



Research article

The influence of phytobiotic feed additive on the gut microbiome of common carp (*Cyprinus carpio*)

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Abstract

Under conditions of intensive fish cultivation, phytobiotic consumption will neutralize such phenomena as a decrease in the immune and antioxidant status of the body, an increase in productivity by improving consumption, digestibility, assimilation of feed, normalization of intestinal microflora, and homeostasis in general. The purpose of this work was to evaluate the influence of the phytobiotic "Probiocid-Phyto" on the growth performance and gut microbiome of common carp (*Cyprinus carpio*). The inclusion of the phytobiotic "Probiocide-Phyto" in the diet of fish had a productive effect on the growth of fish, for eight weeks of the experiment there was recorded an increase in fish weight relative to the control up to 17.3 % ($P \leq 0.05$). An analysis of the composition of the intestinal microbiota showed that changes in the ratio of the main groups of microorganisms may be one of the factors contributing to the rapid growth of fish. The introduction of Probiocide-Phyto into the diet of fish contributed to an increase in the intestinal microbiota of bacteria of the taxa *Pseudomonadota* and *Bacteroidota* by 17.2% and 10.7%, respectively. The main changes in the intestinal microbiota of fish were associated with changes in the number of bacteria of genus *Aurantimicrobium* (- 23%), *unclassified_Bacillota* (- 10.1%), genus *Polynucleobacter* (- 17.3%), genus *Aeromonas* (+ 33.1%) and genus *Pseudaeromonas* (+ 5.97%). A decrease in the number of bacteria was noted in the classes *unclassified_Bacillota*, *Betaproteobacteria* and *Actinomycetota*.

Keywords: Feeding, Fish, Microbiome, Phytobiotics.

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INTRODUCTION

The effectiveness of fish cultivation depends on many factors related to both its content and feeding. At the present stage of aquaculture development, the question of using plant-based feed additives (phytobiotics) as a substitute for synthetic antibiotics in fish feeding is increasingly being raised. However, the effectiveness of their use should be justified. The factors determining the relevance of the use of phytobiotics include the fact that their global use as an alternative to various antimicrobial drugs will not only protect the health and maximize the productivity of animals, but will also provide a solution to public health problems and improve food safety by food safety, by eliminating the cumulative effects of antibiotics in animal products (Kiczorowska et al., 2016).

Phytobiotics contain plant complexes with antimicrobial, antiviral, antifungal, anti-inflammatory, as well as immunomodulatory and growth-stimulating properties, which allows to minimize medication, including the load of antibacterial drugs (antibiotics) on the fish organism. It has been established that a number of phytobiotics contribute to an increase in the overall resistance of the organism, normalization of the intestinal microflora, and an increase in productivity due to better digestibility of feed nutrients. However, it should be noted that the number of phytobiotic preparations is growing every year and a detailed study of their properties and the possibility of using them in fish feeding is necessary.

Medicinal plants have been used all over the world to prevent and treat human and animal diseases for centuries. Common wormwood (*Artemisia vulgaris*), eleutherococcus prickly (*Eleutherococcus senticosus*), yellow milkwort (*Polygala rugellii*), rhodiola rosea (*Rhodiola rosea* L.), echinacea purpurea (*Echinacea purpurea*), laminaria japonica (*Laminaria japonica* Aresch.), milk thistle (*Silybum marianum*) and many other plants have proven to be antimicrobial agents. Numerous literature sources note that the essential oils of oregano (*Origanum vulgare*) and lemongrass (*Cymbopogon flexuosus*), as well as citric and lactic acids, are able to exert an antibacterial effect and modulate the intestinal microbiota (Busti et al., 2020; Rashidian et al., 2021; Sun et al., 2021; Chowdhury et al., 2023). In this regard, the production of more productive and healthy fish should include the assessment of changes in the composition of the intestinal microbiota as one of the priorities in dietary adjustment. The gut microbiome plays an important role in the functioning of the whole organism (Zhang et al., 2020; Wang et al., 2021).

Based on the above, the aim of our study was to evaluate the effect of phytobiotic "Probiocid-Phyto" on growth performance and on the intestinal microbiome of carp.

MATERIALS AND METHODS

Animals

The research was conducted in the conditions of the aquarium stand of the «Biotechnology of animal raw materials and aquaculture» Department of the Orenburg State University. The research objects were carp yearlings reared under the conditions of LLC «Irikla-fish» (Russia, Orenburg). Two groups (n=20) were formed to conduct research by the method of pairs of analogues: control and experimental. The fish of the control group received the main diet (OR) - compound feed "KRK-110" of JSC "Orenburg Feed Plant" (Russia, Orenburg), and the experimental group additionally included the phytobiotic "Probiocid-Phyto" in a dosage of 2 g/kg of feed. The main diet was KRK-110-1 fodder produced by Orenburg Feed Mill (Russia, Orenburg). Phytobiotic feed additive «Probiocid-Phyto» was produced by BIOTROF Ltd.

The daily feeding rate was determined weekly depending on fish weight, water temperature and dissolved oxygen values. Feeding was carried out 6 times

a day. Live weight was monitored weekly by individual weighing in the morning before feeding during the reference period.

Animal care and experimental studies were carried out in accordance with the instructions and recommendations of Russian regulations (1987; Order of the Ministry of Health of the USSR No. 755 of 12.08.1977 "On measures to further improve organizational forms of work using experimental animals") and "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996). During the research, measures were taken to ensure a minimum of animal suffering and to reduce the number of experimental samples studied.

Hematological analysis

Morphological and biochemical parameters of blood (hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), glucose (GLU), total protein (T-Prot)) were evaluated according to standard methods using an automatic hematology analyzer URIT-2900 Vet Plus (URIT Medial Electronic Co., China) and an automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd., China) with using commercial biochemical kits for veterinary medicine (DIAKONDS, Russia; Randox Laboratories Ltd, UK). The results obtained in the studies were processed using the software package "Statistica 10.0," including the determination of the arithmetic mean (M), the standard error of the mean (m). Differences at $p \leq 0.05$ were considered significant. To create an illustration, we used MS Office Excel.

Extraction of total DNA bacteria and archaea

Samples were homogenised on a TissueLyser LT (Qiagen, Hilden, Germany) with a Lysing Matrix Y (MP Biomedicals, Solon, USA). DNA extraction from samples was performed using QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer instruction. The quality of the extracted DNA was assessed with electrophoresis in 1% agarose gel and a Nanodrop 8000 (Thermo Fisher Scientific, Waltham, MA, USA). The DNA concentration was quantified using a Qubit 4 Fluorometer (Life Technologies, Carlsbad, CA, USA) with dsDNA High Sensitivity Assay Kit (Life Technologies, Carlsbad, CA, USA).

Preparation, sequencing and bioinformation processing of DNA

Preparation of the DNA libraries was performed according to the Illumina protocol (Part #15044223, Rev. B.) with primers targeting the V3–V4 regions of the SSU ribosomal RNA (rRNA) gene, S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') as the forward primer and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') as the reverse primer. The reaction mixture (25 μ L) contained both primers, 0.2 μ M each; 80 μ M dNTPs; 0.2 U Q5 High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA). The Following PCR program was used: 95 °C for 3 min, 25 cycles; 95 °C for 30 s, 56 °C for 30 s; 72 °C for 30 s; final extension 72 °C for 5 min. The DNA libraries were cleaned up using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) and were validated with capillary electrophoresis on a Qiaxcel Advanced System (Qiagen, Hilden, Germany) using the QIAxcel DNA Screening Kit (Qiagen, Hilden, Germany). Paired-end 2 $\times$ 251 bp sequencing was performed on the MiSeq platform (Illumina, San Diego, CA, USA) with the Reagent Kit v.2 (Illumina, San Diego, CA, USA).

DNA libraries preparing, sequencing and bioinformatics treatments were performed in the Center of Shared Scientific Equipment "Persistence of microorganisms" of Institute for Cellular and Intracellular Symbiosis UrB RAS (Orenburg, Russia). All stages of preparation, sequencing and bioinformatic processing of DNA libraries are performed according to the previously described methodology (Sizova et al., 2022).

Ethics Statement

The Local Ethics Committee of the Orenburg State University, Orenburg, Russia, has approved the report about this research.

Statistical analysis

The resulting OTUs, after filtering and assigning taxonomic affiliations, were used to calculate alpha (chao1, Fisher's alpha, - statistical method: ANOVA) and beta (ordination method: NMDS; distance method: Bray-Curtis index; statistical method: PERMANOVA) diversity.

RESULTS

The inclusion of the phytobiotic "Probiocide-Phyto" into the diet of fish had a productive effect on the growth of fish starting from the fourth week of the experiment. We found an increase in fish weight relative to control: on the fourth week - by 11% ($P \leq 0.05$), on the fifth week - by 11.1% ($P \leq 0.05$), on the sixth week - by 17.3% ($P \leq 0.05$), on the seventh week - by 12.1% ($P \leq 0.05$), and on the eighth week - by 9.9% ($P \leq 0.05$) (Table 1).

To assess the functional state of the fish organism, an analysis of hematological blood parameters was carried out (Table 2). The number of leukocytes in the experimental group was 55.05% lower than the control ($P \leq 0.001$). Hemoglobin and erythrocyte counts had no significant differences between the groups, while the number of erythrocytes in the experimental group was 27.45% lower than the control. The experimental group recorded an increase in glucose levels by 45.16% ($P < 0.01$) and total protein by 8.81% compared to the control values.

As a result, 39,729 and 47,359 reads were obtained for control and experimental groups, which corresponded to 56 and 46 OTUs. Based on the obtained sequences and OUT, the discharge curves were constructed (Figure 1).

A study of the intestinal microbiota of the control fish showed that the main taxa at the phylum level were *Pseudomonadota* and *Actinomycetota*, which accounted for more than 70% of the total number of bacteria (Figure 2). Among small groups, bacteria of the phyla *Bacillota* (15%) and *Bacteroidota* (11%) were noted.

The main number of classified microorganisms belonged to the classes *Actinomycetes*, *Betaproteobacteria*, *unclassified_Bacillota*, *Chitinophagia*, *Alphaproteobacteria* and *Gammaproteobacteria*. The dominant group of bacteria at the genus level were microorganisms belonging to the classes *Betaproteobacteria* and *Actinomycetes* (Figure 3).

The introduction of Probiocide-Phyto into the diet of fish contributed to an increase in the intestinal microbiota of bacteria of the taxa *Pseudomonadota* and *Bacteroidota* by 17.2% and 10.7%, respectively. The increase in the number of these phyla was directly related to the increase in the number of representatives of the classes *Chitinophagia* and *Gammaproteobacteria*. A decrease in the number of bacteria was noted in the classes *unclassified_Bacillota*, *Betaproteobacteria* and *Actinomycetota* (Figure 2).

The main changes in the intestinal microbiota of fish were associated with changes in the number of bacteria of genus *Aurantimicrobium* (- 23%), *unclassified_Bacillota* (- 10.1%), genus *Polynucleobacter* (- 17.3%), genus *Aeromonas* (+ 33.1%) and genus *Pseudaeromonas* (+ 5.97%) (Figure 3).

Calculations of alpha diversity indices showed greater richness and abundance of taxa in the intestinal microbiota of control fish (Table 3).

Analysis of PERMANOVA (Figure 4) to assess beta diversity did not show any cardinal differences in the organization of bacterial communities in the intestines of fish from the control and experimental groups (p -value = 0.03).

Table 1 Dynamics of fish growth, g

Week of the accounting period	Control	Experimental
The beginning of the Experiment	15.0 ± 1.2	15.1 ± 1.2
1	17.3 ± 1.4	17.4 ± 1.3
2	20.6 ± 1.7	20.7 ± 1.6
3	23.8 ± 2.0	24.8 ± 1.9
4	26.3 ± 2.4	29.2 ± 2.5*
5	30.6 ± 3.1	34.0 ± 3.1*
6	34.6 ± 3.7	40.6 ± 3.6*
7	40.6 ± 4.2	45.5 ± 4.2*
8	47.7 ± 4.7	52.4 ± 4.7*

Note: * - $P \leq 0.05$ compared to the control group

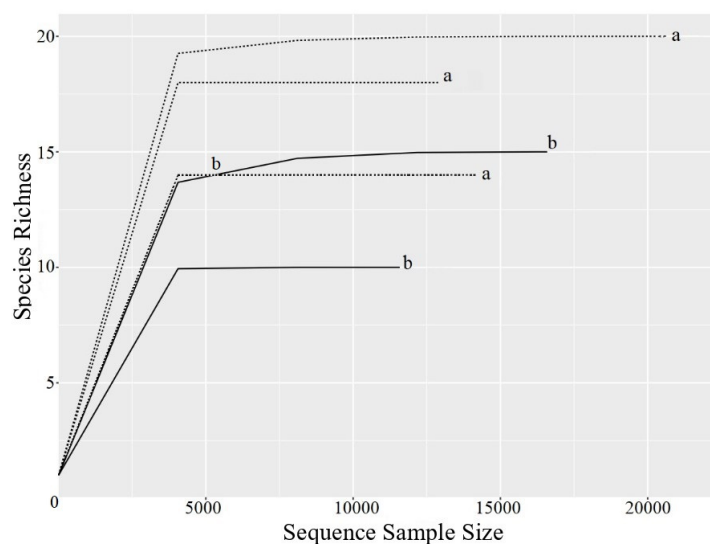
Table 2 Hemato-biochemical parameters of *Cyprinus carpio*

Group	Blood parameters				
	Hb (g/dL)	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	GLU (mg/dL)	T-Prot (g/dL)
Control	11.5±0.8	0.51±0.05	99±5.5	104.04± 2.34	2.78±0,1
Experimental	10.1±0.7	0.37±0.07	44.5±5.0***	151.02±5.3**	3.03±0,2

Note: ** - $P \leq 0.01$; *** - $P \leq 0.001$ compared to the control group

Table 3 Indices of alpha diversity of the intestinal microbiota of fish from the control and experimental groups

Index	Group		P-value
	Control	Experimental	
chao1	18.6	13	0.14
Fisher's alpha	1.85	1.43	0.12

**Figure 1** Sequence-based resolution curves for gut microbiota samples of control (a) and experimental (b) groups

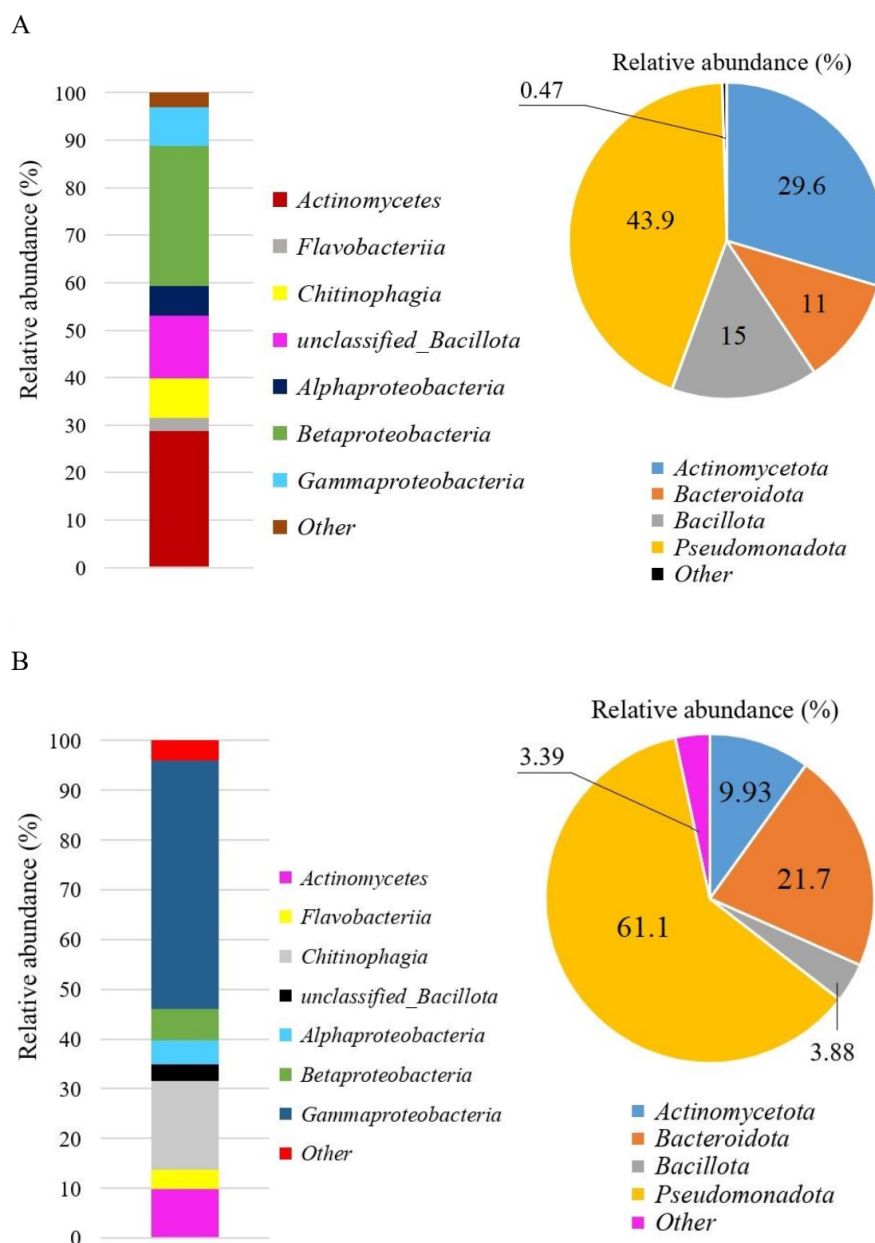


Figure 2 Relative abundance (% of all reads), at various taxonomic levels, A, of identified microorganisms in the gut of control fish; B, of identified microorganisms in the intestines of fish that received Probiocide-Phyto as part of the diet. *Other - taxa, the number of each of which did not exceed 2% of the total number of identified microorganisms

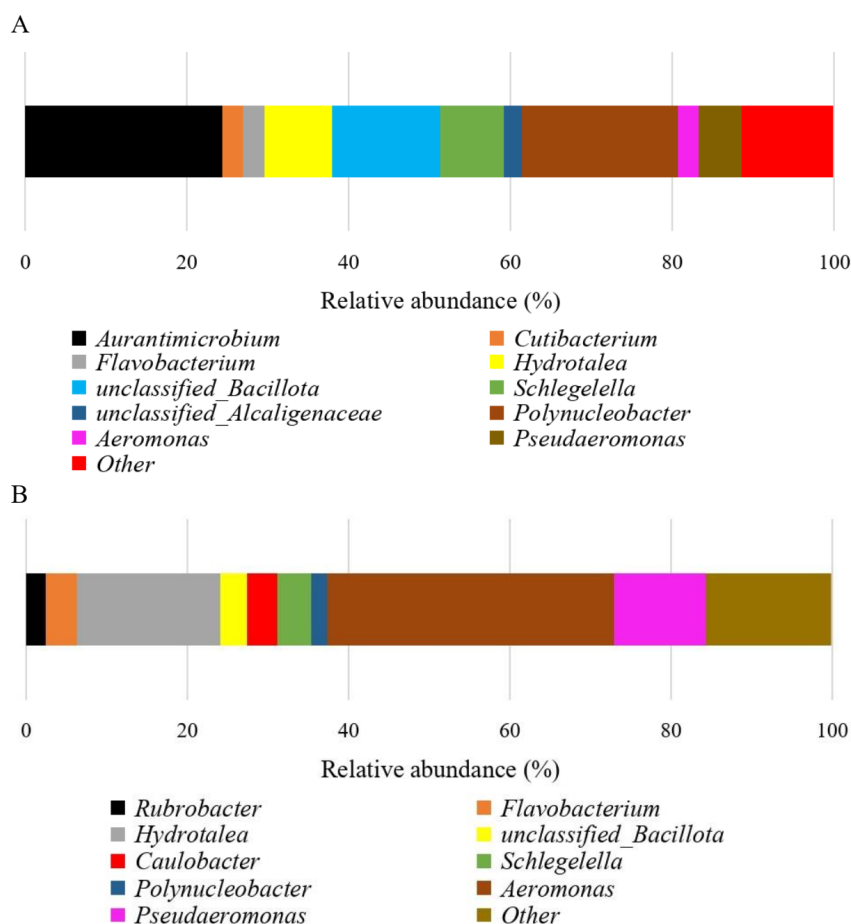


Figure 3 Relative abundance (% of all readings) of the most numerous groups of bacteria at the genus level identified in the intestines of fish from the control group (A); from the experimental group (B). *Other - taxa, the number of each of which did not exceed 2% of the total number of identified microorganisms

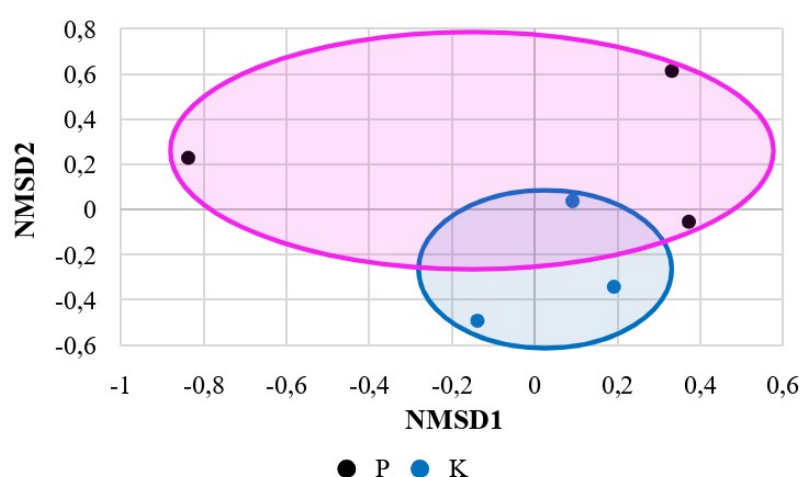


Figure 4 Beta diversity of gut microbes in fish of study groups using the PERMANOVA statistical method, non-metric multivariate scaling, and Bray-Curtis dissimilarity. K - control group; P - group that received the phytobiotic supplement "Probiocide-Phyto" in the diet.

DISCUSSION

The inclusion of a phytobiotic in the diet of fish had a positive effect on the intensity of growth, which may be due to the fact that the phytobiotic "Probiocid-Phyto" contains essential oils in its composition that may improve digestion and metabolism in the body, and which is confirmed by the data on the positive effect of phytobiotics on growth and fish health (Estaiano de Rezende et al., 2021).

A wide range of studies in the field of fish production (Safari et al. 2018; Godrati et al., 2021) highlight the relationship between industrial products and hematological indicators. The established decrease in the number of leukocytes in the experimental group may indicate a low level of inflammatory processes in the body of fish. An increase in blood glucose levels manifests itself in the form of analgesic metabolism and assimilation of nutrients from fish feed (Kondera et al., 2020; Gayed et al., 2021).

In general, the results obtained indicate a positive effect of the phytobiotic "Probiocid-Phyto" on the growth and metabolism of carp. Modern fisheries consider fish health in close relationship with the effective functioning of the intestinal microbiota (Rimoldi et al., 2018; Messina et al., 2023). Analysis of the gut microbiota of carp showed the predominance of four taxa: *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Bacteroidota*, which is similar to the results of other studies and shows a high relationship with the diet used in nutrition (Ke et al., 2020; Fan et al., 2022; Wasu et al., 2023).

The use of phytobiotics in the fish diet stimulated an increase in the number of mainly gram-negative microflora in the intestine. The microorganisms of the *Pseudomonadota* phylum were the most responsive, their number increased markedly. The most pronounced increase was noted in the number of *P. Aeromonas* bacteria, some representatives of which are pathogens of fish (Hayatgheib et al., 2020). Similarly, the most significant decrease in the number of microorganisms was observed in the taxon *Actinomycetota*. Such changes are of interest since changes in the number of these taxa are associated with the development of inflammatory reactions in the intestine and a decrease in the level of beneficial metabolites produced by microorganisms (Litvak et al., 2017; Shelton et al., 2020; Sarmiento-Vizcaíno et al., 2021). In our study, there was no deterioration in the physiological parameters of fish, but an increase in the number of bacteria in the intestines of fish, which are markers of inflammation in the intestine, which suggests that there are no negative consequences of an increase in the number of genus *Aeromonas* in the microbiota. The absence of negative consequences of the increase in genus *Aeromonas* bacteria is probably due to the ability of individual components of the Probiocid-phyto phytobiotic to suppress their metabolic activity (Hayatgheib et al., 2020).

A number of studies have shown that the change in the number of microorganisms in the intestinal microbiota of the *Bacillota* phylum correlates with changes in growth indicators in fish. In mammalian studies it was shown that indicate that high energy absorption in the intestine are associated with an increase in the number of bacteria of the taxon *Bacillota* and a decrease in the number of microorganisms of the phylum *Bacteroidota* (Li et al., 2013; Magne et al., 2020; Fan et al., 2022). In the study on the model of the white cupid (*Ctenopharyngodon idella*), it was shown that in the group with a high weight gain, there was a decrease in the values of the *Bacillota/Bacteroidota* ratio and lower values of alpha diversity than in the fish in the group with a low weight gain (Wang et al., 2020). It is assumed that microorganisms of the *Bacteroidota* phylum may be an important factor in increasing the digestibility of nutrients and increasing weight gain. Similarly, in our studies, a decrease in the values of the *Bacillota/Bacteroidota* ratio and alpha diversity indices was accompanied by an increase in fish growth rates.

Inclusion of phytobiotic "Probiocid-Fito" in the dosage of 2 g/kg feed in carp diet as an alternative to antibiotics promotes the increase of general resistance and

normalization of intestinal microflora, which in turn improves productivity due to better assimilation of feed nutrients.

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AUTHOR CONTRIBUTIONS

Miroshnikova Elena Petrovna; suggested the idea and the experimental design; **Arinzhanov Azamat Ersainovich, Yausheva Elena Vladimirovna and Kilyakova Yulia Vladimirovna** performed the experiment and wrote the draft of manuscript with the support of **Miroshnikova Elena Petrovna**.

All authors discussed the results and contributed to finalize the manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

REFERENCES

- Busti, S., Rossi, B., Volpe, E., Ciulli, S., Piva, A., D'Amico, F., Soverini, M., Candela, M., Gatta, P.P., Bonaldo, A., Grilli, E., Parma, L., 2020. Effects of dietary organic acids and nature identical compounds on growth, immune parameters and gut microbiota of European sea bass. *Sci. Rep.* 10(1), 21321.
- Chowdhury, H., Kumar Bera, A., Subhasmita Raut, S., Chandra Malick, R., Sekhar Swain, H., Saha, A., Kumar Das, B., 2023. In Vitro antibacterial efficacy of *Cymbopogon Flexuosus* essential oil against *Aeromonas Hydrophila* of fish origin and in silico molecular docking of the essential oil components against DNA Gyrase-B and their drug-likeness. *Chem. Biodivers.* 20(3), e202200668.
- Estaiano de Rezende, R.A., Soares, M.P., Sampaio, F.G., Cardoso, I.L., Ishikawa, M.M., Lima Dallago, B.S., Rantin, F.T., Teixeira Duarte, M.C., 2021. Phytobiotics blend as a dietary supplement for Nile tilapia health improvement. *Fish. Shellfish. Immunol.* 114, 293–300.
- Fan, Z., Wu, D., Li, J., Li, C., Zheng, X., Wang, L., 2022. Phosphorus nutrition in Songpu Mirror Carp (*Cyprinus carpio* Songpu) during chronic carbonate alkalinity stress: effects on growth, intestinal immunity, physical barrier function, and intestinal microflora. *Front. Immunol.* 13, 900793.
- Fan, Z., Wu, D., Li, J., Zhang, Y., Cui, Z., Li, T., Zheng, X., Liu, H., Wang, L., Li, H., 2022. Assessment of fish protein hydrolysates in juvenile largemouth bass (*Micropterus Salmoides*) diets: effect on growth, intestinal antioxidant status, immunity, and microflora. *Front. Nutr.* 9, 816341.
- Gayed, M.A., Elabd, H., Tageldin, M., Abbass, A., 2021. Probiotic Zado® (*Ruminococcus Flavefaciens*) boosts hematology, immune, serum proteins, and growth profiles in Nile tilapia (*Oreochromis niloticus*). *Fish. Shellfish. Immunol. Rep.* 2, 100021.
- Ghodrati, M., Islami, H.R., Shekarabi, S.P.H., Masouleh, A.S., Mehrgan, M.S., 2021. Combined effects of enzymes and probiotics on hemato-biochemical parameters and immunological responses of juvenile Siberian sturgeon (*Acipenser baerii*). *Fish. Shellfish. Immunol.* 112, 116–124.
- Hayatgheib, N., Fournel, C., Calvez, S., Pouliquen, H., Moreau, E., 2020. In vitro antimicrobial effect of various commercial essential oils and their chemical

- constituents on *Aeromonas salmonicida* subsp. *salmonicida*. J. Appl. Microbiol. 129(1), 137-145.
- Ke, F., Gao, Y., Liu, L., Zhang, C., Wang, Q., Gao, X., 2020. Comparative analysis of the gut microbiota of grass carp fed with chicken faeces. Environ. Sci. Pollut. Res. Int. 27(26), 32888-32898.
- Kiczorowska, B., Samolinska, W., Al-Yasiry, A., Kiczorowski, P., Winiarska-Mieczan, A., 2017. The natural feed additives as immunostimulants in monogastric animal nutrition – a review. Ann. Anim. Sci. 17(3), 605-625.
- Kondera, E., Bojarski, B., Ługowska, K., Kot, B., Witeska, M., 2020. Effects of oxytetracycline and gentamicin therapeutic doses on hematological, biochemical and hematopoietic parameters in *Cyprinus carpio* juveniles. Animals. 10(12), 2278.
- Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y., Hu, Z., 2013. Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio* L.). PLoS One. 8(5), e64577.
- Litvak, Y., Byndloss, M.X., Tsois, R.M., Bäumler, A.J., 2017. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr. Opin. Microbiol. 39, 1-6.
- Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., Balamurugan, R., 2020. The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients?. Nutrients. 12(5), 1474.
- Messina, M., Iacumin, L., Pascon, G., Tulli, F., Tibaldi, E., Cardinaletti, G., 2023. Effect of feed restriction and refeeding on body condition, digestive functionality and intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 49(1), 169–189.
- National Academy of Sciences, 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
- Rashidian, G., Boldaji, J.T., Rainis, S., Prokić, MD., Faggio, C., 2021. Oregano (*Origanum vulgare*) Extract Enhances Zebrafish (*Danio rerio*) Growth Performance, Serum and Mucus Innate Immune Responses and Resistance against *Aeromonas hydrophila* Challenge. Animals. 11(2), 299.
- Rimoldi, S., Terova, G., Ascione, C., Giannico, R., Brambilla, F., 2018. Next generation sequencing for gut microbiome characterization in rainbow trout (*Oncorhynchus mykiss*) fed animal by-product meals as an alternative to fishmeal protein sources. PLoS One. 13(3), e0193652.
- Saffari, S., Keyvanshokoo, S., Zakeri, M., Johari, S. A., Pasha-Zanoosi, H., Mozanzadeh, M.T., 2018. Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). Fish Physiol. Biochem. 44(4), 1087–1097.
- Sarmiento-Vizcaíno, A., Martín, J., Reyes, F., García, L.A., Blanco, G., 2021. Bioactive natural products in Actinobacteria Isolated in rainwater from storm clouds transported by Western Winds in Spain. Front. Immunol. 12, 773095.
- Sizova, E., Yausheva, E., Marshinskaia, O., Kazakova, T., Khlopko, Y., Lebedev, S., 2022. Elemental composition of the hair and milk of black-spotted cows and its relationship with intestinal microbiome reorganization. Vet. World. 15(11), 2565–2574.
- Shelton, C.D., Byndloss, M.X., 2020. Gut epithelial metabolism as a key driver of intestinal dysbiosis associated with noncommunicable diseases. Infect. Immun. 88(7), e00939-19.
- Sun, S., Xu, X., Liang, L., Wang, X., Bai, X., Zhu, L., He, Q., Liang, H., Xin, X., Wang, L., Lou, C., Cao, X., Chen, X., Li, B., Wang, B., Zhao, J., 2021. Lactic Acid-producing probiotic *saccharomyces cerevisiae* attenuates ulcerative colitis via suppressing macrophage pyroptosis and modulating gut microbiota. Front. Immunol. 12, 777665.

- Wang, J., Jaramillo-Torres, A., Li, Y., Kortner, T.M., Gajardo, K., Brevik, Ø.J., Jakobsen, J. V., Krogdahl, Å., 2021. Microbiota in intestinal digesta of Atlantic salmon (*Salmo salar*), observed from late freshwater stage until one year in seawater, and effects of functional ingredients: a case study from a commercial sized research site in the Arctic region. *Anim. Microbiome*. 3(1), 14.
- Wang, S.T., Meng, X.Z., Zhang, J.H., Dai, Y.F., Shen, Y., Xu, X.Y., Wang, R.Q., Li, J.L., 2020. 16S rRNA sequencing analysis of the correlation between the intestinal microbiota and body-mass of grass carp (*Ctenopharyngodon idella*). *Comp. Biochem. Physiol. Part D Genomics Proteomics*. 35, 100699.
- Wasiu, A.J., Ayodeji, A.A., Olayinka, A.O., Comfort, T.A., Adijat, E.A., Grace, A.O., 2023. Effect of dietary cinnamon supplementation on the growth in length, haematology, serum biochemistry, intestinal microbiota and enzymes and histomorphological changes of the intestine, liver and the kidney of *Heteroclaras* (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis*♂). *Vet. Sci*. 21(3), 973 – 997.
- Zhang, Y., Liang, X.F., He, S., Chen, X., Wang, J., Li, J., Zhu, Q., Zhang, Z., Li, L., Alam, M.S., 2020. Effects of high carbohydrate diet-modulated microbiota on gut health in Chinese Perch. *Front. Microbiol*. 11, 575102.

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