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EFFECTS OF DIETARY COMMERCIAL YEAST GLUCAN ON INNATE IMMUNE RESPONSE, HEMATOLOGICAL PARAMETERS, INTESTINAL MICROBIOTA AND GROWTH PERFORMANCE OF WHITE FISH (*Rutilus frisii*) FRY

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ABSTRACT

This study investigates the effects of different levels of commercial yeast glucan (CYG) on innate immune response, hematological parameters, intestinal microbiota, growth performance and survival rate of white fish (kutum) fry. Specimens (1.1 ± 0.15 g) were randomly allocated into 15 tanks (100 L) at a density of 25 fish per tank and triplicate groups were fed either a basal control diet (0% CYG) or a basal diet supplemented with yeast (0.5, 1, 1.5 or 2% CYG) for 60 days. All dietary CYG levels significantly increased the innate immune responses (IgM levels and lysozyme activity) ($P < 0.05$) and the highest survival was observed in the 1 and 1.5% CYG group. Compared to the control group, CYG had no effects on RBC, Hct and Hb ($P > 0.05$), but WBC levels were significantly higher in CYG fed fish ($P < 0.05$). Microbiological assessment indicated that the viable culturable autochthonous and lactic acid bacteria (LAB) levels significantly elevated in 0.5 and 1% treatments. Our results indicated that low levels of dietary CYG (0.5 and 1 %) improved growth performance, body protein levels and survival rate compared to the control treatment ($P < 0.05$). These results indicate that CYG stimulates innate immune response, improves growth performance and modulates intestinal microbiota of kutum fry. Thus, we suggest that low level CYG may be used as a beneficial dietary supplement in kutum fry culture.

INTRODUCTION

Over the past decade, production of seafood via aquaculture has continued to exhibit sustained expansion around the world (Abdel-Tawwab et al., 2008b, Anonymous, 2005). Kutum (*Rutilus frisii*) is one of the most commercially important fish of the south shores of the Caspian Sea and each year millions of artificially propagated larvae of this fish are released into the Caspian Sea (Abdolhay et al., 2011). Destruction of spawning ground of this species by overfishing has caused remarkable diminution of the stocks in the Caspian Sea (Abdolmaleki, 2006). However, the Iranian Shilat organization has developed propagation and culture methodologies to reduce pressure on natural Caspian Sea populations (Abdolhay et al., 2011).

Intensification of fish farming in aquaculture often leads to deterioration of water quality and this condition makes the cultured organism vulnerable to diseases. Aquaculture faces serious problems due to various adverse effects of antibiotics such as accumulation in the tissue, immunosuppression and emergence of resistant microorganisms (Nayak, 2010) thus stimulation of the innate immune system by dietary supplement is a suitable approach for fortification of an organism against disease (Trichet, 2010). To date, several studies investigated the possible effects of dietary immune stimulant in aquaculture (Sakai, 1999). Among various immunostimulants, the immune modulatory effects of yeast glucan have already been established in several fish species (Meena et al., 2012). The beta-glucans are polysaccharides derived from the cell wall of yeast and fungi. Previous researches revealed that administration of dietary glucan enhanced growth performance of various fish species (Meena et al., 2012). In addition, oral administration of yeast *Saccharomyces cerevisiae*, whose cell wall contains high levels of glucan, induced innate immune response (Ai et al., 2007, Misra et al., 2006a, Selvaraj et al., 2005) (Ogier de Baulny

et al., 1996). Moreover, dietary glucan also improved the resistance against bacterial pathogens in several cultured fish species (Jeney et al., 1997, Misra et al., 2006b, Rodríguez et al., 2009). However, to the authors' knowledge, there is no information on the effect of dietary yeast glucan on kutum. Thus, the aim of the present study was to evaluate the effects of commercial yeast glucan (Hoplite) on innate immune response, hematological parameters, intestinal microbiota and growth performance of Caspain sea white fish *R. frisii.*

MATERIAL AND METHODS

Experimental diets

A basal diet was prepared (for control group) and experimental diets were prepared by supplementing the basal formula with varying levels (0, 0.5, 1.0, 1.5 and 2.0%) of commercial yeast glucan (Hoplit™, Cargill Inc. Minnesota, USA) (Table 1).

Fish culture and feeding trial

Kutum fry (average weight of 1.54 ± 0.06 g) were obtained from Shahid Ansari Hatchery Complex and randomly allocated to 15 fiberglass tanks (80 l; 25 fish per tank). Water temperature, dissolved oxygen and pH were monitored daily and maintained at 23.9 ± 1.01 ºC, 6.85 ± 0.1 mg L^{-1} and 7.5 ± 0.2, respectively. Continuous aeration was provided in each tank through an air stone connected to a central air compressor. During the trial (60 days), fish were fed 3-5% body weight per day (at 06:00, 12:00, 18:00). The feeding ration was corrected every 2 weeks following a 24-h starvation period and batch weighing.

Chemical analysis of experimental diets and fish carcasses

Proximate analysis of the experimental diets and fish carcasses (at the end of trial) were conducted according to the standard protocols of AOAC (Chemists and Cunniff, 1995) in the laboratory of veterinary organization (Rasht, Iran). Gross energy was calculated using the conversion factors of 23.6, 39.5 and 17.0 kJ g^{-1} for protein, lipid and nitrogen-free extracts (NFE), respectively (Brett and Groves, 1978).

Growth performance and survival

The growth performance and survival were calculated according to the following formulae:
Weight gain, WG= (final body weight- initial body weight) × 100/ initial body weight;
Specific growth rate (SGR) = 100 (ln W2–ln W1) T;
Feed conversion ratio (FCR) = feed intake (g) / weight gain (g);
Protein efficiency ratio (PER) = weight gain / protein intake;
Survival rate = (N_f / N_i) × 100;
Where W1 is the initial weight, W2 is the final weight, T is the number of days in the feeding period, N_i is the initial number of fish and N_f is the final number of fish.

Sample collection

At the end of the trial, blood samples from 5 fish per tank were taken from the caudal vein for hematological and immunological analysis. Blood samples were divided into two sets of Eppendorf tubes. One set contained heparin for hematological studies and the other one (non-heparinized) was centrifuged at 6000 rpm for 10 min at 4°C in order to measure biochemical and immunological indices. Isolated serum was stored at -20°C. Haematrocit (Hct) was determined by the microhaematocrit method as described by Brown (1988) and reported as percentage packed cell volume (% PCV). Haemoglobin (Hb) levels were estimated using Sahli's method. Red blood cells (RBC) and white blood cells (WBC) were counted in a Neubauer hemocytometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from RBC, Hct and Hb according to the following formulae: MCV= (PCV×1000) / RBC, MCH = Hb / RBC and MCHC = (Hb×10) / Hct (Lee *et al*., 1998). Differential leukocyte counts (neutrophils, eosinophils, lymphocytes and monocytes) were conducted on May–Grunwald–Giemsa stained blood smears. A minimum of 100 cells per slide were counted.

Immunological assays

Immunoglobulin M (IgM) content was estimated according to the method described by Siwicki and Anderson, 1993. Lysozyme levels were determined based on the method suggested by Sankaran and Gurnani (1972). The lysozyme substrate was a 0.02% (w/v) suspension of *Micrococcus lysodeikticus* made up in phosphate buffer (0.05 M, pH 6.2). Lyophilized hen egg white lysozyme was used as a standard. A new standard curve was prepared for each assay. Standard solutions as well as samples were added to the substrate at 28ºC.

Intestinal microbiota analysis

Enumeration of the autochthonous intestinal microbiota was performed at the start of the trial and end of the trial. Total viable heterotrophic aerobic bacteria and lactic acid bacteria (LAB) levels were determined at the start of trial by random sampling of 15 fish before stocking. At the end of the experiment, 10 fish per tank were randomly sampled from each treatment for microbiological studies and individuals were pre-processed. Sample preparation for intestinal microbiota analysis was performed as described previously (Hoseinifar et al., 2011c). One hundred µl of the

Table 1. Dietary formulations (%) and proximate composition

Ingredients (%)	Experimental feeds				
	0%	0.5%	1%	1.5%	2%
Fish meal	42	42	42	42	42
Soybean meal	30	30	30	30	30
Wheat flour	5	5	5	5	5
Corn flour	5	5	5	5	5
Fish Oil	9	9	9	9	9
meal	3	3	3	3	3
Crustoderma lamarki Cuicuse powder	1	1	1	1	1
*Vitamin Premix	1	1	1	1	1
**Mineral premix	1	1	1	1	1
CYG	0	0.5	1	1.5	2
Proximate analysis (% dry matter basis)					
Moisture	22.5	21.7	22.7	22.2	22.2
Ash (%)	19	19.5	19.2	19.5	19
Crude protein (%)	42	42	42	42	42
Crude lipid (%)	16.5	16.8	17	16.3	16.5
Gross energy (Kcal)	4000	4000	4000	4000	4000

Table 1. Dietary formulations (%) and proximate composition
**Vitamin premix (g 100 g-1 vitamin premix except A, 160000 IU and D3, 40000 IU): E, 4; K3, 0.2; B1, 0.6; B2, 0.8; B3, 1.2; B5, 4; B6, 0.4; B9, 0.2; B12, 0.8; H2, 0.02; C, 6; Inositol, 2; BHT (butylated hydroxyl toluene), 2.*
*** Mineral premix (g 100 g-1 mineral premix): Fe, 2.6; Zn, 1.25; Se, 0.2; Co, 0.048; Cu, 0.42; Mn, 1.58; I, 0.1; Cholin chloride, 1.2.*

sample was spread in triplicate onto the plate count agar (PCA) (Liofilchem, Italy) and de Man, Rogosa and Sharpe (MRS) media (Liofilchem, Italy) for the determination of total viable heterotrophic aerobic bacteria and LAB, respectively.

Statistical analysis

Data are presented as mean ± S.E. Significant differences between groups were determined by a one-way analysis of variance (ANOVA) followed by Tukey's test (Zar, 1999). Prior to statistical analysis, normality and homogeneity of variance were checked. All statistical analyses were performed with SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The effects of dietary CYG on humoral innate immune responses of kutum are shown in Fig. 1a and b. Fish fed 1% and 1.5% CYG displayed significantly elevated lysozyme activity (29.60 ± 2.51 μg mL^{-1} and 28.33 ± 1.42 μg mL^{-1}, respectively) compared to the control (18.16 ± 1.04 μg mL^{-1}), as was also the case for serum IgM levels (1% = 1.10 ± 0.14, 1.5% = 1.15 ± 0.70 and control = 0.85 ± 0.11 mg dL^{-1}) ($P < 0.05$). The effects of dietary CYG supplementation on hematological parameters are presented in Table 2. Erythrocyte levels, Hct and Hb were not affected by dietary CYG ($P > 0.05$). However, the WBC levels were significantly influenced by dietary CYG, as the lowest level was observed in the control group ($P < 0.05$) (Table 2).

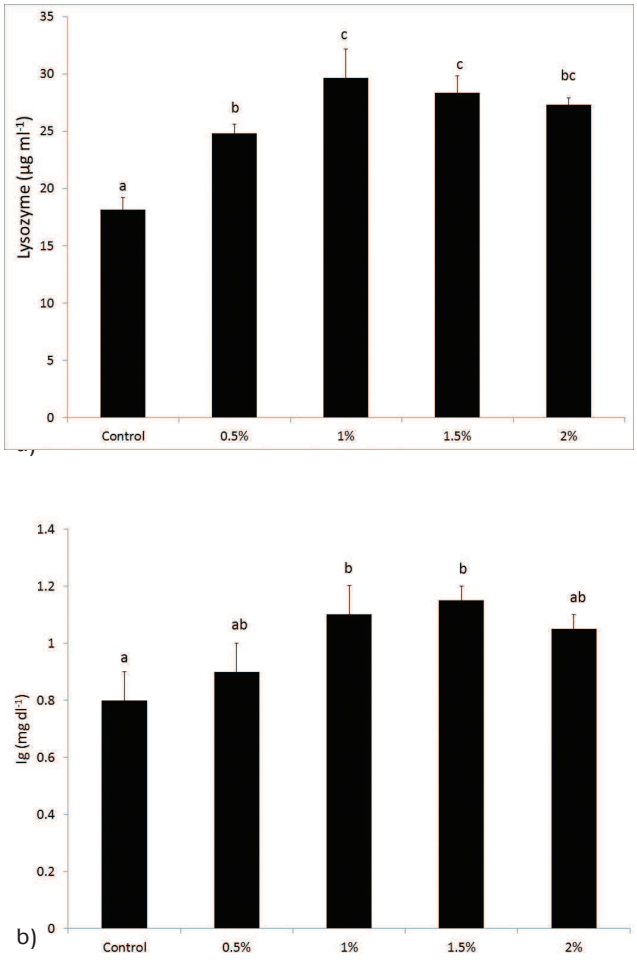

Fig 1. The effects of dietary CYG on humoral innate immune responses of kutum (a= lysozyme activity; b=serum IgM level)

Compared to the control group, total heterotrophic autochthonous bacterial levels in fish fed 0.5% and 1% dietary CYG were significantly higher compared to other treatments ($P < 0.05$) (Table 3). Additionally, autochthonous LAB levels were also significantly elevated in these treatments at the end of trial ($P < 0.05$) (Table 3). Growth performance and feed utilization parameters of kutum fry fed different levels of dietary CYG are shown in Table 2. Fry fed 0.5% and 1%

Table 2. Hematological parameters of kutum fry fed diets containing varying levels of CYG

Groups	Erythrocyte count (× 10^6 µl)	Leukocyte count (×10^3 mm^3)	Haemoglobin (g dl^{-1})	Haematocrit (%)
Control	0.98± 0.31	7.2± 0.18[a]	8.46±0.15	21.3 ± 1.5
CYG 0.5 %	1.14±0.26	8.2± 0.25[b]	8.70± 0.10	22.6 ±2.5
CYG 1 %	1.17± 0.16	9.1 ± 0.10[c]	8.96±0.40	23.0± 1.1
CYG 1.5%	1.07± 0.11	9.3 ± 0.51[c]	8.16± 0.37	21.2± 2.5
CYG 2 %	1.11± 0.14	9.6 ± 0.45[c]	8.56±0.58	21.8± 1.9

Means in the same row with different superscripts are significantly different (P<0.05).

Table 3. Total viable counts (TVC) and lactic acid bacteria (LAB) levels [log colony forming units (CFU) g-1 intestine] in the gut of kutum fry fed diets containing different levels of CYG

Group	TVC Log (CFU/gr)	LAB Log (CFU/gr)	LAB (%)
0 %	6.17± 0.07[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
0.5%	7.67± 0.36[c]	5.69± 0.06[c]	1.06±0.24[b]
1%	6.97± 0.18[c]	3.97±0.17[b]	0.10±0.09[a]
1.5%	6.47± 0.07[b]	3.47±0.07[a]	0.11±0.30[a]
2%	6.05± 0.18[a]	2.90±0.05[a]	0.04±0.07[a]

Means in the same row with different superscripts are significantly different (P<0.05)

dietary CYG displayed significantly higher weight gain, SGR, PER and lower FCR compared to the control group and other CYG groups ($P < 0.05$) (Table 2). Also survival rate in 0.5% and 1% treatments were significantly elevated compared to the control group ($P < 0.05$) (Table 2).

Feeding varying levels of dietary CYG affects kutum fry body composition (Table 4). Fish fed 0.5% and 1% CYG had a significantly higher protein level in carcass composition ($P < 0.05$). However, body lipid, ash and moisture showed no remarkable difference between treatments ($P > 0.05$) (Table 4)

DISCUSSION

Administration of dietary glucan is a beneficial tool for stimulation of immune system of fish and fortification of immune response (Soltanian et al., 2009). Commercial products containing glucan like MacroGard®, Immunoster and ImmunoWall have been recently used in aquatic animal culture (Ringø, 2011). To our knowledge, this study was the first study to investigate the effects of glucan on innate immune response, intestinal microbiotia and performance of Caspian Sea white fish fry (*R. frisii*).

Stimulation of the immune response of fish through dietary supplements is of high interest for commercial aquaculture (Staykov et al., 2007). Aquatic animals are continually vulnerable to numerous opportunistic pathogens and the innate immune system provides the first line of defense for the host (Magnadóttir, 2006). The results of the present study revealed that dietary CYG can modulate the innate immune responses of kutum fry. As shown in Figures 1 and 2, fish fed diets containing CYG had a significantly greater serum IgM and serum lysozyme activity compared to the control group and the highest levels observed in 1% and 1.5% treatments. The enhancement of serum lysozyme activity

Table 4. The effects of dietary CYG on growth performance and survival of kutum fry

	control	0.5%	1%	1.5%	2%
Initial weight (g)	1.13 ± 0.12	1.14 ± 0.01	1.19 ± 0.01	1.24 ± 0.15	1.17± 0.04
Weight gain (g)	0.37± 0.15[a]	0.75± 0.08[b]	0.78 ± 0.04[b]	0.35± 0.04[a]	0.45± 0.10[a]
SGR (%day^{-1})	0.45 ± 0.18[a]	0.8 ± 0.1[b]	1.57± 0.07[b]	1.1±0.19[a]	0.92± 0.23[a]
PER	0.85± 0.2[a]	1.75± 0.28[b]	1.57± 0.07[b]	1.1±0.19[a]	0.92± 0.23[a]
FCR	0.85± 0.2[a]	1.073 ± 0.11[b]	1.32 ± 0.17[b]	1.01 ± 0.01[a]	0.89 ± 0.08[a]
Survival (%)	84.6± 4.51[a]	90.0 ± 3.0[b]	93 ± 2.8[b]	81.6 ± 2.8[a]	86.6 ± 2.8[a]

Means in the same row with different superscripts are significantly different (P<0.05).

Table 5. Carcass proximate composition of kutum fry fed diets containing varying levels of CYG. Data represent means from 3 replicates per treatment.

	Control	0.5%	1%	1.5%	2%
Moisture	65.1± 0.8	67.3 ± 0.8	68.23 ± 0.25	70.3 ± 0.3	63.36 ± 0.12
Crude protein*	20.2 ± 0.26[a]	22.1 ± 0.36[b]	22.36 ± 0.55[b]	19.93 ± 0.60[a]	20.26 ± 0.25[a]
Crude lipid*	6.20 ± 0.72	5.13 ± 0.55	5.73 ± 0.43	6.03 ± 0.50	6.50 ± 0.73
Ash*	3.16 ± 0.54	3.70 ± 0.45	3.67 ± 0.28	3.95 ± 0.16	3.70 ± 0.25

* Dry weight basis

by lower dose of CYG have been reported previously (Ai et al., 2007, Cook et al., 2001, Ortuño et al., 2002, Rodríguez et al., 2009). Also, Bagni et al. (2005) reported that short term (15 days) administration of CYG (MacroGaad; 0.1%) elevated serum lysozyme activity of *Dicentrachus labrax*. In addition, feeding *Sparus aurata* (100-200 g) with brewer's yeast glucan for 4 weeks increased serum IgM levels. It is well-documented that yeast - glucan stimulates innate immune response in fish (Cuesta et al., 2004). Moreover, Ai et al. (2007) and Misra et al. (2006a) stated that lower doses of -glucan were more effective compared to high doses which is in line with the present study. The mechanism by which glucan enhances innate immune response of fish has not been completely elucidated to date (Ai et al., 2007) but some studies have shown that glucan enhances non-specific immunity through direct activation of macrophages (Ai et al., 2007, Sakai, 1999).

Hematological parameters are valuable indicators for monitoring fish health, nutritional status and environmental conditions affecting fish (Hoseinifar et al., 2011b, Cnaani et al., 2004). Our results show that dietary CYG had no remarkable effects on RBC, Htc and Hb levels (Table 2). In accordance to these results, administration of yeast failed to affect hematological parameters (e.g. RBC, Htc, Hb, MCV, MCH, MCHC) of channel catfish (*Ictalurus punctatus*) (Welker et al., 2007) and beluga juvenile (*Huso huso*) (Hoseinifar et al., 2011a). Increases of WBC following administration of - glucan in diet have been reported previously (Ta'ati et al., 2011, Meena et al., 2012), which can be a sign of stimulation of non-specific immunity (Misra et al., 2006a). Moreover, Schiffrin et al. (1995) stated that LAB can stimulate white blood cells proliferation and performance.

LAB have been considered as beneficial components of the fish intestinal ecosystem by producing bacteriocins, lactic acid and other antagonistic compounds which inhibit the growth of certain fish pathogens (Gatesoupe, 2008, Ringø and Gatesoupe, 1998). In the present study, culture-based analysis of the autochthonous intestinal microbiota revealed that presumptive LAB levels were significantly higher in 0.5% and 1% dietary CYG treatments. Although, from the present investigation we conclude that culturable autochthonous LAB are only minor components of the intestine of kutum fry. The exact mechanism behind these observations is not known but it might occur as a result of the provision of minerals, nucleic acid, B-complex, vitamins and/or amino acids provided by dietary yeast (Hoseinifar et al., 2011a).

In the current study, during the feeding trial, growth parameters such as final body weight, weight gain, SGR and FCR were improved by the inclusion of a low dose (0.5% and 1%) dietary CYG (Table 2). Similar benefits have been reported in fish fed dietary glucan (Masahiro, 1999, Meena et al., 2012, Soltanian et al., 2009). Growth rate possibly enhanced due to the energetic benefits obtained through glucan (Ai et al., 2007), microbial manipulation of intestinal microbiota (Ringø et al., 2010), colonization of lactic acid bacteria (as seen in our results; Table 3) in the gut and production of protease and amylase by these bacteria in the digestive tract (Farzanfar, 2006, Gullian et al., 2004, Irianto and Austin, 2002, Waché et al., 2006, Hoseinifar et al., 2011a). In addition, enumeration of LAB increases the concentrations of the volatile fatty acids (VFA_s) which beneficially affect microvillus morphology and nutrient utilization (Daniels et al., 2010, Dimitroglou et al., 2009, Ringø et al., 2010, Salze et al., 2008, Zhou et al., 2010). On the contrary, however, several other studies have reported that dietary yeast had no significant effects on fish growth performance (Li and Gatlin, 2005, Sado et al., 2008, Pooramini et al., 2009, Reza et al., 2009, Sealey et al., 2007, Tovar-Ramırez et al., 2004). Further studies are required to determine why such contradictory results have been obtained.

The supplementation of kutum fry diet with 0.5% or 1% CYG significantly affected the whole-fish body protein content. Similar to our finding, significantly different carcass protein levels were observed in Nile tilapia fed the commercial live bakers' yeast *Saccharomyces cerevisiae* (Abdel-Tawwab et al., 2008). Elevation of body protein content in kutum fry is likely because of changes in their synthesis, deposition rate in muscle and/or growth rate (Abdel-Tawwab et al., 2008).

The present study revealed that the administration of low levels of dietary CYG had significant effects on kutum fry survival (Table 4). In line with our results, beneficial effects have been obtained on the survival of yeast-fed fish under both non-challenged (Lara-Flores et al., 2003, Skjermo et al., 2006, Van Hai and Fotedar, 2009, Hoseinifar et al.,

2011a) and bacterial challenged studies (Abdel-Tawwab et al., 2008, Ai et al., 2007, Li and Gatlin, 2005, Misra et al., 2006b).

In conclusion, the present study indicates that administration of low levels of dietary CYG can be used for growth promotion, stimulation of innate immune system and modulation of intestinal microbiota toward potentially beneficial communities (i.e. LAB). This preliminary study might encourage further research on different aspects of CYG administration in kutum culture.

Sažetak

UČINCI DIJETETSKOG KOMERCIJALNOG KVASCA GLUKANA NA IMUNOLOŠKI ODGOVOR, HEMATOLOŠKE PARAMETRE, CRIJEVNU MIKROFLORU I PERFORMANCE RASTA MLAĐI BIJELE BODORKE (*Rutilus frisii*)

Ova studija istražuje utjecaj različitih razina komercijalnog kvasca glukana (CYG) na imunološki odgovor, hematološke parametre, crijevnu mikrofloru, brzinu rasta i stopu preživljavanja kod mlađi bijele bodorke. Uzorci mlađi (1,1 ± 0,15 g) su slučajnim izborom podijeljeni u 15 spremnika (100 L) pri gustoći od 25 riba po spremniku, a hranjeni su u triplikatu skupina sa osnovnom kontrolnom ishranom (0% CYG) ili osnovnom ishranom nadopunjenom kvascem (0,5, 1, 1,5, ili 2% CYG) 60 dana.

U svim CYG prehrambenim razinama značajno se povećao imunološki odgovor (razine IgM i lizozim aktivnost) (P< 0,05), a najveće preživljavanje zabilježeno je u CYG skupini 1 i 1,5%. U usporedbi s kontrolnom skupinom, CYG nije imala utjecaj na RBC, Hct i Hb (P> 0,05), ali WBC razina bila je znatno viša u riba koje su hranjene s CYG (P <0,05).

Mikrobiološka procjena ukazuje da su razine živih kultura autohtonih i mliječno kiselih bakterija (LAB) značajno povišene u 0,5 i 1% tretmanu. Rezultati pokazuju da niska razina ishrane s CYG (0,5 i 1%) uzrokuje bolju performansu rasta, razinu tjelesnih proteina i stopu preživljavanja u usporedbi s kontrolnom (P <0,05). Ovi rezultati pokazuju da CYG stimulira imunološki odgovor, poboljšava performanse rasta i modulira crijevnu mikrofloru kod mlađi bijele bodorke. Dakle, predlažemo da se niska razina CYG može se koristiti kao koristan dodatak prehrani u proizvodnji mlađi bijele bodorke.

Ključne riječi: Kvasac glukan, imunološki odgovor, crijevna mikroflora, hematologija, rast, Crnomorska bodorka

REFERENCES

Abdel-Tawwab, M., Abdel-Rahman, A. M., Ismael, N. E. M. (2008): Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture, 280, 185-189.

Abdolhay, H. A., Daud, S. K., Ghilkolahi, S. R., Pourkazemi, M., Siraj, S. S., Satar, M. A. (2011): Fingerling production and stock enhancement of Mahisefid (*Rutilus frisii kutum*) lessons for others in the south of Caspian Sea. Reviews in Fish Biology and Fisheries, 21, 247-257.

Abdolmaleki, S. (2006): Trends in stocks fluctuation of *Rutilus* in the Caspian Sea. Iranian Scientific fisheries Journal, 15, 87-99.

AI, Q., Mai, K., Zhang, L., Tan, B., Zhang, W., Xu, W., Li, H. (2007): Effects of dietary β-1, 3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*. Fish & Shellfish Immunology, 22, 394-402.

Anonymous (2005): World aquaculture outlook. Aquaculture Magazine Buyers Guide, 6-12.

Bagni, M., Romano, N., Finoia, M., Abelli, L., Scapigliati, G., Tiscar, P., Sarti, M., Marino, G. (2005): Short-and long-term effects of a dietary yeast [beta]-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). Fish & Shellfish Immunology, 18, 311-325.

Brown, B. (1988): Routine hematology procedures. Hematology: Principles and Procedures, 7-122.

Chemists, A. O. O. A., Cunniff, P. (1995): Official methods of analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists.

Cnaani, A., Tinman, S., Avidar, Y., Ron, M., Hulata, G. (2004): Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *O. niloticus*. Aquaculture Research, 35, 1434-1440.

Cook, M. T., Hayball, P. J., Hutchinson, W., Nowak, B., Hayball, J. D. (2001): The efficacy of a commercial -glucan preparation, EcoActiva™, on stimulating respiratory burst activity of head-kidney macrophages from pink snapper (*Pagrus auratus*), Sparidae. Fish & Shellfish Immunology, 11, 661-672.

Cuesta, A., Meseguer, J. & Esteban, M. (2004): Total serum immunoglobulin M levels are affected by immunomodulators in sea bream (*Sparus aurata* L.). veterinary immunology and immunopathology, 101, 203-210.

Daniels, C. L., Merrifield, D. L., Boothroyd, D. P., Davies, S. J., Factor, J. R., Arnold, K. E. (2010): Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture, 304, 49-57.

Dimitroglou, A., Merrifield, D., Moate, R., Davies, S., Spring, P., Sweetman, J., Bradley, G. (2009): Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of animal science, 87, 3226-3234.

Farzanfar, A. (2006): The use of probiotics in shrimp aqua-

culture. FEMS Immunology & Medical Microbiology, 48, 149-158.

Gatesoupe, F. J. (2008): Updating the importance of lactic acid bacteria in fish farming: Natural occurrence and probiotic treatments. Journal of Molecular Microbiology and Biotechnology, 14, 107-114.

Gullian, M., Thompson, F., Rodriguez, J. (2004): Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture, 233, 1-14.

Hoseinifar, S. H., Mirvaghefi, A., Merrifield, D. L. (2011a): The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (Huso huso). Aquaculture, 318, 90-94.

Hoseinifar, S. H., Mirvaghefi, A., Merrifield, D. L., Amiri, B., Yelghi, S., Bastami, K. (2011b): The study of some haematological and serum biochemical parameters of juvenile beluga (*Huso huso*) fed oligofructose. Fish physiology and biochemistry, 37, 91-96.

Hoseinifar, S. H., Mirvaghefi, A., Mojazi Amiri, B., Rostami, H. K., Merrifield, D. L. (2011c): The effects of oligofructose on growth performance, survival and autochthonous intestinal microbiota of beluga (*Huso huso*) juveniles. Aquaculture Nutrition, 17, 498-504.

Irianto, A., Austin, B. (2002): Probiotics in aquaculture. Journal of Fish Diseases, 25, 633-642.

Jeney, G., Galeotti, M., Volpatti, D., Jeney, Z., Anderson, D. P. (1997): Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. Aquaculture, 154, 1-15.

Lara-Flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E., López-Madrid, W. (2003): Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 216, 193-201.

Li, P., Gatlin, D. M. (2005): Evaluation of the prebiotic GroBiotic®-A and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops*×*M. saxatilis*) challenged in situ with *Mycobacterium marinum*. Aquaculture, 248, 197-205.

Magnadóttir, B. (2006): Innate immunity of fish (overview). Fish & Shellfish Immunology, 20, 137-151.

Masahiro, S. (1999): Current research status of fish immunostimulants. Aquaculture, 172, 63-92.

Meena, D., Das, P., Kumar, S., Mandal, S., Prusty, A., Singh, S., Akhtar, M., Behera, B., Kumar, K., Pal, A. (2012): Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiology and Biochemistry, 1-27.

Misra, C. K., Das, B. K., Mukherjee, S. C., Pattnaik, P. (2006a): Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture, 255, 82-94.

Misra, C. K., Das, B. K., Mukherjee, S. C., Pattnaik, P. (2006b): Effect of multiple injections of [beta]-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish & Shellfish Immunology, 20, 305-319.

Nayak, S. K. (2010) Probiotics and immunity: A fish perspective. Fish & Shellfish Immunology, 29, 2-14.

Ogier De Baulny, M., Quentel, C., Fournier, V., Lamour, F., Le Gouvello, R. (1996): Effect of long-term oral administration of β-glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot *Scophthalmus maximus*. Diseases of aquatic organisms, 26, 139-147.

Ortuño, J., Cuesta, A., Rodríguez, A., Esteban, M. & Meseguer, J. (2002): Oral administration of yeast, Saccharomyces cerevisiae, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). veterinary immunology and immunopathology, 85, 41-50.

Pooramini, M., Kamali, A., Hajimoradloo, A., Alizadeh, M., Ghorbani, R. (2009): Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry. International Aquatic Research, 1, 39-44.

Reza, A., Abdolmajid, H., Abbas, M., Abdolmohammad, A. K. (2009): Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, *Huso huso* (linnaeus, 1758). Journal of the World Aquaculture Society, 40, 771-779.

Ringø, E. (2011): Use of immunostimulants and nucleotides in aquaculture: a review. Journal of Marine Science: Research & Development 1:104.

Ringø, E., Gatesoupe, F.-J. (1998): Lactic acid bacteria in fish: a review. Aquaculture, 160, 177-203.

Ringø, E., Olsen, R., Gifstad, T. Ø., Dalmo, R., Amlund, H., Hemre, G. I., Bakke, A. (2010): Prebiotics in aquaculture: a review. Aquaculture Nutrition, 16, 117-136.

Rodríguez, I., Chamorro, R., Novoa, B. & Figueras, A. (2009): β-Glucan administration enhances disease resistance and some innate immune responses in zebrafish (*Danio rerio*). Fish & Shellfish Immunology, 27, 369-373.

Sado, R. Y., Bicudo, Á. L. J. D. A., Cyrino, J. E. P. (2008): Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, *Oreochromis niloticus*, has no effect on hematological parameters and showed decreased feed consumption. Journal of the World Aquaculture Society, 39, 821-826.

Sakai, M. (1999): Current research status of fish immunostimulants. Aquaculture, 172, 63-92.

Salze, G., Mclean, E., Schwarz, M., Craig, S. (2008): Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture, 274, 148-152.

Sankaran, K., Gurnani, S. (1972): On the variation in the catalytic activity of lysozyme in fishes.

Schiffrin, E., Rochat, F., Link-Amster, H., Aeschlimann, J., Donnet-Hughes, A. (1995): Immunomodulation of human blood cells following the ingestion of lactic acid bac-

teria. Journal of Dairy Science, 78, 491-497.

Sealey, W. M., Barrows, F. T., Johansen, K. A., Overturf, K., Lapatra, S. E., Hardy, R. W. (2007): Evaluation of the ability of partially autolyzed yeast and Grobiotic-A to improve disease resistance in rainbow trout. North American Journal of Aquaculture, 69, 400-406.

Selvaraj, V., Sampath, K., Sekar, V. (2005): Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Fish & Shellfish Immunology, 19, 293-306.

Siwicki, A., Anderson, D. (1993): Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. Fish Disease Diagnosis and Prevention Methods. Olsztyn, Poland, 105-112.

Skjermo, J., Størseth, T. R., Hansen, K., Handå, A., Øie, G. (2006): Evaluation of β-(1→ 3, 1→ 6)-glucans and High-M alginate used as immunostimulatory dietary supplement during first feeding and weaning of Atlantic cod (*Gadus morhua* L.). Aquaculture, 261, 1088-1101.

Soltanian, S., Stuyven, E., Cox, E., Sorgeloos, P., Bossier, P. (2009): Beta-glucans as immunostimulant in vertebrates and invertebrates. Critical reviews in microbiology, 35, 109-138.

Staykov, Y., Spring, P., Denev, S., Sweetman, J. (2007): Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture International, 15, 153-161.

Ta'ati, R., Soltani, M., Bahmani, M., Zamini, A. (2011): Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. Iranian Journal of Fisheries Sciences, 10, 324-335.

Tovar-Ramırez, D., Zambonino Infante, J., Cahu, C., Gatesoupe, F., Vázquez-Juárez, R. (2004): Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture, 234, 415-427.

Trichet, V. V. (2010): Nutrition and immunity: an update. Aquaculture Research, 41, 356-372.

Van Hai, N., Fotedar, R. (2009): Comparison of the effects of the prebiotics (Bio-Mos® and [beta]-1, 3-d-glucan) and the customised probiotics (*Pseudomonas synxantha* and *P. aeruginosa*) on the culture of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). Aquaculture, 289, 310-316.

Waché, Y., Auffray, F., Gatesoupe, F. J., Zambonino, J., Gayet, V., Labbé, L., Quentel, C. (2006): Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. Aquaculture, 258, 470-478.

Welker, T. L., Lim, C., Yildirim-Aksoy, M., Shelby, R., Klesius, P. H. (2007): Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. Journal of the World Aquaculture Society, 38, 24-35.

Zar, J. H. (1999): Biostatistical analysis. Prentice Hall New Jersey, 4.

Zhou, Q. C., Buentello, J. A., Gatlin, D. M. (2010): Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture, 309, 253-257.