

Effects of soluble β -glucan on the immune responses of broiler chickens vaccinated with Newcastle disease vaccine and reared under heat stress

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Summary

The main objectives of this study was to determine the influence of soluble β -glucan extracted from the cell wall of *Saccharomyces cerevisiae* on immune response of broiler chickens reared under heat stress. β -glucan 225 μ g/ml was supplemented in drinking water to broiler chicken vaccinated with Newcastle disease virus (NDV) vaccine. The parameters of the assessment of the immune response was the Heterophil / Lymphocyte ratio as a measure of stress, determination of the serum antibody titer post vaccination with the NDV vaccine by ELISA test and Immunohistochemical detection of macrophages by using monoclonal antibodies (mouse anti-chicken macrophage KUL01). A hundred and twenty (120) Chickens one day old were divided into two equal groups; group under heat stress and group control; each group was divided into two subgroups (G1, G2, G3 and G4) containing thirty chicks. The experiment was conducted for six weeks. The stressed group exposed to heat stress ($\approx 35^{\circ}\text{C}$) starting from the third week of age upto the end of the experiment. While (Group 1) and (G3) chicks were supplemented with 225 μ g/ml of soluble β -glucan in drinking water from day 1 to the end of the experiment, while (G2 and G4) chicks were not supplemented. The result of Heterophil /Lymphocyte ratio indicates that there was a significant ($P < 0.05$) difference within heat stressed treated which showed an elevated H/L ratio at 21,28 and 35 days old. Also there was a significant ($P < 0.05$) difference between groups that were treated with β -glucan (G1, G3) at 21, 28 and 32 days of age compared with a control non treated non stressed group (G4) at same periods. The results of antibody titer to NDV showed that there were significant ($P < 0.05$) differences among all groups at 7, 14 and 21 days of age, and the results of immunohistochemical analysis demonstrated positive staining for duodenal and bursal macrophages labeled with KUL-01 mouse anti-chicken monocyte- macrophages monoclonal antibodies. Tissue sections of duodenum and bursa at 14 and 24 days old stressed and non-stressed groups treated with β -glucan showed a positive result (purple-brown staining macrophages) in G1 and G3 as compared with duodenal and bursal tissues of G2 and G4groups which showed no stained cells.

Key Words: β -glucan, Newcastle disease virus vaccine, immune response, broiler chickens.

Introduction

Heat stress from increased environmental temperature has a significant impact on broiler productivity and welfare and can result in reduced feed intake, body weight gain. Heat stress can also produce what is known as reactive oxygen species within the body (1). Environmental temperature influence poultry immune response. High ambient temperature during rearing is associated with an increase in the stress of broilers (2). All over the world, farmers have to solve a problem of heat stress in poultry during the summer period. Global warming as a result of increased industrialization and environmental degradation has led to continuous increase in

ambient temperature, thereby making heat stress a major problem of livestock farming, particularly in the poultry sector (3).

β -D-glucan is a heterogeneous group of glucose polymers present as structural elements in the cell walls of yeast, fungi and cereals. Biological activities of these molecules have been reported to inhibit tumor formation, enhance defense against bacterial challenge and increase growth performance. β -Glucan belong to a group of physiologically active compounds sometimes called biological response modifiers (4). *In vitro* studies have shown that these molecules influence macrophage morphology, increase nitric oxide

production, and release some kind of cytokines, such as TNF- α , IL-6, IL-1 and IL-2. Glucans could also decrease inflammatory cytokine responses, thereby allowing nutrients to be partitioned toward growth demand (5). Chicken macrophage functions have been shown to be very responsive to several dietary immune modulators include β 1-3, 1-6, glucan (6). Dectin-1 as an important partner for TLR2 on macrophages and dendritic cells for the production of inflammatory cytokines in response to particulate stimuli containing β -glucans (7).

Materials and Methods

The β -glucan used in this study was extracted from local market baker's yeast (*S. cerevisiae*) according to the method published by (8), and modified method by (9). The total concentration of carbohydrates present in the extract was determined according to the (10) that modified by (11); the value was 7.5 mg/ml.

One day old broiler chicks (Breed : Ross, Belgium Origin) were brought in good condition from Al- Baraka Hatchery-Diyala-Kanaan. The experiment was done in the poultry farm of the College of Veterinary Medicine- University of Baghdad, which were maintained in the same condition; after cleaning and disinfection with sodium hypochlorite then fumigated by (Long life 250)^R disinfectant. All chicks were fed on rations formulated to meet the nutrient requirements of broilers (12). Feed and water were provided on an *ad libitum* basis. Broiler chicks were vaccinated with NDV vaccine La Sota strain (AviTtro – Lohmann Animal Health GmbH&Co.KG.Germany) by manual oral drench with drinking water at 10 days old regarding to the ELISA Abs titer that assess the maternal antibodies to NDV, the second NDV vaccine applied after ten days (20 day old). Electrical heaters connected to an electronic temperature controller Eliweli® to achieve heat stress conditions to the heat stressed group that maintain the temperature at $\approx 35^{\circ}\text{C}$. One hundred twenty (120) broiler chicks were divided into two groups, group under heat stress and group control and each group was divided into two subgroups G1, G2,

G3 and G4 containing thirty chicks. The experiment was conducted for six weeks. The stressed groups G1 and G2 exposed to heat stress ($\approx 35^{\circ}\text{C}$) starting from the third week of age until the end of the experiment.

According to a previous study (13); 225 $\mu\text{g/ml}$ of β -glucan was supplied with water to G1 and G3 and it was made available *ad libitum* from day 1 to the end of the experiment.

Blood samples were collected weekly from the wing (brachial vein) from 4 randomly selected birds from each group with ethylenediaminetetra-acetate (EDTA)-tubes as an anticoagulant. One drop of blood was smeared on a glass slide, air-dried and fixed with absolute methanol, as described by (14 and 15). Blood smears were stained with Giemsa stain for 30 min. Birds that were bled were marked for identification. Blood was collected from different birds on each occasion. Heterophil and lymphocyte counts were obtained with a light microscope (100x) in a monolayer-sector of the smear. To avoid differences in the thickness of the layer, the slide was scanned along the short-axis, following (15 and 16).

Four birds for each group were killed at day 1 of age for blood sample collection. At the ages of 7, 14 and 21 days post-vaccination blood samples were collected by disposable syringe, blood was kept in plain glass test tubes and left at 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 to perform according to the manufacturer's instructions listed in the ProFLOK® NDV ELISA Kit (Synbiotics-USA).

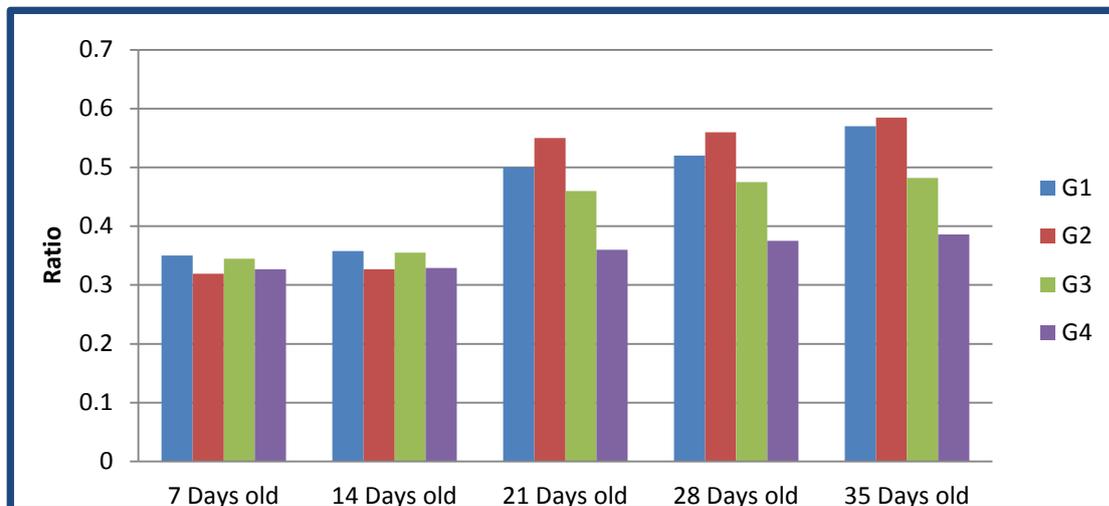
Data were analyzed statistically by using the analysis of variance (ANOVA) and least significant differences (L.S.D.) were used for differentiated among mean of results (17).

Results and Discussion

The result of Heterophil/Lymphocyte ratios that measured at 7, 14, 21, 28 and 35

days of age in heat stressed and non-heat stressed groups that were supplemented with

and without β -glucan in drinking water (Figure,1)



Figure,1: Effect of 225 μ g/ml soluble β -glucan in Heterophil/Lymphocyte ratio of heat stressed and non heat stressed groups at 7,14,21,28 and 35 days old broiler chickens. Means \pm SE (n=4).

G1: Heat stressed treated group. G2: Heat stressed non treated group.

G3: Non heat stressed treated group. G4: Non heat stressed non treated group.

There were no differences among the non-stressed and stressed groups at 7 and 14 days old while there was a significant ($P < 0.05$) difference within heat stressed treated groups shows the elevated H/L ratio at 21,28 and 35 days old.

The results of antibody titers to NDV vaccine measured by Elisa test at 1 ,7 ,14 and 21 days old are shown in (Table ,1). At 14 days old G1 tissue sections of the duodenum and the bursa of fabricius (Fig,2) showed positive stained cells; also with the duodenal section of G3(Fig,4) was positive.G2 tissue sections of the duodenum and the bursa of

fabricius (Fig,3) showed no stained cells; the same result found in the control group G4. At 24 days old tissue sections of the duodenum and the bursa of fabricius in G1 (Fig,5) and G3 (Fig,7) appear positively stained macrophages; While in G2 and G4 the tissue sections showed no stained cells.

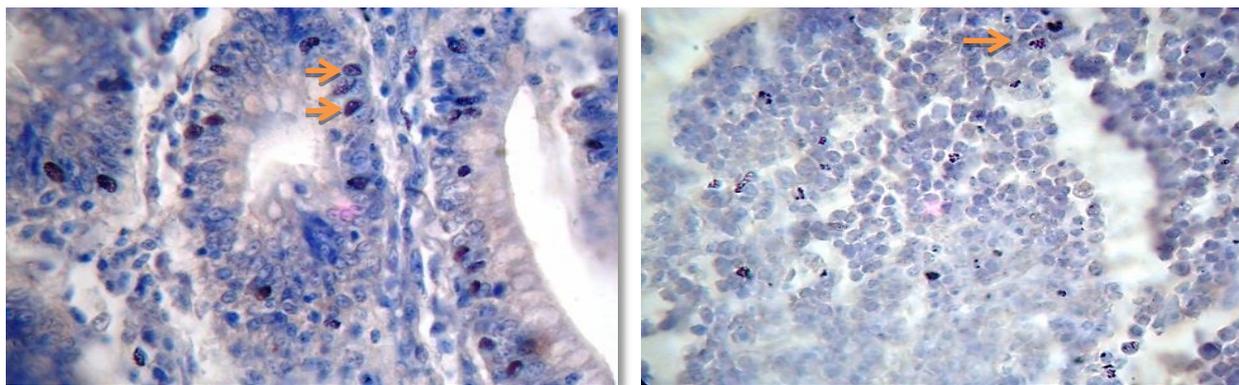
Table,1: Effect of 225 μ g/ml of soluble β -glucan supplementation in drinking water on antibody response of heat stressed and non heat stressed broiler chickens groups to NDV vaccine. Antibody titer to NDV vaccine

Age Groups	NDV Antibody titers by Elisa test (Means \pm SE)			
	1 day	7days	14days	21day
G1	6380.25 \pm 143.02 a	3690.5 \pm 79.33 A c	4353 \pm 135.62 A b	2895.75 \pm 141.64 B d
G2	6380.5 \pm 123.90 a	2468 \pm 131.46 B b	2492.75 \pm 159.69 B b	1775 \pm 61.23 C c
G3	6380 \pm 118.77 a	3669.75 \pm 58.06 A c	4288.5 \pm 130.56 A b	3758.5 \pm 114.99 A c
G4	6380 \pm 136.86 a	2534 \pm 131.41 B b	2467.5 \pm 135.54 B b	2012.75 \pm 70.18 C b

G1: Heat stressed treated group. G2: Heat stressed non treated group. G3: Non heat stressed treated group. G4: Non heat stressed- non treated.

There were significant ($P < 0.05$) differences among all groups at 7 ,14 and 21 days old , but no significant ($P < 0.05$) differences among all groups at 1 day old of maternal antibody titer.

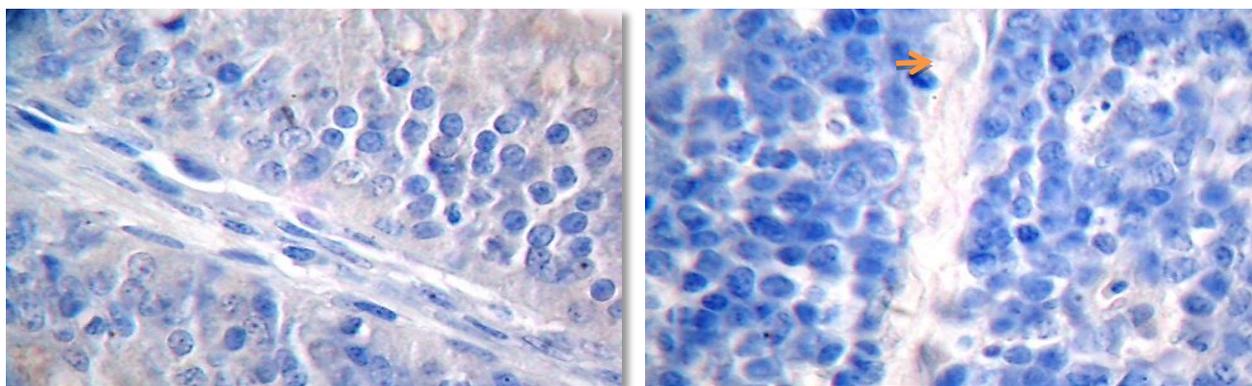
Immunohistochemical detection of duodenal and Bursal macrophages by using (mouse anti-chicken macrophage KUL01).



A

B

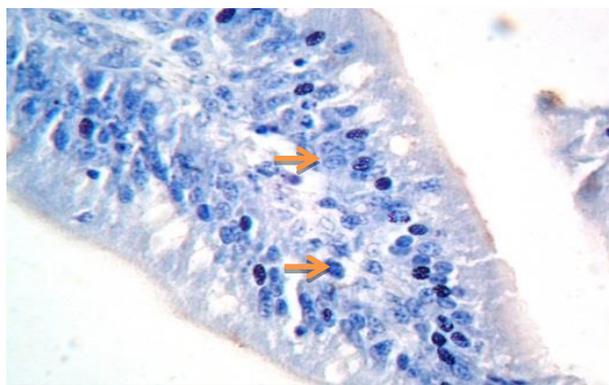
Figure,2: Macrophages in paraffin embedded sections (5µm) were identified by using an anti-chicken monocyte-macrophage mouse monoclonal antibody (KUL01) and an HRP –conjugated secondary antibody A: Macrophages in the duodenum of a heat stressed β -glucan treated 14 days old chick G1. Immunopositive cells are purple-brown (→). B: Macrophages in the bursa of fabricius of a heat stressed β -glucan treated 14 days old chick G1. Immunopositive cells are purple-brown (→). 100 x.



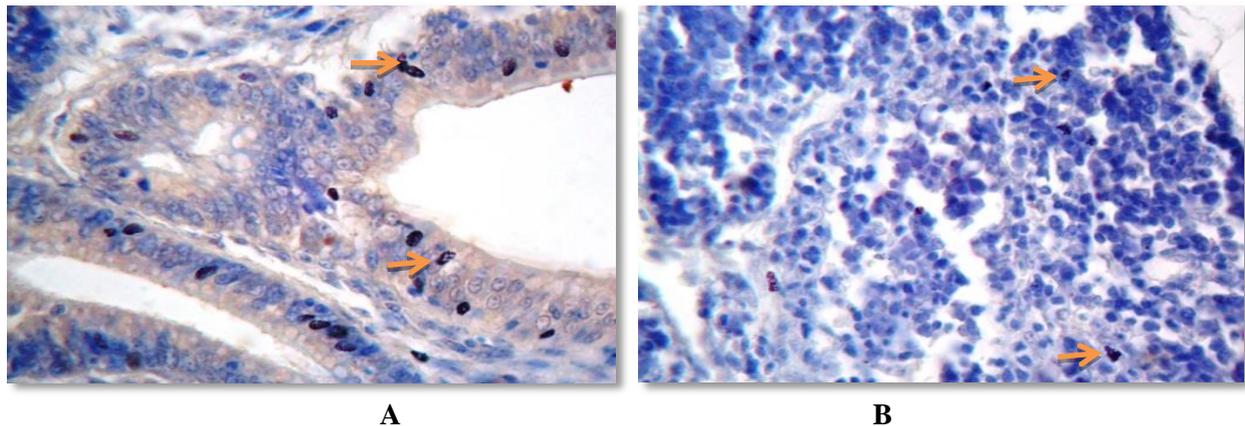
A

B

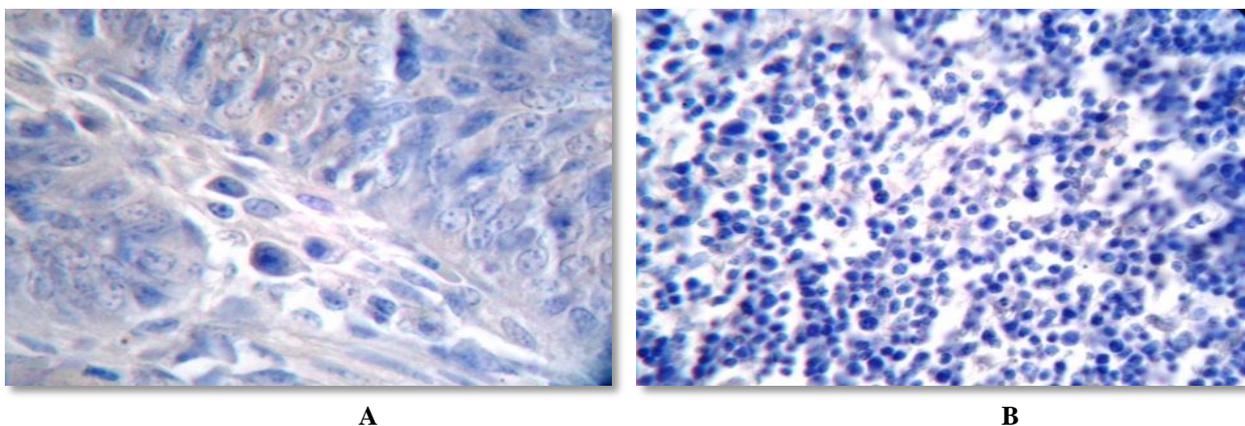
Figure,3-A:Immunohistochemical staining of duodenum in heat stressed non treated 14 days old chick G2 , paraffin embedded sections (5µm) appears no stained cells.B:Immunohistochemical staining of the bursa of fabricius in heat stressed non treated 14 days old chick G2, the paraffin embedded section (5µm) appears no stained cells. 100 x.



Figure,4: Macrophages in the duodenum of non heat stressed treated 14 days old chick G3. Macrophages in paraffin embedded sections (5µm) were identified by using an anti-chicken monocyte-macrophage mouse monoclonal antibody (KUL01) and an HRP – conjugated secondary antibody Immunopositive cells are purple-brown (→) 100 x.



Figure,5: Macrophages in paraffin embedded sections (5µm) were identified by using an anti-chicken monocyte-macrophage mouse monoclonal antibody (KUL01) and an HRP –conjugated secondary antibody A:Macrophages in the duodenum of heat stressed treated 24 days old chick G1. Immunopositive cells are purple-brown (→).B: Macrophages in the bursa of fabricius of heat stressed treated with β-glucan 24 days old chick G1. Immunopositive cells are red-brown (→) 100 X.



Figure,6A:Immunohistochemical staining of the duodenum in heat stressed non treated 24 days old chick G2, the paraffin embedded section (5µm) appears no stained cells. B:Immunohistochemical staining of the bursa of fabricius in heat stressed non treated 24 days old chick G2, the paraffin embedded section (5µm) appears no stained cells. 100 x.

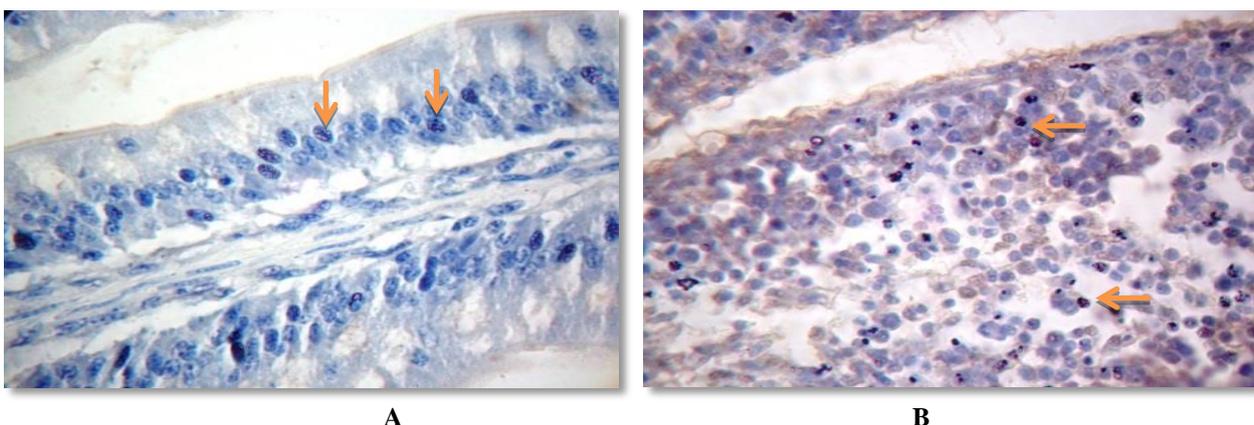
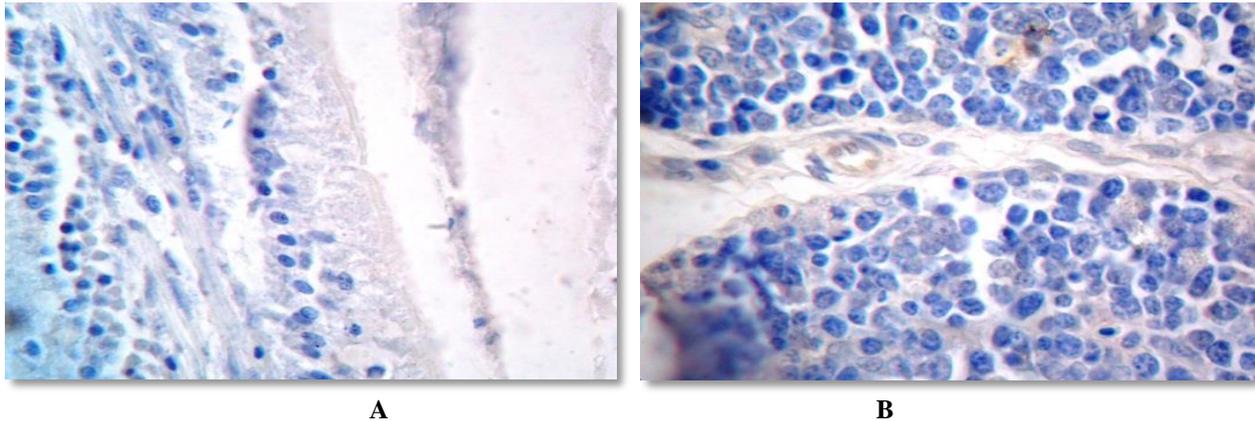


Figure ,7: Macrophages in paraffin embedded sections (5µm) were identified by using an anti-chicken monocyte-macrophage mouse monoclonal antibody (KUL01) and a HRP –conjugated secondary antibody A: Macrophages in the duodenum of non heat stressed treated with β-glucan 24 days old chick G3. Immunopositive cells are purple-brown (→). B:Macrophages in the bursa of fabricius of non heat stressed treated with β-glucan 24 days old chick G3. Immunopositive cells are red-brown. (→) 100 X.



Figure,8: A:Immunohistochemical staining of the duodenum in non-heat stressed non treated 24 days old chick G4, the paraffin embedded section (5 μ m) appears no stained cells..B: Immunohistochemical staining of the bursa of fabricius in non-heat stressed non treated 24 days old chick G4, the paraffin embedded section (5 μ m) appears no stained cells. 100 X.

Broilers chickens exhibit significantly ($P < 0.05$) reduces lymphocyte and raised heterophil ratios. The H/L ratio is a good measure of the chicken's perceptions of stress in its environment and evaluated a variety of parameters, including corticosterone (CS) and heterophil/lymphocyte ratios (H/L), claims about animal welfare based on data regarding the pituitary-adrenocortical system (the physiological stress response system) (18). Corticosterone is an important mediator of the stress-induced changes in the blood leukocyte distribution (19). This reaction conserved adaptive response that might contribute to an enhancement of the immune surveillance in the organs-tissues of the "battle stations" in which leukocytes traffic during stress. Therefore, the ratio of neutrophil (or heterophil) to lymphocyte has been used for years to assess stress in all vertebrates (20). There were no differences among the non-stressed and stressed groups at 7 and 14 days old while there is a significant ($P < 0.05$) difference within heat stressed treated groups showed the elevated H/L ratio at 21, 28 and 35 days old. This is consistent with (21), that the stress challenge dramatically increase oxidative burst and this increased was significantly modulated by Yeast Extract treatment and the number of Heterophils in whole blood was significantly higher in non-stressed control birds fed 1,000 g/ton of Yeast Extract as compared with non-stressed birds fed control feed. Also there is a significant ($P < 0.05$) difference between groups that

treated with β -glucan (G1, G3) at 21, 28 and 32 days of age compared with a control non treated non stressed group (G4) at same periods. This shows agreement with (21). Heterophil numbers, percentages, oxidative burst activity, and the H/L ratio were all increased by Yeast Extract supplementation and resulted in protection from bacterial colonization. The oxidative burst in chicken Heterophils has also been found to be upregulated by β -glucan (22). Dectin-1 as an important partner for TLR2 on macrophages and dendritic cells for the production of inflammatory cytokines in response to particulate stimuli containing β -glucans (7).

The result of humoral response (antibody titer) to NDV vaccine shows in (table-1). The significant ($P < 0.05$) difference in Ab titers at 14 and 21 days old in G2 compared with the same period in G4 showed reduction in Ab titers due to heat stress, this result was in agreement with those of (23, 24 and 25), who showed that heat stress cause a reduction in antibody synthesis. The environmental temperature may influence the immune response of poultry (26). The mechanism by which the environmental temperature may act as an immune suppressor may be due to increased activity of the adrenal gland due to stress increases the level of blood corticosteroids, which cause suppression of cell proliferation factor, or interleukin II (27). The significant differences in Ab titers between the G1 and G2 also G3 and G4 groups were due to β -glucan supplementation

in the drinking water to treated groups (G1 and G3) which lead to elevate the Ab titers to NDV. This results were in agreement with (28) who reported, the dietary supplemented with yeast during heat stress condition could improve the immune response of birds. Also dietary supplementation of β -glucan increased the production of antibodies against NDV and IgA concentrations in the intestine and tracheal mucosa (29). β -glucans increase the concentration of serum IgG, IgG1, IgG2, IgM, and A (30). Also these results are in agreement with (31) who reported that there was a significant increase in antibody titer against ND as a result of administration of β -glucan.

A few researches reported on the determination of the chicken macrophages by using the KUL-01 mouse antibody. The result of Immunohistochemical detection of duodenal and bursal macrophages by using (mouse anti-chicken macrophage KUL01) showed that at 14 days old G1 and G3 duodenal and bursal sections showed positive KUL-01 macrophages labeled cells as a result of β -glucan supplementation compared with G2 and G4 which non supplemented with β -glucan. This is consistent with (32) the results of an in vivo study with C57BL/6 mice showed that oral administration of β -1-3/1-6-glucan isolated from baker's yeast increased the number of intraepithelial lymphocytes in the intestine, and (33) who supports the role of yeast culture in innate immune function that the addition of cell-wall free soluble extract of Yeast culture showed an anti-inflammatory effect in conjunction with activation of natural killer cells and B lymphocytes. Dectin-1 was described as the main β -glucan receptor on leukocytes and generally considered to be responsible for the cellular responses of β -glucan (34). The binding of β -glucan to the receptor may cause macrophage, lymphocyte or other blood cells to secrete greater quantities of lower signal molecules. Accordingly, sIgA production might be enhanced in this manner and secreted by the intestinal macrophages and lamina propria lymphocytes (35).

At 24 days old under heat stress G1 showed a positive result, duodenal and bursal sections were positive KUL-01 macrophages

labeled cells compared with G2 sections that appear negative stained. Heat stress activated the chicken. Hypothalamic-pituitary-adrenal system, increasing corticosterone serum levels and consequently and possibly decreasing food intake, body weight gain, relative immune organ weight and innate immunity. This neuro-immune dysfunction might be influenced the quality of the intestinal-immune barrier, thereby allowing pathogenic bacteria to migrate through the intestinal mucosa and generating an inflammatory infiltrate. Gastric and intestinal lesions are one of the first manifestations of stress (36). β -glucan immune modulation stimulates gut associated and systemic immunity by acting as a nonpathogenic microbial antigen, given an adjuvant-like effect (37). β -1-3/1-6-glucan stimulates the gut associated lymphoid tissue (GALT) to secrete more sIgA in order to enhance the mucosal immunological function (35). Purified β -1,3/1,6 glucans are known to modulate gut-immunity (38). β -glucan can be effective in decreasing environmental stress and this effect may support the immunity (39).

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تأثير البيتا كلوكان على الاستجابة المناعية في أفراخ اللحم الملقحة بلقاح نيوكاسل والمربيات تحت ظروف الإجهاد الحراري

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الخلاصة

الهدف الرئيسي من هذه الدراسة تحديد تأثير البيتا كلوكان الذائب والمستخلص من الجدار الخلوي لخميرة الخبز *Saccharomyces cerevisiae* على الاستجابة المناعية لدجاج اللحم المربي تحت ظروف الاجهاد الحراري. استعمل البيتا كلوكان الذائب بتركيز $225\mu\text{g/ml}$ وتم اعطائه مع ماء الشرب لا فراخ الدجاج الملقحة بلقاح مرض نيوكاسل. تم تقييم الاستجابة المناعية باستعمال عدة مؤشرات منها عن قياس نسبة H/L كمقياس للاجهاد و اختبار الاليزا لتحديد معيار الاجسام المضادة بعد التلقيح بلقاح فايروس مرض نوكاسل. وكذلك الكيمياء النسجية المناعية (IHC) للتحري عن الخلايا البلعمية في الانسجة باستعمال الأضداد وحيدة النسيلة. قسمت 120 فرخ دجاج بعمر يوم واحد الى مجموعتين، مجموعة تحت الاجهاد الحراري و مجموعة سيطرة وتم تقسيم كل مجموعة منها الى مجموعتين فرعية (G1, G2, G3 و G4) تحوي كل منها ثلاثون فرخ. مدة التجربة ستة اسابيع. مجموعة الاجهاد الحراري تم تعريضها لحرارة (35°C) تبدا من الاسبوع الثالث والى نهاية التجربة. المجموعة الاولى والثالثة اعطيت البيتا كلوكان الذائب بتركيز $225\mu\text{g/ml}$ في ماء الشرب من اليوم الاول حتى نهاية التجربة اما المجموعة الثانية والرابعة فتعتبر سيطرة وأعطيت ماء فقط. حيث اظهرت النتائج وجود فروق معنوية ($P < 0.05$) بين المجاميع المعطاة بيثا كلوكان في ماء الشرب وتحت الاجهاد الحراري حيث لوحظ زيادة في نسبة H/L في 28,21 و 35 يوم وكذلك وجد فروق معنوية بين مجاميع المعطاة بيثا كلوكان بالمقارنة مع مجموعة السيطرة الرابعة الغير معطاة بيثا كلوكان في الايام 28,21 و 35. كذلك اظهرت نتائج معيار الاجسام المضادة للقاح نيوكاسل هناك فروق معنوية ($P < 0.05$) بين المجاميع بعمر 7, 14 و 21 يوم. اما نتائج اختبار الكيمياء النسجية المناعية قد ظهرت موجبة حيث ظهرت الخلايا البلعمية المعلمة بالأضداد وحيدة النسيلة في هذه الانسجة في المجموعتين الاولى والثالثة المعطاة بيثا كلوكان في ماء الشرب بعمر 14 و 24 يوم، بينما لم تظهر المجموعتين الثانية والرابعة الغير معطاة بيثا كلوكان اي خلايا مصبغة.

مفتاح الكلمات: بيثا كلوكان , لقاح فيروس مرض نيوكاسل , الاستجابة المناعية , دجاج اللحم.