

Effect of β -1.3/1.6-D-glucan derived from oyster mushroom *Pleurotus ostreatus* on biometrical, haematological, biochemical, and immunological indices in rainbow trout (*Oncorhynchus mykiss*)

Radka DOBŠÍKOVÁ¹, Jana BLAHOVÁ¹, Aleš FRANČ², Juraj JAKUBÍK³,
Ivana MIKULÍKOVÁ¹, Helena MODRÁ¹, Kamila NOVOTNÁ¹, Zdeňka SVOBODOVÁ¹

¹ Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, the Czech Republic

² Department of Pharmaceutics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, the Czech Republic

³ Natures, Slovakia

Correspondence to: Radka Dobšíková, DVM., PhD.
Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tr. 1/3, 612 42 Brno, the Czech Republic.
TEL: + 420 541 562 784; FAX: + 420 541 562 790; E-MAIL: dobsikovar@vfu.cz

Submitted: 2012-09-01 *Accepted:* 2012-11-15 *Published online:* 2012-00-00

Key words: **fish; condition factor; hepatosomatic index; blood parameters; plasma indices; chemiluminescence**

Neuroendocrinol Lett 2012; **33**(Suppl.3):96–106 PMID: 23353851 NEL330912A14 © 2012 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVES: Effect of long-term oral administration of three different concentrations (0.5, 1.0, and 2.0%) of micronized β -1.3/1.6-D-glucan derived from oyster mushroom (*Pleurotus ostreatus*, Hiratake) on biometrical, haematological, biochemical, and immunological indices of half-year-old rainbow trout (*Oncorhynchus mykiss*) was assessed in the study.

DESIGN: Rainbow trout were feed commercial feed pellets containing β -1.3/1.6-D-glucan in the concentrations of 0.5, 1.0, and 2.0% for 85 days. Biometrical indices consisted in total and standard length, body and liver weight, from which derived somatic parameters such as Fulton's condition factor and hepatosomatic index were calculated. Haematological parameters were evaluated according to unified methods for haematological examination in fish. Plasma biochemical profile was analysed using biochemical analyser Konelab 20i and Easy Lyte Analyzer. A phagocyte cells metabolic activity (induced chemiluminescence of phagocytes) was determined as an immunological parameter by a microplate luminometric method on Immunotech LM-01T.

RESULTS: No clinical signs of behavioral, respiratory, or neurologic distress were observed in rainbow trout. Fish showed normal feeding behavior. As for biometric parameters, no significant changes in total and standard length, body weight, liver weight, as well as in condition factor and hepatosomatic index of experimental and control fish were found. In the course of the study, weight gains in rainbow trout were similar and continuous. Shifts in PCV ($p < 0.05$), haemoglobin ($p < 0.05$), and MCHC ($p < 0.01$) were found within haematological indices. Plasma concentration of glucose, lactate, total protein, cholesterol, calcium, natrium, potassium (all $p < 0.05$), albumins and chlorides (both $p < 0.01$), as well as catalytic activities of ALT and AST (both $p < 0.05$) were changed in the course of the study. A phagocyte

cells metabolic activity (luminol-induced chemiluminescence) in rainbow trout was not altered by oyster mushroom β -1.3/1.6-D-glucan administration.

CONCLUSION: After long-term oral administration of three concentrations of micronized β -1.3/1.6-D-glucan derived from oyster mushroom (*Pleurotus ostreatus*, Hiratake) shifts in haematological and biochemical profiling were found in half-year-old rainbow trout (*O. mykiss*) in environmental conditions of a commercial rainbow trout fishery. Biometrical indices were not found significantly altered. No specific effect of β -glucan on immune system response of rainbow trout was found in the study. The use of β -glucan in prosperous, clinically healthy aquaculture is still an issue, nevertheless, its use in breedings endangered by stress stimuli, infectious diseases or adverse environmental factors is indisputable.

Abbreviations:

Alb	- albumins
ALT	- alanine aminotransferase
ALP	- alkalic phosphatase
AST	- aspartate aminotransferase
BG	- β -glucan
BW	- body weight
Ca ²⁺	- calcium
Cl ⁻	- chlorides
CL	- chemiluminescence
Chol	- cholesterol
FCF	- Fulton's condition factor
Glu	- glucose
Hb	- haemoglobin
HSI	- hepatosomatic index
K ⁺	- potassium
Lact	- lactate
LD ₅₀	- lethal dose (50% mortality of fish)
LDH	- lactate dehydrogenase
LW	- liver weight
MCH	- mean corpuscular haemoglobin
MCHC	- mean corpuscular haemoglobin concentration
MCV	- mean corpuscular volume
Na ⁺	- sodium
Phos	- phosphorus
PCV	- packed cell volume, haematocrit
RBC	- red blood cells count
ROS	- reactive oxygen species
SL	- standard length
Trig	- triglycerides
TL	- total length
TP	- total protein
WBC	- white blood cells count

INTRODUCTION

Immunostimulants have been used as feed additives for many years in farm animal husbandry as well as in aquaculture. They augment the innate and adaptive immune responses and are regarded as a promising supplement to vaccination and selective breeding, which remain the key strategies for prevention of diseases in fish aquaculture (Djordjevic *et al.* 2009).

β -glucans are the most commonly used term for a heterogenous group of glucose polysaccharides consisting of a backbone of β -(1.3)-linked β -D-glucopyranosyl units with β -(1.6)-linked side chains of varying length and distribution (Rodríguez *et al.* 2009). They are a major structural component of fungi cell walls and are also found in some bacteria, plants, algae, yeast, and mushrooms. β -glucans can be also extracted from the bran of oat and barley grains, rye and wheat or some species of seaweed. One of the most common sources of β -glucans is derived from the cell wall of baker's yeast *Saccharomyces cerevisiae* (Das *et al.* 2009).

β -glucans bind to specific cell surface receptors of macrophages and neutrophilic granulocytes that promotes the enhancement of organism's protective activity against infections and sepsis through the activation of leukocytes, phagocytic activity, production of inflammatory cytokines, chemokines and reactive oxygen free radicals. The stimulation of receptors also increases the activity of antioxidant enzymes, elimination and killing of microorganisms, and initiates the development of adaptive immunity, all of which contribute to anti-infective and anti-tumorigenic properties of β -glucans (Brown & Gordon 2003).

In fish, β -glucans enhance fish resistance against bacterial and viral infections by means of effective stimulation of non-specific cellular and humoral immune functions such as lysozyme and complement activities. β -glucans have a proven protective effect against fish bacterial pathogens including *Aeromonas salmonicida* and *Edwardsiella tarda* (Nikl *et al.* 1993), and *Vibrio salmonicida* or *Vibrio ruckeri* (Robertsen *et al.* 1990). The administration of β -glucans in fish diet has been shown to enhance the respiratory burst activity (Siwicki *et al.* 1994; Li & Gatlin 2003), phagocytosis (Li & Gatlin 2003), and lysozyme levels (Ogier De Baulny *et al.* 1996). Growth promotion by administration of β -glucan is also reported in fish (Cook *et al.* 2003).

In addition to individual administration, β -glucans have also been used with bacterial vaccine as adjuvant (Figueras *et al.* 1998) or with a lipopolysaccharide as synergists (Cook *et al.* 2001) so as to increase the immune response and to protect fish against pathogens.

The aim of the study was to assess a long-term oral administration of three concentrations of micronized β -1.3/1.6-D-glucan derived from oyster mushroom (*Pleurotus ostreatus*, Hiratake) on biometrical, haematological, biochemical, and immunological indices of half-year-old rainbow trout (*Oncorhynchus mykiss*) in natural environmental conditions of a commercial trout fishery.

MATERIAL AND METHODS

Test animals

In the study, six-month-old (Pd_{1/2}) rainbow trout (*O. mykiss*) of 10.7 cm and 8.5 g of the average total body length and body weight, respectively, in natural envi-

ronmental conditions of a commercial fishery in Ujčov (the Czech Republic, rainbow trout commercial production up to 130 tons a year) were used in the test. Fish in the amount of approximately 1100 individuals were held in 9,000 l concrete tanks in water flow-through system. Examined rainbow trout individuals (eight individuals for each haematological, biochemical, and immunological examination, i.e. 24 individuals in each test group, i.e. 96 individuals in each sampling day) were randomly chosen from one control and three experimental tanks.

Test design

The study was designed to evaluate the effect of three concentrations (0.5, 1.0, and 2.0%) of micronized β -1.3/1.6-D-glucan (Natures, Slovakia) derived from oyster mushroom *Pleurotus ostreatus* Hiratake on half-year-old rainbow trout under natural conditions of a commercial trout fishery. β -glucan was administered orally in feed pellets.

Prior to the experiment, fish were adapted to conditions in the test tanks (t approx. 13.9°C, pH 7.28, oxygen content 9.8 mg L⁻¹) with natural light-dark cycle for 2 weeks. Before the experiment start, fish were fed 1.5 mm (and 2.0 mm since 43rd day) commercial rainbow trout feed pellets (Biomar, Denmark) consisting of fish meal, wheat flour, soyprotein concentrate, cod-liver oil, pea protein, vitamins, and minerals with the content of protein 47%, fat 20%, saccharides 16.4%, fiber 1.1%, ash 9.5%, and total phosphorus 1.3%. Digestible energy of pellets was 4675 Kcal.

The study was performed as follows: in one tank (Control) fish were fed commercial feed pellets (Biomar, Denmark) and in three experimental tanks they were fed the commercial pellets supplemented with either 0.5, 1.0, or 2.0% of micronized oyster mushroom β -1.3/1.6-D-glucan. Feed medicated with β -glucan was prepared by adhesion of micronized (i.e. ground to 5 μ m particles) β -glucan derived from *P. ostreatus* on commercial trout feed pellets. The medicated pellets were then covered with a thin layer of Aerosil 200 (colloid SiO₂ that covers pellet surface and fixes β -glucan uniformly on it, and also prevents the pellets from clustering) and Eudragit E (12.5%, a copolymer of dimethyl-amino-ethyl methacrylate, buthyl methacrylate, and methacrylate in technical ethanol and acetone preventing pellets from abrasion and thus β -glucan from segregation from the pellet surface).

Fish were fed the feed in the range of 1.35 to 4.73% body weight (according to dosage recommended by feed producer) for 85 days. Feed doses were calculated according to actual fish body mass and water temperature.

The study was carried out in autumn season (September – November). Average physico-chemical parameters of the water during the study were as follows: oxygen content of 10.6±0.9 mg L⁻¹ and pH of 7.51±0.30. In the course of the test, water temperature

was measured in each sampling period: 13.0±0.8°C (day 1–14), 13.2±1.1°C (day 15–28), 11.5±1.2°C (day 29–42), 9.0±0.9°C (day 43–63), and 10.8±3.2°C (day 64–84). In the course of the study, five samplings were performed at day 15, 29, 43, 64, and 85.

Biometrical parameters

Biometrical data consisted of total length (TL), standard length (SL), body weight (BW), liver weight (LW), Fulton's condition factor (FCF), and hepatosomatic index (HSI). Condition factor (FCF) and hepatosomatic index (HSI) of each fish were calculated according to formulas as follows: $FCF = BW (g) \times TL^{-3}(cm) \times 100$ and $HSI = \text{liver weight (g)} / \text{body weight (g)} \times 100$, respectively.

Haematological parameters

Blood samples were taken by venepuncture of caudal vein (*vena caudalis*) of control and experimental rainbow trout. Haematological indices including PCV, RBC, Hb, MCV, MCH, MCHC, and WBC (see the list of abbreviations) were determined according to Svobodová *et al.* (1991).

Biochemical profiling

A part of heparinized blood was centrifuged at 855 g for 10 minutes and then plasma was used for biochemical analysis. As for biochemical indices, plasma concentration of glucose, lactate, total proteins, albumins, triglycerides, cholesterol, ammonium, phosphorus, calcium, sodium, potassium, and chlorides as well as catalytic activity of lactate dehydrogenase, aspartate transferase, alanin transferase, and alkaline phosphatase were examined. The levels of Na⁺ and Cl⁻ were analysed using Easy Lyte Analyzer (Medica Corp., Bedford, MA, USA). The other indices were measured using Konelab 20i (ThermoFisher Scientific).

Immunological parameters

A phagocyte cells metabolic activity (induced chemiluminescence of phagocyte cells) was determined. Samples contained a luminophor luminol (Sigma-Aldrich, USA), an activator (opsonized zymosan particles in HBSS, Leino & Lilius 1992) and blood. Also spontaneous chemiluminescence without adding an activator was measured. A microplate luminometric method based on luminol-enhanced phagocyte cells chemiluminescence measured using a luminometer Immuno-tech LM-01T was applied.

Statistical analysis

Statistical analysis of the data was conducted using Unistat (version 5.6, StatSoft, Inc., USA). Values were tested for normal distribution using Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) and Tukey's HSD test were used.

RESULTS

The study on rainbow trout was carried out in environmental conditions of a commercial trout fishery in Ujčov (the Czech Republic). The fishery and fish health status were under continual veterinary surveillance. Fishery water influx from the Svatka river was monitored for physico-chemical parameters (e.g. temperature, pH, oxygen content, heavy metals content etc.) regularly in the course of the study.

During the course of 85day oral administration of 0.5, 1.0, and 2.0% β -glucan no obvious clinical signs of behavioral, respiratory, and neurologic distress were observed in rainbow trout. In all test tanks and the control fish showed normal feeding behavior.

Biometrical examination

Total length (TL), standard length (SL), body weight (BW), liver weight (LW), Fulton's condition factor (FCF), and hepatosomatic index (HSI) of rainbow trout exposed to β -glucan showed no significant differences from untreated fish in all five samplings (Tukey-HSD test, $p > 0.05$). During the study, weight gains as well as total and standard lengths of experimental and control rainbow trout were similar at each sampling and increased depending on time. BW values of experimental and control fish in each sampling are given in Figure 1. From sampling 1 to sampling 5, the average total length, standard length, and liver weight of experimental and control fish increased from 104.55 to 141.07 mm, from 90.77 to 126.38 mm, and from 0.23 to 0.44 g, respectively. HSI ranged from 1.24 to 1.86 in the course of the study, the values were comparable within each sampling. Fulton's condition factor was found 1.09 ± 0.10 in all fish tested.

Haematological examination

Haematological indices significantly altered are given in Table 1. Changes in Hb ($p < 0.05$), PCV ($p < 0.05$), and MCHC ($p < 0.01$) were found in experimental fish

compared to control. Rest of the indices (RBC, MCV, MCH, and WBC) showed no significant differences among experimental and control groups in any sampling (Tukey-HSD test, $p > 0.05$). The average values (\pm SD) of RBC, MCV, MCH, and WBC in the test were as follows: RBC of $0.94 \pm 0.19 \text{ T L}^{-1}$, MCV of $321.8 \pm 54.6 \text{ fl}$, MCH of $66.5 \pm 11.8 \text{ pg}$, and WBC of $13.67 \pm 5.65 \text{ G L}^{-1}$.

Biochemical examination

Plasma biochemical indices significantly changed during the test are listed in Table 2. Some parameters are not given because of plasma shortage (sampling 1 and 2, insufficient plasma volume, small fish) or technical problem in a biochemical analyser (sampling 3). In the course of the test, plasma concentrations of glucose, lactate, total protein, cholesterol, calcium, natrium, potassium (all $p < 0.05$), albumins and chlorides ($p < 0.01$), as well as catalytic activities of ALT and AST ($p < 0.05$) were changed (see Table 2). Direct dose-dependent effect of β -glucan was detected in lactate and TP concentrations in sampling 2 as well as in K^+ and Na^+ levels in sampling 4 and 5, respectively, among all β -glucan concentrations tested. Increase in TP and albumins levels were detected also in the highest β -glucan concentration (2.0%) in sampling 3, unfortunately no comparison to control and 0.5% β -glucan values is available. The other parameters (LDH, ALP, Trig, Phos, and NH_3) were found comparable among all groups in each sampling (Tukey-HSD test, $p > 0.05$), plasma levels of these parameters in the course of the study were as follows: catalytic activities of LDH and ALP were 13.89 ± 6.39 and $2.33 \pm 1.09 \mu\text{kat L}^{-1}$, respectively, and concentrations of triglycerides, phosphorus, and ammonium were $4.34 \pm 1.60 \text{ mmol L}^{-1}$, $4.39 \pm 0.71 \text{ mmol L}^{-1}$, and $308.9 \pm 117.2 \mu\text{mol L}^{-1}$, respectively.

Immunological examination

Luminol-induced chemiluminescence of phagocyte cell was determined by a microplate luminometric method using a luminometer Immunotech LM-01T. Results of

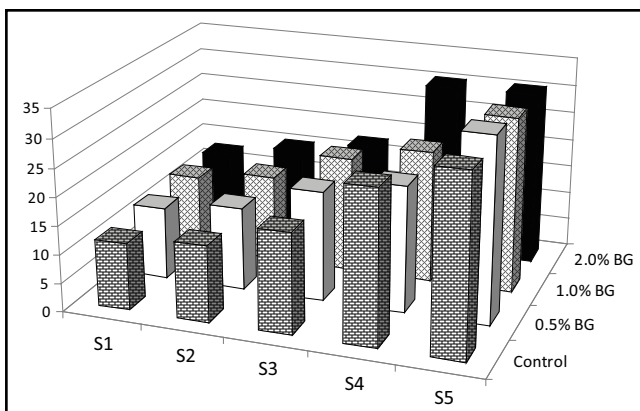


Fig. 1. Body weight (g) of experimental and control trout after long-term administration of β -glucan. (S – sampling)

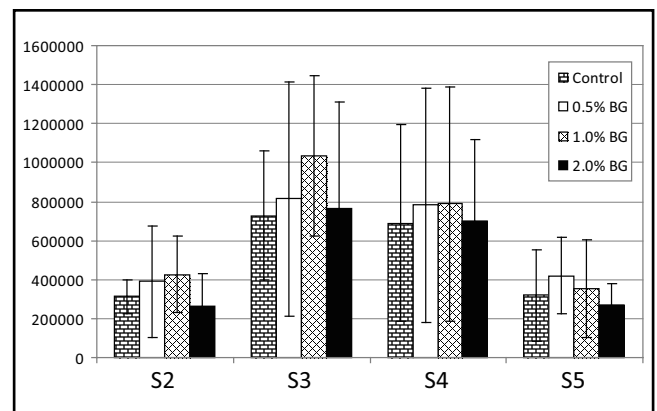


Fig. 2. Luminol-induced chemiluminescence of phagocytes (mV s^{-1}) in experimental and control trout after long-term administration of β -glucan. (S – sampling)

Tab. 1. Haematological indices of rainbow trout after long-term administration of β -glucan.

Fish group	Control	0.5 % BG	1.0 % BG	2.0 % BG
Sampling 1				
Hb (g L ⁻¹)	47.13±3.97 ^{ab}	38.12±7.29 ^a	51.01±3.29 ^b	51.18±5.61 ^{ab}
PCV (l L ⁻¹)	0.265±0.016 ^a	0.284±0.018 ^a	0.292±0.019 ^a	0.285±0.030 ^a
MCHC (g L ⁻¹)	0.181±0.004 ^b	0.139±0.024 ^a	0.172±0.006 ^b	0.183±0.011 ^b
Sampling 2				
Hb (g L ⁻¹)	77.20±15.32 ^a	67.48±12.55 ^a	66.60±6.60 ^a	63.97±7.57 ^a
PCV (l L ⁻¹)	0.301±0.015 ^a	0.299±0.020 ^a	0.294±0.026 ^a	0.301±0.024 ^a
MCHC (g L ⁻¹)	0.259±0.046 ^b	0.230±0.051 ^{ab}	0.226±0.013 ^{ab}	0.207±0.021 ^a
Sampling 3				
Hb (g L ⁻¹)	63.83±6.71 ^a	60.28±7.40 ^a	62.48±4.50 ^a	58.87±5.09 ^a
PCV (l L ⁻¹)	0.284±0.038 ^a	0.283±0.044 ^a	0.286±0.024 ^a	0.273±0.030 ^a
MCHC (g L ⁻¹)	0.228±0.038 ^a	0.217±0.040 ^a	0.219±0.020 ^a	0.217±0.023 ^a
Sampling 4				
Hb (g L ⁻¹)	56.61±6.35 ^a	56.97±8.84 ^a	53.27±6.23 ^a	55.03±8.77 ^a
PCV (l L ⁻¹)	0.283±0.028 ^b	0.266±0.034 ^{ab}	0.233±0.021 ^a	0.266±0.044 ^{ab}
MCHC (g L ⁻¹)	0.201±0.016 ^a	0.215±0.021 ^a	0.230±0.031 ^a	0.207±0.019 ^a
Sampling 5				
Hb (g L ⁻¹)	74.99±14.55 ^{ab}	73.53±12.45 ^{ab}	76.55±11.22 ^b	58.81±14.27 ^a
PCV (l L ⁻¹)	0.381±0.089 ^a	0.410±0.055 ^a	0.349±0.037 ^a	0.333±0.094 ^a
MCHC (g L ⁻¹)	0.201±0.029 ^a	0.181±0.031 ^a	0.220±0.026 ^a	0.181±0.029 ^a

fish blood phagocytes chemiluminescence in sampling 2 to 5 (sampling 1 excluded) are given in Figure 2. No significant shifts in luminol-induced chemiluminescence of phagocytes were detected in any sampling day (Tukey-HSD test, $p>0.05$). An integral of the chemiluminescence slightly increased in each experimental and control fish in the course of the study, but it returned to initial levels at the end of the study.

DISCUSSION

Using immunostimulants in farm animals as well as in aquaculture has been an upcoming area in recent years. They have been reported to be valuable for the control of fish diseases, as they facilitate e.g. the function of phagocytic cells and increase their bactericidal activities. Several substances stimulate the natural killer cells, complement, lysozyme and antibody responses in fish (Couso *et al.* 2003; Sakai 1999).

The study provides data on the effect of long-time exposure of half-year-old rainbow trout to β -glucan from oyster mushroom *Pleurotus ostreatus* Hiratake. The substance was micronized to 5 μ m particles for better absorption from gastrointestinal tract (as stated by producer). In the course of the study, no changes in feeding habits and physiological behavior were found in experimental rainbow trout.

Biometrical examination

Biometrical parameters are regarded as general indicators of fish health and the quality of the aquatic environment. The hepato-somatic index is considered a non-specific biomarker influenced by factors such as sex, season, disease, and nutritional level (Van der Oost *et al.* 2003). It provides an indication on the status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver with less energy reserved in it.

In our study, no significant changes in biometrical parameters (i.e. total length, standard length, body weight, liver weight, condition factor, and hepato-somatic index) in experimental and control rainbow trout were found. It can be found positive as the fish tested were reared in satisfactory, environmentally and nutritionally similar conditions. On the other hand, no positive effect of dietary β -glucan on growth performance or other biometric indicators of experimental rainbow trout was found despite the fact that the producer of β -glucan claims that micronization process, in which β -glucan is ground up to 5 μ m particles, can increase the absorption of the substance by gastrointestinal tract.

Some authors give data on the effect of β -glucan administration on fish biometrical indices. Misra *et al.* (2006) provide results confirming a significant

Tab. 2. Biochemical indices of rainbow trout after long-term administration of β -glucan.

Fish group	Control	0.5 % BG	1.0 % BG	2.0 % BG
Sampling 1				
Glu (mmol L ⁻¹)	-	4.67±0.00	4.75±0.00	-
Lact (mmol L ⁻¹)	-	5.06±2.39	4.04±0.00	-
AST (μkat L ⁻¹)	-	8.10±0.00	7.05±0.00	-
ALT (μkat L ⁻¹)	-	0.522±0.000	0.579±0.000	0.708±0.000
TP (g L ⁻¹)	25.40±4.80 ^a	22.90±2.94 ^a	25.38±4.18 ^a	21.49±7.58 ^a
Alb (g L ⁻¹)	-	2.63±1.58 ^a	2.34±1.34 ^a	5.46±4.47 ^a
Chol (mmol L ⁻¹)	-	6.75±0.08 ^a	6.73±0.97 ^a	-
Ca ²⁺ (mmol L ⁻¹)	-	2.79±0.00 ^a	2.80±0.12 ^a	-
Na ⁺ (mmol L ⁻¹)	145.3±0.0	153.8±3.7 ^a	151.1±3.2 ^a	153.2±2.7 ^a
K ⁺ (mmol L ⁻¹)	2.92±0.00	3.61±0.84 ^a	2.72±1.01 ^a	3.56±0.70 ^a
Cl ⁻ (mmol L ⁻¹)	112.8±0.0	116.2±0.7 ^a	113.0±2.4 ^a	114.2±1.6 ^a
Sampling 2				
Glu (mmol L ⁻¹)	3.65±0.39 ^a	5.52±0.99 ^{ab}	7.44±1.91 ^b	5.76±1.48 ^{ab}
Lact (mmol L ⁻¹)	3.10±0.18 ^a	4.98±1.72 ^{ab}	7.73±1.75 ^b	7.30±1.34 ^b
AST (μkat L ⁻¹)	-	-	-	-
ALT (μkat L ⁻¹)	-	0.440±0.000	0.440±0.141	-
TP (g L ⁻¹)	25.35±2.75 ^a	28.47±3.05 ^{ab}	29.91±2.87 ^{ab}	32.76±2.42 ^b
Alb (g L ⁻¹)	4.65±1.90 ^a	6.06±3.21 ^b	5.38±1.69 ^a	7.16±3.46 ^a
Chol (mmol L ⁻¹)	7.09±1.88 ^{ab}	6.72±0.96 ^a	6.59±0.72 ^a	8.17±0.43 ^b
Ca ²⁺ (mmol L ⁻¹)	2.54±0.20 ^a	2.81±0.18 ^a	2.67±0.07 ^a	2.90±0.16 ^a
Na ⁺ (mmol L ⁻¹)	148.6±4.9 ^a	153.6±4.1 ^a	147.6±3.5 ^a	142.30±9.87 ^a
K ⁺ (mmol L ⁻¹)	3.09±0.62 ^a	2.84±1.17 ^a	3.32±0.69 ^a	3.16±1.02 ^a
Cl ⁻ (mmol L ⁻¹)	114.3±3.1 ^a	112.8±2.5 ^a	109.8±1.1 ^a	107.2±0.7 ^a
Sampling 3				
Glu (mmol L ⁻¹)	-	-	6.31±1.56 ^a	5.36±1.55 ^a
Lact (mmol L ⁻¹)	-	-	3.28±0.90 ^a	3.65±0.72 ^a
AST (μkat L ⁻¹)	-	-	7.40±2.73 ^a	5.71±1.96 ^a
ALT (μkat L ⁻¹)	-	-	0.303±0.134 ^a	0.345±0.124 ^a
TP (g L ⁻¹)	-	-	24.21±2.47 ^a	29.22±4.21 ^b
Alb (g L ⁻¹)	-	-	4.40±2.16 ^a	6.85±2.80 ^b
Chol (mmol L ⁻¹)	-	-	6.25±0.84 ^a	5.49±3.65 ^a
Ca ²⁺ (mmol L ⁻¹)	-	-	2.59±0.08 ^a	2.36±0.49 ^a
Na ⁺ (mmol L ⁻¹)	-	-	154.2±3.2 ^a	155.9±6.2 ^a
K ⁺ (mmol L ⁻¹)	-	-	2.70±0.47 ^a	2.12±1.09 ^a
Cl ⁻ (mmol L ⁻¹)	-	-	116.20±4.5 ^a	118.6±5.4 ^a
Sampling 4				
Glu (mmol L ⁻¹)	5.88±1.38 ^b	4.11±0.59 ^a	5.44±1.23 ^b	4.84±0.56 ^{ab}
Lact (mmol L ⁻¹)	4.00±1.68 ^a	3.98±1.47 ^a	4.39±1.11 ^a	6.34±2.50 ^a
AST (μkat L ⁻¹)	6.04±2.12 ^a	7.25±1.53 ^a	7.19±1.26 ^a	6.67±0.48 ^a
ALT (μkat L ⁻¹)	0.342±0.097 ^a	0.376±0.076 ^a	0.393±0.086 ^a	0.381±0.079 ^a
TP (g L ⁻¹)	31.26±2.64 ^a	29.96±3.69 ^a	30.94±2.23 ^a	33.16±2.19 ^a
Alb (g L ⁻¹)	10.25±2.14 ^a	8.94±2.59 ^a	9.63±2.33 ^a	11.14±1.55 ^a

Tab. 2. (Continued) Biochemical indices of rainbow trout after long-term administration of β -glucan.

Fish group	Control	0.5 % BG	1.0 % BG	2.0 % BG
Chol (mmol L ⁻¹)	7.94±0.88 ^a	8.81±0.90 ^a	9.42±1.21 ^a	9.35±1.33 ^a
Ca ²⁺ (mmol L ⁻¹)	2.68±0.17 ^a	2.69±0.15 ^a	2.88±0.21 ^a	2.88±0.21 ^a
Na ⁺ (mmol L ⁻¹)	157.6±3.0 ^a	157.1±1.7 ^a	159.8±2.0 ^a	158.9±1.6 ^a
K ⁺ (mmol L ⁻¹)	2.29±1.02 ^a	2.52±1.06 ^a	2.27±1.04 ^a	4.40±0.38 ^b
Cl ⁻ (mmol L ⁻¹)	113.2±1.8 ^b	110.3±0.5 ^{ab}	111.1±1.6 ^{ab}	109.5±0.3 ^a
Sampling 5				
Glu (mmol L ⁻¹)	4.98±0.43 ^a	6.25±1.93 ^{ab}	6.29±1.21 ^b	6.13±1.05 ^{ab}
Lact (mmol L ⁻¹)	3.19±1.32 ^a	3.58±1.45 ^a	3.24±0.75 ^a	3.71±1.18 ^a
AST (μ kat L ⁻¹)	8.82±1.81 ^b	5.86±0.72 ^a	7.99±1.18 ^b	7.30±2.66 ^{ab}
ALT (μ kat L ⁻¹)	0.452±0.064 ^b	0.302±0.035 ^a	0.387±0.100 ^{ab}	0.550±0.323 ^{ab}
TP (g L ⁻¹)	32.00±3.53 ^b	28.46±1.39 ^a	30.07±3.87 ^{ab}	35.53±3.19 ^b
Alb (g L ⁻¹)	7.63±3.45 ^a	8.25±3.43 ^a	8.98±2.51 ^a	11.49±5.78 ^a
Chol (mmol L ⁻¹)	8.58±1.37 ^b	7.18±0.72 ^a	8.25±1.60 ^{ab}	9.45±1.46 ^b
Ca ²⁺ (mmol L ⁻¹)	2.48±0.18 ^{ab}	2.46±0.16 ^a	2.54±0.10 ^{ab}	2.73±0.14 ^b
Na ⁺ (mmol L ⁻¹)	150.8±3.2 ^a	157.2±5.6 ^{ab}	155.4±4.8 ^{ab}	157.5±5.4 ^b
K ⁺ (mmol L ⁻¹)	1.66±1.17 ^a	2.27±0.95 ^a	2.11±1.08 ^a	2.30±1.45 ^a
Cl ⁻ (mmol L ⁻¹)	118.9±3.6 ^a	123.5±4.6 ^a	120.4±3.4 ^a	121.3±3.5 ^a

($p < 0.05$) growth stimulation of *Labeo rohita* fingerlings resulting from the administration of β -glucan (Sigma) at 250 or 500 mg kg⁻¹ dry diet (in comparison, fish fed 0 and 100 mg kg⁻¹ showed no differences in growth). Simultaneously, they report no effect of β -glucan on feed conversion ratio in fish tested.

The increase in growth rate of various fish species under certain environmental conditions after administration of β -glucan was also found in studies of Ai *et al.* (2007) and Sealey *et al.* (2008) on large yellow croaker (*Pseudosciaena crocea*) and rainbow trout (*O. mykiss*), respectively. However, in other scientific studies (Bagni *et al.* 2005; Welker *et al.* 2007) no significant increase in growth in fish fed by β -glucans of different origin was found. Dalmo and Børgwald (2008) state that feeding strategies should be developed for each fish species with respect to β -glucan dose and duration to obtain increased growth performance. They also claim that soluble or particulate β -glucans may confer different responses since only soluble ones can be absorbed in the intestine and that there are no indications that particulate β -glucans are taken up by fish intestines or digested by β -glucan degrading enzymes thereby being nutritious.

Kunttu *et al.* (2009) published data on the effect of β -glucan (Macrogard®) at 0.2, 0.6, and 1.8% of feed on mortality of juvenile rainbow trout (*O. mykiss*), in which mortality of fish fed diet supplemented with β -glucan at 1.8% was significantly ($p < 0.05$) higher compared to control. In our study, mortality rates in all

groups were not found significantly different among all β -glucan concentrations tested (0.5, 1.0, and 2.0%) and ranged between 0.08 and 0.25 individual per day.

Haematological examination

Alterations in haematological profiling are usually the first detectable and quantifiable responses to any environmental change (Hawkins & Mawdsley 2006). Review on the effect of acute or chronic stress factors on blood parameters of teleost fish is published by Wendelaar Bonga (1997). Changes in haematological (and biochemical) profile have been reported by many authors testing toxicity of chemical substances and pesticide preparations in fish (Haluzova *et al.* 2011; Modra *et al.* 2008). As β -glucans, being immunostimulants usually of natural origin, are assumed non-toxic, a scarcity of information on the effect of the substance on fish haematological profiling is available.

In our study, oral administration of micronized β -glucan caused shifts in Hb ($p < 0.05$), PCV ($p < 0.05$), and MCHC ($p < 0.01$) (see Table 1). Rest of the indices (RBC, MCV, MCH, and WBC) showed no significant differences among experimental and control groups in any sampling. All haematological parameters were found at relatively low physiological values (according to Svobodova *et al.* 1991) that can be explained by using young individuals (six-month-old trout, Pd_{1/2}) in the study. As for PCV, Hb, and RBC, a mild decrease in the parameters in sampling 4 and mild increase in sampling 5 in all fish groups can be assumed as a response of

fish organism to changes in water temperature. Similar results are given in the study of Dobsinska *et al.* (1980) in which low water temperature was found the actual cause of low Hb, PCV, and RBC values. In opposite, study of Miller *et al.* (1983) presents that low Hb level in rainbow trout was ascribed to the wide temperature variations (3–25°C). Denton and Yousef (1975) state that water temperature is not responsible for variations in haematology of rainbow trout, they also present that the diet composition, metabolic adaptations, and variations in fish activity are the main factors responsible for the seasonal changes in Hb, PCV, MCV, MCH, and MCHC values in trout.

As for WBC, no significant changes among all the groups tested were found in the course of the test. Leukocyte counts were also found at low physiological values for the species (10–60 g L⁻¹, Svobodová *et al.* 1991). Leukocytes are involved in the regulation of immunological function and protective response to stress in fish. The reduction in leukocyte count occurs e.g. through the change in lymphopoiesis or altered release of lymphocytes from lymphoid tissues (Das *et al.* 2009), increased leukocyte counts can result e.g. from immune (inflammatory) response against bacteria (Roberts 1978). Significant increase in WBC was described by Selvaraj *et al.* (2006) in glucan-injected common carp, the increase was directly proportionate to the concentration of yeast glucan administered. They also found that, among the leukocytes, neutrophils increased significantly while basophils declined in number and that lymphocyte counts did not vary much among the control and experimental groups of carp. Significant increase in WBC and proportion of neutrophils and monocytes as an effect of glucan was found in common carp (*C. carpio*) challenged by intraperitoneal injection of LD₅₀ concentration of *Aeromonas hydrophila* in the study of Selvaraj *et al.* (2005). The results are in an agreement with studies of Jeney and Anderson (1993) and Siwicki *et al.* (1994). In opposite, in the study of Gopalakannan and Arul (2010), there was no significant variation in WBC count in carp (*C. carpio*) challenged with *Aeromonas hydrophila* and subjected to oral administration of glucan in the concentration of 10g kg⁻¹ of the basal diet for 60 days, except the day 60 in which WBC was slightly increased compared to control group.

Biochemical profiling

Biochemical profile of blood plasma can provide an important information about the internal environment of the organism. In our study, shifts in glucose concentrations were found in sampling 2, 4, and 5, but no dose-dependent effect of β -glucan administered was proved. In general, shifts in plasma glucose are usually interpreted as a consequence of glycogenolytic activity of catecholamines and gluconeogenic effect of glucocorticoids as an organism reaction to the stress stimuli (Doubek *et al.* 2010). In our study, stress as the cause of

glucose level shifts can be excluded as handling and sampling procedures were done similarly in all test groups in all samplings. It is probable that administered β -glucan became involved in endogenous glucose metabolism.

In our study, shifts in catalytic activities of ALT and AST were found in sampling 5, but no β -glucan dose-dependent manner was found. Nevertheless, it is very likely that β -glucan doesn't give rise to the enzymes changes. Doubek *et al.* (2010) present that the activity of the transaminases is a relevant indicator of stress-based tissue impairment and amplified transamination pathway induced by stress stimuli. In our study, it is unlikely that ALT and AST activities are changed due to oral administration of β -glucan (as a stress stimulus).

In our study, an increase in TP levels in sampling 2 and 3, and significant shifts in TP in sampling 5 were found. Shifts in TP were presented also in the study of Mehrabi *et al.* (2012) in which a synbiotic (Biomin, Austria) was applied to rainbow trout (*O. mykiss*) fingerlings. Ebrahimi *et al.* (2012) found similar changes in TP content in common carp (*C. carpio*) fed a diet supplemented with a commercial prebiotic (Immunogen, Iran) and exposed to *Aeromonas hydrophila*. Increased TP level appears to be a sign of enhanced health status of the prebiotic-fed fish. High concentrations of serum proteins including humoral elements of the non-specific immune system are likely to be results of an enhancement in the non-specific immune response of the fish (Ebrahimi *et al.* 2012).

Immunological parameters

The immune stimulatory effects of β -glucans have been well studied. Many studies proved that β -glucans increase the resistance of fish to pathogens as *Vibrio anguillarum*, *V. salmonicida* and *Yersinia ruckeri* (Robertsen *et al.* 1990), but some studies presented opposite results (e.g. rainbow trout infected with yeast glucan did not show enhanced protection against *V. anguillarum* infection in the study of Thompson *et al.* 1995).

Phagocytes form the major part of the innate immune system (Secombes & Fletcher 1992). They are crucial in the host defense response against infection diseases agents through reactive oxygen species (ROS) production. Measurement of phagocytes ROS production is very important for the determination of physiological consequences resulting from cellular mechanisms leading to oxidative burst. As they are able to produce oxidative anions, chemiluminescence techniques have been used for the measurement of their capability to kill pathogens (Salo *et al.* 1998). Chemiluminescence is based on the amplification of natural luminescence emitted when ROS are released during phagocytosis. Light production is one of the characteristics of the oxidative burst (Alves *et al.* 2003).

In the present study, no dose of orally administered β -glucan (0.5, 1.0, and 2.0%) enhanced significantly the chemiluminescence (CL) response of rainbow trout blood phagocytes in any sampling. The CL of

phagocytes was measured in the form of natural (not published) and luminol-enhanced chemiluminescence (see Figure 2). Integrals of CL slightly increased in all groups during the test, but they returned to initial levels at the end of the test. In our study, the luminol-enhanced CL of phagocytes raised in all groups tested till the sampling 3 (day 43), thereafter it slowly declined. A slight increase and subsequent decrease in CL could be explained as a reaction of immune system to changes in aquatic environment during the test (water temperature particularly) as fish are known to enhance their protective mechanisms at winter season. Final decrease in the parameter could be likely due to an inhibition of metabolic processes in rainbow trout at winter season. No significant differences in the CL of plasma phagocytes in rainbow trout were likely due to the fact, that the fish were clinically healthy and free of pathogenic microorganisms. Therefore, no important effect of β -glucan on innate immune system response was proved. Changes in CL were similar in all groups tested and reflected the environmental conditions (i.e. water temperature, season).

Vainikka *et al.* (2005) tested the effect of β -glucan injected intraperitoneally (150 mg kg^{-1}) on the chemiluminescence response of blood phagocytes of a tench (*Tinca tinca*) and proved no significant shifts in it. There was only an overall (non-significant) enhancement in blood phagocyte chemiluminescence explained as a possible consequence of handling stress. The dietary effect of β -1.3-glucan in the concentrations of 0.09 and 0.18% on innate immune responses of large yellow croaker (*Pseudosciaena crocea*) was studied by Ai *et al.* (2007). In the study, 8week administration of low dose (0.09%) of glucan significantly enhanced phagocytic activity (in head kidney macrophages) while the high concentration (0.18%) did not. Cook *et al.* (2003) tested a commercial β -glucan (EcoActiva™) as a feed supplement for a snapper, *Pagrus auratus*, and found that the immunostimulant increased macrophage oxygen radical production only during the winter time (but not during the summer time). The results suggest that it might be favourable for the snapper to include β -glucans in the feed only during the winter time to increase the fish resistance against diseases.

CONCLUSION

After oral administration of three different concentrations (0.5, 1.0, and 2.0%) of micronized β -1.3/1.6-D-glucan derived from oyster mushroom *P. ostreatus* Hiratake, changes in haematological and biochemical parameters were found in six-month-old rainbow trout (*Oncorhynchus mykiss*) under natural environment conditions of a commercial trout fishery. No shifts in biometrical and immunological parameters were found in experimental fish compared to control.

Inconsistent data on the effect of the administration of β -glucans of various origin to fish are available. Many

studies proved the effect of β -glucans (administered either orally, intraperitoneally or in the form of bath) on especially biometrical (i.e. induction of growth) and immunological (enhancement of immune response, e.g. lysozyme activity, phagocyte metabolic activity) indices, but in opposite, some studies found no changes in the parameters.

Significant changes in haematological and biochemical (except glucose and TP concentration) indices in experimental fish could be likely attributed more to the effect of environmental conditions or other eliciting stimuli than to the direct effect of orally administered micronized oyster mushroom β -glucan. Moreover, as plenty of studies proved, utilization and efficiency of β -glucan in the organism is influenced by many factors such as water temperature, stress, form, dosage, physico-chemical properties (i.e. backbone/branch configuration, solubility, molecular weight), and the route of administration. That is the reason why the use of β -glucan-supplemented feed in clinically healthy fish still remains an issue.

Immunostimulants, chemotherapeutics, and vaccines are used in the control of fish diseases. Immunostimulants are found safer than chemotherapeutics and their range of efficacy is wider than in vaccines, so they have been used for a long time as an important tool to improve immune response in fish. Immunostimulant use in breedings endangered by stress stimuli, infectious diseases or adverse environmental factors is indisputable. Nevertheless, the use of some immunomodulators in prosperous, clinically healthy aquaculture is still questionable.

ACKNOWLEDGEMENTS

The work was realized with the support of MSM Project No. 6215712402 (Veterinary Aspects of Foodstuff Safety and Quality) and project No. QJ1210013 (Technology of the freshwater fish breeding with using of re-circulatory systems of Danish type focused on the methods of effective treatment of environment and veterinary care).

Potential Conflicts of Interest: None disclosed.

REFERENCES

- 1 Ai QH, Mai KS, Zhang L, Tan BP, Zhang WB, Xu W, Li HT (2007). Effects of dietary β -1.3 glucan on innate immune response of large yellow croaker (*Pseudosciaena crocea*). *Fish Shellfish Immun.* **22**: 394–402.
- 2 Alves CMOS, Marzocchi-Machado CM, Carvalho IF, Lucisano-Valim YM (2003). Application of the chemiluminescence systems to evaluate the role of Fcy and complement receptors in stimulating the oxidative burst in neutrophils. *Talanta.* **60**: 601–608.
- 3 Bagni M, Romano N, Finioia MG, Abelli L, Scapiagliati G, Tiscari PG, Sarti M, Marino G (2005). Short- and long-term effects of a dietary yeast beta-glucan (Marcogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). *Fish Shellfish Immun.* **18**: 311–325.

- 4 Brown GD, Gordon S (2003). Fungal beta-glucans and mammalian immunity. *Immunity*. **19**: 311–315.
- 5 Cook MT, Hayball PJ, Hutchinson W, Nowak BF, Hayball JD (2001). The efficacy of a commercial β -glucan preparation, EcoActiva, on stimulating respiratory burst activity of head-kidney macrophages from pink snapper (*Pagrus auratus*), Sparidae. *Fish Shellfish Immun.* **11**: 661–672.
- 6 Cook MT, Hayball PJ, Hutchinson W, Nowak BF, Hayball JD (2003). Administration of a commercial immunostimulant preparation, EcoActiva, as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter. *Fish Shellfish Immun.* **14**: 333–345.
- 7 Couso N, Castro R, Magarinos B, Obach A, Lamas J (2003). Effect of oral administration of glucans on the resistance of gilthead seabream pasteurellosis. *Aquaculture*. **219**: 99–109.
- 8 Dalmo RA, Bøgwald J (2008). β -glucans as conductors of immune symphonies. *Fish Shellfish Immun.* **25**: 384–396.
- 9 Das BK, Debnath C, Patnaik P, Swain DK, Kumar K, Mishra BK (2009). Effect of β -glucan on immunity and survival of early stage of *Anabas testudineus* (Bloch). *Fish Shellfish Immun.* **27**: 678–683.
- 10 Denton JE, Yousef MK (1975). Seasonal changes in haematology of rainbow trout, *Salmo gairdneri*. *Comp Biochem Phys.* **51A**: 151–153.
- 11 Djordjevic B, Škugor S, Jørgensen SM, Øverland M, Mydland LV, Krasnov A (2009). Modulation of splenic immune responses to bacterial lipopolysaccharide in rainbow trout (*Oncorhynchus mykiss*) fed lentinan, a beta-glucan from mushroom *Lentinula edodes*. *Fish Shellfish Immun.* **26**: 201–209.
- 12 Dobsinska E, Kalal L, Kralova D (1980). Haematological values of the trout (*Salmo gairdneri*) fed added technical L-lysine. *Biological Chemical Factors in Animal Production – Veterinaria*. **16**: 357–364 (in Czech).
- 13 Doubek J, Slosarkova S, Rehakova K, Bouda J, Scheer P, Piperisova I, Tomenendalova J, Matalova E (2010). The interpretation of basic biochemical and haematological indices in animals. Noviko, Czech Republic (in Czech). 102 p.
- 14 Ebrahimi G, Ouraji H, Khaledi MK, Sudagar M, Barari A, Zarei Dangesaraki M, Jani Khalili KH (2012). Effects of a prebiotic, Immunogen[®], on feed utilization, body composition, immunity and resistance to *Aeromonas hydrophila* infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. *J Anim Physiol An N.* **96**: 591–599.
- 15 Figueras A, Santarem MM, Novoa B (1998). Influence of the sequence of administration of β -glucan and *Vibrio damsela* vaccine on the immune response of turbot (*Scophthalmus maximus* L.). *Vet Immunol Immunop.* **64**: 59–68.
- 16 Gopalakannan A, Arul V (2010). Enhancement of the innate immune system and disease-resistant activity in *Cyprinus carpio* by oral administration of β -glucan and whole cell yeast. *Aquac Res.* **41**: 884–892.
- 17 Haluzova I, Modra H, Blahova J, Marsalek P, Groch L, Siroka Z, Kruzikova K, Jarkovsky J, Litternerova S, Svobodova Z (2011). The effects of Click 500 SC (terbutylazine) on common carp *Cyprinus carpio* under (sub)chronic conditions. *Neuroendocrinol Lett.* **32**: S15–24.
- 18 Hawkins RI, Mawdsley TL (2006). Fish haematology – a bibliography. *J Fish Biol.* **4**: 193–232.
- 19 Jeney G, Anderson DP (1993). Glucan injection or bath exposure given alone or in combination with a bacterin enhance the non-specific defense mechanisms in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **116**: 315–329.
- 20 Kunttu HMT, Valtonen ET, Suomalainen LR, Vielma J, Jokinen IE (2009). The efficacy of two immunostimulants against *Flavobacterium columnare* infection in juvenile rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immun.* **26**: 850–857.
- 21 Leino L, Lilius EM (1992). The up- and down-modulation of immunoglobulin G Fc receptors and complement receptors in activated human neutrophils depends on mature of activator. *J Leukocyte Biol.* **51**: 157–163.
- 22 Li P, Gatlin DM (2003). Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* x *M. saxatilis*). *Aquaculture*. **219**: 681–692.
- 23 Mehrabi Z, Firouzbakhsh F, Jafarpour A (2012). Effects of dietary supplementation of symbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *J Anim Physiol An N.* **96**: 474–481.
- 24 Miller WR III, Hendricks AC, Cairns J Jr. (1983). Normal ranges for diagnostically important haematological and blood chemistry characteristics of rainbow trout (*Salmo gairdneri*). *Can J Fish Aquat Sci.* **40**: 420–425.
- 25 Misra CK, Das BK, Mukherjee SC, Pattnaik P (2006). Effect of long term administration of dietary β -glucan on immunity, growth and survival of *Labeo rohita* fingerlings. *Aquaculture*. **255**: 82–94.
- 26 Modra H, Haluzova I, Blahova J, Havelkova M, Kruzikova K, Mikula P, Svobodova Z (2008). Effects of subchronic metribuzin exposure on common carp (*Cyprinus carpio*). *Neuroendocrinol Lett.* **29**: 669–674.
- 27 Nikl L, Evelyn TPT, Albright LJ (1993). Trials with an orally and immersion-administered β -1.3 glucan as an immunoprophylactic against *Aeromonas salmonicida* in juvenile Chinook salmon *Oncorhynchus tshawytscha*. *Dis Aquat Organ.* **17**: 191–196.
- 28 Ogier De Baulny M, Quentel C, Fournier V, Lamour F, Le Gouvello R (1996). Effect of long term oral administration of beta-glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot *Scophthalmus maximus*. *Dis Aquat Organ.* **26**: 139–147.
- 29 Roberts RJ (1978). The pathophysiology and systematic pathology of teleosts. In: Roberts RJ, editor. *Fish pathology*. London: Bailliere Tindal, p. 55–91.
- 30 Robertsen B, Rørstad G, Engstad R, Raa J (1990). Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. *J Fish Dis.* **13**: 391–400.
- 31 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 Immun.* **27**: 369–373.
- 32 Sakai M (1999). Current research status of fish immunostimulants. *Aquaculture*. **172**: 63–92.
- 33 Salo HM, Aaltonen TM, Markkula SE, Jokinen EI (1998). Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae). I. Phagocytes. *Photochem Photobiol.* **67**: 433–437.
- 34 Sealey WM, Barrows FT, Hang A, Johansen KA, Overturf K, LaPatra SE (2008). Evaluation of the ability of barley genotypes containing different amounts of β -glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*. *Anim Feed Sci Tech.* **141**: 115–128.
- 35 Secombes CJ, Fletcher TC (1992). The role of phagocytes in the protective mechanism of fish. *Ann Rev Fish Dis.* **2**: 53–71.
- 36 Selvaraj V, Sampath K, Sekar V (2005). Administration of yeast glucan enhances survival and some non-specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. *Fish Shellfish Immun.* **19**: 293–306.
- 37 Selvaraj V, Sampath K, Sekar V (2006). Adjuvant and immunostimulatory effects of β -glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with *Aeromonas hydrophila*. *Vet Immunol Immunop.* **114**: 15–24.
- 38 Siwicki AK, Anderson DP, Rumsey GL (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Vet Immunol Immunop.* **41**: 125–139.
- 39 Svobodova Z, Pravda D, Palackova J (1991). Unified methods of haematological examination of fish. *RIFCH Vodnany, Methods No. 22*. 31 p.

- 40 Thompson KD, Cachos A, Inglis V (1995). Immunomodulating effects of glucans and oxytetracycline in rainbow trout, *Oncorhynchus mykiss*, on serum lysozyme and protection. In: Shariff M, Subasighe RP, Arthur JR, editors. Diseases in Asian Aquaculture, Vol. 11 Fish Health Section, Asian Fisheries Society, Manila, Philippines, p. 433–439.
- 41 Vainikka A, Jokinen EI, Kortet R, Pauku S, Pirhonen J, Rantala MJ, Taskinen J (2005). Effects of testosterone and β -glucan on immune functions in tench. *J Fish Biol.* **66**: 348–361.
- 42 Van der Oost R, Beyer J, Vermeulen NPE (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Phar.* **13**: 57–149.
- 43 Welker TL, Lim C, Yildirim-Aksoy M, Shelby R, Klesius PH (2007). Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell or yeast subcomponents. *J World Aquacult Soc.* **38**: 24–35.
- 44 Wendelaar Bonga SE (1997). The stress response in fish. *Phys Rev.* **77**: 591–625.