation of Poultry House:** The experiment was done in poultry farm of animal resources department at the College of Agriculture\ Baghdad University, Poultry house, after cleaning and disinfection (by Formaline and Sodium Hypochlorite and left for 2 days, finally all doors and windows were opened and ventilator were switched on to assure complete removal of residual toxic gases before chicks admittance, the experiment began at 19/12/2011 to 3/2/2012.
- **Diet Composition and Contents:** The basal diet was formulated for broilers in which yellow corn and wheat were the major sources of energy, whereas the soybean and plant protein were the major sources of protein in this diet. This diet was fed to all group. Other ingredients were same as in the groups (Table 1). Nutritional requirements were adjusted according to the Nutritional Requirements Council (12).

a. Starter: The chicks fed on starter diet from 1 to 20 days at the beginning farm experiment.

b. Final: The chicks used from 21days until the end of the experiment (day47). It was composed of the following:

Table (1) Composition of experiment's diets prepared in this study

Constituents	Percentages of ingredients in Starter	Percentages of ingredients in Final
plant Protein (5% protein)	5 %	5 %
Soybean meal (45% protein)	31.5 %	22 %
Yellow corn	47 %	47 %
Wheat	12 %	20 %
fat	2.5 %	4 %
Dicalcium phosphate	0.5 %	0.5 %
Vitamins	0.2 %	0.2 %
Ca co3	1 %	1 %
Salt (Nacl)	0.3 %	0.3 %
Total	100 %	100 %

- **Total White Blood cell counting cell/ mm³:**Natt and herrick solution was used to dilute blood to count WBC`s by hemocytometer method as mentioned before in RBC`s counting, blood was sucked up to level 0.5, while diluted solution was sucked up to the level 11 then mixed, with neglecting the first drops of the pipette drip one drop on hemocytometer then covered (count 4 corner squares).

  The following equation was applied:

  Number of counted WBC`s/4 × 20 × 10=WBC`s × 50 / **mm³** Blood, (13).

  Natt and Herrich solution was prepared as:-

  1. NaCl ……..….... 3.88 gram
  2. $KH2PO_4$ ……..…… 0.25 gram
  3. formalin ……...…… 7.5 ml
  4. $Na_2HPO_4.12H_2O$ ……..…… 2.9 gram
  5. Na_2SO_4 ……..……..... 2.5 gram
  6. Methyl Violet ……..….. 0.1 gram

  These substances were disolved in 1000 ml of distilled water then filtered by double filter paper (13).

- **Total Red blood cells counting cell/ mm³:** Natt and Herrick solution was used to dilute the blood to count RBCs` by hemocytometer method, blood was sucked up to level 0.5, while diluted solution was sucked up to the level 101 then mixed, with neglecting the first drops of the pipette drip one drop on hemocytometer then covered (Count 5 squares).

  Four squares of four corners and Central Square.

  The number of RBC was measured according to the following equation:

  Number of counted RBCs\cm = N/5 × 25 × 200 × 10

N =Total number of count cells in 5 large square.

5 = Number of the large squares.

25 = Total number of squares.

200 = dilution factor.

10 = slide deep (distance between the cover slip and the slide).

- **Packed cell volume (PCV):** Packed cell volume was measured by using micro-hemocrit capillary tubes. After being filled with blood 2 to 3 up to their length .another side of tube was blocked by clay and set in micro-hematocrit centrifuge for five min (2000\min). Reading was taken by using a micro-hematocrit reader according to the method mentioned by (14).

- **Hemoglobin Concentration** (**HB**): The method to convert hemoglobin to (cyanomethemoglobin) and with drawing 5 ml of solution (drabkin's) and add 0.02 ml of blood, and after mixing wait for a period 5 minutes and then placed in tubes in the centrifuge for 10 minutes to get rid of the packed cells and nuclei were then read by a hemoglobin measurement of the optical spectrum wave length 540 nm, and calculated the amount of hemoglobin applying the following equation: Concentration of hemoglobin (g/dL) = hemoglobin

Concentration/ hemoglobin standard × factor (15).

- **ELISA Test (Synbiotics – USA):** The procedure used in this test was performed according to the manufacturer instructions listed in the ProFLOK ELISA Kit (Synbiotics- USA), which is a rapid serologic test for the detection of antibody in chicken serum samples. It was developed primarily to aid in the detection of pre and post-vaccination antibody levels in chickens.
- **Statistical Analysis:** Data were analyzed by using complete randomize design (CRD) in factorial experimental by the fallowing model.

$Y_{ijk}+G_i+P_j+(GP)_{ij}+eijk$

Where

y_{ijk} = observed variable.

M = common mean.

G_i = the effect of groups(g=1-6).

P_i = the effect of period (p=21,42day).

$(GP)_{ij}$ = the interaction between G_i and P_j.

eijk = random error.

The Data were analyzed by using SPSS (16). To calculate the difference between means was used Duncan-multiple test (17).

Results and Discussion

- **Results of ELISA against NDV Ab titer at day 21 and 42 day old chicks:** Maternal immunity (Antibodies titer against Newcastle disease virus NDV) in this study was measured by indirect method of Enzyme Linked Immunosorbent Assay (ELISA) at one day old and blood samples were collected before dividing broiler chicks into six groups. The results of maternal antibody (MAb) titer of chicks at one day old were recorded, the mean value of NDV was 11853±849.92. The accumulative effects of Ab that produced; several times of vaccination reached to the high titers, thus the eggs height received a high level of MAb from their own breeders, and this was represented in the hatched chicks (18). The Ab titer against NDV showed high level of Ab titer at 21day old in G4, 2501.40, G5, 1926.20, G3, 1751.00 and low level in G1, 949.20, G2, 1273.00, G6, 381.80, the lower titers of Ab in the G6 (no vaccine) which did not receive any supplementation of propolis in contrast to the groups G4, G3 and G5 had the highest Ab titer. At the 42 day old, the result of Ab titer against NDV showed significant differences between mean values of Ab titer in G4 (5786.60), G3 (4999.40), G2 (4745.40) and G5 (4429.00) in contrast to G6 (168.00), the highest Ab titer appearance in groups received propolis (G1, G2, G3, G4 and G5) and the lower titers of Ab present in the G6. These results are shown in Table (2)

Table (2) The means of Ab titer at 21 and 42 day old of chicken against NDV (Mean ± SE)

Gropes / age	G1	G2	G3	G4	G5	G6	Significant level
21	949.20 ±24.6^{c} B	1273.00 ±43.3^{C} B	1751.00 ±16.84^{b} B	2501.40 ±112.6^{a} B	1926.20 ±67.8^{b} B	381.80 ±7.07^{d} B	p≤0.01
42	3851.00 ±120.75^{c} A	4745.40 ±121.83^{ab} A	4999.40 ±28.05^{a} A	5786.60 ±173.67^{a} A	4429.00 ±22.06^{b} A	168.00 ±16.8^{d} A	p≤0.01
Significant level	p≤0.01	p≤0.01	p≤0.01	p≤0.01	p≤0.01	p≤0.01	

Small letters between groups (raw) denoted significant differences (p≤0.01).
Capital letters between period (column) denoted significant differences (p≤0.01). G1 basal diet+0.5mg/kg propolis, G2 basal diet + 1g/kg propolis, G3 basal diet + 2g/kg propolis, G4 basal diet + 3g/kg propolis, G5 basal diet (vaccinated) and G6 basal diet (non vaccinated). SE = standard error.

Serum antibody level is the indicator of humoral immunity. These results showed that serum IgG levels in the group receiving 3 g/kg feed of propolis were significantly higher than those of G5 and other groups, suggesting that 3 g/kg of propolis could promote humoral immunity in broiler. A similar, but not significant, trend was observed for 1 and 2g/kg of propolis. Several studies have showed that propolis activates the immune system, increasing macrophage activity (19) and increasing interleukin-1, interleukin-2, and interleukin-4 levels (19). These cytokines stimulate B lymphocytes and then they are changed to plasma cells, which would be able to produce immunoglobulins (20). Therefore, in These study, the increased levels of IgG in birds of groups given 3 g/kg of propolis may be related to the stimulation of B lymphocytes by these cytokines.

- **Result of Red blood cell (RBCs)**: The results of red blood cells (RBCs) count of the chicks is presented in Table (3). There was no significant difference (P ≥0.01) in erythrocyte count between all group at 21 day old, but there was a significant (P ≤ 0.01) increase in erythrocyte count at 42 day old of chicks given 2 and 3 g/kg of propolis compared with the control and other treated groups. However, interestingly, the erythrocyte count was not significantly altered by doses of 0.5, 1, of propolis.

Table (3) Means of the chicks RBC×10^{6} (cells/mm^{3}) (Mean ± S.E)

Gropes / age	G1	G2	G3	G4	G5	G6	Significant level
21	2.39 ±0.05^{a} A	2.39 ±0.10^{a} A	2.32 ±0.11^{a} B	2.53 ±0.07^{a} A	2.54 ±0.05^{a} A	2.43 ±0.05^{a} A	N.S
42	2.52 ±0.17^{ab} A	2.54 ±0.12^{ab} A	2.78 ±0.10^{a} A	2.72 ±0.17^{b} A	2.39 ±0.09^{c} A	2.51 ±0.20^{ab} A	p≤ 0.01
Significant level	N.S	N.S	p≤0.01	N.S	N.S	N.S	

Small letters between groups (raw) denoted significant differences (p≤0.01).
Capital letters between period (column) denoted significant differences (p≤0.01).
G1 basal diet+0.5mg/kg propolis,G2 basal diet + 1g/kg propolis, G3 basal diet + 2g/kg propolis, G4 basal diet + 3g/kg propolis, G5 basal diet (vaccinated) and G6 basal diet (non vaccinated). SE = standard error. N.S = non significant.

In this experiment, a significant elevation of the of erythrocytes count in chicks of groups given 2 and 3 g/kg of propolis suggests that propolis might have a stimulatory effect on the synthesis of these cells in bone marrow.

- **Result of White blood cell (WBC):** The effect of dietary supplementation of propolis on some hematological parameters of the Broiler is presented below. The result of White blood cells (WBCs) count showed significant differences (P≤0.01) between all groups at 21 and 42 day of old table (4). In these study, a significant elevation of the count of leukocyte in chicks at G4 at 21 and 42 day of old compared with other groups, suggests that propolis acts directly on hematopoietic bone marrow cells and enhances their growth and differentiation into colony-forming cells.

Table (4) Means of the chicks WBC ×10^3 (cells/mm^3) (Mean ± S .E)

Gropes / age	G1	G2	G3	G4	G5	G6	Significant level
21	19.98 ± 0.74^b A	19.64 ± 0.49^b A	19.74 ±0.62^b A	21.50 ±0.78^a A	19.14 ±0.99^b A	19.96 ±0.65^b A	p≤0.01
42	19.88 ±0.82^b A	18.56 ±1.16^b B	19.98 ±1.02^b A	20.96 ±1.02^a A	19.62 ±1.14^b A	17.62 ±1.33^c B	p≤0.01
Significant level	N.S	p≤0.01	N.S	N.S	N.S	p≤0.01	

Small letters between groups (raw) denoted significant differences (p≤0.01).
Capital letters between period (column) denoted significant differences (p≤0.01).
G1 basal diet + 0.5 mg/kg propolis, G2 basal diet + 1 g/kg propolis, G3 basal diet + 2g/kg propolis, G4 basal diet + 3g/kg propolis, G5 basal diet (vaccinated) and G6 basal diet (non vaccinated). SE = standard error, N.S=non significant.

- **Packed cell volume (PCV %) and hemoglobin (Hb g/dl):** Table (5 and 6) show the result of (PCV and Hb). Although the increase was determined in hematocrit and hemoglobin values at 21 and 42 day in group receiving 2 and 3 g/kg of propolis in the diet, this increases was found to be statistically significant (P ≤ 0.01) in comparison to the G6 and other treatment groups, respectively.

Table (5) Means of the chicks PCV (%) (Mean ± S.E)

Gropes / age	G1	G2	G3	G4	G5	G6	Significant level
21	29.60 ±0.88^c B	31.08 ±0.52^b A	30.52 ±1.15^b B	33.54 ±0.49^a B	33.40 ±0.50^a A	29.07 ±0.63^C A	p≤0.01
42	31.76 ±2.02^C A	31.32 ±1.31^C A	33.82 ±0.87^b A	38.56 ±0.84^a A	29.72 ±0.85^d B	30.54 ±1.75^{cd} A	p≤0.01
Significant level	p≤0.01	N.S	p≤0.01	p≤0.01	p≤0.01	N.S	

Small letters between groups (raw) denoted significant differences (p≤0.01).
Capital letters between period (column) denoted significant differences (p≤0.01).
G1 basal diet +0.5 mg/kg propolis, G2 basal diet + 1g/kg propolis, G3 basal diet + 2g/kg propolis, G4 basal diet + 3 g/kg propolis, G5 basal diet (vaccinated)and G6 basal diet(non vaccinated).SE=standard error N.S=non significant.

Table (6) Means of the chicks HB (g/dl) (Mean ± S.E)

Gropes / age	G1	G2	G3	G4	G5	G6	Significant level
21	9.78 ±0.26^b A	9.88 ±0.19^b A	10.06 ±0.50^{ab} B	10.46 ±0.21^a B	10.32 ±0.13^a A	9.28 ±0.32^c A	p≤0.01
42	10.22 ±0.60^b A	10.40 ±0.43^b A	11.32 ±0.28^a A	12.40 ±o.98^a A	9.90 ±0.36^b A	9.32 ±0.62^c A	p≤0.01
Significant level	N.S	N.S	p≤0.01	p≤0.01	N.S	N.S	

Small letters between groups (raw) denoted significant differences (p≤0.01).
Capital letters between period (column) denoted significant differences (p≤0.01).
G1 basal diet + 0.5 mg/kg propolis, G2 basal diet + 1g/kg propolis, G3 basal diet + 2g/kg propolis, G4 basal diet + 3g/kg propolis,G5 basal diet (vaccinated) and G6 basal diet (non vaccinated). SE = standard error. N.S=non significant.

The increased hemoglobin level, in response to 2 and 3 g/kg of propolis supplementation observed in this study, could be explained by assuming that propolis improves the digestive utilization of iron and the regeneration efficiency of hemoglobin (21). The above findings suggest that dietary propolis supplementation at 2 and 3 g/kg of diet may help prevention of anemia.

References

1. Ghisalberti, E. L. 1979. Propolis: a review. Bee World, 60:59-84.
2. Burdock, G. A. 1998. Review of the biological properties and toxicity of bee propolis. Food Chem. Toxicol., 36:347-363.
3. Fischer, G. & Vidor, T. 2008. Propolis as an immune system modulator substance. In: Orsolic, N. & Basic, I. (eds) Scientific Evidence of the Use of Propolis in Ethnomedicine, Transworld Research Network, Kerala.
4. Fischer, G.; Conceicao, F. R.; Leite, F. P.; Dummer, L. A.; Vargas, G. D.; Hubner Sde, O.; Dellagostin, O. A.; Paulino, N.; Paulino, A. S. & Vidor, T. 2007. Immunomodulation produced by a green propolis extract on humoral and cellular responses of mice immunized with SuHV-1. Vaccine, 25:1250-1256.
5. Vatansever, H. S.; Sorkun, S. K.; Gurhan, I. D.; Kurt, F. O.; Turkoz, E.; Gencay, O. & Salih, B. 2010. Propolis from Turkey induces apoptosis through activating caspases in human breast carcinoma cell lines. Acta Histochem., 112: 546-556.
6. Gregoris, E. & Stevanato, R. 2010. Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. Food Chem. Toxicol., 48: 76-82.
7. Serkedjieva, J.; Manolova, N. & Bankova, V. 1992. Anti-influenza virus effect of some propolis constituents and their analogues (esters of substituted cinnamic acids). J. Nat. Prod., 55: 294-297.
8. Amoros, M.; Sauvager, F.; Girre, L. & Cormier, M. 1992. In vitro antiviral activity of propolis. Apidologie, 23:231-240.
9. Schnitzler, P.; Neuner, A.; Nolkemper, S.; Zundel, C.; Nowack, H. & Sensch, K. H. 2010. Antiviral activity and mode of action of propolis extracts and selected compounds. Phytother. Res., 24: S20-S28.
10. Nolkemper, S.; Reichling, J.; Sensch, K. H. & Schnitzler, P. 2010. Mechanism of herpes simplex virus type 2 suppression by propolis extracts. Phytomedicine, 17: 132-138.
11. Aghdam Shahryar, H.; Namvari, M.; Nourollahi, H. & Shaddel Tili, A. 2011. Effect of Alcoholic Extract Propolis on Immune System in Broiler Chickens. J. Basic and Appl. Sci. Res., 1(11): 2094-2097.
12. NRC. 1994. Nutrient requirements of poultry, National Research.
13. Pierson, F. W. 2000. Laboratory techniques for avian hematology. In: Weiss, D. J. & Wardrop, K. J. Schalm's Veterinary Hematology. 5th ed. Wolters Kluwer Company. Pennsylvania. USA., PP. 1145-1152.
14. Campbell, T. W. 1988. Avian Hematology and Cytology. 1st ed. Iowa State University. Press. Amess. Iowa, 1: 23- 43.
15. Varley, H.; Growenlock, A. & Bell, M. 1980. Practical clinical biochemistry. 5th. ed. London. William Heimemann Medical Ltd.
16. SPSS For windows. 2008. Base system Users Guide, Chicago, USA.
17. Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrica, 11: 1- 42.
18. Hamal, K. R.; Burgess, S. C.; Pevzner, I. Y. & Erf, G. F. 2006. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poult. Sci., 85:1364-1372.
19. Park, J. H.; Lee, J. K.; Kim, H. S.; Chung, S. T.; Eom, J. H.; Kim, K. A.; Chung, S. J.; Paik, S. U. & Oh, H. Y. 2004. Immunomodulatory effect of caffeic acid phenethyl ester in Balb/c mice. Int. Immunopharmacol., 4: 429-436.
20. Diker, S. K. 1998. İmmunoloji. 1st ed. Medisan Yayınevi, Ankara. Dimov V., Ivanovska N., Manolova N., Bankova V., Nikolav N. and Popov S. (1991). Immunomodulatory action of propolis. Influence on antiinfectious protection and macrophage function. Apidologie, 22:155-162.
21. Haro, A.; Aliaga, I. L.; Francisco, L.; Barrionuevo, M.; Alfe'rez, M. J. M. & Campos, M. S. 2000. Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. J. Agric. Food Chem., 48:5715-5722.