



## Immunomodulation of broiler chicks to live NDV vaccine by natural supplements

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### ABSTRACT

This study aims to determine the effect of using propolis and Echinacea Extract as immunomodulators against live Newcastle disease (ND) virus vaccination on broiler chicks. 120 One-day-old broiler Cupp chicks were randomly divided into four groups (30 chicks /group). Group A was not treated with any immunomodulators and used as control, while groups B,C and D were supplemented with propolis in ration 1gm/kg, propolis in water 1gm/liter, and Echinacea extract in water 2cm/liter, respectively. Birds of group B had the highest mean antibody titers against live ND vaccination begins at 21<sup>th</sup> and lasts for 42 days, while birds of group C had the heaviest body weight, followed by group B and D in comparison to the birds of group A. Thus, supplementing immunomodulators as Propolis in diet had a significant positive effect on immune response against live ND vaccination in broiler chickens as well as gain in body weight.

**Keywords:** *immunomodulators, propolis, Broiler chicks, Newcastle live vaccines.*

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### 1. INTRODUCTION

Newcastle Disease (ND) is one of the most economically important avian viral diseases as it causes a lot of losses in poultry industry as well as trade restriction (Zhao et al., 2017). It is an enveloped virus belonged to order *Mononegavirales*, Family *paramyxoviridae*, Subfamily *Paramyxovirinae* and Genus *Avulavirus*. Its genome consists of negative sense single strand RNA genome (Amarasinghe et al., 2017) containing six genes encoding six structural proteins from the 3' to 5', nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and RNA dependent RNA polymerase (L) (Dortmans et al., 2011). ND virus is classified into 2 classes, class I

which is one genotype and isolated from water fowl and shorebirds and class II including 18 genotypes including the virulent and Avirulent vaccine strains Lasota and Hitchener B1 which used as vaccines around the world (Ewies et al., 2017; Susta et al., 2018). ND is notifiable to OIE (OIE 2012). ND is endemic in Egypt since 1960 and still causing a lot of economic losses in many places like Benisuef, Minia, Ismalia and Menofia in broiler (Ewies et al., 2017).

Vaccination is the most important control method and most time effective (Abraham-Oyiguh et al., 2014) but if unperformed, performed incorrectly or performed irregularly will cause a lot of losses. Any vaccine has three

main goals to control viral disease: i) decrease or eliminate clinical signs, ii) decrease the amount of shedding of the virus, iii) increase the infectious dose of the challenge virus (Kapczynski et al., 2013). So, Nowadays the concern to overcome a lot of diseases is the immunomodulators which enhancing the immune system as general to overcome any disease (Shukla et al., 2014). Using natural plant or its extract as immunomodulators having a lot of advantages including safety, lack of side effects, low cost and availability (Sharma et al., 2017).

Propolis is a resinous material that bees collect from different trees (Seven et al., 2010) and use it to seal the pores in the inner side of the hive after mixing it with wax to protect the hive from any pathogenic microorganisms (Simone-Finstrom and Spivak, 2010). Propolis contains a lot of active principles which give it the antimicrobial and immunostimulatory effect like flavonoids, poly phenols, minerals and vitamins (Schnitzler et al., 2010). It was reported that it has antiviral effect against HIV (Harish et al., 1997), Herpesviruses (Yildirim et al., 2016) and influenza viruses (Shimizu et al., 2008) and also has effect against bacteria including staphylococci (Lu et al., 2005), streptococci (Duailibe et al., 2007), E. Coli (Ugur et al., 2000) and K. pneumoniae (Pavilonis et al., 2008) and fungi as candida albicans (de Lima et al., 2018).

Echinacea dry extract is one of the commercial products available in the Egyptian market as immunostimulant for human. Echinacea is perennial plant related to family Asteraceae, the most characteristic feature of this family is it has purple cone flower (Binns et al., 2002). It has been reported that Echinacea has immunomodulatory activity (Turner et al., 2000). It has antioxidant substances which simulate immune system and cytokine production (Mishima et al., 2004), the first antioxidant substance is arabinogalactan which affects tumor necrosis factor (TNF) release

which increases macrophage level, interleukin-1 and interferon beta-2, Heteroxylan which another polysaccharide of Echinacea increases phagocytic activity, Alkyl amide and Chicoric acid glycosides potentiate phagocytosis (Kumar and Ramaiah, 2011). Echinacea also has a role in activation cytotoxic activity of phagocytes against microorganism (Pullaiah, 2006), and it was reported that it has antiviral effect against influenza virus (Hudson, 2012), Herpes simplex virus 1 and 2 (Hudson, 2012), HIV (Birt et al., 2008), Rhinovirus (Hudson, 2012), and against (SARS-CoV) (Hudson, 2012). Our study aimed to determine the effect of using propolis and Echinacea extract as immunomodulators on the immune response of broiler chicks to live ND virus vaccination.

## 2. Materials and methods

### 2.1. Chickens and Experimental design:

A total of 120 one-day-old broiler chicks (male and female cup) were weighed and randomly divided into four groups on arrival. All groups received live ND vaccines; group A is control (vaccinated nontreated birds); group B was treated with Propolis in ration in a dose 1 gm / kg; group C was treated with Propolis in drinking water in a dose 1 gm / liter; group D was treated with Echinacea extract in drinking water in a dose 2 ml / liter along the period of experiment 42 days. Chicks were raised on floor for 6 weeks and had access to feed (Feed Mix, Egypt) and water throughout the entire experimental period. The lighting program was 23h light and 1 h Dark. The temperature was beginning 33°C and decreased gradually 25°C on 21 day and then constant.

### 2.2. Vaccination schedule:

The birds of all groups were vaccinated with live ND virus vaccines via drinking water at 7<sup>th</sup> day of age (HitchnerB1 Vaccine<sup>®</sup>, SerVac, Egypt), 18<sup>th</sup> day of age (Lasota Vaccine<sup>®</sup>, SerVac, Egypt), 28<sup>th</sup> day of age (Lasota<sup>®</sup>, SerVac, Egypt). Both types of vaccines were

used with a titer of  $7 \log_{10}$  embryo infective doses (EID<sub>50</sub>) per dose in drinking water.

### 2.3. Immunomodulators:

#### *Propolis:*

Propolis was obtained kindly from department of plant protection, Faculty of Agriculture-Moshtohor, Benha University (Prof. Dr. Reda Omar). Propolis was kept in refrigerator at 4 °C until used. Extraction and sample preparation was done as one gram of each sample was cut into small pieces and extracted at room temperature with 50 ml of 70% ethanol. The alcoholic extract was evaporated under vacuum at 50°C until dryness. The percentage of extracted matter was 0.8 gm/dry weight (Omar et al., 2014).

#### 2.4. *Echinacea extract:*

The aqueous preparation contains each 100 ml contains Echinacea dry extract 1.67 gram (Squibb's materia medica, 1916).

#### 2.5. *Serum samples:*

Blood samples were collected from the wing veins of 5 birds randomly selected from each treatment at time intervals of the experiment at 0,7,14,21,28,35 and 42 days into tubes without anticoagulant. Blood samples were dated and labeled according to number of chickens and groups then were centrifuged for 30 min. at 1500 rpm to obtain sera. Sera were stored in Cryo tubes at – 20 °C until subjected for hemagglutination Inhibition (HI) test to determine the antibody titer against ND virus following vaccination.

#### 2.6. *Hemagglutination Inhibition (HI) test:*

Serial two-fold dilutions of the collected sera (50 µl) were prepared, then equal volume of 4 HA units of ND virus were prepared and added to each serum dilution. Subsequently, 30 minutes of incubation, 50 µl of 0.5% washed chicken red blood cells (RBCs) was added on each well. The blend was incubated for 30 min at room temperature. Hemagglutination inhibition endpoint (the highest dilution of serum causes complete inhibition of viral hemagglutination) were scored and recorded as

reciprocal  $\log_2$  values of the highest dilution of HI (OIE, 2012).

#### 2.7. *Performance of broiler chickens:*

The experiment period duration was 42 days. On day 0,7,14,21,28,35 and 42 birds were weighed by group for weight measurement, 10 individual birds per each group were weighed, and the mean weight of each group was calculated.

#### 2.8. *Statistical analysis:*

Data were analyzed by One-way ANOVA using SPSS14 and Duncan's multiple range test that were used to compare the means.

## 3. RESULTS

### *Impacts of propolis and echinacea extract on the humoral immune response of broiler chicks against live ND vaccination (table 1):*

Maternal antibodies were high in all groups then after 14 days the antibodies drop and Group B which has given Propolis in diet 1gm/kg along the experimental period has the highest mean antibody titer on 21, 28, 35 and 42 days of the experiment (titers of antibodies measured with HI test were  $1.6 \pm 0.24 \log_2$ ,  $6.2 \pm 0.37 \log_2$ ,  $9.8 \pm 0.97 \log_2$  and  $9.2 \pm 0.49 \log_2$ , respectively) when compared with group C which has given propolis in water 1gm/1liter (titers of antibodies measured with HI test were  $1 \log_2$ ,  $3.8 \log_2$ ,  $6.2 \log_2$  and  $6.2 \log_2$ ), group D which has given Echinacea extract in water 2 ml/1liter (titers of antibodies measured with HI test were  $1.40 \log_2$ ,  $2.80 \log_2$ ,  $4 \log_2$  and  $6.80 \log_2$ ) and group A which was non-treated with immunomodulators (titers of antibodies measured with HI test were  $1.4 \log_2$ ,  $2.2 \log_2$ ,  $4.2 \log_2$  and  $7.2 \log_2$ ).

### *Effects of different immunomodulators on weight gain of broilers at different ages (table 2):*

Group B which has given Propolis in diet 1gm/kg along the experimental period has the highest weight gain on zero, 7, 14, 21, 28, 35 and 42 days of the experiment (body weight

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gain measured with kilo gram were 0.04, 0.25, 0.56, 1.00, 1.50, 1.95 and 2.15 Kg, respectively) when compared with group C which has given propolis in water 1gm/1liter (body weight gain measured with kilo gram were 0.04, 0.20, 0.48, 0.92, 1.49, 2.10 and 2.31 Kg, respectively), group D which has given Echinacea extract in water 2 ml/1liter

(body weight gain measured with kilo gram were 0.04, 0.24, 0.54, 0.96, 1.45, 1.88 and 2.07, respectively) and group A which was non-treated with immunomodulators (body weight gain measured with kilo gram were 0.04, 0.22, 0.49, 0.88, 1.32, 1.72 and 1.89, respectively).

**Table1: Mean log<sub>2</sub> antibody titers against live ND virus using HI test in vaccinated broiler chicks treated with Propolis and Echinacea extract as immunomodulators.**

Chicken group	Mean log <sub>2</sub> antibody titer ± Standard Error against ND virus using HI test / days of treatment						
	Zero day	7 days	14 days	21 days	28 days	35 days	42 days
Group A	9.40 <sup>a</sup> ± 0.24	7.00 <sup>a</sup> ± 0.32	3.80 <sup>ab</sup> ± 0.37	1.40 <sup>a</sup> ± 0.24	2.20 <sup>cd</sup> ± 0.37	4.20 <sup>c</sup> ± 0.37	7.20 <sup>b</sup> ± 0.37
Group B	9.60 <sup>a</sup> ± 0.24	6.20 <sup>a</sup> ± 0.37	4.80 <sup>a</sup> ± 0.20	1.60 <sup>a</sup> ± 0.24	6.20 <sup>a</sup> ± 0.37	9.80 <sup>a</sup> ± 0.97	9.20 <sup>a</sup> ± 0.49
Group C	9.60 <sup>a</sup> ± 0.24	6.20 <sup>a</sup> ± 0.20	3.60 <sup>b</sup> ± 0.51	1.00 <sup>a</sup> ± 0.00	3.80 <sup>b</sup> ± 0.37	6.20 <sup>b</sup> ± 0.20	6.20 <sup>b</sup> ± 0.58
Group D	9.40 <sup>a</sup> ± 0.24	7.00 <sup>a</sup> ± 0.32	3.80 <sup>ab</sup> ± 0.37	1.40 <sup>a</sup> ± 0.40	2.80 <sup>bc</sup> ± 0.58	4.00 <sup>c</sup> ± 0.32	6.80 <sup>b</sup> ± 0.92

Group A was not treated with any immunomodulators and used as a control; Group B was supplemented with propolis in diet; Group C was supplemented with propolis in water; Group D was supplemented with Echinacea with water.

Results are expressed as mean ± S.E.M. Different superscripts (a,b,c) within the same rows indicate significant differences at P ≤ 0.05.

**Table 2: Average body weights of broiler chickens treated with Propolis and Echinacea extract as immunomodulators.**

Chicken group	Mean body/Kg ± Standard Error / days of treatment						
	Zero day	7 days	14 days	21 days	28 days	35 days	42 days
Group A	0.04 <sup>a</sup> ±0.00	0.22 <sup>ab</sup> ±0.01	0.49 <sup>ab</sup> ±0.02	0.88 <sup>b</sup> ±0.04	1.32 <sup>b</sup> ±0.06	1.72 <sup>c</sup> ±0.08	1.89 <sup>c</sup> ±0.09
Group B	0.04 <sup>a</sup> ±0.00	0.25 <sup>a</sup> ±0.01	0.56 <sup>a</sup> ±0.02	1.00 <sup>a</sup> ±0.03	1.50 <sup>a</sup> ±0.05	1.95 <sup>ab</sup> ±0.06	2.15 <sup>ab</sup> ±0.07
Group C	0.04 <sup>a</sup> ±0.00	0.20 <sup>b</sup> ±0.02	0.48 <sup>b</sup> ±0.03	0.92 <sup>ab</sup> ±0.04	1.49 <sup>a</sup> ±0.07	2.10 <sup>a</sup> ±0.04	2.31 <sup>a</sup> ±0.05
Group D	0.04 <sup>a</sup> ±0.00	0.24 <sup>a</sup> ±0.01	0.54 <sup>ab</sup> ±0.01	0.96 <sup>ab</sup> ±0.03	1.45 <sup>ab</sup> ±0.04	1.88 <sup>bc</sup> ±0.05	2.07 <sup>bc</sup> ±0.06

Group A was not treated with any immunomodulators and used as a control; Group B was supplemented with propolis in diet; Group C was supplemented with propolis in water; Group D was supplemented with Echinacea drinking water.

Results are expressed as mean ± S.E.M. Different superscripts (a,b,c) within the same rows indicate significant differences at P ≤ 0.05.

**4. DISCUSSION**

Our study revealed that firstly maternal antibodies were high in all chick groups then after 14 days the antibodies drop. Results showed the priority of group B chicks (given propolis in diet 1gm/kg) that has higher mean

serum antibody titers (1.6 log<sub>2</sub>, 6.2 log<sub>2</sub>, 9.8 log<sub>2</sub> and 9.2 log<sub>2</sub> on 21, 28, 35 and 42 days of the experiment, respectively) when compared with group C chicks (given propolis in water 1gm/liter) showed antibody titers 1 log<sub>2</sub>, 3.8 log<sub>2</sub>, 6.2 log<sub>2</sub> and 6.2 log<sub>2</sub>) and group A chicks

(non-treated with immunomodulators) that showed antibody titers  $1.4 \log_2$ ,  $2.2 \log_2$ ,  $4.2 \log_2$  and  $7.2 \log_2$ . These results came in agreement with that showed addition of propolis at 100 mg/kg in poultry diet was an effective immunopotentiator for cell-mediated responses during the starter phase (Eyng et al., 2013). Broilers receiving propolis 0.7, 0.8 and 0.9 g/kg of diet have serum IgG and IgM concentration higher than control birds (Zafarnejad et al., 2017); it also result in increasing the relative weight of bursa and spleen in comparison to the control group that may attributed to chemical constituents of propolis such as benzene or flavonoids which cause a greater immune response because increases the phagocytic, Macrophage, more cytokines which result in proliferation of other immune cells T and B cells. Also, the ability of propolis in increasing the levels of antibodies may be due to several effects it may be due to increase expression of IL-2 and interferon- $\gamma$  which stimulate antibodies production in chicks (Khan, 2017), or due to increasing weight of bursa and spleen which are the sites of B and T cells differentiation. Propolis supplementation causing increase in antibody response to Newcastle disease virus, avian influenza and infectious bursal disease, there is a negative effect of high concentration of propolis on the humoral immunity of broilers against Newcastle and bursal virus on days 21 and 42 the antibody concentration increased with a dose of propolis up to 100 mg/kg but the antibody concentration declined when the dose of propolis increased (Taheri et al., 2005; Ziaran et al., 2005). Propolis also has effect on other species (Babaei et al., 2016) stated that 1.0 gm/kg propolis causing increase in ND antibody titer when compared with control group in Quail.

Regarding group D chicks which has given Echinacea extract in water 2 ml/1liter, showed no significant changes in titers of antibodies measured with HI test ( $1.40 \log_2$ ,

$2.80 \log_2$ ,  $4.00 \log_2$  and  $6.80 \log_2$ ) in comparison to group A chicks non-treated with immunomodulators ( $1.4 \log_2$ ,  $2.2 \log_2$ ,  $4.2 \log_2$  and  $7.2 \log_2$ ). These results disagreed with the studies proved that Echinacea contain polysaccharides which effect on immune system like Arabinogalactan which activates cytotoxic activity of phagocytes. It also induces tumor necrosis factor (TNF) which increases macrophage level, interlekin-1 and interferon beta-2. Heteroxylyan is other polysaccharides from Echinacea that increase phagocytic activity (Pullaiah, 2006). Alkylamide and Chicoric acid glycosides also enhance phagocytosis (Kumar and Ramaiah, 2011). Schraner et al., (1989) studied the effect of the complex drug (Influx) and Echinacea angustifolia extract in humoral immune response of intact and immunodeficient chickens administered in two oral doses. It induced a rise in the serum immunoglobulin concentration, as well as increase in the three classes of antibody. In immunodeficient chickens, the complex drug caused a slight production of IgG. Intermittent application of Echinacea purpurea in feed of layer for 2 days (12 days interval) resulted in highest antibody titers and increased number of lymphocytes and total leucocytes but reduced phagocytic activity in groups receiving for 5 days (Böhmer et al., 2008). Respecting the effect on body weight of the broiler chicken our result revealed that group C which had given propolis in water 1gm/liter had a significant effect on the live body weight of broilers table (2) (2.31kg) followed by propolis in diet group B (2.15 kg), then group D Echinacea (2.07kg) in comparison with group A control (1.89kg), Many scientists have suggested that addition of propolis in broiler chickens feed causing increase in the growth performance (Shalmany and Shivazad, 2006; Seven et al., 2010; Tekeli et al., 2010). In Experiment, broilers fed with 0.1% propolis result in increasing Body weight (BW) by

2.03% when compared with the control group (Zhi-jiang et al., 2004). Different experiments with different supplementation levels of propolis / kg broiler's diet like 0.05g (Kleczek et al., 2014), 0.25g (Roodsari et al., 2004), 0.5 and 1.5 g (Abbas, 2014), 0.6 to 0.9 g (Zafarnejad, et al., 2017), 3.0g (Hosseini et al., 2016) and 5.0g (Seven et al., 2010) had proved that propolis greatly improved BW, BWG and feed conversion ratios (FCR). Other study also reported that propolis has been greatly improved the BW and BWG of broiler chickens when feeding propolis at 0.05, 0.10, 0.15, 0.20, 0.25, 0.5, 1.5 and 2.0 g/kg diet (Shalmany and Shivazad, 2006; Shaddel-Tili et al., 2016). Other species like Japanese quail also had improved body weight when fed with 1.0g propolis/kg diet between 14 to 35 days of age (Denli et al., 2005), this effect of propolis on weight may be due to its rich composition in vitamins, Enzymes and minerals (Lotfy, 2006; Kurek-Gorecka et al., 2014), also containing Flavonoides, Cinnamic acid, amino acids and fatty acids (Wagh, 2013). Propolis also had effect on normal gastrointestinal microflora which enhancing the beneficial bacteria and decreasing the pathogenic ones (Kačániová et al., 2012). I think that variation in results with propolis may be due to difference in the origin, dose, duration, extraction, method of administration, viruses and species of animals.

Echinacea were administered through drinking water (Nasir and Grashorn, 2007a,b and 2008) and maintained meat quality during storage (Koreleski and Swiatkiewicz, 2007).

Considering effect of Echinacea on body weight gain, better feed conversion ratio was observed in treated group as compared to control but its application at the rate of 0.25 ml/kg BW<sup>0.75</sup> resulted in nonsignificant improvement of feed conversion ratio and performance (Nasir and Grashorn, 2007b, 2008).

The variation in results between supplementation of propolis in diet and water and Echinacea extract in water may be attributed to its palatability and the differences in studies may be due to difference in the origin, dose, duration, extraction, method of administration, viruses and animals. Finally, it is concluded that supplementing propolis as immunomodulator in diet had a significant positive effect on immune response against live ND vaccination in broiler chickens as well as on body weight gain.

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