УДК 639.3.043.2:579.8-026.81 *https://doi.org/10.31861/biosystems2022.01.029*

INFLUENCE OF PROBIOTICS, INTRODUCED IN THE COMPOSITION OF THE FEED SUBSTRATE, IN PARTICULAR HEMATOLOGICAL INDICATORS OF CARASSIUS GIBELIO BLOCH

L.M. VASINA, V.O. STARIKOVA, L.V. KHUDA

Yuriy Fedkovych Chernivtsi National University,
Ukraine, 58012, Chernivtsi, Kotsiubynsky 2 Str.
E-mail: l.vasina@chnu.edu.ua

Significant anthropogenic influence, the emergence of a large number of pollutants of various origins create an adverse effect on the vital activities of organisms inhabiting water bodies. Often, the consequence of such impacts is a violation of the qualitative and quantitative composition of the microbiota - the autochthonous specific water body and the one that forms the microbiocenosis of the organism of aquatic animals, which leads to the development of diseases. Recently, the number of studies indicating the positive effect of the use of probiotics as preventive, therapeutic agents and biological factors affecting water quality has been increasing. Probiotic microorganisms not only perform a protective function against pathogens through the production of short-chain fatty acids, bacteriocins, lysozyme, hydrogen peroxide, but also synthesize numerous enzymes and metabolites to provide digestive, regulatory, trophic, immunomodulatory and immunostimulatory functions. The range of studied probiotics is represented not only by microorganisms traditional for aquaculture (for example, Bacillus), but also by representatives of the genus Lactobacillus, the presence of which is recorded in individual biotopes of fish. We have studied the effect of Lactobacillus casei probiotic microorganisms introduced into the feed on some individual hematological indicators of Carassius gibelio Bloch and analyzed changes in general behavioral indicators under the influence of modified feed. The effect of probiotics was studied after 15-day acclimatization of fish in aquariums.

It has been proven that the introduction of probiotics as part of the feed substrate for 28 days has a positive effect on rapid adaptation to external stress factors, stabilization and weight gain of fish, physiological state of external mucous membranes, behavior and general condition of fish, which may indirectly indicate the role of probiotics in mobilization of body systems in the event of a change in living conditions. An increase in the number of leukocytes, an increase in the level of phagocytic activity of neutrophils, in particular the phagocytic index, was noted, which confirms the direct effect of probiotics in increasing the body's reactivity.

Ключові слова: Lactobacillus casei, Carassius gibelio Bloch, probiotics, leukocytes, phagocytic activity

Introduction. One of the reasons that leads to the development of pathological conditions of the fish organism is the increase in the content of pollutants in reservoirs due to the intensification of various industries, including fish farming. Not only highly virulent pathogens, but also representatives of the saprophytic microflora are often released from the organs of sick fish, which indicates a significant decrease in the overall reactivity of the organism. To overcome or prevent bacterial infection of aquatic organisms, alternative means – probiotics – are increasingly used to replace the active use of antibiotics, which leads to the selection and further circulation of opportunistic and pathogenic microorganisms with increased resistance (Simon R., et al., 2021; Sayes C. et al., 2017; Cruz P. M. et al., 2012).

It is known that probiotics are living cultures of microorganisms that are used to normalize microbiocenoses in the case of pathologies associated with dysbiotic conditions and the prevention of infectious diseases. Among the main requirements for probiotics are: safety of use, significant adhesive and antagonistic potential, stimulating effect on the development of indigenous microflora, etc. Among the probiotic cultures actively used in aquaculture, the share of Lactobacillus, which is not only native to the digestive tract of warm-blooded animals, but also inhabits various biotopes of the fish body, has recently been increasing (Yasmin N. et al, 2022). The beneficial effect of probiotics is associated with colonization resistance, biotransformation of toxins, regulatory and digestive functions. Particular attention among them deserves immunomodulatory (Gupta S. et al, 2019). It is known that immunological reactions are powerful homeostatic mechanisms that ensure the stability of the body's internal environment when it is disturbed by substances of an antigenic nature. The immune system of fish is a combination of cellular (lymphocytes, granulocytes, Kupffer cells, Langerhans cells, etc.) and humoral (immunoglobulins, system of complement components, lysozyme, interferon, hemolysins, etc.) immunity factors (Ringo E.et al, 2018). A significant part of immunocompetent cells is an integral part of blood and lymph. Cellular link of nonspecific resistance of fish organism, as well as mammals, is characterized by phagocytic activity of blood. Phagocytosis is considered the main process of nonspecific re-

sistance. According to the literature, fish are characterized by the dominance of non-specific protective factors, and in the early stages of ontogenesis, they are decisive.

Therefore, the purpose of this work was to study individual hematological indicators of the body of fish under conditions of 28-day exposure to probiotics introduced as part of the feed substrate. At the same time, the dynamics of fish mass, leukocyte content, indicators of phagocytic activity were determined, and general behavioral reactions were evaluated.

Materials and methods. *Carassius gibelio Bloch* fish with an average initial weight of 60 g were kept in glass aquariums 40x27x40 cm (water volume was 40 l) at a water temperature of 14°C and a 16-hour photoperiod. The water in the aquariums was changed daily by 3/4 of the volume using a siphon.

For 15 days, the fish were acclimatized, receiving a standard feed ration. The amount of feed was 1% of the total weight, taking into account the temperature regime, the features of the component composition and the size of the feed.

Further, the fish were divided into 2 experimental groups: I – fish of the control group, which were on a standard feed ration (Aller Bronze feed with a diameter of 3 mm); II - fish that received probiotic cultures in the feed substrate. To create probiotic-containing feed, Aller Bronze with a diameter of 3 mm was used as a basis, after wetting with water, *Lactobacillus casei* was added (the latter kindly provided to us by the employees of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine) in the amount of CFU $1x10^{11}$/g, and the modified feed was regranulated (the diameter of the granulator holes is 3 mm). The obtained granules were dried for 48 hours.

The experiment lasted 28 days. Fish were weighed every 7 days of the experiment to assess changes in fish weight.

Determination of individual indicators of the immune system was carried out in whole blood. Blood sampling was carried out from the site of a live incision at the intersection of the tail fin of fish, having previously removed the scales from the puncture site and treated it with alcohol to avoid mucus getting into the blood (Lawrence M.J. et al., 2020). The instruments were pre-treated with an anticoagulant.

The total number of leukocytes was determined and the phagocytic activity of neutrophils was analyzed by a modified method (Simpson D. W. 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 0C, 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 phagocytic index (the percentage of neutrophils involved in phagocytosis) and the phagocytic number (the average number of yeast captured by one neutrophil) were calculated.

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 $p \leq 0,05$ according to the Student's criterion.

Results and discussion. The diversity of action of probiotics confirms their importance in key areas of successful fish farming, including nutrition of hydrobionts, control of the environment and strengthening of resistance of organisms (Wuertz S. et al., 2021). The introduction of probiotics under the conditions of aquaculture can be carried out in a variety of ways, which will be manifested in a different mechanism of their beneficial effect - from inhibiting pathogens, strengthening the immune response to improving water quality (Pandey A.A. et al., 2022; Niamphithak P. et al., 2017). They are often used to directly add them to water or encapsulate them in live feed. In the case of using probiotics for the needs of large fish farms, it is rational, in our opinion, to use probiotic cultures as part of feed.

Approbation of this type of introduction of probiotics under the conditions of aquarium cultivation of *Carassius gibelio Bloch* revealed a number of positive changes.

It should be noted that after removing the fish from their permanent habitat and transferring them to aquariums (during the acclimatization period), the fish lost weight, on average, it decreased to 87% of the initial weight. The 28-day use of probiotic-containing feed contributed to a gradual increase in the weight of the experimental fish (Fig. 1). At the terminal stages of the experiment, it exceeded the initial levels, on average, by 16%. It is worth noting that a similar tendency was not detected when using a regular fodder diet. In the group of control fish, a stable decrease in biomass was noted throughout the experiment. Visual observations made it possible to state that the fish of the experimental group demonstrated active behavioral reactions (fast and ori-ented swimming, immediate reaction to food), high efficiency of food consumption and moder-ate sliminess of the skin and no signs of its dark-ening.

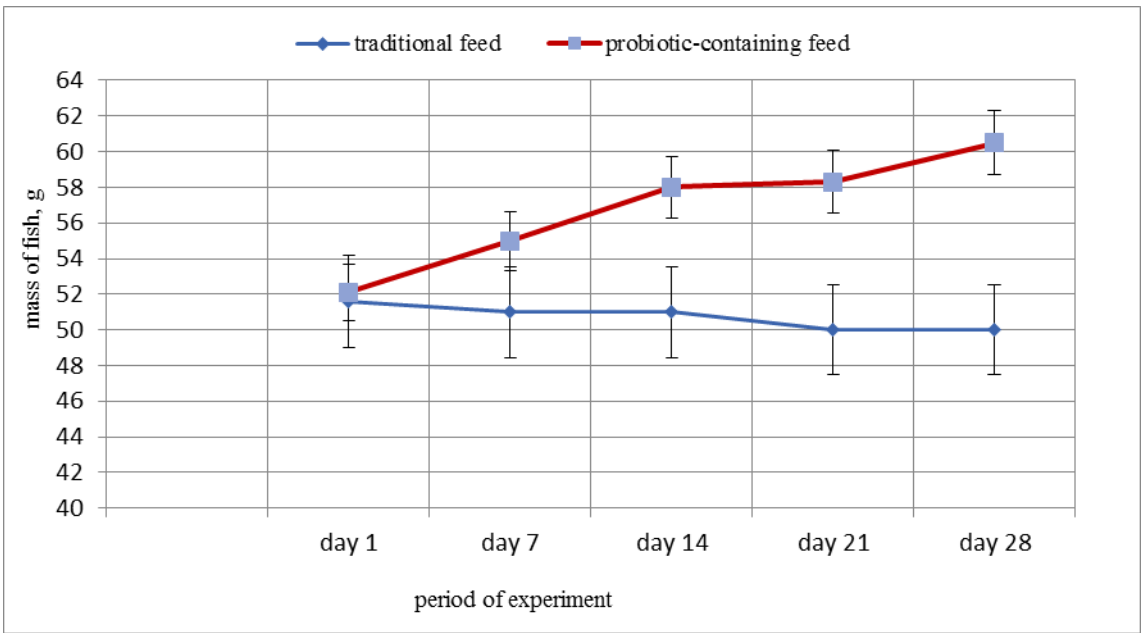

Fig. 1. Amount of biomass of A. dimorphus under different nitrogen supply

The characteristics of similar indicators, which are important for the analysis of fish health, may indicate the indirect beneficial effect of probiotics on the speed of adaptation to stress-ful factors (changes in living conditions).

It is known that probiotics, in addition to the protective function (creating colonization re-sistance of mucous membranes), provide a num-ber of other important functions - lytic, synthetic, trophic, antimutagenic. One of the most important of them is the stimulation and modulation of im-mune defense. Therefore, the next stage of our research involved the analysis of individual he-matological indicators, the change of which is associated with the action of prophylactic or ther-apeutic agents based on microorganisms - in par-ticular, the total number of leukocytes and their physiological activity. It is known that the num-ber of leukocytes affects the efficiency of regen-eration of damaged tissues, destruction of foreign bodies, production of antibodies, neutralization of parasites. According to experimental data, the use of probiotic-containing feed caused an in-crease in the total number of leukocytes (by 1.3 times, on average from 2.9*1010/l to 3.8*1010/l), and also caused changes in the phagocytic activi-ty of neutrophils (Fig. 2).

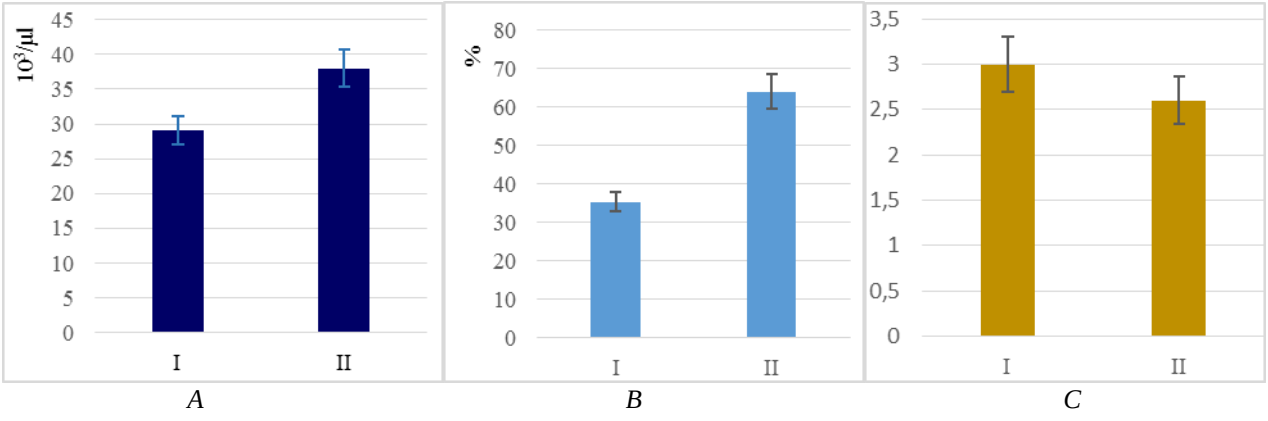

Fig. 2. Hematological indicators of Carassius gibelio Bloch under the conditions of using traditional (I) and probiotic-containing feed substrate (II): A – leukocyte content; B – phagocytic index; C – phagocytic number

To characterize the lat-ter, we took into account: the phagocytic index, which indicates the intensity of phagocytosis, and the phagocytic number, which indicates the ab-sorptive capacity of the neutrophil. In particular, we registered an increase in the phagocytic index - in the control group it was 35%, in the experi-mental group - 64%, but no significant changes in the phagocytic number were recorded - 2.6 as opposed to 3 for the control fish.

Conclusions. The use of probiotic-containing feed for 28 days led to a change in individual hematological indicators - an increase in the number of leukocytes, an increase in phagocytic activity (phagocytic index) and caused an increase in the biomass of fish, an increase in feed-eating activity, a positive effect on the physiological state of the external mucous membranes, the behavior of fish.

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ВПЛИВ ПРОБІОТИКІВ, ВВЕДЕНИХ У СКЛАДІ КОРМОВОГО СУБСТРАТУ, НА ОКРЕМІ ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ CARASSIUS GIBELIO BLOCH

Л.М. Васіна, В.О. Старікова, Л.В. Худа

Значний антрогенний вплив, поява великої кількості полютантів різноманітного походження створюють несприятливий вплив на життєдіяльність організмів, що населяють водойми. Нерідко, наслідком подібних впливів є порушення якісно-кількісного складу мікробіоти – аутохтонної конкретної водойми та тієї, що формує мікробіоценози організму водних тварин, що призводить до розвитку хвороб. Останнім часом зростає кількість досліджень, що вказують на позитивний ефект застосування пробіотиків як профілактичних, лікувальних засобів та біологічних чинників, що впливають на якість води. Пробіотичні мікроорганізми не лише здійснюють захисну функцію щодо патогенів шляхом продукції коротколанцюгових жирних кислот, бактеріоцинів, лізоциму, пероксиду водню, а й а синтезують чисельні ферменти й метаболіти для забезпечення травної, регуляторної, трофічної, імуномодулюючої та імуностимулюючої функцій. Спектр досліджуваних пробіотиків представлений не лише традиційними для аквакультурами мікроорганізмами (наприклад Bacillus), а й представниками роду Lactobacillus, наявність яких фіксують в окремих біотопах риб. Нами досліджувався вплив пробіотичних мікроорганізмів Lactobacillus casei, введених у складі корму, на окремі гематологічні показники Carassius gibelio Bloch та проаналізовані зміни загальних поведінкових показників за дії модифікованого корму. Вивчення дії пробіотиків здійснювали після 15-денної акліматизації риб у акваріумах.

Доведено, що введення пробіотику у складі кормового субстрату впродовж 28-ми днів позитивно впливає на швидку адаптацію до зовнішніх стресових факторів, стабілізацію та приріст маси риб, фізіологічний стан зовнішніх слизових покривів, поведінку та загальний стан риб, що може опосередковано свідчити про роль пробіотиків у мобілізації систем організму у випадку зміни умов існування. Відмічено збільшення кількості лейкоцитів, зростання рівня фагоцитарної активності нейтрофілів, зокрема фагоцитарного показника, що підтверджує безпосередню дію пробіотиків у посиленні реактивності організму.

Keywords: Lactobacillus casei, Carassius gibelio Bloch, пробіотики, лейкоцити, фагоцитарна активність

Отримано редколегією 12.04.2022 р.