Effects of using soluble Beta-glucan on immune responses against infectious bronchitis disease in broiler chicks

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Abstract

The study was conducted to investigate the effects of soluble β-glucan derived from yeast cell wall isolated from *Saccharomyces cerevisiae* in drinking water on immune response post routine vaccination and challenge with local isolates of infectious bronchitis virus. Four hundred Ross 308 broiler chicks (from 1 to 40 days old) were divided randomly into four equal groups (diet and drinking water offered ad libitum). G1 was given 225µg/ml of soluble β-glucan in drinking water from day 1 to the end of the experiment, and vaccinated with H120 vaccine at one day old by intranasal and ocular drop methods, and Ma5 strain vaccine at 14 days old by spray method. G2 was received 225µg/ml of soluble *β*-glucan in drinking water from one day to the end of the experiment and considered as control positive. G3 was vaccinated as G1 but without given soluble *β*-glucan, and G4 considered as control negative (not vaccinated not treated). Results showed that G1 was given significant ($P<0.05$) increase of antibody titer and gave high production against challenge and increased in IL2 level compared to other groups. In conclusion, we found that the soluble β-glucan enhanced the cellular and humoral immunity against IBV.

Key words: Infectious bronchitis, β-glucan, ELISA, immune response, broiler chicks.

تأثير استخدام مادة البيتاكلوكان على الاستجابة المناعية ضد مرض التهاب القصبات المعدي في فروج اللحم

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير بيتاكلوكان السائل المستخلص من الخميرة على الاستجابة المناعية قبل وبعد التحدي بالحمة الضارية لمرض التهاب القصبات المعدي المعزولة محليا. تم استخدام 400 فرخه اللاحم من سلالة روس 308 من عمر (1-40) يوم تم تقسيمها عشوائيا الى اربعة مجاميع متساوية. المجموعة الاولى: تم إعطائها بيتاكلوكان السائل من عمر يوم إلى نهاية التجربة مع ماء الشرب بجرعة 225 ملغ / مل ولقحت بعترة H120 باليوم الأول بواسطة التقطير بالعين والمنخر وعترة Ma5 بواسطة الرش بعمر 14 يوم. المجموعة الثانية أعطيت بيتاكلوكان السائل فقط من عمر اليوم الأول إلى نهاية التجربة بنفس جرعة المجموعة الأولى واعتبرت مجموعة سيطرة موجبة. المجموعة الثالثة: لقحت بنفس برنامج المجموعة الأولى ولكن بدون بيتاكلوكان السائل. المجوعة الرابعة: جعلت مجموعة سيطرة سالبة (لم تلقح ولم تعالج) .اشارة النتائج الى ان استخدام مادة بيتاكلوكان السائل مع ماء الشرب في فروج اللحم في المجوعة الأولى الملقحة أعطت مستوى عالي من الأضداد وكذلك أعلى استجابة مناعية ضد حمة التهاب القصبات المعدي، كما لوحظ زيادة معنوية في مستوى IL2 .مما يدل على ان مادة بيتاكلوكان السائل تسبب زيادة في المناعة الخلوية والخلطية في فروج اللحم.

الكلمات المفتاحية: التهاب القصبات المعدي ، بيتاكلوكان ، الاليزا ، الاستجابة المناعية ، فروج اللحم.

Introduction

Infectious bronchitis (IB) is first described by Schalk and Hawn in 1931 in North Dakota as a new respiratory disease of chickens. It is an acute highly contagious disease of chickens caused by a virus belonging to the genus Corona virus of the

family *Coronaviridae* characterized by depression, tracheal rales, sneezing, coughing, wet frothy eyes with conjunctivitis and high morbidity and mortality as well as secondary infections (1). In layer the disease cause poor of weight gain, feed efficiency, decrease in egg production, egg quality and mortality causing significant economic loses to the poultry industry in worldwide (2). Out breaks of IB frequently occurs in the field in many countries because the virus has a tendency of frequent mutations many variants strain with changes in the genome have been identified (3). The mutations are natural mechanisms of defense of the virus, trying to avoid the immune system defenses in chickens. These resulting "variant" strains have new neutralizing epitopes that may not be recognized by the antibodies exerted by the use of the current vaccines the presence of cross-protection produced by some IBV serotypes against antigenically unrelated strains (variants) is unpredictable (4). In spite of use of many vaccination programs include both commercial live and inactivated oil adjuvant vaccines there is continued outbreak of infectious bronchitis disease in reared poultry farms which give raise for need of more effective vaccination program in protecting birds against challenge (5). β-glucan derived from yeast cell wall isolated from *Saccharomyces cerevisiae* are promising alternatives to antibiotics, as they have been shown to improve growth performance has been shown to act as a potent non-specific immune-activator, and further efforts resulted in the development of a water-soluble pharmaceutical grade yeast β-glucan whose biological effects have been extensively studied in vivo. The best known effects of β-glucan consist of the direct stimulation of phagocytosis of professional phagocytes (6). β-glucan can modulate the immune response in chick to stress (disease, heat stress, and transport) as evidence by maintenance of immune function (7).The aim of the present study to determine the protective efficacy provided with vaccinal program that supplemented versus non supplemented with soluble β-glucan by evaluate the cellular and humoral immune responses.

Materials and methods

Four hundred, one day old (Ross 308) broiler chicks from Sallam hatchery/Babel province, were divided randomly into four equal groups supplemented all management requirements as poultry hygiene standardization. G1 was given 225µg/ml of soluble β-glucan in drinking water from day 1 to the end of the experiment, and vaccinated with H120 vaccine at one day old by intranasal and ocular drop methods, and Ma5 strain vaccine at 14 days old by spray method**.** G2 was given 225µg/ml of soluble β-glucan in drinking water from one day to the end of the experiment, without vaccination and considered as control positive. G3 was vaccinated as G1 but without given soluble β-glucan, and G4 considered as control negative (not vaccinated not treated). Blood samples were collected from jugular vein at 1, 7, 14, 21, 28, 35 and 40 days to determine the antibody titer against IBV by ELISA test (Indirect method), and at 18, 28 and 35 determine the chicken IL2 by ELISA kit used sandwich-ELISA method to determined of Chicken IL-2 concentrations in serum according to manufacturer instructions (Elabscieence-Catalog No: E-EL-Ch0120 96T www.Elab-science). All groups were challenge with virulent local isolate of IBV $1\times10^{6.5}$ ELD50/ml at 28 days old.

Results

Antibody response by ELISA against IBV

Results were showed that the titer of antibody in chickens vaccinated with commercial IB vaccines at different ages during the period of 40 days were highly variable. The antibody titers in G1 and G3 revealed significant ($P<0.05$) differences compared to the control, G2 and G4 groups. After challenge at the age of 28 days, G1 group showed the highest antibody titer (high production) at the age of 35 and 40 days (2790.8 and 4160.4) among other groups;

these differences were highly significant (P<0.05) (Table 1).

clinical signs

G1 was display the least clinical signs and the low mortality percentage, followed by G3 which recorded medium percentage of signs in comparison with G2 and control groups which registered the high percentage in clinical signs and mortality (Table 2).

IL2 detection by ELISA test

Table 3 show presence of significant (P<0.05) difference between all groups in IL2 titer at (18, 28 and 35) days old chicks. At day 18th the higher means (191) was recorded in G1, followed by (165.2) in G3, but the less mean values were recorded in G2 and G4 which were (150.2 and 145.8) respectively. Also the results at day 28th revealed a significant increase, so the higher mean (186.4) was recorded in G1, followed by 163.4, 145.8 and 135.2 in G3, G2, and G4 respectively. These results reflected a significant decrease than the results of the day 18th, while it revealed a significant increase among all groups at day 35th compared with 18th and 28th days old chicks. So the highest mean level (220.2) was given by G1, followed by (197.8 and 192.6) in G3 and G2 respectively, compared to (162.6) in G4.

Table (1): Antibody titer by ELISA test against IB in different ages (per days).

IBV antibody titer (Means ± SE) n=10						
Age / Groups	7 Days	14 Days	21 Days	28 Days*	35 Days	40 Days
G1	3844.2±67.6 A	2314.4±23.5 A	3398.6±24.4 A	3877.2±33.8 A	2790.8±42.3 A	4160.4±64.4 A
G2	2495±10.1 C	1781.8±33.7 C	986.4±22 C	469±3.7 C	0±0 C	1105.6±33.6 C
G3	3224.6±85.05 B	2046.4±42.7 B	2860.2±15.7 B	3167±29 B	1884.6±31.3 B	3742.4±62.07 B
G4	2464±22.17 C	1626.6±25.3 D	506.2±25.9 D	449.2±25.9 C	0±0 C	637.4±41.5 D

The different capital litter in columns refer to significant differences (P<0.05) among groups.

Table (2): Development of clinical signs and mortalities during 5 days post challenge with IBV at 28 days of age.

Groups / Days (C.S.)	G1	G2	G3	G4
1dpc	0	0	0	0
2dpc	0	0	0	0
3dpc	0	2	1	6
4dpc	1	8ab	3a	6abc
5dpc	3a	10cde	6b	10def
Death NO.	1	5	2	6
Sign %	30	100	60	100
Mortality %	10	50	20	60

pc = post-challenge, Days (C.S.) = day of clinical signs, N=10. Clinical signs = include one or more signs of depression, lacrimation, slight shake head, swollen head, soft dropping, respiratory signs. (a, b, c, d, e, f, g) chicks were died in day post challenge.

Table (3): Serum levels of IL2 of the experiment detected by sandwich-ELISA method

Serum levels of IL-2 (Means ± SE) n=5			
Age groups	18 days	28 days*	35 days
G1	191±1.43 A	186.4±2.12 A	220.2±2.23 A
G2	150.2±1.65 C	145.8±1.23 C	192.6±2.53 B
G3	165.2±1.3 B	163.4±1.2 B	197.8±3.43 B
G4	145.8±1.4 C	135.2±1.7 D	162.6±2.92 C

The different capital litter in columns refer to significant differences (P<0.05) among groups.

Discussion

Maternal antibody titer that evaluated by ELISA test to IB vaccine was detected in the chicks of the experiments. Result of 20 serum samples (before division into groups)

demonstrated good level of antibodies; the mean values of antibody titer was 4320±43.4. These findings agree with data obtained by Gharaibeh *et al*. (8) who found that the IBV had the second highest transfer rate (maternal Ab titer). Also the hens were vaccinated by IBV attenuated vaccine at early breeding stages followed by oil emulsion at 18 weeks, demonstrated that several serotypes of IBV activate the mucosal immunity in the reproductive tract causing direct secretion of IBV antibodies into the eggs (9).The results of present study showed significant increase in antibody titer against IBV in the experiment especially in G1 that's received β-glucan in drinking water this finding agree with (10), who suggest that β-glucan may also influence systemic or humoral immunity of birds, increased IgM in β-glucan feed birds, It was proposed that oligosaccharides in the yeast cell wall could bind to viruses and work as adjuvants of vaccines to increase the titers of antibody in β-glucan treated birds. These finding interpreted the increment in antibody titer in G1 in comparison with G3 that received vaccine without the β-glucan also, Cheng *et al.*, (11) suggested that β-glucan feeding enhanced some cell mediated immune responses of broiler chickens by modulating macrophage activity. After challenge with virulent IBV at 28 days old chicks, a more pronounced systemic response to the live vaccine was detected by ELISA 9 day post-challenge, indicating a secondary immune response (12). in addition, H120 induced a local humoral response, as demonstrated by an increase in IgA levels in tracheal washes of vaccinated birds, vaccination with Ma5 induced cross-reactive antibody production as well as was detected 9 days post challenge, in accordance with previous reports (13). Also due to the percentage of β-glucan contents in inactivated *Saccharomyces cerevisiae* about 18.2% which consequently stimulating broiler immune system (14). Oral administration of *β*-glucan also enhanced the activities of natural killer cells and peritoneal macrophages. In addition, *β*-glucan stimulated cytotoxic T-lymphocytes, B cells, and macrophages in mice (15). Summarily, our results indicate marginal benefits of *β*-glucan supplementation on avian lymphocyte subpopulations. there was a significant increase in antibody titer against ND as a result of administration of beta-glucan also Beta-glucan cause significant increase in phagocytic activity (7). Clinical signs percentages observed on chicks in groups challenged with Iraqi isolate, so H120 and Ma5 (Mass serotype) could provide partial protection against it. Regarding the remaining Iraqi isolates based on the S1 sequence, there was no homology reported between them and the vaccine used so the vaccine provide only little protection against them. This results in agreement with finding that the reports of in *vivo* cross protection often declines with decreasing S1 sequence homology between vaccinal strain and strains used in challenge (16). Also low percentage of clinical signs in the group one related to the effect of *saccharomyces cerevisiae* on stimulating the immune system and compete with other pathogenic microbes for adhesive sites in intestine (17). After challenge with virulent IBV at 28 days old chicks it was clear that the groups supplied by *Saccharomyces cerevisiae* showed low mortality rate than control group and this may related to the effect of *Saccharomyces cerevisiae* on stimulating the immune system reducing intestinal pH and release bacteriocins (18). this reflect on the birds immunity and resistance to diseases, as recorded by (10). Also it may be due to the percentage of *ß*-glucan contents in inactivated *Saccharomyces cerevisiae* about 18.2% which consequently stimulating broiler immune system (17). The increment of IL-2 level especially in G1 at 18 days that's return to the role of *β*-glucan in enhanced the immunity against IBV vaccine after booster dose these finding agree with (19) who showed that the proliferative responses of spleen cells from β-glucan administered mice to T-cell and B-cell mitogens were higher than those from normal mice. Also the result of this study agree with

(20) that revealed orally administered yeast beta glucan results in signaling processes leading to activation of macrophages and other cells and subsequent secretion of cytokines and other substances initiating inflammation reactions like interleukins IL-1, IL-2, IL-6, TNF. After challenge the increment in IL-2 level in all groups specific in G1 these finding agree with Al-zubaidy (21), she reported orally supplementation of β-glucan increased the IL-2 serum levels after challenge with *Salmonella typhimurium*. Also (22), which focused on the stimulation of IL-2 production by spleen cells in vitro and found that whereas all glucans (with the exception of Senseiro) stimulated production of IL-2. Results also are in agreement with (23) who found that the Zymosan is a particulate yeast preparation that elicits high levels of IL-2 and IL-10 from dendritic cells (DC) and engages multiple innate receptors, including the Syk-coupled receptor dectin-1 and the MyD88-coupled receptor TLR2.the obtained results from this study that's soluble β-glucan enhanced the cellular and humoral immunity against IBV.

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