Polish Journal of Veterinary Sciences Vol. 26, No. 1 (2023), 109–118

DOI 10.24425/pjvs.2023.145012

Original article

Effects of β-1,3/1,6 glucan dietary supplements on some immunological and hematological health markers in Siberian sturgeon (*Acipenser baerii*) infected with *Aeromonas hydrophila*

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Abstract

This experimental study aimed to investigate some hematological and immunological changes as a result of *Aeromanas hydrophila* infection in Siberian sturgeon (*Acipenser baerii* Brandt, 1869). Their feeds were supplemented with β-1,3/1,6 glucan at different ratios, 250 mg/kg (βG_{250}); 500 mg/kg (βG_{500}) and 750 mg/kg (βG_{750}). To create an experimental infection, 4×10^6 cfu/ml *Aeromonas hydrophila* inoculum was intraperitoneally injected to fish. 0.1 ml intraperitoneal bacteria injection was given to the fish in 12 of 15 tanks, each consisting of 10 fish (the fish in the control group were not given bacteria). Considering the βG_{500} and βG_{750} group fish as positive (C+) and negative control (C−) groups in terms of hematological parameters, it was found that RBC (erythrocyte) and Hb (hemoglobin) values, as well as RBC indices (MCV, MCHC, MCH) significantly increased. The immunological parameters, including WBC (leukocyte), leukocyte cell percentages (lymphocyte, monocyte, neutrophil, eosinophil), as well as cytokines, including IL-1β, IL-6, IL-8, and TNF-α values showed similar increases in the βG_{500} and βG_{750} groups. It was found that the addition of 500 and 750 mg/kg doses of β-1,3/1,6 glucan to the feed stimulated non-specific immunity of fish against bacterial agents and/or septicemic diseases and beta glucan at this dosage range was determined to be ideal for fish health and that it may be a herbal immunostimulant that can be an alternative to many medicaments.

Key words: *Acipenser baerii*, *Aeromanas hydrophila*, β-1.3/1.6 glucan, hematology, non-specific immune parameters.

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Table 1. Design of the control (C+, C-) and β-1,3/1,6 glucan (βG) Siberian sturgeon groups.

	A. hydrophila	β-1,3/1,6 Glucan (βG)
Control Groups		
Positive Control (C+)	+	-
Negative Control (C-)	-	-
βG Groups		
250 mg/kg β-1,3/1,6 glucan (βG_{250})	+	+
500 mg/kg β-1,3/1,6 glucan (βG_{500})	+	+
750 mg/kg β-1,3/1,6 glucan (βG_{750})	+	+

Introduction

Bacteria, viruses, fungi, and parasitic pathogens are among the primary problems that cause significant losses in aquaculture. Antibiotics used improperly in preventing bacterial infections in aquaculture have led to increases in the antibiotic resistance of fish over time. Therefore, the use of immunostimulants in aquaculture is a promising method to increase the disease and stress resistance and also to avoid the side effects of medicaments used improperly. Immunostimulants are preferred in the aquaculture industry and livestock industry to reduce the mortality caused by infections and to improve the overall performance of the animal (Sakai 1999). Pathogens such as *Aeromonas hydrophila, A. salmonicida, Edwardsiella tarda, E. ictaluri, Vibrio anguillarum, V. vulnificus, V. salmonicida, Yersinia ruckeri* and *Streptococcus* spp.; viruses such as infectious hematopoietic necrosis, yellow head virus, viral hemorrhagic septicemia and the parasite *Ichthyopthirius multifiliis* in fish/shrimp have been successfully controlled using immunostimulants (Barman et al. 2013). Immunostimulants used as dietary supplements increase the defense resistance against pathogens (Sakai 1999, Magnadottir 2010). Glucans are immunostimulants used in fish farming and show good results. They are obtained from the cell wall of yeast and some plants. Glucan is a highly effective immunostimulant which strengthens the non-specific defense mechanism and provides protection against disease-causing pathogens (Jeney and Anderson 1993).

The β-1,3/1,6 glucan used in this research is a herbal immunostimulant and, in various studies, it has been found to activate the non-specific immune system and provide resistance against pathogens in different fish species struggling with the disease (Jeney et al. 1997, Hastuti 2012, Aramli et al. 2015, Kazuń et al. 2020). Fish, like all living things, become sick due to their interactions with the environment. Success in aquaculture can be achieved by preventing fish from becoming sick and treating sick fish as soon as possible. Accordingly, hematological and immunological techniques, which are the methods achieving the fastest and definitive diagnosis in fish diseases, have important roles in fighting the infection, especially in the early stages (Duman and Şahan 2018).

The purpose of this research was to reveal the effects of feeding Siberian sturgeon (*A. baerii*) with β-1,3/1,6 glucan fishmeals against *Aeromanas hydrophila* on hematological and non-specific immune parameters and also to determine the most effective dose of beta-glucan.

Materials and Methods

Experimental animals and design

In the study, 150 sturgeon (*A. baerii*) of approximately 250 to 300 g (weight 274.2±13.8 g and length 40.7±3.1 cm) supplied from a private fish farm were stored in 15 concrete ponds of 1.5×1×1 m in the same facility with stocking density 1.8±0.31 kg/m^3. The study was designed to have 5 groups with 3 repetitions, including 2 control groups as one positive and one negative (C+; C−) and 3 experimental groups (βG_{250}, βG_{500}, βG_{750}) and 10 fish were placed in each pond (Table 1). Before the fish were put into the ponds, they were subjected to macroscopic (external visual examinations) and microscopic (parasitological and bacteriological examinations) health screenings. The supplied spring water flow rate for each of the 15 concrete ponds was 3 L min^{-1}. During the experiment, temperature of the water was 19±0.91 ºC, pH was 7.8±0.58 and the amount of dissolved oxygen was 7.1±0.92 mg/L. No photoperiod was applied during the trial. The study was approved by the Animal Experiments Local Ethics Committee (Ç.Ü-SABİDAM) dated 10.12.2020 (meeting number: 9, decision no: 11). The measurements for the water temperature, oxygen, and pH of the concrete ponds were measured daily with a YSI 6600-brand CTD multiparameter instrument.

Table 2. Formulation of basic sturgeon diet (feed formulation g/100g).

Basic Content	Amounts
Wheat	15
Dehulled Extracted Toasted Soya	13
Poultry Meal	5
Fish meal	37
Blood Meal	7
Wheat gluten	8
Lipids	
Fish oil	12
Vitamins and Minerals	
Vitamin A	10020 IU/kg
Vitamin C	500 mg/kg
Vitamin E	200 mg/kg
Vitamin D3	1137 IU/kg
Phosphorus	0.9 %
Calcium	1.5 %
Sodium	0.3 %
Antioxidants	
Ethoxyquin	100 mg/kg
Butylated hydroxytoluene	32 mg/kg

Bacterial strain and challenge study

The *A. hydrophila* strain (ATCC 7966 code number, *A. hydrophila* Microbiologics, Eastern Fish Disease Laboratory, Leetown, WV, USA) used in the present study was stored at -80 °C in a glycerol solution. This strain was then inoculated on Tryptone Soy Agar (TSA), and the bacterial culture incubated for 24 hours at 27°C. This strain was then inoculated in Tryptone Soy Broth (TSB). The bacterial density in physiological saline of *Aeromanas hydrophila* inoculum, which was the appropriate predetermined dose to establish the experimental infection, was adjusted to a concentration of 4×10^6 cfu/mL (Güven et al. 2013). The bacterial suspension was then stored.

Feed preparation with β-1,3/1,6 glucan and basic diet

The β-1,3/1,6 glucan (the commercial name is ImmunexR) was purchased from Turkey and added to basic sturgeon feeds. Feed mixes and design were made according to Jeney et al. (Jeney et al. 1997). Basic sturgeon feed was ground, and β-1,3/1,6 glucan in powder form was mixed into this feed at three different rates (250 mg/kg, 500 mg/kg and 750 mg/kg). The feeds were stirred for 20 minutes, pelleted in the appropriate sizes, and dried in an oven at 40 °C. The dried feed was stored at +4 °C in glass jars until the time of analysis (Jeney et al. 1997, Düğenci and Candan 2003). The control fish (C+, C−) were fed with basic sturgeon feed while the experimental fish (βG_{250}, βG_{500}, βG_{750}) were fed with the feeds including different ratios of β-1,3/1,6 glucan. Control and experimental fish were fed at 2% of their body weight two times a day during the 10-week feeding period. The feed formulations of the control and experimental groups are shown in Table 2.

Experimental infection with *Aeromonas hydrophila*

The experiment consisted of 2 control groups one positive (C+; *A. hydrophila* infection available; β-1,3/1,6 glucan not available) and one negative (C−; *A. hydrophila* infection not available; β-1,3/1,6 glucan not available). Also, it consisted of 3 experimental groups of β-1,3/1,6 glucan at different ratios of 250 mg/kg (βG_{250}), 500 mg/kg (βG_{500}), and 750 mg/kg (βG_{750}) (Table 1). To create an experimental infection, *A. hydrophila* inoculum of 4×10^6 cfu/mL, which was determined to be optimum infective dose, was intraperitoneally injected to 120 fish (except C−) within 12 ponds in groups of 10 in the amount of 0.1 mL at the 10^{th} week when feed applications were terminated. Physiological saline solution, i.e. blank

injection, was intraperitoneally applied to C- group fish at the same ratios to create similar stress to other groups. The fish showed signs of disease 5 days after injection and fish that died afterward were dissected. *A. hydrophila* was re-isolated from all the diseased and healthy skin, gill, and gut tissues of fish using Tryptic Soy Agar (TSA) medium (Austin and Austin 2012). Bacterial colonies were confirmed as *A. hydrophila* by Polymerase Chain Reaction (PCR) (Dewi and Koesharyani 2017).

Immunological analyses

The fish, which were given an *A. hydrophila* injection after beta-glucan feeding for 10 weeks, were subjected to immunological and hematological analyses on the 5th day after symptoms appeared. The fish, including the control group, continued to be fed until the onset of symptoms on the 5th day after injection. Before the immunological and hematological analyses, 2-phenoxyethanol of 0.01 mL/L concentration was applied to the fish (Priborsky and Velisek 2018).

For non-specific immunological analysis, leukocyte cell formulas (lymphocyte, monocyte, eosinophil, neutrophil), cytokines and phagocytic activity levels were considered from the blood samples taken from fish. In determining leukocyte cell formulas, a drop of blood without anticoagulant obtained from the tail of a fish on a slide was spread with the help of a second slide. In preparations stained using the May-Grünwald-Giemsa staining technique, the whole region was stained and 100 leukocyte cells were counted in the shaded area and the percentage of leukocyte cells (monocyte, lymphocyte, neutrophil, eosinophil) was then determined (Blaxhall 1972, Fujimaki and Isoda 1990). Moreover, the blood cells were photographed with Olympus DPI25 digital camera for the examinations performed at 100× magnification of the light microscope.

The phagocytic activities of leukocyte cells were determined using the spectrophotometric method (Jeney et al. 1997). Accordingly, congo-red-stained yeast cells, which were previously phagocytized, were measured. Leukocyte solution (250 µL) was mixed with 500 µL of congo-red stained and autoclaved yeast cell suspension (providing a yeast cell: leukocyte ratio of 40:1). The mixtures were left to incubate at room temperature for 60 minutes. Following the incubation, 1 mL ice-cold HBSS was added and 1mL Histopaque (1.077) was injected to the bottom of each sample tube. The samples were centrifuged at 850 ×g for 5 minutes to separate macrophages from free yeast cells. The macrophages were harvested and washed twice in HBSS. The cells 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 the blank (Jeney et al. 1997).

For the serum TNF-α, IFN-γ, IL-1β, IL-6, and IL-8 cytokine levels, enzyme-linked immunosorbent assay (ELISA) kits were used and the results were assesed according to the double-antibody sandwich method (Voller et al. 1978). The double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) is preferably used to detect the concentration of an obscure antibody in a specimen. Native antigen is not required here, but the use of a reporter-labeled determination antibody is necessary (Kohl and Ascoli CA 2017). ELISA commercial kits were used for the analyses. Accordingly, the Fish Interleukin 1beta (IL- 1beta) ELISA Kit (Catalog no. MBS700230), Fish Interferon-gamma (IFN-gamma) ELISA Kit (Catalog no. MBS702530), Fish Tumor Necrosis Factor-alpha ELISA Kit (Catalog no. MBS024441), Fish Interleukin 6 (IL-6) ELISA Kit (Catalog no. MBS702353), and Fish Interleukin-8 (IL-8) ELISA Kit (Catalog no. MBS700055) were used. Catalog numbers for specified ELISA kits were obtained from MyBioSource, Inc. company.

Hematological analyses

Blood samples were taken from the caudal vein using a syringe (Gomułka et al. 2015), transferred into tubes with EDTA for hematological analysis. In the research, the Natt-Herrick solution was preferred to determine RBC and WBC amounts. With this solution, erythrocyte and leukocyte cells could be simultaneously counted with an Olympus BX 51 light microscope at 400× magnification. The results are given as $\times 10^6/mm^3$ for erythrocyte and $\times 10^3/mm^3$ for leukocyte (Blaxhal and Daisley 1973, Kocabatmaz and Ekingen 1984). Cyanmethaemoglobin and microhaematocrit methods were used to determine Hb and Hct (Kocabatmaz and Ekingen 1984). Leukocyte cell percentages were determined on blood smears from each fish. Peripheric blood smears (PBS) were stained with the mixture of May-Grünwald and Giemsa (Kocabatmaz and Ekingen 1984). The photographs were taken from stained blood cells.

RBC indices determined are as follows; MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin); MCHC (Mean Corpuscular Haemoglobin Concentration) The formulations for these indexes are as given:

MCV (μ^3)= (Hct) (%) / RBC ($10^6/mm^3$) × 10

MCH (pg) = Hb (g/100 mL)/ RBC ($10^6/mm^3$) ×10

MCHC (g/dL)= Hb (g/100 mL) ÷ (Hct) (%) × 100 (Schreck and Moyle 1990).

Fig. 1. A – General view of internal organs in Siberian sturgeons infected with *Aeromanas hydrophila*; B – Fins, gill filaments and general appearance of the mouth in Siberian sturgeon infected with *A. hydrophila.*

Disease resistance

Mortalities in the concrete pools were recorded daily from all the groups for 15 days after the infection has been created. Relative percent survival (RPS) was determined according to the Baulny et al. (Ogier de Baulny et al. 1996) formula;

$$PS\ (\%) = \frac{\text{Mortality (\%) of untreated controls} - \text{Mortality (\%) of treated}}{\text{Mortality (\%) of untreated controls}} \times 100$$

Statistical analyses

For the evaluation of the results, One-Way ANOVA and Duncan tests were performed using the SPSS 10.0 statistical program for statistical analysis of each parameter belonging to the control and infected group (Hayran and Ozdemir 1996).

Table 3. Non-specific immune parameters in Siberian sturgeons fed with different rates β-1,3/1,6 Glucan.

	Control	Groups	Beta	Glucan	Groups
Parameters	C-	C+	βG_{250}	βG_{500}	βG_{750}
Lymphocyte (%)	67.54±4.7[a]	69.21±5.2[a]	70.21±6.1[a]	79.93±5.8[b]	76.74±5 .7[b]
Monocyte (%)	9.25±0.7[a]	8.72±1.3[a]	8.63±1.2[a]	16.39±1.0[b]	15.11±1.4[b]
Neutrophil (%)	12.98±1.5[a]	10.46±1.9[a]	13.34±1.5[a]	22.93±1.2[b]	21.61±1.7[b]
Eosinophil (%)	3.75±0.8[a]	2.82±0.6[a]	3.12±0.7[a]	3.88±0.3[a]	3.79±0.5[a]
Phagocyt. act. (O.D. 510 nm)	0.23±0.0[a]	0.28±0.0[a]	0.21±0.01[a]	0.39±0.0[b]	0.37±0.0[b]
TNF-α (pg/mL)	25.38±4.1 [a]	27.14±6.2 [a]	22.13±5.7 [a]	39.42±1.0 [b]	46.24±8.3 [b]
IFN-γ (pg/mL)	50.84±1.1 [a]	60.87±13.5 [a]	107.49±2.9 [b]	59.13±1.5 [a]	66.57±1.2 [a]
IL-1β (pg/mL)	1.21±0.4 [a]	1.18±0.2 [a]	1.15±0.2 [a]	1.75±0.7 [b]	1.82±0.4 [b]
IL-6 (pg/mL)	13.7±1.4 [a]	12.04±3.1 [a]	14.2±2.3 [a]	21.8±5.6 [b]	23.4±5.8 [b]
IL-8 (pg/mL)	35.47±8.9 [a]	39.82±9.8 [a]	40.32±9.5 [a]	68.7±12.1 [b]	69.1±13.6 [b]

Data are represented as mean ± SD. Values in the same line with different letters are significantly different ($p<0.05$).

Table 4. Hematological parameters in Siberian sturgeons fed with different rates of β 1,3/1,6 Glucan. WBC – Leukocyte, RBC – Erythrocyte, Hb – Hemoglobin, Hct – Hematocrit, RBC Indices (Erythrocyte indices); MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Hemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration.

	Control	Groups	Beta	Glucan	Groups
Parameters	C-	C+	βG_{250}	βG_{500}	βG_{750}
WBC($\times 10^3$/mm^3)	12.88±0.3[a]	11.45±0.5[a]	13.12±0.6[a]	15.97±0.8[b]	15.69±0.8[b]
RBC ($\times 10^6$/mm^3)	0.71±0.1[a]	0.78±0.1[a]	0.74±0.1[a]	0.97±0.1[b]	0.95±0.1[b]
Hb (g/dL)	6.41±0.5[a]	7.01±0.3[a]	6.94±0.5[a]	7.11±0.8[a]	6.73±0.7[a]
Hct (%)	23.17±0.8[a]	21.75±0.9[a]	21.54±1.1[a]	28.38±1.3[b]	26.95±1.1[a]
RBC Indices					
MCV (μ^3)	326.33±21.3[a]	278.71±23.1[b]	291.08±18.7[b]	292.57±22.5[b]	283.68±20.4[b]
MCH (pg)	90.28±8.2[a]	91.15±9.6[a]	93.78±8.8[a]	72.26±6.1[b]	70.84±5.9[b]
MCHC (g/dL)	27.66±3.5[a]	32.68±4.8[a]	32.21±3.1[a]	24.71±2.6[b]	24.97±3.3[b]

Data are represented as mean ± SD. Values in the same line with different letters are significantly different ($p<0.05$).

Results

Clinical and necropsy findings of fish

On the fifth day after the injection, in all groups except the C- group, the clinical symptoms of the pathogen intensified and deaths were observed in the C+ group. In the clinical evaluations of the C+ group fish, lethargy and anorexia were observed at 48 hours. In the C+ group, hyperemic areas in the fin base, erosions in the caudal fin and fin tips, and discoloration of skin were observed on day 5. No symptoms were detected in the βG_{500} and βG_{750} groups in necropsy examinations. However, in the dead or dying fish in C+ and βG_{250} groups, bright-red large spots around the mouth, hemorrhage areas in the gill filaments (Fig. 1A) were observed. Also, hyperemic areas, enlarged internal organs, common hemorrhages, and bloody intestinal contents were determined (Fig. 1B).

Immunological parameters

In the study, leukocyte cell percentages and phagocytic activity values examined in terms of non-specific immune system parameters are compared in Table 3 for experimental and control groups. According to the table, when comparing these values for C+ and C− groups, significant increases were observed in the βG_{500} and βG_{750} group fish; on the other hand, TNF-**α,** IL-1β, IL-6, and IL-8 values showed similar increases in the same group of fish ($p<0.05$).

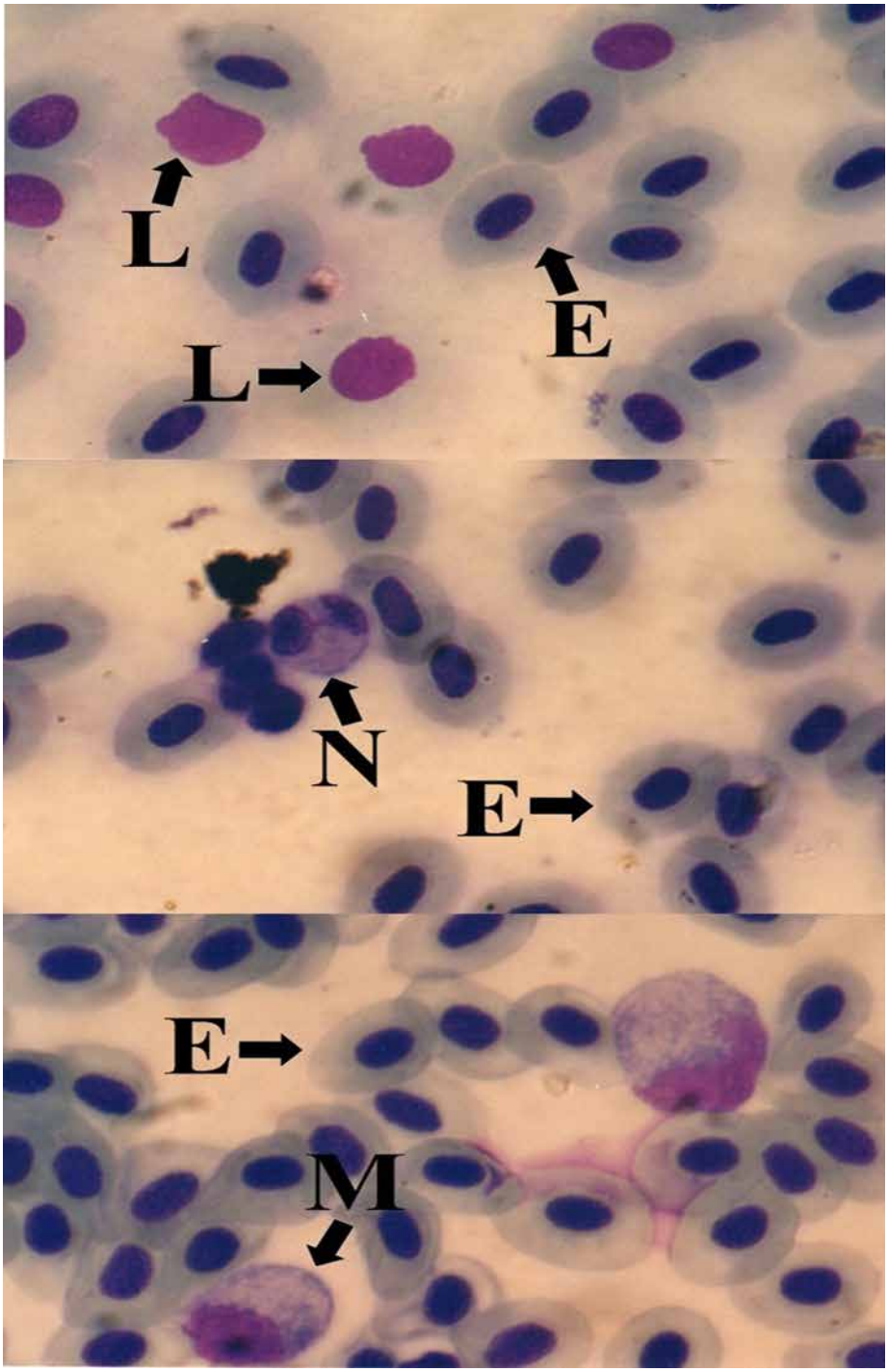

Fig. 2. General view of blood cells in Siberian sturgeons fed with β-1,3/1,6 Glucan (βG 500, βG 750). E – Erythrocyte, L – Lymphocyte, M – Monocyte, N – Neutrophil (May Grünwald Giemsa; ×100).

Hematological parameters

In our study, when comparing WBC, Hb and RBC and erythrocyte indices for βG_{500}, βG_{750} and C+ group fish, both beta-glucan groups showed significant increases very close to each other ($p<0.05$) (Table 4). The βG_{500} and βG_{750} groups showed highly effective results on immune parameters, and also did not reveal any swelling, shrinkage or other deformations in blood cells (Fig. 2).

Disease resistance

Mortalities of 40.0% and 30.0% were recorded in fish treated with βG_{500} and βG_{750} groups supplementation diets to provide maximum protection. The highest RPS was statistically significant in βG_{750} group fish ($p<0.05$) (Table 5). However, the cumulative mortality was determined as high as 60.0% in C+ and βG 250 group fish for 10-15 days.

Table 5. Mortality rate, survival and relative percentage survival (RPS) of Siberian sturgeons infected with *Acipenser baerii* fed with beta-glucan (βG) at different ratios. C+: Positive control, C-: Negative control, βG: Beta-glucan experimental groups.

		Numbers of sampled fish	Mortality (%)	Survival (%)	RPS (%)
Control	C+	10	6 (60.00)[a]	4 (40.00)[a]	-
Groups	C-	10	-	-	-
Beta	βG_{250}	10	6 (60.00)[a]	4 (40.00)[a]	
Glucan	βG_{500}	10	4 (40.00)[b]	6 (60.00)[b]	33.33[a]
Groups	βG_{750}	10	3 (30.00)[b]	7 (70.00)[b]	50.00[b]

Values in the same line with different letters are significantly different ($p<0.05$).

Discussion

Today, sturgeon breeding has become quite important due to the high commercial value of their eggs (caviar) and their meat (Duman 2020). Siberian sturgeon is recommendable as a potential species to increase species diversity in the Turkish aquaculture sector. Siberian sturgeon, which are still raised in Turkey, is one of the most widely cultured sturgeon species. As in all other farm fish, it is considered as a very important species for health status, diseases, and their prevention, as well as the sustainability, economy, and profitability of cultures.

Beta-glucan plays an important role in developing resistance against pathogens during stressful periods and increasing innate and adaptive immune responses due to increased body resistance in experimental infections. *Aeromonas* species are one of the most common pathogenic bacterial species isolated from fish (Simide et al. 2016). In our study, significant increases were observed in the RPS rates of the groups with βG_{500} and βG_{750} of *A. hydrophila*-infected Siberian sturgeons when compared to the C+ group. In particular, the lowest RPS rate (33.33%) was recorded in the βG_{500} group fish while the highest RPS rate (50.0%) was recorded in fish treated with βG_{750}. Misra et al. (2006) in their study where β-glucan was provided as a feed supplement in *E. tarda* and *A. hydrophila* - infected *L. rohita* fingerlings, emphasized that a dose of 250 mg β-glucan was the most effective dose in the bactericidal activities of fish during the 42-day period. In our study, a dose of 750 mg was reported to give the lowest (30.0%) mortality rate during the trial period for *A. hydrophila*-infected Siberian sturgeon. In the study performed by Misra et al. (2006), the importance of a lower dose of β-glucan (250 mg/kg) was emphasized in terms of mortality while the effective dose obtained in our study was higher (750 mg/kg). Misra et al. (2006) reported that the efficacy of immunostimulants on fish was directly related to the application method, fish type, size, intensity, and duration of infection.

In various studies with beta-glucan investigating the effect of different immunostimulants on *Aeromonas* species, it has been reported that levamisole, chitosan, boron compounds and also especially β-glucan supported the immune systems of fish and decreased the mortality in different fish species (Ardó et al. 2008, Ray et al. 2016, Sherif and Mahfouz 2019). In our study, statistically significant similar increases were determined in the percentages of lymphocyte cells (from peripheric blood smears) and WBC in the βG_{500} and βG_{750} group fish compared to the C+ group. These cells form the first step of the body's defense and are the basic elements and indicators of the non-specific immune system (Stanley and Omerebele 2010). On the other hand, for RBC, Hb, and RBC indices, similar increases were observed in the βG_{500} and βG_{750} groups as in the immune system parameters. Hematological and immunological techniques have important roles in determining fish health and their physiological status and also identifying the metabolism and diseases of fish in different ecological environments (Simide et al. 2016). Sherif and Mahfouz (2019) examined the effects of 1,3 β-glucan and levamisole on the immune system in *A. hydrophila*-infected *O. niloticus*. According to the results, while there were no significant changes in RBC and Hb amounts in the chronic infection of fish with *A. hydrophila*, increases were determined in the amounts of leukocyte cells and interleukin.

It is known that immunostimulants both had an active role in the immune system in *O.niloticus* and served as inducers for immune parameters. In our study, the immunological parameters obtained from *A. hydrophila*-infected Siberian sturgeon in βG_{250}, βG_{500} and βG_{750} groups were compared with the control groups and it was found that the βG_{500} and βG_{750} groups showed statistically significant similar differences. Moreover, although leukocyte cell, as well as phagocytic activity values, increased in βG_{500} group fish, similar differences were determined with the results of βG_{750} group fish.

The immune system protects the organisms attacked by opportunistic microorganisms such as bacteria, viruses, fungi, and parasites with various responses such as phagocytosis, lysozyme, and antibody activity.

Phagocytosis in particular is an important mechanism in eliminating extra-cellular bacterial pathogens in all living things (Secombes 1994). On the other hand, in our study, cytokines such as TNF-α and interleukin IL-1β, IL-6, and IL-8 were found to be higher in the βG_{750} group fish compared to the C+ group but statistically similar results with the βG_{500} group fish were also determined. The IFN-γ value, which achieved the regulation of the immune response and activation of phagocytic cells, was found to be significantly higher even in the βG_{250} group fish where the lowest of the beta-glucan dose was applied. To increase the efficacy of cells participating in the immune response, the cytokines synthesized by stimulated lymphocytes, monocytes, macrophages, and some other somatic cells regulate the immunity and its phenomena, including systemic responses to cell growth, recovery, and injury. Bacterial antigens stimulate the release of TNF- α, IL-1, IL-6, and IL-8 by affecting especially T lymphocytes (Akdoğan and Yöntem 2018). In several studies, MacroGard, a type of β-1,3 glucan, obtained from the cell wall of yeast, was injected to salmon and the increases in their phagocytic activities, lysozyme production IL-1, TNF produced by macrophage, antibody production, and complement production in the blood were determined (Engstad et al. 1992, Stanley and Omerebele 2010). Stanley and Omerebele (2010) have reported that β-glucan, applied by mixing with feed, was the most effective and best supplement immunostimulant on the immune system. It is considered in our study that the percentages of leukocyte cells, as well as IL-1β, IL-6, IL-8 and TNF-α levels of β-1,3/1,6 glucan used against the bacterial agent *A. hydrophila* increased in the βG_{500} and βG_{750} fish group as a result of the stimulation of the non-specific cellular defense system. On the other hand, according to the results of numerous scientific studies carried out by feeding fish with β-1,3/1,6 glucan, it was reported that non-specific immunity was stimulated in septicemic diseases against the bacterial agent, it decreased the mortality rate as a result of forming resistance in fish, and also it was prophylactically applicable in fish farms (Aramli et al. 2015, Sherif and Mahfouz 2019). It was noted that the cytokine and leukocyte values obtained from our study were similar to the results in previous studies using different fish species fed with beta-glucan feeds. It was determined in our study that Siberian sturgeon fed with both 500 mg/kg and 750 mg/kg beta-glucan feeds had similar high antibacterial performances against *A. hydrophila* infection in their health indicators and defense-oriented hematological parameters and also 500 mg/kg and 750 mg/kg beta-glucan feeds decreased the mortality level against pathogen by supporting the immune system.

Also, it was emphasized in our study that there was no significant difference between the results obtained from both βG_{500} and βG_{750} groups and thus these doses could be defined as ideal dose ranges.

Conclusion

The effect of beta-glucan added feeds on the immune system in *A.hydrophila*-infected Siberian sturgeon has been discussed and reported for the first time both in the national and international aquaculture sectors. When we consider the study from this point of view, it is thought that it may be important in terms of guiding and illuminating similar studies on the subject in the future.

The study was found to be important in terms of reducing the losses caused by the disease in Siberian sturgeon farming, rasing disease-resistant fish, preventing the abuse of drugs, and determining the most effective β-1,3/1.6 glucan dose with hematological and immunological parameters.

Acknowledgements

This study was supported by Cukurova University Scientific Research Projects Unit. Project Number: FBA-2015-3302.

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