



**ASSESSMENT OF WATER ADMINISTRATION OF BACTERIAL IMMUNOSTIMULANT (BRONCHO-VAXOM®) ON PERFORMANCE, BLOOD BIOCHEMICAL, ANTIOXIDATIVE STATUS AND HUMORAL IMMUNE RESPONSE OF GROWING AND LAYING JAPANESE QUAIL**

**Abdel-Moneim Eid Abdel-Moneim<sup>\*1</sup>, E. M. Sabic<sup>1</sup>, A. M. Abu-Taleb<sup>1</sup>, N. S. Ibrahim<sup>1</sup>**

<sup>1</sup>Biolog. App. Dept., Nuclear Res. Cent., Atomic Energy Authority, Abou-Zabael 13759, Egypt

**\*Corresponding author:** Abdel-Moneim Eid Email : [aeabdelmoneim@gmail.com](mailto:aeabdelmoneim@gmail.com)

Received: 07/12/2019

Accepted: 14 /01/2020

**ABSTRACT:** A straight run experiment was used to investigate the effects of water administration of lyophilized bacterial lysates Broncho-Vaxom® on performance, blood biochemical, antioxidative status and humoral immune response of growing and laying Japanese quail. At the fattening trial, 400 -21-day-old quails were allotted equally into two experimental groups (eight replicates each); the first served as a control and the second received 3.5g Broncho-Vaxom®/ 4L drinking water. The same arrangement was sustained in the laying trial except the reduction of initial number to 160 female quails in each group. No significant alternations were observed in growth performance traits at the end of fattening trial. However, prolongation of treatment time revealed the enhancement effect of Broncho-Vaxom® on final body weight, ovary index and egg production performance. Yolk (%) and yolk-to-albumin ratio were significantly increased, while, albumin (%), egg shape index and Haugh unit were decreased in Broncho-Vaxom® group. Serum liver enzymes and uric acid levels were reduced, while serum concentrations of T<sub>3</sub> and HDL-cholesterol were elevated in Broncho-Vaxom® group at the end of growing trial. None of the aforementioned parameters were affected by the treatment at the end of laying trial. Antioxidant status was significantly improved only at 42 days of age, while total antibodies against-NDV and IgG were improved while IgM was not affected at 42 and 98 days of age. In conclusion, administration of bacterial immunostimulant Broncho-Vaxom® enhanced laying productive performance, egg quality, antioxidant status and humoral immune response of Japanese quail at the end of growing and laying periods.

**Key words:** Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail

## **INTRODUCTION**

Immunomodulatory represents a highly active area in the field of scientific medical and veterinary research. Immunomodifiers could be defined as substances or agents that can affect the responsiveness of the immune system either negatively (immunosuppression) or positively (immunostimulation) (Hadden, 1993). The ideal immunostimulant is a safe substance (recombinant, synthetic or natural) which has the ability to increase the total activity of a healthy immune system as well as restore the function of an impaired one (immunorestitution) (Eichelberg and Schmutzler, 1983). Unlike adjuvants, immunostimulants do not need to be co-administrated with antigens. Classification of immunostimulants may be according to their origin, chemical, nature, mode of action and identification of use in the veterinary practice where safety considerations are less strict. Thus, wider range of immunostimulants could be used particularly in animals' food or water aiming to enhance protection against infective pathogens to augment vaccination programs to substitute banned antibiotic feed additives and to maintain high feed efficiency and growth parameters (Raslan, 2012). Poultry model is an ideal representative for the animals that have high economic importance and in the same time subjected to stress factors associated with the current intensive poultry production systems such as over crowding, contagious infections, nutritional imbalances, climatic and environmental factors which all have a direct or indirect immunosuppressive effect on the bird immune system. Hence effective immunostimulation can help the immune system of such birds

to overcome immunosuppression or even to improve the health condition of normal birds (Raslan, 2012). Vaccination against viral diseases has become a routine practice in poultry industry to avoid heavy economic losses caused by viral infections such as infectious bursal disease virus (IBDV) and Newcastle disease virus. Unfortunately, vaccination itself may become a considerable immunosuppressive stress factor either during the process of vaccination (depending on the route of administration) or because of direct damaging effect on immune system of vaccinated birds in particular the bursal damage associated with IBDV vaccines (Faragher et al., 1972).

Broncho-Vaxom® is a lyophilized bacterial lysate prepared from eight various species of bacteria frequently related to respiratory diseases. Specific and unspecific cell-mediated immune response in man kind are induced by this bacterial preparation (Clot and Andary, 1980). Moreover, Broncho-Vaxom® triggers the production of secretory IgM and IgG in serum and of IgA in saliva in healthy volunteers (Puigdollers et al., 1980). Furthermore, orally application of Broncho-Vaxom® in animal models significantly diminished the infection rate of salmonella and influenza virus (H1N1) by non-specific activation of immunoreactive cells (Bessler et al., 2010), and engaged in the induction of the formation and activation of FoxP3+ T-cell (Navarro et al., 2011). In rat model, regulation of immunomodulatory effect of Broncho-Vaxom® may be also conducted by the elevation of Th-1 cytokines in favour of Th-2 cytokines (Bowman and Holt, 2001). Furthermore, Broncho-Vaxom® reduced mice response to allergens by lessening the expression

## **Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

of the high affinity IgE receptor (Huber et al., 2005; Han et al., 2014). In allergic patients, this latter impact was confirmed, where Broncho-Vaxom® significantly decreased the circulating IgE level in patients with allergies (Emmerich et al., 1990; Antonova et al., 2008).

So far, to our knowledge no trials have been conducted in poultry using Broncho-Vaxom®. The present study aimed to evaluate the effect Broncho-Vaxom® on performance, blood biochemical, antioxidant status and humoral immune response of growing and laying Japanese quail birds.

### **MATERIALS AND METHODS**

The bird management followed the regulations of the Animal Care and Ethics Committee at Nuclear Research Center, Atomic Energy Authority, Egypt.

#### **Experimental design, husbandry and diets**

In a straight run experimental design, water supplementation of Broncho-Vaxom® at concentration of 3.5 g/ 4 L drinking water was tested from 21 to 98 days of age. Broncho-Vaxom® was produced by Memphis For Pharmaceuticals and Chemical Industries, Cairo, Egypt under licence from OM PHARMA, Meyrin, Switzerland. Each 3.5 g of Broncho-Vaxom® contain lyophilized bacterial lysates of *Haemophilus influenza*, *Diplococcus pneumoniae*, *Klebsiella pneumonia*, *Klebsiella ozaenae*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus viridians*, *Neisseria catarrhalis*, glutamate (E621), indigotine (coloring gent, E132), propyl gallate (an antioxidant, E310) and other adjuvants. Experimental diets in mash form were offered during the growing and laying periods (21-42 and 43- 98 days of age, respectively) are presented in Table 1.

Feed were formulated to meet the nutrient requirements of Japanese quail (NRC, 1994) and were available with water *ad libitum*. At the fattening trial, 400 Japanese quail birds with approximately the same weight were allotted equally into two experimental groups (eight replicates each) the first group serves as a control and the second groups received the respective dose of Broncho-Vaxom®. The same arrangement was sustained in the laying trial except the reduction of the initial number of to 80 female quails in each experimental group. Quails were housed in battery brooders (100 cm L x 60 cm W x 25 H cm) during the fattening period and wire cages (50 cm L x 45 cm W x 23 H cm) during the laying period. Quails were housed in room temperature maintained at 27°C during the 3<sup>rd</sup> week of age, and reduced by 3°C per week till reach 24°C. A photoperiod cycle was 23L:1D up to 42 days of age then gradually adjusted to reach 16L:8D during the laying period.

#### **Productive performance**

Initial and final body weight were recorded during each experimental period. Egg weight, to the nearest 0.1 g, and egg number were recorded daily to calculate daily egg mass (egg number × average egg weight) and hen-day egg production percentage.

#### **Egg quality criteria**

Thirteen eggs per group were collected at the end of laying trial and used to determine egg quality according to Abd El-Moneim and Sabic (2019).

#### **Carcass traits**

At 42 and 98 d of age, 8 quails per group were starved for 12 hr, weighed and slaughtered by the Islamic method then defeathered. Dressed weight was determined by weighing the carcass without the head, shanks, viscera, heart,

liver, digestive tract, spleen and abdominal fat. Dressing percentage was calculated using the following equation (dressed weight/live weight  $\times$  100). The liver, heart, empty gizzard, proventriculus, intestine, bursa (birds of fattening trial only), ovary and oviduct (birds of laying trial only) and spleen were weighed for each individual and their percentages to live body weight were calculated. Carcass yield was calculated according to Abd El-Moneim and Sabic (2019).

#### **Blood biochemical traits**

At slaughtering, blood samples were collected from eight quails/group at 42 and 98 days of age. The unheparinized blood samples were centrifuged at 4500 rpm for 15 min to obtain sera. Serum concentrations of total protein, albumin, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analyzed using spectrophotometer (Spectronic 1201, Milton Roy, Ivyland, PA, USA) using commercial kits (Spinreact Co., Girona, Spain) according to the manufacturer's instructions. The reduced glutathione (GSH) and malondialdehyde (MDA) contents, and glutathione peroxidase (GPx) activity in the serum were analyzed using commercial kits (Cell Biolabs Inc., San Diego, CA, USA). Serum concentration of triiodothyronine (T3) was measured in all blood samples using radioimmunoassay (RIA) kits.

Regarding humoral immune response, eight birds from each group were randomly chosen, separately housed in cages and eye dropped with live lasota strain of Newcastle disease virus (NDV)

(Live Lasota strain; KBNP, Inc.; Hungnam, Korea). Blood samples were collected during slaughtering from each bird at 7 days post-immunization, allowed to clot and then centrifuged immediately to separate serum samples which were stored at -80 °C until the determination of the total primary antibody titer against NDV by the haemagglutination inhibition test using ELISA test kit (FLOCKTYPE recNDV, Labor Diagnostik, Leipzig, Germany)(Allan and Gough, 1974). Sera samples then were analyzed for mercaptoethanol-resistant (MER-IgG) and mercaptoethanol-sensitive (Presumably IgM) anti-NDV antibodies after their heat-inactivation at 56°C for half hours described by Qureshi and Havenstein (1994).

#### **Statistical analysis**

Collected data were analyzed by student's t-test procedure of the statistical software SPSS (ver. 18.0; IBM Corp., Armonk, NY, USA). All the percentages were adjusted to normalize data distribution. Statistically differences were observed at a significance level of  $P < 0.05$ .

## **RESULTS**

#### **Productive performance**

Data presented in Table (2) showed that the water treatment with Broncho-Vaxom® did not affect the final body weight (FBW) of growing Japanese quail at 42 day of age, while FBW of laying Japanese quail at 98 day of age was significantly increased. Dressing percentage, relative weight of heart, gizzard, liver, proventriculus, intestine, bursa, spleen and oviduct and carcass yield were not significantly altered. Relative weight of ovary was increased significantly at the end of laying trial in response to the treatment with Broncho-Vaxom®. This increase in ovary weight was associated with the elevation in egg

## **Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

production where egg mass, egg weight and hen-day egg production were significantly increased during all experimental periods (8-12, 12-16 and 8-16 weeks of age) (Table 3).

### **Egg quality criteria**

Egg quality traits were greatly affected by the water supplementation of Broncho-Vaxom® at the end of laying trial (Table 3). Yolk (%) and yolk-to-albumin ratio were increased significantly, while, albumin (%), egg shape index and Haugh unit score were reduced compared with control group.

### **Blood biochemical indices**

As presented in Table (4), blood biochemical indices were varied in their response to the treatment with Broncho-Vaxom® at the end of growing and laying experiment. Serum levels of AST, ALT, ALP and uric acid were significantly reduced in treated group compared with the control. However, serum T<sub>3</sub> and HDL-cholesterol concentrations were elevated at the end of the fattening period. Nevertheless, all the abovementioned parameters were insignificantly affected with the administration of Broncho-Vaxom® at the end of laying trial except serum level of ALT.

### **Antioxidant status and humoral immune response**

In the growing experiment the antioxidant status and humoral immune response were greatly enhanced in the treated group. Serum content of GSH and GPx activity were significantly elevated while serum content of MDA was reduced compared with the control group. Total antibody titer against NDV and IgG were significantly increased in the Broncho-Vaxom® treated group while serum concentration of IgM was not altered. However, at the end of laying trial GSH

and GPx were numerically increased and MDA was numerically decreased in treated group compared with the control. Furthermore, Total antibody titer against NDV and IgG were significantly increased while IgM was numerically elevated in the Broncho-Vaxom® treated group compared to the control group during this experimental period.

### **DISCUSSION**

To our knowledge, no previous studies were conducted to investigate the effect of Broncho-Vaxom® administration in poultry productive performance, antioxidant defense system and immune response. We attempted in the present study to shed light on the role of Broncho-Vaxom® as a potential immunostimulant in poultry production sector. Broncho-Vaxom® is fundamentally consisted of three constituents' namely bacterial lysates, glutamate and propyl gallate. Bacterial lysates were considered as effective inducers of host specific immune response that stimulate the production of antibodies against antigenic structure of bacteria (Braido et al., 2007). Glutamate is an essential amino acid plays a significant role in neurotransmission (Zhou and Danbolt, 2014), preservation of gut integrity (Larson et al., 2007), stimulation of gut-associated lymphatic tissue production (Alverdy et al., 1992) and proliferation, migration and maturation of crypt cells (Wu et al., 2011). The previous properties indicate the potential growth enhancing impacts of glutamate for poultry. Additionally, Propyl gallate is a polyphenolic antioxidant extensively used in the prevention of oxidative deterioration in human and animal food because its ability to chelate iron ion (catalyzer in the oxidation process) and to enhance vegetable oil stability during

storage (Lu et al., 2014). Obviously, these ingredients are responsible for giving Broncho-Vaxom® its distinctive properties and ability to improve production and immune response of birds. However, our results suggested that the beneficial effect of Broncho-Vaxom® may depend on the treatment duration. During the fattening period no alternations in final body weight and all carcass traits were observed, whereas, when the duration was extended to the end of the laying experiment, significant increases in final body weight and ovary relative weight were recorded. Egg weight, mass and production, yolk (%), and yolk-to-albumin ratio were also elevated significantly in Broncho-Vaxom® treated group while albumin (%), egg shape index and Haugh unit were decreased. Our results are in close agreement with other reports used other immunostimulants like Natstim® (Oblakova et al., 2015), Levamisole® (Miran et al., 2010) and  $\beta$ -glucan (Moon et al., 2016).

It is likely that the beneficial effects of Broncho-Vaxom® constituents may also have reduced the microbial burden on the liver and kidneys of birds through the activation of innate and acquired immune systems (Braido et al., 2007; Cazzola et al., 2012) in addition to antibacterial properties of propyl gallate (Daniel, 1986; Kubo et al., 2001; Medina et al., 2013). In the present study we noticed a significant in activities of liver enzymes and uric acid value in the treatment group at 42 days of age. Serum lipid profile of birds administrated with Broncho-Vaxom® was mostly not altered except significant elevation in HDL compared with untreated group. The previous observations indicated that water administration of Broncho-Vaxom® did not exert

physiological stress on the function of body's vital organs and blood homeostasis.

The significant reduction in serum MDA content and increased GSH and GPx levels are outstanding indications for the enhancement in host's antioxidant defense system. This improvement in quail antioxidant defense system may be attributed to the role of Broncho-Vaxom®, or at least one of its components, as an antioxidant agent. Propylgallate, like other phenolic antioxidants, is a chain breaking antioxidant that transfers a hydrogen atom from its hydroxyl group to lipid peroxy radicals or lipid radicals in order to inhibit lipid peroxidation (Medina et al., 2013). Gallates were considered as effective antioxidant because during lipid peroxidation process lipid radicals effectively bind to gallate semiquinone free radicals forming structures exhibited relatively high stability (Terao et al., 1994; Miran et al., 2010). These roles of propyl gallate improve cellular defense system against free radicals and reactive oxygen species and eliminate the oxidative degradation of lipids in cell membrane.

The concentrations of immunoglobulins in the serum were expected to be greater in the Broncho-Vaxom® treated group than the control. The remarkable ability of Broncho-Vaxom® to activate immune cells in each of innate and adaptive immune response attributed to its content of bacterial lysates and their derivatives. Structures belonging to bacterial antigens (e.g., lipopolysaccharide or peptidoglycan) are specific directed to some receptor structures (e.g., toll like receptors) expressed in monocytic-macrophage membrane which cause activation of resting monocytes.

## **Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

---

Consequently, activated monocytes differentiate to immature dendritic cells which in turn mature and form professional antigen presenting cells (Braido et al., 2007). Presenting mature dendritic cells of bacterial antigens results in the activation of B lymphocytes with a consequent maturation of plasma cells and secretion of specific antibodies directed to the administered antigens. Mature dendritic cells also stimulate the T lymphocyte compartment with a following induction of tremendous helper function (Braido et al., 2007; Cazzola et al., 2012). Supplementation of Broncho-Vaxom® bacterial lysates in quail drinking water is able to induce production powerful T helper lymphocytes, maturation of specific B lymphocytes resulting in the secretion of antibodies and activation of monocytic-macrophagic cells of the sub-mucosa.

### **CONCLUSION**

Water administration of lyophilized bacterial lysates Broncho-Vaxom® did

not affect quail growth performance during the fattening period, however, laying productive performance and egg quality were significantly affected. Our results suggested that the stimulating effects of Broncho-Vaxom® may depend on the treatment duration for healthy birds or higher doses are needed. Antioxidant defense system of treated quails was improved and circulating immunoglobulins were significantly elevated. However, further investigations are needed to clear the effects of Broncho-Vaxom® on healthy and challenged birds.

### **Conflict of interest**

The authors declare that they have no conflict of interest.

### **ACKNOWLEDGMENT**

The authors acknowledge the Biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority for their cooperation.

**Table (1):** Composition and calculated analysis of experimental diets of Japanese quail birds

| Item                                    | The basal diets |        |
|---|-----------------|--------|
|   | Growing         | Laying |
| <b>Ingredients, %</b>                   |                 |        |
| yellow maize                            | 50.00           | 53.88  |
| soybean meal (44%)                      | 44.29           | 34.50  |
| dicalcium phosphate                     | 0.80            | 1.20   |
| Limestone                               | 1.30            | 5.70   |
| sodium chloride                         | 0.30            | 0.30   |
| vitamin-mineralpremix <sup>1</sup>      | 0.30            | 0.30   |
| dl-methionine                           | 0.11            | 0.12   |
| soybean oil                             | 2.90            | 4.00   |
| <b>Calculated values<sup>2</sup>, %</b> |                 |        |
| crude protein                           | 24.00           | 20.04  |
| metabolizable energy (ME) MJ/kg         | 12.18           | 12.20  |
| crude fibre                             | 4.20            | 3.60   |
| Lysine                                  | 1.42            | 1.14   |
| Methionine                              | 0.50            | 0.45   |
| methionine + cysteine                   | 0.88            | 0.80   |
| calcium                                 | 0.81            | 2.51   |
| available phosphorus                    | 0.31            | 0.36   |

<sup>1</sup> vitamin-mineral premix provided per kg diet: VA 8,000 IU, VD3 1,000 IU, VE 20 IU, VK 0.5 mg, VB1 3 mg, VB2 9 mg, VB6 7 mg, VB12 0.03 mg, niacin 35 mg, D-pantothenic acid 10 mg, folic acid 0.55 mg, biotin 0.18 mg, Fe 100 mg, Cu 8 mg, Zn 100 mg, Mn 120 mg, I 0.7 mg, Se 0.3 mg; <sup>2</sup> calculated according to National Research Centre (NRC, 1994).

**Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

**Table (2):** Effect of water administration of Broncho-Vaxom® on productive performance of Japanese quails birds(mean ± SE)

| Indices                           | Fattening trial (FT) |                | Laying trial (LT)        |                          | P-value |        |
|-----------------------------------|----------------------|----------------|--------------------------|--------------------------|---------|--------|
|                                   | Control              | Broncho-Vaxom® | Control                  | Broncho-Vaxom®           | FT      | LT     |
| <b>Body weight, g</b>             |                      |                |                          |                          |         |        |
| initial <sup>1</sup>              | 73.77±0.34           | 72.93±0.37     | 220.5±5.22               | 219.2±6.01               | 0.115   | 0.871  |
| final <sup>2</sup>                | 222.8±4.27           | 215.6±5.26     | 243.8 <sup>b</sup> ±3.49 | 258.3 <sup>a</sup> ±3.86 | 0.285   | 0.007  |
| <b>Carcass characteristics, %</b> |                      |                |                          |                          |         |        |
| Dressing                          | 67.59±1.93           | 66.77±0.82     | 70.22±2.48               | 70.17±2.47               | 0.702   | 0.990  |
| Liver weight                      | 2.22±0.30            | 1.79±0.23      | 3.02±0.17                | 3.62±0.49                | 0.282   | 0.296  |
| Gizzard weight                    | 2.07±0.10            | 2.07±0.05      | 2.64±0.26                | 2.53±0.13                | 0.974   | 0.708  |
| Heart weight                      | 0.88±0.05            | 0.77±0.04      | 0.83±0.06                | 0.78±0.03                | 0.112   | 0.487  |
| Proventriculus weight             | 0.48±0.02            | 0.41±0.03      | 0.40±0.02                | 0.46±0.02                | 0.108   | 0.054  |
| Intestine weight                  | 5.33±0.43            | 5.71±0.72      | 7.42±0.30                | 7.56±0.46                | 0.664   | 0.798  |
| Bursa weight                      | 0.168±0.03           | 0.099±0.02     | atrophied                |                          | 0.068   | -      |
| Spleen weight                     | 0.26±0.14            | 0.06±0.01      | 0.07±0.01                | 0.06±0.01                | 0.193   | 0.309  |
| Edible giblets weight             | 5.12±0.33            | 3.64±0.26      | 6.54±0.31                | 6.92±0.55                | 0.277   | 0.558  |
| Ovary weight                      | -                    | -              | 2.04 <sup>b</sup> ±0.08  | 4.10 <sup>a</sup> ±0.08  | -       | <0.001 |
| Oviduct weight                    | -                    | -              | 2.58±0.17                | 3.05±0.25                | -       | 0.194  |
| Carcass yield                     | 72.76±2.00           | 71.39±0.44     | 76.71±2.19               | 77.10±2.30               | 0.832   | 0.872  |

<sup>1</sup>initial weight was at 21 day of age for the fattening trial and at 43 day for the laying one.

<sup>2</sup>final weight was at 42 day of age for the fattening trial and at 98 day of age for the laying one.

a–b– means with different superscripts are significantly different.

**Abdel-MoneimEid Abdel-Moneim et al.**

**Table (3):** Effect of water administration of Broncho-Vaxom® on egg production and egg quality of laying Japanese quails birds at the end of the laying trial (mean ± SE)

| <b>Indices</b>                   | <b>Control</b>           | <b>Broncho-Vaxom®</b>    | <b>P-value</b> |
|----------------------------------|--------------------------|--------------------------|----------------|
| <b>Egg weight, g</b>             |                          |                          |                |
| weeks 8-12                       | 12.00 <sup>b</sup> ±0.34 | 12.96 <sup>a</sup> ±0.21 | 0.033          |
| weeks 12-16                      | 13.01 <sup>b</sup> ±0.06 | 13.12 <sup>a</sup> ±0.05 | 0.021          |
| Overall                          | 12.50 <sup>b</sup> ±0.17 | 13.09 <sup>a</sup> ±0.09 | 0.009          |
| <b>Hen-day egg production, %</b> |                          |                          |                |
| weeks 8-12                       | 62.86 <sup>b</sup> ±1.84 | 74.29 <sup>a</sup> ±3.69 | 0.022          |
| weeks 12-16                      | 74.37 <sup>b</sup> ±3.77 | 91.43 <sup>a</sup> ±4.04 | 0.009          |
| Overall                          | 68.62 <sup>b</sup> ±2.37 | 82.86 <sup>a</sup> ±1.84 | <0.001         |
| <b>Egg mass, g egg/hen/day</b>   |                          |                          |                |
| weeks 8-12                       | 7.55 <sup>b</sup> ±0.34  | 9.62 <sup>a</sup> ±0.48  | 0.004          |
| weeks 12-16                      | 9.66 <sup>b</sup> ±0.46  | 12.01 <sup>a</sup> ±0.55 | 0.005          |
| Overall                          | 8.59 <sup>b</sup> ±0.37  | 10.84 <sup>a</sup> ±0.19 | <0.001         |
| Albumen, %                       | 55.32 <sup>a</sup> ±0.75 | 51.19 <sup>b</sup> ±1.27 | 0.008          |
| Yolk, %                          | 31.01 <sup>b</sup> ±0.82 | 34.58 <sup>a</sup> ±1.23 | 0.022          |
| Shell, %                         | 13.66±0.26               | 14.23±0.50               | 0.288          |
| Egg shape index                  | 80.92 <sup>a</sup> ±0.65 | 74.84 <sup>b</sup> ±1.40 | <0.001         |
| Yolk index                       | 39.35±1.73               | 43.32±3.28               | 0.265          |
| Shell thickness, mm              | 0.329±0.01               | 0.334±0.02               | 0.789          |
| Yolk: albumen ratio              | 0.564 <sup>b</sup> ±0.02 | 0.682 <sup>a</sup> ±0.04 | 0.013          |
| Haugh unit score                 | 87.58 <sup>a</sup> ±0.40 | 82.98 <sup>b</sup> ±0.85 | 0.001          |

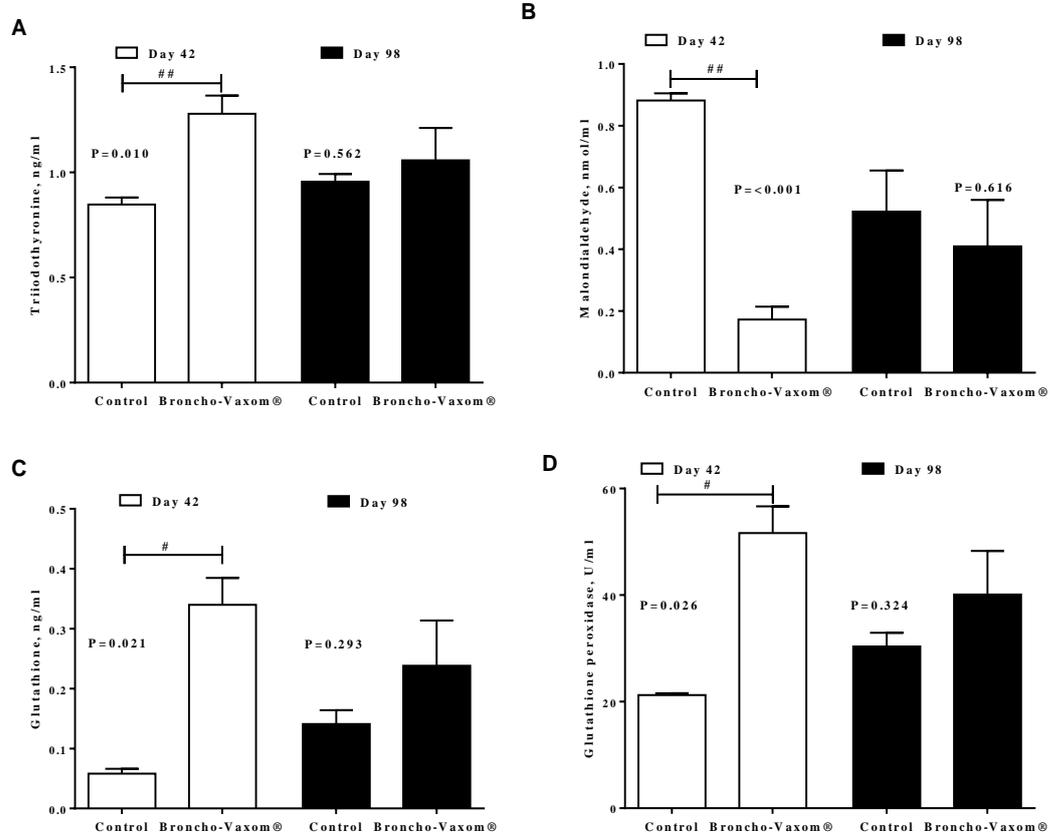
a–b– means with different superscripts are significantly different.

**Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

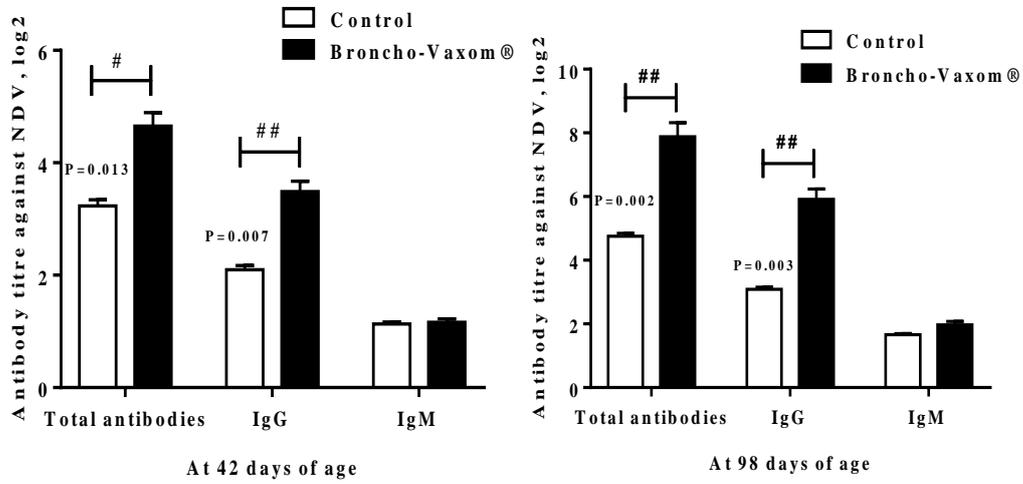
**Table (4):**Effect of water administration of Broncho-Vaxom® on serum biochemical components of Japanese quails birds (mean ± SE)

| Indices                                 | Fattening trial (FT)     |                          | Laying trial (LT)        |                          | P-value |       |
|---|--------------------------|--------------------------|--------------------------|--------------------------|---------|-------|
|   | Control                  | Broncho-Vaxom®           | Control                  | Broncho-Vaxom®           | FT      | LT    |
| <b>Protein fractions, g/dl</b>          |                          |                          |                          |                          |         |       |
| total protein                           | 3.78±0.80                | 3.41±0.268               | 3.31±0.18                | 3.52±0.30                | 0.687   | 0.581 |
| Albumin                                 | 1.58±0.23                | 1.43±0.13                | 1.53±0.16                | 1.65±0.26                | 0.616   | 0.735 |
| <b>Liver enzymes activity, U/L</b>      |                          |                          |                          |                          |         |       |
| AST                                     | 56.30 <sup>a</sup> ±2.86 | 24.16 <sup>b</sup> ±2.14 | 35.27±11.00              | 18.73±1.66               | 0.001   | 0.270 |
| ALT                                     | 28.42 <sup>a</sup> ±0.43 | 15.08 <sup>b</sup> ±1.67 | 25.29 <sup>a</sup> ±3.24 | 16.10 <sup>b</sup> ±1.67 | 0.002   | 0.041 |
| ALP                                     | 353.1 <sup>a</sup> ±20.9 | 205.4 <sup>b</sup> ±10.9 | 228.8±56.9               | 222.6±32.7               | 0.003   | 0.922 |
| <b>Renal function biomarkers, mg/dl</b> |                          |                          |                          |                          |         |       |
| uric acid                               | 8.12 <sup>a</sup> ±1.09  | 3.43 <sup>b</sup> ±0.49  | 4.52±0.57                | 3.49±0.51                | 0.017   | 0.238 |
| Creatinine                              | 0.70±0.02                | 0.45±0.09                | 0.46±0.12                | 0.50±0.06                | 0.056   | 0.781 |
| <b>Lipid profile, mg/dl</b>             |                          |                          |                          |                          |         |       |
| triglycerides                           | 1519.0±256.8             | 1329.5±82.6              | 1117.0±183.9             | 1049.7±134.3             | 0.521   | 0.773 |
| total cholesterol                       | 293.0±47.3               | 239.4±19.0               | 279.3±45.99              | 241.2±18.64              | 0.353   | 0.429 |
| HDL- cholesterol                        | 22.84 <sup>b</sup> ±4.80 | 51.37 <sup>a</sup> ±2.41 | 44.35±8.13               | 48.52±6.44               | 0.006   | 0.318 |
| LDL- cholesterol                        | 112.1±8.68               | 122.2±31.7               | 128.26±10.85             | 101.01±19.31             | 0.774   | 0.700 |

AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, HDL – high-density lipoprotein, LDL – low-density lipoprotein; a–b– means with different superscripts are significantly different.



**Fig. (1):** Effect of water administration of Broncho-Vaxom® on serum concentrations of Atriiodothyronine, Bmalondialdehyde, C glutathione andD glutathione peroxidase activity of Japanese quail birds at 42 and 98 days of age. Data presented as mean values with their standard errors. # $P < 0.05$  and ## $P < 0.01$ .



**Fig. (2):** Effect of water administration of Broncho-Vaxom® on serum concentrations of totalantibody titre against Newcastle disease virus (NDV), IgG and IgM of Japanese quail birds at 42 and 98 days of age. Data presented as mean values with their standard errors. # $P < 0.05$  and ## $P < 0.01$ .

REFERENCES

- Abd El-Moneim, A. E. and E. M. Sabic, 2019.** Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails. *Journal of Animal and Feed Sciences* 28, 52-61.
- Allan, W. and R. Gough, 1974.** A standard haemagglutination inhibition test for Newcastle disease.(1). A comparison of macro and micro methods. *Veterinary Record* 95, 120-123.
- Alverdy, J.; E. Aoy; P. Weiss-Carrington and D. Burke, 1992.** The effect of glutamine-enriched TPN on gut immune cellularity. *Journal of Surgical Research* 52, 34-38.
- Antonova, L.; V. Romanov and M. Averbakh, 2008.** Experience with bronchomunal used in the combined treatment of patients with bronchial asthma and chronic obstructive pulmonary disease. *Problemy tuberkuleza i boleznei legkikh*, 8-11.
- Bessler, W. G.; U. vor dem Esche and N. Masihi, 2010.** The bacterial extract OM-85 BV protects mice against influenza and Salmonella infection. *International immunopharmacology* 10, 1086-1090.
- Bowman, L. and P. Holt, 2001.** Selective enhancement of systemic Th1 immunity in immunologically immature rats with an orally administered bacterial extract. *Infection and immunity* 69, 3719-3727.
- Braido, F.; F. Tarantini; V. Ghiglione; G. Melioli and G. Canonica, 2007.** Bacterial lysate in the prevention of acute exacerbation of COPD and in respiratory recurrent infections. *International Journal of Chronic Obstructive Pulmonary Disease* 2, 335.
- Cazzola, M.; A. Capuano; P. Rogliani and M. G. Matera, 2012.** Bacterial lysates as a potentially effective approach in preventing acute exacerbation of COPD. *Current opinion in pharmacology* 12, 300-308.
- Clot, J. and M. Andary, 1980.** Immunostimulation induite par un lysat bacterial lyophilise. Etude in vitro des responses specifiques et non specifiques. *Med. Hug*, 2776-2782.
- Daniel, J., 1986.** Metabolic aspects of antioxidants and preservatives. *Xenobiotica* 16, 1073-1078.
- Eichelberg, D. and W. Schmutzler, 1983.** Pharmacological aspects of immunostimulation. *Immunitat und Infektion* 11, 109-122.
- Emmerich, B.; H. P. Emslander; K. Pachmann; M. Hallek; D. Milatovic and R. Busch, 1990.** Local immunity in patients with chronic bronchitis and the effects of a bacterial extract, Broncho-Vaxom®, on T lymphocytes, macrophages, gamma-interferon and secretory immunoglobulin A in bronchoalveolar lavage fluid and other variables. *Respiration* 57, 90-99.
- Faragher, J.; W. Allan and G. Cullen, 1972.** Immunosuppressive effect of the infectious bursal agent in the chicken. *Nature New Biology* 237, 118.
- Hadden, J. W., 1993.** Immunostimulants. *Immunology today* 14, 275-280.
- Han, L.; C.-P. Zheng; Y.-Q. Sun; G. Xu; W. Wen and Q.-L. Fu, 2014.** A bacterial extract of OM-85 Broncho-Vaxom prevents allergic rhinitis in mice. *American journal of rhinology & allergy* 28, 110-116.
- Huber, M.; H. Mossmann and W. Bessler, 2005.** Th1-orientated

## **Broncho-Vaxom; bacterial lysates; performance; antioxidant; immunity; quail**

- immunological properties of the bacterial extract OM-85-BV. *European Journal of Medical Research* 10, 209-217.
- Kubo, I.; P. Xiao and K. i. Fujita, 2001.** Antifungal activity of octyl gallate: structural criteria and mode of action. *Bioorganic & Medicinal Chemistry Letters* 11, 347-350.
- Larson, S. D.; J. Li; D. H. Chung and B. M. Evers, 2007.** Molecular mechanisms contributing to glutamine-mediated intestinal cell survival. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 293, G1262-G1271.
- Lu, T.; A. Harper; J. Zhao and R. Dalloul, 2014.** Effects of a dietary antioxidant blend and vitamin E on growth performance, oxidative status, and meat quality in broiler chickens fed a diet high in oxidants. *Poultry science* 93, 1649-1657.
- Medina, M. E.; C. Iuga and J. R. Alvarez-Idaboy, 2013.** Antioxidant activity of propyl gallate in aqueous and lipid media: a theoretical study. *Physical Chemistry Chemical Physics* 15, 13137-13146.
- Miran, S. N. K.; M. A. K. Torshizi; M. R. Bassami and H. Jandaghi, 2010.** Effect of three immunostimulants on some of indicators of broilers' immune response. *The journal of poultry science* 47, 321-325.
- Moon, S. H.; I. Lee; X. Feng; H. Y. Lee; J. Kim and D. U. Ahn, 2016.** Effect of dietary beta-glucan on the performance of broilers and the quality of broiler breast meat. *Asian-Australasian journal of animal sciences* 29, 384.
- Navarro, S.; G. Cossalter; C. Chiavaroli; A. Kanda; S. Fleury; A. Lazzari; J. Cazareth; T. Sparwasser; D. Dombrowicz and N. Glaichenhaus, 2011.** The oral administration of bacterial extracts prevents asthma via the recruitment of regulatory T cells to the airways. *Mucosal immunology* 4, 53.
- NRC, 1994.** Nutrition Requirements of Poultry. National Academy Press, Washington, DC.
- Oblakova, M.; L. Sotirov; M. Lalev; P. Hristakieva; N. Mincheva; I. Ivanova; N. Bozakova and T. Koynarski, 2015.** Growth performance and natural humoral immune Status in broiler chickens treated with the immunomodulator natstim. *International Journal of Current Microbiology and Applied Sciences* 4, 1-7.
- Puigdollers, J. M.; G. R. Serna; I. H. Del Rey; M. T. Barruffet and J. J. Torroella, 1980.** Immunoglobulin production in man stimulated by an orally administered bacterial lysate. *Respiration* 40, 142-149.
- Qureshi, M. and G. Havenstein, 1994.** A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. *Poultry science* 73, 1805-1812.
- Raslan, I. M., 2012. The use of immunomodulators in chicken. Cairo University, Cairo, Egypt.
- Terao, J.; M. Piskula and Q. Yao, 1994.** Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Archives of Biochemistry and Biophysics* 308, 278-284.
- Wu, G.; F. W. Bazer; G. A. Johnson; D. A. Knabe; R. C. Burghardt; T. E. Spencer; X. Li and J. Wang, 2011.** Triennial Growth Symposium: important roles for L-glutamine in swine nutrition and production. *Journal of Animal Science* 89, 2017-2030.
- Zhou, Y. and N. Danbolt, 2014.** Glutamate as a neurotransmitter in the healthy brain. *Journal of neural transmission* 121, 799-817.

### الملخص العربي

تقييم المعاملة المائية بالمحفز المناعي البكتيري (Broncho-Vaxom®) على الأداء و بيوكيمياء الدم و حالة مضادات الأكسدة و الاستجابة المناعية الخلطية للسمان الياباني النامي والبياض

عبد المنعم عيد عبد المنعم<sup>1</sup> - عصام محمد سابق<sup>1</sup> - عادل محمد ابوظالب<sup>1</sup> - نشأت سعيد ابراهيم<sup>1</sup>

<sup>1</sup> - قسم التطبيقات البيولوجية - مركز البحوث النووية - هيئة الطاقة الذرية - مصر

تم استخدام تصميم تجريبي على المدى الطويل لدراسة تأثير المعاملة المائية بالبكتيريا المحللة المجفدة Broncho-Vaxom® على الأداء ، والمؤشرات البيوكيميائية للدم ، وحالة مضادات الأكسدة والاستجابة المناعية الخلطية للسمان الياباني النامي والبياض. في تجربة التسمين ، تم توزيع 400 من طيور السمان اليابانية عمر 21 يوم بالتساوي في مجموعتين تجريبتين (ثمانية مكررات لكل منهما) تمثل المجموعة الأولى الكنترول في حين تم معاملة المجموعة الثانية بـ 3.5 جرام من Broncho-Vaxom® / 4 لتر ماء شرب. تم الحفاظ على نفس الترتيب في تجربة البياض باستثناء خفض العدد الأولي إلى 160 من السمات الإناث في كل مجموعة تجريبية. أظهرت نتائج هذه الدراسة عدم وجود اختلافات معنوية في أداء النمو في نهاية تجربة التسمين. ومع ذلك ، فإن إطالة مدة المعاملة أظهرت التأثير المحفز لـ Broncho-Vaxom® على وزن الجسم النهائي والوزن النسبي للمبيض والأداء الإنتاجي للبيض. كما لوحظ زيادة نسبة الصفار (%). ونسبة الصفار إلى البياض معنوية، في حين انخفض نسبة البياض (%). ومؤشر شكل البيضة ودرجة Haugh Unit مقارنة مع مجموعة الكنترول. كما لوحظ انخفاض في قيم السيرم من انزيمات الكبد وحمض اليوريك في حين ارتفعت تراكيز السيرم من T3 و HDL كوليستيرول في مجموعة المعاملة بـ Broncho-Vaxom® في نهاية تجربة النمو. لم تتأثر أي من القياسات المذكورة أعلاه بالمعاملة في نهاية تجربة البياض. كما تحسنت حالة مضادات الأكسدة عند اليوم 42 من العمر فقط بينما لوحظ ارتفاع مستوي الاجسام المضادة الكلية ضد فيروس النيوكاسيل و الجلوبيولين المناعي من النوع G بينما لم يتأثر مستوي الجلوبيولين المناعي من النوع M عند اليوم 42 و 98 من العمر. مما سبق نستخلص ان المعاملة بالمحفز المناعي البكتيري Broncho-Vaxom® في مياه شرب طيور السمان اليابانية ادت الى تحسين الأداء الإنتاجي للسمان البياض ومعايير جودة البيض وحالة مضادات الأكسدة و الاستجابة المناعية الخلطية في نهاية فترات النمو و البياض.