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Humoral Immunity of Broilers is Affected by Oil Extracted Propolis (OEP) in the Diet

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Abstract: This study was conducted to determine the effect of different levels of oil extracted propolis (OEP) on humoral immunity of broilers from 1st to 7th week of age. The experiment started with 672 chicken (Ross 308, 336 marked male and female), in a completely randomized design test with 7 treatments, 4 replicates and 24 chicken (12 male, 12 female) per treatment. Chicken received the normal soybean meal-corn diet supplemented with 0 (control), 40, 70, 100, 400, 700 and 1000 mg/kg of OEP. Immunization program included vaccination against infectious bronchitis virus (IB, H_{120} on days one and 8 spray; H_{52} on day 30 drinking), Newcastle disease (ND, B_1, day 10, eye drop; Lasota days 20 and 32 drinking) and infectious bursal disease (BD, or Gumboro, D_{78}, days 12 and 24, eye drop). Blood samples were collected two times on days 21 and 42 of age, via brachial vein from one male and one female of each replicate and plasma was separated by centrifugation. Antibody concentration against IB, ND, BD and avian influenza (AI) were measured by ELISA method. Results indicated that antibody titer against AI, ND and BD were significantly ($P<0.05$) increased with OEP supplementation, without any effect on IB. Relatively negative effect of higher concentration of OEP on humoral immunity of broilers, concluded that broiler's immune system may respond to OEP on a crucial dosage.

Key words: Broiler, immunity, humoral, propolis

Introduction

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 (Croft, 1998; Hassig *et al*., 1999) and is stored in different forms such as propolis (Giurgea *et al*., 1981; Dobrowolski, *et al*., 1991). Propolis or bee glue is collected by bee which is rich in polyphenols, flavonoids, phenolic acids, cafeic acid and their related esters.

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 (Havsteen, 1983; 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^+_4/CD^+_8 ratio and macrophages activation (Kimoto *et al*., 1998; Dimov *et al*., 1991; Borrelli *et al*., 2002; Park *et al*., 2004). The objective of the present study was to evaluate the effect of oil extracted propolis on humoral immune response of the broilers which is important in broiler production.

Materials and Methods

In a completely randomized design test 672 one day old broiler chicken (Ross 308) were divided into 7 treatments with 4 replicates and 24 (12 male and 12 female) chickens per cage. Chicken had free access to water and food *ad labium*. The diets were based on soy-bean corn (Table 1 and 2) regarding the 1994 Council procedure (NRC, 1994). Propolis content of the diet were 0 (control), 40, 70, 100, 400, 700, and 1000 mg/kg of diet. The propolis was collected from Ziaran district of Qazvin province in Iran. The extraction procedure included; dissolving 15 gram of propolis in 85 gram of 85°C heated sunflower oil for 20 minutes, and then filtering the solution through a 1.5 mm sieve and removing non-dissolved solids.

The chicken were immunized against diseases with the

Table 1: Nutrient content of the basal diet over different periods of production

Nutrient (%)	1-21 day	21-42 day	42-49 day
Corn	61	66.23	71
Soya meal	34.45	29	23.83
DCP	1.4	1.4	1.4
Shell fish	1.3	1.3	1.3
Salt	0.2	0.2	0.2
Na bicarbonate	0.28	0.48	0.66
Methionin	0.17	0.19	0.23
Lysine	0	0	0.18
Sunflower oil	0.7	0.7	0.7
Mineral	0.25	0.25	0.25
Vitamins	0.25	0.25	0.25
Total	100	100	100

Table 2: Analysis of the ration over the different periods of production

	1-21 days	21-42 days	42-49 days
Metabolizable energy (Kcal/kg)	2873	2927	2981
Protein (%)	20.44	18.5	16.87
Energy protein ratio	140.55	158.21	176.7
Linoleic acid (%)	1.47	1.57	1.65
Calcium (%)	0.91	0.89	0.88
Available phosphorus (%)		0.39	0.39
Calcium phosphorus ratio	2.2	2.2	2.2
Methionin+Cystein (%)	0.82	0.79	0.78
Lysine (%)	1.08	0.95	0.96
Arginine (%)	1.31	1.16	1.18
Tryptophan (%)	0.41	0.38	0.36
Na (%)	0.17	0.22	0.27
K (%)	0.87	0.78	0.69
Cl (%)	0.17	0.17	0.16
Anion cation balance (meq/kg)	250.9	250.9	249.6

program included vaccination against infectious bronchitis virus (IB, H_{120} on days one and 8 spray; H_{52} on day 30 drinking), Newcastle disease (ND, B_1, day 10, eye drop; Lasota days 20 and 32, drinking) and infectious bursal disease (BD, or Gumboro, D_{78}, days 12 and 24, eye drop), and no vaccination against avian influenza, leaving them for natural contamination and immunization by existing subtypes. On days 21 and 42 two chicks (one male and one female) of each cage were randomly selected and their blood samples were collected via brachial vein. The separated serums by centrifugation (3000 RPM, 10 minutes) were used for antibody titration against Newcastle, influenza, infectious bronchitis and Gumboro (ELISA method) viruses by standard procedures. The data were analyzed by GLM procedure for completely randomized design test with 7 treatment and 4 replicates with using the SAS software (SAS, 1989), and the mean±SE were also compared by Duncan's range test. Because the effect of sex was not significant on any of characterized data it was not included in the model.

Results and Discussion

The results are shown in Table 3. As monitored in this Table there is significant difference ($p<0.05$) between antibody content of the serum against avian influenza (AI), Newcastle disease (ND), and bursal disease (BD). Highest concentration is related to 400, 700 and 1000 mg/ kg propolis treatments for AI, but 40, 70 and 100 mg/kg propolis were not different from control group. Comparing the treatments for antibody against Newcastle and bursal virus on days 21 and 42 revealed that the antibody concentration increases with propolis concentration up to 100 mg/kg and with higher concentration declines ($p<0.05$). There was no effect of propolis observed on 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; Kong *et al*., 2004). Not only in broilers, but also in rodents these effect of propolis has been confirmed (Blonska *et al*., 2004; 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 have revealed different actions on immune system. For example, increasing the macrophage activity (Dimov *et al*., 1991), increasing the IL1 (Bratter *et al*., 1999; Havsteen, 1983; Ivanovska *et al*., 1995; Orsolic and Basic, 2003), IL2 (Ivanovska *et al*., 1995; Park *et al*., 2004) 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; Russo *et al*., 2002) and anti-inflammatory (Borrelli *et al*., 2002; Dimov *et al*., 1991) effects, and these are related to inhibition of prostaglandin synthesis (Namgoong *et al*., 1994; Toma *et al*., 1981) as an anti-immune substance and resulting better humoral response. One point which should be

Table 3: Comparison between antibody titrations against different viruses by ELISA method on 21 and 42 days age of broilers

		Propolis content (mg/kg diet)							
		0	40	70	100	400	700	1000	
Age (day)	Virus								SEM
21	IB	740	800	850	800	720	750	600	140
	ND	560^{c}	640^{c}	1020^{b}	1540^{a}	1100^{b}	800^{bc}	740^{bc}	190
	AI	450^{b}	570^{b}	650^{b}	700b	1200^{ab}	1300^{a}	1400^{a}	250
	BD	800^{b}	1100^{a}	1050^{a}	1200^{a}	750^{b}	1100^{a}	900^{ab}	150
42	IB	650	740	770	800	720	800	700	120
	ND	600^{b}	720^{b}	1100^{ab}	1500^{a}	1050^{ab}	700^{b}	750^{b}	210
	AI	540^{b}	600^{b}	720^{b}	840^{b}	1000^{ab}	1200^{a}	1600^{a}	310
	BD	700^{b}	850^{b}	1050^{a}	1200^{a}	1020^{a}	1000^{ab}	950^{ab}	120

IB (infectious bronchitis), ND (Newcastle disease), AI (avian influenza) and BD (bursal disease or Gumboro).
Different superscript letters in each row indicate significant difference ($p<0.05$)

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 it's real action on immune system.

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