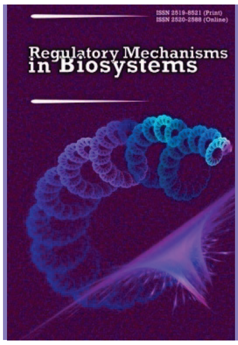

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Method for obtaining ecological products from rainbow trout *Oncorhynchus mykiss* farming

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In aquaculture, the period of active development of aquatic organisms is considered one of the most important for the formation of potential in the future. The issue of using natural components when growing aquatic organisms and feeding young fish is given special attention. This research investigated *Spirulina* (25%) + Vermiculture (50%) + *Hermetia illucens* (25%) as an additional source of functional active substances and protein that affect the body performance of rainbow trout in early ontogenesis. All objects were cultured in a special bioreactor, which was a component of the recirculating aquaculture model system as well as in the laboratory, where an installation was made for cultivating natural food. The results of the scientific and practical part of the work showed that after the end of the experiment, body weight was higher in the experimental fish by 7.2% than in the control group. This article presents the results of feeding rainbow trout as one of the cases of improving the morpho-functional and biochemical composition of the blood of this species: an increase in the total number of red blood cells by 11.0%, hemoglobin content by 9.7%, total protein at 22.7%, albumin by 24.2%, and creatinine by 15.5%, compared with the control group. The presented positive changes in the functional status of the rainbow trout organism were in harmony with the parameters of body weight, as well as histological indicators. Histological indicators of the liver and muscle tissue of the fish supplemented the obtained positive result of the influence of natural and active components of the feed. Feeding fish according to the scheme in the experimental group promotes the formation of layers of special adipose tissue in the endomysium. The total amount of the stromal component in the muscle tissue of rainbow trout was 25% of the mass of the entire tissue. The control group showed that the muscle fibers were polygonal in cross section, separated from each other within the bundle by delicate layers of endomysium. When using a standard feeding regimen (control group), the stromal component is a moderately expanding structure that replaces the space between the fibers. The experimental group had the most significant indicators. The intensification of individual aspects of homeostasis achieved with the help of feed components (experimental group) promotes the growth of muscle fibers in thickness due to the formation of new fibrils, but not an increase in the proportion of sarcoplasm (watery meat). As a result, neurohumoral regulation and substance synthesis processes improved in the fishes' body, which helps to improve ontogenesis rates. The presented ingredients in the diet of rainbow trout are natural stimulants of metabolic processes and sources of nutrients. The method allows aquaculture to be defined as being as close as possible to "environmentally safe". The next stage will be a comprehensive analysis of the qualitative and quantitative characteristics of rainbow trout meat under the influence of the feed factor.

Keywords: biochemical composition of the blood; endomysium; growth of muscle fibers; *Spirulina*; vermiculture; *Hermetia illucens*.

Introduction

In aquaculture, the rational use of resources, including feed, is one of the cases of successful management of the industry. Undoubtedly, the symbiosis of abiotic and biotic factors is the optimal model for the introduction of innovative technologies against the background of the rapid development of demand for environmentally safe products (Gould et al., 2019; Honcharova et al., 2020). Cultivation of hydrobionts using natural feeds, without chemical preparations and hormonal growth stimulants, is relevant in the field of aquaculture. In this context, we can also pay attention to the fact that in recent years more and more attention, and even priority, has been paid to the issue of "welfare" (Fr. "Bien-etre", En. "Welfare") of fish under different forms of cultivation (Ashley, 2006). The concept of "welfare" of fish can be broadly classified into "welfare" in industrial conditions and adaptation of fish after stocking of rivers. In addition, the concept is considered as a set of optimal components of technological conditions (additional feeding, feeding, quality water ecosystem, humane relationship with fish during technological processes, etc.) (North et al., 2006; Zonghang et al., 2023). This work considers the factor of feeding fish and additional feeding with natural feed, observing not only quantitative, but also qualitative characteristics of products at the end of cultivation. The use of chemicals to stimulate the productivity of hydrobionts is also negative and identifies aquaculture products as "ecologically dangerous" for the ecosystem and for consumers.

Modern conditions, global climate transformations, the impact of man-made pollution, the introduction of extensive innovative technologies – all these aspects ultimately form the concept of "aquaculture product quality" and its compliance with standards. Using recirculation systems as the main form of fish farming, and as an additional stage, is an important technological aspect. For many types of hydrobionts, feeding parameters (diet composition, additional feeding with various biologically active, phytosubstances, etc.) contribute to the improvement of feed conversion, increase of their growth in ontogenesis. Of course, there are other generally accepted factors that can be used to regulate the parameters of effective management of the fishing industry. There is a study by the authors that emphasizes the importance of physiological and biochemical processes, the quality of water and the availability of feed using the example of rainbow trout (Skov et al., 2011). At the same time, there are publications in which the authors note that physiological stress has different origins, in particular, the density of planting, the crowding of fish, which can be the main reason for the decrease in the growth rates of hydrobionts and feed consumption (North et al., 2006). Summarizing most of the available results, it can be noted that different fish rearing conditions can affect specific growth rates and feed conversion efficiency.

In this context, let us note that imported feed production can destabilize the development of aquaculture. The conditions for growing fish on a Ukrainian farm are not always the same as the standards adopted in foreign countries, including the European Union. In addition, imported feed is mainly intended for certain types of fish, typical for the geographical zone of a particular country (for example, in France there are local fish species that are not available for breeding in Ukrainian aquaculture). Therefore, the search for new and alternative ways to optimize feed mixtures, their quality, and cost reduction is relevant and one of the main tasks of aquaculture. Given that natural components and biologically active substances activate metabolic processes in hydrobionts' organisms in a neurohumoral way, competent correction of active processes and the functional status of the organism can achieve high results. Taking into account the outlined positive consequences, it is worth noting that the issue of feeding hydrobionts with such natural components is of practical importance and has scientific value.

Currently, in modern aquaculture there are many vectors for optimizing the technological process of growing and breeding aquatic organisms. If a method of optimizing a technological map is used in practice, then practitioners take into account the level of "environmental safety of products". The authors present in their scientific and practical manuscripts ways to increase the growth rate of aquatic organisms, which are widely practiced using metabolites – chemical and hormonal growth stimulants. It is important to consider that such use will affect the quality characteristics of the product in the future. At the end of growing fish using this method after stocking, this can lead to a deterioration in the ecological condition of water bodies, and thereby to an imbalance in the ecosystem as a whole. As hormonal or chemical substances accumulate in the muscle and other parts of fish, which is physiologically impossible to avoid, more and more attention is being paid to the issue of feeding. As is known from the literature, the high activity of digestive enzymes when rainbow trout enters the body contributes to the digestion of a larger amount of animal proteins (from 30% to 50%).

In the context of obtaining ecologically safe aquaculture products for the population, the question arises of the influence of biologically active additives on the body of hydrobionts during the growing period. One of the promising ways to improve the physiological and biochemical status of fish is the use of non-traditional feeds, probiotics (Hakim et al., 2021; Damodaran et al., 2023). The study of the role of microalgae as an alternative source of protein is receiving attention from scientists and practitioners (Honcharova & Tushnytska, 2018; Nagappan et al., 2021).

Modern abiotic and biotic factors form a modified adaptive and compensatory system in the organism of hydrobionts. At the same time, physiological-biochemical, histological, zootechnical, genetic and other parameters are subject to certain transformations. If the organism of hydrobionts is not able to go through each link of these complex and complex processes, then it most likely enters the stage of stress, productivity and development decrease since the organism is a coherent system that correlates with other parameters, processes and is reflected in the formation of the potential for productivity and development in ontogenesis (Storey, 1996).

Studies by most authors demonstrate information about the importance of balancing the diet for fish at each stage of ontogenesis. For example, the carbohydrate content in food, which is the main source of energy for fish, is recommended for complete nutrition of rainbow trout: no more than 9–12% of easily digestible carbohydrates. At the same time, their total content in granulated feed should not exceed 25–30%. There are authors who recommend supplementing the diet with, for example, high-protein microalgae for trout, such as spirulina (Velichkova et al., 2020). Scientists have experimentally established and explained metabolic processes by the physiological characteristics of fish, showing that catalytic functions depend on a complete diet. At the same time, an unbalanced diet or lack of protein, vitamins, microelements or other ingredients in feed causes profound metabolic disorders, which leads to illness and death (Dehtiarov et al., 2008).

It is physiologically proven that the fish body's need for protein depends on the amino acid composition of the protein, which contains essential amino acids supplied with food. Protein in fish food is considered complete if it contains all the necessary amino acids and is in a balanced state. It is known that insufficient content, for example, of valine in the diet of fish leads to a lack of coordination, and a lack of lysine in food leads to impaired blood circulation, a decrease in the number of red blood cells and hemoglobin concentration, nitrogen balance (Dehtiarov et al., 2008). The imbalance of other metabolisms is also physiologically and biochemically justified, for example, the fish feed does not have enough fat for the body, since more protein is consumed to compensate for energy needs. As a consequence of these processes, fish growth decreases and feed consumption increases. In literary sources, the authors demonstrate the results of experimental studies on obtaining a positive effect from the use of phytopreparations in aquaculture, among which there is experience in using seeds in aquaculture of *Hibiscus sabdariffa*. The authors note that the fish fed with phytopreparations had the best weight gain, feed conversion ratio and characteristic specific growth rate (Usman et al., 2023).

Conducting comprehensive studies on the use of natural substances in aquaculture has demonstrated the positive effect of such plants as *Mitracarpus scaber* and *Alstonia boonei*. The authors note positive results, for example, at the end of the experiment, the final weight (FW), weight gain (WG), specific growth rate (SGR) and average daily growth rate (ADGR) of fish fed with *Alstonia boonei* and *Mitracarpus scaber* were significantly higher than that of control. The values of RBC and haemoglobin of the *Mitracarpus scaber* group were significantly higher than the other groups including the control (Ajadi et al., 2022). Scientific research is presented on how the addition of plant extracts (phytogenic additives or herbal medicinal mixtures) to fish diets changes physiological responses to stress, increased resistance, adaptation and the body's capacity (Honcharova et al., 2021; Yang et al., 2022).

In addition, in literary sources, the authors note the positive effect on the condition of the gastrointestinal tract of the intestinal walls of fish and the general state of functional processes. The manuscripts emphasize that conventional feed additives or red crayfish meal, dried microbial biomass have a stimulating effect as a feed additive in diets without fishmeal for rainbow trout. Among the ingredients, soybean meal is also provided, which is widely used in the preparation of aquatic feed due to its high protein content, near-optimal amino acid profile and digestibility (Yang et al., 2022). However, there are studies in which the authors note that high levels of adding soybean meal to the diet of hydrobionts negatively affects their growth performance (Collins et al., 2013), intestinal and liver integrity. Thus, there are debatable aspects in the literature, which is a positive thing, because there is an opportunity for objectivity and analysis of both negative and positive sides.

The use of phytopreparations and natural components is also practiced in Ukrainian aquaculture. In particular, there is research on the positive effect of determining the biological effect of milk thistle oil in different amounts of its introduction to the diet on the antioxidant status of the organism of young fish when reared in conditions of recirculating aquaculture systems (Koryliak et al., 2023).

Herbal medicines are increasingly used for their effects on the immune system. Silymarin, a mixture of flavonolignans from the seed of milk thistle (*Silybum marianum*), is used to improve liver function. An experimental study was designed to study in more detail the effect of rose thistle on hematological parameters in rainbow trout, as well as immunological. Silymarin (rose thistle), a mixture of flavonolignans from the seeds of *S. marianum*, is used to improve cellular metabolism and liver synthesis processes. Haematological studies recorded increases in RBC and Hb following the administration of the silymarin-supplemented feed (Ahmadi et al., 2012).

In recent years, the use of feed antibiotics has been prohibited in the countries of the European Union. Thus, there is a need to find a rational alternative to growth stimulants and antibiotics. In agriculture, the aquaculture sector is no exception, experts have begun to intensively implement the use of prebiotics, phytosupplements, natural feeds, conceptually borrowed from human medicine (Ringø et al., 2010). Thus, the technological aspects of combining several such components for additional feeding of hydrobionts are relevant (Cho et al., 1982; Hajen et al., 1993). This method provides an opportunity to more fully and comprehensively enrich and satisfy the fish body's need for protein, active substances for synthesis, metabolism and accumulation of body weight, as a whole, to increase the body's potential in ontogenesis. Such results (a complex combination of several ingredients in the diet of fish) are not often found in literary sources. That's why the topic is becoming more relevant. Synthetic agents as growth promoters in aquaculture are becoming unpopular, hence the need to find alternatives. In this context, the subject of the article is of scientific and practical interest.

Materials and methods

Scientific-practical basis of work and ethical aspects of the experimental part. The practical modular parts of the research work were carried out on the basis of the "Kherson Production and Experimental Plant for Breeding of the Ordinary Fish" (Ukraine); "Production-Experimental Dnipro Sturgeon Fish Breeding Plant named after acad. S. T. Artyushchyka" (Ukraine) and on the basis of the Scientific Research Laboratory "Aquaculture Perspectives", Research Laboratory of Physiological and Biochemical Research named after S. Pentelyuk", Scientific Research Laboratory on Ecological and Chemical Analysis and Water Monitoring" (Kherson State Agrarian and Economic University (KSAEU, south of Ukraine). The entire experimental part was organized and implemented in strict accordance with aquaculture standards (Yevtushenko & Khyzhniak, 2019). The principles of bioethics were entirely adhered to in the research. The experiments presented in this work were approved by the Academic Council of the Kherson State Agrarian and Economic University in accordance with the section of the Recommendations of the Council of the European Union (2010/63/EU) on the use of experimental animals and the principles of the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) (Grath et al., 2010). The conditions for keeping aquatic organisms were completely favorable, maintaining the density of fish stocking and the conditions of the hydrochemical regime. All work was carried out in strict compliance with the provisions of the framework of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986); International Principles of the Declaration of Principles of Tolerance (28th session of UNESCO, 1995), the Rules of Conducting Studies using Experimental Animals, according to the Order of the Ministry of Healthcare of Ukraine No. 281 as of 1 November 2000, The Measures of Further Improvement of Organizational Forms of Study using Experimental Animals and according to the Law of Ukraine On the Protection of Animals from Abuse (No. 3447-IV as of 02.21.2006, Kyiv).

Object of research, growing conditions. Rainbow trout *Oncorhynchus mykiss* were fed in a recirculating fish culture system for intensive multi-species culture with an average dissolved oxygen level of 9.45 mg/L and an average water temperature of 13.1 ± 0.2 °C (according to technological recommendations "Trout feed for RAS"). The initial weight of the rainbow trout was 1.46 ± 0.16 g. Rainbow trout were grown in recirculating fish tanks; as the fish developed, the volume of the pools was increased: volume – 135, 250, 550 m^3. The stocking density of fish depended on body weight and water level in the RAS basins. Rainbow trout were placed in two pools of a recirculating system. Each of them corresponded to the Control and Experimental groups (50 specimens of rainbow trout). The technological map for growing rainbow trout provided for additional feeding with ingredients high in natural components. Also, a mini solar panel was used as additional energy. Rainbow trout were hand-fed thrice during the day from the beginning every two 1.5 hours (08:20 h, 12:30 h and 17:30 h). Feeding rates corresponded to the rainbow trout age group.

Feeding conditions of the diet. In order to assess the quality of the diet, the following physical and chemical indicators of the functionality of the feed were determined: moisture content of the granules, water resistance. Rainbow trout in the control group were fed a general diet without additives. All ingredients in the diet were balanced in terms of nutrients and energy. In the experimental group, the fish received the main diet and additional feed: *Spirulina* (25 %) + Vermiculture (50 %) + *Hermetia illucens* (25 %). In the control group, fish received the basic diet without additives.

Spirulina was cultivated in the laboratory in special tanks, with oxygenation and illumination (Fig. 1). We used our own installation (bioreactor), developed by a co-author, for which a declarative patent had already been obtained (Kutishchev & Honcharova, 2022). According to the schedule, filtration was done, the mother culture was selected, special preparation was carried out, the mass of spirulina was separated, excess moisture was removed. As a result, a paste-like mass was obtained, which was then dried to be added to fish during feeding. *Hermetia illucens* and vermiculture were also cultivated in the conditions of two fish enterprises in compliance with all rules and sanitation requirements. Special cabinets with a barrier were used for cultivation. The insects were specially processed, dried on laboratory equipment and packaged for future use as part of the fodder mass.

All ingredients were cultivated in the laboratory of aquaculture and biological resources of KSAEU. All the resulting feed mixture was filtered and processed for feeding rations for rainbow trout.

When the first stage of the experiment was organized, rainbow trout were fed small pellets (according to standards in trout farming). As the fish grew, pelleted food was used. The size of the granules corresponded to the mass of the rainbow trout (1.2 mm – 1.5 g). Ingredient content (%): for starter (juvenile fish) – crude protein (45–48), crude fat (11–13), carbohydrates (15–20), ash (10–11), fibre (2.0–2.5); gross energy (kcal/kg feed): 4500–5000, digestible energy (kcal/kg): 3000–3500. For after starting – crude protein (38–43), crudefat (7–9), carbohydrates (25–30), ash (10–15), fibre (3–5); gross energy (kcal/kg feed): 4000–4500, digestible energy (kcal/kg): 2500–3000. The experimental group of rainbow trout received the same diet as the control group. When feeding, natural ingredients were added to the experimental group.

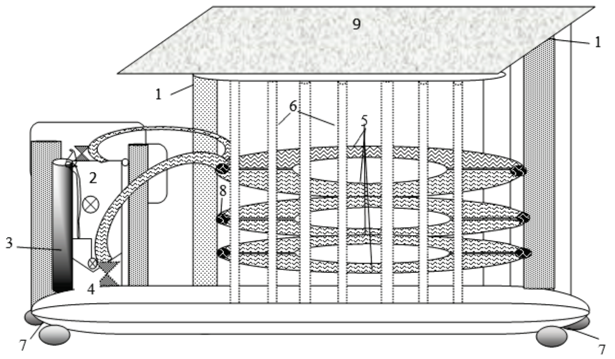

Fig. 1. Device for cultivation of microalgae by using energy-saving technologies: mobile platform (*1*); bioreactor (*2*); carbon dioxide cylinder (*3*); recirculation pump (*4*); glass tubes for recirculation of microalgae culture (*5*); fluorescent lamps (*6*); wheels (*7*); pipe retainer (*8*); monocrystalline solar panels (*9*)

Studying growth and development of O. mykiss. To study the level of development of rainbow trout, systematic weighing of the fish was carried out. For this purpose, a random selection of 25 specimens from each pool was made. To study the functional state of the rainbow trout's body, the composition of the blood, biochemical, morphological, and histological was studied. For the research, 12 samples were selected. Rainbow trout were weighed on a scale with an accuracy of 0.001, and the body weight of the fish was expressed in g. The survival rate of rainbow trout in each experimental group was determined during the experimental part of calculating the final number of rainbow trout specimens in relation to the initial number of rainbow trout specimens, based on percentage (%). Also, the rate of development of rainbow trout was studied in the ontogenesis of several periods under the influence of additional feeding: at the beginning of the experiment, after 3 weeks, 2, 3, and 4 months.

Morphological and biochemical studies. To study blood composition, blood samples from rainbow trout were obtained from the tail vein. For this purpose, a puncture and a medical syringe were used. During the research, two test tubes were used for the experimental and control groups. To do this, in each group two test tubes were prepared according to the following scheme: for the first test tube, a solution of 2% heparin was added to prevent the blood from clotting. We studied the hematological profile of the blood of rainbow trout (total number of red blood cells (RBC), hemoglobin content (HGB), hematocrit concentration (Ht)). The corpuscular indices of trout erythrocytes were also studied: mean red cell volume (MCV), mean red cell hemoglobin (MCH), mean red blood cell hemoglobin content (MCHC). The second tube was used to obtain coagulation, followed by rainbow trout serum. In this test tube, the state of the biochemical profile was examined (these were the following parameters of protein and carbohydrate metabolism: the amount of total protein, albumin, creatinine, as well as glucose content). The biochemical profile of rainbow trout was determined on Humalyzer 3000 (Germany, 2010) biochemical analyzer (in combination with using standard unified kits by Human GmbH (Germany). The hematological profile of rainbow trout blood by hemoglobin was determined by the cyanmethemoglobin method. We also used quantitative analysis using a spectrophotometer (with wavelength 530 nm), UNICO 1201 (United States of America (USA), 2010). The study of hematocrit values and corpuscular blood parameters (MCV, MCH, MCHC) were calculated based on the total number of formed elements – red blood cells and the hemoglobin content in the blood of rainbow trout.

Histological studies. The histomaterial was processed using proprietary equipment and original techniques specifically designed for histological diagnosis of tissues of aquatic animals of animal origin (Kozij, 2009) using optical equipment "E. Leitz «Diaplan» Wetzlar" (Germany), halogen illuminator "Linvatec-2" (United States of America) with a nominal power of 10–240 W. General morphometric studies of tissue structures were performed using a detachable eyepiece micrometer. Contrasting histological preparations were performed using correction filters "ZhZM 2.5x", "MONOCHROM 2.5x" (Belarus). Microphotography of histological sections was performed with a digital camera Nikon D-60 (Austria). The microanatomical structure of the somatic muscles of trout and the morphological parameters of the liver were studied and compared.

Studies of the hydrochemical regime of basins. Studies of the parametric hydrochemical regime in the basins where rainbow trout were raised were carried out systematically according to the analysis plan. An express method was used and also studied in laboratory conditions in accordance with generally accepted recommendations in fish farming (Alekin, 1970). Hydrochemical analysis was carried out using a Palintest 7500 photometer (United Kingdom, 2018), using the appropriate Palintest reagents (United Kingdom, 2018). When water samples were taken from the pools, the following parameters were determined in each test tube: dissolved oxygen content, pH, hardness, oxidation, nitrate and nitrite concentration. Water temperature was measured before the beginning of the experiment three times a day. After this, the concentration of dissolved oxygen and water temperature was studied twice a day.

Statistical analysis. The values studied were expressed as mean (x) ± standard deviation (SD) using one-way analysis of variance (ANOVA), considering that $P < 0.05$ is statistically significant.

Results

A comprehensive study of the functional status of the organism in rainbow trout under the influence of the feeding factor demonstrates a tendency to activate metabolic processes in trout which were additionally fed with natural ingredients of the general diet. First of all, before interpreting the results of the experiment, we note that all parameters corresponded to physiological values in aquaculture. The normal course of all physiological processes and the increase in the weight of rainbow trout (in the experimental and control groups) prove that nutritional needs were fully satisfied. This means that all the substances and compounds necessary for the body were present in the feed, and the feed had the necessary nutritional value. But in the experimental group these processes occurred more actively than in the control group.

A visual inspection showed that all the fish had the following appearance indicators: the body surface was clean, the color was natural, typical of this type of fish with a thin layer of mucus, there were no signs of disease, the gills were red. The eyes were bright, transparent, without damage. The smell was characteristic of living fish of this species, without any foreign odors. Examination in rainbow trout in both the experimental and control groups showed that the position, shape and color of the internal organs were within the physiological norm, no changes were observed. No cardiac obesity was detected, the tissue structure of the liver and spleen was dense, the kidneys were without tissue growths. The results of a study on the development rate of rainbow trout under the influence of natural ingredients that were added to the experimental group demonstrated a positive effect (Fig. 2).

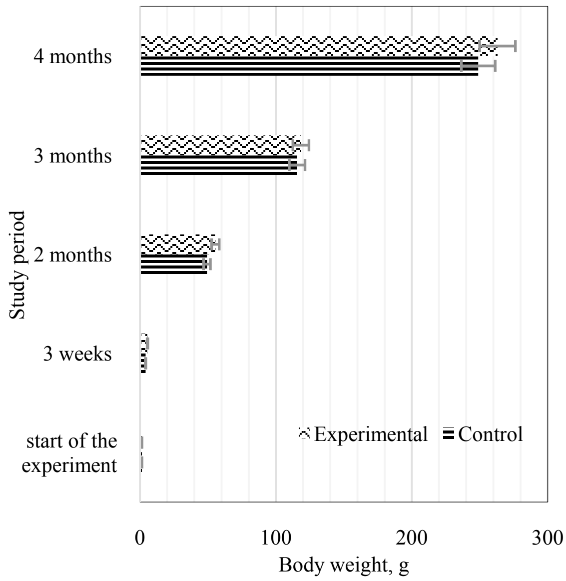

Fig. 2. Study of the growth rate of rainbow trout *Oncorhynchus mykiss* in ontogenesis under the influence of technological factors (x ± SD, n = 25)

Analysis of body weight indicators of rainbow trout in average values in the experimental group after 3 weeks of using natural food showed body weight to be 33.6% higher than that of the control group. The use of additional feeding in the trout diet with natural feed and active substances helped stimulate metabolism.

As a result, metabolic processes in the experimental group were more active. A study of the rate of fish development after 2 months showed that the weight of rainbow trout which additionally received natural ingredients when feeding (experimental group), was higher than the control group by 15.4% ($P < 0.01$).

After 3 months of feeding, the body weight of the experimental group of rainbow trout had better values and was 14.1 % greater than in the control group. It can be assumed that improving parameters by stimulating processes of a physiological and biochemical nature also contributed to an increase in feed conversion and maximum use of potential in the body of experimental fish. After using additional feeding, the average body weight in the experimental group exceeded the value in the control group by 7.2% ($P < 0.01$). There were similar positive dynamics in fish survival; on average, this parameter was 4.9% higher than the control values for the entire period of the experiment.

Analysis of comparison of blood parameters showed that among all indicators there were significant differences between the control and experimental groups. The processes of erythropoiesis in the body of rainbow trout which were additionally fed were more active. The total number of red blood cells and hemoglobin was higher than the control group by 11.0% ($P < 0.05$) and 9.7% ($P < 0.05$) respectively (Table 1). Comparison of corpuscular blood parameters reflects the morphological and functional characteristics of blood. Among the MCH indices, MCHC, as well as MCV, there was no significant difference.

Thus, the morpho-functional parameters of the blood showed better parameters in the body of fish which additionally received natural components and active substances to the main diet. The fish that received standard feeding as the main diet (without additional feeding) were smaller in comparison with the fish in the experimental group.

The transformation of physiological and biochemical processes can be explained by the more active processes of nutrient metabolism. Under the influence of natural components, it is possible that simple sugar compounds rapidly decompose in the blood of rainbow trout from the experimental group. Analysis of glucose concentrations in the blood of rainbow trout demonstrated a difference of 11.2% between the control and experimental groups. This difference demonstrates the dynamics of active carbohydrate metabolism. The state of protein metabolism demonstrated active synthesis reactions (Table 2).

Table 1
Morpho-functional blood parameters in rainbow trout under the influence of food factor (x ± SD, n = 6)

Parameters	Control group	Experimental group
Red blood cells (RBC), $^{x}10^{6}$/L	1.428 ± 0.027	1.586 ± 0.042*
Hemoglobin (HGB), g/L	149.0 ± 3.8	163.5 ± 4.3*
Hematocrit (Ht), %	41.4 ± 2.2	44.8 ± 1.8
Mean red cell hemoglobin (MCH), pg	104.29 ± 0.96	103.21 ± 2.38
Mean cell hemoglobin concentration (MCHC), %	35.29 ± 1.14	36.61 ± 0.86
Mean corpuscular volume (MCV), μm^3	289.0 ± 10.7	282.7 ± 9.0

Note: * – P < 0.05 between the control and expermental groups.

Table 2
Biochemical composition of blood in rainbow trout under the influence of food factors (x ± SD, n = 6)

Parameters	Control group	Experimental group
Total protein, g/L	48.4 ± 1.4	59.4 ± 1.9***
Albumin, g/L	20.7 ± 1.3	25.7 ± 1.3*
Creatinine, μmol/L	50.9 ± 1.2	58.8 ± 1.8**
Glucose, mol/L	3.12 ± 0.15	3.47 ± 0.24

Note: * – P < 0.05;** – P < 0.01;*** – P < 0.001 between the control and expermental groups.

Perhaps natural ingredients were a source of protein, biologically active substances, and the body of the experimental rainbow trout most effectively used the potential in the organism. The dynamics of positive changes were reflected in the content of total protein and albumin in the blood of the fish. The level of total protein and albumin in the blood of fish from the experimental group was higher than in the control group by 22.7% (P < 0.001) and 24.2% (P < 0.05) respectively. An analysis of creatinine in the blood of rainbow trout under experimental conditions showed that its content was higher in rainbow trout that received additional natural components in the diet. Perhaps it can be assumed that physiological and biochemical processes occurred more actively. The level of creatinine was within the physiological norm in two groups. But in the experimental group its amount exceeded the control group by 15.5% (P < 0.01). At the same time, the body weight of the fish was also greater in the experimental group. Such positive dynamics can be explainned by the higher muscle tissue in fish from the experimental group (Fig. 2). Determination of the dependence of biochemical and morpho-functional parameters in rainbow trout on body weight showed that the coefficient of determination was in the range 0.742–0.949 (Table 3).

Table 3
Coefficient of determination correspondences for dependence of blood parameters on body weight of rainbow trout

Parameters	Logarithmic equation of connection	Coefficient of determination, R^2
Creatinine	y = 371.7ln(x) – 2013	0.888
Total protein	y = 431.1ln(x) – 2343.1	0.847
Albumin	y = 244.1ln(x) – 1335	0.742
RBC (Red blood cells)	y = 8.8ln(x) – 47.519	0.949
HGB (Hemoglobin)	y = 836.5ln(x) – 4499.7	0.814

Note: x – body weight of rainbow trout; y – blood parameters of rainbow trout.

The results of comparison and analysis of the histological characteristics of the biological material of trout from the control and experimental groups also demonstrated the difference between the groups depending on the feeding factor. When studying the influence of feed composition in the diet on the morphological parameters of the liver, the role of a kind of marker is assigned to the marginal zone, since a number of changes primarily occur within its boundaries. The results of histological analysis of the liver parenchyma of fish from the experimental group fully confirm this fact (Fig. 3). Optimization of fish feeding conditions contributes to the variability of lipid content within one given zone of the liver, which compensates for the deficiency of glycogen in the somatic muscles.

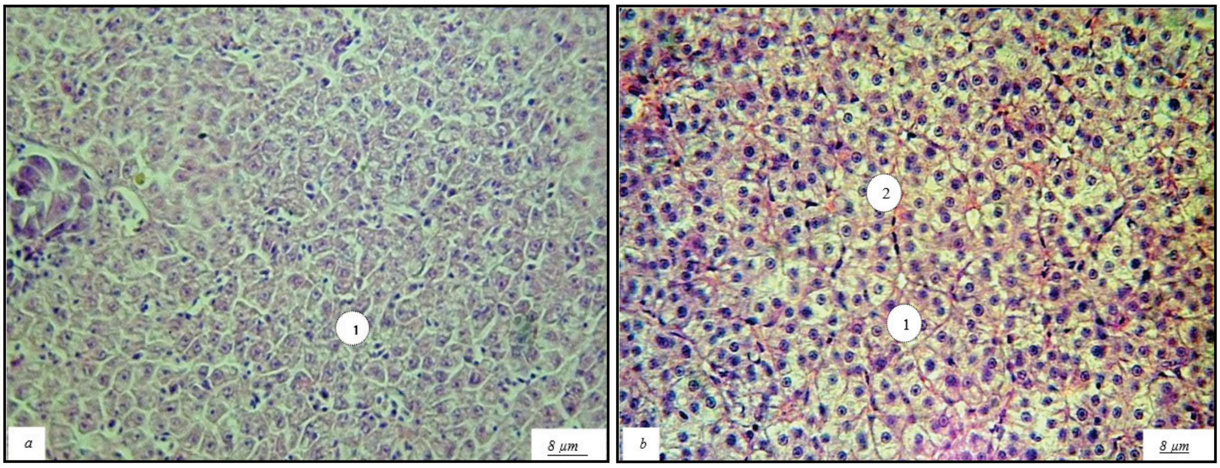

Fig. 3. The structure of the marginal zone of the rainbow trout liver parenchyma: *a* – control group; *b* – experimental group: *1* – hepatocytes; *2* – sinusoidal capillary

An exception is the cell population of rainbow trout, which can be explained by the species-specific lipid metabolism characteristic of Salmonidae fish. Against the background of an increase in the values of linear cell sizes in rainbow trout – 0.96, 3.63 μm, the values of nuclear parameters 1.57, 2.35 μm adequately change, which is an indicator of a change in the nature of intracellular metabolism.

The resulting changes in the number of intracellular cavities in the hepatocytes of rainbow trout from the experimental group is evidence of a decrease in lipostatic function. A slight change in the value of the "nucleolus/nucleus" diameter ratio in the liver cells of rainbow trout from the experimental group indicates an increase in glycogen synthetic function, which is visually determined by the appearance of specific granularity and "saturation" of the cytoplasm. This fact is also confirmed by the disappearance of typical monochromasy in cells. Feeding fish according to the scheme in the experimental group promotes the formation of layers of special adipose tissue in the endomysium. The total amount of the stromal component in the muscle tissue of rainbow trout was 25 % of the mass of the entire tissue (Fig. 4). When studying the microanatomical structure of the somatic muscles of rainbow trout, wide muscle segments (myomeres) were recorded, separated by strands of connective tissue. The connective tissue of the muscle (myocommata) is functionally similar to the internal and external endomysium. The control group showed that the muscle fibers were polygonal in cross section, separated from each other within the bundle by delicate layers of endomysium. Individual fibers have a small diameter, which is normal. When using a standard feeding (control group), the stromal component is a moderately expanding structure that replaces the space between the fibers.

The experimental group has the most significant indicators, comparing the histograms, we can say that in the species under study there is a noticeable decrease in the proportion of the stromal component with an increase in the diameter of the muscle fibers (Fig. 4). Based on the data, muscle fibers in a cross section, on the contrary, demonstrate sufficient "fibrillar filling". Obviously, the intensification of individual aspects of homeostasis achieved with the help of feed mixture components (experimental group) promotes the growth of muscle fibers in thickness due to the formation of new fibrils, but not an increase in the proportion of sarcoplasm (watery meat).

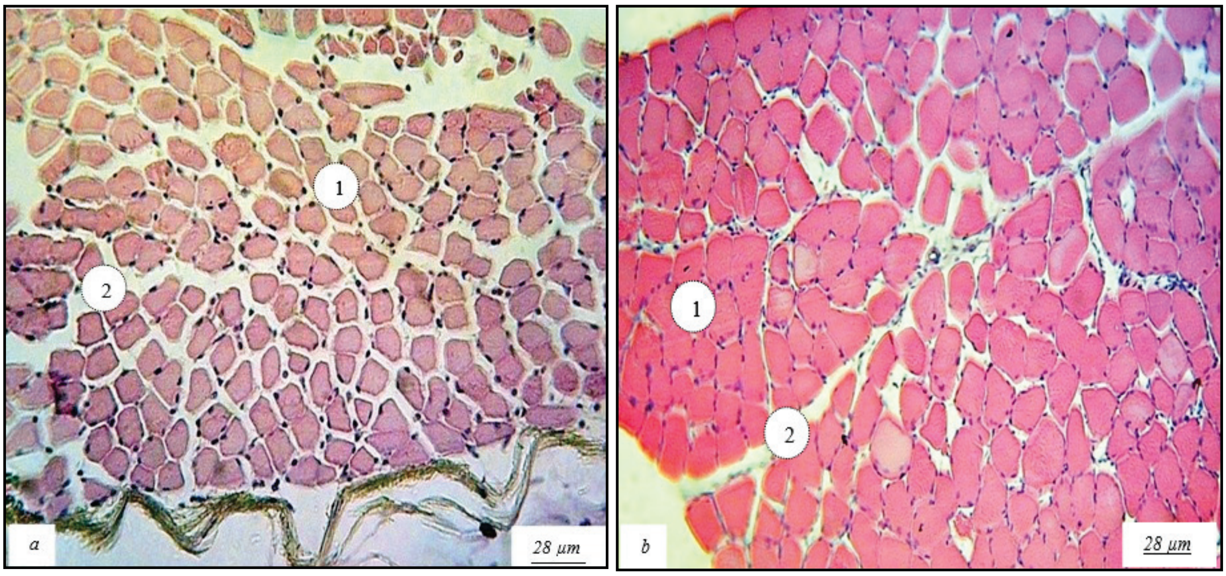

Fig. 4. The structure a cross section of a fragment of the muscle bundle of the dorsal part of the rainbow trout body: *a* – control group; *b* – experimental group: *1* – muscle fiber; *2* – endomysium

Monitoring of hydrochemical parameters demonstrated compliance with aquaculture standards for cold-water fish. Systematic monitoring of hydrochemistry parameters throughout the experiment showed slight fluctuations, the pH changed from 7.6 to 7.8, but remained within the pH range of 6.9–7.3, which was optimal for the life support of the fish. The concentration of nitrates and nitrites during the entire feeding period increased slightly and was within the normal range.

The dynamics of development in ontogenesis changed, which is associated, first of all, with an increase in the mass of fish, and, accordingly, the biological load on the recirculation system as a whole. An analysis of the hydrochemical regime of the basins of the control and experimental recirculation system demonstrated that the actual values of all parameters corresponded to the permissible limits and limits of fluctuations for trout farming. The results of measuring the concentration of free hydrogen ions (pH) were at the level of 7.1 against the background of an oxygen concentration of 89% to 98% (from 9.1 to 9.8 mg/L). Temperature measurements showed optimal values, which were at the level of 12–14 °C, the mineralization value in the pools was 365 mg/dm^3 against the background of nitrite and nitrate content – 0.05 and 1.10 respectively.

Discussion

Analyzing the majority of scientific and practical material with the vector of topics presented in this work, we can emphasize that the dominant focus is on the issue of constantly searching for ways to optimize the technology for growing planting material in aquaculture. At the same time, the technology for growing aquatic organisms largely depends on the areas of aquaculture and the characteristics of the geographical description. Drawing a general conclusion, the entire array of information described in scientific and practical literature is most often adapted for the relevant aquaculture areas. The issue of the quality of fish seeding material for hydrobionts is always important, since the effect of stocking reservoirs with fish depends on it in the future. Therefore, research works on the harmonization of technological cases of adaptation of innovative technologies to the biological features of hydrobionts are relevant today since those substances that are added to the diet of hydrobionts are transformed in the body and after complex metabolic processes are excreted to the outside, into the water ecosystem. However, it is important to emphasize that this scientific work examines the level of effectiveness of the use in practice in the modern context of natural biotechnology ingredients in aquaculture. Considering that biotechnologies using photoautotrophs, in particular microalgae, have recently been developing, the topic remains relevant. The experience of effective use of phytoplankton is presented in scientific works, where the author notes the positive effects of chlorella (Alagawany et al., 2021).

Also, results of a positive effect in aquaculture from the use of natural feeds and phytocomponents in aquaculture are shown in the works of authors where the emphasis is on enhancing processes at the level of physiology and biochemistry in the body of aquatic organisms (Nya & Austin, 2009; Yevtushenko & Khyzhniak, 2013; Bekh et al., 2020). Of course, thanks to neurohumoral regulation and other factors, blood composition is maintained at a certain level. Changes in the blood system are the corresponding reaction of the fish body to changes in internal and external factors (Honcharova & Sherman, 2023). Thus, the morphological analysis of blood is one of the objective methods of monitoring the physiological state of the fish organism. Therefore, it reinforces the obtained results and makes them more reasonable. The results of experimental studies demonstrate the effect of a stimulator of metabolic processes in the body of hydrobionts under the conditions of the use of herbal preparations, non-traditional preparations or probiotics (Borquez et al., 2011; Zheltov et al., 2016).

The development of aquaculture at the global level contributes to the development and use of methods for increasing the production of feed resources for the needs of aquatic organisms. In turn, such transformations will entail the need to increase the amount of feed ingredients, in the foreground these will be protein sources. This has stimulated research into alternatives to fishmeal to provide protein in fish feed. Therefore, the development of possible methods of alternative protein and optimization of production with an emphasis on reducing the pressure on ecosystems, development of filtration methods is relevant. It should be noted that this method of feeding hydrobionts with additional natural components ensures the maximum approximation to the standards of "environmentally safe" products. It provides advantages for aquaculture producers and makes them more competitive in harmony with the modern realities of aquaculture development. Among them, for example, are hydrochemical conditions, breeding conditions – fish stocking density, filtration level, genetics, etc. However, based on the results of the majority of authors who carried out scientific and experimental studies on the use of natural components in the general diet of hydrobionts, this factor can be singled out as one of the effective ones. For example, there are reliable data in literary sources, which note a better metabolic rate in fish, better energy processes under the conditions of additional feeding with microalgae, plants, zooplankton, biologically active mixtures (Pivovarov et al., 2017). At the same time, the hematological profile of blood in fish correlates with metabolic processes, affecting the oxidation-reduction capabilities of blood, oxygen transport, etc. (Sherman & Honcharova, 2022).

The authors note in their results that the physiological-biochemical, morphological and histological parameters improve with the additional use of natural substances, ingredients to the general diet of fish (Antache et al., 2014). Of course, such changes may not occur reactively compared to the use of chemical or hormonal drugs. However, it is important to consider that the qualitative and quantitative parameters of products at the end of cultivation are important for the aquaculture industry. Today, abiotic and biotic factors are transforming, climatic conditions, man-made loads on the ecosystem most often have a negative impact or make it difficult to obtain ecologically safe products (Honcharova & Bekh, 2023). When using vegetable proteins, amino acid imbalance problems may arise. Plant proteins contain 5–8 times less methionine and 2–3 times less lysine than fish body proteins. Therefore, the cultivation of juvenile Salmonidae fish on a diet with vegetable proteins gives poor results if it is not enriched with proteins of animal origin. Therefore, the topic of this manuscript, reflecting the result of the combination of several components, sources of protein, active substances necessary for the development of fish, is relevant, the results acquire scientific and practical value.

Summarizing, it is appropriate to note that environmentally safe aquaculture products require the absence of aggressive substances, hormonal preparations, etc. in the technological scheme. Therefore, phytopreparations, substances with a natural high protein content, biologically active substances of humic nature, natural vitamin complexes provide an opportunity to raise the level of aquaculture to a higher quality. The main results of the manuscript demonstrate the positive trend of this direction and certain advantages of "organic aquaculture", which to a certain extent corresponds to European standards and general norms of high-quality, and provides useful aquaculture products for consumers of any country.

Conclusion

Research has established that, based on the general diet, the additional use of *Spirulina*, *Hermetia illucens*, vermiculture, when growing rainbow trout contributed to positive ontogenesis results. The results presented in this work for study of the influence of natural food factors on the physiological, histological and biochemical parameters of trout demonstrated an improvement in the overall functional status and activation of homeostasis. A positive dynamic of increased growth rate was obtained, which was supported by better blood parameters (within the physiological norm). In the experimental group, the oxygen capacity of the blood of rainbow trout improved due to increased processes of hemoglobin synthesis and erythropoiesis. The established parameters correlated with the rate of development of trout in ontogenesis, which contributed to an increase in the average daily gain, increased safety.

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