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INFLUENCE OF FEEDING TYPE ON GROWTH AND BLOOD PARAMETERS OF BLACK BARBUS, *Puntius nigrofasciatus*

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Abstract

A twenty-five weeks study of Black Barbus, P. nigrofasciatus fry was conducted to determine the effectiveness of various combinations of feed and its effect on growth, survival, and physiological and hematological parameters composition of fish blood. To achieve this goal, Black Barbus, P. nigrofasciatus fry were kept in three identical 120-liter aquariums, and fed different diets. The first diet included industrial feed in the form of flakes. The second diet included live microorganisms and crustaceans. The third diet was a 1:1 combination of industrial feed and live organisms. The results of the study showed a higher growth rate in Black Barbus, P. nigrofasciatus, which consumed combined feed, than peers consumed dry feed by 0.60 cm or 10.53% (p<0.05) and then peers consumed live feed by 0.20 cm or 3.28% (p<0.05). The content of hemoglobin in the blood was the same in fish of three diets. The level of erythrocytes was higher by 0.12 million×μl-1 in the blood of fry, whose diet was combined.

Key words: *aquaculture, feed, fry, hemoglobin, hydro-chemical parameters.*

INTRODUCTION

One of the important factors of effective fisheries, which combines high productivity with economically justified costs and high nutritional value of fish products is scientifically sound feeding of fish (Cottrell, 2022; James, 2013; Verma & Satyanarayan, 2016). Intensification fish farming involves the use of balanced and cost-effective feeds for feeding all age groups of fish. The main task in commodity fish farming is to ensure maximum growth of fish products in the shortest possible time. The solution of this problem is based on meeting the nutritional needs of fish (Brune et al., 2003; Kong et al., 2009). Scientifically based use of vitamins, minerals and enzymes in combination with other biologically active substances can significantly increase the efficiency of fish feeding by increasing the availability and digestibility of feed nutrients (Lall & Kaushik, 2021). The latest achievements in the field of biological sciences, combined with the growing capabilities of modern technology in the near future will contribute to the improvement of fish farming technologies, in which fish feeding will maintain a leading position (Pradeepkiran, 2019). With feed, the body of fish receives a variety of energy-rich substances, which are further broken down into simpler substances. The energy released in this case ensures the flow of various physiological processes. In addition, substances entering the body are used to repair worn out and build new cells and tissues, for the formation of hormones and enzymes (Bogdan, 2005).

It is important to choose the right quality feed with a huge variety of nutrients and biologically active substances, which at least slightly correspond to the natural diet of fish (Hamre et al., 2013; Prabhu et al., 2019). Mastering the principles of rational use of feed and modern methods of feeding fish opens up the possibility of significantly reducing the cost of feed per unit of fish production. This is not only an economically positive result, but also a circumstance that has a certain environmental significance, which logically follows from energy conservation, improving the environmental situation by significantly reducing the pressure on the environment (Velasco-Santamaría & Corredor-Santamaría, 2011). The process of growing high-quality standard fish stocking material, especially using the technologies of using fry, involves the use of live feed or starter feed mixtures enriched with biologically active substances (Krepych et al., 2021). Improperly selected feed can lead to various diseases and death of fish. For the normal functioning of fish, their feed must contain a complex of nutrients in certain quantities and ratios that will create its balance of feed. The need of fish in certain feed ingredients is not constant and depends on the age, size and sexual maturity of fish (Virtanen et al., 2008; Zhao et al., 2015), season, body weight, fatness and the quality of others environmental factors (Aubin et al., 2019; Tselu & Klei, 2017). Lack of nutrients (proteins, fats, carbohydrates, minerals and vitamins) that come with feed, leads to pathology in fish. Therefore, if possible, it is necessary to avoid the use of feed that is unbalanced in terms of basic nutrients (Vilain et al., 2016; Wang et al., 2016). In addition to the imbalance of feed, we should not forget about such a criterion as poor-quality feed and feed with violations of storage conditions of its ingredients. This is especially true of feed or its components that contain large amounts of fat, as this leads to its oxidation with the formation of highly toxic free fatty acids and peroxides. The use of such feeds can lead to the development of pathological processes and the death of fish (Mwihia et al., 2018; Oliveira & Vasconcelos, 2020; Pietsch, 2020).

The study of the influence of feed type on the growth and survival of fish in the laboratory allows the use of Black Barbus, *Puntius nigrofasciatus* (Günther, 1868), which is found both in natural waters of different regions and is widely used in aquaristics. Its homeland is the island of Sri Lanka. It lives in slow-flowing rivers and stagnant ponds with dense vegetation. The natural population of it is small, which was the reason for listing in the Red Book, but the fish is easily breeds in captivity (Carosi et al., 2017).

Mortality of Black Barbus, *P. nigrofasciatus* (Günther, 1868) during fattening and intensive growth under artificial conditions is associated with inappropriate feed (Rutaisire et al., 2015). Studies of the effect of feed on blood characteristics in Black Barbus *P. nigrofasciatus* have not been previously published. However, the results of the experiment (Prusińska et al., 2020) were found, which indicated that the content of neurophiles in the blood of Barbel Larvae *Barbus barbus* probably depended on feeding.

The behavior of Black Barbus, *P. nigrofasciatus* (Günther, 1868) during fattening, which will allow optimal survival and growth in artificial growing conditions, needs a clear understanding. It is necessary to study the intensity of growth and survival of fry in unnatural growing conditions. Barbus is usually limited to the river environment at the beginning of life, but adult fish are capable of high mobility and adapted to survive in both river and lake environments (Ondhoro et al., 2016**)**. From this it is possible to make assumptions about the possibility of applying different survival strategies taking into account the impact of feed on different age groups. Barbus is known to be an omnivorous fish species (Aruho et al., 2018; Corbet, 1961**)**, so its breeding requires knowledge of its diet and growth in a limited environment.

The study of the problem of efficient fish feeding is becoming more relevant than before with the growing feed demand for protein of fish origin and the high need to restore natural resources of fish farming. Studying the impact of different feeds on the growth of Black Barbus, *P. nigrofasciatus* (Günther, 1868) in aquarium conditions can transfer the experience gained to the process of breeding other related fish species in terms of their fish farming in natural and artificial reservoirs. The results will also be useful in the field of aquaristics, which is gaining popularity again and needs constant study to improve the conditions of fish, taking into account their physiological needs, adequate provision and humane treatment.

The aim of our study was to investigate the effect of different types of feeding on the growth of fish and their physiological using Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry.

MATERIALS AND METHODS

This study was conducted for 25 weeks in 2022. The scientific experiment was staged at the Bila Tserkva National Agrarian University, Kyiv region, Ukraine (49.7631997,30.0764052).

Three 120-liter aquariums were used to determine the dynamics of Black Barbus, *P. nigrofasciatus* (Günther, 1868) growth. Ninety 0.7 cm Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry were used for the experiment. Thirty fries were placed in each aquarium. Later they continued to be used as growing aquariums. The planting density of fry was based on the prospect of their growth. During the study, the hydrochemical conditions of water for the maintenance and cultivation of young Black Barbus, *P. nigrofasciatus* (Günther, 1868) were maintained at a constant level. Illumination in aquariums was in the range of 4-6watts per one liter of water. Duration of lighting was ten hours per day. For the reliability of the research, the parameters and conditions in the 3 aquariums were similar. Water for filling aquariums was used from the city watercourse, which had previously been defended and aerated for 4 days. Water samples for analysis were taken every day. The value of water index (pH), concentrations of biological elements (ammonium, nitrate and nitrate nitrogen), temporal hardness of water and the presence of dissolved oxygen were determined in the studied water.

Based on the purpose of the work, different types of feeding were used in each aquarium, namely (Table 1): the first aquarium included dry feed industrial production of the company Tetra, Sweden; the second aquarium included live Nauplii Cyclops, *Cyclops strenuus strenuous* (Fischer, 1851), live Artemis, *Artemia salina* (Linnaeus, 1758), live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833), live Tubeworm, *Tubifex tubifex* (Müller, 1774), live Moth, *Chironomidae* (Newman, 1834), live Koretra, *Chaoborus* (Lichtenstein, 1800); the third aquarium included a combined type of feeding. Fish kept in the first aquarium were taken in group I, those kept in the second and third aquariums were classified according to II and III groups. Fish in the experiment received feed in quantities not exceeding 2.0% of the total weight of all fish in each aquarium twice a day in the morning and in the evening for 1 aquarium: 1-5 weeks – 21.42-61.2 g; 6-10 weeks – 61.2-91.8 g; 11-15 weeks – 91.8-134.6 g, 16-20 weeks – 134.6-162.18 g, 21-25 weeks – 162.18-174.42 g. Every seventh day of the week the fish did not receive feed at all.

The presented structure of the diet allows us to identify that in the first aquarium for feeding Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry dry feed was used as a substitute, in the second was used live feed and in the third was used a combined type of feed (industrial feed and live feed).

Table 1. The use of feed depending on the fish age

Weeks	**Type of feed**		
	Group I	**Group II**	**Group III**
1-5	Dried Cyclops Nauplii, *Cyclops strenuus strenuus* (Fischer, 1851) – 35% Dried Artemia, *Artemia salina* (Linnaeus, 1758) – 35% Dried Rotifer, *Brachionus urceolaris* (Müller, 1773) – 30%	Live Nauplii Cyclops, *Cyclops strenuus strenuous* (Fischer, 1851) – 35% Live Artemis, *Artemia salina* (Linnaeus, 1758) – 35% Live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833) – 30%	Dried Cyclops Nauplii, *Cyclops strenuus strenuous* (Fischer, 1851) – 25% Dried Artemia, *Artemia salina* (Linnaeus, 1758) – 25% Live Ciliate Shoe, *Paramecium caudatum* (Ehrenberg, 1833) – 50%
6-10	Dried Daphnia, *Daphnia magna* (Straus, 1820) – 50% 'Tetra Mikro Min' feed – 50%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 35% Live Moth, *Chironomidae* (Newman, 1834) – 35% Live Koretra, *Chaoborus* (Lichtenstein, 1800) – 30%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 25% Live Moth, *Chironomidae* (Newman, 1834) – 25% 'Tetra Mikro Min' feed – 50%
11-25	'Tetra Min' feed – 100%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 35% Live Moth, *Chironomidae* (Newman, 1834) – 35% Live Koretra, *Chaoborus* (Lichtenstein, 1800) – 30%	Live Tubeworm, *Tubifex tubifex* (Müller, 1774) – 25% Live Moth, *Chironomidae* (Newman, 1834) – 25% 'Tetra Min' feed – 50%

The biochemical composition and energy value of the fish diet for each individual period of retention was reflected in terms of its multicomponent composition (Table 2).

Table 2. Biochemical composition and energy value of Black Barbus, *P. nigrofasciatus* (Günther, 1868) diet

Indicator	1-5 weeks	6-10 weeks	11-25 weeks
Group I (dry feed)			
Moisture content, %	9.00	9.00	8.00
Protein content, %	56.73	48.85	46.00
Fat content, %	13.67	10.20	12.20
Ash content, %	15.07	11.35	3.20
Nitrogen-free extractives content, %	14.53	29.60	42.00
Energy value of dry matter, $kJ \times g^{-1}$	20.48	39.82	44.95
Group II (live feed)			
Moisture content, %	87.47	34.07	62.43
Protein content, %	57.70	51.30	49.70
Fat content, %	20.73	8.40	6.20
Ash content, %	12.37	6.30	6.80
Nitrogen-free extractives content, %	9.20	34.00	37.30
Energy value of dry matter, $kJ \times g^{-1}$	23.54	36.30	39.45
Group III (combined feed)			
Moisture content, %	87.47	34.04	33.40
Protein content, %	57.70	51.30	51.30
Fat content, %	20.73	8.40	8.07
Ash content, %	12.37	6.30	6.30
Nitrogen-free extractives content, %	9.20	34.00	34.33
Energy value of dry matter, $kJ \times g^{-1}$	45.19	40.83	40.66

Oxygen, temperature and hydro-chemical regimes were systematically monitored during the experimental works in the aquariums with the help of Tetra reagents. The water temperature during the study was kept within 24-25°C, which was provided by automatic heating. Water temperatures were within optimal limits for the growth and assimilation of Black Barbus, *P. nigrofasciatus* (Günther, 1868) feed. A pH meter PH-037 (0.00 to 14.00 pH, ±0,1 pH) (Kelilong Electron, China) and an oximeter AKT EZODO 7031 (0.0. ~ 20.00 $mg \times l^{-1}$ (ppm), 0.00 ~ 200.00%, ±0.20 $mg \times l^{-1}$ (ppm), ±2.00%) (EZODO, Taiwan) were used as control devices. The body length of fry at different ages was measured with a measuring tape (measuring range 0-100 mm, accuracy ±0.5) every 5 weeks. Body length was the length of the fish measured from the tip of the snout to the tip of the longest lobe of the caudal fin. This was a rectilinear measure that does not measure along the curve of the body. The rate of maturation was observed visually by behavior, changes in mating attire in males and physiological features in females were noted, and the influence of feed type on hematological parameters of Black Barbus, *P. nigrofasciatus* (Günther, 1868) was assessed. Every fish was weighed individually on electronic scales Radwag PS 200/2000.R1, (Axis, Poland) (measuring range 0.02-600.00 g, accuracy ±0.01). When weighing the fish, narcotization or anesthesia of the fry was not used. Water hardness was measured using Hach Method 8123 – Digital Titration using EDTA. Total ammonia nitrogen was measured using Hach Method 8038 – Nessler. Nitrite nitrogen was measured using Hach Method 8507 – Diazotization. Nitrate nitrogen was measured using Hach Method 8171 – Cadmium Reduction. Total nitrogen was measured using Hach Methods 10071, 10072 – Persulfate Digestion Method.

Fish feeding was stopped 24 hours before blood sampling. Anesthesia of the fish was done using tricaine methanesulfonate (MS-222; 0.3 $g \times l^{-1}$) at the time before blood sampling. Blood samples were collected from the tail vein using sterile heparinized capillary tubes. The samples were then transferred to microtubes (Ningbo Siny Medical Technology Co., Ltd 0.6 ml Shanghai, China) containing ethylenediaminetetraacetic acid (EDTA, 1.26 mg $(0.6\ ml)^{-1}$) for anticoagulation. The content of hemoglobin in the blood of Black Barbus, *P. nigrofasciatus* (Günther, 1868) was determined by hemoglobin-cyanide method. The principle of the method was that hemoglobin in interaction with iron-blue potassium is oxidized to methemoglobin, which forms with acetone cyanhydrin colored hemoglobin cyanide, the intensity of which is proportional to the hemoglobin content. Cyanmethemoglobin absorption was measured at 540 nm in a photoelectric calorimeter KFK-2 (Spectral range of the photocolorimeter from 315.00 to 980.00 nm, ±0.30 of the main error) (ZOMZ, Russia) against a standard solution (Bhaskaram et al., 2003). The number of erythrocytes in the blood of frys was counted in Goryaev's cell.

Data on the length of fry growth, hemoglobin content and erythrocyte content in the blood were presented as averages. In our experiment, a p-value of 5% is considered statistically significant. Significance of differences in the results of the length, weight and content of hemoglobin and erythrocytes in the blood was assessed using Kruskal-Wallis test. All statistical analyzes were processed using the standard package of statistical programs 'Microsoft Excel 2010'.

RESULTS AND DISCUSSIONS

The chemical values obtained in this study are presented in Table 1. According to the results of the study, the content of ammonium nitrogen did not exceed the normative limits and ranged from 0.03 to 0.64 mg×l^{-1} in the first aquarium, 0.03 to 0.55 mg×l^{-1} in the second aquarium and 0.03 to 0.43 mg×l^{-1} in the third aquarium (Table 3).

Table 3. Hydro-chemical parameters of water in aquariums for keeping Black Barbus, *P. nigrofasciatus*

Group	NH_4^+ (mg×l^{-1})	pH	Hardness temporary (dH)	O_2 (mg×l^{-1})	NO_2 (mg×l^{-1})	NO_3 (mg×l^{-1})
I (dry feed)	0.03-0.64	6.5-8.3	5-18	7.6-6.4	0.01-0.07	0.1-0.3
II (live feed)	0.03-0.55	6.5-8.0	5-14	7.6-8.0	0.01-0.06	0.1-0.2
III (combined feed)	0.03-0.43	6.5-7.4	5-10	7.6-8.9	0.01-0.06	0.1-0.2

The oxygen concentration did not fall below the normative values and averaged 6.4-8.9mg×l^{-1}, which was ensured by continuous aeration of water in aquariums. The temporal hardness ranged from (dH) 5-18. The hydrogen index was optimal for biochemical processes and retention of young Black Barbus, *P. nigrofasciatus* (Günther, 1868) (6.5-8.3). The water was not contaminated with nitrites, which were present in small concentrations. The content of nitrites in water did not exceed the normative values and ranged from 0.01 to 0.07 mg×l^{-1} (up to 0.10 mg×l^{-1}). Accordingly, the content of nitrates in water ranged from 0.1 to 0.3 mg×l^{-1}, which does not exceed the normative values (up to 2.0 mg×l^{-1}).

According to the determination of hydro-chemical parameters, in three aquariums where Barbus were kept, the condition of this species of fish was satisfactory, which indicates their individual and species adaptation to a given range of hydro-chemical parameters.

According to the research, it was found that the growth rates of fish for different types of feeding in each experimental aquarium using different types of feeding depending on the age of the fish differed from each other. During the study, Barbus fry were fed small portions and care was taken to ensure that uneaten feed did not accumulate in the aquarium.

Depending on the type of diet, we observed a tendency of different growth intensity of Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry (Figure 1).

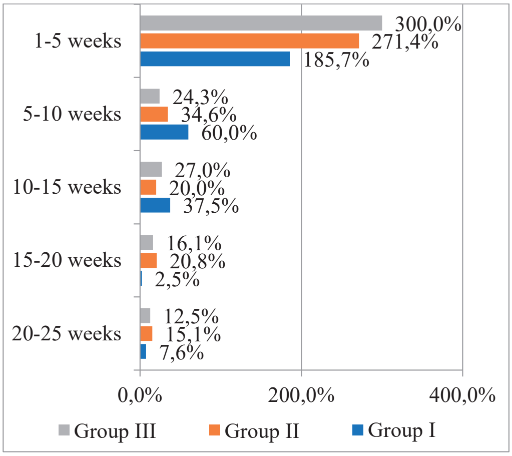

Figure1. The growth rate of the body length (cm) of Black Barbus, *Puntius nigrofasciatus* (Günther, 1868) fry every five weeks, %

Thus, Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry grew the fastest from 1 to 5 weeks. Moreover, the most intensive growth was in fish that ate compound feed during this period. The slowest growth was from 20 to 25 weeks in all fish. Black Barbus, *P. nigrofasciatus* (Günther, 1868), which consumed dry feed, grew more intensively from 5 to 15 weeks. The fry, which consumed live feed, showed a higher growth rate from 15 to 25 week.

Analysis of fish growth dynamics showed that Barbus consuming live feed and compound feed had a higher growth rate than their counterparts consuming dry feed after the first 5 weeks by 0.60 cm or 30.00% (p<0.05), and 0.80 cm or 40.00% (p<0.05) (Table 4).

Table 4. The growth rate of the body length of the Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry depending on the type of feeding, n = 90

Age (weeks)	Body length (cm)		
	Group I (dry feed)	**Group II (live feed)**	**Group III (combined feed)**
1	0.7±0.01[a]	0.7±0.02[a]	0.7±0.01[a]
5	2.0±0.04[a]	2.6±0.01[b]	2.8±0.02[b]
10	3.2±0.01[a]	3.5±0.05[b]	3.7±0,06[b]
15	4.4±0.09[a]	4.2±0.01[a]	4.7±0.04[b]
20	5.3±0.07[a]	5.3±0.03[a]	5,6±0,09[b]
25	5.7±0.11 [a]	6.1±0.10[b]	6.3±0.02[b]

The difference between the averages shown with different letters in the same row is significant (p<0.05).

The predominance of fish of the experimental groups over the fish of the control in terms of body length at 10 weeks of retention was slightly less and amounted to 0.30 cm or 9.38% (p<0.05) in II group and 0.05 cm 15.63% (p<0.05) in III group. At week 15, there was no significant difference between dry-fed and live-fed fish, but mixed-fed fish significantly outperformed group I peers by 0.30 cm 6.82% and group II peers by 0.50 cm or 11.90%. (p<0.05). At the end of the 20th week of detention, fish consumed compound feed were longer than their counterparts consuming both dry and live feed by 0.30 cm or 5.66%.

Using different types of feeding, it can be observed that on the 25th week in the III aquarium (combined type of feeding) Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry reached a maximum size of 6.3 cm, which is more than peers of the first control group by 0.60 cm or 10.53% (p<0.05) and peers of the second experimental group by 0.20 cm or 3.28% (p<0.05). It was also interesting that during the feeding of live feed (II aquarium) the growth rate of fry at 25 weeks (6.1cm), although higher than the fry in the first aquarium (5.70 cm) by 0.40 cm or 7.02% (p<0.05), but in the period from 10 by week 15 they had growth retardation. Thus, the use of a combined type of feeding contributed to the best results of growth of Black Barbus, *P. nigrofasciatus* (Günther, 1868).

Live weight of fish during the first week of rearing was the same in all groups. At the end of the 5th week, the excess weight of the fry that consumed the combined feed was found to be higher than the counterparts that received live feed by 0.2 g or 8.0% (p<0.05) and over the counterparts that received dry commercial feed by 0.7 g or 28.0% (p<0.05). Weighing the fry at the end of the 10th week showed that they weighed the same in group II and group III, but the fish from group I that consumed dry food weighed less than their peers that consumed live food by 0.3 g or 10.71% (p<0.05) and less than their counterparts, who consumed combined feed by 0.4 g or 14.29% (p<0.05). The analysis of the weight of Black Barbus fry *P. nigrofasciatus* (Günther, 1868) on the 15th week of research showed that fish from group I exceeded fish from group II by 0.2 g or 5.13% (p<0.05), but weighed less than fish from group III by 0.3 g or 7.69% (p<0.05). On the 20th week of rearing fry, their weight in group I and group II was equal, however, fish from group III that consumed combined feed equally exceeded their counterparts that consumed dry and live feed by 0.3 g or 6.52% (p<0.05). The end of the experiment at the 25th week showed the lowest weight in Black Barbus fish that consumed dry food, which was less than that of counterparts receiving combined feed by 0.5 g or 9.09% (p<0.05) and less than that of counterparts given live food by 0.3 g or 6.0% (p<0.05) (Table 5).

Table 5. The growth rate of the body weight of the Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry depending on the type of feeding, n = 90

Age (weeks)	Body weight (g)		
	Group I (dry feed)	**Group II (live feed)**	**Group III (combined feed)**
1	0.6±0.01[a]	0.6±0.01[a]	0.6±0.02[a]
5	1.8±0.02[a]	2.3±0.03[b]	2.5±0.02[c]
10	2.8±0.02[a]	3.1±0.04[b]	3.2±0.03[b]
15	3.9±0.06[a]	3.7±0.03[b]	4.1±0.05[c]
20	4.6±0.05[a]	4.6±0.06[a]	4.9±0.06[b]
25	5.0±0.09[a]	5.3±0.09[b]	5.5±0.08[b]

The difference between the averages shown with different letters in the same row is significant (p<0.05).

The study of hematological parameters did not show a significant difference in the number of erythrocytes and hemoglobin content in the blood of Black Barbus, *P. nigrofasciatus* (Günther, 1868) from the first and second aquariums, which indicates the normal physiological development of fish when fed all types of feed. In the blood of fish of the third aquarium there was a tendency to increase the number of erythrocytes, which was 1.132±0.053 million×μl^{-1}, which is 12.08% ($p<0.05$) more than in the first aquarium. No differences in the blood of fish relative to the second aquarium were found (Table 6).

Table. 6. Physiological and hematological parameters of Black Barbus, *P. nigrofasciatus* (Günther, 1868), n = 90

Group	Hemoglobin content, g%	The number of erythrocytes, million×μl^{-1}
I (dry feed)	5.97±0.812[a]	1.010±0.041[a]
II (live feed)	6.04±0.531[a]	1.101±0.046[a]
III (combined feed)	5.91±0.627[a]	1.132±0.039[b]

The difference between the averages shown with different letters in the same row is significant ($p<0.05$).

The survival of the fry is the most urgent thing in the cultivation and breeding of any species of fish. Very often in the fry of schooling fish, including Black Barbus, *P. nigrofasciatus* (Günther, 1868), is different growth rates of representatives. Therefore, larger and stronger specimens suppress or completely kill smaller and weaker members of the pack. One of the stages of our research was to analyze the survival of Black Barbus, *P. nigrofasciatus* (Günther, 1868) in different types of feeding (Table 7).

Table 7. Survival of Black Barbus, *P. nigrofasciatus* (Günther, 1868) depending on the type of feeding for 25 weeks

Group	Quantity at the beginning of the experiment	Including		Quantity at the end of the experiment	Survival,%
		males	females		
I (dry feed)	30	10	20	21	70
II (live feed)	30	12	18	25	83
III (combined feed)	30	5	25	29	97

Thus, according to our research, when feeding Black Barbus, *P. nigrofasciatus* (Günther, 1868) fry was using only dry feed, the greatest death of fry was observed. This fact indicates that very often this type of feeding in fish is obese and liver degeneration, which in the future leads to their death. The best results were with the use of a combined type of feeding. Only one specimen died in the third aquarium.

Modern fish farming and aquaristics offer fish-balanced artificial feeds. The results of our research on the influence of the type of feed on the physiological state of fish did not coincide with the reports of other authors, who indicated that feeding fish only dry feed is impractical because long-term feeding can worsen the physiological condition of fish and the ability of broodstock to reproduce (Davidson et al., 2013; Bayrak et al., 2009).

The results of our experiment coincided with the reports of other authors who reported that Black Barbus, *P. nigrofasciatus* (Günther, 1868) are quite unpretentious in terms of diet, in the aquarium they are actually omnivorous aquatic organisms, happy to consume both live and dry feed (Viskushenko, 2021).

The published results show a significant effect of feed types on the relative growth of Javanese Barbus, *P. orphoides* (Valenciennes, 1842). It also became known the highest increase was given by the use of feed types in combination with 35.0% soybean feed, 35.0% *I. Aquatica* (Forssk) leaf and 30.0% dry feed pellets.

We found results similar to those previously published data (Jagtap & Kulkarni, 2013), who says the growth rate was higher in fish using a compound feed that included pellets and mosquito larvae. However, our results did not coincide with this report on the survival of 100% of fish, as we got reduced survival of fish kept on all types of feed.

Our results did not coincide with reports (Ortega-Salas et al., 2009) indicating that a diet of live ingredients improves the growth and survival of aquarium fish compared to a dry or combined diet.

The data we found coincided with conclusions of experiment that showed fish fry fed with the combined diet showed significantly better survival rate (54.80±2.43%) than those fed with other feed types ($p<0.001$) (Alavi et al., 2009; Kami´nski et al., 2010).

Scientists report (Thandile & Akewake, 2022) that live feed helps reduce the time required for the process of organogenesis of aquarium fish, and allows the early formation of a functional digestive system, which then optimizes the growth of fry. However, we found that the best feed for Black Barbus, *P. nigrofasciatus* (Günther, 1868) was live feed in combination with industrial dry feed.

Our findings also coincided with a report (David & Aaron, 2022) indicating that commercial pelleted feeds showed lower fish growth outcomes than natural feeds in terms of growth on a fixed diet.

The authors (Kim et al., 2016) report that fish fed diets with a protein content of 45.0%, 50.0% and 60.0% had higher feed efficiencies and specific growth rates than fish fed diets with a protein content of 40.0% and below. Our result was similar to these data and shows Barbus fish, that had higher protein content in the third aquarium using the combined type of feeding, showed the best growth result.

It is known that environmental parameters affect the number, morphological composition and structure of blood cell distribution (Srivastava & Choudhary, 2010). The published results (Haghbayan & Mehrgan, 2015) showed that growing fish on an artificial diet with high bone meal had a positive effect on growth rates (p<0.001), but it reduced hematocrit and hemoglobin. Our studies did not show a difference in the level of hemoglobin in the blood of fish when using artificial, live and combined feed.

The number of erythrocytes is closely related to the activity of fish, water temperature fluctuations and the concentration of dissolved oxygen in the aquarium. Other environmental factors have a definite impact and are manifested along with seasonal variability. The number of erythrocytes also depends on age, sex, diet and reproductive status and can range from $0.5–1.5\times10^6$ $(mm^3)^{-1}$ in less active species to $3.0–4.2\times10^6$ $(mm^3)^{-1}$ in more active species (Witeska 2013). But in our experimental aquariums, the oxygen content was the same throughout the study period, but the level of erythrocytes was higher in Barbus fry, which consumed compound feed. We attribute this to an increase in the metabolic rate of fish on the combined feed.

CONCLUSIONS

Our results showed that the combined feeding allowed us to produce in the 25th week of the experiment Black Barbus, *P. nigrofasciatus* (Günther, 1868), with a maximum body length of 6.30 cm and a body weight of 5.5 g, which was more compared to the analogues that consumed dry industrial feed and live feed of microorganisms and crustaceans. The haemoglobin content in the blood of the fish did not differ significantly among the different diets, but the erythrocyte content was 12.08% higher in the fish that consumed combined diets. The survival rate of Black Barbus, *P. nigrofasciatus* (Günther, 1868), was also higher in fish fed combined diets and was 97.00%.

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