Effect of inclusion of fish protein hydrolysate in diet for european whitefish (*coregonus lavaretus linnaeus*, 1758) juveniles on their hematological parameters

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Abstract. Prior studies have measured the effects on the Growth and Survival of supplementing fish diets with fish protein hydrolysate (FPH) as an alternative protein source. Initial results have suggested a positive impact on fish health and this study aimed to investigate specifically the impact of a 5% substitution of FPH on European Whitefish juveniles hematological parameters. A 56-day manual feeding trial was conducted on two triplicate groups of 500 fish, consulting of a control diet group and 5% FPH diet group. A feeding rate of 3% of total biomass was applied with feeding being carried out three times a day. The higher concentration of hemoglobin (60.73±3.55 g/L vs. 51.47±2.52 g/L), color index (1.62±0.15 vs. 1.23±0,07) and mean corpuscular hemoglobin (56.64±3.49 g/L vs. 45.75±2.11 g/L), as well as the lower level of oxyphilic normoblasts (1.33±0.26 % vs. 2.08±0.32 %) in the blood of fish in the experimental group, indicate active metabolic processes occurring in their bodies and better oxygen supply to their tissues. The significantly higher concentration of total protein in the blood serum (48.29±1.48 g/L vs. 35.35±1.43 g/L) of this group of fish suggests better food assimilation in their organism. The hematological data obtained during the study indicate the positive influence of the fish protein hydrolysate on the fish organism.

1 Introduction

Aquaculture production, which is the fastest growing industry in animal farming worldwide, still depends on high-quality fish feeds containing fishmeal, a crucial protein source for the growth of many farmed aquatic species [1]. However, the availability of fishmeal is limited because it is mainly derived from marine pelagic fish. The excessive use of fishmeal not only has potential negative environmental impacts but also poses economic challenges due to its unstable supply and increasing prices over the past decade [2]. Therefore, it is important to investigate alternative protein sources and explore substitutes for fishmeal to ensure the sustainability of aquaculture [2].

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One potential alternative to fish meal are protein hydrolysates, which can be included into aquaculture diets [3]. The inclusion of aquatic protein hydrolysates in feed has been shown to enhance growth performance what indicates the feasibility and positive effects of incorporating these hydrolysates into aquaculture feeding practices [4, 5, 6]. However, only limited research has been conducted on the impact of incorporating fish protein hydrolysate into diets on hematological parameters of fish [7, 8, 9].

The European whitefish *Coregonus lavaretus* (Linnaeus, 1758), is a highly sought-after species within the Coregonus genus and is extensively utilized in commercial aquaculture and stock enhancement programs [6, 10]. Whitefish cultivation is prevalent in Russia, Northern Europe and the Baltic Sea region [11–13]. However, there is a significant research gap regarding the impact of protein hydrolysates in the diets of whitefish species.

This study aims to assess the influence of fish protein hydrolysate derived from smoked sprat heads on the hematological parameters (hemoglobin values, red blood cells, white blood cells, platelets, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, and color index) of European whitefish juveniles.

2 Material and methods

2.1 Experimental diets

Two experimental diets, formulated to contain 40% crude protein and 22% crude lipid, were manufactured at the Center for Advanced Technologies of Protein Use, Kaliningrad State Technical University, Russia. The diet protein lipid contents were made to be similar to commercial diets used for whitefish and rainbow trout. Of these, the control diet (CD) contained fish meal (61.7% crude protein; 12.7% crude lipid) as the main protein source, whereas 5% of the fish meal was substituted with the fish protein hydrolysate (82.7% crude protein; 2.0% crude lipid) in the experimental diet (FPHD). The formulation and nutritional composition of the experimental diets are shown in Table 1.

Table 1. The formulation and nutritional composition of the experimental diets.

Ingredient, %	**CD**	**FPHD**
Fish meal	66.5	63.0
Wheat meal	20.0	19.0
Fish protein hydrolysate	0.0	5.0
Fish oil	9.5	9.0
Carboxymethyl cellulose (CMC)	2.0	2.0
Vitamin and mineral premix	1.0	1.0
Gelatine	1.0	1.0
Feed composition, %		
Crude protein	39.4	40.8
Crude lipid	22.7	21.7
Crude fiber	5.0	5.0
Ash	12.9	12.5

The main protein source in the diets was a commercial fish meal and the fish protein hydrolysate was obtained by hydrothermal treatment hydrolysis. The process involved mixing homogenized smoked sprat heads with hot water prior to treatment in an autoclave at 130-160 °C for 60 minutes at a pressure of 0.15-0.20 Mpa.

All dry ingredients were mixed with fish oil using a drilling machine and a food mixer to guarantee a uniform mixture. A hand operated meat grinder with a 1.5-mm die was then used to repeatedly cold-pellet the mash.

2.2 Fish and feeding

An experimental recirculating aquaculture system (RAS) consisting of six rectangular fiberglass tanks of 500 L capacity was used to carry out the 8-week feeding trial. Prior to the experiment, the juvenile whitefish were fed commercial diets (Aller Aqua A/S, Christiansfeld, Denmark). In total 3000 juvenile whitefish, with initial mean weight of 1.03±0.38 g were randomly distributed in triplicate groups. The fish were kept under a 24 hour light regime over the course of the experiment and fed manually three times per day with a feeding rate of 3% of the total biomass. Daily measurements of the water parameters in the system were taken.

The water parameters in the system were measured daily and were as follows: average water temperature of 21.9±1.4 °C, dissolved oxygen level of 7.5±1.0 mg/L, nitrite (as NO^{2-}) of 0.08±0.03 mg/L and nitrate (as NO^{3-}) of 6.6±1.8 mg/L.

2.3 Hematological analyses

Five fish, clinically healthy individuals without visible damage, from each tank were randomly selected for blood sampling. The blood was drawn from the tail vein by syringes, which contain heparin used to separate plasma samples.

Values of hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets (PLT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and color index (CI) were determined. The differential WBCs: neutrophils, lymphocytes, eosinophils, pseudo-eosinophils, pseudobasophils, and monocytes were identified and counted in a blood smear, stained according to Pappenheim's panchromatic method. The nuclear shift index of neutrophils and leukocytes as well as the percentage ratio of immature and pathological forms of RBCs were determined and counted with a microscope.

The concentration of Hb was determined by the hemiglobincyanide method, the RBCs were counted in a hemocytometer, and MCHC was measured using a refractometer by a refractive index of blood serum. MCH and CI were evaluated according to the formulas (1) and (2) respectively:

$$MCH\ [pg] = \frac{Hb\ [g/L]}{RBC\ count\ [10^{12}/L]} \quad (1)$$

$$CI = \frac{Hb\ [g/L] \times 3}{RBC\ count\ [10^{12}/L]} \times 10^2 \quad (2)$$

WBCs, PLTs, polychromatophilic and oxyphilic normoblasts and pathological forms of RBCs (erythrocyte hemolysis) were counted out of 500 RBCs in a blood smear and expressed as a percentage of the RBCs.

2.4 Statistical analysis

The means of the groups were compared by the Student's t test using Microsoft Excel. The differences were considered significant when the value of P<0.05.

3 Results and discussion

Hematological parameters of juvenile whitefish after a 56 day feeding trial are presented in Table 2, where [1, 2, 3] – differences between the two groups are significant at P < 0.05; 0.01 and 0.001 respectively.

The statistical differences of the values of RBC count, WBC count and PLTs were not significant between treatments. However, the fish fed the FPHD had significantly higher Hb levels (P<0.05), MCHC (P<0.001), MCH content (P<0.01) and CI (P<0.01) than fish in the CD group. Additionally, statistically significant differences were determined in metamyelocytes (P<0.01), pseudo-eosinophils (P<0.01) and oxyphilic normoblasts (P<0.05).

Generally, the Hb concentration in the blood of the examined whitefish juveniles was lower lower compared to that of whitefish species in natural waters, where it typically ranges from 80 to 130 g/L [14; 15]. The lower concentrations observed in this study can be attributed to the young age of the fish, which is consistent with values reported for whitefish reared under artificial conditions in RAS [16]. Conversely, the higher Hb value in the FPHD (60.73 g/L) compared to the CD (51.47 g/L) suggests that the inclusion of hydrolysate in the diet resulted in improved oxygen transport throughout the bodies during the period of active growth of juveniles. A study by Javaherdoust [17] showed that juvenile rainbow trout fed a diet containing 2% fish viscera protein hydrolysate exhibited increased Hb levels. Similarly, Buddhi E. Gunathilaka et al. [18] reported significant improvement in Hb levels of red seabream when fish meal was supplemented with 5% shrimp hydrolysate. The presence of specific bioactive peptides resulting from the hydrolysis processes is likely responsible for promoting the enhanced Hb levels, which may explain the observed effects [19]. However, no significant effects were observed in other species.

The decrease in the levels of MCH and CI values observed in the blood of the CD group is likely linked to an intensification of erythropoiesis in the bodies of these fish, as indicated by the higher percentage of immature RBCs. Notably, significant differences were found among immature erythrocytes, particularly in the percentage of oxyphilic normoblasts. The number of these cells was significantly higher in whitefish juveniles from the CD group compared to the FPHD group, which can be attributed to the increased oxygen demand during the digestion of artificial feed. Furthermore, in both groups, the level of oxyphilic normoblasts exceeded 0.5%, indicating the overall high level of metabolic processes occurring in the bodies of these fish.

The concentration of WBC and PLT in the blood of the studied groups of whitefish juveniles was at a low level, which is suggestive that the immune system had low activity.

The MCHC was significantly higher in the FPHD group than in juveniles from the CD group. The MCHC levels in fish are influenced by nutrition and food availability. The obtained data suggest improved food assimilation in the experimental group, as higher feeding intensity correlates with higher MCHC levels. Moreover, MCHC is a crucial biochemical indicator that reflects the functional state of the fish organism.

In the peripheral blood of the studied groups, nine forms of white blood cells (WBCs) were found. Among the neutrophils, immature forms (myelocytes and metamyelocytes) predominated in the CD group, while mature forms (band neutrophils and segmented neutrophils) prevailed in the FPHD group. Since the main function of neutrophils is to protect the body from infections and toxic effects, the increased production of neutrophils indicates a response of the hematopoietic organs to the occurring changes in the body of the CD group of fish. This is further supported by significant differences observed in the WBCs of the studied groups, specifically in the percentage of neutrophilic metamyelocytes. The percentage of these cell forms was significantly higher (6.40%) in the blood of whitefish from the CD group compared to the FPHD group (4.03%).

Table 2. Hematological parameters of juvenile whitefish.

Items	**CD**		**FPHD**	
	M ±m	**SD**	**M ±m**	**SD**
Complete blood count				
RBC Red blood cells, 10^{12}/L	1.09±0.05	0.17	1.13±0.03	0.11
WBC White blood cells, 10^{9}/L	9.67±2.00	7.49	8.83±1.38	5.16
PLT Platelets, 109/L	23.00±5.66	21.18	18.50±2.28	8.55
Hb Hemoglobin, g/L	51.47±2.52 [1]	9.42	60.73±3.55 [1]	13.30
Total protein serum, g/L	35.35±1.43 [3]	3.77	48.29±1.48 [3]	4.61
MCH Mean corpuscular hemoglobin, pg	45.75±2.11 [2]	7.91	56.64±3.49 [2]	13.04
CI Color index	1.23±0,07 [2]	0.24	1.62±0.15 [2]	0.55
White blood cell parameters, %				
Neutrophils:	16.03±2.58	9.67	13.13±1.62	6.07
Myelocytes	2.13±0.58	2.18	1.87±0.44	1.64
Metamyelocytes	6.40±0.90 [2]	3.38	4.03±0.59 [2]	2.20
Band neutrophils	4.57±0.85	3.19	4.57±0.67	2.49
Segmented neutrophils	2.93±0.58	2.19	2.67±0.37	1.38
Lymphocytes:	80.30±3.31	12.40	83.13±1.92	7.18
Large lymphocytes	1.37±0.27	1.03	1.27±0,41	1.52
Small lymphocytes	78.93±3.35	12.53	81.87±2,07	7.74
Pseudo-eosinophils	0.43±0.14 [2]	0.53	1.23±0.29 [2]	1.08
Pseudobasophils	3.03±0.82	3.07	2.27±0.33	1.25
Monocytes	0.20±0.11	0.41	0.23±0.10	0.37
Neutrophil shift index	6.56±1.37	5.14	6.07±2.34	8.75
Leukocyte shift index	0.23±0.05	0.20	0.18±0.03	0.10
Red blood cell development, %				
Polychromatophilic normoblasts	0.43±0.11	0.41	0.37±0.08	0.29
Oxyphilic normoblasts	2.08±0.32 [1]	1.21	1.33±0.26 [1]	0.97
Pathology of red blood cells, %				
Erythrocyte hemolysis	1.77±0.45	1.70	1.33±0.44	1.65

The total number of neutrophils in the blood of whitefish from the FPHD group fell within the range typical for healthy fish (3-15%) [16] and amounted to 13.13%. In the CD group, this parameter slightly exceeded the upper value of the normal range, reaching 16.03%.

Significant differences also extended to the percentage of pseudo-eosinophils. In the FPHD group, the values showed an increase of 1.23%, while in both groups, the overall percentage remained low and fell within the lower range observed in healthy fish (0.5-5%) [16].

4 Conclusion

The hematological data obtained during the study indicate the positive influence of the fish protein hydrolysate on the fish organism. The higher concentration of hemoglobin, color index, and mean corpuscular hemoglobin, as well as the lower level of oxyphilic normoblasts in the blood of fish in the FPHD group, indicate active metabolic processes occurring in their bodies and better oxygen supply to their tissues. The significantly higher concentration of total protein in the blood serum of this group of fish suggests better food assimilation in their organism. All the studied blood parameters of the fish in the FPHD group were within the range of values typical for healthy fish, in contrast to the CD group.

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