

Study the Immunomodulatory effects of Beta - Glucan in broiler chickens

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Summary

This study was carried out to investigate the immunomodulating activity of β -glucan which extracted from the cell wall of *Saccharomyces cerevisiae*. β -glucan of 225 μ g/ml was supplemented in drinking water of broiler chicken vaccinated with Newcastle disease virus (NDV) vaccine. The parameters of the immunomodulating activity employed were the body weight, Phagocytic activity and determination of antibody titer to NDV vaccine. Chicks one day old (120), were divided into four equal groups, G1 treated with β -glucan for six weeks and vaccinated with NDV vaccine; G2 treated with β -glucan for three weeks and vaccinated with NDV vaccine; G3 not given β -glucan but vaccinated with NDV vaccine (control group); while G4 was not treated with β -glucan and not vaccinated with NDV vaccine (second control). The results of the body weight indicated that; there were significant differences ($P < 0.05$) between treated groups (G1 and G2) compared to the control groups (G3 and G4) at 21,28 and 35 day of age. The results of phagocytic activity showed that treated group had significantly ($P < 0.05$) higher clearance of carbon particles from blood circulation than did the control groups; and the antibody titer to NDV showed significant differences ($P < 0.05$) between treated and non treated groups at 14d and 28d. The data presented in this study contribute for the first time in Iraq; that β -glucan given via drinking water to chicks from day one for 35 days improves the immune responses and body weight.

دراسة تأثير البيتا كلوكان كمعدل مناعي في أفرخ اللحم

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الخلاصة

أجريت هذه الدراسة لمعرفة فعالية المعدل المناعي بيتا كلوكان المستخلص من جدار الخلية لخميرة *Saccharomyces cerevisiae* عند إعطائه مع ماء الشرب لأفرخ الدجاج الملقحة بلقاح فيروس مرض نيوكاسل. أستخدم البيتا كلوكان بتركيز 225 μ g/ml, وأستخدم مقياس وزن الجسم, الفعالية البلعمية ومعيار الاجسام المضاده بعد التلقيح بلقاح النيوكاسل كمؤشر لفعاليتيه كمعدل مناعي. قسمت 120 فرخه بعمر يوم الى أربعة مجاميع متساوية. المجموعه الأولى أعطيت البيتا كلوكان لمدة 6 أسابيع ولقحت بلقاح النيوكاسل, المجموعه الثانيه أعطيت البيتا كلوكان لمدة 3 أسابيع ولقحت بلقاح النيوكاسل, المجموعه الثالثه (سيطره) لم تعطى البيتا كلوكان ولكن لقحت بلقاح النيوكاسل أما المجموعه الرابعه (مجموعه السيطره الثانيه) لم تعطى البيتا كلوكان ولقاح النيوكاسل. أوضحت نتائج أوزان الجسم وجود فرق معنوي بمستوى $P < 0.05$ بين المجاميع الاولى والثانيه مقارنة مع مجموعتي السيطره بعمر 21,28 و35 يوم؛ وبينت نتائج الفعالية البلعمية أن المجاميع المعامله كانت أكثر قابليه لأزاله جزيئات الكربون من الدوره الدمويه عن فعالية الخلايا في مجاميع السيطره كذلك أظهرت نتائج معيار الاجسام المضاده للقاح النيوكاسل فرق معنوي بمستوى $P < 0.05$ بين المجاميع المعامله ومجموعتي السيطره بعمر 14 و28 يوم. الإثباتات التي توصل اليها البحث ولأول مره في العراق؛ أن أعطاء البيتا كلوكان مع ماء الشرب للأفرخ ابتداء من اليوم الأول لمدة 35 يوم أدى الى تحسين الاستجابة المناعية ووزن الجسم.

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 has been of great assistance to human particularly in immune modulation and prebiotic β -1,3/1,6-glucans have been characterized as "biological response modifiers" (1,2,3,4,5).

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 (6). It can bind to various types of cell surface receptors including lectins, scavenger receptors and

intergrins on monocytes, macrophages, neutral killer cells, neutrophils and lymphocyte populations, resulting in activation of lymphocyte, production of inflammatory cytokines and chemokines and microbial killing. This lead to the development of adaptive immunity (7,8). β - glucan can stimulate the neutrophil function, leading to disease resistance. This has been reported in different animal species such as mammals, amphibians, fish and crustaceans. It has been found out, highly purified β - 1,3/1,6-glucans in diets which had extracted from baker's yeast, stimulate cellular and humoral immune responses and increase disease resistance in a number of fish species (9). Improving the disease resistance of animals grown without antibiotics will not only benefit the animals' health, welfare, and production efficiency but is also a key strategy in the effort to improve the microbiological safety of poultry products. Newcastle disease is one of the serious infectious diseases. There is no treatment for Newcastle disease yet; vaccination is the only major measure for the control of the disease (10) vaccination is either by using live vaccines or inactivated vaccines. Fungal cell wall components have been shown to have immunomodulating effects in humans and animals, and may have potential as alternatives to antibiotic growth promoters for poultry production (11, 12). The aim of this study focuses on studying the effect of the β -glucan extracted from the cell wall of the yeast *Saccharomyces cerevisiae*, that supplemented with the drinking water of broiler chickens to improve the immune response against NDV vaccine; and its effect on the growth performance.

Materials and Methods

β -glucan

The β -glucan used in this study was extracted from baker's yeast (*S. cerevisiae*) according to the method published by (13), and the modified method by (14). The total concentration of carbohydrates present in the extract was determined according to the method (15) that modified by (16); the value was 7.5 mg /ml.

Animals and Experimental Design

Day old broiler chicks (Breed: Rose, Belgium Origin) were brought in good condition from Sarmmed Hatchery-Wasit- Al-swera. The chicks weight 37 gm. The experiments were done in the animal house of the College of Veterinary Medicine-Baghdad University, which were maintained in the same condition; after cleaning and disinfection with sodium hypochlorite then fumigated by (Long life 250)^R. All chicks were fed on rations formulated to meet the nutrient requirements of broilers (17). Feed and water was provided on an *ad libitum* basis.

Broiler chicks were vaccinated with NDV vaccine La Sota strain (Intervet – netherland, holland) by manual oral drench 10 days old regarding to the ELISA Abs titer against NDV (maternal immunity), the second NDV vaccine applied after ten days (20 day old) .

Experiment 1

This experiment was a preliminary trial to determine the inclusion rate (percentage V/V) of the β -Glucan extract in the drinking water to determine if there is any adverse effect on the performance of chicks for 3weeks. At 7, 14 and 21 day, the performance index was carried out to determine the feed intake and body weight.

Forty day-old chicks were randomly divided into 4 groups of 10 birds each. Chicks in groups G1, G2 and G3 were given drinking water supplemented with 1% (75 μ g/ml), 3% (225 μ g/ml) and 5% (375 μ g /ml) of β -Glucan extract, respectively; while G4 served as a control(drinking water only).

Experiment 2

This experiment was designed to determine the effect 225 μ g/ml β -glucan in drinking water on the body weight, the Phagocytic activity and NDV antibody titer.

One hundred and twenty chicks one day old were divided into four groups each group (30) birds and treated as follow for 6 weeks:

Group 1: β -glucan added directly to drinking water for 6 weeks and vaccinated with NDV vaccine.

Group 2: β -glucan added directly to drinking water for 3 weeks only and vaccinated with NDV vaccine.

Group 3: drinking water only (control 1) for 6 weeks and vaccinated with NDV vaccine.

Group 4: drinking water only (control 2) for 6 weeks and not vaccinated with NDV vaccine.

Phagocytic activity by Carbon Clearance Assay

Carbon (black India ink) was injected (100 μ L/bird) into the brachial vein of 6 chicks/group at 7, 14 and 21 day of age. The concentration of carbon particles at 0 and 15-min intervals was measured in plasma samples via a microplate reader at 650 nm, as previously described (18).

Blood Samples for the detection of NDV antibody

Five birds/ group were killed at 2 days of age for blood sample collection. At ages of 14, 21 and 28 day post-vaccination blood samples were collected from wing vein by disposable syringe, blood was collected in plain glass test tubes and left in room temperature for 2 hours then centrifuged at 3000 rpm for 10 min; after that the serum was collected and stored at -20°C until used to determine the NDV antibody.

Indirect ELISA test for the detection of NDV antibody

The procedure used in this test was performed according to the manufacturer instructions listed in the ProFLOK® NDV ELISA Kit (Synbiotics–USA).

Statistical Analysis

Data were analyzed statistically by using the analysis of variance (ANOVA) and least significant differences (L.S.D.).

Results

Results of Experiment 1

The result in Table-1 show; there was a difference in food intake between groups supplied with different concentration (75 μ g/ml), (225 μ g/ml) and (375 μ g /ml), respectively.

Table-1: Effect of difference concentration of β -glucan extract on average of Feed intake (gram /bird weekly) .

weeks Groups	Gram / bird weekly		
	<u>1 week</u>	<u>2 week</u>	<u>3 week</u>
G 1	228	405	479
G2	233	416	490
G3	230	414	484
G4	220	400	463

G1= treated with 75 μ g/ml β -glucan. G2= treated with 225 μ g/ml β -glucan
G3= treated with 375 μ g /ml β -glucan G 4= control not treated

Table - 2 show the result of the body weight in treated groups with different concentration of β -glucan supplementation in drinking water as compared to the control group. There was no difference between groups at one day old; but there was difference between treated and non treated control at 7 days of age with significant difference $P < 0.05$ at 21 days; also there was significant difference between treated G2 compared with other treated groups .

Table-2: Effect of different percentage of β -glucan extract on body weight.

Groups	Body weight(g) Means \pm SD		
	1 day	7 days	22 day
G1	38.8 \pm 0.58 A c	129 \pm 0.54 B b	724.4 \pm 2.6 C a
G 2	38.7 \pm 1.64 A c	135.4 \pm 1.28 A b	826.00 \pm 2.12 A a
G 3	38.4 \pm 0.67 A c	132.6 \pm 0.87 A b	814.4 \pm 3.13 B a
G 4	38.8 \pm 1.48 A c	121 \pm 1.14 C b	688.40 \pm 5.46 D a

The different capital letters in column refer to significant difference ($P < 0.05$) among groups; and different small letter refer to significant difference ($P < 0.05$) among period. Means \pm SD (n=6). G1= treated with 75 μ g/ml β -glucan. G2 =treated with 225 μ g/ml β -glucan.G3 =treated with 375 μ g /ml β -glucan. G4 =control not treated.

Results of experiment 2

Body weight

The data in Table -3 show there was a significant difference ($P < 0.05$) in the body weight of treated groups (G1 and G2) as compared to control groups (G3 and G4) at 21, 28 and 35 day of age; with a high significant difference in G1 at 28 and 35 day of age.

Table- 3: Effect of β -glucan extract on body weight of chicks that supplemented with β -glucan in drinking water as compared to control group.

The different capital letters in column refer to significant difference ($P < 0.05$) among groups and different small letter refer to significant difference ($P < 0.05$) among period. Means \pm SD (n=6)

Day Groups	Body weight (g) Means \pm SD				
	7 days	14 day	21day	28day	35day
G1	132.16 \pm 1.35 A e	399.83 \pm 2.61 A d	845.66 \pm 3.47 A c	1576.5 \pm 5.81 A b	2485 \pm 2.23 A a
G 2	129.50 \pm 2.83 A e	402.16 \pm 2.56 A d	839.66 \pm 6.9 A c	1463 \pm 6.01 B b	2231 \pm 7.74 B a
G 3	127.33 \pm 1.14 B e	382.5 \pm 2.18 B d	790.16 \pm 5.02 B c	1405 \pm 3.92 C b	2091 \pm 4.69 C a
G 4	128. \pm 2.18 B e	369.83 \pm 1.7 C d	736.33 \pm 3.62 C c	1379 \pm 4.21 D b	1996 \pm 3.2 D a

G 1= treated with β -glucan 225 μ g/ml for 6 week and vaccinated with NDV vaccine.

G2 =treated with β -glucan 225 μ g/ml for 3 week and vaccinated with NDV vaccine.

G3= control vaccinated with NDV vaccine only.

G 4= control not treated and not vaccinated with NDV vaccine

Phagocytic activity

Phagocytic activity was determined at 7, 14 and 28 day of age, and the data are summarized in Figure -1. A comparative decline in the OD of carbon particles in the plasma indicates higher carbon clearance by the cells phagocytic system. The treated group was significantly ($P = 0.05$) higher clearance of carbon particles at 15 min post carbon injection than did the control groups at 7, 14 and 28 days.

Furthermore, in treated group G1 demonstrated an increase clearance of carbon from the blood circulation than G2 at 28 day of age.

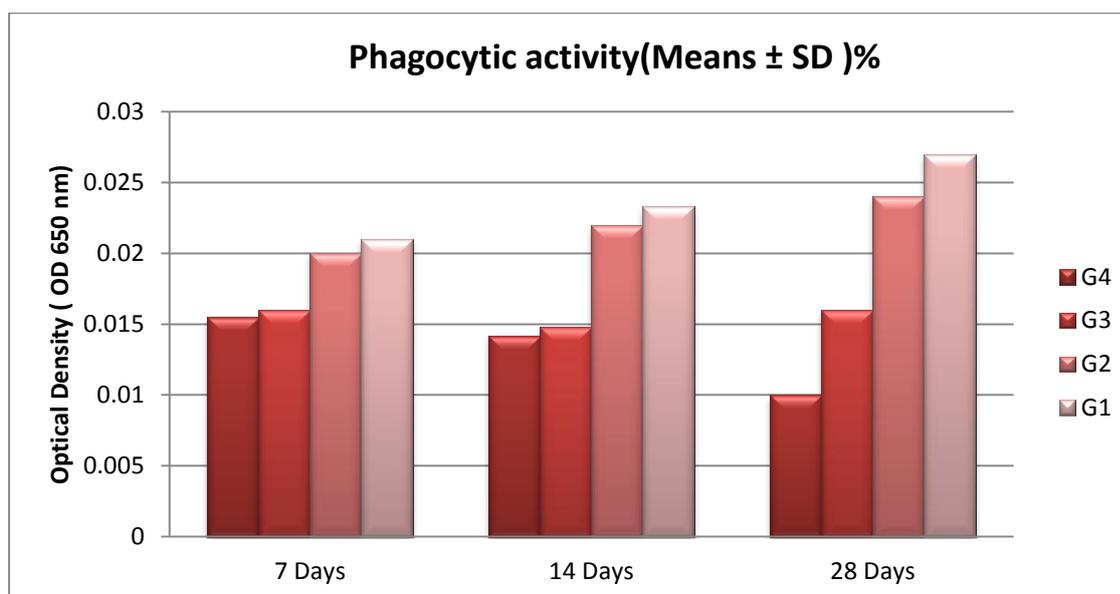


Figure -1: Percentage carbon particles in the blood circulation of treated group as compared to non treated control groups. Means ± SD (n=6)

Antibody titer to NDV vaccine

Table -4 show the mean antibody titers to NDV vaccine measured at 2, 14 and 28 day of age. The vaccine antibody titers of G1, G2 and G3 have increased with age and have shown a significant difference ($P < 0.05$) at days 14 and 28 days as compared to G4; also there was a significant difference ($P < 0.05$) between the treated groups (G1 and G2) in comparable to G3 at 14 and 28 days; as well as between the treated groups (G1 and G2) at 28 day.

Table-4: Effect of supplementation β -glucan (225 $\mu\text{g/ml}$) on antibody titer for NDV vaccine for each group.

Days	NDV antibody titers (Mean±SE)		
	2 day	14 days	28 days
Group 1	5434 A	2728 ± 3.42 B c	3479 ± 6.34 C b
Group 2	5434 A	2731 ± 0.92 B c	3072 ± 8.95 D b
Group 3	5434 A	2129 ± 2.09 C c	2911 ± 7.55 B b
Group 4	5434 A	1838 ± 2.41 A b	171.25 ± 5.73 A c

The different capital letters in column refer to significant difference ($P < 0.05$) among groups and different small letters refer to significant difference ($P < 0.05$) among period. Means ± SD (n=6).

Discussion

In the current study; the results of experiment 1 were in agreement with the study in 1995 (19); who reported that β -glucan has an increase in the average feed intake and weight gain. The mean body weight in G3 was significantly lower than G2, because the higher level of β -glucan does not always lead to the better production parameters (20). The result of experiment 2 indicated an improvement in body weight in G1 and G2; this may be due to the reducing the incidence and severity of subclinical infections (21). The β -glucan components might stimulate the gut-associated immune system by acting as a nonpathogenic microbial antigen, giving an adjuvant-like effect; the importance of digestive microbial antigen stimulation on the development of lymphoid organ tissue (22, 23). β -glucans could enhance

broiler performance especially under unhygienic conditions (1, 24, 25). The enhancement of β -glucan can be explained in part by the improvement of intestinal function or gut health through the increase of villi height, uniformity and integrity. β -glucans have been shown to improve immune response and to block bacterial adhesion (especially enteric pathogens) to gut lining (26).

The results of Phagocytic activity showed that G1 demonstrated an increased clearance of carbon from the blood circulation than G2 at 28 day of age; such a β -glucan effect on macrophages is lost quickly because of the reduction of membrane associated β -glucan receptor (27). This means that treated groups have maintained the nonspecific first line of defense mechanisms, as indicated by a significantly better carbon clearance from circulation when compared with non treated groups. The main action of β -glucan is to enhance phagocytosis and proliferation of monocytes and macrophages (28). The β -glucan has the ability to activate phagocytic activity of macrophages and PMNCs by binding to specific receptors either CR3 or Dectin-1 (29). In addition, β -glucan binding triggers intracellular processes, characterized by the respiratory burst after phagocytosis of invading cells (formation of reactive oxygen species and free radicals), the increase of content and activity of hydrolytic enzymes, and signaling processes leading to activation of other cells and secretion of cytokines (30).

The results of antibody titers against NDV vaccine as shown in Table-4; there was no significant difference ($P>0.05$) in the maternal antibody titre between all groups. The maternal antibodies have reduced in all groups during the time before vaccination; this finding is in agreement with other studies (31, 32) that each two fold decay in maternally derived HI antibody titre takes about 4.5 days. After vaccination, the vaccine antibody titers of G1, G2 and G3 have increased with age and have shown a significant difference ($P<0.05$) at days 14 and 28 days as compared to G4; also the vaccine antibody titer have shown a significant difference ($P<0.05$) between the treated groups (G1 and G2) in comparable to G3 at 14 and 28 days; as well as in groups (G1 and G2) at 28 day. Therefore it can be suggested that soluble β -glucan supplemented in drinking water showed high immunomodulatory property at a 225 $\mu\text{g/ml}$ concentration. In studies (9, 33); have been found out that highly purified β -1,3-1,6-glucans in diets stimulate cellular and humoral immune responses and increase disease resistance in a number of fish species and weaned piglets. Dietary supplementation of β -glucan increased the production of antibodies against NDV and IgA concentrations in the intestinal and tracheal mucosa (34); also β -glucans increase the concentration of serum IgG, IgM, and A (35); these results, taken together, provide new and clear evidence that β -glucan has an immunoregulatory effect at the local and systemic level (36).

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