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RESEARCH ARTICLE

Effects of functional feeds on hematological and biochemical indicators of juvenile sea trout (*Salmo trutta* m. *trutta* L.)

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Abstract. The effects functional additives had on blood hematological and biochemical indicators of sea trout (body weight 74.6 g, standard length 17.2 cm) were studied. The fish were divided into four experimental groups. Two groups were fed experimental feeds (group TU basic feed; group TUB basic feed with Bioimmuno), and two groups were fed commercial feeds (group TB commercial feed; group TBF commercial feed with FOCUS Plus®). After 14 and 28 days of rearing (the end of the experiment) specimens were measured and blood was drawn from seven fish from each group for hematological and biochemical tests. No effects were confirmed on rearing parameters. After 14 days of rearing, decreased values of WBC, RBC, HGB, and HCT were noted in groups TU and TB, while after 28 days lower values of RBC and HCT persisted in these groups. Lower values of these parameters were also noted in group TUB. Increased ALP activity was noted in group TUB after 14 days, while in groups TU and TUB increased levels of TP and ALB were noted after 28 days. A significant increase in ALB was also noted in group TBF. The functional feeds positively affected the physiological state of the sea trout. The results indicated that it is necessary to conduct studies to determine the optimal dose of Bioimmuno for juvenile sea trout and the period during which functional feeds should be given.

Keywords: β-glucan, functional feed, biochemical indicators, hematological indicators, rearing indicators, sea trout

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Introduction

One of the most important issues focused on in today's aquaculture is improving the quality and survival of cultured fishes; this refers to the production of both stocking material and culture material for producing fish for consumers. To this aim, functional feed additives are increasingly being used (Dawood et al. 2018). An important group among these include prebiotics, probiotics, synbiotics, and

immunostimulants, which are additives that affect fish resistance to stress and disease (Encarnação 2016, Terech-Majewska 2016). Immunostimulants not only mobilize the immune system and increase immunological responses, they also affect the condition and growth rates of cultured fishes (Ringø et al. 2012). Promising immunomodulators include β-glucan that is extracted primarily from yeast cell walls (above all from *Saccharomyces cerevisiae*) and from some cereal grains (Eicher et al. 2006). The effects of β-glucans, yeasts, and various yeast-derived additives have been studied in several fish species, including the following: Atlantic salmon (*Salmo salar* L.) (Paulsen et al. 2001, Bridle et al. 2005); rainbow trout (*Oncorhynchus mykiss* (Walbaum)) (Djordievic et al. 2009); pikeperch (*Sander lucioperca* (L.)) (Jarmołowicz et al. 2018); silver seabream (*Pagrus auratus* (Forster), (Cook et al. 2003); African catfish (*Clarias gariepinus* (Burch.)) (Yoshida et al. 1995, Kazuń and Siwicki 2013); Nile tilapia (*Oreochromis niloticus* (L.)) (Dawood et al. 2020); sea bass (*Dicentrarchus labrax* (L.)) (Bonaldo et al. 2007, Bagni et al. 2000). In many cases, these studies revealed that supplementing feed with these additives advantageously affected fish growth (Cook et al. 2003), increased enterocyte surface area (Jarmołowicz et al. 2018, Dawood et al. 2020), improved survival and pathogen immunity (Welker et al. 2007), and increased antibody counts (Selvaraj et al. 2005). Despite the wide range of studies on the effects of these additives (including β-glucans) on fishes, studies focusing on the effects these immunostimulators have on the physiological state and health of this group of animals are few. The most reliable method for assessing physiological state and health is the analysis of hematological and biochemical indicators (Collins et al. 2016). Determining the values of basic blood morphology parameters such as hemoglobin, hematocrit, and red and white blood cell and thrombocyte counts permits diagnosing anemia, inflammation, infection, or other disease processes (Clauss et al. 2008). Analyzing hematological profiles is also often used in studies of various toxic substances (Javed and Usmani 2015), while biochemical parameters are good indicators of the proper functioning of various organs (e.g., liver, kidneys, heart) and glands, nutritional status and systemic hydration (total protein, magnesium, calcium), and the occurrence of stress (e.g., cortisol, glucose) (Haluzova et al. 2010, Brinn et al. 2012).

The aim of the study was to determine the effects of two functional additives (Bioimmuno and FOCUS Plus®) added to feed on the blood serum hematological and biochemical indicators of juvenile sea trout (*Salmo trutta m. trutt*a L.).

Materials and methods

Fish and rearing conditions (initial study phase)

Sea trout eggs were obtained in late October and early November from broodstock females at the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn. The eggs and then alevins were held in horizontal apparatuses connected to an open system with a steady supply of fresh water from the Radunia River (Kashubian Lake District, Northern Poland). After yolk sac resorption at the end of March, the free-swimming fry were transferred to tanks with internal measurements of 195×195×45 cm that were connected to the same open system in which incubation and hatching were done. Optimal environmental conditions for sea trout were maintained during rearing (S. Dobosz, unpublished materials). Commercial feed for salmonids was used (S. Dobosz, personal information). For the two months preceding the experiment proper, the fish were fed Aller Bronze (Aller Aqua A/S, Christiansfeld, Denmark), a 3 mm granulate size feed and containing 45% crude protein, 15% crude fat, 22.3% nitrogen-free extracts (NFE), 3.2% fiber, 6.5% ash with a gross energy of 21.2 MJ kg^{-1}. The feed was delivered manually three times daily.

Dividing fish into groups, feed and feeding, environmental conditions, and the rearing phase proper

The experiment was performed on 480 fish aged 18-months post-hatch. The fish were divided into four groups (120 specimens in each group). Each group was stocked into three rearing tanks (40 specimens per tank, n = 3). The factor tested was feed. The following 3 mm granulate commercial feeds from BioMar A/S (Aarhus, Denmark) were used: standard – BioMar EFICO Enviro (group TB); functional – EFICO Enviro FP (group TBF with FOCUS Plus®). The other two feeds (TU and TUB) were prepared at the Feed Science Laboratory, Department of Ichthyology and Aquaculture, University of Warmia and Mazury in Olsztyn (Niewiadomski et al. 2016) (Table 1). TUB feed was supplemented with Bioimmuno (1,3/1,6 β-glucan – 96 g 100 g^{-1}; Biolex®, Leiber, Germany) and methisoprinol (4 g 100 g^{-1}; Polfa, Grodzisk Mazowiecki, Poland) at doses of 20 g kg^{-1} feed (Kazuń and Siwicki 2013; IFI Olsztyn, Poland). The proximate composition of the feed was determined according to standard procedures (AOAC 2007). The gross energy of the feeds was calculated based on their proximate composition using the energy conversion factors of 39 kJ g^{-1} fat, 24 kJ g^{-1} protein, and 17 kJ g^{-1} NFE (Jobling 1994). The NFE values were calculated with the following equation: NFE = (100 – (crude protein + crude fat + water + ash)) (Table 1). The fish were fed manually every four hours (08:00, 12:00, 16:00; to satiation) according to the feeding program in the D-journal Freshwater Farm® software (From and Rasmussen 1984). Every seven days, the weight of the fish in the tanks was determined in vivo to adjust feed rations. The experiment ran for 28 days (four weeks).

Table 1
Proximate composition (% wet weight) and the ingredients (g 100 g^{-1}) of the feeds tested

	Feeds tested			
Description	TB	TBF	TU	TUB
Proximate composition				
Crude protein	44.37	42.84	48.68	46.29
Crude lipid	22.38	21.33	18.74	19.98
Nitrogen-free extracts (NFE)[#]	22.64	23.89	16.18	17.47
Ash	5.21	6.06	8.34	8.03
Water	5.40	5.88	8.06	8.23
Gross energy (MJ kg^{-1} feed)[$]	23.22	22.66	21.74	21.87
Ingredients				
Fish meal[a]	Manufacturer's Data: fish meal; poultry meal; soy concentrate; blood meal; fish oil; rapeseed oil; fava bean; wheat; hydrolyzed feather meal; sunflower cake; guar protein; wheat gluten; monosodium phosphate; monocalcium phosphate; yeast; FOCUS Plus (TBF feed)		30.0	30.0
Poultry meal[b]			18.0	18.0
Soy concentrate[c]			5.0	5.0
Blood meal[d]			5.0	5.0
Wheat flour[e]			10.0	10.0
Yeast[f]			5.0	5.0
Fish oil[g]			18.0	18.0
Rapeseed oil[h]			6.0	4.0
Bioimmuno[i]			0.0	2.0
Premix[jk]			3.0	3.0

[#]NFE = 100 – (crude protein + crude fat + ash + water); [$]gross energy calculated based on proximate composition using the following energy conversion factors: 24 MJ kg^{-1} protein, 39 MJ kg^{-1} lipid, 17 MJ kg^{-1} NFE (Jobling 1994); [a]FF SKAGEN, Denmark; [b]SONAC, Poland; [c]HP 300 HAMELT, Denmark; [d]SONAC, Poland; [e]CASTELLO, Poland; [f]ARTEX, Poland; [g]AGROFISH, Poland; [h]ZT Kruszwica S.A., Poland; [i]IFI Olsztyn, Poland; [j]DOLFOS, Poland; [k]premix ingredients (dry weight): vitamin A – 7,0000 IU kg^{-1}; vitamin D – 200,000 IU kg^{-1}; vitamin E – 17,500 IU kg^{-1}; vitamin K – 867 mg kg^{-1}; vitamin C – 28,500 mg kg^{-1}; vitamin B1 – 1,067 mg kg^{-1}; vitamin B2 – 2,000 mg kg^{-1}; vitamin B5 – 5,334 mg kg^{-1}; vitamin B6 – 1,334 mg kg^{-1}; vitamin B12 – 400 mg kg^{-1}; biotin – 200 mg kg^{-1}; niacin – 12,000 mg kg^{-1}; folic acid – 800 mg kg^{-1}; inositol – 20,000 mg kg^{-1}; choline chloride – 120,000 mg kg^{-1}; betaine – 75,000 mg kg^{-1}; $FeSO_4 \times H_2O$ – 4,334 mg kg^{-1}; KI – 734 mg kg^{-1}; $CuSO_4 \times 5H_2O$ – 267 mg kg^{-1}; MnO – 734 mg kg^{-1}; $ZnSO_4 \times H_2O$ – 1,250 mg kg^{-1}; ZnO – 750 mg kg^{-1}; Na_2SeO_4 – 34 mg kg^{-1}.

The feeding tests were performed in tanks of identical size (195 L × 195 W × 45 cm H) that were adapted for the initial rearing phase and supplied with water from the Radunia River (Kashubian Lake District, northern Poland). During rearing, water temperature (± 0.1°C) and oxygen concentration (± 0.01 mg O_2 l^{-1}) were measured daily at the rearing tank inflows and outflows, while the concentrations of the other water parameters of total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N; ± 0.01 mg TAN l^{-1}), nitrites (± 0.01 mg NO_2-N l^{-1}), and water pH were measured at the rearing tank outflows every seven days. The mean water temperature was 12.0 ± 0.1°C. Oxygen concentration at the tank outflows did not decrease below 7.43 mg O_2 l^{-1} (83.9% saturation). The oxygen level at the tank inflows was maintained within a range of 90–98% saturation. Concentrations of TAN and NO_2-N at the tank outflows did not exceed 0.52 mg TAN l^{-1} or 0.008 mg NO_2-N l^{-1}, respectively. The water pH was within the range of 6.91–7.15.

Experimental and sampling procedures

Before the tanks were stocked and the experiment began, the mean standard length (SL ± 0.1 cm), mean caudal length (CL ± 0.1 cm), and mean body weight (BW ± 0.01 g) were determined for 30 fish sampled randomly. Their mean initial length SL was 17.2 cm and the mean initial weight BW was 74.6 g. Individual fish measurements were taken on days 14 and 28 of rearing (d14 and d28). These data were used to calculate the values of the following indicators: daily growth rate – DGR (g d^{-1}) = (BW_2 – BW_1) × t^{-1}; specific growth rate – SGR (% d^{-1}) = 100 × (ln BW_2 – ln BW_1) × t^{-1}; Fulton's condition factor – F = 100 × BW × SL^{-3}; feed conversion ratio – FCR = TFS × (FB - IB)$^{-1}$, where: BW_1 – initial fish body weight (g); BW_2 – final fish body weight (g); t – rearing period (days); SL – fish standard length (cm); FB – final stock biomass (g); IB – initial stock biomass (g); TFS – total feed supply (g). Fish mortality in the tanks was monitored daily.

On the first day of the experiment and then at 14-day intervals (d0, d14, d28) blood was drawn from seven fish selected randomly from each of the groups with heparinized syringes (Smiths Medical International ASD, Inc., Minnesota, USA). Approximately 1 ml of blood was drawn directly from the caudal vein of each specimen. Prior to the measurements and blood sampling, the fish were anesthetized in an aqueous solution of tricaine methanesulfonate (MS-222) (Sigma-Aldrich Co., Missouri, USA) at a concentration of 100 mg l^{-1}.

The biological materials were used to perform hematological and biochemical tests. The hematological indicators analyzed were: white blood cell count (WBC); red blood cell count (RBC); hemoglobin (HGB); hematocrit (HCT); thrombocytes (PLT). The following red blood cell indicators were also tested: mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC). The remaining blood samples were centrifuged at 4,000 rpm for 3 min (Fresco 17, Thermo Scientific, Waltham, USA). The material obtained was used to determine the following biochemical indicators: cortisol (CORT); glucose (GLU); triglycerides (TG); cholesterol (CHOL); total protein (TP); albumin (ALB); globulins (GLOB); total bilirubin (Bil-T); ammonia (NH_3); C-reactive protein (CRP); alanine aminotransferase (ALT); aspartate aminotransferase (AST); alkaline phosphatase (ALP); lipase (LIP); amylase (AMYL); sodium ions (Na^+); chloride (Cl^-) ions. The hematological analyses were done with a BC-2800 VET semi-automated hematology analyzer (Mindray, Shenzhen, China). The readings from the analyzer were calibrated for several fish species, including sea trout, and were based on the results of tests performed with traditional hematological methods (Dacie and Lewis 2001). Stamar (Dąbrowa Górnicza, Poland) calibrated the analyzer. The biochemical indicators of blood plasma were analyzed on a BS-120 automated biochemistry analyzer (Mindray, Shenzhen, China). Cortisol was determined with an ELISA enzyme-linked immunosorbent assay test (Cayman Chemical Company, Michigan, USA). The hormone was extracted from the plasma with ethyl ether according to the method in Hermelink et al.

(2011). The analyses were conducted in 96-well plates onto which the following were applied: EIA test buffer; cortisol standards; the samples tested (each in two replicates). The optimal dilution of the samples analyzed was determined earlier based on a series of tests. After incubation and rinsing the well, the samples were read at a wavelength of 412 nm. According to manufacturer instructions (Cayman Chemical Company, Michigan, USA), the cortisol levels in given samples were calculated using the standard curve.

Statistical analyses

Statistical analyses were performed with Statistica 12 (StatSoft, Inc., USA). The data were verified for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test). Statistical comparisons of the data were performed with one-way analysis of variance (ANOVA). When statistical significance was determined, further statistical analyses were performed with Tukey's test. Differences were significant at $P \leq 0.05$.

Results

None of the feeds tested affected sea trout growth or condition or the FCR of the feeds ($P > 0.05$; Table 2). Only in the first two weeks of the experiment were single fish deaths noted, and the final survival exceeded 95% (Table 2).

After 14 days of rearing, significantly lower WBC counts were noted in the group of fish fed the Biomar feed without any functional additive (group TB; $P \leq 0.05$; Table 3). For the other hematological parameters (RBC, HGB, and HCT), statistically significant differences were determined in both groups fed feed without functional additives (groups TU and TB; $P \leq 0.05$; Table 3). After 28 days, groups TU and TB both still had decreased RBC and HCT values. Lower values for these parameters were also recorded in the group given the experimental feed with Bioimmuno but only after 28 days of receiving the additive (group TUB; $P \leq 0.05$; Table 3).

No statistically significant differences were noted among the groups with regard to the basic stress indicators of cortisol and glucose ($P > 0.05$; Table 4). As regards the other biochemical parameters, after 14 days of the feeding experiment statistically significant differences were only noted in ALP activity in group TUB ($P \leq 0.05$; Table 3). After 28 days, in the groups fed the two experimental feeds (groups TU and TUB) increased levels of total protein and albumin were noted. Significantly increased albumin levels were also noted in the group fed the Biomar feed with the functional additive (group TBF) ($P \leq 0.05$; Table 3). Elevated values for sodium ions ($P \leq 0.05$; Table 4) were noted after 28 days of the feeding experiment in three groups of fish (TUB, TB, and TBF).

Discussion

The ingredients of commercial functional additives are not revealed by manufacturers, but, Bioimmuno, which was used to supplement the experimental feed, contains 1,3/1,6 β-glucan (Kazuń and Siwicki 2013). This additive has been used successfully with several other fish species (Terech-Majewska 2016), for example, carp (*Cyprinus carpio* L.) and African catfish (Siwicki et al. 2009, Kazuń and Siwicki 2013).

The present study indicated that applying functional and commercial feeds and experimental feeds supplemented with immunomodulatory additives (FOCUS Plus® and Bioimmuno) can affect the values of sea trout hematological indicators. Similar conclusions are reported for other fish species. Nguyen et al. (2016) also reported increased RBC, HGB, and HCT values in juvenile specimens of *Pangasianodon hypophthalmus* (Sauvage) (BW 16.2 ± 0.7 g; fed twice daily at 08:00 and 16:00 to satiation) that were given β-glucan in quantities of 1 g kg^{-1} feed. These authors showed that these effects depended on the dose of β-glucan in the feed since the other doses of this immunostimulant (0.5, 1.5, 2.0, and 2.5 g kg^{-1}) were not shown to effect changes in the hematological indicators tested (Phu et al. 2016). In the current study, quite high doses of Bioimmuno (20 g kg^{-1} feed) were

Table 2
Rearing indicators of sea trout fed feeds without or with functional additives (groups TU and TB and groups TUB and TBF, respectively) on subsequent days of rearing (d0 – initial day of the test, d14, d28, respectively 14 and 28 days of rearing) (mean values ± SD; n = 3). Details in Materials and methods. No statistically significant differences were noted (P > 0.05)

	Fish group			
Parameters	group TU	group TUB	group TB	group TBF
Caudal length (CL; cm)				
0d	18.80 (± 1.01)	18.80 (± 1.01)	18.80 (± 1.01)	18.80 (± 1.01)
28d	19.85 (± 0.32)	20.02 (± 0.14)	19.86 (± 0.44)	19.75 (± 0.15)
Standard length (SL; cm)				
0d	17.20 (± 0.95)	17.20 (± 0.95)	17.20 (± 0.95)	17.20 (± 0.95)
28d	18.33 (± 0.35)	18.43 (± 0.14)	18.29 (± 0.36)	18.22 (± 0.12)
Body weight (BW; g)				
0d	74.60 (± 15.29)	74.60 (± 15.29)	74.60 (± 15.29)	74.60 (± 15.29)
28d	93.70 (± 6.30)	95.46 (± 2.50)	94.35 (± 10.62)	92.27 (± 0.30)
Fulton's condition factor (F)				
0d	1.45 (± 0.13)	1.45 (± 0.13)	1.45 (± 0.13)	1.45 (± 0.13)
28d	1.50 (± 0.02)	1.51 (± 0.02)	1.53 (± 0.09)	1.50 (± 0.03)
Daily growth rate (DGR; g d^{-1})				
0–14d	0.97 (± 0.19)	0.90 (± 0.11)	0.88 (± 0.14)	0.98 (± 0.17)
15–28d	0.95 (± 0.13)	0.95 (± 0.05)	0.88 (± 0.07)	0.93 (± 0.07)
Specific growth rate (SGR; % d^{-1})				
0–14d	0.96 (± 0.19)	0.90 (± 0.10)	0.87 (± 0.13)	0.98 (± 0.17)
15–28d	0.95 (± 0.12)	0.95 (± 0.05)	0.88 (± 0.07)	0.93 (± 0.07)
Feed conversion ratio (FCR)				
0–14d	1.05 (± 0.19)	1.12 (± 0.15)	1.05 (± 0.15)	0.99 (± 0.09)
15–28d	1.29 (± 0.25)	1.30 (± 0.14)	1.28 (± 0.22)	1.20 (± 0.12)
Survival (%)				
0–14d	95.83 (± 3.82)	98.33 (± 1.44)	95.83 (± 3.82)	95.00 (± 4.33)
15–28d	100.00 (± 0.00)	100.00 (± 0.00)	100.00 (± 0.00)	100.00 (± 0.00)

used as are recommended for fish (Kazuń and Siwicki 2013). The reaction of the fish to the functional diet supplemented with immunomodulatory additives illustrates species specificity, and it can also depend on the type of additive used (Ringø et al. 2012, Meena et al. 2013). A study of channel catfish (*Ictalurus punctatus* (Raf.)) that were given feed supplemented with 1,3/1,6β-glucan for four weeks indicated that RBC and HCT values were significantly lowered (Sánchez-Martínez et al. 2017). A similar effect was observed in the present study in the groups of sea trout fed feed with Bioimmuno, while feeding the sea trout the commercial feed with Focus Plus® did not affect the hematological indicators. It should also be noted that in another study on sea trout of an initial BW of 2.3 g no significant effects from the β-glucan additive (1 or 3 g kg^{-1} feed; test period – 6 months) were noted with regard to HCT values (Revina et al. 2019).

The main task for which yeast and yeast-derivatives are used in fish nutrition is to strengthen the fish immune response (Ringø et al. 2012, Meena et al. 2013). However, no increased WBC counts were noted in the peripheral blood of sea trout fed feed with functional additives (groups TUB and TBF). In other fish species a reaction to diets supplemented with β-glucan is increased WBC

Table 3
Effects of feeding sea trout feeds with and without functional additives (groups TU and TB and groups TUB and TBF, respectively) on hematological parameters after 14 and 28 days of feeding with these feeds (mean values (± SD); n = 7). Details in Materials and methods

			Feeding period/groups							
			14 days				28 days			
Parameter	Unit	Initial sample	group TU	group TUB	group TB	group TBF	group TU	group TUB	group TB	group TBF
WBC	10^3 µl^{-1}	270.73 (± 8.38)^b	257.37 (± 9.54)^b	261.35 (± 11.04)^b	254.13 (± 7.21)^a	263.14 (± 10.04)^b	261.57 (± 9.25)^b	255.25 (± 7.84)^b	269.86 (± 7.03)^b	265.01 (± 6.36)^b
RBC	10^6 µl^{-1}	0.77 (± 0.05)^b	0.63 (± 0.05)^a	0.65 (± 0.08)^b	0.61 (± 0.05)^a	0.65 (± 0.05)^b	0.61 (± 0.09)^a	0.57 (± 0.07)^a	0.62 (± 0.09)^a	0.68 (± 0.07)^b
HGB	g l^{-1}	50.02 (± 3.56)^b	44.09 (± 3.41)^a	45.85 (± 3.24)^b	44.18 (± 1.53)^a	45.92 (± 3.30)^b	46.45 (± 4.64)^b	45.34 (± 2.98)^b	49.24 (± 2.77)^b	46.97 (± 2.97)^b
HCT	%	21.52 (± 1.30)^b	17.86 (± 1.66)^a	18.68 (± 2.24)^b	17.55 (± 1.33)^a	18.57 (± 1.62)^b	17.40 (± 2.61)^a	16.18 (± 1.96)^a	17.86 (± 2.66)^a	19.38 (± 2.05)^b
MCV	fl	208.50 (± 2.33)^a	211.63 (± 2.86)^a	214.77 (± 2.09)^b	212.99 (± 3.39)^a	213.34 (± 2.74)^a	213.06 (± 2.73)^a	211.85 (± 3.50)^a	212.84 (± 3.30)^a	210.79 (± 4.14)^a
MCH	pg	107.00 (± 6.65)^a	115.40 (± 5.34)^a	116.88 (± 8.16)^a	118.87 (± 11.94)^a	116.67 (± 8.40)^a	126.49 (± 10.99)^a	132.22 (± 17.02)^b	131.57 (± 19.56)^b	114.14 (± 17.24)^a
MCHC	g l^{-1}	514.29 (± 29.07)^a	546.86 (± 23.22)^a	545.67 (± 34.33)^a	560.29 (± 57.92)^a	548.71 (± 39.32)^a	595.14 (± 48.25)^a	626.33 (± 75.71)^b	620.00 (± 89.63)^b	542.14 (± 78.33)^a
PLT	10^3 µl^{-1}	52.71 (± 16.52)	42.43 (± 4.65)	34.33 (± 2.50)	57.57 (± 55.44)	39.14 (± 5.30)	37.14 (± 29.28)	30.17 (± 10.46)	50.86 (± 38.99)	28.43 (± 6.02)

Explanations: WBC – white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – thrombocytes.
Values in the same row with different letter indexes differ significantly statistically ($P \leq 0.05$).

Table 4

Effects of feeding sea trout feeds with and without functional additives (groups TU and TB and groups TUB and TBF, respectively) on blood plasm biochemical parameters after 14 and 28 days of feeding with these feeds (mean values (± SD); n = 7). Details in Materials and methods

			Feeding period/groups							
			14 days				28 days			
Parameter/ Unit		Initial sample	group TU	group TUB	group TB	group TBF	group TU	group TUB	group TB	group TBF
CORT	ng ml^{-1}	1.08 (± 1.00)	0.22 (± 0.07)	0.37 (± 0.21)	0.21 (± 0.06)	0.62 (± 0.62)	1.33 (± 0.93)	3.97 (± 5.61)	65.70 (± 91.81)	17.89 (± 32.98)
GLU	mg dl^{-1}	87.43 (± 6.90)	80.43 (± 15.51)	86.29 (± 12.68)	98.29 (± 27.80)	92.43 (± 13.34)	88.86 (± 16.12)	91.43 (± 15.16)	102.00 (± 17.53)	90.57 (± 11.59)
TG	mg dl^{-1}	279.29 (± 93.36)	251.00 (± 97.39)	279.57 (± 48.28)	268.29 (± 33.49)	275.71 (± 87.22)	197.86 (± 49.16)	214.14 (± 57.64)	271.86 (± 103.73)	259.71 (± 42.60)
CHOL	mg dl^{-1}	328.71 (± 23.34)	349.71 (± 49.82)	338.14 (± 30.53)	316.86 (± 62.77)	267.57 (± 67.02)	350.86 (± 42.35)	378.86 (± 53.30)	289.29 (± 56.77)	286.14 (± 60.15)
TP	g dl^{-1}	3.40 (± 0.37)^{a}	3.63 (± 0.24)^{a}	3.47 (± 0.27)^{a}	3.37 (± 0.28)^{a}	3.50 (± 0.49)^{a}	3.71 (± 0.20)^{a}	3.96 (± 0.24)^{b}	3.52 (± 0.26)^{a}	3.75 (± 0.32)^{a}
ALB	g dl^{-1}	1.87 (± 0.18)^{a}	2.00 (± 0.10)^{a}	1.99 (± 0.12)^{a}	1.96 (± 0.07)^{a}	1.91 (± 0.09)^{a}	2.17 (± 0.24)^{b}	2.15 (± 0.12)^{b}	2.10 (± 0.15)^{a}	2.22 (± 0.26)^{b}
GLOB	g dl^{-1}	1.54 (± 0.31)	1.64 (± 0.18)	1.48 (± 0.19)	1.41 (± 0.23)	1.60 (± 0.45)	1.54 (± 0.20)	1.80 (± 0.26)	1.42 (± 0.29)	1.53 (± 0.31)
Bil-T	mg dl^{-1}	0.77 (± 0.59)	0.15 (± 0.14)	0.17 (± 0.06)	0.12 (± 0.04)	0.24 (± 0.23)	0.05 (± 0.01)	0.08 (± 0.04)	0.15 (± 0.07)	0.77 (± 1.74)
NH_3	µg dl^{-1}	360.49 (± 178.46)	230.11 (± 70.58)	265.29 (± 62.19)	310.86 (± 135.32)	297.86 (± 83.69)	326.66 (± 93.32)	251.37 (± 78.21)	410.80 (± 138.14)	379.94 (± 150.02)
CRP	g ml^{-1}	0.66 (± 0.37)	0.60 (± 0.38)	0.60 (± 0.44)	0.63 (± 0.47)	0.69 (± 0.27)	0.54 (± 0.47)	0.40 (± 0.23)	0.30 (± 0.16)	0.40 (± 0.40)
ALT	U l^{-1}	3.86 (± 6.09)	1.86 (± 2.04)	1.00 (± 1.15)	0.71 (± 0.76)	0.71 (± 1.25)	7.86 (± 4.45)	6.14 (± 6.23)	9.00 (± 2.77)	3.86 (± 3.76)
AST	U l^{-1}	455.67 (± 305.27)	370.57 (± 67.26)	367.43 (± 85.83)	350.29 (± 74.61)	380.20 (± 157.33)	337.14 (± 64.35)	330.43 (± 33.64)	390.86 (± 50.36)	330.43 (± 108.54)
ALP	U l^{-1}	132.86 (± 14.98)^{a}	203.71 (± 43.40)^{a}	226.71 (± 39.17)^{b}	176.71 (± 39.24)^{a}	154.14 (± 33.00)^{a}	190.14 (± 27.71)^{a}	207.71 (± 56.95)^{a}	151.71 (± 42.91)^{a}	140.00 (± 30.39)^{a}
LIP	U l^{-1}	96.43 (± 57.68)	101.86 (± 62.76)	97.43 (± 36.75)	111.14 (± 15.50)	127.86 (± 16.32)	99.00 (± 39.10)	106.71 (± 45.59)	101.43 (± 51.83)	99.86 (± 43.08)
AMYL	U l^{-1}	423.14 (± 91.63)	559.00 (± 174.00)	581.71 (± 58.81)	534.43 (± 118.61)	512.00 (± 126.71)	525.86 (± 116.75)	739.00 (± 441.51)	674.71 (± 137.11)	540.71 (± 223.03)
Na+	mmol l^{-1}	149.73 (± 2.89)^{a}	152.10 (± 3.91)^{a}	155.36 (± 3.18)^{a}	154.63 (± 4.75)^{a}	157.38 (± 4.04)^{a}	154.95 (± 5.20)^{a}	159.35 (± 3.44)^{b}	160.47 (± 3.55)^{b}	161.19 (± 3.84)^{b}
Cl^-	mmol l^{-1}	296.46 (± 19.06)	293.84 (± 20.75)	286.74 (± 14.19)	275.00 (± 20.71)	270.10 (± 34.47)	280.77 (± 17.66)	300.23 (± 12.34)	270.81 (± 18.63)	277.19 (± 16.52)

Explanations: CORT – cortisol; GLU – glucose; TG – triglycerides; CHOL – cholesterol; TP – total protein; ALB – albumin; GLOB – globulins; Bil-T – total bilirubin; NH_3 – ammonia; CRP – C-reactive protein; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; LIP – lipase; AMYL – amylase; Na+ – sodium ions; Cl^- – chloride ions. Values in the same row with different letter indexes differ significantly statistically ($P \leq 0.05$).

counts. However, the reaction of the fish depends on the dose of the additive and the period for which the fish are fed functional feeds. Phu et al. (2016) tested doses of β-glucan from 0 to 2.5 g kg^{-1} feed in a 30-day test in fry of *P. hypophthalmus* (BW 16.2 ± 0.7 g). Significant increases in WBC counts during their experiment were observed only in the groups fed this additive in doses of 1.0 and 1.5 g kg^{-1}. These changes occurred seven days after the beginning of the test and were maintained until the end of the experiment. Symptomatically, increased WBC counts were not noted in the groups of fish fed the lowest and highest doses of the additive. It cannot be ruled out that the dose of Bioimmuno tested in the current study was inappropriate for sea trout. This dose was, however, verified experimentally and was within the dose range recommended for fish from the families Siluridae, Cyprinidae, and Salmonidae (Terech-Majewska 2016). In other studies, significant increases in WBC counts were observed in juvenile carp (*C. carpio*) (BW 50.0 ± 4.2 g) that were administered β-glucan at a does of 10 g kg^{-1} feed. Higher values of this indicator were observed after 30 days of rearing the fish on functional feeds. It should be noted that in the longer term (after 45 days) these differences were no longer noted (Gopalakannan and Arul 2010).

The diets used in the current feed experiment, both with and without functional additives, were not found to affect the stress markers of cortisol or glucose concentrations in the blood plasma. Observations of *P. hypophthalmus* differed; juvenile specimens of this species were fed feed supplemented with β-glucan at a dose of 1 g kg^{-1} feed, and after just seven days of the test lowered levels of blood plasma cortisol were noted and maintained until the end of the experiment (30 days). It must be emphasized that the other β-glucan concentrations tested, which ranged from 0 to 2.5 g kg^{-1} feed, did not effect changes in cortisol levels. In the same study, identical observations were made for glucose, the second parameter that provides information about body stress reactions. The blood plasma concentration of this simple sugar was also the lowest in the group fed feed supplemented with β-glucan at 1 g kg^{-1} feed. This was noted at both seven and 14 days of the experiment (Phu et al. 2016).

Total protein level, mainly of albumin and globulin, is one of the most important indicators of nutritional status in fish (Folmar 1993). In none of the groups fed feed with functional additives (TUB and TBF) were negative effects noted in the nutritional status of the fish. After four weeks of the feeding, increased levels of TP and ALB (group TUB) or only ALB (group TBF) were recorded. Increased concentrations of these parameters could most likely have resulted from increased immunoglobulin concentrations in the blood plasma. Other authors report that including β-glucan in fish diets led to a significant increase in this type of protein in the blood plasma (e.g., Kazuń and Siwicki 2013, Phu et al. 2016). Nevertheless, in another study on juvenile pikeperch (*S. lucioperca*) (BW 10.18 ± 0.25 g) that were fed feed with an extract of the yeast *Saccharomyces cerevisiae* for 60 days, no significant changes in the concentrations of TP, ALB, or GLOB were noted by the end of the experiment (Jarmołowicz et al. 2018). Increased ALP activity after 14 days of feeding (group TUB) could also indicate that the sea trout immune system was mobilized after administering β-glucan in the feed. One characteristic property of this enzyme is its support, regulation, and acceleration of phagocytosis (Chen et al. 2007).

Including the additives Bioimmuno and FOCUS Plus® in the sea trout diet was safe and did not induce stress or have negative effects on the nutritional status, metabolism, or health of juvenile specimens of this species. Although the recommended doses of the immunostimulatory additives were administered in the feed, they were not observed to affect WBC counts, which, inter alia, correspond to the body's immune response. However, the increased levels of TP and ALB in the blood plasma and increased ALP activity could indicate that the immune system was stimulated. Given the reaction of other fish species to these types of immunostimulatory additives, we concluded that modifying the recommended dose for sea trout and/or the period of time for which additives are administered should be considered, which

undoubtedly requires conducting further studies on this topic.

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