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INFLUENCE OF BISPHENOL A AND PROBIOTIC-CONTAINING FEED *CARASSIUS GIBELIO* BLOCH INDICATES SEPARATELY

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One of the most common pollutants of water bodies is bisphenol A (BPA), a plastic monomer used to synthesize polycarbonate plastics, epoxy resins and thermal paper (Barboza L. et. al., 2020). BPA enters freshwater and marine ecosystems as a result of leaching from BPA-based composites, as well as with water discharges from manufacturing plants, wastewater treatment plants and landfills. BPA is characterized by a low accumulation potential in freshwater environments, a short half-life under aerobic conditions, but due to the continuity of exposure, it is a serious problem (Wu N. C., Seebacher F., 2020). The ways BPA enters the fish body are diverse: through the digestive tract, gills, skin. Numerous studies have shown the adverse effects of BPA on the behavioral and morpho-physiological parameters of fish, including swimming patterns, coordination of movements, appetite, and dysfunction of many systems (endocrine, reproductive, nervous).

It is possible to prevent the negative effects of hunting through the prophylactic use of agents that enhance the overall reactivity of the body. In this sense, our attention was drawn to probiotics - microorganisms that have a stimulating effect on the development of indigenous microflora, have a high antagonistic, synthetic, immunomodulatory, regulatory potential. We have studied the effect of bisphenol A and probiotic microorganisms Lactobacillus casei, introduced in the feed, on certain indicators of Carassius gibelio Bloch (general behavioral reactions, certain morpho-physiological parameters, qualitative and quantitative characteristics of erythrocytes and leukocytes). The study was carried out after 15-day acclimatization of fish in aquariums at a water temperature of 14°C, appropriate aeration regime and 16-hour photoperiod.

It was found that 96-hour exposure to bisphenol A at a concentration of 1.5 mg/l causes darkening of the skin, increased mucus secretion, uncharacteristic motor activity, the appearance of unusual morphotypes of red blood cells, a slight increase in the number of leukocytes, and a decrease in the phagocytic activity of Carassius gibelio Bloch. Preventive introduction of probiotic cultures in the feed contributed to the correction of behavioral reactions and individual hematological parameters.

Key words: Carassius gibelio Bloch, bisphenol A, probiotics, erythrocytes, leukocytes, phagocytic activity

Introduction. An urgent problem of today is the rapid increase in the number of pollutants in the environment, especially anthropogenic ones. In aquatic ecosystems, organisms are at greater risk compared to terrestrial ones, as a large number of chemicals of various origins easily settle in the hydrosphere and enter the body.

An example of such hazardous compounds that pose a threat to the environment and its inhabitants is plastic and its components. Modern plastic materials may contain a wide range of additives that provide specific properties and improve its performance, stability, durability. Most often, plasticizers, flame retardants, antioxidants, acid absorbers, lubricants, light and heat stabilizers, pigments and dyes, etc. are used for these purposes. Bisphenol A (BPA) is the most representative representative of this group of chemicals (Barboza et. al., 2020). It is a xenobiotic and an exclusively man-made compound used for the synthesis of polycarbonate plastics, epoxy resins and thermal paper. Large-scale production of bisphenol A in different countries of the world has led to its significant release into the environment of all continents, including the hydrosphere (Repossi et. al., 2016).

According to meta-analysis data, the BPA content in fresh water is on average 42.3 ng/l (Wu, Seebacher, 2020). In the aquatic environment, BPA usually decomposes in 0.5-6 days, but due to continuous ingestion, many organisms, especially fish, are constantly exposed to this toxic substance (Bhandari et. al., 2015). At the same time, the following are noted: disruption of the reproductive system, embryo development, larval and juvenile growth, obesity due to modulation of pathways involved in adipocyte differentiation and lipid accumulation (Sun et.al, 2019); damage to the nervous system and impaired motor functions due to penetration through the blood-brain barrier, induction of the pro-inflammatory process through inhibitory/activating effects on kinases; development of oxidative stress due to an increase in reactive oxygen species (ROS) and nitric oxide (NO) (Afzal et.al., 2022; Akram et.al., 2021).

To prevent the above-mentioned changes, it is possible to use means based on the strengthening of specific and non-specific resistance of fish. Probiotics are the most promising among them. Probiotics, which are representatives of the autochthonous microflora of organisms, provide numerous functions - protective, digestive, synthetic, regulatory, trophic

(Hoseinifar et. al., 2018). They can act not only as an alternative to antibiotics in the case of overcoming fungal and bacterial infections, but also show immunostimulating and immunomodulating effects (Pandey et. al., 2022; Chauhan and Singh, 2019). These effects of probiotic bacteria are realized through cell-associated mechanisms and the production of biologically active substances with immunoregulatory properties. On the other hand, the broad enzymatic activity of probiotics, which provides biotransformation of toxic compounds, encourages their use as bioremediation and biocontrol factors (Hasan and Banerjee, 2020).

Therefore, the aim of this work was to study the influence of bisphenol A and the action of probiotics introduced into the fodder substrate on individual indicators of the body of *Carassius gibelio Bloch*. At the same time, the content of leukocytes was determined, the qualitative and quantitative composition of erythrocytes, indicators of phagocytic activity were analyzed, general behavioral reactions and changes in fish weight were evaluated.

Materials and methods. Experimental studies were conducted on *Carassius gibelio Bloch* fish, the initial weight of which, on average, was 60 g. The fish were transferred from the pools of the closed water supply system to glass aquariums 40x27x40 cm in size and left for acclimatization for 15 days. During this time, the fish were on a standard feed ration (daily in the amount of 1% of the total weight). Throughout the experimental period, the following conditions were observed: water temperature - 14 °C, photoperiod - 16-hour, oxygen supply - appropriate through the functioning of oxygenators.

Subsequently, the fish were divided into 3 experimental groups: I - fish of the control group (group - control), which were on a standard food diet (Aller Bronze feed with a diameter of 3 mm); II - fish that were on a standard food diet for 28 days and were exposed to a toxic 96-hour exposure to bisphenol A, concentration of 1.5 mg / l (group - bisphenol) at the last stages of the experiment; III - fish that received probiotic cultures as part of the feed substrate for 28 days and were further exposed to 96-hour exposure to bisphenol (probiotics + bisphenol). Probiotic-containing feed was created on the basis of Aller Bronze with a diameter of 3 mm, followed by the introduction of Lactobacillus casei (kindly provided to us by the staff of the Institute of Microbiology and Virology named after D.K. Zabolotny of the National Academy of Sciences of Ukraine) in the amount of 1×1011 cfu/g and granulation of the modified feed (Vasina, 2022). The experiment lasted 32 days. During the period of acclimatization and 28-day study of the preventive effect of probiotic feed, water in the aquariums was replaced daily by 3/4 of the volume. When studying the short-term effect of bisphenol A on Carassius gibelio Bloch, the concentration of the polutant in water was maintained daily at a constant level.

Determination of some hematological parameters was carried out in whole blood. After pretreatment of the live incision site, blood was collected with a Pasteur pipette from the tail vein (Lawrence et al., 2020). The instruments were pretreated with anticoagulant. The total number of erythrocytes and leukocytes was determined, and the phagocytic activity of neutrophils was analyzed by a modified method (Simpson et al., 1979) using a previously prepared yeast suspension. 50 μl of working yeast solution was added to 100 μl of heparin-stabilized blood and mixed gently. After a 60-minute incubation at 37 °C, a smear was prepared by carefully removing 10 μl of leukoconcentrate from the upper layer. Fixation was carried out with 96% ethyl alcohol for 15 min and stained according to Pappenheim.

The results were calculated using a microscope using an immersion lens (×100), the total magnification of the microscope was 1500 times.The results of the experimental data were processed statistically using software Microsoft Excel. At the same time, the results were reliable at the level of reliability p≤0,05 according to the Student's criterion.

Results and their discussion. Hydrobionts, including fish, are very sensitive to the appearance of various pollutants in the environment. BPA enters the body of fish through the skin, gills or alimentary way. It is known that the toxic effect of bisphenol A on the body is associated with impaired functioning of many systems - endocrine, reproductive, nervous, etc. The effect of BPA on the growth, behavior, morphological characteristics of fish, its genotoxicity has been characterized (Afzal et.al., 2022; Frenzilli G. et.al., 2021). Both long-term and short-term exposure to polutant causes changes in various tissues and organs such as liver, kidneys, gills, brain, etc. (Akram et al., 2022; Faheem et al., 2019; Huang et.al., 2018).

During the experimental study, we did not record significant changes in the weight of fish of groups I and II. During the acclimatization period, fish of both groups lost weight, which did not reach the initial values even after 32 days of feeding with traditional feed substrate. Instead, as in previous studies, the stabilization (with subsequent increase) of the biomass of fish of the third group under the conditions of 28-day use of probiotic-containing feed was stated. On the 32nd day of the experiment, the weight of fish in the "control" and "bisphenol" groups averaged 51±6.2 g, and in the "probiotic + bisphenol" group 62±8.1.

Experimental studies have shown that four-day exposure to bisphenol A caused some morphological and behavioral changes in fish. In particular, we observed darkening of the skin and increased external mucus formation (Fig. 1), as well as a decrease in the efficiency of feed consumption, increased frequency of swallowing movements, accelerated movement of gill covers, trembling of fins, decreased motor activity, and deterioration of coordination of movements. Such changes were not characteristic of fish of group III, which were fed with probiotic-containing feed for 28 days: fish actively moved, effectively consumed feed; the color of their skin did not differ from the control group; increased mucus formation was not observed; atypical respiratory movements were not detected.

Bisphenol

Probiotic + bisphenol

Fig. 1. Coloration of the skin of the fish of the research groups

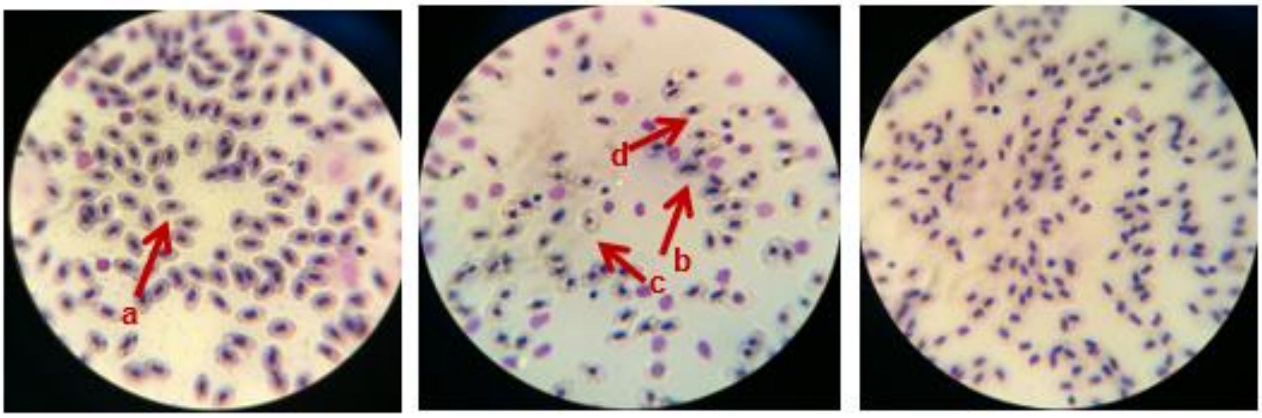
The long-term action of probiotics introduced in the feed substrate obviously not only contributed to the stimulation of the autochthonous microflora of fish, but also enhanced the biotransformation of xenobiotics due to the broad enzymatic activity of bacteria. It is known that BPA entering the body through food can be absorbed by the cells of the gastrointestinal tract and liver. Bisphenol deactivation reactions occur here with the formation of bisphenol A-glucuronide (Mit et. al., 2022), less often bisphenol A-sulfate (reactions have been studied in detail in humans and primates (Dekant end Völkel, 2008)), which can be excreted from the body. All of the above may indirectly indicate the rapid adaptation of fish to new conditions of keeping and increased reactivity of the body.

It is known that both chronic and short-term exposure to bisphenol A causes various disorders in the body of animals, as evidenced by changes in indicators that act as biomarkers of the functional state of the liver, kidneys, gills, muscles, and formed blood elements (Akram et. al., 2022; Afzal et. al., 2022; Dile et.al., 2022; Akram et. al., 2021; Faheem et. al., 2019). It is suggested that BPA exhibits genotoxic activity, and oxidative stress may be one of the mechanisms of its manifestation (Sharma end Chadha, 2021; Abdel-Tawwab end Hamed, 2018). On the other hand, probiotics have significant synthetic and immunomodulating /stimulating properties (Maldonado Galdeano et al., 2019), which allows mobilizing various links of the body's defenses. Therefore, the next stage of the experiment was dedicated to the study of individual indicators characterizing the qualitative and quantitative changes of erythrocytes and leukocytes in the blood of experimental groups of fish.

The results of the experiment proved the absence of reliable changes in the number of erythrocytes of all experimental groups, but allowed to ascertain the morphological changes of erythrocytes in the blood of fish exposed to BPA. At the same time, the appearance of erythrocytes was detected, which were characterized by a pear-shaped, disk-shaped, rod-shaped shape. Similar morphotypes of erythrocytes were not observed in the blood of fish of other groups (Fig. 2).

According to literature data (Sharma and Chadha, 2021), under the influence of toxic doses of bisphenol A for 24-96 hours, abnormalities were detected in the erythrocytes of C. punctatus, in particular, lysed cells, binucleated cells, cells with micronuclei, karyolyzed cells, erythrocytes with a dentate nucleus, fusion of cells.

It is worth noting the tendency to increase the level of leukocytes in the blood of fish exposed to bisphenol A, and in the case of preliminary long-term feeding of fish with probiotic-containing feed, these changes were significant (Fig. 3, A).



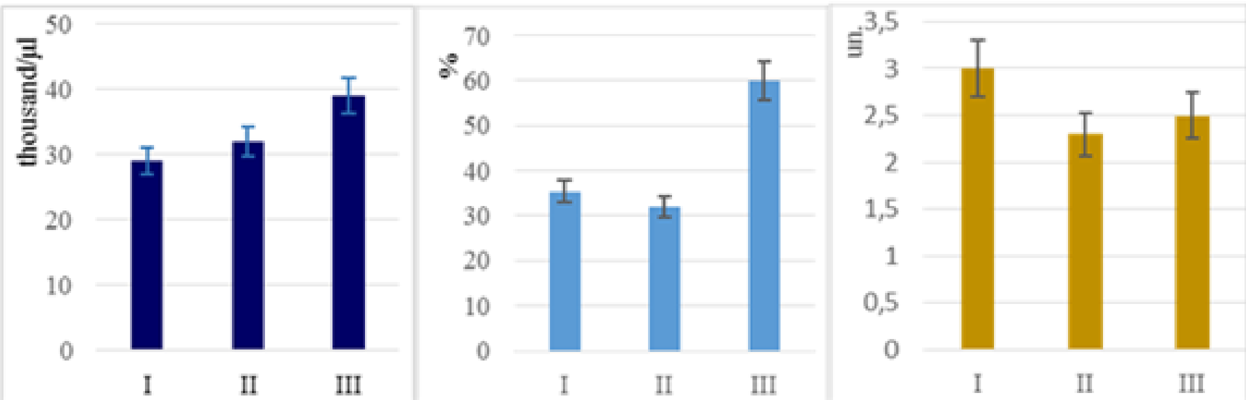
Control *Bisphenol* *Probiotic + bisphenol*

Fig. 2. Morphotypes of fish erythrocytes: a – normocyte, b – pear-shaped, c – disc-shaped, d – rod-shaped

The number of leukocytes in the blood of fish of group III was, on average, 3.9*1010 / l and slightly differed from the established for fish fed for a long time with probiotic-containing feed. Obviously, this indicates the dominant contribution to the activation of leukocytopoiesis of the appearance of probiotics in the feed rationThe analysis of phagocytic activity was carried out, considering two indicators of neutrophil functioning - phagocytic index, indicating the percentage of neutrophils involved in phagocytosis and phagocytic number, which characterizes the average number of yeast cells captured by one neutrophil.

We registered an increase in the phagocytic index in fish of the III group (Fig. 3, B). For example, if in the control group it was, on average, 35%, then in the III group of fish it was equal to 60%.

On the other hand, there was a tendency to decrease the phagocytic index in fish of the II group - up to 32% on average.The highest value of the phagocytic number was noted for animals of the control group (Fig. 3, C). For the other two groups that were exposed to short-term toxic effects of bisphenol, a slight decrease in the level of phagocytic number was shown, and it was significant only in the case of the study of fish that were fed with feed without probiotics.



A **B** **C**

Fig. 3. Leukocyte content (A), phagocytic index (B), phagocytic number (C) in Carassius gibelio Bloch

*Note: I – control, III – bisphenol, III – probiotic + bisphenol; * - statistically significant difference compared to the control, p≤0.05*

Conclusions. A 96-hour exposure to bisphenol A at a concentration of 1.5 mg/l causes skin darkening, increased mucus secretion, unusual motor activity, the appearance of unusual erythrocyte morphotypes, a slight increase in the number of leukocytes, and a decrease in the phagocytic activity of *Carassius gibelio Bloch*. Prophylactic introduction of probiotic cultures as part of the feed contributed to the correction of behavioral reactions and individual hematological indicators.

References:

1. Barboza L. G. A., Cunha S. C., Monteiro C. et al. Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. *Journal of Hazardous Materials*. 2020; 393. https://doi.org/10.1016/j.jhazmat.2020.122419
2. Wu N. C., Seebacher F. Effect of the plastic pollutant bisphenol A on the biology of aquatic organisms: a meta-analysis. *Global Change Biology*. 2020; 26 (7): 3821–3833. https://doi.org/10.1111/gcb.15127
3. Repossi F., Farabegoli F., Gazzotti T. et.al. Bisphenol A in edible part of seafood. *Ital J Food Saf*. 2016; 5(2): 5666. doi: 10.4081/ijfs.2016.5666
4. Bhandari R. K., Deem S. L., Holliday D. K. et. al. Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. *Gen Comp Endocrinol*. 2015; 214: 195-219. doi: 10.1016/j.ygcen.2014.09.014.
5. Sun S.-X., Zhang Y.-N., Lu D.-L. et.al. Concentration-dependent effects of 17β-estradiol and bisphenol A on lipid deposition, inflammation and antioxidant response in male zebrafish (Danio rerio). *Chemosphere*. 2019; 237. doi:10.1016/j.chemosphere.2019.124422
6. Afzal G., Ahmad H.S., Hussain R. et.al. Bisphenol A induces histopathological, hematobiochemical alterations, oxidative stress, and genotoxicity in common carp (Cyprinus carpio L.). *Oxid Med Cell Longev*. 2022. doi: 10.1155/2022/5450421.
7. Akram R., Iqbal R., Hussain R. et. al. Evaluation of oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (Aristichthys nobils) fish at low concentrations. *Environmental Pollution.* 2021; 268. doi: 10.1016/j.envpol.2020.115896
8. Chauhan A., Singh R. Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis*. 2019; 77: 99–113 https://doi.org/10.1007/s13199-018-0580-1
9. Hoseinifar S.H., Yun-Zhang Sun Y.-Z., Wang A. et. al. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. *Front. Microbiol. Sec. Aquatic Microbiology.* 2018. https://doi.org/10.3389/fmicb.2018.02429
10. Hasan K.N., Banerjee G. Recent studies on probiotics as beneficial mediator in aquaculture: a review. The *Journal of Basic and Applied Zoology*. 2020; 81. https://doi.org/10.1186/s41936-020-00190-y
11. Pandey A.A., Tyagi A., Khairnar S. O. Oral feed-based administration of *Lactobacillus plantarum* enhances growth, haematological and immunological responses in *Cyprinus carpio. Emerging Animal Species.* 2022; 3. https://doi.org/10.1016/j.eas.2022.100003
12. Vasina L.M., Starikova V.O., Khuda L.V. Influence of probiotics, introduced in the composition of the feed substrate, in particular haematological indicators of Carassius gibelio Bloch. *Biol. syst*. 2022; 14 (1): 29-32 https://doi.org/10.31861/biosystems2022.01.029
13. Lawrence M.J., Raby G.D., Teffer A.K. et al. Best practices for non-lethal blood sampling of fish via the caudal vasculature. *J Fish Biol.* 2020; 97(1): 4-15. doi: 10.1111/jfb.14339
14. Simpson D. W., Roth R., Loose L. D. A rapid, inexpensive and easily quantified assay for phagocytosis and microbicidal activity of macrophages and neutrophils. *J Immunol Methods*. 1979; 29(3): 221-226. doi: 10.1016/0022-1759(79)90309-0.
15. Frenzilli G., Martorell-Ribera J., Bernardeschi M. Bisphenol A and bisphenol S induce endocrine and chromosomal alterations in Brown Trout. *Front. Endocrinol.* 2021. https://doi.org/10.3389/fendo.2021.645519
16. Faheem M., Khaliq S., Lone K. P. Effect of bisphenol-a on serum biochemistry and liver function in the freshwater fish, Catla catla. *Pak. Vet. J.* 2019; 39(1): 1–5.
17. Huang Q., Liu Y., Chen Y. et.al. New insights into the metabolism and toxicity of bisphenol A on marine fish under long-term exposure. *Environmental Pollution*. 2018; 242 (Pt A): 914-921 DOI: 10.1016/j.envpol.2018.07.048
18. Akram R., R. Iqbal R., Hussain R. et al. Effects of bisphenol a on hematological, serum biochemical, and histopathological biomarkers in bighead carp (Aristichthys nobilis) under long-term exposure. *Environmental Science and Pollution Research*. 2022; 29(15): 21380-21395. doi: 10.1007/s11356-021-17329-1.
19. Diler Ö., Özil Ö., Nane İ. D. et.al. The Effects of Bisphenol A on Oxidative Stress, Antioxidant Defence, Histopathological Alterations and Lysozyme Activity in Narrow-Clawed Crayfish (Pontastacus leptodactylus). *Turkish Journal of Fisheries and Aquatic Sciences.* 2022; 22(10): TRJFAS19877. https://doi.org/10.4194/TRJFAS19877
20. Abdel-Tawwab M., Hamed H. S. Effect of bisphenol A toxicity on growth performance, biochemical variables, and oxidative stress biomarkers of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Applied Ichthyology.* 2018; 34 (5): 1117-1125 https://doi.org/10.1111/jai.13763
21. Mit C., Bado-Nilles A., Daniele G. et.al. The toxicokinetics of bisphenol A and its metabolites in fish elucidated by a PBTK model. *Aquatic Toxicology*. 2022;247.https://doi.org/10.1016/j.aquatox.2022.106174
22. Dekant W., Völkel W. Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures. *Toxicology and Applied Pharmacology.* 2008; 228: 114–134. doi: 10.1016/j.taap.2007.12.008.
23. Sharma P., Chadha P. Bisphenol A induced toxicity in blood cells of freshwater fish Channa punctatus after acute exposure. *Saudi Journal of Biological Sciences*. 2021; 28 (8): 4738-4750 https://doi.org/10.1016/j.sjbs.2021.04.088
24. Maldonado Galdeano C., Cazorla S.I., Lemme Dumit J.M. et al. Beneficial effects of probiotic consumption on the immune system. *Ann Nutr Metab*. 2019; 74: 115–124

ВПЛИВ БІСФЕНОЛУ А ТА ПРОБІОТИКОВМІСНОГО КОРМУ НА ОКРЕМІ ПОКАЗНИКИ CARASSIUS GIBELIO BLOCH

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Одним із найпоширеніших полютантів водойм є бісфенол А (ВРА) – пластиковий мономер, що використовується для синтезу полікарбонатних пластмас, епоксидних смол і термопаперу (Barboza L. et. al., 2020). ВРА потрапляє в прісноводні та морські екосистеми внаслідок вимивання з композитів на основі ВРА, також зі скидами вод виробничих підприємств, очисних споруд та сміттєзвалищ. ВРА характеризується низьким потенціалом накопичування в прісноводних середовищах, нетривалим періодом напіврозпаду в аеробних умовах, проте через безперервність потрапляння складає серйозну проблему (Wu N. C., Seebacher F., 2020). Шляхи потрапляння ВРА в організм риби – різноманітні: через травний тракт, зябра, шкіру. Чисельні дослідження показали несприятливий вплив ВРА на поведінкові та морфо-фізіологічні параметри риб, включаючи модель плавання, координацію рухів, апетит, порушення функціонування багатьох систем (ендокринної, репродуктивної, нервової).

Попередити негативні ефекти полютанта можна через профілактичне застосування засобів, що сприяють посиленню загальної реактивності організму. У цьому сенсі нашу увагу привернули пробіотики – мікроорганізми, що виявляють стимулюючий вплив на розвиток індигенної мікрофлори, володіють високим антагоністичним, синтетичним, імуномодулюючим, регуляторним потенціалом. Нами досліджувався вплив бісфенолу А та пробіотичних мікроорганізмів Lactobacillus casei, введених у складі корму, на окремі показники Carassius gibelio Bloch (загальні поведінкові реакції, певні морфо-фізіологічні параметри, якісно-кількісну характеристику еритроцитів та лейкоцитів). Дослідження здійснювали після 15-денної акліматизації риб у акваріумах за температури води 14°С, відповідного режиму аерації та 16-годинного фотоперіоду.

Встановлено, що 96-годинна дія бісфенолу А, концентрацією 1,5 мг/л, викликає потемніння шкірних покривів, посилене слизовиділення, нехарактерну рухову діяльність, появу незвичних морфотипів еритроцитів, незначне збільшення кількості лейкоцитів, зниження показника фагоцитарної активності Carassius gibelio Bloch. Профілактичне введення пробіотичних культур у складі корму сприяло корекції поведінкових реакцій та окремих гематологічних показників.

Ключові слова: Carassius gibelio Bloch, бісфенол А, пробіотики, еритроцити, лейкоцити, фагоцитарна активність

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