

## Impact of *Ocimum basilicum* leaves powder on immune response of chicken vaccinated against Newcastle Disease Virus

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### Abstract

In this research, we study the effect of adding the *Ocimum basilicum* leaves powder (OBLP), as broilers chicken feed additive and study its effects on the innate and adaptive immune responses as well as the antioxidant effects in broilers vaccinated with Newcastle disease virus (NDV) vaccine. A total of 100 one-day old broiler were randomly divided into 4 equal groups, the first group (G1) was served as normal control group, the second group (G2) was served as vaccination control group. Groups 3 (G3) and 4 (G4) fed 0.5% and 1% OBLP in diets, respectively, and vaccinated against NDV. The obtained results showed that the adding of OBLP to diets improved the broilers innate immune response which resulted in significant increases in peripheral blood monocyte cells phagocytic activity, lysozyme levels and lymphoid organs / body weight ratios. Adaptive immune response has also been improved as the hemagglutination inhibition (HI) antibody titers against NDV vaccine in G3 and G4 reached the peak earlier, declined slowly and gave significantly higher titer in G4 than other groups at the end of the experiment. The antioxidant effects of OBLP were evident particularly by using high concentration of OBLP as the serum catalase enzyme (CAT) levels were significantly increased whereas malondialdehyde (MDA) were significantly reduced. It could be concluded that the use of OBLP as a feed additive can boost the innate and adaptive immune response to NDV vaccination and improve the antioxidant activity of broilers in a dose dependent manner.

**Keyword:** Ocimum, Newcactle, innate, adaptive, immunity.

### Introduction

In Egypt, poultry sector is the most important industry due to it provides the greatest part of meat production and job opportunities for approximately 3 million people (Sabry, 2019). Newcastle disease (ND) is considered as one of the most important infectious diseases that affects poultry causing major economic losses in the poultry industry due to high mortality rate, decrease in egg production and cost of treatment (Musa, et al.,2010). It is classified as a List A by the World Organization for Animal Health (OIE) (3). In Egypt, NDV was first recorded in 1948 (Daubney and Mansy, 1948), then the virus become endemic in Egypt causing several outbreaks until now (Osman, et al., 2014, Mohamed, et al., 2011). Prevention and control of NDV depend mainly on the vaccination and application of biosecurity measures

(Alexander, 2000). There are two main types of vaccines used against NDV: live vaccines and inactivated vaccines. The live vaccine is more effective than the inactivated vaccine, antigenic similar, better immunity and decrease virus shedding (Dimitrov *et al.*, 2017; Perozo *et al.*, 2012, Marangon and Busani, 2007; Miller *et al.*, 2009). On the other hand, several outbreaks were reported in Egypt in the vaccinated flocks due to viral mutation in the antigenic site or improper vaccination, lack of biosecurity measures (Kattenbelt, *et al.*, 2006; Ke, *et al.*, 2001) and reduced immune responses (Arivuchelvan *et al.*, 2012). The immuno-suppressed flocks may have poor response to commonly used vaccines (Sharma *et al.*, 2000), therefore the application of immunostimulants is an essential requirement to improve the immunity of broilers.

The immunostimulant substances are agents innately non-specific in nature as they envisage enhancing body's resistance against infections. They are anticipated to function as prophylactic and promoter agents by augmenting elementary level of immune response. Immunostimulants can act through innate and adaptive immune response (Alfons and Patrick, 2001). Numerous medicinal plants displaying immunomodulatory activity have been used because of their sufficient absorption and ability to reach the objective organ without much degradation by digestive enzymes and low toxicity for the host system (Arivuchelvan *et al.*, 2012). Also, the therapeutic uses of plant are safe, economical and effective (Kumar *et al.*, 2011).

Among the plants known for medicinal importance, the plants of genus *Ocimum* belonging to family Lamiaceae are very important for their therapeutic potentials (Kadian and Parle., 2012). *Ocimum basilicum* (OB), is commonly grow in tropical and warm temperate regions of the world. It is known as Sweet Basil and also named as Rehan (Adam and Omer, 2015). It is classified taxonomically as, Kingdom: Plantae, Phylum: Magnoliophyta, Class: Magnoliopsida, Order: Lamiales, Family: Lamiaceae, Genus: *Ocimum*, Species: *basilicum* (Khair *et al.*, 2012). Many chemical components are isolated from the OB plant including flavonoids, alkaloids, tannins, terpenoids, glycosides, saponin and ascorbic acid. The *Ocimum basilicum* leaves extracts have potent antioxidant, anti-aging, anticancer, antiviral, antimicrobial properties, immunostimulatory, hepatoprotective, immunomodulatory, antihyperglycemic, hypolipidemic, antitoxic and anti-inflammatory effects (Chiang *et al.*, 2005, Bozin *et al.*, 2006, Manosroi *et al.*, 2006, Almeida *et al.*, 2007, Khair *et al.*, 2012 and Zahid *et al.*, 2017).

Little information is available regarding the effect of OBLP as feed additive on the immune status of broiler chickens in response to Newcastle disease vaccination. Hence, the present study was designed to evaluate the effect of the OBLP feed additive on broiler innate and adaptive immune responses to ND vaccination, as well as its effect on final body weight, lymphoid organ/ body weight ratio and antioxidant activity.

## Materials and methods

**Plant materials:** Dried OB leaves were purchased from Unit of Aromatic and Medicinal Plants, Faculty of Pharmacy, Cairo University. Then the leaves were crushed manually to make it powder.

### **Phytochemical analysis of some constituents of dried *Ocimum basilicum* leaves powder:**

A weight of 10 g sample was extracted twice with 70 mL of Dimethyl sulfoxide (DMSO) for three times. The combined extracts were evaporated to dryness in a rotary evaporator at room temperature. The precipitates were weighed, dissolved in DMSO, and kept in the dark at 4°C for further analysis.

### **Determination of Total Phenolic Content:**

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method as described previously (Medini *et al.*, 2014). The total phenolic content was expressed as mg of gallic acid equivalent (mg GAE/g) per gram sample.

### **Determination of Total Flavonoid Content.**

Total flavonoid content was determined using a colorimetric method described by Abu-Bakar *et al.* (2009). Results were expressed as milligrams of quercetin equivalent in 1 mL of sample (mg QE mL<sup>-1</sup>).

**Extraction of volatile oil** The dried OBLP was subjected to hydro distillation using a Clevenger-type apparatus for 3 hours to extract the oils. The obtained oils were decanted and dried over anhydrous sodium sulfate then stored in sealed vial in a refrigerator (6°C) before being analyzed. Gas Chromatography analysis (GC) of the oils was performed on a Hewlett-Packard Gas Chromatograph (GC 5890 II; Hewlett-Packard GmbH, Bad Homburg, Germany) and identification of the components of the volatile oils was based on the comparison of their spectral data and retention indices with Wiley Registry of Mass Spectral Data 8<sup>th</sup> edition. (Adams, 2007).

### **Chemicals and Biological Reagents:**

*Micrococcus lysodeikticus* bacteria, agarose, and Roswell Park Memorial Institute media 1640 media (RPMI) with L-Glutamine, fetal calf serum (FCS) were obtained from Sigma-Aldrich Co. Germany. *Candida albicans* (*C. albicans*) kindly supplied by Department of Mycology, (AHRI).

**Experimental Design:** One hundred, 1-day-old broiler chicks of both sexes (Hubbard local breed) were obtained from a local hatchery and divided into 4 groups, 25 chicks each: Group (1): Chicks fed normal diet and kept as normal control. Group (2): Chicks fed normal diet and kept as vaccination control. Group (3): Chicks fed normal diet supplemented with 0.5 % OBLP (low dose). Group (4): Chicks fed normal diet supplemented with 1 % OBLP (high dose). Groups 2, 3 and 4 were vaccinated via drinking by Hitchner B1 NDV –IB bivalent

vaccine at 7<sup>th</sup> day of age, then by LaSota NDV vaccine at 21<sup>st</sup> day of age with the recommended doses. All broiler chicks were offered feed and water ad libitum during the experimental period. The composition and calculated analysis of the basal diets Ingredients are illustrated in Table-1, which were formulated according to the National Research Council (NRC, 1994). All experimental procedures involving chickens were conducted following the animal ethics guidelines and approved protocols of Animal Health Research Institute, Dokki, Giza, Egypt.

**Table 1: Diet ingredients and chemical composition of basal diets:**

<b>Ingredients %</b>	<b>Starter</b>	<b>Grower</b>	<b>Finisher</b>
Yellow corn	52.05	53.95	57.25
Soybean meal (44%)	32.00	30.00	28.00
Corn gluten meal (60%)	9.00	7.00	5.00
Vegetable oil	2.50	5.00	6.00
Limestone	1.10	1.10	1.10
Dicalcium phosphate	2.10	1.80	1.70
Vitamin and Mineral mix*	0.30	0.30	0.30
Salt	0.35	0.35	0.35
L-Lysine HCl	0.35	0.25	0.15
DL-Methionine	0.25	0.25	0.15
Total	100	100	100
<b>Calculated Composition %**</b>			
Crude protein	24.20	22.15	20.15
ME (kcal kg-1)	3030	3190	3260
Lysine	1.43	1.27	1.10
Methionine	0.67	0.64	0.50
Methionine + Cystine	1.07	1.02	0.84
Calcium	0.99	0.91	0.88
Nonphytate P	0.55	0.47	0.44

\* Each 3 g of vitamins and mineral premix contained Vitamin A: 12000 IU, Vitamin D<sub>3</sub> : 2200 IU, Vitamin E: 10 mg, Vitamin B<sub>1</sub> :1mg, Vitamin B<sub>2</sub>: 4 mg, Vitamin B<sub>6</sub> :1.5 mg, Vitamin B<sub>12</sub>: 0.01 mg, Vitamin K<sub>3</sub>: 2 mg, Choline: 500 mg, Pantothenic acid: 10 mg, Folic acid: 1mg, Biotin: 0.05 mg, Niacin: 20mg, Manganese: 55 mg, Zinc: 50 mg, Iron: 30 mg, Iodine: 1mg, Copper: 10 mg and Magnesium and Selenium: 0.1 mg.\*\*Calculated values based on feed composition Tables of **NRC (1994)**.

**Blood Samples:** Heparinized blood samples were taken at 1<sup>st</sup> week post 1<sup>st</sup> vaccination and at 1<sup>st</sup> and 3<sup>rd</sup> weeks post 2<sup>nd</sup> vaccination (1<sup>st</sup> and 3<sup>rd</sup> WP2<sup>nd</sup> V) for separation of mononuclear cells used in assay of phagocytosis. Blood samples for serum separation were taken from all groups (8 samples /group) at 0 time (7 day of age) then weekly to measure NDV HI antibody titers and lysozyme activity. Catalase (CAT) and malondialdehyde (MDA) were measured in serum samples taken at the 3<sup>rd</sup> week post the 2<sup>nd</sup> dose vaccination.

## Evaluation of Innate Immunity

**Phagocytic activity:** measurement of phagocytic activity of chicken peripheral monocytes was carried out by consuming *C. albicans* according to (Boyum, 1968), Anthony *et al.* (1985) and Chu and Dietert (1989). Briefly, heparinized blood samples were collected from chickens, diluted with sterile phosphate buffer saline (PBS), layered on lymphocyte separation medium and centrifuged at 2400 rpm for 30 min at 4°C. The formed layer between the plasma and the lymphocyte separation medium was aspirated, washed 3 times and re-suspended in 20% FCS in RPMI. The cells concentration was adjusted to  $1 \times 10^7$  viable cells/ml, located in cell culture and staining chamber with sterile rounded cover, incubated for 1 h at 37 °C in 5% CO<sub>2</sub> incubator. The adherent monocyte cells were incubated with 1 ml of 20% FCS in RPMI for 24 h then washed 3 times. One ml volume of *C. albicans* ( $1 \times 10^6$ ) suspension was added to the chambers and incubated at 37 °C in a humidified CO<sub>2</sub> incubator for 1 h. Finally, the cover slips were washed, and stained with Giemsa stain. One hundred phagocytic cells were counted under the oil immersion lens to calculate the phagocytic percent and index by the following equations :-

Phagocytosis percent = No. of phagocytic macrophages X100 / Total No. of macrophages including non-phagocytic macrophages.

Phagocytic index = No. of engulfed *C.albicans* spores / Total No. of phagocytic macrophages cells

**Lysozyme Assay:** Lysozyme activity of broilers sera was estimated by agarose gel plate lyses assay following Peeters and Vantrappen (1977). Briefly, *Micrococcus lysodeikticus* (50 mg) was dispersed in 100 ml of 1% agarose in 0.06 m PBS (pH 6.3). Then the agarose was allocated in petri dishe plates in which 25 µl of serum samples, as well as, the standard lysozyme were tested in separate wells. After 18 hours, the diameter of cleared zones were measured. The lysozyme concentration was calculated from logarithmic curve prepared using standard lysozyme solutions.

## Evaluation of Humeral Immune Response:

Detection of NDV HI antibody titers was done by performing the HI test with chicken red blood cells by Plate HA. Test was done to measure the 1HA unit. Dispense the 25 µl of PBS into each well and then 25 µl of virus suspension (i.e. infective allantoic fluid) then twofold dilutions of 25 µl volumes of the virus suspension were done across the plate. Then a volume of 25 µl of 1% (v/v) chicken RBCs was dispensed to each well. Mixing was done by tapping the plate gently and then the RBCs were allowed to settle for about 40 minutes at room temperature. HA is determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and calculated accurately from the initial range of dilutions then measured 4HAU. (OIE, 2018). HI test was done by putting 0.025 ml of PBS into each well of a plastic V-bottomed micro- titer plate then 0.025 ml of serum were added in the first well of the plate and twofold dilutions of 0.025 ml of the serum

were put in the plate then virus/antigen was added in conc. 4 HAU in 0.025 ml to each well and incubated for 30 minutes at 20°C then chicken 1%RBCs was added in 0.025 ml in each well, incubated at room temperature for 40 minutes to settle RBCs. plates were tilted to read. The HI titer is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The negative control well containing 0.025 ml RBCs and 0.05 ml PBS only be considered to show inhibition. (OIE, 2018).

#### **Measurement of lymphoid organs /body weight ratios:**

The living broiler chicks from all groups were weighted at the end of the experiment then the weights of bursa of fabricious, spleen, and thymus were detected after slaughter of the broilers and the lymphoid organs to body weight ratios were calculated by the equation: organ weight in grams x 1000 / final body weight in grams (Sharma *et al.*, 1989).

**Estimation of catalase enzyme:** The levels of CAT enzyme were estimated at the end of the experiment in serum samples of all groups by using colorimetric method according to Aebi (1984).

**Estimation of Malondialdehyde level:** MDA levels were estimated at the end of the experiment in serum samples by using colorimetric method according to Ohkawa *et al.*, (1979).

**Statistical Analysis:** The obtained results were statistically analyzed using IBM SPSS. Results were expressed as the mean values  $\pm$  standard error (SE) and compared by oneway ANOVA ( $P \leq 0.05$ ).

## **Results**

### **Results of the phytochemical analysis of some constituents of dried *Ocimum basilicum* leaves powder:**

The concentration of total phenolics and flavonoids were illustrated in table (2) and the concentration of the different volatile oil of OBLP were shown in table (3).

### **Effect of the dietary supplementation of *Ocimum basilicum* leaves powder on phagocytic activity of broilers peripheral blood monocyctic cells:**

The results of phagocytosis assay of broilers peripheral blood monocyctic cells illustrated in Table-4, indicated that, supplementation of high concentration of OBLP (G4) induced significant increase of the phagocytic parameters (percent and index) compared to the other groups at most of the time intervals. The effect of OBLP on phagocytic assay was dose dependent in most of the experimental time intervals.

**Table 2: Concentration of total phenolics and flavonoids compound in *Ocimum basilicum* leaves powder**

Test	Results
Total phenolics (mg GAE/g)	39.62±0.25
Total flavonoids (mg QE/ml)	3.84±0.13

mg GAE /g : mg of gallic acid equivalent/ gram of sample. Mg QE /ml : mg of quercetin equivalent in 1 mL of sample.

**Table 3: Concentration of the different volatile oil of *Ocimum basilicum* leaves powder**

Volatile compounds	Concentration	Retention index
1,8- Cineole	5.21 <sup>a</sup>	1035
Linalool	25.23	1096
γ- Terpineol	6.03	1152
Eugenol	2.82	1358
β – Farnesene	7.42	1442
α –Bisabolol	4.80	1681

<sup>a</sup> Values are expressed as relative area percentage to the total identified volatile compounds on DB-5 column (5% phenyl-methyl polysiloxane).

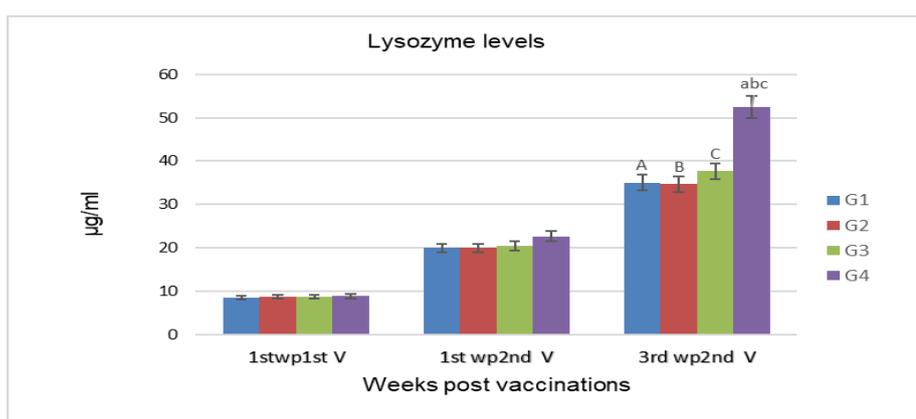
### **The levels of serum lysozyme of broiler chickens fed on ration supplemented with *Ocimum basilicum* leaves powder.**

The results of lysozyme assay (Figure1) Showed higher values in G 3 and G4 compared to other groups in a dose dependent manner. At 1 % concentration (G4), OBLP induced significantly higher values at 3<sup>rd</sup> WP 2<sup>nd</sup> V.

**Table 4: Phagocytic % and index of broilers peripheral blood monocytes.**

Phagocytic parameter	Times post vaccination	Groups			
		G1	G2	G3	G4
Phagocytic %	1 <sup>st</sup> WP1 <sup>st</sup> V	44.25 ± 0.63 A	40.5 ± 1.66 B	43.25 ± 0.53 C	48.25 ± 0.84 abc
	1 <sup>st</sup> WP2 <sup>nd</sup> V	61.5 ± 0.87 A	55.75 ± 1.71 Ba	59.75 ± 1.03 bC	62.25 ± 0.25 bc
	3 <sup>rd</sup> WP2 <sup>nd</sup> V	52.33 ± 5.36	50.67 ± 5.36 A	51.0 ± 3.46 B	58.33 ± 5.04 ab
Phagocytic index	1 <sup>st</sup> WP1 <sup>st</sup> V	0.56 ± 0.037 A	0.55 ± 0.035 B	0.62 ± 0.036 C	0.75 ± 0.012 abc
	1 <sup>st</sup> WP2 <sup>nd</sup> V	1.07 ± 0.065 A	0.98 ± 0.012 B	1.05 ± 0.047 C	1.35 ± 0.053 abc
	3 <sup>rd</sup> WP2 <sup>nd</sup> V	1.0 ± 0.07	0.88 ± 0.20	1.02 ± 0.11	0.96 ± 0.07

G 1: Control group non vaccinated and fed on ration free from OBLP. G 2: Vaccinated group with ND vaccines and fed on ration free from OBLP. G 3: Vaccinated group with ND vaccines and fed on ration contain 0.5% OBLP. G 4: Vaccinated group with ND vaccines and fed on ration contain 1% OBLP. Means with capital letters show significantly different against the small of the same litter in the same row ( $P \leq 0.05$ ). Values represent means  $\pm$  SE.

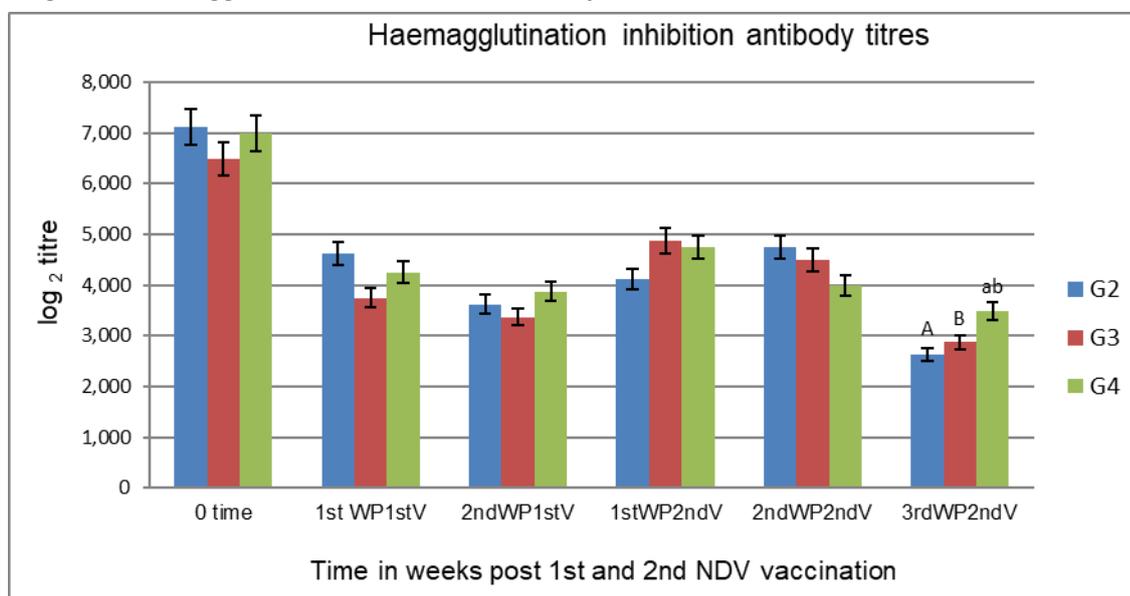
**Figure 1: Effect of the low and high concentration of *Ocimum basilicum* leaves powder on lysozyme ( $\mu\text{g/ml}$ ) in sera of broiler chickens.**

Columns with capital letters indicate significantly different against the small of the same litter on columns of the same time ( $P \leq 0.05$ ). The significant highest lysozyme value was recorded in G 4 (1% OBLP) at the end of the experiment.

### Effect of the dietary supplementation of *Ocimum basilicum* leaves powder on hemagglutination inhibition antibody Log<sub>2</sub> titers in sera of broiler chickens:

The humoral immune response against NDV vaccine was determined in broiler sera by HI test (figure 2). HI maternal derived antibody titers in G1 (control group) showed gradual decrease to negligible values at 28<sup>th</sup> day of age. In all vaccinated groups (G2, 3 and 4), HI antibody titers decreased gradually in 1<sup>st</sup> WP1<sup>st</sup> V and 2<sup>nd</sup> WP1<sup>st</sup> V (7<sup>th</sup> and 14<sup>th</sup> day of age) then began to rise again after 2<sup>nd</sup> vaccination and reached the peak earlier at 1<sup>st</sup> WP 2<sup>nd</sup> V in OBLP groups (G3 and G4) then decline slowly and gradually in relative to G2. Whereas G4 induced significant elevation in HI antibody titers compared to groups G2 and G3 at the last week of the experiment. While in control vaccinated group (G2) HI titers reached the peak at 2<sup>nd</sup> WP 2<sup>nd</sup> V then decline sharply at 3<sup>rd</sup> WP2<sup>nd</sup> V.

**Figure 2: Hemagglutination inhibition antibody titers in sera of broiler chickens**



Columns with capital letters indicate significantly difference against the small of the same letter on columns of the same time ( $P \leq 0.05$ ).

### Results of Lymphoid organs /body weight ratios and final body weight.

The obtained results (table 5) revealed that, high concentration of OBLP (G4) caused higher ratios than other groups. And there were significant increases in bursa of fabricious, spleen and thymus body weight ratios in G4 compared to other groups.

The mean of broiler final body weight before slaughter ranged from 1840 to 1973 grams without significant differences between groups and it decreased with the increases of OBLP concentrations.

**Table 5: Lymphoid organs /body weight ratios and the average final body weight in grams.**

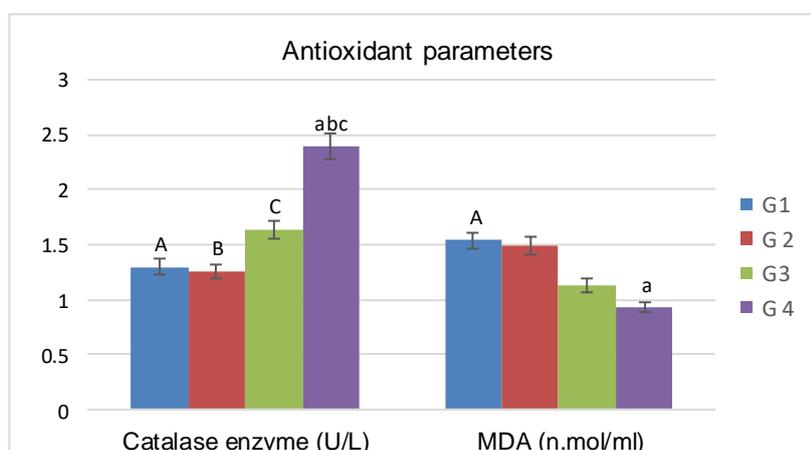
Times post vaccination	Groups			
	G1	G2	G3	G4
Bursa	1.31 ± 0.36 A	1.12 ± 0.29 B	2.6 ± 0.8	3.5 ± 0.25 ab
Spleen	1.76 ± 0.38 A	1.73 ± 0.11 B	1.78 ± 0.14 C	2.69 ± 0.25 abc
Thymus	3.3 ± 0.70	2.56 ± 0.67 A	2.82 ± 0.37	3.52 ± 0.39 a
Average body weight	1973 ± 112	1963 ± 64	1903 ± 52	1840 ± 32

Means with capital letters indicate significantly different against the small of the same litter in the same row ( $P \leq 0.05$ ). Values represent means  $\pm$  SE.

### The results of dietary supplementation of *Ocimum basilicum* leaves powder on Catalase and Malondialdehyde in sera of broiler chickens

Results of feed supplementation of both concentrations of OBLP (Figure 3) indicated that, both concentrations have good antioxidant effects as the values of CAT enzyme were increased in G3 and G4. It was clear that G4 (high concentration of OBLP) gave significant increase compared to other groups.

The results of MDA assay revealed that G3 and G4 showed lower values compared to other groups and the lowering was significant between G4 and control group (G1).

**Figure 3: Levels of Catalase (U/L) and Malondialdehyde (MDA) (n.mol/l) in sera of broiler chicken**

Columns with capital letters indicate significantly different against the small of the same litter on columns of the same antioxidant parameters ( $P \leq 0.05$ ).

### Discussion

The ND is one of the most important respiratory diseases affecting the poultry industry and represents a real challenge to the Egyptian poultry industry (Musa, *et al*, 2010). The prevention and control of NDV depend mainly on vaccination, even with this preventive measure ND outbreak are still known to occur in the vaccinated flocks in Egypt hence we suggest improving the immunity with the vaccination program. Lentogenic Newcastle disease (ND) virus-strains most commonly used as vaccine strains, the Hitchner B I and the LaSota are the

best known. Voeten *et al.* (1977) reported that one of the lentogenic ND-vaccines was remarkable for its long elution time so we use the most applicable and protective vaccine program in the field by using Hitchner B1 NDV –IB bivalent vaccine at 7th day of age, then by LaSota NDV vaccine at 21st.

In this study, we attempted to investigate the effect of OBLP as broiler chickens feed additive, on the immune responses and antioxidant activity of broilers in response to NDV vaccination.

To evaluate the effect of OBLP on the innate immune response, peripheral blood monocytes phagocytic assay and serum lysozyme activity were examined.

Phagocytic cells such as macrophages play an essential role in host immune system and contribute to the nonspecific (innate immunity) defense through phagocytosis and secretion of lysozyme, anti- and pro-inflammatory cytokines (Nagashima *et al.*, 1996). Help also to initiate the specific defense mechanisms (adaptive immunity) by recruiting other immune cells including lymphocytes (Hirayama *et al.*, 2017). The obtained results of the phagocytosis assay revealed that, OBLP in high concentration (G4) induced significant higher values than other groups in the phagocytic percent and indexes of the broilers macrophages. These results agree with previous findings which recorded that, addition of OBLP or seed oils to broiler basal diet, increased the phagocytic index and activity of the macrophages and it could be used safely as a natural growth promoters to improve growth and immune response of broiler chick (ELnaggar and El-Tahawy, 2018). Also, oral administration of aqueous and ethanolic leaves extracts of OB to mice was found to evoke a significant increase in the carbon clearance index in mononuclear macrophage which indicated the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity (Dashputre and Naikwade, 2010)

Lysozymes are vital components of the innate immune system of birds that elaborated from polymorph nuclear and mononuclear cells and hydrolyze peptidoglycan, the main bacterial cell wall constituent. (Moore *et al.*, 2006). Because of the immune stimulatory effect of OBLP, the level of lysozyme was obviously increased by supplementing OBLP particularly in high concentration (G4) at 3rd WP 2nd V compared to the other groups. These results come in accordance with Mohamad and Abasali (2010), who found that OB extract in diet mixture enhanced lysozyme activity in common carp and resulting in a marked augmentation in the immune system of fish to avoid and control microbial diseases.

Concerning to the effect of OBLP on broiler adaptive immune response, the humeral antibody response was tested in sera of broiler chicken by measurement of HI antibody titer values against NDV vaccine. The humoral immunity involves interaction of B cells with the antigen and their consequent proliferation and differentiation into antibody-secreting plasma cells (Neelam and Nilofer, 2010). Whereas the level of serum antibody titers can exactly reflect the humoral immunity efficacy which can neutralize and exclude extracellular microbes and microbial toxins (Abbas and Lichtman, 2004).

The obtained results of measurement of HI antibody titers revealed that the using of OBLP at both concentration induced an improvement in the HI antibody titers and reached the peak earlier than G2 (vaccination control) at 1st WP 2nd V then decline slowly than G2 until the end of the experiment whereas the high concentration of OBLP (1% OBLP) induced significant higher HI antibody titers relative to groups G2 (vaccination control) and G3 (0.5% OBLP). However, the maternal HI antibody titers showed high levels at beginning of the experiment and decreased gradually to negligible levels at 28 day of age.

These results agreed with that of Zahid et al., (2017), who found that supplementation of OB seeds in broiler diets increased significantly serum HI antibody titers against ND and IB viruses at 6th weeks of age. Also, ELnaggar and El-Tahawy (2018), found that, diet with different levels of sweet basil, thyme and their oils fed to broiler chick significantly increased total protein,  $\gamma$ -globulin, globulin, IgM, IgG.

Regarding the effect of OBLP on the lymphoid organ body weight ratios and the final body weight, it was observed that, the bursa, spleen and thymus body weight ratio were improved significantly by supplementation of high dose of OBLP (G4). These data come in accordance with Ahmed et al., (2015), who concluded that *Ocimum* seeds had immune stimulant effects on broiler chicks as it was capable to improve the bursa, spleen and thymus gland relative weight. However, the current study did not evoke any significant differences in final body weight at slaughter age by adding OBLP. While the broilers weight were decreased by increasing the OBLP percent in the food (0.5% and 1%), these results are in agreement with those of Gurbuz1 and Ismael (2016), who found numerical decrease in the final weight by increasing the percent of the OBLP in the diet (0.5% ,1% and 1.5%). This decreasing in body weight may be due to the presence of a large number of poly phenolic and flavonoids compounds in the OB (Chen et al., 2015) which act as anti-hyperglycemic and hypolipidemic effects which may accompanied by decrease in body weight (Zeggeevagh et al., 2007 and Arfa and Rashed, 2008).

Oxidative stress is the major cause of increased incidence of infectious and metabolic diseases in poultry, which can be minimized by the use of anti-stress such as the antioxidant compounds (Vara Prasad Reddy et al., 2009). Antioxidants are substances that protect the living cells against oxidative damage caused by the formation of free radicals and reactive oxygen species during metabolic activity (Nantitanon et al., 2007). Catalase enzyme is one of the antioxidant defense mechanisms enzymes that elaborate from cells and control the damage effects of reactive oxygen species by the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Goyal and Basak, 2010 and He et al., 2017). MDA is the principal final product of lipid peroxidation and has been often used for determining oxidative damage which is indicated by increase its level (Ciftci et al., 2010).

The results obtained from the measurement of CAT and MDA enzymes indicated that both OBLP concentration (0.5% and 1%) induced a potent antioxidant effect specially in G4 (OBLP high concentration) where, the CAT level increased significantly and MDA decreased significantly. Similar observation were recorded by Kahilo et al., (2015) who concluded that feeding of OBLP to broilers was found to have good antioxidant and immunostimulant effects

against the effects of gibberellic acid and auxin. Also, the addition of OB seeds to broiler could increase the CAT and decrease the MDA in thymus, bursa, and spleen (Ahmed et al., 2015).

The immunostimulatory and antioxidant effects reported in the current study can be attributed to the presence of various phytochemical active compounds in the plant as evidenced by the phytochemical analysis performed in the current study. The obtained data indicated that the OBLP contain high amount of total phenolics and flavonoids compounds in concentration of 39.62 mg GAE/g and 3.84 mg QE/ml respectively and some of the predominant components of the volatile oil were, linalool, farnesene, terpineol, cineole, bisabolol and eugenol in concentration of 5.21, 25.23, 6.03, 2.82, 7.42 and 4.8 respectively. These results showed some variation in the types and concentrations of the chemical compounds than the previously reported by many researchers (Ma and Le, 2019 and Gebrehiwot et al., 2015), which can be largely attributed to the differences in experimental conditions, age, plant genetic difference, local climate and seasonal variations.

The phytochemical compounds in OB have been found to augment the immunostimulant activity on specific and nonspecific levels (Nahak and Sahu, 2014). Where its flavonoids have been able to modulate TLR-mediated signaling pathways and induces the expression of many immune and inflammatory genes by stimulating the nuclear factor  $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinases (MAPKs) (McGettrick and O'Neill 2010). Furthermore, some of OB constituents such as caffeic and p-coumaric acid have been previously found to stimulate DNA synthesis of PBM and increase lymphocyte proliferation which was correlated to the increase of the cell fractions of total B and activated T cells (Tsai et al., 2011). Whereas the antioxidant activities of OB were correlated to the redox properties of their phenolic compounds, which can perform an essential role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides ( Osawa , 1994 and Liu et al., 2008). In addition, the presence of other antioxidant secondary metabolites, such as carotenoids and vitamins can potentiate the antioxidant activity of OB plants (Rice-Evans et al., 1995).

In conclusion, this study provides evidence that the adding of OBLP to broiler chickens ration has beneficial effects on the innate immune response that reflected as significant increases in peripheral blood monocyte cells phagocytic activity, lysozyme levels and lymphoid organs / body weight ratios especially when using 1% OBLP.

Adding of OBLP can improve the adaptive immune response where the HI antibodies to NDV vaccines were reached the peak earlier and declined slowly. The use of 1% OBLP increased significantly HI antibodies to NDV vaccines at the end of the experiment. OBLP administration can improve the anti-oxidative activity of broiler chickens and the high concentration of OBLP increased significantly the CAT and decreased significantly MDA enzyme. It was clear that the adding of 1% OBLP to broiler ration could be more advantageous than 0.5% and could be used in areas with a high risk of viral infection as it will help in maximizing the benefits of vaccines.

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