

Protective immunity induced by co-injection of mixture of Mannan and B-glucan immunostimulant substances with the inactivated Bivalent AI-ND vaccine in broiler chickens

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Received: 29/09/2009

Accepted: 09/02/2010

SUMMARY

In the present study, the effect of a mixture of some immunostimulant substances on the immune response of broiler chicks to bivalent AI-ND vaccine was determined. Four groups of chicks were used. Group 1 was vaccinated with one dose of AI-ND vaccine and simultaneously injected with the mixture of immunostimulant substances. Group 2 was vaccinated with one dose of the bivalent AI-ND vaccine. Group 3 was vaccinated with 2 doses of live ND vaccines (Hitchner then Lasota strains) and group 4 was kept none vaccinated as control chicks. All birds were monitored weekly for the humoral and cellular immune response then challenged with the virulent

NDV at 35 days of age. HI test was used for titration of antibodies for both AIV and NDV, phagocytic activity, Nitric oxide; Lysozyme activity and total antioxidant in serum were used to determine the cellular immune response of the chicks. Protective immunity induced in the vaccinated groups varied. The injected immunostimulant mixture demonstrates its effect on the immune response to the bivalent AI-ND vaccine in group 1 with 100% protection against the challenge NDV. Whereas, 80 and 60 % protection were obtained in chicks vaccinated either with AI-ND vaccine (group 2) or live ND vaccines (group 3); respectively. The present study reports the effect of injection of some immunostimulant

substances in augmentation of the immune response to the inactivated vaccines.

INTRODUCTION

B-glucans, are glucose polymers found in the cell walls of yeast, fungi and cereal plants. Studies on B-glucan molecule have been focused on its beneficial effects on the immune system, lack of its toxic or adverse effects (Carrow, 1996). Currently, B-glucans are accepted to be one of the most powerful immune response modifiers (Brown and Gordon, 2003). Several studies have demonstrated its inhibitory effect on tumor development or stimulatory effect in enhancing the defense against bacterial, viral, fungal, parasitic challenge (Kemodde et al., 1998 and Nakagawa et al., 2003), activate macrophages (Liang et al., 1998 and Cleary et al., 1999), inducing production of cytokines (Engstad et al., 2002 and Sherwood et al., 2001); effect on nitric oxide (NO), arachidonic acid metabolites (Ljungman et al., 1998 and Hashimoto et al., 1997), increases hematopoiesis, exerts radio protective effects, improves wound healing by inducing the macrophage release of wound growth factors (Wei et al., 2002 and Delatte et al., 2001). B-glucan enhance the functional activity of macrophages and activate

antimicrobial activity of mononuclear cells and neutrophils in vitro (Williams, D.L. 1997., Zekovic et al., 2005). This enhanced immune response is accomplished by an increased pro-inflammatory cytokine production (Olson et al., 1996; Adachi et al., 1994), oxidative burst, and chemokine production (Williams, D.L., 1997). In addition, human whole blood incubated with soluble yeast B-glucan showed an enhanced production of TNF α , IL-6, IL-8 and monocytes tissue factor (TF). Furthermore, when LPS was added together with this B-glucan TNF α , IL-8, IL-10 and TF concentrations were strongly increased (Engstad et al., 2002). Stimulated leukocytes from animals that were treated with B-glucan showed an increased pro-inflammatory cytokine production (Adachi et al., 1994; Abel, and Czop, 1992), oxidative burst (Muckosova et al., 2001) and chemotaxis (Leblanc et al., 2006). Stimulatory effects of B-glucan on both specific and non specific immune responses have been demonstrated in mice (Suzuki et al., 1990), in fish (Robertsen et al., 1990; Jeney and Anderson, 1993), beneficial effects on growth performances in pigs and poultry (Chae et al., 2006).

Mannan, a polysaccharide isolated from yeast binds to C-type lectins of the mannose receptor family, expressed by antigen-presenting cells (APCs) including dendritic cells (DCs) and macrophages. As these

receptors mediate endocytosis, they have been targeted with ligands to deliver antigens into APCs to initiate immune responses Sheng et al., (2006) and Taylor et al., (2005). Mannan also stimulated up regulation of inflammatory cytokines including interleukin-1B and differential T helper1(Th1)/ Th2 cytokines (Tada et al., 2002). The adjuvant effect of mannan and its derivatives on antigens greatly enhanced antigen- up take and major histocompatibility complex (MHC) classII-restricted antigen presentation by DCs (Engering et al., 1997). Mannan was capable of stimulating mouse bone marrow-derived DC in vitro, eliciting enhanced allogenic T-cell proliferation and T-cell responses (Sheng et al., 2006). On the other hand, studies on macrophages have identified mannan as a ligand for Toll-like receptor-4 (TLR4) that stimulates Tumor necrosis factor α (TNF- α) production.

The aim of the present study is to investigate the immunostimulatory effect of co-injection of mixture of immunostimulant substances with the inactivated bivalent AI-ND vaccine in broiler chicken.

MATERIAL AND METHODS

A. Materials:

1. **Experimental birds:** A total of 120 one-day-old chicks were obtained from Wadi poultry production company, Egypt. They were floor reared, fed on a commercial Poultry ration, and kept under good hygienic conditions throughout the experiment.
2. **Mixture of Mannan digosaccharides** (3000mg/liter) and B-glucan (25000mg/liter) was used.
3. **Antigen:**
 - A. **Challenge NDV strains:** Velogenic viscerotropic strain of NDV was obtained from Serum and Vaccine Research Institute, Abbasia, Egypt.
 - B. **NDV vaccine:** Hitchner and Lasota strains of NDV were used. It was obtained from Intervet local agency, Egypt.
4. **Candida albicans:** It was supplied by the Dept. of Mycology, Animal Health Research Institute, Giza-Egypt. 24 hours old subculture of candida albicans was used as antigen for evaluation of macrophages phagocytic activity.
5. **Media, reagents and chemicals:** RPMI 1640, Ficoll-hypaque, fetal calf serum, Giemsa stain, heparin preservative free

(500 I-u/ ml) were obtained from Sermend Lab., Germany.

6. **Micrococcus lysodeikticus:** Sigma chemical Co., St.louis, USA
7. **Griess reagent:** Sulphanimide, Naphthyl ethaylene diamine – di – hydrochloride , H3PO4.
8. **Totalantioxidant capacity kit:** It was obtained from Biodiagnostic Company. CAT. No TA2513. Egypt.

Methods:

1- Measurment of phagocytic activity of peripheral blood monocytes using candida albicans:

Separation of peripheral blood mononuclear cells using ficoll hypaque density gradient was carried out as described by Boyum (1968). Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% fetal calf serum and viability was done after Hanks and Wallace, (1985). The test was performed according to procedure described by Anthony et al., (1985) and Chu and Dietert, (1989). Phagocytic percentage and index were estimated as follow:

Phagocytic % =

$\frac{\text{No. of macrophages ingesting candida}}{\text{Total No. of Macrophages}} \times 100$

Phagocytic index =

$\frac{\text{No. of macrophages ingesting more than 3 blastospore}}{\text{Total No. of macrophages with ingested blastospores}}$

2- **Haemagglutination inhibition test (HI):** It was done as described by Beard (1989). It was used to evaluate humoral immune response.

3- **Lysozyme:** Lysozyme activity was measured by agarose gel lysis assay according to the method described by Schltz (1987). Briefly, lysoplates were prepared by dissolving 1% agarose in 0.06 MPBS at pH 6.3. 500 mg of uniform suspension of Micrococcus lysodeikticus in 5ml saline were added to 1 liter of agarose, plates were poured. Then, 25ul of serum samples and standard lysozyme were added in each wells. After 18 hours the cleared zones diameter were measured to both standard lysozyme and serum sample and the concentration was estimated.

4- **Nitric oxide:** Determination of serum nitric oxide was carried out according to Green et al., (1982) and Rajaraman et al., (1998). Briefly 100ul of serum sample was transferred into flat-bottom 96-well ELISA plate and 100ul of Griess reagent were added to each well. The optical density was determined at 570nm with an ELISA plate reader. Absorbance of test samples was converted to 10um of nitrite by comparison with absorbance values of sodium nitrite standard curve within linear curve fit.

5-Total antioxidant capacity: It was determined according to Koracevic et al., (2001). Total antioxidant concentration $mM/L = A_B - A_{SA} \times 3.33$. Where A_B = absorbance of blank; A_{SA} = absorbance of sample.

6- Experimental design: One hundred and twenty one-day old commercial chicks were used in this study and were divided into 4 groups 30 chicks each:

Group (1): birds vaccinated with AI-NDV bivalent vaccine and co-injected with the mixture of immunostimulant substance.

Group (2): birds vaccinated with AI-NDV bivalent vaccine.

Group (3): birds vaccinated with 2 doses of live NDV vaccine.

Group (4): birds non vaccinated and non treated.

Two blood samples (a and b) were taken from 5 birds from each group weekly intervals for 5 successive weeks via heart puncture.

a- Sample was taken in sterilized plastic centrifuge tube containing heparin for separation of mononuclear cells used in phagocytic activity.

b- Sample was taken without anticoagulant for serum separation and used for detection of hemagglutination titer (HI), lysozyme activity, Nitric oxide and total antioxidant capacity.

At the of the experiment, 10 chickens from each group were challenged intramuscular with 0.2 ml suspension containing 10^6 virions of NDV Velogenic strain (challenge test). Birds were kept under observation for 3 weeks with daily recording of symptoms and deaths.

7. Statistical analysis:

Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at $P < 0.05$ according to Petrie and Watson (1999) and computerized using SPSS (1999).

RESULTS AND DISCUSSION

The present study demonstrates that B-glucan and mannan have immunostimulatory effect on avian immune response. The macrophage activity of chicken treated with B-glucan and mannan exhibited significant increase in phagocytic % and phagocytic index along the experiment (Table 1). These results are in accordance with those previously reported by others who described that B-glucan stimulated human macrophages and increases the number of CD4 positive lymphocytes in lymph node of gastric cancer patients (Browder et al., 1990 and Takeshita et al., 1993). Also,

Krakowski et al., (1999) recorded significant increase in phagocytic activity of neutrophil, bactericidal with 1,3/ 1,6 glucan. Similarly, Lowry et al., (2005) found that B-glucan feed additive induce significantly increased in heterophil phagocytosis, bactericidal killing and oxidative burst in chicken. In addition, Vetvika et al., (2007) showed that B-1,3 glucan induced significant stimulation of phagocytic activity of mice peritoneal macrophages as well as synthesis and release of IL-2 by splenocytes. Indeed, various glucans are well known to stimulate phagocytosis (Abel et. al., 1989). It has been suggested that B-glucans bind to scavenger receptors and inhibit the interactions of monocyte membrane with classical scavenger ligands (Brown and Gordon, 2003). B-glucan receptor activity has also been reported on a variety of leukocytes, including macrophages, neutrophils, eosinophils and natural killer cells, as well as non immune cells including endothelial cells. Likewise, previous researches reported that mannan as a ligand for TLR2-TLR4 (Toll-like receptor) which are present in a wide spectrum of cell types including antigen presenting cells at various levels (Roeder et al., 2004) stimulating TNF- α production (Tada et al., 2002). Also, mannan might be useful ligand for to target dendritic cells to improve immune response (Foged et al., 2004). Functionally, DCs pulsed with mannan

exhibit enhanced capacities in stimulating allogenic T- cell proliferation (Wilson et al., 2003). On the other hand, mannan is recognized by the C-type lectin receptors (e.g MR) which are considered as pattern recognition receptors involved in host defense and innate immunity (Apostolopoulos and Mc Kenzie., 2001).

In the present study, chicken treated with the mixture of immunostimulant and co-vaccinated with AI-ND vaccine (group 1) showed high level of antibodies in different times at 3rd, 4th, 5th weeks and one week after challenge in comparison to other groups (Table 2). Stimulation of both IgM and IgG formation 4 weeks of oral administration of phycarine in mice has been reported (Vetvika et al., (2007). Also, Suzuki et al., (2001) reported that isolated splenocytes from SSG (B-glucan) administered mice showed an increase in IgG2a production and decreased IgG1 production, these antibodies responses are respectively regarded as Th1 and Th2. Indeed, results of the current study and others demonstrate that B-glucan-mannan are good T helper inducer which subsequently reflect on the specific immune response. Additionally, Gou et al., (2008) reported that mannan functionalized nanoparticles obtained by simple protocol might enhance the humoral immune response against target antigen and it might have great

potential application in vaccine delivery system.

Lysozymes are proteins of low molecular weight found in polymorph nuclear cells and synthesized also in mononuclear cells. They are present in most tissue fluid except cerebrospinal fluid, sweat and urine. Lysozymes are considered as a member of innate humoral factors that elaborated from body and showed domestic increase in their concentration (Weir, 1983). Our results showed significant increase in chicken treated with B-glucan-mannan compared to non treated groups. This is in harmony with the results of Paulsen et al., (2001) who demonstrated that B-glucan induced an increased in lysozyme activity and gene transcription in culture supernatant macrophage of Atlantic salmon at 3, and 6-days post intra peritoneal injection of B-glucan. Contrary, Karkowski et al., (1999) reported non significant difference in serum lysozyme activity of pregnant mare injected with (1-3)(1-6) b-glucan. Indeed, an increase in lysozyme activity was reported in this study which may be due to the direct effect of the substance used in the injection on the macrophage. The serum nitric oxide level was low in chicken vaccinated and treated with B-glucan-mannan (group 1) compared to (group 2) at 2nd week whereas at 3rd, 4th and 5th weeks did not show any significant change. Nitric

oxide is a chemical messenger which has been recognized as important effectors molecules for macrophages in their cytostatic activity in fighting against invading pathogens and tumor cell target (Liew, 1995). In addition, it does not regulate host immunity as a modulator of T-lymphocyte response (Albina et al., 1991). The results are resemble to those obtained by Pacheco-Sauche et al., (2007) who found that B-glucan extracted from mushroom *C-drophila* induces significant inhibition of nitric oxide production, they suggested that B-glucan exerts its effect by inhibiting inducible nitric oxide synthase protein (iNOS) and mRNA expression. In contrast, Chen et al., (2003) found that lentinan (typical B-glucan) at doses 40, 80 and 160 microgram/ml, increased splenocyte nitric oxide production after incubation 20 minutes. Also, Jung et al., (2004) reported increase in nitric oxide production in broncho-alveolar lavage fluid from pig pre administered with B-glucan and infected with swine influenza. Pre treatment of bone marrow macrophages and denderitic cells with lentinan resulted in increased in nitric production after *Listeria monocytogens* infection in vitro.

Total antioxidant capacity was increased in chicken vaccinated and treated with B-glucan-mannan (group 1) at 4th week compared to other groups. Beta glucan has antioxidant effect (Krizkova et al., 2003). Also, Sheng et

al., (2006) demonstrated that administration of Beta glucan following methotrexate in rats abolished the depletion glutathione (GSH) and inhibited the increase in malondialdehyde. Furthermore, Kayali et al., (2005) used Beta-glucan against spinal cord injury and demonstrated that it works like scavenger and has antioxidant effect on lipid peroxidation in spinal cord injury.

In respect of protection test, chicken vaccinated and treated with B-glucan-mannan compound showed the highest protection level among groups. The protection rate was 100% against velogenic strain of NDV. Our data indicate that (Beta-glucan-mannan) providing a good protection from avian pathogens. The present results agree with those obtained by Yun et al., (2003) who stated that B-glucan supplied significant protection against challenge with *Staphylococcus aureus*, and the enhanced resistance was suggested to be related to increased gamma interferon production in accordance with present result. Lowry et al., (2005) recorded a purified B-glucan feed additive significantly decreasing the incidence of *Salmonella enterica* (SE) organ invasion in immature chickens and up regulating the functional abilities of heterophils isolated from immature chickens against an invading pathogen, SE. Also, bacterial counts in blood of *Staphylococcus-aureus* challenged rats treated

intramuscular with PGG-glucan were lower than control rats. In addition, subcutaneous injection of yeast B-glucan and whole glucan increased survival rate diminished bacterial load in the lungs and increased the proportion of bacterial-free animals after infection with anthrax in mice (Kournikakis et al., 2003). Jung et al., (2004) reported administration Beta-glucan in pigs infected with Swine influenza produced decreased in pulmonary lesion score and viral replication rate in infected pigs. In contrast, Dritz et al., (1995) found that supplementation nursery pigs diets with 0.25% B-glucan increased the susceptibility to *Streptococcus suis* infection.

Taken together, the protective effect of B-glucan-mannan in the present study may be attributed to activation of numerous immune cells and immune mediators which reflect in cellular immune response subsequently provide good protection against pathogenic invasion. This is supported by previous reports who demonstrated that oral administration of B-glucan enhanced the activities of natural killer cells, peritoneal macrophage and cytotoxic T-lymphocytes in mice (Cross et al., 2001). CD8 and TCR were significantly higher in B-glucan group as compared to control chickens (Chae et al., 2006). Another possibility for protection effect of B-glucan is the antioxidant capacity of it against lipid peroxidation. Kayali et al.,

(2005) stated that B-glucan has antioxidant effect against oxidative stress, subsequently

On the other hand, reports on the effect of mannan on the immune response support the results of the present study. Mannan is an example from prebiotic, addition of prebiotic to animal diets can improve mucosal immune system function, particularly increase levels of immunoglobulin both in serum of pigs (White et al., 2002) and in the intestinal lumen of mice (Hosono et al., 2003). Also, Peng et al, (2004) found that Brewers yeast that contains various compounds such as B-glucan and Mannan when used as feed additive to fish enhanced growth performance, increased intracellular superoxide anion production of head kidney macrophages as well as enhanced survival after bath exposure to *Streptococcus* infection

preserving cellular integrity.

compared to fish fed the basal diet. Furthermore, Shashidhara and Devegowda (2003) reported that the antibody responses against infection bursal disease virus were significantly higher in mannan treated group and the maternal antibody titers in progeny were also influenced by mannan supplementation. Recently, Sang et al., (2009) recorded dietary supplementation of mannan to marron, improve the health status and immunity of marron under the bacterial infection and stress condition caused by air and NH₃ exposure.

Finally, the present study demonstrates the great potential application of a mixture of glucan-mannan in vaccine delivery system.

Table (1) Phagocytic % and index of chicken macrophages treated with B-glucan-mannan and/ or vaccinated with AI-NDV or ND vaccines

Group / Time	2 rd week		3 rd week		4 th week		5 th week	
	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index
Group (1) Vaccinated + B-glucan	A 63 ± 1.9	aB 0.16 ± 0.01	aB 63 ± 1.4	aB 0.21 ± 0.01	B 67 ± 3.8	aB 0.17 ± 0.01	aB 53 ± 2.3	aB 0.18 ± 0.01
Group (2) Vaccinated and non treated	A 48 ± 4.4	B 0.10 ± 0.02	B 54 ± 2.1	BC 0.12 ± 0.01	b 55 ± 1.6	b 0.10 ± 0.008	b 41 ± 1.2	bC 0.12 ± 0.01
Group (3) Control vaccinated with live NDV	58 ± 1.4	A 0.11 ± 0.01	A 57 ± 1.1	A 0.10 ± 0.05	A 59 ± 0.8	A 0.10 ± 0.05	A 42 ± 1.8	A 0.09 ± 0.005
Group (4) Control Non vaccinated Non treated	a 51 ± 0.8	b 0.08 ± 0.005	B 54 ± 2.0	bC 0.08 ± 0.008	ab 47 ± 3.7	b 0.08 ± 0.008	b 37 ± 1.6	bC 0.08 ± 0.008
F Calculated	7.1	6.2	6.3	38.9	8.3	22.8	15.0	17.5

Aa,Bb significantly different between two comparison groups in the same column against capital letter at $P \leq 0.05$ using least significant difference (LSD)

Chicken vaccinated with Bivalent vaccine and treated with B-glucan-mannan (group 1) revealed significant increase in phagocytic% and phagocytic index at 2nd, 3rd, 4th and 5th weeks compared to chicken vaccinated with Bivalent non treated (group 2). Also (group 2) showed significant increase in phagocytic % and phagocytic index compared to non vaccinated non treated chickens (group 4) through the experiment times. As well as (group 1) showed significant increase in phagocytic % at 3rd and 5th weeks, also at 2nd, 3rd, 4th and 5th weeks in phagocytic index compared to chicken vaccinated with live NDV (group 3).

Table (2): Haemagglutination inhibition titers for NDV in chickens treated with B-glucan-mannan expressed as Geometric mean titer.

Group	Group (1)(vaccinated treated with glucan-mannan)	Group (2)(vaccinated, non treated)	Group (3)(vaccinated with live NDV, non treated)	Group (4)(non vaccinated non treated)
Age				
1 st week of age	2.3	4.6	4	3
2 nd week of age	0	2.3	4.9	2.5
3 rd week of age	16	6.1	4	0
4 th week of age	16	4	3.5	0
5 th week of age	6.8	2	3	0
Challenge	73.3	13.9	48.5	0

Group (1): vaccinated chickens and treated with b-glucan-mannan
 Group (2): vaccinated chickens and non treated
 Group (3): vaccinated chickens with live NDV and non treated
 Group (4): control non vaccinated non treated chickens

Chickens vaccinated and treated with B-glucan-mannan (group 1) showed the highest antibody titers against NDV vaccine among the groups. Higher antibody titers were obvious at 3rd, 4th weeks and after challenge among groups. Chickens vaccinated with live NDV vaccine (group 3) revealed parallel level of antibody titer at 3rd week only compared to group (1).

Table (3) Serum Lysozyme ($\mu\text{g} / \text{ML}$), nitric oxide Serum total antioxidant (mMIL), in different groups chickens.

Group / Time	2 nd week			3 rd week			4 th week			5 th week		
	Antioxidant	Lysozyme	Nitric oxide	Antioxidant	Lysozyme	Nitric oxide	Antioxidant	Lysozyme	Nitric oxide	Antioxidant	Lysozyme	Nitric oxide
Group (1) Vaccinated + B-glucan- mannan	1.0 ± .27	12 ± 2.5	14 ± 0.4 A	1.0 ± 0.10 A	38 ± 5	16 ± 0.2	2.1 ± 0.19 aB	33 ± 7.3 A	15 ± 0.06 a	1.9 ± 0.25 A	28 ± 3.7	16 ± 1.1 aB
Group (2) Vaccinated and non treated	1.1 ± .0.23	18 ± 4.9	19 ± 1.7 aB	1.0 ± 0.10 B	37 ± 5	15 ± 0.8	1.5 ± 0.14 bc	20 ± 4.3 a	18 ± 1.2	1.8 ± 0.19 B	24 ± 4.3	21 ± 0.5 b
Group (3) control vaccinated non treated	0.7 ± 0.13	17 ± 0.5	15 ± 0.20	0.9 ± 0.02	14 ± 2.3	17 ± 1.7	1.2 ± 0.21 A	28 ± 3.7	20 ± 0.9 A	1.5 ± 0.12	28 ± 3.7	23 ± 1.3 A
Group (4) Non Vaccinated Non treated	0.5 ± 0.21	20 ± 3.7	16 ± 0.2 b	0.7 ± 0.14 a b	20 ± 3.7	16 ± 0.2	0.9 ± 0.16 bc	24 ± 4.3	18 ± 1.6	1.0 ± 0.08 ab	24 ± 4.3	19 ± 1.0 a

Aa, Bb significantly different between two comparison groups in the same column against capital letter at $P \leq 0.05$ using least significant difference (LSD).

Serum lysozyme activity was significant elevated at 4th week in group (1) compared to group (2). On the other hand concerning to serum nitric oxide, chicken vaccinated and treated with B-glucan-mannan (group 1) revealed significant decrease in serum nitric oxide concentration at 2nd and 5th weeks compared to chicken vaccinated non treated (group 2). Total antioxidant capacity in chicken vaccinated and treated with B-glucan-mannan (group 1) was significantly increase at 4th week compared to chicken vaccinated non treated (group 2). Also, significant increase at 3rd, 4th and 5th weeks compared to chicken non vaccinated non treated (group 4).

Table (4): Protection rate of different groups of chickens against challenge with NDV (strain vWVDV74):

Group No.	Total no. of birds	Challenge test at 35 days of age		Protection %
		No. of dead birds	No. of survival	
Group (1)	10	0	10	100
Group (2)	10	2	8	80
Group (3)	10	4	6	60
Group (4)	10	10	-	0

Group (1): vaccinated chickens and treated with Glucan-mannan

Group (2): vaccinated chickens and non treated

Group (3): vaccinated chickens with live NDV and non treated

Group (4): control non vaccinated non treated chickens

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الحماية المناعية المستحدثة بحقن خليط من المواد المناعية المنشطة مع اللقاح المزدوج الميت من النيوكاسل الإنفلونزا

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في هذه الدراسة تم تقصي تأثير خليط من المواد المناعية المنشطة على الاستجابة المناعية لكتاكت
التسمين المحصنة بلقاح مزدوج من الإنفلونزا النيوكاسل.

المجموعة الأولى : تم التحصين بلقاح مزدوج من الإنفلونزا النيوكاسل وفي نفس الوقت متزامنا تم
حقنها بخليط من المواد المناعية المنشطة.

المجموعة الثانية : تم تحصينها فقد باللقاح المزدوج.

المجموعة الثالثة : تم تحصينها بلقاح النيوكاسل الحي (هتشنرولاسوتا).

المجموعة الرابعة : مجموعة ضابطة سالبة غير محصنة وغير محقونة بالمواد المنشطة.

جميع المجموعات تم تقييمها أسبوعياً لقياس الاستجابة المناعية السائلة والخلوية وعند عمر 35 يوم تم
عمل اختبار التحدي النيوكاسل شديد الضراوة وتم قياس الأجسام المناعية لللقاح النيوكاسل وكفاءة خلايا
الماكروفاغ الابتلاعية (Phagocytosis) ومستوى أكسيد النيتريك والليوسوزيم في السيرم وقياس مجموع
مضادات الأكسدة وأظهرت اختبارات الحماية الآتي: المجموعة الأولى مستوى حماية 100% المجموعة
الثانية والثالثة 80% و60% على التوالي بالترتيب وتأكد من النتائج أن حقن المواد المنشطة المناعية يعضد
من الاستجابة المناعية لللقاح المزدوج الميت من النيوكاسل و الإنفلونزا.