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# Immune Response to A Killed *Salmonella enteritidis* Vaccine with β-Glucan Supplementation in Broiler Chicks

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**Abstract:** This study was performed to evaluate the effect of β-glucan on white blood cell profiles, humoral and cellular immune response and cytokines in chickens vaccinated with killed *Salmonella enteritidis* (SE) vaccine. One day old ninety Saso chickens were divided into three equal groups, group (1) kept as normal control, group (2) vaccinated with SE killed vaccine 0.5 cm S/C at neck region at 12 days old, group (3) supplemented with β-glucan 0.3gm/L water from day one till the end of the experiment and vaccinated with SE killed vaccine at 12 days old. All groups were challenged with SE strain $5.5\times10^8$cfu at 26 days old by oral gavage. Blood samples were collected at 23, 33, 40 and 47 days old. β-glucan supplementation with killed SE vaccine regime lead to heterophilia, lymphocytosis and monocytosis with increased Ab titre against Newcastle and also, significant increase in serum IL6 and TNF-α levels with decreased IL10 level. The results showed that β-glucan in combination with SE vaccine displays an efficacy in chicks challenged with SE and can improve the immune response of the chicks.

**Key words:** Chickens · B-Glucan · Vaccination · *Salmonella enteritidis*

## INTRODUCTION

Immunostimulants comprise a group of biological and synthetic compounds that enhance the non–specific cellular and humoral defense mechanisms in animals [1]. They also, increase resistance to infectious diseases and the use of these immunostimulants is an effective means of increasing the immunocompetence and disease resistance of animals, birds and fishes.

β-glucan is a group of glucose polymers that consist of β-1, 3 and β-1, 6 glycosidic linkages. It is a main cell wall structural component of fungi, plants and some bacteria [2]. It can bind to various types of cell surface receptors including lectins, scavenger receptors and intergrins on monocytes, macrophages, natural killer cells, neutrophils and lymphocyte populations resulting in activation of lymphocyte production of inflammatory cytokines and chemokines and microbial killing. These lead to development of adaptive immunity. Therefore it has suggested that β-glucan has anti-infective and anti-tumorigenic properties [3].

Salmonella is an enteric pathogen that colonizes the intestinal tract of poultry and humans and accounts for millions of cases of gastroenteritis and food-borne illness each year [4]. The most common causes of human Salmonella foodborne disease are *S. enteritidis* and *S. typhimurium* [5].

Salmonella vaccines can act by distinct mechanisms as, killed vaccines vastly adopted in many countries for vaccination of commercial table-egg layers. Most of these vaccines contain SE antigens and adjuvants and stimulate an enhanced humoral immune response with variable levels of protection [6]. The aim of this work is to evaluate the effect of β-glucan supplementation with *Salmonella enteritidis* killed vaccine on immune response of broiler chicks to *Salmonella enteritidis* infection.

## MATERIALS AND METHODS

**Experimental Birds:** Ninety broiler Saso chickens one day old purchased from Al-Kahira Poultry Company. Chickens were reared on litter under standard

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environmental and hygienic conditions. The temperature was adjusted (30°C at the first week of age and decreased 2°C each week). Chickens were maintained on a commercial well balanced ration. All chickens were vaccinated against ND by Hitchner B1 via eye drop at 7 days old and against IBD via drinking water at 12 days old [7].

**Bacterial Strain and Vaccine (Make All Titles like Each Other):** A strain of *Salmonella enteritidis*: 5.5 $\times 10^8$ CFU /ml [8] was obtained from Animal Health Research Institute, Dokki, Cairo to be used for experimental infection. *Salmonella enteritidis* killed vaccine (Bacterine) was obtained from Vet. Serum and Vaccine Research Institute, Abbassia, Cairo. Β-glucan powder extract batch. No. 2809/15 was obtained from Pharma-health Company.

**Experimental Design:** Chickens were classified equally into 3 groups each of 30 chicks. *Group 1:* Kept as normal control and fed normal ration. *Group 2:* Chicks of this group received vaccine against *Salmonella enteritidis* at age of 12 days old by injection 0.5cm S/C at neck region. *Group 3:* Chicks of this group received vaccine against *Salmonella entertidis* at age of 12 days old and also supplemented with β-glucan in drinking water from day one of age till the end of the experiment by 0.3g/L water.

Chickens of group 2 and 3 were vaccinated against *Salmonella entertidis* at 12 days old 0.5 cm S/C injection at neck region according to Liu *et al.* [9]. Then post vaccination by 2 weeks the chickens of group 2 and 3 were challenged by *Salmonella entertidis* strain 1ml of (5.5 cfu$\times 10^8$/ml) administered orally by oral gavage.

**Blood Sampling:** Five chickens from each group were sacrificed at 3 days pre-infection and at 7, 14 and 21 days post-infection after collecting blood samples from the wing vein. Three blood samples were collected, the first one on EDTA as anticoagulant for total leukocytic count (0.5 mg/ml blood), the second one was collected for serum separation and kept in an inclined position for 20 minutes at room temperature then the samples were centrifuged at 3000 rpm for 10 minutes, the separated clear sera were stored in Eppendorf tubes at -20°C until use and the third sample was collected on heparin for estimation of phagocytic activity.

**Laboratory Examinations**

**Total Leukocytic Counting:** It was performed by manual method according to Feldman *et al.* [10] using improved Neubauer hemocytometer and Natt and Herrick solution as diluting fluid [11].

**Differential Leukocytic Count:** Blood films were stained by Giemsa stain to be used for differential leukocytic count according to Anderson and Latimer [12].

**Hemagglutination Inhibition Antibody Titer (HIA):** The hemagglutination test (HA) was carried out according to Anon [13]. Micro-technique of hemagglutination inhibition test according to Majujabe and Hitchner [14] was adopted to detect the hemagglutination inhibition antibody to Newcastle disease virus in serum samples.

**Lysozyme Activity:** The lysozyme activity was determined according to Schltz [15] using agarose gel lysis assay.

**Phagocytic Activity and Phagocytic Index:** Measurement of phagocytic activity of peripheral blood monocytes using *Candida albicans* was adapted as described by Anthony *et al.* [16] and Chu and Dietert [17]

**Interleukin 6 (IL6), Interleukin 10 (IL10) and Tumor Necrosis Factor α (TNF-α):** Estimated by ELISA Kit, cat. No. MBS2512036 (96 tests), MBS2020250 and MBS260419 respectively, according to Dowlati *et al.* [18].

**Statistical Analysis:** The data obtained from this investigation were statistically analyzed by F-test according to Tamhane and Dunlop [19] using "MSTAT-C" computer program.

## RESULTS

Regarding leukogram data presented in Table (1) revealed that at 3 days pre-infection and at 7 and 14 days post-infection chickens in both supplemented and non-supplemented groups showed significant leukopenia and at 21 days post- infection only chickens in supplemented group showed significant leukopenia. At 3 days pre-infection and at 7 days post-infection chickens in both groups showed lymphocytosis and at 14 days post-infection chickens in supplemented group (3) only showed lymphocytosis. At 7 days post-infection chickens in both groups showed significant heterophilia, monocytosis and eosinophilia and at 14 days post-infection chickens in both groups showed heterophilia.

At 3 days pre-infection chickens in group (3) showed significant increase in Lysozyme activity compared to group (2) but, at 7 days post-infection table (1) revealed that chickens in both groups showed significant increase in lysozyme activity compared to control group.

Table 1: Effect of β-glucan supplementation with vaccination against *S. enteritidis* on some hematological and immunological parameters

| Parameters | Groups | Time | | | |
|---|---|---|---|---|---|
| | | At 23 days old | At 33 days old | At 40 days old | At 47 days old |
| Total leukocytic count (x10$^3$/µl) | 1 | 20.44±0.26$^b$ | 19.84±0.39$^b$ | 21.24±0.9$^b$ | 22.72±0.43$^b$ |
| | 2 | 22.56±0.64$^a$ | 23.68±0.54$^a$ | 23.36±0.45$^a$ | 23.44±0.28$^{ab}$ |
| | 3 | 23.56±0.41$^a$ | 24.12±0.39$^a$ | 24.32±0.52$^a$ | 24.12±0.34$^a$ |
| Heterophils (x10$^3$/µl) | 1 | 7.85±0.16 | 7.16±0.23$^b$ | 7.78±0.39$^b$ | 8.45±0.21 |
| | 2 | 7.88±0.24 | 8.76±0.36$^a$ | 8.97±0.22$^a$ | 8.77±0.21 |
| | 3 | 8.14±0.19 | 9.12±0.23$^a$ | 9.33±0.21$^a$ | 8.87±0.1 |
| Lymphocytes (x10$^3$/µl) | 1 | 9.68±0.14$^b$ | 10.04±0.31$^b$ | 10.51±0.35$^c$ | 11±0.42 |
| | 2 | 11.42±0.42$^a$ | 11.43±0.54$^a$ | 11.06±0.20$^{bc}$ | 11.49±0.31 |
| | 3 | 11.94±47$^a$ | 11.44±0.39$^a$ | 11.53±0.36$^{ab}$ | 11.83±0.39 |
| Monocytes (x10$^3$/µl) | 1 | 1.64±0.11 | 1.55±0.1$^b$ | 1.62±0.15$^b$ | 1.81±0.09 |
| | 2 | 1.85±0.17 | 1.98±0.07$^a$ | 1.87±0.13$^{ab}$ | 1.78±0.09 |
| | 3 | 1.97±0.13 | 1.97±0.08$^a$ | 1.95±0.12$^{ab}$ | 1.88±0.07 |
| Eosinophils (x10$^3$/µl) | 1 | 0.90±0.06 | 0.84±0.05$^b$ | 0.9±0.08 | 1±0.05 |
| | 2 | 0.90±0.03 | 1.08±0.04$^a$ | 1.03±0.07 | 0.98±0.05 |
| | 3 | 1.03±0.05 | 1.06±0.05$^a$ | 1.07±0.06 | 1.01±0.04 |
| Basophils (x10$^3$/µl) | 1 | 0.37±0.08 | 0.31±0.07 | 0.43±0.08 | 0.45±0.07 |
| | 2 | 0.5±0.09 | 0.42±0.09 | 0.43±0.09 | 0.42±0.09 |
| | 3 | 0.47±0.07 | 0.53±0.09 | 0.44±0.1 | 0.53±0.09 |
| Lysozyme activity (ug/ml) | 1 | 34.70±8.24$^{ab}$ | 13.28±2.28$^b$ | 23.17±5.87 | 30.36±6.74 |
| | 2 | 23±3.68$^b$ | 61.02±9.73$^a$ | 26.17±5.85 | 23±3.68 |
| | 3 | 45.06±5.32$^a$ | 55.22±8.07$^a$ | 30.52±8.11 | 37.71±7.10 |
| Ab Titre | 1 | 3.2±0.37$^b$ | 2.4±0.24$^b$ | 3.2±0.37$^a$ | 4±0.55$^{ab}$ |
| | 2 | 3.4±0.51$^b$ | 2.4±0.24$^b$ | 2.8±0.37$^{ab}$ | 3±0.32$^{bc}$ |
| | 3 | 5.2±0.37$^a$ | 4.2±0.37$^a$ | 4±0.45$^a$ | 4.2±0.58$^{ab}$ |
| Phagocytic index | 1 | 4.62±0.23$^b$ | 5±0.2$^a$ | 5.08±0.16$^a$ | 4.72±0.15$^a$ |
| | 2 | 4.62±0.14$^b$ | 4.10±0.35$^b$ | 3.84±0.46$^b$ | 3.90±0.18$^b$ |
| | 3 | 5.76±0.15$^a$ | 4.52±0.24$^{ab}$ | 4.4±0.3$^{ab}$ | 4.5±0.17$^a$ |
| Phagocytic % | 1 | 72.20±2.03$^b$ | 71.6±1.36$^a$ | 69±2.35$^a$ | 66.8±2.75$^{ab}$ |
| | 2 | 74.40±1.36$^{ab}$ | 61±3.62$^b$ | 61.2±2.08$^b$ | 63±1.82$^b$ |
| | 3 | 79.40±1.21$^a$ | 63.80±2.6$^{ab}$ | 70.6±1.86$^a$ | 71.6±1.29$^a$ |
| IL6 (pg/ml) | 1 | | 21.58±1.68$^b$ | 26.84±0.88$^b$ | 26.24±2.16 |
| | 2 | | 44.77±5.10$^a$ | 30.82±2.06$^b$ | 28.94±1.16 |
| | 3 | | 47.94±5.22$^a$ | 26.31±1.76$^b$ | 26.89±2.41 |
| IL10 (pg/ml) | 1 | | 26.92±1.61$^a$ | 27.51±1.38$^a$ | 22.81±1.29$^a$ |
| | 2 | | 15.2±2.41$^{bc}$ | 21.83±1.21$^b$ | 18.03±1.29$^b$ |
| | 3 | | 20±2.20$^b$ | 25.34±1.73$^{ab}$ | 21.08±1.42$^{ab}$ |
| TNFα (pg/ml) | 1 | | 19.16±1.85$^b$ | 27.31±0.97 | 24.07±2.11 |
| | 2 | | 50.80±7.19$^a$ | 24.67±3.65 | 24.31±2.52 |
| | 3 | | 60.17±5.88$^a$ | 24.63±1.67 | 23.84±1.48 |

Values are: means ± S.E, values in the same raw with different superscripts are significantly different (P < 0.05) (n=5)

Anti-body titre against ND showed significant increase in group (3) compared to other groups at 3 days pre-infection and at 7 days post-infection.

Results of the present study showed that at 3 days pre-infection chickens in supplemented group (3) showed significant increase in phagocytic index and phagocytic % but, at 7, 14 and 21 days post-infection chickens in non-supplemented group (2) showed significant decrease in both parameters.

At 7 days post-infection chickens in both supplemented and non-supplemented groups there was significant increase in serum IL6 and TNF-α level. On the other hand at 7 days post-infection chickens in both supplemented and non-supplemented groups there was significant decrease in serum IL10 level and at 14 and 21 days post-infection chickens in non-supplemented group (2) only showed significant decrease in IL10 level.

## DISCUSSION

Regarding the leukogram our results revealed increased total leukocytic count with lymphocytosis along the experimental period and after infection by 7 days results revealed heterophilia, lymphocytosis,

monocytosis and eosinophilia in supplemented group but returned to control levels by 21 days. Our results are in line with Hassan *et al.* [20] and Alena *et al.* [21] who reported increase in WBCs and monocyte count in broiler chicks whose diet supplemented with 40 g/t β-1, 3/1, 6-D-glucan for 42 days. The increased lymphocyte populations may be indicative of higher activity of humoral immunity in chicks fed yeast supplemented diets as reported by Paryad and Mahmoudi [22]. Heterophilia and monocytosis may occur due to bacterial invasion of the intestinal mucosa which causes rapid inflammation of the intestinal mucosa followed by infiltration of heterophils and then macrophages [23]. Eosinophilia was recorded in the present work in both groups after infection by 7 days and this is in agreement with Oladele *et al.* [24] who found eosinophilia in chicks after infection with IBDV and they attributed this increase to tissue destruction especially tissues such as skin, lungs, gastrointestinal tract and female genitalia.

In our study *Salmonella enteritidis* infection resulted in increased lysozyme activity at 7 days post-infection. Our result is in agreement with Abdel Hamid *et al.* [25] (put family name in the text and family name followed by her name in references list) who reported increase in lysozyme activity in chicks supplemented with probiotic at one week post infection with *Salmonella enteritidis*. The increase in lysozyme activity may occur either due to probiotics treatments which indicated an immune stimulation or due to infections or invasion by foreign material [26].

The present study showed increased Ab titre in group supplemented with β-glucan before infection and this in agreement with Wang *et al.* [27] who mentioned that feeding chickens on sulfated glucan from *Saccharomyces cerevisiae* resulted in increased serum antibody titer after vaccination with Newcastle disease vaccine. In our study after challenge with SE by 14 and 21 days the group which was not supplemented with b-glucan Ab- titre has decreased compared to control and supplemented groups and this is similar to those of Sadeghi *et al.* [28]. This decrease in Ab-titre may be due to release of corticosterone which has been found to be inhibiting the production and action of antibodies [29].

For Phagocytic activity (PA) and phagocytic index the present study revealed that there is an increase in phagocytic activity among chicks supplemented with b-glucan before infection and after infection chicks supplemented with b-glucan revealed increase in PA compared to non-supplemented chicks. The increase of the macrophage phagocytic activity was observed also in the study by Guo *et al.* [30] in broiler chicks which were fed a diet containing 20 and 40 mg/kg yeast b-glucan in the starter and 20 and 20 mg/kg in the grower diet.

Muthusamy *et al.* [31] reported an enhanced phagocytic activity of intestinal intra-epithelial leukocytes (iIEL) in mushroom glucan fed birds. Increased phagocytic activity may be due to the action of β-glucan in enhancement phagocytosis and proliferation of monocytes and macrophages [32].

IL6 is one of the pro-inflammatory cytokines which is a multifunctional cytokine that helps to resolve innate immunity and develop acquired immunity as an important function of pro-inflammatory cytokines is preventing translocation of bacteria invading the intestinal mucosa into systemic compartments of the organism by recruiting bacteria phagocytizing cells (Neutrophils, macrophages and dendritic cells) to the site of infection [33].

The present study revealed that there is an increase in the IL6 level among chicks infected with SE and this is in agreement with Kogut *et al.* [34] who said that in vitro *S. enteritidis* exposure of heterophils from outbred Rhode Island Red chickens resulted in upregulation of both pro-inflammatory (IL-6) and anti-inflammatory cytokine mRNA expression. Okamura *et al.* [35] showed significant increased serum level of IL6 at 7 days post-vaccination with killed *Salmonella enteritidis* vaccine.

Increases IL6 level may be as a result of initiation of an acute phase response occur in avian cells in response to invasion by salmonella as reported by Kaiser *et al.* [36]. In contrast to our result, Kaiser *et al.* [37] reported that both pro-inflammatory (IL-6) and anti-inflammatory cytokines in chicken peripheral blood mononuclear cells (PBMC) were decreased as a result of *S. enteritidis* exposure.

IL10 is a cytokine with potent immunoregulatory and anti-inflammatory properties and is produced by activated macrophages and T-cells [38]. IL10 suppresses T-cell proliferation and the release and function of many pro-inflammatory cytokines such as IL1 and IL6 [39].

Our study showed significant decrease in IL10 levels in supplemented and non-supplemented chicks at 7, 14 and 21 days post-infection with more decrease in non-supplemented groups and these are in line with Filho *et al.* [40] who reported decreased level of IL10 after challenge with *S. enteritidis* in all chickens vaccinated either by killed or live vaccine against *Salmonella* and explained this decrease as an important shift to increase antigen presentation and the pro-inflammatory response. TNF-α is a cytokine capable of enhancing expression of neutrophil adhesion molecules on the endothelium and amplifying many other inflammatory processes [41]. Our result showed increased level of TNF-α in all groups

ither supplemented or non-supplemented with b-glucan at 7 days post-infection, our result is in agreement with Young *et al*. [42].

## CONCLUSIONS

In conclusion β-glucan supplementation has a positive effect on modulating the broiler chickens performance and stimulates its immune response after vaccination against *Salmonella enteritidis* without altering blood picture or biochemical parameters.

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