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Evaluation of Some Immunostimulants on the Immune-response of Broiler Chickens Against Avian Influenza and Newcastle Diseases Vaccination

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Abstract

Immunostimulants get an increasing importance to enhance the immune system and allowing maximum performance in poultry production. The effects of using different immunostimulants on the immune response of broiler chickens to Newcastle disease (ND) and Avian influenza (AI) vaccination were investigated in this study. 100 day-old Ross broiler chicks were randomly divided into 4 groups, 25 birds for each group. Group A was not treated with any immunostimulants and used as a control, while groups B, C and D were supplemented with Imutrix® (oregano oils plus β-glucan), Evit liquid® (Vitamin E plus selenium) and inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides), respectively. Statistical analysis results demonstrated significant divergence ($P < 0.05$) in body weight (BW) and feed conversion ratio among the applicable treatments. Birds of group B had the heaviest body weight (2.03 kg), followed by group C (1.87 Kg) and D (1.82 Kg) in comparison to the birds of group A (control, 1.79 Kg). Bursa of Fabricius weight showed non-significant differences among groups except in group B that showed an increase in the Bursa of Fabricius weight more than other groups especially the control group (1.15 g more). Comparable immune response was recorded for both AI and ND vaccination. The highest mean antibody titers were recorded for birds in group B at 14, 28 and 35 days old, while those in group D had the lowest antibody titre values. Thus, supplementing immunostimulants especially Imutrix® and Evit liquid® had a significant positive effect on performance characteristics and immune response against AI and ND vaccination in broiler chickens in contrast to non-treated group, which had low levels of immune response, rapid decreasing and no persistence for keeping high HI titer for long time.

Keywords: Immunostimulants, Immune response, Broiler chickens, Avian influenza, Newcastle, Vaccination.

Introduction

Infectious diseases are the chief challenge in the commercial poultry industry. Newcastle disease (ND) and avian influenza (AI) are considered the most somber worldwide diseases affecting poultry flocks which causing stark economic losses to the poultry industry [1]. Both of them can be controlled by bird vaccination using both live and inactivated virus vaccines [2,3]. Despite the use of the available vaccines, disease outbreaks are still common due to variety of stresses of intensive production systems, high density, nutritional and antibiotic administration as well as immunosuppression that adversely affect the immune status of the birds [4,5]. Thus, there is a need to improve the immune response of ND and AI vaccines to produce respectable antibody titers that can reduce viral replication. This enhancement can be achieved by using of adjuvants and/or immunostimulant supplements. Therefore, immunostimulants have been used as adjuvants to give long lasting humoral and cellular immune responses and improving the response of vaccines [6,7].

Immunostimulants consist of a cluster of biological and synthetic compounds that boost the non–specific cellular immunity by activating macrophages which consequently stimulate the immune response such as antigen engulf, cell killing, cytokine release and creation of antibody as well as humoral defense mechanisms in animals [8] which increases the sustainability of immune

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response to the infectious agent and resistance to disease infections [9,10]. Antioxidants, vitamin E and selenium (Se), play an important role in caring cells from reactive oxygen (RO) by free radical reduction and stopping the lipid peroxidation [11]. Vitamin E is the main lipid soluble antioxidant present in the cell membrane [12] and provides the first defense line against oxidative damage. Selenium (Se) is an essential component of the enzymes, Se-dependent glutathione peroxidases, which reduce hydrogen peroxide and lipid hydroperoxides [13]. Selenium addition to chickens infested with coccidia created changes in the numbers of blood leucocytes, revealing that Se possibly improves resistance to infection [14]. Therefore, it is not astonishing that deficiency of Se or vitamin E seriously affects immunological functions [15,16]. A combination of selenium and vitamin E play a major role in the development and maintenance of defense systems.

Inmunair 17.5® (*Propionibacterium acnes*, and *E. coli* lipopolysaccharides) is one of the commercial products available in the Egyptian market as nonspecific immunostimulant for the chicken farms. *Propionibacterium acnes* is a Gram positive, non-spore forming opportunistic bacteria [17]. *P. acnes* is an effective activator of macrophage, lymphocyte, natural killer cells and cytokine release in the examined lab animals [18]. Lipopolysaccharides are the main components of the outer membrane of Gram-negative bacteria, induce strong immune responses. Lipopolysaccharides of *Escherichia coli* (LPS) had a role in releasing IL-1, IL-6, or tumor necrosis factor (TNF) by macrophages [19].

Oregano essential oils (OEO) are extracted from *Origanumvulgare* plants (leaves and flowers). They consist of more than 30 ingredients, most of which are carvacrol and thymol that constitute 78-82% of oregano essential oil [20]. Oregano essential oils have potent antioxidant effects, which inhibit the oxidative reactions that exert on phospholipid membranes [21]. They support increasing the proportion of differentiation (CD) molecules of lymphocytes CD4+ and CD8+ (T lymphocyte), in pigs fed oregano in contrast to control ones [22]**.** β-Glucans are glucose molecules that considered the base components of cell wall of several bacteria, fungi, algae and yeast [23]. β-glucans activated the immune cells, macrophages, dendritic cells neutrophils, B cells, T cells, and natural killer cells [24]. The intensification of specific immunity (humoral and cell-mediated) will increase the animal's ability to resist diseases. Therefore, the present study was undertaken to investigate the immunomodulatory effects of the different immunostimulants on antibody response and performance against routine vaccination of ND and AI in broiler chickens.

Material and Methods

Chickens and experimental design

A total of 100 one-day-old broiler chicks (male and female Ross), were weighed and randomly divided into four groups on arrival. All groups received AI and ND vaccines; group A is control (vaccinated non treated birds); group B was treated with (imutrix®) in a dose of 1.5ml/L to drinking water for 24 hours at the first three days of every week along the period of experiment. Group C was treated by Vit E and selenium with the same regimen of administration and group D was similarly supplemented with inmunair 17.5. Chicks were raised on floor for 5 weeks and had free access to feed (Elwadi feed Co., Egypt) and water throughout the entire experimental period. The lighting program was 23 h light and 1 h of darkness. The temperature was gradually decreased from 33 to 25°C on day 21 and then was kept constant.

Vaccination schedule

The birds of all groups were vaccinated against ND via eye drop at 7^{th} day of age (HB1, Pfizer), 15^{th} day of age (LaSota, Pfizer) and at 21 days of age (clone30, Izovac). AI (Nobilis, H5N2) vaccination was given one S/C shot at the 5^{th} day of age using killed (H5N2). Gumboro (Pfizer) disease vaccination was applied at 11^{th} day of age with intermediate strain vaccine via eye drop route.

Immunostimulants

Purified β- glucan 11 g and oregano oils essential oils 120 g (carvacrol 60 g + thymol 3.6 g) in demineralized water up to 1 liter emulsifiers produced commercially under the trade name "Imutrix®" (ELT Co., South

Korea) were used. Vitamin E and selenium produced commercially under the trade name "EVIT LIQUID®" (Tecnozoo Co., ITALIA) were used. The Inmunair 17.5® (LABORATORIOS CALIER SA) which consists of (inactivated cells of propionibacterium acnes 0.17 mg, lipopolysaccharide from *E. coli* 0.05 mg, Thiomersal, 0.10 mg and Excipient q.s 1 ml).

Immunological measurements

Sera for determing the HI titre

Blood samples were collected from the wing and brachial veins of 5 birds randomly selected from each treatment at the 7, 14, 21, 28, 35 days into tubes without anticoagulant to determine the antibody titre following vaccination against ND and AI. Blood samples were dated and labeled according to number of chickens and groups. Samples were centrifuged for 10 min at 3000 rpm to obtain serum then were divided into aliquots and stored in Eppendorf tubes at -20ºC until analysis.

HI test

Two fold serial dilutions of the collected sera (50 µL) were prepared, then 4 HA units of NDV and AIV were prepared and added to each dilution. Subsequently, 30 minutes of incubation, 50 µL of 0.5% washed chicken red blood cells (RBC) was added on each well, the blend was incubated for 30 min at room temperature. Hemagglutination inhibition endpoint (the highest dilution of serum cause complete inhibition) were scored and recorded as reciprocal $\log_2$ values of the highest dilution of HI.

Bursa index

Birds were weighed and then slaughtered. The bursa of Fabricius was directly removed and weighed from 5 birds per group. The immune organ index was calculated: organo-somatic index=organ weight (g)/body weight (kg) [25].

Performance

The experimental period duration was 35 days. On day 7, 14, 21, 28 and 35 birds were weighed by group. For weight measurement, individual birds were weighed and the mean weight of each group was calculated. Feed conversion ratio (FCR) was estimated as the amount of feed it takes to grow a kilogram. (https://en.wikipedia.org/wiki/Feed_conversion_ratio#Poultry).

Statistical analysis

Data were analyzed by one-way ANOVA using SPSS14 and Duncan's multiple range test was used to compare the means ($P<0.05$).

Results

Impacts of different immunostimulants on weight gain of broilers at different ages were presented in Table (1). The average of total BW of treated groups was higher than that observed in the control group along the experimental period but the difference was non-significant except group B; the body weights tended to significantly increase ($P>0.05$) at days 28 and 35 in comparison to group A (table 1). As presented in Table (2), the FCR of control group (1.503) was higher than treated groups (1.33, 1.406 and 1.42 for group B, C and D, respectively), which was significantly lower in group B at day 35 in comparison to control group. According to the results shown in Table (3), There was an increase in the bursa weight of birds that were treated with imutrix (group B) at day 35 of age (2.78 g) which was 1.15 g increased from the weight of control group.

Table 1: Average body weights of broiler chickens (g) during the experimental period

	7 days	14 days	21 days	28 days	35 days
Group A[1]	160±0.12	420±0.17	825±0.43	1278±0.32[a]	1795±0.37[a]
Group B[2]	179±0.37	454±0.42	855±0.22	1426±0.38[b]	2030±0.15[b]
Group C[3]	180±0.25	445±0.19	850±0.12	1340±0.26[a]	1877±0.63[a]
Group D[4]	172±0.14	462±0.23	825±0.54	1300±0.13[a]	1825±0.46[a]

1: group A was not treated with any immunostimulants and used as a control. 2: groups B was supplemented with Imutrix® (oregano oils plus β-glucan). 3: group C was supplemented with Evit liquid® (Vitamin E plus selenium). 4: group D was supplemented with inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides). The means within the same column with common letter, do not have a significant difference (P>0.05).

Effects of different immunostimulants treatments on humoral immune response against ND and AI vaccination were presented in Table (4). The evaluated antibody response at all intervals of the experiment starting from the day 14 revealed the AI and ND titer in control group had low levels of immune response and rapid decrease which reflected as no persistence for keeping high HI titer for long time (Figure 1A and 1B). Improved immunological response of AI was observed in group B at day 21, 28 and 35 by HI. At day 14, group C was found in the second category in the response to immunostimulant, while for response to ND vaccination, as shown in Table (4) and Figure (1B). There was a significant improvement ($P< 0.05$) of antibody titer against ND, especially in group B at day 21, 28 and 35 followed by group C which significantly increased especially at day 35 of age in birds. Group D had the lowest positive effect by increase the antibody response to ND but not statistically significant ($P> 0.05$) in comparison to control group.

Table 2: Average Feed conversion ratio (FCR) of broiler chickens during the experimental period

	7 days	14 days	21 days	28 days	35 days	Average
Group A[1]	1.13±0.33	1.49±0.32	1.50±0.19	1.60±0.14	1.80±0.12[a]	1.503±0.32[a]
Group B[2]	1.00±0.02	1.27±0.19	1.33±0.25	1.45±0.22	1.60±0.53[b]	1.33±0.34[b]
Group C[3]	1.00±0.10	1.35±0.27	1.40±0.37	1.55±0.36	1.73±0.14[a]	1.406±0.56[a]
Group D[4]	1.00±0.02	1.30 ±0.45	1.44±0.51	1.60±0.21	1.76±0.27[a]	1.42±0.11[a]

1: group A was not treated with any immunostimulants and used as a control. 2: groups B was supplemented with Imutrix® (oregano oils plus β-glucan). 3: group C was supplemented with Evit liquid® (Vitamin E plus selenium). 4: group D was supplemented with inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides). The means within the same column with common letter, do not have a significant difference (P>0.05).

Discussion

It is a significant to get a good immune response to prevent viral diseases in poultry. So, the use of immune stimulants is a resolution to enhance bird's resistance to infectious diseases. Our results revealed that, the average of total BW and FCR of treated groups were higher and lower, respectively in comparison with that observed in control non treated birds along the experimental period. Group B which was supplemented with imutrix® as a source of β-glucan and oregano oil, is the only group that differed statistically in BW gain ($P < 0.05$) at day 28 and 35 in comparison to the control group. These results are in agreement with a broad idea of Iren [26] who found the submission of immune stimulating substances lead to increase the immune response that could result in enhanced growth rate and performance may be due to the decrease in the load of infectious causes and give chance to maximum performance. Also, increased the body weight of broiler chickens treated with immunostimualnts may be due to an increase in the digestive enzymes as trypsin and amylase [27-29].

Table 3: Mean bursal weight (g) and relative weight to body weight of the experimental groups at day 35

	A[1]	B[2]	C[3]	D[4]
Body weight (kg)	1.79±0.37[a]	2.03±0.15[b]	1.87±0.63[a]	1.82±0.46[a]
Bursa weight (gm)	1.63±0.12[a]	2.78±0.23[b]	1.47±0.34[a]	1.58±0.39[a]
At 35 days*	0.91	1.31	0.78	0.86

1: group A was not treated with any immunostimulants and used as a control. 2: groups B was supplemented with Imutrix® (oregano oils plus β-glucan). 3: group C was supplemented with Evit liquid® (Vitamin E plus selenium). 4: group D was supplemented with inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides). The means within the same row with common letter, do not have a significant difference (P>0.05). * Relative weight of bursa to body weight of the experimental groups at day 35.

In particular, with Imutrix® treated group (a combination between β-glucan and oregano oil), our results were in concordance with Mocar *et al*. [30] who observed that oregano essential oils help in improving the BW gain of broilers through improvements of feed consumption and encouragement of proteins deposition in muscle**.** There was a positive effect of oregano oils on body weight gain through an increase in the feed conversion efficiency [29,31,32].

In group D which supplemented with Inmuonair17.5® (*Propionibacterium acnes* +lipopolysaccharides of *E. coli)*, the increase in BW was low that was in agreement with Gaines *et al*., [33] and Yang *et al*., [34] who found that the LPS administration is a potent inflammatory inducer which elicit the pro-inflammatory cytokines that have an effect on BW gain as a consequence of inflammatory response. Also, Korver and Klasing [35] noted the decrease in growth rate that resulted from LPS due to the rationing of nutrient supply from growth and directed toward inflammatory immune response.

In this study, the bursal weights (as a percentage to the BW) were not significantly different for experimental supplemented immunostimulants when measured at 5th weeks of age except in group B in which there was an increase in the weight of bursa in comparison to the control group. Broilers supplemented with feed containing thyme had no considerable diversity in the spleen and bursa relative weights in comparison to control groups which was disagreed with our results [36]. In this study, the highest AI and ND HI-antibody responses were emerged in birds of group B followed by group C and the least response was of group D in comparison to control group.

Table 4: Average HI log_2 titers of immune response against Avian Influenza and Newcastle Diseases vaccination

Groups	AI					ND				
	day 7	day 14	day 21	day 28	day 35	day 7	day 14	day 21	day 28	day 35
A[1]	5.0±0.21	4.0±0.42	4.3±0.66^{a}	3.5±0.45^{a}	2.0±0.19^{a}	6.0±0.52	4.7±0.36	2.6±0.44^{a}	4.6±0.71^{a}	4.0±0.48^{a}
B[2]	4.8±0.34	4.3±0.36	5.4±0.56^{b}	4.6±0.61^{b}	3.5±0.34^{b}	6.7±0.19	5.4±0.55	6.3±0.10^{b}	6.7±0.57^{b}	6.2±0.21^{b}
C[3]	5.2±0.47	4.4.±0.53	5.1±0.76^{a}	4.2±0.51^{a}	3.2±0.54^{b}	6.3±0.61	5.0±0.61	5.6±0.27^{b}	6.2±0.74^{b}	5.1±0.37^{a}
D[4]	4.8±0.65	4.5±0.12	4.9±0.32^{a}	3.9±0.11^{a}	3.0±0.56^{a}	6.0±0.43	4.6±0.38	5.1±0.54^{b}	6.0±0.66^{b}	4.75±0.22^{a}

1: group A was not treated with any immunostimulants and used as a control. 2: groups B was supplemented with Imutrix® (oregano oils plus β-glucan). 3: group C was supplemented with Evit liquid® (Vitamin E plus selenium). 4: group D was supplemented with inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides). The means within the same column with common letter, do not have a significant difference (P>0.05).

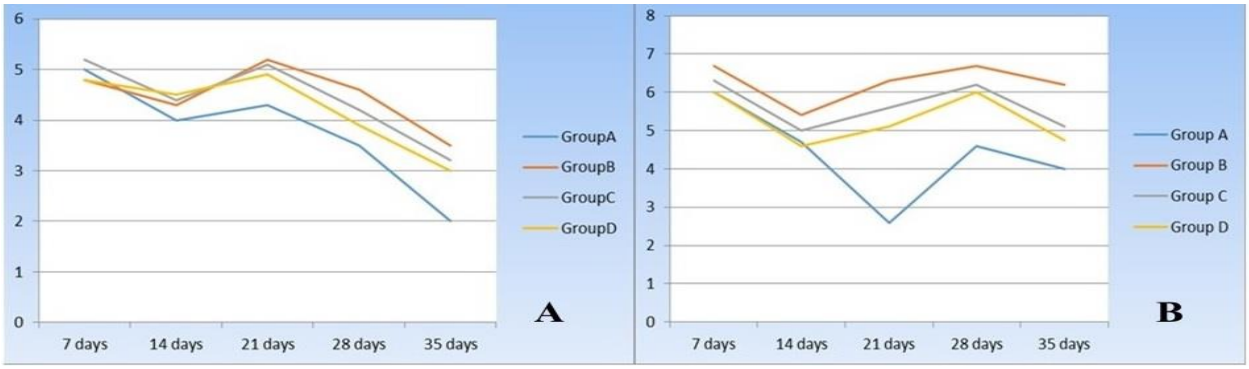

Figure 1: The immune profile of AI vaccination with immunostimulant supplementation in broiler chickens. A: The HI log_2 titers after AI vaccination. B: The HI log_2 titers after ND vaccination. The birds in group A were not treated, while, groups B, C and D were supplemented with Imutrix® (oregano oils plus β-glucan), Evit liquid® (Vitamin E plus selenium) and inmunair®17.5 (*Propionibacterium acnes* and *E. coli* lipopolysaccharides), respectively.

Similar to our results immune stimulants have antioxidant property such as thyme and Vit. E plus selenium could improve immune responses [37-39]. Acamovic and Brooker [37] investigated the mechanism of how thyme could raise the immune response and stated the immune-stimulating activity of thymol and oregano essential oil hence to the phagocyte system, cellular and humoral immunity. Also, continuous dietary application of thymol + carvacrol has a potential humoral immunostimulant activity in broilers [29,40]. Moreover, another study was carried to see the effect of thymol and carvarcol combination on NDV-HI specific antibody of broiler chickens and they found that those oils induced NDV-HI specific antibody titers in superior manner than that mentioned with levamisole [41].

In accordance with our results of group C, the humoral immune response was improved in broilers supplemented with 132mg/kg of vitamin E than the control group [42,43]. Explaining the mechanism of Vitamin E in intensification of the immune response in birds, Moriguchi *et al*. [44] and Tampieri *et al*. [45] noticed an enhancement in macrophage phagocytic function, declining in prostaglandin E_2 production, increasing IL-1 secretion by macrophages, and enhancing IL-2 production and T-cell in broilers fed diets with high levels of Vitamin E. In particular to group D, there was a little effect on the immune response in comparison to other treated groups, this may be in accordance with the previous researchers who found a poor humoral immune response in goats treated with *P. acnes* and attributed this to the method of administration (oral route) [46]. Also, Flaminio *et al*. [47] did not detect any improvement on IgG and IgM levels on foals treated with *P. acnes.*

Conclusion

In conclusion, there was a noteworthy effect of oregano essential oils with β-glucan (Imutrix®) on BW gain at the end of the experiment. However, birds supplemented the vitamin E plus selenium (Evit®) had a lower feed intake and a better FCR than birds in the control group. Regarding the serological data, immunomodulators (Oregano oil plus β-glucan and Vitamin E plus selenium) used in present study were revealed a slight positive effect on antibody production against AI and ND vaccines. However, *Propionibacterium acnes* plus LPS of *E. coli* did not significantly change the serum IgG levels of broilers.

Conflict of interest

None of the authors have any conflict of interest to declare.

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الملخص العربى

تقييم مقارن لبعض المحفزات المناعية على رد الفعل المناعى في بدارى التسمين ضد تحصينات انفلونزا الطيور والنيوكاسل

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إن استخدام المحفزات المناعية جذب مزيد من الإهتمام لتقوية الجهاز المناعى و تحسين الاداء فى انتاج الدواجن. تناولت هذه الدراسة تأثير استخدام المنشطات المناعية المختلفة على الاستجابة المناعية للقاحات ضد مرضي الانفلوانزا والنيوكاسيل. أجريت هذه التجربة باستخدام عدد ١٠٠ كتكوت تسمين من سلالة روص عمر يوم حيث تم تربيتهم حتى عمر ٣٥ يوم مع تقسيمهم منذ اليوم الاول الى اربعة مجموعات (أ، ب، ج، د) كل مجموعة عبارة عن ٢٥كتكوت. المجموعة الاولى (أ)الضابطة بدون إضافة اي منشطات مناعية. بينما المجموعات ب، ج، د تم اضافة منشط المناعة اميو تركس (عبارة عن زيوت الاوريجانو وبيتاجلوكان) و فيتامين هـ+سيلنيوم و منشط المناعة اميونير (وهو عبارة عن ليبو بولي سكرايد لميكروب الايكولاى بالاضافة الى بكتيريا اكنى) علي التوالي. وقد اظهر التحليل الاحصائي للبيانات وجود فروق معنوية بين المعاملات بالنسبة لوزن الجسم وكذلك معامل التحويل الغذائي حيث سجلت المجموعه (ب) اعلى وزن نهائي للجسم (٢.٠٣كجم) بينما طيور المجموعة (ج) ١,٨٧كجم يليها المجموعة (د) ١,٨٢كجم وقد جاءت النتائج كلها اعلى من المجموعة الضابطة (١,٧٩كجم). أوضح وزن البيرسا عدم وجود اي فروق معنوية بين المجموعات ماعدا المجموعة (ب) والتي زاد وزن البرسا بها عن باقي المجموعات والذي وصل بفارق ١.١٥ جرام عن المجموعة الضابطة .وقد كانت الاستجابة المناعية متناغمة مع هذه النتائج وكانت ايجابية حيث سجلت القياسات لمستوى الاجسام المضادة الناتجة ضد تحصين انفلونزا الطيوروالنيوكاسل أعلى درجة ايجابية في المجموعة (ب) وخاصة فى ايام ٢١و ٢٨و ٣٥ بينما سجلت أقل مستوى ايجابى بدون فرق معنوى عند درجة ٠.٠٥ في المجموعة (د). من كل ماسبق يتضح أن استخدام منشطات المناعة خاصة اميوتركس او فيتامين هـ+س في قطعان التسمين له تاثير ايجابي واضح على اداء واوزان الطيور المعالجة.كما ان استخدام هذه المحفزات المناعية يعظم من قدرة واستجابة الجهاز المناعي لبدارى التسمين ضد تحصينات الانفلونزا والنيوكاسل كما يزيد من الحفاظ على مستوى الحماية للاجسام المضادة المتكونة ومنع انحسارها بصورة سريعة كما شوهد فى طيور المجموعة الضابطة.