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Influence of β -1,3/1,6 glucan applications on some non-specific cellular immune response and haematologic parameters of healthy Nile tilapia (*Oreochromis niloticus* L., 1758)*

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Abstract: In this study, the effects of β -glucan administration at different dosages on non-specific immune response and haematological parameters in healthy Nile tilapia (*Oreochromis niloticus* L.) were investigated. Fish were fed with diets containing 0.5% and 0.1% glucan (experiment) and without glucan (control) for 2 weeks. After feeding for 2 weeks with 2 different dosages, erythrocyte (RBC), leukocyte (WBC), hematocrit (Hct), haemoglobin (Hb) amount, leukocyte cell types (percentage of lymphocyte, monocyte, neutrophil, eosinophil), the values of phagocytic activities, erythrocyte cell indexes (MCV and MCH) and blood cell sizes were determined. WBC and phagocytic activity amounts, percentages of lymphocyte and monocyte of fish fed with the diets containing 0.1% glucan were higher than 0.5% glucan and control groups ($P < 0.05$). RBC amount was found to be higher in the experimental groups than in the control group. There were no differences between groups in terms of Hb, Hct, MCV, MCH, neutrophil, eosinophil, and cell sizes values ($P > 0.05$).

Key words: Nile tilapia (*Oreochromis niloticus* L.), immunostimulant, β -1,3/1,6 glucan, haematology, non-specific immune system

β -1,3/1,6 glukun uygulamalarının sağlıklı Nil tilapia (*Oreochromis niloticus* L., 1758)'lerinde bazı hematolojik parametreler ve özel olmayan hücresel immün yanıt üzerine etkisi

Özet: Bu araştırmada, Nil tilapia (*Oreochromis niloticus* L.)'lerinde farklı dozlarda β -glukan uygulamasının özel olmayan immün yanıt ve hematolojik parametreler üzerine etkisi araştırılmıştır. Balıklar iki hafta boyunca % 0,5 ve % 0,1 oranlarında (deney) glukun içeren ve glukun içermeyen (kontrol) yemlerle beslenmişlerdir. İki farklı dozla iki haftalık besleme sonrasında, eritrosit (RBC), lökosit (WBC), hematokrit (Hct), hemoglobin (Hb) miktarı, lökosit hücre tipleri (lenfosit, monosit, nötrofil, eosinofil yüzdeleri), fagositik aktivite miktarı, eritrosit hücre indeksleri (MCV, MCH) ve kan hücre büyüklükleri tayin edilmiştir. Denemenin sonunda, % 0,1 oranında glukun ile beslenen balıklarda WBC ve fagositik aktivite miktarları, lenfosit ve monosit yüzdeleri, % 0,5 oranındaki glukun ve kontrol gruplarından daha yüksek bulunmuştur ($P < 0,05$). RBC miktarı da deney gruplarında, kontrol grubundan daha yüksek bulunmuştur. Gruplar arasında Hb, Hct, MCV, MCH, nötrofil, eosinofil yüzdeleri ve hücre büyüklüklerinde herhangi bir farklılık gözlenmemiştir ($P > 0,05$).

Anahtar sözcükler: Nil tilapia (*Oreochromis niloticus* L.), immunostimülant, β -1,3/1,6 glukun, hematoloji, özel olmayan immün sistem

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Introduction

Immune responses have important role at the beginning of parasitic and bacterial infections and also against phagocytosis, natural killer cell (NK), complement, lysozyme, and antibody activity. Specific and non-specific immune-responses fight against infections (e.g. parasitic, bacterial) (1).

Immunostimulants are used to support general health performance of animals and decrease mortality rates in animal farming, including aquaculture (2).

In this study, β -1,3/1,6 glucan, a polysaccharide group obtained from the cell wall of bread yeast (*Saccharomyces cerevisiae*), are used as immunostimulant. They also reduce the side effects of vaccination and antibiotics (3). Immunostimulants are chemical substances that activate leukocyte cells which make animals more resistant to viral, bacterial, fungal, and parasitic infections (4). It was reported in various aquaculture studies that, immunostimulants used in fish pellets increase the number and functions of leukocyte cells and stimulate non-specific immune system (5-7).

In this study, β -1,3/1,6 glucan was used as an immunostimulator on the healthy Nile tilapia (*Oreochromis niloticus* L., 1758). β -1,3/1,6 glucan was added to pellet feeds at the rates of 0.1%, 0.5%, and 0.0%. The aim of this study was to determine the changes on some haematological parameters and non-specific cellular response of Nile tilapia.

Materials and methods

Fish

Healthy Nile tilapia were obtained from the Freshwater Fish Culture Unit of Fisheries Faculty, Çukurova University.

Immunostimulant

β -1,3/1,6 glucan (commercial name is Immunex®) was purchased from Mustafa Nevzat Ltd. in Turkey, and added to pellet feed (Pınar-Çamlı Feed Company) at the rates of 0.1% and 0.5%. Determined dosages of glucan were mixed with feed for 20 min, and dried and stored at +4 °C in a glass jar until used (7,8).

Experimental and sampling methods

Ninety fish were stocked as 3 groups: 2 experiment (0.1%: group I and 0.5%: group II) and one control group (0.0%: group III) (N = 45). Before the experiment, a total of 45 fish (15 fish each) were stocked into 3 concrete ponds (100 × 170 × 75 cm). The weight and length of each fish was measured using a ruler and a balance (0.001 g sensitivity). Average length and weight of fish were found as 18.17 ± 2.7 cm and 111.78 ± 9.6 g, respectively.

The fish were fed with at the rate of 2% of body weight during the experiment period (15 days). Experiment was conducted in triplicate. During the experiment, each pond was aerated and their temperature was measured with a digital thermometer (YSI 3010 USA).

Haematologic examinations

After the feeding period was completed, fish in all groups were anaesthetized with MS-222 and blood samples were taken from the caudal vein using a syringe (8,9).

Blood samples were transferred into tubes with EDTA and stored at 4 °C. All the samples were taken into analysis in the same day. Red and white blood cells were counted using Natt-Herrick solution and Thoma microslide (10,11). Cyanmethaemoglobin and microhaematocrit methods were used to determine Hb and Hct (10,12).

Leukocyte cell types were determined on blood smears from each fish. Peripheral blood smears (PBS) were stained with the mixture of May-Grünwald and Giemsa. Percentages of leukocyte cell types were determined using these preparations (13,14). The diameters of lymphocyte, monocyte, neutrophil, and eosinophil were measured with ocular micrometer. The photographs were taken from stained blood cells.

Erythrocyte indexes, which are known as health indicator in human and veterinary medicine, were used. These indexes were calculated as:

$$\text{MCV (Mean Corpuscular Volume)} (\mu\text{3}) = (\text{Hct} (\%) \div \text{RBC}(10^6/\text{mm}^3)) \times 10$$

$$\text{MCH (Mean Haemoglobin Concentration)} (\text{pg}) = \text{Hb}(\text{g}/100 \text{ mL}) \div \text{RBC}(10^6/\text{mm}^3) \times 10 (15,16).$$

Phagocytic activity

Phagocytic activities of leukocyte cells were determined with a spectrophotometric method. In this method, congo-red-stained yeast cells, which have been phagocytised, were measured. Leukocyte solution (250 µL) was mixed with 500 µL congo-red stained and autoclaved yeast cell suspension (providing a yeast cell: leukocyte ratio of 40:1). The mixtures were incubated at room temperature for 60 min. After incubation, 1 mL ice-cold HBSS was added and 1mL Histopaque (1.077) was injected into the bottom of each sample tube. The samples were centrifuged at 850 ×g for 5 min to separate macrophages from free yeast cells. Macrophages were harvested and washed twice in HBSS. The cells then were resuspended in 1 mL trypsin-EDTA solution (5.0 g/L trypsin and 2.0 g/L EDTA, Sigma) and

incubated at 37 °C overnight. The absorbance of the samples was measured at 510 nm using trypsin-EDTA as blank (17).

Statistical analysis

Experiments were conducted in triplicate and data obtained from the 3 replicates were pooled and analysed with t-test using SPSS 10.0 at the significance level at P = 0.05 (18).

Results

During practice, mean daily water temperature and oxygen level were measured in each tank as 26.0 ± 2.0 °C and 6.1 ± 1.5 mg/L, respectively.

The results of haematological analyses are presented in Tables 1, 2 and 3. The photographs are presented in Figures 1 and 2.

Table 1. RBC, Hb , Hct, MCV, and MCH counts of Nile tilapia.

Parameters	0.1% (Glucan) Group I	0.5% (Glucan) Group II	0.0% (Control) Group III
RBC ($10^6/\text{mm}^3$) X ± SD	1.998 ± 314.17^a	2.295 ± 292.81^a	614 ± 162.42^b
Hb (g/dL) X ± SD	8.47 ± 0.2	8.58 ± 0.4	8.52 ± 0.2
Hct (%) X ± SD	40.6 ± 1.80	38 ± 1.4	38 ± 1.8
MCV (μ^3) X ± SD	1.81 ± 0.71	1.50 ± 0.54	2.10 ± 0.78
MCH (pg) X ± SD	3.76 ± 1.46	3.80 ± 0.40	5.32 ± 0.54

X ± SD: Mean Value ± Standart Deviation

a, b : Means signed with different letter at each line are statistically different. P < 0.05

Table 2. WBC amount, cell types rates, and phagocytic activity amount of Nile tilapia.

Groups	WBC ($10^3/\text{mm}^3$) X ± SD	Lymphocyte (%) X ± SD	Monocyte (%) X ± SD	Neutrophil (%) X ± SD	Eosinophil (%) X ± SD	Phagocytic activity (O.D. 510 nm) X ± SD
0.1% (Glucan)	4.350 ± 581.66^a	38.50 ± 1.71^a	31.0 ± 1.49^a	2.00 ± 0.81	1.50 ± 0.70	0.55 ± 2.4^a
0.5% (Glucan)	4.200 ± 182.57^a	17.80 ± 1.39^b	29.0 ± 4.66^a	2.10 ± 0.87	1.90 ± 0.73	0.44 ± 2.1^b
0.0% (Control)	3.660 ± 254.73^b	20.10 ± 4.14^b	7.30 ± 1.63^b	2.90 ± 0.99	2.40 ± 0.69	0.40 ± 1.6^b

X ± SD: Mean Value ± Standart Deviation

O.D. : Optical Density

a, b : Means signed with different letter at each column are statistically different. P<0.05

Table 3. Leukocyte cell sizes of Nile tilapia.

Groups	Lymphocyte (μm) X \pm SD	Monocyte (μm) X \pm SD	Neutrophil (μm) X \pm SD	Eosinophil (μm) X \pm SD
0.1% (Glucan) Group I	10.0 \pm 1.15	11.0 \pm 1.05	11.6 \pm 1.83	9.90 \pm 1.19
0.5% (Glucan) Group II	9.90 \pm 1.10	11.10 \pm 0.87	10.40 \pm 1.07	10.50 \pm 0.52
0.0% (Control) Group III	8.90 \pm 1.28	8.70 \pm 1.15	9.0 \pm 1.24	8.70 \pm 0.82

X \pm SD: Mean Value \pm Standart Deviation

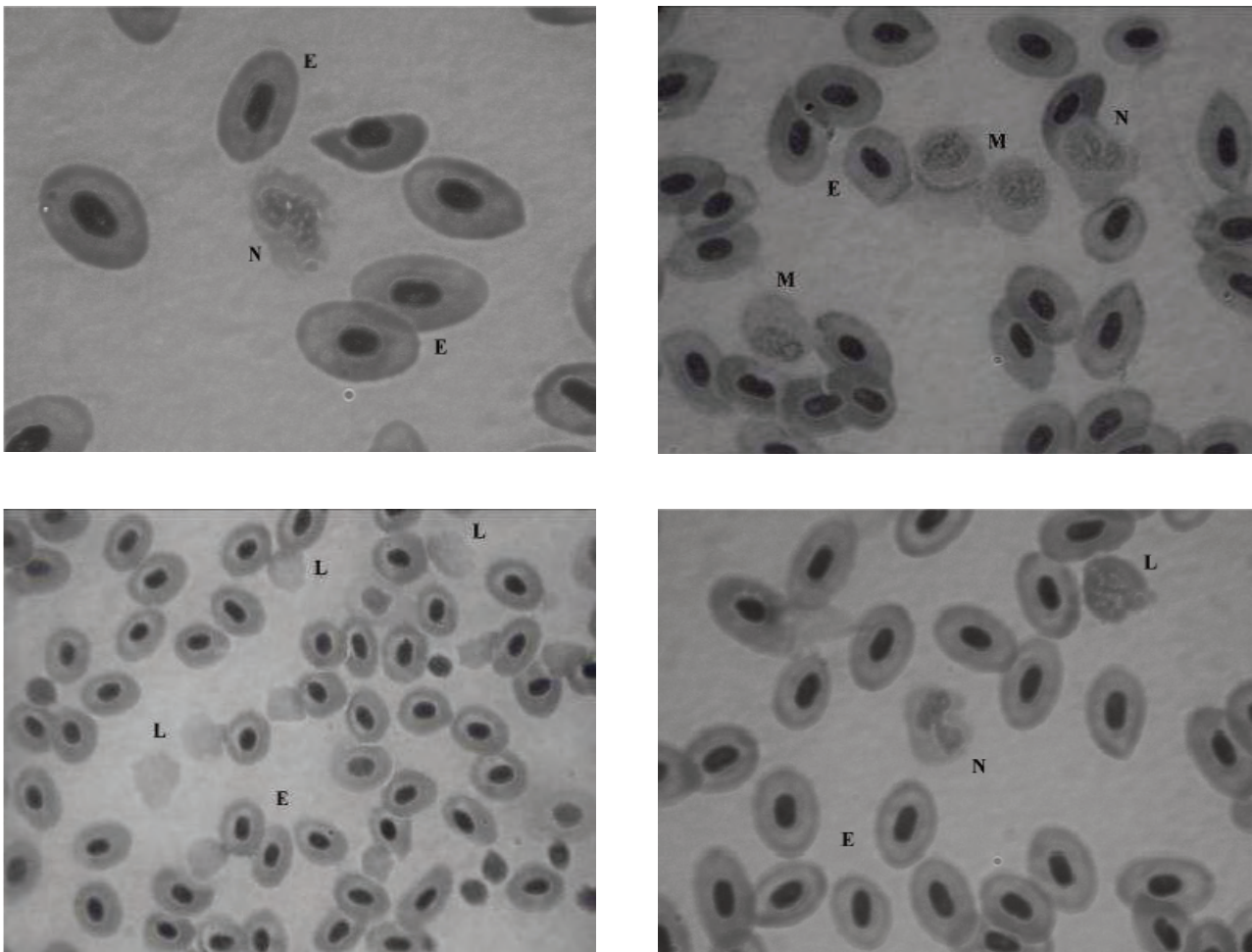


Figure 1. Blood cells of Nile Tilapia (with 0.1% glucan). L. lymphocyte; M.monocyte; N. neutrophyl; E. erythrocyte.

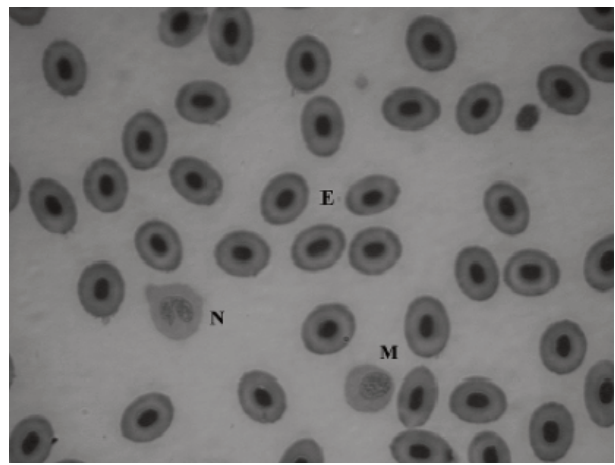
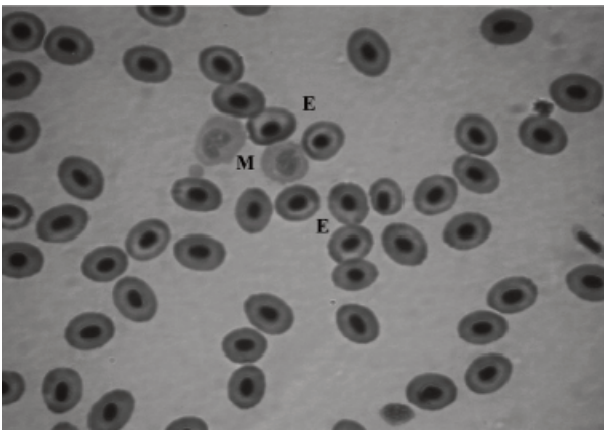
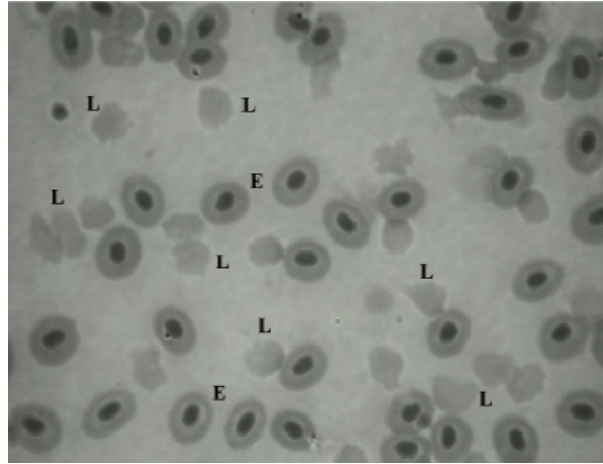


Figure 2. Blood Cells of Nile Tilapia (with 0.5% glucan). L. lymphocyte; M. monocyte; N. neutrophil; E: erythrocyte.

Results showed that there were no differences ($P > 0.05$) in the amounts of Hb, Hct, and the percentages of neutrophil, eosinophil, and cell sizes between groups (Tables 1, 2 and 3). Percentage of lymphocyte, phagocytic activity, monocyte cell, and WBC amounts of fish in group I was higher compared to other groups (groups II and III) ($P < 0.05$) (Table 2). The number of RBC significantly increased ($P < 0.05$) in group II. No significant differences were detected between groups for MCH and MCV counts (Table 1). Furthermore, there were no significant differences in the size of the leukocyte between groups (Table 3).

Discussion

It is known that well supported immune system protects the fish against diseases and increases the rate of survival. Immunostimulants increase non-specific immune response when they are applied alone, but if they are applied with vaccines, they increase both specific and non-specific immune response.

Increases on phagocytic cells, lymphocyte activities, and macrophages can be listed as the common observations on fish treated with immunostimulants. For the effective use of immunostimulants, timing, dosages, the method of administration, and the physiologic condition of fish need to be taken into consideration (2,3).

The β -1,3/1,6 glucan is the most promising among the immunostimulants since they have a well defined chemical structure and mode of action on the immune system (4). Earlier studies focused on the immunostimulatory effects of glucan (2).

In this study, it was found that the percentages of leukocyte, lymphocyte, and monocyte cells were significantly higher in group I in comparison to group II and group III. It is known that the amount of leukocyte cells is normally lower in healthy fishes and can be used as a significant indicator for infectious diseases. The leukocytes, which have an important role in the defence of the host, are blood cells showing phagocytic effects on yeast cells. It was reported that the activities of leukocytes were increased against bacteria, viruses, fungi, and parasites, which entered the organisms, when they were stimulated with a certain amount of glucan (19). Various studies showed similar results for β -1,3/1,6 glucan; lower doses (0.1%, 0.2%, 0.3%) induced immune response and overdoses (1.0%, 2.0%) caused immune suppressing (2,3,6,8). In our study, the application of group I had an effect on non-specific immune system. An effect on phagocytosis also occurred significantly ($P < 0.05$).

Previous studies indicated that immunostimulators decrease the number of erythrocyte (1,5,7,8). Increase in RBC was reported among the effects of β -1,3/1,6 glucan on channel cat fish in comparison with group III (20,21). In the present study, increases on RBC levels are assumed as health indicator of fish.

Erythrocyte cell indexes, such as MCV and MCH, are used to diagnose the classification of anaemia. They are also used in the identification of erythrocyte

cell activities (11,15). There were no significant differences for MCH and MCV values between groups (I, II, and III).

In our study, no significant difference was observed in terms of Hb amount in group III ($P > 0.05$). There was no significant difference between groups in terms of neutrophil and eosinophil cell rates. In some studies, differences in blood cell size or deformations in blood cells due to bacterial diseases, blood parameters, different kinds of medicaments, and chemicals poured in water were reported (22,23). In this study, it was determined that glucan has no effect on blood cell size or shape (Figures 1 and 2; Table 3).

In our study, glucan application in the fish led to the changes in leukocyte, monocyte, lymphocyte, and phagocytic activity (Tables 1-3); (Figures 1 and 2). Result obtained in the present study supported other studies.

In this study, considering the activation of some physiological events evaluated in immuno-haematology, the diet with β -1,3/1,6 glucan was effective. β -1,3/1,6 glucan, which is a strong immunostimulant, may aid in the replacement and reduction of the use of conventional medicines and thus their deleterious effects, such as damage to the environment and resistance.

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