BLOOD HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS AND HUMORAL IMMUNITY AS AFFECTED BY ADDED DIETARY PROPOLIS SUPPLEMENTATION OF COBB BROILER CHICKS

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SUMMARY

This experiment was carried out at El-Takamoly Poultry Project. The objective of this study was to evaluate the effect of dietary propolis supplementation on immune response of broiler chicks. A total number of 200 unsexed one day-old Cobb broiler chicks were used in this experiment; the chicks were divided into five groups, with four replicates of ten chicks each. Chicks were fed a starter diet without propolis supplementation during the first 6 days of age. At the 7th day, birds were fed diets containing different levels of propolis (0, 200, 400, 600 and 800 mg/kg) till the end of 6th weeks of age. Ethanolic extract of propolis was added to mixed diets. At 42 dy the blood hematological and biochemical parameters were determined, also , the hemagglutination- inhibition (HI) test was applied for determination of antibodies response in plasma and Commercial kits were used for detection of antibodies against nucleoprotein and matrix against of Newcastle disease (NDV) and avian influenza (AIDV). The present result revealed that the hematocrit level of chicks fed diet containing 400, 600 or 800 mg propolis was significantly ($P \leq 0.05$) higher than that of control-group. With respect to plasma total protein and globulin, it could be speculated that the supplemental propolis at 400, 600 or 800 mg significantly increased plasma total protein and globulin compared to control group, also there was highest antibodies concentration is related to 400, 600 and 800 mg/ kg propolis treatments for AI and ND, but 200 mg/kg propolis were not different from control group. There was no effect of propolis observed on Infectious bronchitis (IB) titration. In conclusion, the propolis may have positive effect on blood biochemical and humoral immunity of broilers.

Keywords; *Propolis, Broiler chicks' nutrition, Hematological, Biochemical Humoral immunity.*

INTRODUCTION

Many countries tend to prohibit use of antibiotics as growth promoters because of their adverse effects as their residual problems in tissues and eggs of birds. Supplementation of natural components in poultry ration is now widely distributed in the world. These components are served as growth promoting, which are healthful and help to improve the production performance of animal and poultry without any harmful effect (El-Ghamry., *et al* 2002 and Abdelsalam *et al.,* 2018). There are considerable reports which confirm the positive effects of natural flavonoids on immune system of different species. These studies are almost focused on antibody synthesis, T lymphocyte stimulation, increasing blood lymphocytes, phagocytosis activity, thymus and bursa of fabricious weight are several factors which have been considered in this relation (Kong *et al.,* 2004). Beginning of the humoral and cellular immune response is mainly related to the cytokines released from activated T cells stimulated by ethanol extract of propolis (Scheller *et al.,* 1988) .

In addition to its immune system booster, propolis is an excellent natural antibiotic (Bratter *et al.,* 1999). The addition of propolis to the diet produce a positive effect on weight gain and improve the digestive utilization of iron and the regeneration efficiency of hemoglobin (Haro *et al.,* 2000). Propolis has a strong anti-bacterial activity, in addition to antifungal, antiviral and antiprotozoal properties (Scheller *et al.,* 1999) Thus, the objective of the present study was to evaluate the effect of extracted propolis on blood parameters and humoral immunity of the broilers.

MATERIALS AND METHODS

The experimental work of the present study was carried out at El-Takamoly Poultry project, Fayoum Governorate**.** A total number of 200 of unsexed one day-old Cobb broiler chicks were used in this experiment; the chicks were grown in floor brooder and were fed on experimental diet without propolis supplementation for one week of age.Chicks were fed a starter diet without propolis supplementation during the first week, at the 7^{th} day of age, birds were fed diets containing different levels of propolis (0, 200, 400, 600 and 800 mg/kg) till the end of 6^{th} weeks of age. Ethanolic extract of propolis was added to mixed diets.

Parameters tested

Hematological and blood biochemical parameters:

At the end of experiment, Blood samples were collected from birds (5 ml from brachial vein). Each blood sample from each individual was divided into two samples, one in a heparinized test tube for blood hematological parameters, and the other in a non-heparinized test tube for biochemical parameters, and then serum was separated by centrifuging at 4000 rpm for 15 minutes. The clear serum samples were carefully drawn and transferred to dry, clean, small glass bottles and stored at -20^{o}C in a deep freezer until the time of chemical determinations. The biochemical characteristics of blood were calorimetrically determined using commercial kits.

Humoral immunity

At 42 days of age, hemagglutination- inhibition (HI) test was applied for determination of antibodies response in plasma samples according to OIE Manual (2005). Commercial kits were used for detection of antibodies against nucleoprotein and matrix against of NDV and AIDV.

Hemagglutination- inhibition (HI) test titter regarded as positive if there is inhibition at serum dilution of 1/16 (4 log2) . as well as titter against IB (infection bronchitis disease) were estimated in the serum of immunized birds using The enzyme-linked immunosorbant assay (ELISA) technique (ELISA is an extremely sensitive test that is used to detect antibodies or specific antigens).

Statistical analysis:

Data were analyzed using general linear model procedure of SPSS software SPSS, (1999). Significant differences among treatment means were determined using Duncan's multiple range test Duncan, (1955) According to the following model:

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

Where; Y_{ij}= Trait measured,, μ = Overall means,, T_i = Treatment effect (i= 1-5), e_{ij} = Experimental error.

RESULTS AND DISCUSSION

Blood parameters:

Hematological parameters:

Effects of experimental diets on broilers' blood parameters are presented in table (1). The hematological parameters tested in current study include red and white blood cell counts, hemoglobin concentration and hematocrit percentage. Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. For example, leucocytes are known to increase sharply when infection occurs, as they are one of the first lines of defense of the body **(Ganong, 1999).**

The hematological values obtained in this study indicated no detrimental impact of Propolis on RBCs, WBCs counts and hemoglobin content. The present result revealed that there was no significant difference between control and 200 mg propolis diet for hematocrit level. Inversely, the hematocrit level of chicks fed

diet containing 400, 600 or 800 mg propolis was significantly (P≤0.05) higher than that of control-group. The higher hematocrit level may have enhanced oxygen delivery to the tissue **(Zongo and Petitjean, 1990).** Also, this increase is supposed to be caused by increased blood volume as a reaction to increasing body oxygen requirement. Reports on the effect of propolis supplementation on blood hematological parameters are very scarce.

Table (1): Hematological parameters (Means ± SE) of birds fed different levels of propolis supplementation.

Item	Propolis (mg/kg diet)					SE
	0	200	400	600	800	
Hemoglobin	10.03	10.15	10.38	9.85	10.23	0.39
RBC	2.73	2.91	2.88	2.72	2.76	0.17
WBC	12.64	13.62	13.73	14.03	13.20	0.39
Hematocrit level,%	36.25^{b}	36.25^{b}	38.75^{a}	39.00^{a}	38.50^{a}	0.70

a,…. b values in the same row within the same item followed by different superscripts are significantly different (at P ≤ 0.05 for a to).

Biochemical parameters:

Effect of supplemental propolis on some blood constituent of broiler chicks are summarized in table (2). With respect to plasma total protein, it could be speculated that the supplemental propolis at 400, 600 or 800 mg significantly (P≤0.01) increased plasma total protein compared to control group. Similar reports were drawn by Giurgea *et al.* (1981). They indicated that daily administration of propolis extract to chickens changed the blood concentration of cholesterol, total proteins and amino acid. Also, Propolis stimulated mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured *in vitro* and it enhanced protein biosynthesis (Gabrys *et al.*, 1986). Similar trend was not observed for albumin, whereas there was no significant difference among treated groups for plasma albumin. Concerning plasma globulin, our result revealed that the plasma globulin was significantly (P≤0.01) increased when the propolis was added to the diet at 400, 600 or 800 mg. The globulins are composed of three fractions, designated alpha, beta and gamma. Alpha-globulins are a group of proteins manufactured almost entirely by the liver. Normally, these proteins increase with acute nephritis, severe active hepatitis, usually systemic inflammation, malnutrition and in nephritic syndromes (Galal *et al.*, 2008). The gammaglobulin fraction contains most of the immuno-proteins, including IgM, IgA, IgE and IgG. These usually elevate with ongoing antigenic stimulation, usually from infectious agents (Galal *et al.*, 2008). In accordance to A/G ratio, Non significant differences have been recorded. But numerical differences were obtained. These findings in turn have influenced the A/G ratio as it is declined in treated groups 400,600 and 800 mg compared to 0 and 200 mg .This reduction may reflect an enhancement of bird's immunity. The A/G ratio has been well known as an indicator for the metabolic activities and immune resistance. In birds, the low A/G ratio indicates more disease resistance and immune response (Griminger, 1986).

With respect to liver function, it could be noticed that supplemental Propolis at 400, 600 and 800 mg significantly reduced ALT (P≤0.01) and AST (P≤0.05) concentration compared to control-group. Hegazi *et al.* (1997) showed that, administration of Egyptian and Bulgarian propolis induces an antibacterial activity *in vivo* as well as *in vitro*. The ethanolic extract of propolis has a weak general effect on estimated parameters in normal rats and it is not a toxic substance. Both types of propolis exerted an anabolic effect for protein synthesis by liver cells. Both types of infections with *S. aureus* and *E. coli* caused an increase in the activity in serum AST & ALT and consequently decrease their activity in the liver. On the other hand, the activity of ALT and AST returned to the control level after administration of propolis in rats infected with *S. aureus* and *E. coli*

Table (2): Biochemical parameters (Means ± SE) of birds fed different levels of propolis supplementation.

Item	Propolis (mg/kg diet)					SE
	0	200	400	600	800	
Total Protein, (g/dl)	2.73^{C}	2.80^{BC}	3.03^{AB}	3.08^{A}	3.25^{A}	0.08
Albumin, (g/dl)	1.63	1.65	1.70	1.73	1.90	0.09
Globulin (g/dl)	1.10^{B}	1.15^{B}	1.33^{A}	1.35^{A}	1.35^{A}	0.05
A/G ratio	1.48	1.45	1.30	1.28	1.42	0.11
ALT,U/L	9.33^{A}	9.17^{A}	7.67^{B}	7.33^{B}	7.67^{B}	0.42
AST, U/L	210.33^{a}	210.50^{a}	192.17^{ab}	190.33^{b}	188.17^{b}	6.01

a, …b, and A,.. C, values in the same row within the same item followed by different superscripts are significantly different (at P ≤ 0.05 for a to b ; P ≤0.01 for A to C).

Humoral immunity:

The results are shown in table (3). As monitored in this Table, there is significant difference (P≤0.01) between antibody content of the serum against avian influenza (AI), and significant difference (P≤0.05) between antibody content of the serum against Newcastle disease (ND). Highest concentration is related to 400, 600 and 800 mg/ kg propolis treatments for AI and ND, but 200 mg/kg propolis were not different from control group. There was no effect of propolis observed on infectious bronchitis (IB) titration. These results indicate that propolis may have positive effect on humoral immunity of broilers.

Stimulation of immune system by natural products has already been reported (Hegazi *et al.,* 1995 and Kong *et al.,* 2004). Not only in broilers, but also in rodents. These effects of propolis have been confirmed (Blonska *et al.,* 2004 and Giurgea *et al.,* 1983). The effect of natural products such as propolis on 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 indirect effect via changing the microbial population of the lumen of GIT. At the moment there is no specific answer to this question, but it is very obvious that propolis is able to enhance the immune response to different antigenic stimulants even in mouse (Scheller *et al.,* 1988). Propolis is a natural product which in numerous experiments has revealed different actions on immune system. For example, increasing the macrophage activity (Dimov *et al.,* 1991), changing microbial populations in the stomach and intestine lumen and stimulating lymphatic tissues (Taheri *et al.,* 2005), increasing the IL 1 (Bratter *et al.,* 1999; Havsteen, 1983; Ivanovska *et al.,* 1995 and Orsolic and Basic, 2003), IL2 (Ivanovska *et al.,* 1995) and IL4 (Park *et al.,* 2004). In this relation increasing the humoral response in broilers might be related to 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 have anti-oxidant (Kumazawa et al., 2004; Nagei *et al.,* 2003 and Russo *et al.,* 2002) and anti-inflammatory (Borrelli et al., 2002 and Dimov et al., 1991) effects, and these are related to inhibition of prostaglandin synthesis (Namgoong *et al.,* 1994 and Toma *et al.,* 1981) as an anti-immune substance and resulting better humoral response. One point which should be mentioned is about the influenza antibody that was raised against natural infection from the environmental serotypes without any vaccination, which the response might be much lower than forced vaccination. It seems interesting to fractionate the propolis and study the effect of each fraction individually, to realize its real action on immune system (Taheri *et al.,* 2005).

Table (3): Antibody titrations against different viruses of birds fed different levels of propolis supplementations

Virus	Propolis (mg/kg diet)					SE
	0	200	400	600	800	
AI*	6.25^{C}	6.25^{C}	7.50^{AB}	8.25^{A}	8.00^{AB}	0.23
ND**	6.25^{b}	6.25^{b}	7.00^{ab}	7.50^{a}	7.50^{a}	0.30
IB***	3771.25	3079.75	3101.50	2951.00	4735.75	545

a,…. b, and A,…C values in the same row within the same item followed by different superscripts are significantly different (at P ≤ 0.05 for a to b ; P ≤ 0.01 for A to C).

** Avian influenza, ** Newcastle disease, *** Infectious bronchitis*

CONCLUSION

Generally, it can be concluded that propolis as a natural feed additive have positive effect on humoral immunity of broilers and blood parameters.

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تأثر مكونات الدم الخلوية والبيوكيميائية والمناعة المصلية باضافة البروبليس في علائق سلالة دجاج الكب

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تم اجراء هذه الدراسة بمشروع الدواجن التكاملى بالعزب – الفيوم فى الفترة من نوفمبر 2010 حتى يناير 2011 وقد اجرى هذا البحث لدراسة تأثير اضافة مستويات مختلفة من البروبوليس (صمغ النحل) الى علائق بدارى التسمين على الاداء الانتاجى والاستجابة المناعية .حيث تم استخدام عدد 200 كتكوت من سلالة كب عمر يوم غير مجنس وقسمت على عمر 7 ايام الى 5 مجموعات تجريبية كل مجموعة 4 مكررات . 10 طائر بكل مكرر وغذيت المجموعات على علائق نباتية مضافا اليها مستخلص البروبوليس بمستويات (0 ، 200 ،400 ، 600 ، 800 ملجم / كجم عليقة). عند عر 42 يوم تم تقدير قاييس الدم الخلوية البيوكيميائية وايضا تم تقدير الاستجابة للاجسام المضاده ضد مرض النيوكاسل ومرض انفلونزا الطيور. اظهرت نتائجنا أن المجموعات المغذاه على 400 ، 600 ، 800 ملجم نتائج معنوية (P0.05≥) كانت أعلي لمستوى الهيماتوكريت مقارنة بمجموعة الكونترول بالنسبة للبروتن للكلي في البلازما, اوضحت النتائج وجود فروق معنوية (P≤0.01) بين المعاملات المختلفة بالنسبة لمستوى كلا من البروتين الكلى والجلوبيولين حيث لوحظ أن المجموعات المغذاه على 600 ، 800 ملجم أعطت أفضل النتائج وايضا من النتائج يتضح أن تغذية بدارى التسمين على علائق تحتوى على البروبوليس بمستويات 600 ، 800 ملجم أدى الى تحسين الاستجابة المناعية ضد كل من فيروس النيوكاسيل (P0.05≥) ، وانفلونزا الطيور(P0.01≥) مقارنة بمجموعة الكنترول فى حين لم يكن هناك أى تأثير على الحالة المناعية ضد فيروس التهاب القصبة الهوائية. في النهاية يكن تلخيص ان اضافة البروبليس له تأثير ايجابي علي مكونات الدم البيوكيميائية والمناعة المصليه.

الكلمات الداله: *بروبليس – تغذية دجاج التسمين – المكونات الخلويه– البيوكيميائيه – المناعة المصليه*