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Original Article

Effect of β Glucan on Haematology of Common Carp (*Cyprinus Carpio*) Infected by Ectoparasites

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Abstract

In this study, the effects of beta-glucan on haematological parameters of common carp, *Cyprinus carpio*, which were naturally infested with ecto-parasites were determined. In the experimental group, infested fish were fed with 0.3% beta-glucan containing pellets during thirty days. In control, infested fish were fed with fish pellets without beta-glucan. Blood samples were collected from each group after feeding. Mortality rates, haemoglobin (Hb) (g/dl), haematocrite (hct) (%), erythrocyte (RBC) ($x10^6/mm^3$), leukocyte (WBC) ($x10^3/mm^3$) amounts and leukocyte cell types (%) were determined. In comparison with the control group, Hct and WBC amounts were increased in the fish fed with beta glucan ($P < 0.05$), while there was no significant difference ($P > 0.05$) for Hb and RBC amounts. Lymphocytes were decreased, while monocytes and neutrophils were increasing among the leukocyte cell types. Survival rate values were 91.12 and 77.78% for fish fed with β-1,3/1,6 glucan and the control group, respectively.

Key Words: Ecto-parasite, Common carp (*Cyprinus carpio*), Immunostimulant, β-1,31,6/Glucan, Haematology.

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Introduction

As in all animal production types, among the most important problems encountered in aquaculture are diseases and harmful organisms. Parasites are the most dangerous of these organisms, and unless necessary precautions are taken, they can quickly infest the body and spread. They frequently lead to weight loss, less fecundity, lack of appetite, difficulty in respiration and higher mortality. Especially ecto-parasites cause mechanical defects and

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bruises on gills and skin and more serious result in severe injures (Wootten, 1989). All parasites begin to damage the hosts, and even cause its death when they reach to a certain density. There are some chemical or herbal therapeutics used against these parasites in aquaculture, but some of these are thought to be harmful for environment, and thus for aqua life and humans due to their pollution load. Therefore, some less harmful alternative treatment methods have been taken place. Immunostimulants, one of the alternative ways in dealing with the diseases, are easy to use and very effective (Sakai, 1999). They are known, in aquaculture and general animal production, to decrease mortality rate which are stemmed from infections, strengthen the immune system, and thus improve growth and general performance and protect production loss (Raa, 1996).

The β-1,3/1,6 glucan, which we used in our study, is an immunostimulant and actually a polysaccharide derived from the cell wall of bread yeast (MacroGard, Betafectin). It has a specific structure which can be used with drugs safely and improve the effects of vaccinations and antibiotics. It is also commonly used in fish farms after added into the therapeutics (Sakai, 1999). Especially, in the studies related to immunostimulant in aquaculture, it led to remarkable increases in the amount and function of leukocyte cells as it stimulated non-specific immune system after it added to diet and taken orally by fish (Jeney and Anderson, 1993; Cleary, et al. 1999; Cook, et al. 2001 and Dugenci and Candan, 2003). In the present study, determine effects of dietary β-1,3/1,6 glucan on blood cells which have phagocytic roles in non-specific cell response in parasite infected common carp.

Materials and Methods

Fish

Parasite infested common carps, *C.carpio*, were obtained from earthen ponds of Freshwater Fish Research Unit (FFRU) of Fisheries Faculty, University of Cukurova. Ninety fish were found naturally infected with *Lerneae cyprinacea* (80%), Argulus foliaceus (12.22%). After that, total length and body weight of fish were measured (With milimetric ruler and the scale in 0.001g sensitivity). Average length and weight of fish were found as 42.9±5.7g and 14.7±0.9cm, respectively.

Immunostimulant and Feed Preparation

β-1,3/1,6 glucan obtained from S.cerevisiae (Macrogard®) was added carp feed (PINAR-ÇAMLI Feed Company, Turkey) at the rate of 0.3% by mixing with feed during 20 minutes. After that, the mixture was dried in an oven at +40^{o}C until it was stored at +4^{o}C in a glass jar (Jeney, et al. 1997 and Dugenci and Candan, 2003).

Experimental and Sampling Methods

Fish were examined for parasitic infestation amount and determination of parasites. Firstly, body surface, gills and fins of fish were observed as macroscopic and the parasite were counted, recorded and identified (Bikhovskaya Pavlovskaya, 1962; Fryer, 1982; Bauer, 1987 and Wootten, 1989). During the parasitic controls, the fish were anaesthetized using quinaldine sulphate (SIGMA Chemical Company, Germany) at a dose of 20ml/L quinaldine sulphate (Yanar and Genc, 2004). After that, 45 fish for the trial and 45 for control groups and in total 90 parasitized fish were used for the experiments.

Fish were fed twice in a day as 2% of thier live body weight for 30 days. Six aquarium (80x40x50cm, LxWxH, respectively) were used in triplicate, to contain 15 fish in each experiment aquarium. Through trial, each aquarium was aerated and its temperature was measured with digital thermometer (YSI 3010 USA). Treatment groups were fed with diet containing β-1,3/1,6 glucan while control groups were fed with diet without glucan.

After the end of experiment, all groups were observed for blood analysis. To reduce stress resulting for transformation and handling process, during the measurements, the fish were

anaesthetized using quinaldine sulphate (Yanar and Genc, 2004). Following anaesthetized application, blood was taken from a caudal vein using syringe (Blaxhall and Daisley, 1973 and Kocabatmaz and Ekingen, 1984). Also, mortality rates of fish were registered until the end of the experiment.

Blood Analysis

Blood samples were taken from caudal vein of each fish by means of an injector and put into the tubes with EDTA. Red and white blood cells were counted by using Natt-Herrick solution and thoma micro-slide (Konuk, 1981 and Stolen, et al. 1994). Syanmethaemoglobin method was used in Hb and also, microhaematocrit technique was used in Hct (Blaxhall and Daisley, 1973). Leukocyte cell types were determined on blood smears from each fish. Peripheric blood smears (PBS) were dyed with the mixture of May-Grünwald and Giemsa. Percentages of leukocyte cell types were determined using these preparations (Kocabatmaz and Ekingen, 1984 and Fujimaki and Isoda, 1990).

Statistical Analysis

Data obtained from the blood parameters were analysed with t-test using SPSS 10.0 packet programme at the significant level at (P<, >0.05).

Results

Water temperature and oxygen level were measured in each aquarium as 20.33±3.08°C and 7.10±0.99 mg/L, respectively.

Fish were examined for parasite and 90 of them were found infested with *Lerneae cyprinacea* (Copepoda) and/or Argulus foliaceus (Branchiura) classified in Arthropoda phylum (Table 1). *Lerneae cyprinacea* was identified in 80% of fish and the percentage of Argulus foliaceus was found as 12.22% among 90 fish.

Treatments and the control groups were shown in (Table 2 and 3). Results showed that there were no differences (P >0.05) in amounts of erythrocyte, haemoglobin and eosinophil between treatment and the control groups. However, the amounts of haematocrit, leukocyte and the percentage of monocyte and neuthrophil cells were significantly increases (P <0.05) between treatment and the control groups. A significant decrease in the percentage of lymphocyte in treatment group was observed. The rate of mortality was 22.22% in the control group while it was 8.88% in treatment group. In addition, the photograph of blood smear were taken and presented in (Figure 1 and 2).

Table 1: Parasite Species and Amounts in Common carp (*C.carpio*) (n: 90):

Parasite Species	Regions of the Infestation in Fish			Infested	Total Parasite
	Gill	Fin	Skin	Fish Amount	Amount
Lerneae cyprinacea	49	22	6	72(80%)	77(85.55%)
Argulus foliaceus	-	-	18	11(12.22%)	18(20.0%)
Lerneae cyprinacea +	3	-	5	7(7.77%)	8(8.88%)
Argulus foliaceus	-	-	-	-	-
Toplam	-	-	-	90	103

Table 2: Leukocyte Cell ($10^3/mm^3$) and Leukocyte Cell Types (%) Amount in Groups:

Groups	Leukocyte ($10^3/mm^3$) X±SD	Lymphocyte (%) X±SD	Monocyte (%) X±SD	Neutrophil (%) X±SD	Eosinophil (%) X±SD
β-Glucan	5.49±0.69*	21.90±1.39*	25.43±2.54*	4.36±1.01*	3.72±0.54
Control	3.62±0.29	40.58±1.58	12.38±1.17	2.94±0.87	3.71±0.60

X±SD: Mean Value ± Standart Deviation
***:** P <0.05 significant level

Table 3: Erythrocyte, Haemoglobin and Haematocrit Amount in Groups:

Groups	Erythrocyte (10^6/mm^3) X±SD	Haemoglobin (g/100ml) X±SD	Haematocrit (%) X±SD
β-Glucan	1.643±24.65	4.53±0.47	35.61±2.47*
Control	1.606±30.70	4.41±0.29	28.80±2.50

X±SD: Mean Value ± Standart Deviation
***:** P <0.05 significant level

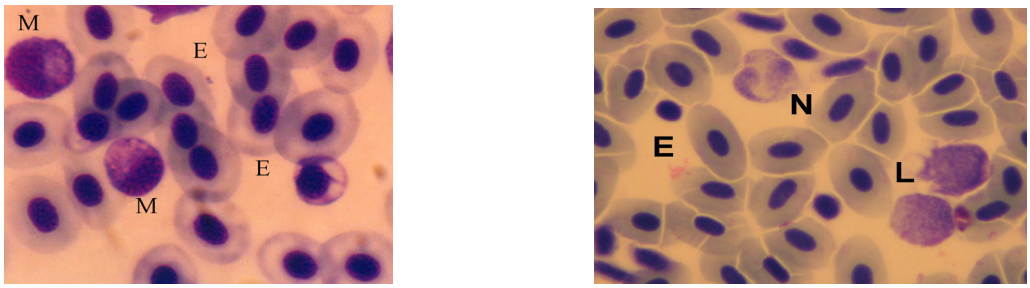

Fig. 1: The Blood Cells in Control Group.
A: E. Erythrocyte M. Monocyte
B: L. Lymphocyte N. Neuthrophil

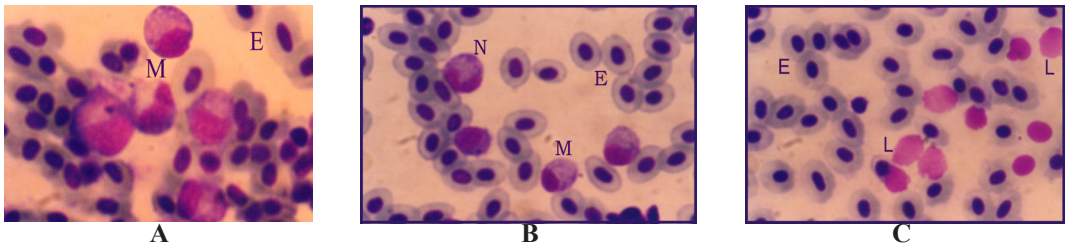

Fig. 2: The Blood Cells in Glucan Treatment.
A: E. Erythrocyte M. Monocyte
B: N. Neuthrophil
C: L. Lymphocyte

Discussion

Immunostimulants are chemical substances that activate white blood cells and thus may render animals more resistant to infections by viruses, bacteria, fungi and parasites (Raa, 2000). The Immunostimulants, such as chitin, lactoferrin and levamisol, including Beta-Glucan, takes great part in some disease control, increasing the bactericidal, fungicidal and phagocytic activities. Especially in parasitic infections, the development of parasites which attach to skin and mucosal membranes are inhibited by complement factors, enzymes and enzymes inhibitors. These chemical defense weapons are produced by specialized cells in the surface tissues and are continuously secreted (Putz and Bowen, 1964 and Robinson and Avenant Oldewage, 1996). Certain immunostimulants, eg. β-1,3/1,6 glucans, stimulates these cells and thereby the defense against surface parasites. Using immunostimulants, in addition to chemotherapeutic agents and vaccines, has been widely accepted by fish farms.

The β-1,3/1,6 glucan is among the most promising immune stimulants since they have a well defined chemical structure and

mode of action on the immune system (Raa, 2000). However, in various studies, overdoses of several immunostimulants were seen to induce immunosuppression in fish. For the effective use of immunostimulants, the timing, dosages, method of administration and the physiologic condition of fish need to be taken in to consideration (Raa, 1996 and Sakai, 1999). Various studies show similar results for β-1,3/1,6 glucan, too: lower doses (0.1; 0.2; 0.3%) inducing immune response, overdoses (1.0; 2.0%) causing immune suppressing (Raa, 1996 and Jeney, et al. 1997). In this study, β-1,3/1,6 glucan was chosen for its defensive features for parasitic attacks on skin and mucosa, and, taking the previous studies into consideration, a dose of 0.3% was preferred. By the 30th day, there were no difference in the amount of RBC and Hb, but Hct increased (Treatment group, 35.61±2.47%; control, 28.8±2.5%). In a study about non-specific immunity on trout, Siwicki et al. (1994) reported some decreases in the Hct amount under stress, but an increase with glucan treatment. In another study, Hct amounts of control were reported to become higher with different doses of immunostimulant treatment on seabass (Sealey and Gatlin, 2002). Although it was carried our for a different species, the increases in haematocrit amounts with glucan treatment in our study can be said to support the previous studies (Table 3)

In our study, the effects of glucan treatment were also examined on the changes in leukocyte cell types due to the physiological stress of Copepoda and Branchiura parasites on fish. Glucan treatment was seen to cause remarkable increases in leukocyte cells.

It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases. The leukocytes, which have an important role in the defense 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 parasitic pathogens which entered the organisms when they were stimulated with a certain amount of glucan (Keles, et al. 2002).

In our study, the percentage of lymphocytes in the peripheral blood decreased, while the relative populations of monocyte and neutrophils increased (Table 2). In addition to macrophage activity of β-1,3/1,6 it has also important effect on increasing the neutrophil number in circulation and on B-lymphocytes and T-suppressor cells. In a study, glucan (β-1,3/1,6) was seen to remarkably improved macrophages function and host resistance against bacterial, viral, fungal and parasitic infections. Lymphocytes are blood cells responsible for antibody production in the circulation against any infection. However, the decrease in the lymphocytes (Treatment group, 21.90±1.39%; control, 40.58±1.58%) and the increase in the monocytes responsible for phagocytosis and neutrophils in the fish groups. It can be said that, decreasing of lymphocyte percentage in the experimental group shows that it is not activited by using beta-glucan dose in this study. And also, increasing of monocytes and neutrophil were regarded as an indication of the effect mechanism of glucan on phagocytic cells. Jeney et al. (1997) made a related study on trout and examined the effects of different doses of glucans on stress prevention. They reported low doses of glucans (0.1%) increased neutrophil and macrophage cells, which are responsible for non-specific immune responses. In the haematological studies on fishes infected with parasites, RBC amount was reported to decrease, whereas WBC and neutrophil were increased in infected fish (Cengizler and Şahan, 2000). Boon et al. (1990) studied the effects of various infestation levels of A. crassus on the hematology of European eels. WBC quantities were reported to reach the highest level in fishes infected with this parasite.

The reports in various previous studies related to the increases in the amount of WBC of the fishes infected with parasites, which is

considered as a part of defense mechanism of the fish body against the diseases, are in harmony with our findings for control. β-1,3/1,6 glucan molecules, known to be special receptors found in white blood cells of all animals, ranging from invertebrate to humans, and to inhibit the most essential defense mechanism in the living beings (Raa, 2000), are given orally to the fish in our study. In the group fed with glucan supplement in our study, defensive leukocyte cells were seen to be activated and their amount was increased. Jeney et al. (1997) reported higher mortality in trout with higher doses of glucan application (1.0 and 5.0%), but lower mortality with lower doses. In our study with 30 days of experiment period, control had 22.22% mortality rate, while the experiment group with β-1,3/1,6 glucan had 8.88% mortality rate. A lower dose of glucan was also observed to lead to a lower mortality rate. Moreover, the blood cells of the experiment group were seen to have dense leukocyte cells nearly in every viewable part (Table 2).

In the study, the therapeutic effects of β-1,3/1,6 glucan on Copepoda parasites commonly seen in both sea and fresh water aquaculture were studied. In this aim, β-1,3/1,6 was added into the diet and given to the fish orally and was seen to increase monocyte and neutrophil cells as well as leukocyte cell, which have phagocytic roles in non-specific cell response. Especially the increases in the cells of non-specific defense system and lower mortality rates are good signs that β-1,3/1,6 glucan is a good immunostimulant.

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