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Trophic Modulation of Homeostasis and Adaptive Plasticity in Rainbow Trout (*Oncorhynchus mykiss*) under Industrial Environmental Conditions

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Abstract: The present study examined the mechanisms of physiological adaptation and maintenance of homeostasis in rainbow trout (*Oncorhynchus mykiss*) to the conditions of an industrial environment. The central focus of the study was the evaluation of the role of the trophic factor as a modulator of stress-limiting systems in the organism. The relevance of the research is due to the fact that industrial aquaculture represents a fundamentally new environment that differs from the species' evolutionarily formed range. Under conditions of chronic allostatic load caused by anthropogenic stressors, the fish's adaptive reserves are depleted, and key physiological parameters exceed the species' norm. We used the trophic factor as a modulator of the mechanisms maintaining homeostasis to compensate for the gap between the species' adaptive programs and the demands of the anthropogenic environment. The aim of the work is to expand the understanding of the limits of the physiological plasticity of rainbow trout (*Oncorhynchus mykiss*), the mechanisms of maintaining homeostasis under the influence of anthropogenic stressors, and the role of the trophic factor in modulating these mechanisms. The study was conducted on rainbow trout raised under industrial aquaculture conditions. The effect of the trophic poly-nutrient matrix (TPM), which includes eubiotics and corrective nutraceuticals, on a set of physiological parameters was assessed: hematological markers, cortisol levels, amino acid SCOR, fatty acid composition (omega-3 and omega-6 content), as well as cytogenetic stability. The results, which reflect the adaptive potential of the species in the industrial environment, were assessed according to the species norm. The use of TPM significantly improved hematological parameters: the hemoglobin content increased from 8.42 to 11.9 g/dl, red blood cells from 1.06 to 1.39×10^6/mm^3, platelets from 25.6 to 42.2×10^6/mm^3 ($p<0,05$); the cortisol level decreased to normal against the background of TPM, which indicates the suppression of excessive activation of the interregnal system, amino acid score was optimized, the proportion of omega-3 and omega-6 polyunsaturated fattyacids increased, which demonstrates the restoration of the body's ability to maintain metabolic balance and form energy reserves; the protein and lipid content increased, the water level in muscle tissue decreased; an increase in cytogenetic stability was noted. The results demonstrate that trophic modulation via a poly-nutrient matrix helps restore key homeostatic parameters -- hematological, neuroendocrine, metabolic, and cytogenetic — to the range of the species' norm. The study expands the understanding of the adaptive plasticity of *Oncorhynchus mykiss* and substantiates the effectiveness of using a trophic factor as a tool for maintaining the physiological well-being of aquatic organisms in an industrial environment.

Keywords: *Oncorhynchus mykiss*, Industrial environment, Homeostasis, Adaptation, Trophic modulator, Cortisol, Hematological markers, Cytogenetic stability

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Introduction

Industrial aquaculture creates a fundamentally new environment for aquatic organisms, which is radically different from the species' evolutionarily formed range in terms of a range of abiotic and biotic parameters. High stocking density, limited locomotor activity, man-made fluctuations in water quality, and handling stress create a situation of chronic allostatic stress for fish, overcoming the natural limits of the species' adaptive plasticity. Under these conditions, the study of how the trophic factor, the most evolutionarily conservative and controllable element of the environment, can modulate the physiological systems that ensure homeostasis and compensate for the gap between species' adaptive programs and the requirements of the anthropogenic environment becomes particularly important. Solving this problem has not only practical but also general theoretical significance for understanding the mechanisms of vertebrates' adaptation to extreme conditions.

Rainbow trout (Oncorhynchus mykiss) is one of the key species in modern aquaculture, combining high productivity with pronounced sensitivity to environmental factors (Neissi *et al*., 2020, 2024; Huang *et al*., 2023). From a zoological perspective, this species is of particular interest as a typical representative of the salmonid family, evolutionarily adapted to living in clean, oxygen-rich watercourses in temperate latitudes. The natural habitat of rainbow trout is characterized by relatively stable abiotic parameters: low water temperature, high dissolved oxygen content, moderate mineralization, and absence of sharp fluctuations in hydrochemical indicators (Zargar *et al*., 2020). Under artificially created conditions, particularly in an industrial environment, these parameters undergo significant transformation, and an anthropogenically altered environment only partially corresponds to the ecological optimum of the species (Lyubomirova *et al*., 2024).

As a result, fish activate adaptive reactions aimed at maintaining physiological homeostasis; however, with prolonged exposure to adverse factors, compensatory mechanisms may become depleted, which manifests as disruptions in metabolic and hematological parameters, as well as an increase in the stress reaction (Tomás-Almenar *et al*., 2020; Neissi *et al*., 2024).

The most sensitive indicator of well-being in fish is the blood system, which provides oxygen transport, immune defence, and maintenance of acid-base balance. Hematological parameters such as red blood cell concentration (RBC), haemoglobin (Hb) content, haematocrit (Hct), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) serve as reliable markers of adaptive changes and reflect the ability of organism to cope with hypoxic and other stress loads (Lyubomirova *et al*., 2024).

In rainbow trout, as an oxygen-loving species, hematological parameters are particularly informative: a decrease in hemoglobin and hematocrit often indicates a disruption of oxygen-carrying function, while an increase within the normal range reflects a compensatory enhancement of erythropoiesis in response to chronic oxygen deficiency or other adverse effects (Lyubomirova *et al*., 2024).

In industrial conditions, fish hematological parameters are often recorded at the lower limits of reference ranges, which indicates a strained state of compensatory mechanisms and a potential decrease in the overall resistance of the fish (Park *et al*., 2023; Lyubomirova *et al*., 2024).

Identifying and maintaining the species-specific norm of hematological parameters under

artificial conditions is important not only for practical fish farming but also has fundamental significance, as it allows us to assess the limits of a species' physiological plasticity and the degree of its adaptation to an anthropogenically created environment (Blouin *et al*., 2010; Nenciu *et al*., 2022; Lyubomirova *et al*., 2024).

A significant indicator of the physiological status of rainbow trout is the level of cortisol, a key glucocorticoid, that regulates the stress response in bony fish. Unlike mammals, whose cortisol is synthesized in the adrenal cortex, in fish the main source of this hormone is the interrenal cells. Its secretion is due to the activation of the hypothalamic-pituitary-interrenal axis (Barton *et al*., 1987; Saulnier *et al*., 2021).

Cortisol performs a wide range of functions: it mobilizes energy reserves, modulates the immune response, regulates the water-salt balance, and influences behavior. In aquaculture, cortisol levels serve as a universal marker of stress load caused by such technological factors as high stocking density, frequent handling of fish, sudden changes in temperature and water quality, as well as noise and light exposure (Barton *et al*., 1987; Cassidy *et al*., 2016).

For adult rainbow trout, cortisol concentrations in the range of 10–20 nmol/L are considered normal; exceeding these values indicates the development of an acute or chronic stress response, which can lead to a decrease in growth rates, impaired feed conversion, increased susceptibility to infections, and, ultimately, reduced survival. It is important to note that individual variability in cortisol levels can be high, reflecting the heterogeneity of the physiological state of individuals in the population (Culbert and Gilmour, 2016; Saulnier *et al*., 2021).

In recent years, increasing attention has been paid to finding ways to minimize stress load and optimize the physiological status of fish and other hydrobionts using the trophic factor. We have developed a synergistic complex of nutraceuticals (Turaeva and Romanova, 2022; Lyubomirova *et al*., 2024) with matrix properties.

For convenience, we have introduced the term trophic poly-nutraceutical matrix (TPM), and to highlight its specific features, we have designated it as a matrix that represents a synergistic complex of eubiotics and nutraceuticals, including: a probiotic + an adaptogen + vitamins + amino acids. This complex is capable of programming and correcting physiological processes by modulating the metabolism of fish. This matrix optimizes the diet, modulates the body's physiological responses, stabilizes hematological, biochemical, and cytogenetic parameters, and alleviates the manifestations of stress reactions.

The objective was to investigate the effects of TPM on homeostasis indicators in rainbow trout and to assess its ability to shift the physiological state of the fish closer to the species norm, thereby increasing their resistance to adverse factors of the industrial environment. As part of the study, hematological parameters (RBC, Hb, Hct, MCV, MCH, leukocytes, platelets), the cortisol content in fish blood, and the content of protein, fats, amino acids, and polyunsaturated omega-3 and omega-6 fatty acids in the muscles of fish raised under TPM conditions were examined as indicators of adaptive plasticity.

The obtained results were interpreted from the perspective of species biology, considering them as manifestations of adaptive reactions and mechanisms for maintaining homeostasis in an anthropogenically created industrial habitat.

The relevance of the study is determined by the need for an in-depth understanding of the physiological adaptations of rainbow trout to aquaculture conditions (since fish do not have evolutionarily developed mechanisms for adapting to an industrial habitat) and the development of scientifically grounded approaches to managing its well-being.

From a fundamental perspective, the study aims to expand the understanding of the limits of the physiological plasticity of *Oncorhynchus mykiss*, the mechanisms of maintaining

homeostasis under the influence of technological stressors, and the role of nutrition as a factor modulating these mechanisms.

The results obtained will not only make it possible to clarify the species-specific norms for hematological and metabolic parameters and stress levels under industrial conditions but also to propose objective criteria for assessing the well-being of rainbow trout based on integral physiological markers.

The practical significance of the work lies in proving the possibility of using TPM as a tool for stabilizing physiological homeostasis and enhancing the adaptive potential of fish in aquaculture. The study is devoted to addressing an important fundamental problem: identifying the patterns of organisms' adaptation to changing environmental conditions and the quantitative characteristics of species-specific norms of physiological reactions that ensure this process.

The aim of the present study was to expand the understanding of the limits of physiological plasticity in *Oncorhynchus mykiss*, the mechanisms of maintaining homeostasis under the influence of technological stressors, and the role of the trophic factor that modulates these mechanisms.

Materials and Methods

Healthy trout with an average weight of 300 g, of the same age and size, were selected for the experiment from the Yantarny Ruchey farm, Bogdashkino, Ulyanovsk Oblast, Russia. The trout underwent a one-month adaptation period under standard conditions of industrial aquaculture. The fish were kept in autonomous fish-rearing modules at a water temperature of 16–18 °C; the oxygen content in the water was 80–100 % saturation, and the pH was about 7.0.

From the fish that had undergone quarantine and adaptation, experimental and control groups were formed, each consisting of 30 individuals. Blood sampling was carried out under sedation to minimize stress and avoid injuring the fish. For this purpose, clove oil (eugenol) was used at a concentration of 0.05% until the fish reached a loss of balance and reduced motor activity.

Microchips were used to tag the fish. Blood was taken from the caudal vein; for hematology and micronucleus testing into Lab-Vac vacuum tubes with K3 EDTA, purple lid, 2 ml, 13x75 mm, 143021375. Hematological parameters were determined by standardized methods. To determine cortisol, test tubes without anticoagulant RHN 2019/8488 (red lid) were used.

To assess cytogenetic stability, a micronucleus test was used on fish erythrocytes (Anbumani and Mohankumar, 2012; Ting Goh *et al*., 2023). To carry out the test, blood smears were prepared and fixed in a mixture: 1:1 — ethyl alcohol/diethyl ether for 20 min to stabilize cellular structures and prevent chromatin degradation; then they were stained with azure-eosin, rinsed with distilled water, and dried. Microscopy and identification of micronuclei types were performed under a light microscope at magnification ×1000 (oil immersion).

Micronuclei are structures in the cytoplasm that are separate from the main nucleus or connected to it by a chromatin filament; they have a round or oval shape and are stained like the main nucleus; they do not exceed 30% of the main nucleus and do not have a nuclear membrane, which the main nucleus does have. For each fish, 3 smears were prepared. At least 2,000 red blood cells were examined in each preparation. The frequency of micronuclei was expressed in per mille (‰) of the total number of examined red blood cells.

Micronuclei were differentiated based on their morphology. Type A micronuclei were small, round, isolated structures that had no connection to the nucleus but were stained like the nucleus. They are located autonomously in the cytoplasm, their diameter does not exceed 1/20 of the diameter of the main nucleus, and they are formed from acentric chromosome fragments that remained outside the daughter nuclei due to a

disruption of mitosis.

Type B micronuclei have the structure and staining characteristics of the main nucleus (homogeneous chromatin, clear outlines). This more complex type of nuclear pathology is associated with the delay of entire chromosomes in anaphase. This is how the aneugenic effect manifests itself. This type of nuclear pathology is also considered a sign of chromosome segregation disorders due to spindle dysfunction.

Type C was characterized by large micronuclei, reaching 1/3 of the diameter of the main nucleus. Their size is due to the inclusion of several fragments, entire chromosomes, or the fusion of small micronuclei. Sometimes such structures are the result of severe or chronic genome damage that the cell has not had time to eliminate.

Protein, fat, water content, fatty acids, and amino acids were determined in the fish muscle tissue. The contents of protein, water, fat, and fatty acids were determined in trout muscle at RSBU Simbirsk Reference Center for Veterinary Medicine and Food Safety using generally accepted methods, in accordance with the current standards.

The determination of the fatty acid composition was carried out in accordance with GOST 31663-2012 Interstate Standard. Determination of the mass fraction of methyl esters of fatty acids was performed by gas chromatography. When studying fatty acids, a combined sample was used in accordance with GOST 31339-2006 – Interstate Standard Fish, non-fish objects and products from them. When preparing the fish for analysis, it was filleted, the internal organs, skin, and bones were removed, and the fillet was homogenized. The separation and identification of the fatty acid phase were carried out using the chromatograph (Khromatek-Crystal 5000.1, Russia). For identification, a mixture of methyl esters of fatty acids Supelco 37 component FAME MIX (Sigma-Aldrich, Germany) was used. Special software was used to control the analysis modes, record chromatograms, and process the obtained information.

The amino acid content was determined at the FSBEI HE, Saratov State University of Genetics, Biotechnology and Engineering in accordance with the Interstate Standard GOST 31480-2012, Compound feeds, compound feed raw materials. Determination of amino acid content (lysine, methionine, threonine, cystine and tryptophan) was done by capillary electrophoresis method and GOST R 55569-2013 Feeds, compound feeds, compound feed raw materials. Determination of proteinogenic amino acids (Arginine, Lysine, Tyrosine, Phenylalanine, Histidine, Leucine and Isoleucine, Methionine, Valine, Proline, Threonine, Serine, Alanine, Glycine, Glutamic acid and glutamine, Aspartic acid and asparagine, Cystine) was done by using capillary electrophoresis In the study of amino acids, a combined sample was used in accordance with GOST 31339-2006 Separation and identification were performed using the Kapel-105M capillary electrophoresis system (analyzer) (manufactured by Lumex-Marketing LLC, Russia).

The calibration curve was constructed using a set of 18 L-amino acids (Ala, Arg, Asn, Val, His, Gly, Glu, Leu, He, Lys, Met, Pro, Ser, Tyr, Thr, Phe, Cys-Cys) from LLC Lumex-Marketing.

The tryptophan content was determined in accordance with GOST. 31480—2012. The amino acid score (AAS) was calculated using the generally accepted method: AAS (Amino Acid Score) = (the amino acid content in 1 g of trout protein is related to the content of the same amino acid in 1 g of the reference protein according to FAO/WHO) × 100% (Storebakken, 2025).

The cortisol level was determined at the Research Medical-Biological Center, Ulyanovsk State University. The cortisol concentration was determined using a competitive enzyme-linked immunosorbent analysis (ELISA) with a commercial Salimetrics kit (State College, PA, USA). The analysis was performed in 3 replicates.

Table 1: The effect of the trophic poly-nutrient matrix (TPM) on the hematological parameters of rainbow trout

Parameters	Reference values	Control group	TPM group
Red blood cells (RBC)	1.01 – 2.04 × 10^6/µl	1.06±0.06	1.39±0.09*
White blood cells (WBC)	31.32 – 90.60 × 10^3/µl	32.23±0.63	56±1.2*
Hematocrit (Hct / PCV)	29 – 40 %	32±1.3	36±2.1
Mean corpuscular volume (MCV)	~270 fL	302±7.3	259±4.8*
Mean corpuscular hemoglobin (MCH)	60 – 90 pg	79.4±6.2	85.6±7.3
Hemoglobin (Hb)	8.32 – 12.28 g/dl	8.42±0.41	11.9±0.54*
Thrombocytes (TC)	23.00 – 68.00 × 10^6/µl	25.6±1.4	42.2±1.9*

*p<0.05

A standard hormone solution was used to construct the calibration curve; the kit sensitivity was less than 0.007 µg/dl (Ghassemi Nejad *et al.*, 2019). The results are expressed as nmol/L.

The trophic poly-nutraceutical matrix included: the probiotic Aquasporin – 2 g/kg, the adaptogen Trekrezan 0.04 mg/kg, and the vitamin-amino acid complex Chiktonik 2 ml/kg. All calculations for eubiotics and nutraceuticals were carried out per 1 kg of feed.

The research results were subjected to biometric analysis using traditional reliability criteria.

Results

In the first stage of the research, we investigated the effect of the trophic poly-nutrient matrix (TPM) on the blood parameters of rainbow trout; the results obtained are presented in Table 1. The research results revealed a positive effect of TPM on indicator hematological parameters. In particular, red blood cells and hemoglobin content, as well as the mean corpuscular volume of red blood cells, increased significantly. Before using the feed matrix, these indicators were at the lower limits of the normal range. The content of leukocytes and platelets also increased as compared to normal values.

In the next stage, the nutritional value of meat of fish that received TPM was studied. The experimental data were compared with the average values for trout. The main components of fish muscle tissue that determine its properties are water, protein, and fat; water determines the moisture level of fish meat, affects the taste and quality of the fillet, and its content decreases as the fat content of the fish increases (Crouse *et al.*, 2023).

Trout is classified as a protein-rich and high-protein fish, depending on its protein content. Depending on its fat content, marketable trout can be classified as either low-fat or medium-fat fish. The results are presented in Table 2.

Table 2 shows that TPM (trophic poly-nutraceutical complex) when fed to rainbow trout provides it with the category of a high-protein, medium-fat product and reduces the moisture content of its meat, increasing the gross and true energy value.

In the present study amino acid analysis was carried out on the muscle tissue of fish that had received TPM as part of the feed. The research results are expressed in the form of amino acid score (AAS) (Fig. 1).

The control group of trout received traditional compound feeds, and two essential limiting amino acids, methionine and tryptophan, were detected in AAS. In the experimental group treated with TPM, the levels of methionine and tryptophan increased above the levels recommended by the

Table 2: Protein, fat, and water content in rainbow trout muscles with and without TPM application

Parameters	Overall reference range	Control group	TPM group
Moisture, %	71–74	75±0.5	72±0.4*
Protein, %	19.0–21.0	18.3±0.2	21.2±0.3*
Lipid, %	3.0–6.0	4.8±0.4	6.6±0.4*
Protein content category	High-protein (> 19% protein) Medium-protein (< 19% protein)	Medium-protein	High-protein
Lipid content category	Low-fat (3–5% lipid) Medium-fat (6–7% lipid)	Low-fat	Medium-fat

*p<0.05

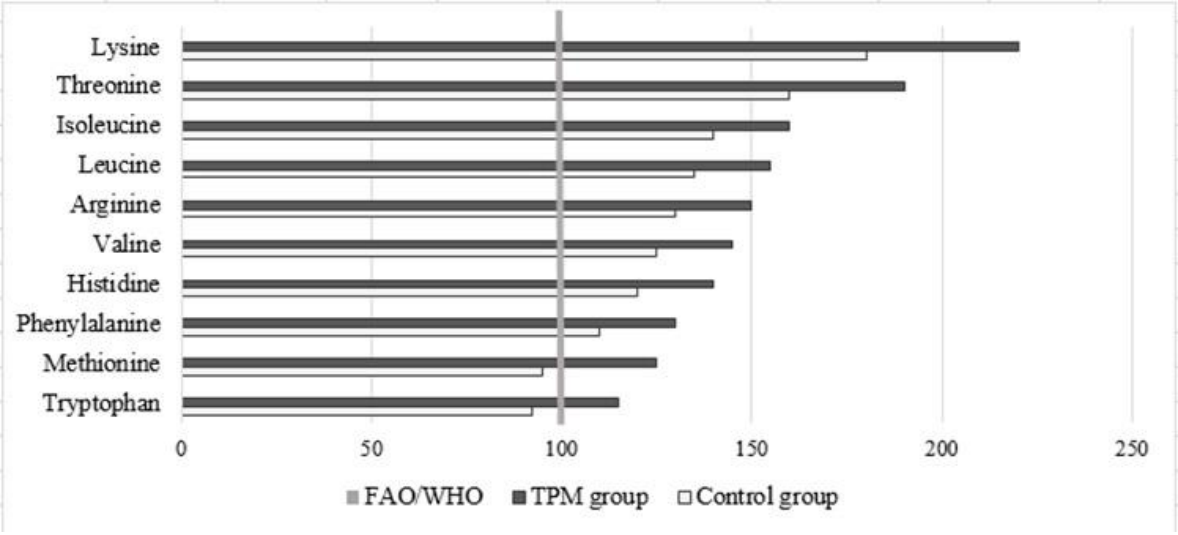


Fig. 1: The amino acid score of rainbow trout when using TPM.

FAO/WHO (the levels of amino acids recommended by the FAO/WHO are assumed to be 100%).

Rainbow trout have 10 essential amino acids: Lys, Arg, Met, Thr, Leu, Ile, Trp, His, Phe and Val. Valine and isoleucine are usually well-balanced in classic compound feeds, and arginine is present in sufficient quantities in feeds based on fish meal protein. Recent studies have shown that lysine, arginine, threonine, leucine, and tryptophan are better absorbed when a probiotic is added to fish feeds (Liu *et al.*, 2021; Shadyeva *et al.*, 2023).

Trout is considered a species in which the AAS for almost all essential amino acids exceeds the FAO and WHO standards; however, in an industrial environment, as we have demonstrated, this depends on the composition of the feed.

Methionine and tryptophan (Fig. 1) were found to be deficient in the profile of essential amino acids in the trout of the control group. Due to the ideal amino acid profile of fish protein, even a slight deficiency of one essential amino acid sharply reduces the absorption of the others, this is the so-called first-limiting amino acid effect. Therefore, any deficiency of an amino acid must be eliminated. In our study, this problem was solved by using TPM, largely due to the probiotic in its composition (Shadyeva *et al.*, 2023).

The leading role of a probiotic in correcting the amino acid score has been reported by Niu *et*

Table 3: The ω-3/ω-6 ratio in intact trout and against the background of using a poly-nutrient matrix

PUFAs	Control group	TPM group
Total PUFA ω-3, %	6.729	7.143
Total PUFA ω-6, %	16.913	17.087
ω-3/ω-6 ratio	0.3979	0.418
In the experimental group, the ω-3/ω-6 ratio of PUFAs exceeded that in the control group by 5.05%		

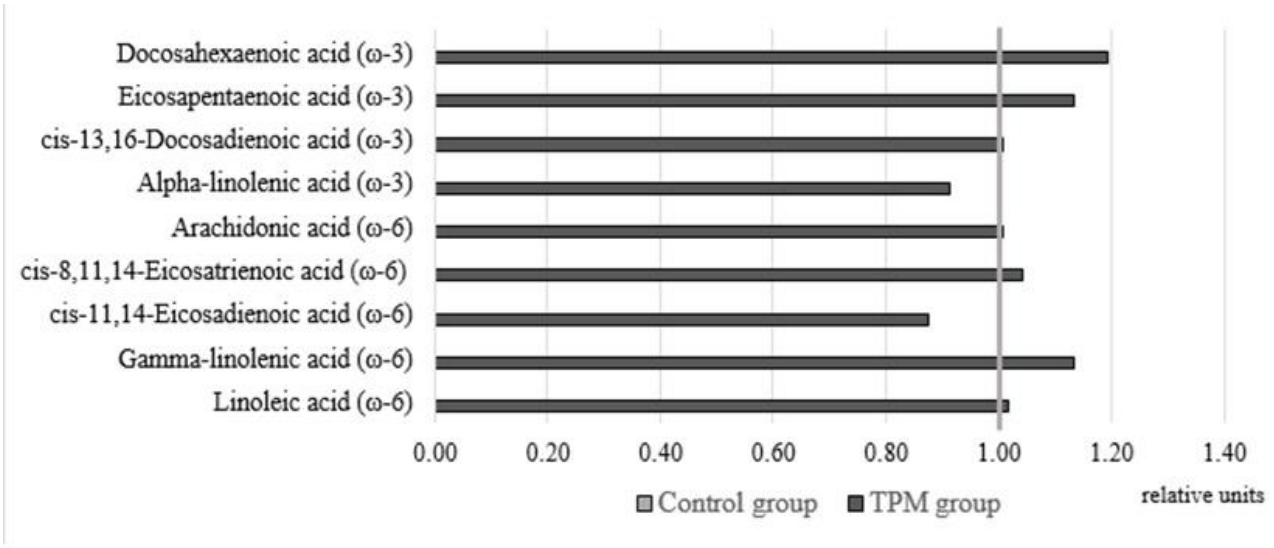


Fig. 2: The content of PUFAs of the ω-3 and ω-6 families against the background of using a feed poly-nutrient matrix.

al. (2024), who found that a probiotic increases the level, digestibility and bioavailability of amino acids which affects the expression of genes of transport proteins.

At the next stage of the research, the indicators of fatty acid metabolism were studied; the composition of these acids, along with amino acids, determines the biological and nutritional value of fish and its importance in the prevention of human cardiovascular diseases.

The focus was on the content of polyunsaturated fatty acids (PUFAs) of the ω-3 and ω-6 families, as well as their ratio. The results are presented in Table 3 and Figure 2.

As the contents of the studied amino acids vary significantly in absolute value, the data were normalized to construct a generalized graph: the values of the control group were taken as 1, and the data for the experimental group were expressed proportionally in relative units.

The results of the present study showed that the use of TPM increased the ω-3/ω-6 ratio by 5.05%. Perhaps the observed increase is not very significant, but it confirms the fact of a modulating positive effect on lipid metabolism parameters.

In the next stage of the work, the effect of TPM on cytogenetic stability in the trout organism was studied. For this purpose, a micronucleus test was

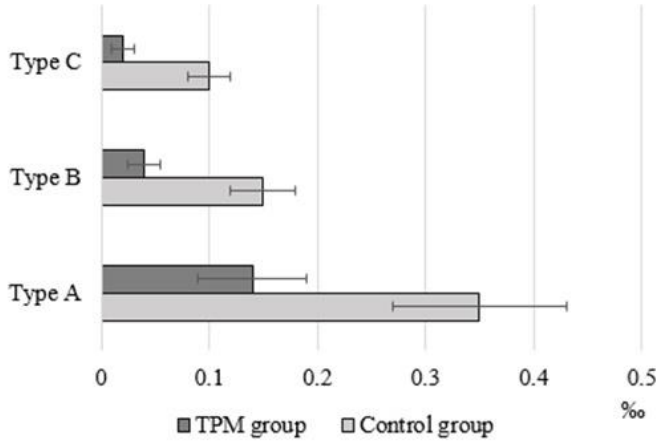


Fig. 3: Indicators of cytogenetic stability in trout when using a feed matrix ($p<0.05$).

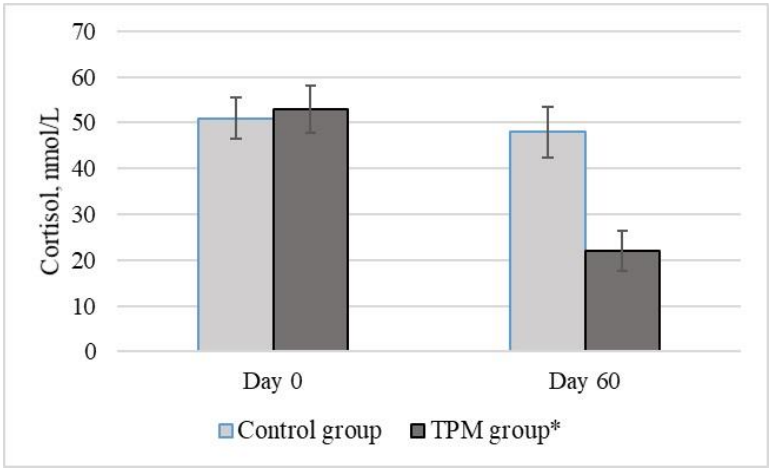


Fig. 4: The effect of TPM on cortisol levels in the blood of rainbow trout.

used. The research results are presented in Figure 3.

Erythrocytes were used as a cellular model. The appearance of micronuclei in cells is a sign of cytogenetic disorders: they are formed from chromosome fragments or entire chromosomes that end up outside the daughter nuclei during cell division. A high proportion of such cells indicates genotoxic stress and is regarded as a violation of cytogenetic homeostasis of varying severity.

The present study showed that the proportion of cells with type A micronuclei at the start of the experiment in intact fish and in fish from the control group receiving a standard diet averaged 0.35‰, while against the background of TPM, the pathology rate decreased to 0.14‰. In the control group, cells with type B and C micronuclei occurred at an average frequency of approximately 0.15‰ and 0.1‰, respectively, while in the group receiving the probiotic, this nuclear pathology occurred at a frequency of 0.04‰ and 0.02‰, respectively.

Thus, the results showed that in fish, the enteral administration of TPM normalizes cytogenetic homeostasis and more than doubles the resistance to the genotoxic effects of the environment.

A decrease in the proportion of cells with micronuclei is considered an indicator of fish health and viability: less DNA damage means

better growth and higher resistance to diseases and stress.

In the present study, the effect of TPM on the level of the stress hormone cortisol in fish was assessed. In fish, cortisol is considered an indicator of the stress response. Normally, the level of the stress hormone in adult rainbow trout is 10-20 nmol/L (Pérez-Sánchez *et al.*, 2021).

According to the results of our studies, the cortisol levels in fish at the start ranged from 45 to 60 nmol/L and amounted to 51±4.6 nmol/L in the control group and 53±5.1 nmol/L in the experimental group (Fig. 4).

In fish, cortisol is considered an indicator of the stress response: its concentration increases rapidly when exposed to adverse environmental factors, during manipulations with fish, under conditions of poor water quality, etc. The high level of cortisol in the control group did not change significantly during the experiment (48±5.6 nmol/L), which indicates a pronounced stress load due to the technological factors of industrial aquaculture.

In fish, against the background of TPM, the cortisol level was significantly lower and practically dropped to the normal level, amounting to 22±4.3 nmol/L. The effect of the matrix manifested itself in a stress-protective effect.

Discussion

The biology of any fish species in an industrial environment undergoes significant changes (Ma *et al.*, 2023). Industrial fish farming requires constant monitoring to regulate both environmental parameters and the trophic factor (Pang *et al.*, 2026).

In this study, we investigated the possibility of systemic correction of homeostasis in *Oncorhynchus mykiss* in an industrial environment using trophic complex that can modulate physiological processes in fish and called it a trophic polynutraceutical matrix (TPM), in which the total effect exceeds the sum of the effects of individual substances. The matrix includes the probiotic Aquasporin (based on strains of *Bacillus subtilis* and *Bacillus licheniformis*), the adaptogen Trekrezan, and the vitamin-amino acid complex Chiktonik. When designing the matrix, we were guided by the mechanism of action of the substances included in it.

Probiotics suppress pathogenic microflora, synthesize exogenous enzymes (proteases. lipases). This increases the feed conversion ratio (FCR) and releases free amino acids for deposition in muscles. Adaptogens reduce cortisol levels in fish, and the released stress energy is redirected by the body to maintain homeostasis and support anabolic processes.

Vitamins B, C, and E are coenzymes in many processes. Vitamin E is a powerful antioxidant; it protects polyunsaturated fatty acids from oxidation in fish tissues. Vitamin C also neutralizes free radicals and restores other antioxidants, such as vitamin E. Both vitamins enhance each other's effects, protecting the fish's body from technological stressors; B-group vitamins are critical for fat utilization.

Free amino acids act as chemoattractants, increasing feed palatability, and are quickly incorporated into muscle protein synthesis, bypassing the deamination stage.

First, let us consider the influence of the trophic factor on hematological parameters from the perspective of reflecting oxygen transport adaptation to the technological regimes for keeping fish. Hematological parameters were considered as quantitative characteristics of an evolutionarily formed adaptation to oxyphilic conditions. Indicators such as RBC, Hb, Hct, MCV, and MCH reflect the species' ability to supply tissues with oxygen under fluctuations in temperature and O_2 content in the water (Latief *et al.*, 2025).

During the study, it was found that in trout, the hemoglobin content and its total amount in the erythrocyte increased against the background of TPM. The number of erythrocytes, the level of leukocytes and thrombocytes increased. This

means that nutraceutical support allowed the species *Oncorhynchus mykiss* to reach a higher level of oxygen-transport function, characteristic of its ecological niche.

In the control group, the indicators were at the lower limit of the norm, which indicates that the compensatory mechanisms were strained and that the fish were existing in a state of minimally sufficient oxygen supply without the use of TPM.

TPM does not stimulate individual parameters; rather, it promotes the normalization of the entire range of hematological indicators, restoring the typical blood picture for the species.

There is reason to believe that vitamins and amino acids have a greater influence on hematological parameters in TPM, while probiotics and adaptogens have an indirect effect (Ahmed *et al.*, 2020).

Let's consider how the correction of physiological processes using TPM affected the protein, fat, and water content in the fish. The results showed that the protein and fat content in the trout muscles increased against the background of TPM, while the water content decreased.

The decrease in water content in the muscles (from 75% to 72%) and the increase in protein content (from 18.3% to 21.2%) and fat content (from 4.8% to 6.6%) indicate a redistribution of resources between growth, fat accumulation, and the maintenance of physiological functions; this is an indicator of how a species redistributes resources under new conditions. They make it possible to compare the trophic status of fish in different environments and assess how well the diet complies with the species norm.

Let us consider how the use of TPM affected the cytogenetic stability and resistance of the species to genotoxic environmental factors. It was established that against the background of TPM, the proportion of micronuclei of all types — A, B, and C — significantly decreased.

The main role in this process belongs to vitamins and TPM amino acids; to a lesser extent, probiotics and adaptogens contribute to it. Many vitamins work as antioxidants or participate in DNA repair processes: vitamins C, E, B2, B5, B7, B9, B12. There is reason to believe that it is vitamins in general that provide protection of genetic material, since a link has been identified between their levels in the body and the frequency of nuclear pathology in the form of micronuclei (Serrano-García *et al.*, 2018; Li *et al.*, 2022).

Amino acids also exhibit an antimutagenic effect. Among amino acids, cysteine, glycine, tryptophan, lysine, and arginine have the most pronounced antimutagenic effect. These amino acids are capable of reducing the clastogenic effect of chemical substances by decreasing the proportion of micronuclei in the experiment; the mechanism is often associated with modulation of the pro-oxidant–antioxidant balance or support of repair systems (Ahmed *et al.*, 2020).

The role of probiotics in the antimutagenic effect is rather supportive. They improve the state of the bowel microbiota, reduce systemic inflammation and toxic load, creating favorable conditions for genome stability. But themselves, they do not save from DNA damage. Direct evidence that adaptogens reduce the proportion of micronuclei has not been found in the available scientific literature.

A decrease in the frequency of micronuclei is an indicator of increased viability and adaptive potential. The less DNA damage there is, the higher the resistance to diseases and stress, and the more effective the growth and survival.

Let us analyze the nature of TPM's effect on amino acid metabolism in fish. Against the background of TPM, due to the modulation of endogenous synthesis, the level of limiting amino acids in trout muscles returned to normal values.

Compared to TPM, traditional feeds often provide an excess of non-essential amino acids (glycine, alanine) and create a deficiency of essential ones, such as lysine, methionine, and threonine (Sorgeloos *et al.*, 2003).

The limiting amino acids (methionine and tryptophan) we identified in the control group are considered not as a feeding standard but as key components of the species' protein metabolism strategy. Methionine is involved in antioxidant defense and lipid metabolism, reducing the load on nitrogen metabolism; tryptophan, as a precursor of serotonin, affects the regulation of stress and feeding behavior. Therefore, eliminating their deficiency through TPM allows the fish to use resources more efficiently for growth, maintaining immunity, and adapting to stress.

Amino acids with branched side chains (valine, isoleucine) play a special role: in fish, a significant part of their metabolism occurs not in the liver, but in the muscles and gills, which reflects the species-specific distribution of metabolic loads in the industrial environment.

Our results are in agreement with the studies of Niu *et al.* (2024), who showed that the addition of probitotic to young trout feed increases the digestibility and level of amino acids in serum, and affects the expression of transport protein genes. Against the background of probiotics, AAS evens out, and the bioavailability of amino acids increases (Niu *et al.*, 2024), apparently due to the fact that probiotics loosen the peritoneal membrane of the intestine of aquatic organisms, increasing the absorption area of protein monomers (Sorgeloos *et al.*, 2003).

From the perspective of fish physiology, the effect of the complex of nutraceuticals we used on amino acid metabolism should be considered in the context of adaptive responses, maintenance of homeostasis, and the implementation of normal physiological responses.

When fish are deficient in essential amino acids in their diet, their bodies experience metabolic stress, which is accompanied by the activation of stress signaling pathways (GCN2/ATF4) and a disruption of nitrogen balance, thereby taking the system out of the physiological norm.

The nutraceutical complex replenishes the limiting trophic factor, helps eliminate the bottleneck in metabolic pathways, and triggers an adaptive restructuring that restores the transport and regulatory mechanisms of amino acid metabolism, normalizes protein synthesis and nitrogen metabolism indicators, and brings key physiological parameters back to normal.

The balance of nutraceutical correction is critically important, as an excessive intake of certain components can induce new deviations from homeostasis, whereas an optimally selected complex ensures stable adaptation of the body to the industrial environment and shifts limiting essential amino acids into the category of physiologically provided ones, normalizing homeostasis indicators.

Now it is necessary to assess the effect of TPM on lipid metabolism. It has been established that the nutraceutical complex successfully transforms the fatty acid profile, significantly increasing the Omega spectrum. In this case, the fish body is essentially a «living bioreactor» that synthesizes the long–chain EPA eicosapentaenoic acid and DHA-docosahexaenoic acid from its precursors, alpha-linolenic acid (ALA). Adaptogens and vitamins in this process act as catalysts for the work of desaturases and elongases.

Due to the optimization of lipid metabolism, the atherogenicity index decreases, since the proportion of saturated fatty acids decreases: myristic and palmitic, which increase cholesterol in humans. In our studies, the ratio of ω−3/ω−6 increased by– 5.05%. Vitamin E - tocopherol in the matrix prevents lipid peroxidation in fish during its lifetime. This ensures that a person consuming fish raised under industrial conditions will receive ω−3 and ω−6 in a biologically active form, rather than their breakdown products. This is important because consuming fish with a high ω−3 and ω−6 index is correlated with a reduced risk of cardiovascular diseases.

It has been established that arginine, the content of which increases in high-quality fish

protein, is a substrate for the synthesis of nitric oxide (NO). NO dilates blood vessels, regulates blood pressure, and prevents platelet aggregation. High-quality protein provides the building material for the vascular wall, and DHA/EPA ratio inhibits pro-inflammatory cytokines. This is how anti-inflammatory synergy is achieved, delivering a double blow to the pathogenesis of atherosclerosis.

We have also obtained results regarding the effect of TPM on the stress level in fish. Against the background of the nutraceutical complex, the level of the stress hormone cortisol decreased by 54% compared to the control; therefore, the effect of the matrix manifested itself in a stress-protective action.

In aquaculture, the issue of reducing cortisol levels — the main stress hormone in fish — has been extensively studied. Cortisol levels are regulated by the hypothalamus- pituitary-interrenal axis, or HPI axis (Herrera *et al.*, 2019).

In TPM, adaptogens have the most pronounced and direct effect in suppressing cortisol production. They contain biologically active compounds that directly modulate the functioning of the HPI axis and have a powerful anti-inflammatory effect (Santos *et al.*, 2022).

Probiotics occupy the second most important position, but their mechanism of action is fundamental. They work through the microbiota-bowel-brain axis, strengthening the intestinal epithelial barrier and preventing endotoxins from entering the bloodstream. This reduces systemic inflammation, which is a trigger for cortisol production. Bacteria themselves produce neuroactive substances (gamma-aminobutyric acid, serotonin) that inhibit the HPI axis. Probiotics are most effective in reducing basal (background) cortisol levels and increasing overall stress resistance under prolonged (chronic) exposure to aquaculture-related technological factors.

Amino acids such as tryptophan, arginine, and glutamine reduce stress by exerting a strong targeted effect. Tryptophan is a direct precursor of serotonin and melatonin; an increase in serotonin levels in the brain directly inhibits (slows) the activity of the HPI axis, reducing cortisol release. Arginine stimulates the production of nitric oxide (NO), which modulates the stress response. Glutamine supports intestinal integrity and serves as fuel for immune cells, indirectly reducing stress. Adding tryptophan to the feed reduces cortisol levels in salmon and carp. The effect largely depends on the balance of other amino acids in the diet (Herrera *et al.*, 2019).

Vitamins play a supporting role; they are critically important for combating the effects of stress. High levels of cortisol cause powerful oxidative stress, which is accompanied by the formation of free radicals that destroy cells. Vitamins C and E act as powerful antioxidants. The TPM we have developed includes four levels of stress protection.

From an ecological perspective, cortisol levels are an integral indicator of how well environmental conditions match the ecological tolerance of a species.

Summarizing the results obtained, it should be emphasized that TPM affects on not just one, but several metabolic pathways, ensuring optimization of hematological parameters, cytogenetic stability, reduction of stress levels, modulation of the metabolism of essential amino acids, polyunsaturated fatty acids, and overall maintenance of health.

Conclusion

The theoretical significance of the study lies in the fact that it demonstrates that the comprehensive nutraceutical effect of TPM can bring the key indicators of fish health closer to the species-specific norm typical of *Oncorhynchus mykiss* in its natural habitat.

All identified changes are interrelated and reflect the integrative nature of the adaptations of *Oncorhynchus mykiss* to an artificial habitat in industrial aquaculture. A reduction in stress levels

creates conditions for more efficient use of nutrients, which is manifested in the normalization of hematological parameters and the improvement of amino acid and fatty acid profiles, as well as an increase in cytogenetic stability. In turn, a balanced supply of essential nutrients in the form of TPM helps maintain cellular homeostasis and stress resistance, forming a positive feedback loop in the fish's body.

In the present study, we used a systemic interpretation of the processes and considered not a set of individual indicators of the species *Oncorhynchus mykiss*, but their biological integrity at the organismal level, which has a certain reaction norm and limits of adaptive plasticity of metabolic processes.

The obtained results expand the understanding of the limits of the adaptive plasticity and ecological tolerance of *Oncorhynchus mykiss* in an industrial environment and bring together physiological criteria for assessing the well-being of fish, which is an important task for modern fundamental and applied zoology and ecological physiology.

The study demonstrates that the combined synergistic effect of eubiotics and nutraceuticals in an industrial environment can bring key indicators of physiological well-being closer to the species-specific norm characteristic of *Oncorhynchus mykiss* in its natural habitat.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the FSBEI HE Ulyanovsk State Agrarian University 432017, Ulyanovsk, Novyi Venets Boulevard 1, Russia.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

References

Ahmed I, Reshi QM and Fazio F. (2020) The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquacult Int. 28(3): 869-899.

Anbumani S and Mohankumar MN. (2012) Gamma radiation induced micronuclei and erythrocyte cellular abnormalities in the fish *Catla catla*. Aquat Toxicol. 122-123: 125-132.

Barton BA, Schreck CB and Barton LD. (1987) Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis Aquat Org. 2: 173-185.

Blouin MS, Virginie T, Cooper B, Amarasinghe V, Cluzel L, Araki H and Grunau C. (2010) No evidence for large differences in genomic methylation between wild and hatchery steelhead (*Oncorhynchus mykiss*). Canadian J Fisheries Acquat Sci. 67(2): 217-224.

Cassidy AA, Saulnier RJ and Lamarre SG. (2016) Adjustments of protein metabolism in fasting Arctic charr *Salvelinus alpinus*. PLoS One 11: e0153364.

Crouse C, Knight A, May T, Davidson J and Good C. (2023) Performance, processing yields, and fillet composition of specific United States diploid and triploid rainbow trout (*Oncorhynchus mykiss*) lines reared in a semi-commercial scale freshwater recirculating aquaculture system. Aquacult Rep. 33: 101794.

Culbert BM and Gilmour KM. (2016) Rapid recovery of the cortisol response following social subordination in rainbow trout. Physiol Behav. 164: 306-313.

Ghassemi Nejad A, Bertotto D and Mazzi D. (2019) Cortisol analysis in market-frozen rainbow trout (*Oncorhynchus mykiss*). Fisheries Aquacult. 29(6): 404-412.

Herrera M, Mancera JM and Costas B. (2019) The use of dietary additives in fish stress mitigation: Comparative endocrine and physiological responses. Front Endocrinol (Lausanne) 10: 447.

Huang X, Dalsgaard J, Aalto SL, Lund I and Pedersen PB. (2023) Influence of dietary phosphorus onorthophosphate accumulation in recirculating aquaculture systems with rainbow trout (*Oncorhynchus mykiss*). Aquacult Engineer. 103: 102363.

Latief T, Farooz AB, Tasaduq HS, Adnan A, Bilal AB, Kumar A, Ishrat M, Hudisa B and Junaid K. (2025) Age and sex related variations in hematological

profile of rainbow trout (*Oncorhynchus Mykiss*) from Kashmir aquaculture systems. J Adv Biol Biotechnol. 28 (8): 1650-1661.

Li Y, Wang X, Zhang Y and Liu Y. (2022) Dietary vitamin E deficiency induces micronuclei in erythrocytes of common carp (*Cyprinus carpio*). Ecotoxicol Environ Saf. 240: 110087.

Liu Y, Wang X, Zhang Y and Li X. (2021) Effects of a probiotic on growth, digestive enzyme activity and amino acid absorption in rainbow trout (*Oncorhynchus mykiss*). Aquacult Int. 29(4): 1237-1247.

Lyubomirova VN, Romanova EM, Romanov VV and Vasiliev AV. (2024) Evaluation of the effectiveness of "Aquasporin" feed additive application when rearing carp in industrial aquaculture conditions. Vestnik Ulyanovsk State Agricult Acad. 1(65): 178-184.

Ma Z, Zhang J, Zhang X, Li H, Liu Y and Gao L. (2023) Effects of temperature and photoperiod on growth, physiological, and behavioral performance in steelhead trout (*Oncorhynchus mykiss*) under indoor aquaculture condition. Front Mar Sci. 10: 1114662.

Neissi A, Rafiee G, Farahmand H, Rahimi S and Mijakovic I. (2020) Cold-resistant heterotrophic ammonium and nitrite-removing bacteria improve aquaculture conditions of rainbow trout (*Oncorhynchus mykiss*). Microb Ecol. 80(2): 266-277.

Neissi A, Majidi Zahed H and Roshan R. (2024) Probiotic performance of *B. subtilis* MS. 45 improves aquaculture of rainbow trout *Oncorhynchus mykiss* during acute hypoxia stress. Sci Rep. 14(1): 3720.

Nenciu M, Nita V, Nicolae C and Akbulut B. (2022) Boosting biomass gain and meat quality of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) - an approach for fostering Romanian aquaculture. Agro Life Sci J. 11(1): 145-156.

Niu X, Li Y, Zhang Y, Wang Y and Liu J. (2024) The effects of using hydrolyzed feather meal, amino acids, and probiotics in the diet of juvenile rainbow trout on growth, digestibility, and expression of growth-related genes. Aquacult Int. 32: 9671-9693.

Pang CV, Courtman N and Samsing F. (2026) Comparative study of blood biochemistry in clinically healthy farmed barramundi (*Lates calcarifer*) and rainbow trout (*Oncorhynchus mykiss*) from freshwater recirculating aquaculture systems. Aust Vet J. 104(6): 385-394.

Park K, Choi J, Lee Y and Park J. (2023) Evaluation of the optimal dissolved oxygen level for rainbow trout (*Oncorhynchus mykiss*) in the recirculating aquaculture system. J Korean Soc Fisheries Technol. 59(4): 387-398.

Pérez-Sánchez J, Tort L and Planas JV. (2021) Endocrine and metabolic responses to stress in fish: Rainbow trout as a model species. Gen Comp Endocrinol. 301: 113667.

Santos CM, Fonseca AM and Gonçalves AM. (2022) Effects of dietary supplementation with a plant extract (*Salvia officinalis*) on stress response and HPI axis activity in juvenile rainbow trout (*Oncorhynchus mykiss*). Aquacult. 552*:* 737845.

Saulnier RJ, Best C, Kostyniuk DJ, Gilmour KM and Lamarre SG. (2021) Chronic social stress alters protein metabolism in juvenile rainbow trout, *Oncorhynchus mykiss*. J Comp Physiol B. 191(3): 517-530.

Serrano-García L, Montero-Montoya R and Ostrosky L. (2018) Protective effect of a multivitamin supplement on micronuclei induction in peripheral erythrocytes of Nile tilapia (*Oreochromis niloticus*) exposed to cadmium. Ecotoxicol Environ Saf. 198: 114-120.

Shadyeva LA, Romanova EM, Vasiliev AV and Turaeva EV. (2023) Influence of Pravad biologically active food additive on the antioxidant protection system and amino acid composition of the muscle tissue of African clarium catfish. Vestnik Ulyanovsk State Agricult Acad. 3(63): 194-199.

Sorgeloos P, Kaushik SJ and Tacon AGJ. (2003) Sustainable fish feeds: The challenge of reducing reliance on fishmeal. Aquacult. 228(1-4): 1-18.

Storebakken T. (2025) Amino acid nutrition of salmonids: dietary requirements and bioavailability. Aquacult Int. 33(3): 457-472.

Ting Goh VS, Fujishima Y, Nakayama R, Takebayashi K, Yoshida MA, Kasai K, Ariyoshi K and Miura T. (2023) Manual scoring with shortened 48 h cytokinesis-block micronucleus assay feasible for triage in the event of a mass-casualty radiation accident. Radiat Res. 199(4): 385-395.

Tomás-Almenar C, Toledo-Solís FJ, Larrán AM, de Mercado E, Alarcón FJ, Rico D, Martín-Diana AB and Fernández I. (2020) Effects and safe inclusion of narbonne vetch (*Vicia narbonensis*) in rainbow trout (*Oncorhynchus mykiss*) diets: Towards a more sustainable aquaculture. Animals (Basel) 10(11): 2175.

Turaeva EE and Romanova EM. (2022) Effect of Pravad feed additive on growth and development of *Macrobrachium rosenbergii* shrimp larvae. Vestnik Ulyanovsk State Agricult Acad. 2(70): 196-202.

Zargar A, Taheri Mirghaed A, Saeed Mirzargar S, Ghelichpour M, Yousefi M and Hoseini SM. (2020) Dietary ginger administration attenuates oxidative stress and immunosuppression caused by oxytetracycline in rainbow trout (*Oncorhynchus mykiss*). Aquac Res. 51: 4215-4224.