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Effect of Alcoholic Extract Propolis on Immune System in Broiler Chickens

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ABSTRACT

This experiment was done to evaluate the effects of different levels of alcoholic extract propolis on immune system in broiler chickens. In a completely randomized design, 225 one-day old cobb 500 broiler were randomly assigned in 5 dietary treatments containing 0, 150, 300, 450 and 600 mg of alcoholic extract propolis per kilogram of experiment diets. Each dietary treatment was replicated 3 times with 15 birds each. Feed and water were provided ad libitum. In the end of experiment period, two birds of each replicate were randomly selected and the blood samples were taken from wing vein with syringes. Data were pooled and analyzed using the general linear model of SAS, and means were separated by Duncan multiple range test ($p<0/05$). As a result, diet supplementation with alcoholic extract propolis hadn't significantly effects on broiler chickens immune system ($P>0/05$).

KEY WORDS: alcoholic extract propolis, immune system, broiler chickens.

1. INTRODUCTION

In recent years there has been renewed interest in the composition of propolis, a substance that can be regarded as a potential natural source in folk medicine and in the chemical industry. Propolis is a natural resinous substance collected by bees from parts of plants, buds and exudates (Ghisalberti, 1979). Bees use it as a sealer for their hives (Garcia-viguera et al., 1993) and, more importantly, to prevent the decomposition of creatures which have been killed by bees after an invasion of the hive (brumfitt et al., 1990). Propolis is a well known substance that beekeepers find in their hives. There are many factors affecting propolis composition such as collecting location, time and plant source (Markham et al., 1996). Propolis according to research has shown to be effective againsta variety of bacteria (Velikova *et al*., 2000), viruses (Amoros *et al*., 1994), fungi (Murad *et al*., 2002) and molds (Miyataka *et al*., 1997). It has been shown to be a non-specific immunostimulant (Dimov *et al*., 1991). Hegazi *et al.* (1995) studied the effect of some bee products on immune response of chicken infected with virulent NDV. They found that, the mortality rate was reduced in-groups infected with virulent NDV and subsequently treated either with Propolis or honey if compared with the infected groups only. It was clear that, Propolis acts actively as antiviral agent than honey. The treatment with Propolis and honey of NDV infected chicken groups induced increase in the antibody titres and phagocytic percentage. The anti-oxidative, cytostatic, anti-mutagenic and immunomodulatory properties of propolis are based on its rich, flavonoid, phenolic acid and terpenoid contents (Prytzyk et al. 2003; Wang et al. 2004). Over the several last years using the prebiotics, probiotics and natural products is going to be substituted 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. One of the regarded candidates in natural products is flavonoids, which are naturally produced in plants (Hassig *et al*., 1999) and is stored in different forms such as propolis (Giurgea *et al*., 1981). 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 (Toma *et al*., 1981; Giurgea *et al*., 1982; Konig, 1986; Hegazi *et al*., 1995; Kong, 2004). T lymphocyte stimulation, increasing blood lymphocytes, phagocytosis activity, thymus and bursa of fabricious weight are several factors which have been considered in this relation (Giurgea *et al*., 1981; Toma *et al*., 1981; Giurgea *et al*., 1982; Giurgea *et al*., 1984; Konig, 1986; Hegazi *et al*., 1995; 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). There are numerous confirmed studies which believe using propolis or its extracts activate immune system in mouse and human which include; increasing IL-1 (Ivanovska *et al*., 1995; Bratter *et al*., 1999; Orsolic and Basic, 2003), IL-2 (Ivanovska *et al*., 1995; Park *et al*., 2004), IL-4 (Park *et al*., 2004) antibody response (Scheller *et al*., 1988; Park *et al*., 2004), T lymphocyte proliferation, increasing CD+ /CD+ ratio and macrophages activation (Dimov *et al*., 1991; Borrelli *et al*., 2002; Park *et al*., 2004). The objective of the present study was to evaluate the effect of alcoholic extracted propolis on immune system of the broilers which is important in broiler production.

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MATERIALS AND METHODS

In a completely randomized design test 225 one day old broiler chicken (Cobb 500) were divided into 5 treatments with 3 replicates and 15 (male chicken) chickens per cage. Chicken had free access to water and food *ad libitum*. The diets were based on soy bean meal and corn regarding the 1994 Council procedure (NRC, 1994). Diets were formulated as starter, grower and finisher diets (Table 1). Propolis content of the diet were 0 (control), 150, 300, 450 and 600 mg/kg of diet. The propolis was collected from Neyriz township of Fars province in Iran. Hand collected propolis samples were kept desiccated in dark until the processing. Collected propolis was extracted for a week with 100 ml of 70% ethanol at room temperature to obtain the extract. After filtration, the extract was evaporated using a vacuum evaporator at 50 °C and then used in the experiment. In end of experiment two chicks (two male) of each cage were randomly selected and their blood samples blood samples were taken from the brachial vein into heparinized tubes for all birds. However, serum separated and some blood parameters assay including heterophils, lymphocytes, globulin, albumin, monocytes, basophils, eosinophils, WBC and RBC.

Table 1. Ingredients and nutrient Composition of experimental diets (%)

Nutrient (%)	1-10 day	11-24 day	24-42 day
Corn	61.48	65.54	66.90
Soybean meal	34.22	28.72	26.47
Sunflower oil	0.23	1.74	2.84
DCP	1.83	1.80	1.67
Oyster Shell	1.22	1.19	1.13
Salt	0.20	0.20	0.20
Na bicarbonate	0.11	0.07	0.07
Methionine	0.10	0.12	0.11
Lysine	0.11	0.12	0.11
Mineral[1]	0.25	0.25	0.25
Vitamins[1]	0.25	0.25	0.25
Total	100	100	100
ME(kcal/kg)	2894	2987	3176
CP %	20.30	18.30	18

[1]Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12.000 IU; cholecalciferol 1.500 IU; vitamin E, 30 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 µg; Ca-D- panthotenate, 10 mg; folic acid, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg

RESULTS AND DISCUSSION

A comparison of some hematological parameters of chicks fed a diet supplemented with alcoholic extract propolis is presented in Table 2. As monitored in Table 2, there is no significant difference between different levels of alcoholic extract propolis in blood parameters and immune system ($p>0.05$). Higher level of Albumin is in 450 mg alcoholic extract propolis per kg diet group and lower level is 300 mg alcoholic extract propolis per kg diet group. Many reports indicating that alcoholic extract propolis has immunostmulant effect and maintaining good health (Bratter et al., 1999; Dimov et al., 1991 and Giurgea et al., 1982) but we not fount it in this experiment. It was found that the addition of alcoholic extract propolis didn't improve broilers immune system. The analysis of the effect of a diet supplemented with alcoholic extract propolis on erythrocyte parameter showed that the chick fed a diet supplemented with alcoholic extract propolis hadn't a significantly increase in rbc count than those in control group, wheras omar(2002) with diet supplementation to water extract propolis, showed that the Sasso chicks fed a diet supplemented with propolis had a significantly increase in RBC count than those in control group. In our experiment, diet contain 450 mg alcoholic extract propolis per kg diet was higher level in RBC count. With regard to the total leukocytic count, results showed that there was no significant difference in the total leukocytic count between the five groups. The same trend was obtain in the presentages of heterophils, eosinophils and basophils. On other hand, the chicks fed a diet supplemented with propolis showed no significant higher percentage of lymphocytes than those in control group. Moreover, the birds in propolis group had no a significant lower percentage of monocytes than those in control group, but it appear that with increase propolis level, decrease in monocytes percentage than those in control group will be significantly. Concerning the globulins, 450 mg alcoholic extract propolis per kg diet group in between experimental groups was higher levels. Omar (2002) sowed that the improvement of Hb%, PCV%, RBC count, serum protein and its fractions in propolis and Nigeria sativa seed oil may be due to the direct effect on the haemopoietic tissue and the stimulating effect of propolis and Nigeria sativa seed oil to the liver exhibiting an anabolic action favouring protein synthesis on due to their preserving effect to the body protein from degeneration. Effect of a material on synthetic action liver show with Albumin level. Since, there is a toxically

substance in body; serum Albumin level will decrease, because liver found toxically case. The propolis improves the digestive utilization of iron and the regeneration efficiency of hemoglobin and it has a prophylactic immunostimulating effect (Bratter et al., 1999 and Har et al., 2000).

Table 2. Dietary of alcoholic extract propolis on immune system in broiler (mg/kg diet)

Parameters	0	150	300	450	600	SEM	P Value
Albumin(mg/dl)	1.13	1.06	1.03	1.23	1.17	0.05	0.16
Globulin (mg/dl)	2.26	1.87	2.03	2.56	2.20	0.12	0.19
Lymphocyte %	71.66	72.33	71.00	68.00	72.66	2.02	0.52
Monocyte %	4.33	5.66	4.33	3.66	3.66	1.14	0.70
Heterophils %	22.00	23.00	21.00	24.00	20.66	1.2	0.37
Basophils %	0.01	0.00	0.34	0.34	0.67	0.25	0.38
Eosinophils %	2.00	1.66	2.00	1.33	1.66	0.68	0.90
WBC ($\times 10^9$/lit)	10.43	9.93	10.13	10.03	9.86	0.4	0.87
RBC($\times 10^6$/µlit)	2.55	2.48	2.56	2.62	2.58	0.17	0.98

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