Vet. Med. J., Giza Vol. 57, No. 4 (2009): 649 – 667.

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

Hussein, H. A.*, Shereen, M. A.**, Sultan, H. A.***, Samah, H. M.**, Nada, A. A.** and El Sanousi, A. A.*

* Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza-Egypt.
** Department of Immunology, Animal Health Research Institute-Dokki Giza.
***Department of Poultry Diseases, Faculty of Veterinary Medicine, Menoufia University-Sadate City-Egypt.

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 (Kemoddle 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 hematopeosis, 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 TNFα, IL-6, IL-8 and monocytes tissue factor (TF). Furthermore, when LPS was added together with this B-glucan TNFα, 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 dendretic 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 interlukin-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 histocompitability 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 strain, 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 =

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

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}×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 heamagglutination 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 denderitic 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 3^{rd} ,4^{th} ,5^{th} 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 2^{nd} week whereas at 3^{rd}, 4^{th} and 5^{th} 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-Sauchez 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 4^{th} 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 signifacntly 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 preserving cellular integrity.

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, particulary 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 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 %**	**Phagoytic index**
Group (1) Vaccinated + B-glucan	A 63 ± 1.9	a B o.16 ± 0.01	a B 63 ± 1.4	a B 0.21 ± 0.01	B 67 ± 3.8	a B 0.17 ± 0.01	a B 53 ±2.3	a B 0.18 ± 0.01
Group (2) Vaccinated and non treated	A 48 ± 4.4	B 0.10 ± 0.02	B 54 ± 2.1	B C 0.12 ± 0.01	b 55 ± 1.6	b 0.10 + 0. 008	b 41 ± 1.2	b C 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	b C 0.08 ± 0.008	a b 47 ± 3.7	b 0.08 ± 0.008	b 37 ± 1.6	b C 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): Heamagglutination inhibition titers for NDV in chickens treated with B-glucan-mannan expressed as Geometric mean titer.

Group Age	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)
1st week of age	2.3	4.6	4	3
2nd week of age	0	2.3	4.9	2.5
3rd week of age	16	6.1	4	0
4th week of age	16	4	3.5	0
5th 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 (μg / ML), nitric oxide Serum total antioxidant (m MIL) , in different groups chickens.

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

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 vvNDV74):

Group No.	Total no. of birds	Challenge test at 35 days of age		
		No.of dead birds	No. of survival	Protection %
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

REFERENCES

Abel, G; Zolosi, J; Chihara, G and Fachet, J. (1989): Effect of lentinan and mannan on phagocytosis of fl. Internationalouroscent latex microbeads by by mouse peritoneal macrophages: flow cytometric study. Int. J. immunopharmacol II : 615.

Abel, G; Czop, J. K. (1992): Stimulation of human monocytes beta-glucan receptors by glucan particles induces production of TNF-alpha and IL-1 beta. Int. J. Immunopharmacol 14: 1363-73.

Adachi, Y, Okazaki, M, Ohno, N, and Yadomae, T. (1994): Enhancement of cytokine production by macrophages stimulated with (1-3)- beta-D-glucan, grifolan (GRN) isolated from Gryolafronosa. Biol Pharm Bull. 17: 1554-60.

Albina, J. E; Abat, J. A and Henry, W. L. (1991): Nitric oxide production is required for murine resident peritoneal macrophages to suppress mitogen-stimulated T-cell proliferation: Role of INF-Y in the induction of nitric oxide synthesizing pathway. J. immunol. 147: 144-18.

Anthony, T.W.C., Trwin, K.M.L., Erin, M.W and Micheal, E.M. (1985): phagocytic and killing capacities of uterine derived leukocytes from more resistant and susceptible to chronic endometritis. Am. J.Vet. Res., 46(9): 1938-1940.

Beard, C.W. (1989): serological procedures. In : laboratory manual for the isolation and identification of avian pathogens published by the American Association of avian pathologists, 3rd Ed., PP 192-200.

Boyum, A.(1968): Isolation of mononuclear cells and granulocytes from human blood Cand. J. clin. Invest., 21: 77-89.

Browder, W; Williams, D; Pretus, H; Olivero, G; Enrichens, F; Mao, P and Franchello, A. (1990): Beneficial effects of enhanced macrophage function in the trauma patient. Ann. Surg. 211, 605-612.

Brown, G.D, and Gordon, S. (2003): Fungal B-glucans and mammalian immunity. Immunity 19:311-5.

Carrow, D.J.(1996): B-1,3-Glucan as a primary immune activator. Townsend Lett 86-91.

Chae, B. J; Lohakare, J. D; Moon, W.K; Lee, S. L; Park, Y. H and Hahn, T. W. (2006): Effects of supplementation of B-glucan on the growth performance and immunity in broilers. Research in veterinary science 80, 291-298.

Chen, H. L; Li, D.F; Chang, B.Y; Gong, L.M; Piao, X. S; Yi, G.F and Zhang, J. X. (2003): Effect of lentinan on broiler splenocyte proliferation, interleukin-2-production, and signal transduction. Poult. Sci. 82 (5): 760-6.

Chu, Yi and Dietert, R.R (1989): Monocyte function in chickens with hereditary dystrophy. Poult. Sci., 68: 226-232.

Clearly, J.A, Kelly,G.E, and Husband, A.J. (1999): The effect of molecular weight and B-1,6-linkages on priming of macrophage function in mice by (1,3)B-D-glucan. Immun Cell Biol. 77:395-403.

Cross, G. G; Hennings, H. J; Whitfied, D. M; Peny, C. L; Zacharie, B and Gagnon, L. (2001): Immunostimulant oxidized-B-glucan conjugates. International immunopharmacology 1, 539-550.

Delatte, S.J, Evans, J, Hebra, A, Adamson, W, Othersen, H.B, and Tagge, E.P. (2001): Effectivness of beta-glucan collagen for treatment of partial-thickness burns in children. J. Pediatr .Surg. 36: 113-8.

Dritz, S.S; Shi, J; Kielian, T.L; Goodband, R. D; Nelsen, J. L; Tokach, M. D; Chengappa, M. M; Smith, J. E and Blecha, F. (1995): Influences of diatery B-glucan on growth performance, nonspecific immunity and

resistance to Streptococcus suis infection in weanling pigs. Journal of animal Science 73, 3341-3350.

Engering, A.I, Cella, M, Fluitsma, D.M, Hoefsmit, E. C, Lanzavecchia, A, and Pieters, J.(1997): Mannose receptor mediated antigen uptake and presentation in human denderitic cells. Adv. Exp. Med. Biol, 417:183-7.

Engstad, C.S, Engstad, R.E, Olsen, J.O, and Osterud, B. (2002): The effect of soluble B-1,3- glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. Int. Immunopharmacol. 2: 1585-97.

Foged, C; Arigita, C; Sundblad, A; Jiskoot, W; Storm, G and Frokjaer, S. (2004): Interaction of dendritic cells with antigen containing liposomes: effect of bilayer composition. Vaccine 22: 1903-1913.

Gou, M; Dai, M; Li, X; Yang, L; Huang, M; Wang, Y; Kan, B; Lu, Y; Wei, Y and Qian, Z. (2008): Preparation of mannan modified anionic PCL-PEC. PCL nanoparticles at one step for bFGF antigen delivery to improve humoral immunity colloids and surface B: Biointerfaces 64: 135-139.

Green, L.C, Wagner, D.A, Glogowski, J, Skipper, P.L, Wishonk, J.S and Tannebaum, S.R. (1982): Anal. Biochem. 126 (1): 131-8.

Hanks, D.H and Wallace, O.H. (1985): Determination of cell viability. Proc. Soc. Exp. Biol. Med., 98: 183-192.

Hashimoto,T, Ohno, N, Adachi, Y, and Yadomae, T. (1997): Enhanced production of inducible nitric oxide synthase by B-glucans in mice. FEMS Immunol Med Microbiol. 19: 131-5.

Hosono, A; Ozawa, A; Kato, R; Ohnishi, Y; Nakanishi, Y; Kimura, T and Nakamura, R. (2003): Dietary fructo-oligosaccharides induce immunoregulation of intestinal IgA secretion by murine peyers patch cells. Biosci-Biotechnol. Biochem. 64, 758-764.

Jeney, G, and Anderson, D.P. (1993): Glucan injection or bath exposure given alone or in combination with a bacterin enhance the non-specific defense mechanisms in rainbow trout (Oncorhynchusmykiss) Aquaculture. 116, 315.

Jung, K; Ha, Y; Ha, S.K; Han, D.U; Kim, D.W; Moon, W.K and Chae, C. (2004): Antiviral effect of Saccharomyces cerevisiae beta-glucan to swine influenza virus by increased production of interferon-gamma and nitric oxide. J. Vet. Med. B. infect. Dis. Vet. Public Health 51 (2): 72-6.

Kayali, H; Ozdag, M.F; Kahrman, S; Aydin, A; Gonul, E; Sayel, A; Odabasi, Z and Timurkayanek, E. (2005): The antioxidant effect of beta-glucan on oxidative stress status in experimental spinal cord injury in rats. Neurosurg. Rev., 28, 298-302.

Kemoddle, D.S, Gates,H, and Kaiser, A. B. (1998): Prophylactic anti-infective activity of poly-{1-6}-B-D-glucopyranosyl-[1-3]-B-D-glucopyranose glucan in a guinea pig model of staphylococcal wound infection. Antimicrobe Agents Chemother. 42:545-9.

Koracevic, D., Koracevic, G., Diordjevic, V., Andreievic, S and Cosic, V. (2001): Methods for the measurement of antioxidant activity in human fluids. J. clin. Pathol. 54(5) 356-361.

Kournikakis, B; Mandeville, R; Brousseau, P and Ostroff, G. (2003): Anthrax-protective effects of yeast beta 1,3 glucan. Med. Gen. Meb 5:1.

Krakowski, L; Krzyzanowski, J; Wrona, Z and Siwicki, A. (1999): The effect of nonspecific immunostimulation of pregnant mares with 1,3/ 1,6 glucan and Levamisole on the immunoglobulins levels in colostrums, selrcted indices of nonspecific cellular and humoral immunity in foals in neonatal and postnatal period. Veterinary

immunology and immunopathology 68, 1-11.

Krizkova, L; Durackova, Z; Sendula, J; Slamenova, D; Sasinkova, V; Sivonova, M and Krajcovic, J. (2003): Fungal beta-(1-3)-Dglucan derivatives exhibit high antioxidative and antimutagenic activity in vitro. Anticancer Research. 23, 2751-2756.

Leblanc, B.W, Albina, J.E, and Reichner, J. S. (2006): The effect of PGG-{beta}- glucan on neutrophil chemotaxix in vivo. J. Leukoc. Biol.

Liang, J; Melican, D; Cafro, L; Palace, G; Fisette, L and Amstrong, R. (1998): Enhanced clearance of multiple antibiotic resistance Staphylococcus aureus in rats treated with PGG-glucan in associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int. J. immunopharmacol. 20: 595-614.

Liew, F. Y. (1995): Regulation of lymphocytes function by nitric oxide. Curr. Opin. Immunol. 7: 396-400.

Ljungman, A.G, Leanderson,P, and Tagesson, C. (1998): (1-3)-B-D-glucan stimulates nitric oxide generation and cytokine mRNA expression in macrophages. Environ Toxicol. Pharmacol. 5: 273-81.

Lowry, V.K; Farnell, M.B; Ferro, P. J; Swaggerty, C. L; Bahl, A and Kogut, M. H. (2005): Purified B-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against Salmonella enterica serovar Enteritidis. International Journal of food Microbiology 98: 309-318.

Mucksova, J, Babicek, K, and Pospisil, M. (2001): Particulate 1,3- beta- D- glucan, carboxymethylglucan and sulfoethyl glucan- influence of their oral or intrperitoneal administration on immunological respondence of mice. Folia Microbiol (Praha) 46: 559-63.

Nakagawa, Y, Ohno, N, and Murai, T.(2003): Supression by candida albicans B-glucan of cytokine release from activated human monocytes and from T cells in the presence of monocytes. J. Infect. Dis. 187:710-3.

Olson, E. J, Standing, J.E, Griego-Harper, N, Hoffman, O. A, and Limper, A. H. (1996): Fungal beta-glucan interacts with vitronectin and stimulates tumor necrosis factor alpha release from macrophages. Infect. Immun. 64: 3548-54.

Pacheco-Sanchez, M; Boutin, Y; Angers, P; Gosselin, A and Tweddell, R. J. (2007): Inhibitory effect of CDP, a polysaccharide extracted from the mushroom collybia dryophila, on nitric oxide synthase expression and nitric oxide production in macrophages Eur. J. Pharmacol. 19;555 (1): 61-6.

Paulsen, S.M; Engstad, R and Robertsen, B. (2001): Enhanced lysozyme production in Atlantic Salmon (Salmo salar L.) macrophages treated with yeast B-glucan and bacterial lipopolysaccharide. Fish and shellfish immunology 11, 23-37.

Peng, L.i; Delbert, M and Gatlin III. (2004): Dietary brewers yeast and the prebiotic Grobiotic ™ AE influence growth performance, immune responses and resistance of hybrid striped bass (Morone chrysops XM. Saxatilis) to streptococcus iniae infection. Aquaculture 231, 445-456.

Petrie, A and Watson, P. (1999): Statistics for Veerinary and Animal Science. 1st Ed., PP. 90-99, the Blackwell science Ltd, United Kingdom.

APostolopoulos, V; Mc Kenzie, I.F. (2001): Role of mannose receptor in the immune response. Curr. Mol. Med. 1:469-74.

Rajaraman, V., Nonnecke, B., Franklin, S., Hammell, D and Horest, R (1998): Effect of vit A and E on nitric oxide production by blood mononuclear leukocytes from

neonatal calves fed milk replacer. J. Dairy. Sci., 81: 3278-3285.

Robertsen, B, Rorstad, G, Engstad, R, and Raa, J. (1990): Enhancement of non- specific disease resistance in Atlantic salmon, solmo salar L., by a glucan from saccharomyces verevisiae cell walls. Journal of fish disease. 13: 391-400.

Roeder, A; Kirscning, C. J; Rupec, R.A; Schaller, M; Weindl, G and Korting, H. C. (2004): Toll-like receptors as key mediators in innate antifungal immunity. Med. Mycol. 42: 485-98.

Sang, H.M; Ky, L. T and Fotedar, R. (2009): Dietary supplementation of mannan oligosaccharide improved the immune responses and survival of marron, cherax Tenuimanus (Smith 1912) when challenged with different stressors. Fish, shellfish immunology 27, 341-348.

Sheng, K.C; Dodie, S. P; Mark, D.W; Choon Kit Tang; Eliada Lazoura; Geoffrey, A.P and Vasso ,A (2006): Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. Immunology, 118: 372-383.

Schltz, L.A. (1987): Methods in clinical chemistry. The C.V. Masby Lost Louis 742-746.

Shashidhara, R. G and Devegowda. (2003): Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. Poultry Science, valu 82, (8), 1319-1325.

Sherwood, E. R, Varma, T.K, Framm, R.Y, Koutrouvelis, A.P, and Toliver-Kinsky, T.E. (2001): Glucan phosphate potentiates endotoxin-induced interferon-γ expression in immunocompetent mice, but attenuates induction of endotoxin tolerance. Clin. Sci. 101: 541-50.

Suzuki, T, Tanaka, H, Kinoshita, A, Oikawa, S, Osawa, M, and Yadomae, T. (1990): Effect of orally administered beta-glucan in macrophages function in mice, International Journal of Immunopharmacology. 12: 675-684.

Suzuki, Y; Adachi, Y; Ohno, N. I and Yadomae, T. (2001): Th1/ Th2- balancing immunomodulating activity of gel-forming (1-3) beta-glucan from fungi. Biol. Pharm Bull; 24:811-9.

Tada, H, Nemoto, E, Shimauchi, H et al.,(2002): Saccharomyces cerevisiae and candida albicans-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. Microbial Immunol. 46:503-12.

Takeshita, K; Watanuki, S; Iida, M; Saite, N; Maruyama, M; Sunagawa, M; Habu, H and Endo, M. (1993): Effect of Lentinan on lymphocyte subsets of peripheral blood, lymph nodes and tumor tissues in patients with gastric cancer. Surg. Today 23, 125-129.

Taylor, P. R, Gordon, S, Martinez-Pomares, L.(2005): The mannose receptor: linking homeostasis and immunity through sugar recognition. Trends Immunol, 26: 104-10.

Vetvicka, V; Dvorak, B; Vetvickova, J; Richter, J; Krizan, J; Sima, P and Vin, J. (2007): Orally administered marine (1-3) B-D-glucan phycarine stimulates both humoral and cellular immunity. International Journal of Biological Macromolecules 40, 291-298.

Wei, D, Williams, D, and Browder, W. (2002): Activation of AP-1 and SP1 correlates with wound growth factor gene expression in glucan-treated human fibroblasts. Int. Immunopharmacol. 2: 1163-72.

Weir, D. M. (1983): Immunology: an outline for students of medicine and biology: 5th Ed 15-16, Churchil Livingstone, London. Melbourne, New York.

White, L.A; Newman, M.C; Cromwell, G.L and Lindemann, M.D. (2002): Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. J. Anim-Sci. 80, 2619-2628.

Williams, D.L(1997): (1-3) beta-D-glucan immunobiology. Med. Inflamm. 6: 247-50.

Wilson, N. S; EL-Sukkari, D; Belz, G. T; Smith, C.M; Steptoe, R. J; Heath, W.R; Shortman, K and Villadangos, J. A. (2003): Most lymphoid organ dendiritic cell types are phenotypically and functionally immature. Blood 102: 2187-94.

Yun, C. H; Estrade, A; Vankessel, A; Park, B. C and Laarveld, B. (2003): Beta-glucan extracted from oat, enhances disease resistance against bacterial and parasitic infection. FEMS Immunol. Med. Microbiol. 35, 67-75.

Zekovic, D.B, Kwiatkowski, S, Vrvic, M.M, Jakovljevic, D, and Moran, C.A. (2005): Natural and modified (1-3)-beta-D-glucan in health promotion and disease alleviation. Crit. Rev. Biotechnol. 25: 205-30.

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

حسين على حسين ،*شيرين محمد عبد الحميد ،هشام سلطان ،*سماح حلمي ،*عبد الفتاح عبد الحميد ندا ، أحمد عبد الغني السنوسي**

قسم الفيروسات - كلية طب بيطري - جامعة القاهرة
* قسم المناعة- معهد بحوث صحة الحيوان- الدقى
** قسم الدواجن - كلية طب بيطري – جامعة المنوفية (السادات)

في هذه الدراسة تم تقصي تأثير خليط من المواد المناعية المنشطة على الاستجابة المناعية لكتاكيت 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 (هتشنروالاسوتا).

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

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