

## Egyptian Propolis 9- Its Effect on Chicken Productivity and Immune Response Against Newcastle Disease Vaccine

<sup>1</sup>Ahmed Hegazi, <sup>2</sup>Amr M. Abdou and <sup>3</sup>Fyrouz Abd Allah

<sup>1</sup>Department of Zoonotic Diseases,

National Research Center, Dokki, Giza, Egypt

<sup>2</sup>Department of Microbiology and Immunology,

National Research Center, Giza, Egypt

<sup>3</sup>Department of Parasitology and Animal Diseases,

National Research Center, Giza, Egypt

**Abstract:** We determined the effect of propolis on immune response and performance status of broiler chicks previously vaccinated with Newcastle disease virus (NDV) vaccine. Our results revealed the growth promoting effect of propolis extract. At the same time propolis extract decreased the mortality rate in chicks throughout the experiment. It was also clear that propolis extract caused an increase in the weight of lymphoid organs of chicks. Furthermore, propolis extract treated group was the highest in the (NDV) antibodies titer (4.9, 6.4 and 7.7) when compared with control group (2.7, 2.2 and 1.9) on 14, 21 and 28 days respectively.

**Key words:** Propolis • Newcastle Disease Virus • Immune Response

### INTRODUCTION

Propolis (bee glue) is a natural dark-colored, resinous sticky substance produced by honeybees by mixing their own waxes with resins collected from plants and is used as a sealant and sterilant in their nests. Propolis has been used since ancient times as a medicine [1, 2] because of its biological properties as an antimicrobial [3, 4] antifungal [5] antiprotzoa, antiparasitic [6] and antiviral agent [7-10]. Its chemical composition includes more than 300 compounds [3,11-13] that induced antioxidant [14-16] hepatoprotective [17] immunostimulating [3, 18-20] and cytostatic effects [21, 13]. The aim of this study was to determine the effect of propolis on immune response and performance status of broiler chicks vaccinated with Newcastle disease virus (NDV).

### MATERIALS AND METHODS

**Propolis:** Propolis material was collected from apiary farm near El-Mansoura City, Dakahlia Province. The resinous materials were kept in dark bag in the refrigerator till being extracted with ethyl alcohol.

**Extraction and Sample Preparation:** One gram of each sample was cut into small pieces and extracted at room temperature with 50 ml of 70% ethanol (twice after 24 hours) according to Hegazi *et al.* [6]. The alcoholic extract was evaporated under vacuum at 50°C until dryness. The percentage of extracted matter was 0.8 gm/dry weight.

**Chicks:** One hundred and twenty commercial broiler chicks (Ross 308), purchased from Agricultural Development Company Ltd. (ADC), were housed in wire cages (60 cm×100 cm) in rooms lighted for 24 h at the beginning of pretrial period. The temperature was gradually declined to the room temperature. Broiler chicken fed commercial ration and water ad libitum and reared under strict hygienic measures.

### Newcastle Disease Virus Vaccines:

- HB1 vaccine: Hebra, batch no.40M/2, 1000 dose.
- La Sota vaccine – Intervet - batch no. 12836KJ01, 1000 doses and titer of  $10^{7.5}$  EID<sub>50</sub> /dose.

**Blood Samples:** Blood samples were drawn by heart puncture into centrifuge tubes and left to clot, for 30 minutes, at room temperature for collection of chicken sera. Sera were kept at -20°C in the deep freezer for analysis.

**Haemagglutinating Antigen:** It was prepared according to methods of Allan *et al.* [22].

**Serum Samples for HI Test:** Serum from blood samples was collected from wing vein, labeled and kept at -20°C till used.

**Chicken Red Blood Cells:** Red blood cells (RBCs) from susceptible adult birds were collected on 4% sodium citrate as anticoagulant. The RBCs were washed three times with phosphate buffered saline (PBS) at PH 7.0 - 7.2.

**Physiological Saline:** Prepared and autoclaved according to Cruickshank *et al.* [23], then stored at 4°C till used.

**Haemagglutination Inhibition (HI) Test:** The test was carried out according to the standard procedure described by Majiyagbe and Hitchner [24]. The end point was estimated according to scheme described by Kaleta and Siegmann [25].

**Determination of Body and Lymphoid Organ Weights:** The body weight and lymphoid organ weight of each broiler chick were determined using digital balance at second and fourth week of the experiment, the mean body weight of each group was measured.

**Experimental Design:** One hundred and twenty broiler chicks (one day old) were randomly distributed. Chicks were divided into three groups. The first group served as normal control. The second group served as control vaccinated group while the third and fourth groups were injected intraperitoneally (IP) 0.1 ml propolis extract day after day till the end of the experiment (28 days). The second and third groups were vaccinated with Hitchener B1 by ocular route at 5 days of age followed by booster dose using la Sota live vaccine at 14 days of age in drinking water. Mortality rate was recorded. Five chicks from each group were sacrificed after 7, 14, 21 and 28 days where serum samples were taken for analysis using HI test for ND antibody titers.

**Statistical Analysis:** The results obtained in the present work are represented as means  $\pm$  standard error and were analyzed using analysis of variance (ANOVA). The significance of difference between means at  $P < 0.05$  was calculated using the Duncan Multiple Range Test [26].

## RESULTS

Data displayed in Table (1) summarized the differences in live body weights between broiler chicks received propolis and vaccinated with NDV vaccines from one day till 28 days of age through the experiment. The results revealed that the growth promoting effect of propolis. Propolis extract and vaccinated with NDV vaccines showed the highest increase in body weight followed by those received NDV vaccines and control negative groups respectively.

Results in Table (2) summarized the differences in mortality rates between broiler chicks in different groups weekly for 4 weeks of age through the experiment. The results indicated that the fewest deaths through all the experimental periods was in propolis group if compared with two other groups.

Data showed in Table (3) summarized the differences in lymphoid organs relative weights between broiler chicks in different groups in second and fourth weeks. It was found that there were no significant differences in lymphoid organ relative weights during the experiment. It was clear that the propolis vaccinated group showed the highest lymphoid organ weight all over the experimental period.

The differences in antibodies titer of broiler chicks in different groups were summarized in Table (4). The results of effect of propolis on (NDV) antibodies titer showed an increase in the HI titer when compared with control group through all the experimental periods. However, propolis treated group was the highest in the (NDV) antibodies titer when compared with control group from the 2<sup>nd</sup> week to the end of experiment.

## DISCUSSION

The differences in live body weights between broiler chicks received propolis and vaccinated with NDV vaccines from one day to 28 days of age through the

Table 1: Average body weight gain / grams of treated and control negative broiler chicken groups

Treatment	Average body weight in grams at days of age			
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Control negative	164	404.2	723	1019
Vaccinated control	166	410.4	739	1038
Propolis	165	418.2	743	1026
Propolis + vaccinated	165	435.2	799.2	1269.68

Table 2: Means of mortality rate (%) for broiler chicks treated and control negative broiler chicken groups.

Treatments	Mortality rate (%)			
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Control negative	0.00 %	3.13 %	3.00 %	2.15 %
Vaccinated control	0.00 %	1.23 %	2.15 %	0.00 %
Propolis	0.00 %	1.22 %	1.95 %	0.00 %
Propolis + vaccinated	0.00 %	0.00 %	1.13 %	0.00 %

Table 3: Means of relative lymphoid organs weights (spleen, thymus, bursa) (gm/100 gm L.B.W.) for broiler chicks treated and control negative broiler chicken groups

Treatments	Experimental periods					
	2 <sup>nd</sup> week			4 <sup>th</sup> week		
	Spleen	Thymus	Bursa	Spleen	Thymus	Bursa
Control negative	0.08	0.10	0.24	0.09	0.10	0.19
Vaccinated control	0.07	0.13	0.17	0.12	0.11	0.23
Propolis	0.08	0.15	0.27	0.16	0.12	0.23
Propolis + vaccinated	0.09	0.18	0.32	0.18	0.14	0.25

Table 4: Determination of logarithmic (log 2) mean antibody titers among sera of chicks vaccinated with NDV vaccine and subsequently treated with propolis

Treatment	Mean HI titer / days		
	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Control negative	2.7	2.2	1.9
Vaccinated control	3.6	5.7	7.1
Propolis	3.9	6.0	7.4
Propolis + vaccinated	4.9	6.4	7.7

experiment were studied. Results revealed the growth promoting effect of propolis. Propolis extract and vaccinated with NDV vaccines has the highest body weight followed by those received NDV vaccines and control negative groups respectively (Table 1). The treatment with propolis in propolis and vaccinated group acted as a growth promotor. The increase of body weight was due to the stimulatory effect of propolis [27, 28]. Hegazi and Abd El Hady [29] found that there was increase in the final chicken weight gain of chicken vaccinated with NDV vaccine and treated with Egyptian propolis. Also, Hegazi *et al.* [30] found that Egyptian propolis increased body weight gain in chicken infected with NDV.

Regarding to the results indicated the fewest deaths through all the experimental period in propolis group if

compared with two other groups which were summarized through the differences in mortality rates between broiler chicks vaccinated only and propolis vaccinated with NDV vaccines as well as control normal chicks weekly for 4 weeks of age through the experiment. This finding was due to the antiviral activity of propolis. This activity is attributed to the flavone and flavonoid constituents of propolis as induced by Serkedjieva *et al.* [31] in influenza virus, [32] in herpes simplex virus, [33] in smallpox, [8, 34-36]. Abd El Hady & Hegazi [37] in NDV and Machado *et al.* [38] in various animal viruses.

The differences in lymphoid organs relative weights between broiler chicks vaccinated only and propolis vaccinated with NDV vaccines as well as control normal chicks weekly for 4 weeks of age through the experiment. It was found that there were no significant differences in

lymphoid organ relative weights during the experiment. It was clear that the propolis vaccinated group showed the highest lymphoid organ weight all over the experimental period. The increase in the lymphoid organs weight (Thymus, spleen bursa) of chicks injected with propolis was due to the action of propolis on cellular element of these organs. Similar results were observed by Hegazi and Abd El Hady [29]. These results agree with the speculation of some authors as Frankiewicz and Scheller [39] who found that propolis increases the T cell and activation of mitosis as well as activation of granulocytes. Giurgea, *et al.* [40] suggested that propolis has anabolic effect and that it also stimulated body immune response. Fan *et al.* [10] demonstrated that EPI (propolis) at high and medium doses could significantly promote lymphocyte proliferation and enlarge immune organ index as compared with model control group.

The result of HI titer to ND vaccine was higher in all vaccinated groups (groups 2 and 3) than negative control group. On the other hand propolis vaccinated group had higher titer than the group received vaccine only. The significant increase of antibody titer in vaccinated chicks and treated with propolis or vaccinated only was due to the ability of propolis to give a chance to immune response. Giurgea *et al.* [40] suggested that propolis had an anabolic effect and also stimulated body immune response. The efficiency of the propolis was to induce significant nonspecific protection and had an immunostimulatory action against vaccination by NDV [29, 31, 41- 44]. Yuan *et al.* [45] showed that three doses of propolis flavonoids liposome (PFL) could significantly enhance antibody titer, concentrations of IgG, IgM and promote lymphocyte proliferation, interferon- $\gamma$  and interleukin-2 secretion. Fan *et al.* [10] demonstrated that EPI (propolis) at high and medium doses could significantly enhance antibody titer and IgG, IgM, IFN- $\gamma$  and IL-6 concentrations, promote lymphocyte proliferation and enlarge immune organ index as compared with model control group.

From over mentioned results, it could be concluded that propolis extract improves poultry productivity, humoral immune response to live Newcastle vaccine and weight gain.

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