



The impact of β glucan on the Immune Response of Broiler Chickens Vaccinated with NDV and AI H9V Vaccines

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ABSTRACT

To evaluate the effects of β glucan on innate and adaptive immune responses of broilers vaccinated for routine vaccination with Newcastle disease virus (NDV) and Avian Influenza H9N2 (AIV) vaccines. A total of 180 chicks of one-day old Hubbard local breed they were divided into three groups (sixty chicks for each group). The first group fed with normal broiler diet without any additives and served as control group, while the other two groups consumed diets contained (200mg , 400mg pure β glucan /kg diet respectively for 42 days of the experimental period. We noticed that, the administration of β glucan to broiler chickens early in life increased significantly ($p < 0.05$) the nitric oxide levels, lysozyme activity, phagocytic activity, and phagocytic index , in addition improved the oxidative state by decreasing malondialdehyde (MDA) and increasing glutathione (GSH) and. High concentration of β glucan improves immune response to NDV and AIV vaccines.

Keywords: β glucan, Immune response, Broiler chickens NDV, AIV, Vaccines.

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1. INTRODUCTION

Poultry production is a growing and economically an important industry, and therefore, the interest in improving the production results through improved health of the poultry. Fungal biotechnology greatly assists human particularly in immune modulation, and prebiotic β glucans have been characterized as “biological response modifiers” (Huff *et al.*, 2016; Leung *et al.*, 2010; Volman *et al.*, 2018, Novak and Vetvicka, 2017; Soltanian *et al.*, 2009). β - glucan is a group of glucose polymers that consist of β -1, 3 and the β -1, 6 glycosidic linkages. It is a main cell wall structural component of fungi, plants and some bacteria (Jorgensen and Robertsen, 1995). It can

activate lymphocyte, production of inflammatory cytokines and chemokines and microbial killing. This makes the adaptive immunity to be developed (Brown and Gordon, 2013; Brown *et al.*, 2003). β - glucan can stimulate leukocytes and neutrophil function, leading to increase resistance to diseases. This was reported in different animal species such as mammals, amphibians, fish and crustaceans. It has been found that a highly purified β - 1,3/1,6-glucans in diets which extracted from baker's yeast, stimulate the humoral and cellular immune responses and increase disease resistance in many animal species (Sakai, 1999).

Newcastle disease is one of the serious infectious diseases. There is no treatment for Newcastle disease yet, and vaccination is the only way for the control of the disease (Huang *et al.*, 2004) vaccination is either by using active vaccines or inactivated vaccines. Components of the Fungal cell wall have been shown to have immunomodulating effects in humans and animals, and may have implicit as alternatives to antibiotic growth promoters for poultry - β glucan production (Novak and Vetvicka, 2018; Thompson *et al.*, 2019). Little information is available regarding the effect of adding - β glucan to broiler diets on the immune status of broiler chickens. Based on this concept, this study was designed to evaluate the effect of β glucan on innate and adaptive immune responses to routine vaccination with NDV and AIV vaccines, as well as oxidant/antioxidant balance.

2. Materials and methods

2.1. β -glucan:

β -glucan was extracted from baker's yeast (*S. Cerevisiae*) according to the method published by (Williams *et al.*, 1991), and the modified method by (Chaunget *et al.*, 2009). The total concentration of carbohydrates present in the extract was determined according to the method (Dubois *et al.*, 1956) that modified by (Masuko *et al.*, 2015).

2.2. Experimental Design:

One hundred and eighty, 1-day-old broiler chicks (Hubbard local breed) of both sexes, were obtained from a local hatchery and divided into 3 groups 60 chicks each:

Group (1): Chicks fed on normal diet and kept as control.

Group (2): Chicks fed on normal diet mixed with β glucan 200mg /kg diet (low dose).

Group (3): Chicks fed on normal diet mixed with β glucan 400mg /kg diet (High dose).

All groups were vaccinated by bivalent AI-NDV vaccine at 7 day of age, then by Lasota vaccine at 21st day of age.

2.3. Blood Samples:

Heparinized blood samples were taken (5 samples/group) at 5th & 12th day post 1st & 2nd vaccination for separation of mononuclear cells used in phagocytic activity and for detection of malondialdehyde & glutathione at the end of the experiment. Blood samples for serum separation were taken from all groups (5 samples /group) at 2nd day, 1st & 2nd week post 1st vaccination and 2nd day, 1st, 2nd and 3rd week post 2nd vaccination, to detect and titrate antibodies against ND & AI vaccine and for measurement of lysozyme and nitric oxide. The antibody titers against ND and AI H9 must be measured before vaccination to be ensured that the detected antibodies either high or low is due to the effect of vaccination not to the maternal antibodies.

2.4. Evaluation of Innate Immunity:

Assessment of innate immune response by evaluation of phagocytic activity, Lysozyme and nitric oxide production as the following:

2.4.1. Assay of Phagocytosis:

The test was performed according to (Bos and Souza, 2000), peripheral blood mononuclear cell layer was collected, washed and resuspended in RPMI-1640 media supplemented with 15% FCS. Then monolayer of macrophages was obtained by seeding 1ml 5×10^6 mononuclear cells in culture and staining chambers with cover slip and incubated for 1hr at 37° in 5% CO₂ and 99% humidity. Removing the non-adherent cells by washing 3 times, then incubate for 24 hrs, after that the adherent macrophages were incubated at the same condition with 1 ml *Candida Albicans* (10^7 /ml RPMI with 15% FCS), washed 3 times, fixed and stained. Finally calculate the phagocytic% (number of phagocytic macrophages/total number of macrophages) and phagocytic index (number

of macrophages engulf ≥ 3 Candida spores/total no of phagocytic macrophages.

2.4.2. Lysozyme Assay:

Lysozyme activity was measured by agarose gel plate lyses assay according to (Peeters and Vantrappen, 1977). Briefly, Lysoplates were prepared by dissolving 1% agarose in 0.06 mPBS at pH 6.3 in which *Micrococcus lysodeikticus* (50 mg/100 ml agarose) had been dispersed. Then 25 μ l of serum samples and standard lysozyme were added in each well. After 18 hours the cleared zones diameter were measured. The concentration of lysozyme was obtained from logarithmic curve prepared using standard lysozyme solution.

2.4.3. Nitric Oxide Assay:

Carried out according to (Jose *et al.*, 1998) and (Yang *et al.*, 2010) briefly 100 μ l of serum sample was mixed with 80 μ l of 375mM ZnSO₄ and 120 μ l of 275 Mm NaOH, then centrifuged at 13000 rpm for 20 min to remove proteins. Supernatant was obtained and added to 400 mg of Cu plated Cd, then shook for 2.5h at room temperature after adding 100 μ l of 0.2 M glycine buffer. 100 μ l Supernatant was added into 96-well ELISA plate then added 100 μ l of Griess reagent. The optical density was determined at 545 nm with an ELISA plate reader. Nitric oxide concentration was calculated from standard curve using NaNO₂.

2.5. Evaluation of Humeral Immune Response:

2.5.1. Detection of Antibodies Titer to ND&AI: Using Haemagglutination inhibition test (HI) according to (Beard, 1989).

The tested serum samples were serially diluted in PBS (Double fold dilution of 25 μ l volumes starting with 1/2 dilution) using a U-shaped micro titer plate.

Twenty five μ l volumes containing 8 H.A. units of NDV or IV (prepared in PBS) were added to all individual wells of the diluted serum. Serum-virus mixture were allowed to stand at room temperature for 30 minutes to permit antigen-antibody reaction to occur.

Fifty μ l of 0.5% pre-washed chicken RBCs were added in the wells containing 8 H A units of virus. The whole mixture was kept at room temperature for 30 minutes.

The wells containing 100 μ l of PBS-RBCs served as a negative control.

Antibody titer was determined as the reciprocal of the highest dilution of the tested serum that completely inhibits the hemagglutination of the cells with 8 H A units of the virus (button like pattern).

Quality Control

- Known positive serum
- Known negative serum
- Serum and cells without antigen (to detect nonspecific agglutination)
- Back titration of hemagglutination activity of the antigen (to ensure that 8 hemagglutinating virus (HAU) were tested).

2.5.2. Detection of Malondialdehyde and Glutathione:

Malondialdehyde was measured according to (Ohkawa *et al.*, 1979) and glutathione was measured according to (Ellman, 1959) at the end of experiment.

2.6. 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.

3. RESULTS

In this study, we examined the effect of the β glucan on Peripheral blood mononuclear cells, the phagocytic % & index of broiler chickens (Table1) exhibited significant increase in groups (2) and (3) compared to control group at 5th day post 1st & 2nd vaccination. Also at 12th day post 2nd vaccination in group (3), receiving 400mg pure β glucan /kg diet (high concentration).

The impact of β glucan on the Immune Response of Broiler Chickens Vaccinated with NDV and AI H9V Vaccines

Our results for lysozyme (Table 2) showed significant increase at 2 day post 1st vaccination in group3 and at 2 weeks post 2 vaccinations in groups 2, 3 comparing with control group.

Regarding to nitric oxide level in serum (Table 2) there is significant increase at 2nd day post 1st and 2nd vaccination in groups (2, 3).

Concerning to humeral immune response, high dose of β glucan (group3) improve the HI antibody titers for NDV and AIV comparing with that of control group surprised the result

of group (2), broiler chickens fed on diet containing 200mg β glucan/kg diet (low concentration), are fluctuated above and below that of the control group. We notice high level of maternal antibody for NDV and AIV which declined to negligible levels at 21 days of age (Table 3).

Regarding to the effect of β glucan on oxidant and antioxidant status, there is a significant increase in GSH level and decrease in MDA level in 2&3 groups comparing to control group (Table 4).

Table1: Effect of the dietary supplementation of β glucan on phagocytic %& index of Peripheral blood mononuclear cells of broiler chickens.

Parameterstime	Phagocytic %			Phagocytic index		
	Group1	Group2	Group3	Control	Low	High
5 th day post 1st vac.	54±1.67A	59±0.33Ba	65±0.88Ca	0.40±0.03A	0.56±0.02Ba	0.62±0.02Ca
12 th day post 1st vac	53±1.22	54±3.18b	55±2.40b	0.37±0.04	0.36±0.051b	0.41±0.05b
5 th day post 2nd vac.	55.±1.86A	61±0.33Bac	66±0.67Cac	0.41±0.03A	0.60±0.01Bac	0.62±0.02Ba
12 th day post 2nd vac	54±1.67A	56±0.98Aab	62±0.36Bad	0.43±0.04A	0.41±0.02Abd	0.58±0.02Ba
LSD	4			0.05		

Means with different capital letters are significant different between groups

Means with different small letters are significant different between time intervals

Table 2: Effect of the dietary supplementation of β glucan on Serum lysozyme (μ g/ml) and nitric oxide (μ mol/ml) of broiler chickens.

Parameterstime	Lysozyme			Nitric oxide		
	Group1	Group2	Group3	Group1	Group2	Group3
2 nd day post 1st vac.	9.27±1.23Aa	11.12±0.58a	15.5±0.98Ba	20.36±0.33	19.90±0.58a	21.40±0.64a
1 st week post 1st vac	18.76±1.87b	20.93±1.59b	19.56±1.34b	18.96±0.73a	18.96±0.07a	20.33±0.58a
2 nd day post 2nd vac.	23.74±0.87c	26.85±4.77c	24.95±2.77c	21.33±1.71Ab	24.62±1.10Bb	26.70± 0.26Cb
1 st week post 2nd vac	32.72±2.18d	32.75±2.52d	33.28±4.11d	18.90±2.03a	19.43±0.98a	20.09± 0.36a
2 nd t week post 2nd vac	33.96±2.19Ad	43.68±3.97Ba	46.65±3.61Ce	20.13±1.35	21.26±0.03a	21.03±0.76a
LSD	5.41			1.84		

Means with different capital letters are significant different between groups

Means with different small letters are significant different between time intervals

Table 3: Effect of the dietary supplementation of β glucan on HI titer of NDV and AIV in broilers chickens.

Parameter's time	NDV			AIV		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
0 day	7.2 \pm 0.16Ca	4.80 \pm 0.25A	5.20 \pm 0.25A	5.40 \pm 0.16A	5.39 \pm 0.26b	5.43 \pm 0.27b
7 th day post 1 st vac.	4.20 \pm 0.25b	4.29 \pm 0.23Ba	4.9 \pm 0.61Ca	3.40 \pm 0.15A	4.0 \pm 0.25Ba	4.2 \pm 0.17Ca
14 th day post 1 st vac.	4.6 \pm 0.28Ba	5.1 \pm 0.38b	5.8 \pm 1.40b	3.00 \pm 0.21A	2.89 \pm 0.21b	3.15 \pm 0.15b
1 st week post 2 nd vac.	7.1 \pm 0.26A	6.7 \pm 0.31Bac	7.9 \pm 0.54Cac	3.20 \pm 0.19A	3.01 \pm 0.19Bac	5.43 \pm 0.61Ba
2 nd week post 2 nd vac.	7.4 \pm 0.22A	7.8 \pm 0.48Aab	8.2 \pm 0.34Bad	5.10 \pm 0.20A	5.00 \pm 0.22Abd	5.60 \pm 0.12A
3 rd week post 2 nd vac.	7.5 \pm 0.20b	7.7 \pm 0.27A	8.4 \pm 0.29b	5.12 \pm 0.24A	5.18 \pm 0.24A	6.98 \pm 0.26b
LSD	1.2			0.09		

Means with different capital letters are significant different between groups

Means with different small letters are significant different between time intervals

Table 4: Effect of the dietary supplementation of β glucan on Glutathione and Malondialdehyde.

Parameters-groups	Glutathione Mmol/l	Malondialdehyde Mmol/l
Group 1	3.82 \pm 0.09a	16.19 \pm 0.02a
Group 2	4.43 \pm 0.05b	10.63 \pm 0.31b
Group 3	4.49 \pm 0.09b	11.23 \pm 0.34b

Means with different small letters are significant different between groups

4. DISCUSSION

Recent researches and development of β glucan products have been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection and improved immune status in broiler chicks. The consumption of a probiotic in combination with a suitable prebiotic (β glucan) can result in synergistic effects (Zanoni *et al.*, 2008).

In this study, we examined the effect of the β glucan on Peripheral blood mononuclear cells, the phagocytic % & index of broiler chickens (Table1) exhibited significant increase in groups (2) and(3) compared to control group at 5th day post 1st& 2nd vaccination. Also at 12th day post 2nd vaccination in group (3), receiving 400mg pure β glucan /kg diet (high concentration). These results agree with previous findings (Schiffirin *et al.*, 1997; Panigrahi *et al.*, 2014; Diaz-Rosales *et al.*, 2016) which recorded that probiotic including β glucan increases the activities of phagocytes,

also with (Shimada *et al.*, 2009) who reported that β glucan act on macrophages activity in a dose dependent manner. The activities of phagocyte may be explained as, the bacterial cell or bioactive peptide released during fermentation by lactic acid bacteria activate the immune response through a dynamic interaction with specific Toll-like receptors on the surface of macrophage (it was known that the phagocytosis by macrophages is Toll-like receptors dependent) this interaction between host cells and pathogens or their structural components may play a critical role in the early innate immune response. The activation of the TLRs starts signaling cascades that involve the activation of proteins and transcription factors inducing the secretion of proinflammatory & effectors cytokines which farther activate macrophage cells (Blander and Medzhitov, 2004).

Lysozyme was known to be one of Lysosomal enzyme which attacks mucoprotein in cell

walls of various bacteria and a member of innate humeral factors that elaborated from polymorph nuclear and mononuclear cells (Moore *et al.*, 2006). Our results (Table 2) showed significant increase at 2 day post 1 vaccination in group3 and at 2 weeks post 2 vaccinations in groups 2, 3 comparing with control group. These results are in agreement with (Schiffrin *et al.*, 1997) and (Weir, 1983) who recorded that, the β glucan increases the activities of lysozyme due to activation of phagocytic macrophage.

Regarding to nitric oxide level in serum (Table 2) there is significant increase at 2nd day post 1st and 2nd vaccination in groups (2, 3). Nitric oxide is generated during immune and inflammatory response; it is involved in innate immunity as a toxic agent towards infectious organisms and can induce or regulate death and function of host immune cells (Coleman, 2011). It is produced at high levels by macrophages through its activation (Aouatef *et al.*, 2002).

Concerning to humeral immune response, high dose of β glucan (group3) improve the HI antibody titers for NDV and AIV comparing with that of control group while the result of group (2), broiler chickens fed on diet containing 200mg β glucan/kg diet (low concentration), are fluctuated above and below that of the control group. We notice high level of maternal antibody for NDV and AIV which declined to negligible levels at 21 days of age. These results are in agreement with that of (Maassen *et al.*, 2000) who recorded that, oral administration of β glucan significantly enhance IgG response, also to (Haghighi *et al.*, 2016) who found that β glucan enhance the systemic antibody response to some antigens in chickens and (Talebi *et al.*, 2008) who found that administration of β glucan improve the antibody responses to ND. It is possible that, binding of structural components of Commencal bacteria to Toll-like receptors (TLRS) expressed on the surface of

macrophage and dendritic cells in the lamina propria may lead to their activation and differentiation. Upon its activation, they promote the activation and differentiation of different subsets of other immune system cells, leading to the production of cytokines such as IL4, IL10 and transforming growth factor β , that are important for antibody production and isotope switching (Di Giacinto *et al.*, 2005; Mohamadzadeh *et al.*, 2005). Regarding to the effect of β glucan on oxidant and antioxidant status, there is a significant increase in GSH level and decrease in MDA level in 2&3 groups comparing to control group (Table 3). Our results may be due to antioxidative activity of polysaccharides (constituent of β glucan), these results were partially agree with studies that described antioxidative activity of yeast cell wall polysaccharides (Songisepp *et al.*, 2004; Kullisaar *et al.*, 2012) and are confirmed by (Kai Truusalu *et al.*, 2008) who showed that the administration of the mannan-oligosaccharide significantly reduce MDA values. Also by (Hutt *et al.*, 2009) that showed increase of GSH after the consumption of the β glucan.

5. Conclusion

This study provides evidence that the oral administration of β glucan (low & high doses) to broiler chickens early in life enhances innate immunity which represented by significantly increase phagocytic activity, lysozyme activity and nitric oxide in dose dependent manner. The administration of β glucan in high dose improves humeral immune response represented by increase antibody response to NDV and AIV vaccines. The administration of β glucan in both doses improved the oxidative state of broiler chickens by increase GSH and decrease MDA due to antioxidative activity of the used product.

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