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Morphological and functional parameters and species composition of *Cyprinus carpio* microbiocenoses

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Abstract. The results of studies of the quantitative and species composition of microorganisms of microbiocenoses *Cyprinus carpio* are presented. During the species identification of microorganisms isolated from the intestinal contents of Cyprinus carpio, a total of 27 (71.1%) isolates were identified, including 13 (48.1%) *E. coli*; 4 (10.5%) *K. pneumoniae*; 3 (7.9%) *P.vulgaris*; 2 (5.3%) *E. cloacae*; 1 (2.7%) *P. aeruginosa*; 1 (2.7%) *S. aureus*; 1 (2.7%) *S. epidermidis*; 2 (5.3%) *C. albicans*; 1 (2.7%) *C. parapsilosis*. Gill histology is the preferred method for monitoring the effects of environmental factors affecting farmed fish in fish farms. In the gills, gill petals and branchial lamellae extending from them were identified. The main mass consisted of respiratory cells, characterized by small size, located in the center of the nucleus, basophilic cytoplasm. Myocardiocytes and blood vessels were identified in the heart. In the gastrointestinal tract, the integumentary epithelium and goblet cells of the villi and crypts of the intestinal mucosa were observed. The liver capsule and interlobular connective tissue were clearly distinguishable, uniform staining of the cytoplasm of hepatocytes was observed in the hepatic lobules, the nuclei had a clear outline and were located in the central part of the cells. In the spleen, the interstitial tissue of the stroma was observed, a clear border between the cortical and medulla of the lymphatic follicles.

Keywords: *microbiocenoses, microorganisms, myocardiocytes, lymphocytes, lamellae, epithelium, villi, crypts*

Introduction

The rapid growth of anthropogenic loads contributes to the pollution of water bodies, the immunosuppressive effect of antigens determines the risks of the development of pathology of susceptible species of aquatic organisms [1]. In a microbiological study, the number of

microorganisms of carp gill filaments was $3.3\pm3.8\times10^6$–$7.9\pm5.6\times10^6$ CFU/ml; intestinal contents – $1.4\pm2.9\times10^{10}$ –$1.7\pm6.0\times10^{11}$ CFU/ml. Among the identified microorganisms, *Aeromonas hydrophila* was dominant, accounting for 32.0% of the total number of isolates. In addition, the following species were identified: *Shewanella putrefaciens*, *Vibrio cholerae*, *Staphylococcus* spp., *Vibrio vulnificus*, *Pasteurella pneumotropica*, *Corynebacterium urealyticum* and *Micrococcus* spp. [2]. In the microbiocenoses of the carp intestine, the dominant microorganisms were *Fusobacterium spp*., *Proteobacterium spp*., *Bacteroides spp*. *Firmicutes spp* [3]. Of 84 fish samples, 32 samples (38.09%) contained *Salmonella spp*. Bacteria, 12.5% of isolates possessed the virulence genome - *invasin A* [4].

Infection of carp with *pathogenic bacteria revealed* pathological *processes* with abundant absorption vacuole, degenerative processes with signs of inflammation of the middle and distal parts of the intestine, fish mortality rates were 46.7–63.3% [5, 6]. To develop effective methods for assessing the physiological and immunological status of aquatic organisms, the priority task is to study the morphological parameters, quantitative and species composition of microorganisms of microbiocenoses *Cyprinus carpio*, which determined the relevance of research.

Materials and methods

The studies were carried out in compliance with the international requirements of the "Declaration of Helsinki on the Humane Treatment of Animals", "Declaration of Helsinki Ethical Principles", 2008 [7], "Directives of the Council of the European Community on the protection of animals used for experimental and other scientific purposes" [8].

The object of the research was fish *Cyprinus carpio*, three to four years old, weighing 1.0–1.5 kg, grown in a pond farm (n=5).

To study the quantitative and species composition of microorganisms, the contents of the intestine were examined for this, 0.5 g of each sample was placed in test tubes containing 10.0 ml of sterile 0.85% *NaCl* solution. To precipitate large particles, the samples were kept for 10–15 min at 18–20°C, then the supernatant was inoculated onto the surface of the differential diagnostic media; for the accelerated identification of microorganisms, the "*API 20E"* test system (*BioMereux*, France) was used. Determination of morphological, cultural and biochemical properties of microorganisms was carried out by conventional methods in accordance with the guidance "*Bergey's Manual of Systematic Bacteriology" (1984-1989)* and "Identifier of pathogenic and opportunistic fungi" [9,10]. For histological studies, the material was fixed in a 10.0% solution of neutral formalin, embedded in paraffin, sections were stained with hematoxylin and eosin.

The results of the experimental data were processed by the method of statistical analysis using the Student's t-test, the results were considered reliable at $p\leq0.05$.

Research results

Taking into account the quantitative and species composition of microorganisms of the intestinal microbiocenoses of *Cyprinus carpio*, the growth of microorganisms was observed in all the studied differential diagnostic media.

On *Brilliance agar* medium, due to the presence of sodium dodecyl sulfate in the medium, the growth of gram-positive bacteria was suppressed, the number of gram-negative bacteria was: 7.15 ± 0.12 - 9.33 ± 0.26. Taking into account the differential characteristics of 20 isolated gram-negative pure cultures of microorganisms, 19 (95.0%) isolates of bacteria of the *Enterobacteriaceae* family were identified, of which 12 (60.0%) were *Escherichia coli*, 4 (20.0%) - *Klebsiella pneumoniae*, 2 (10.0%) - *Proteus vulgaris*, 1 (5.0%) - *Enterobacter cloacae*.

On the *Cetrimide Agar* medium, the number of microorganisms was: 1.01 ± 0.12 - 2.01 ± 0.10. Out of 6 isolated pure cultures of microorganisms, 1 isolate (16.7%) of gram-negative aerobic bacteria *Pseudomonas aeruginosa* was identified.

The number of microorganisms on the *Yolk Salt Agar* medium containing 10.0% sodium chloride was 0.83 ± 0.07 - 1.36 ± 0.09. From 6 isolated pure cultures of microorganisms, 2 isolates (33.3%) of gram-positive bacteria were identified: *S. aureus* - 1 (16.7%), *S. epidermidis* - 1 (16.7%).

The number of colonies of microorganisms on *Hi Crome Candida Agar* medium was 1.74 ± 0.13 - 2.18 ± 0.03. Of the 6 isolated pure cultures of microorganisms, 3 isolates (50.0%) of yeast-like fungi were identified: *Candida albicans* - 2 (33.3%), *Candida parapsil osis* - 1 (16.7%).

During the species identification of microorganisms isolated from the intestinal contents of *Cyprinus carpio*, a total of 27 (71.1%) isolates were identified, including (48.1%) *E.coli*; 4 (10.5%) *K.pneumoniae*; 3 (7.9 %) *P.vulgaris*; 2 (5.3 %) *E. cloacae*; 1 (2.7%) *P.aeruginosa*; 1 (2.7%) *S. aureus*; 1 (2.7%) *S.epidermidis*; 2 (5.3%) *C.albicans*; 1 (2.7%) *C. parapsilosis*.

In the gills, gill lobes and branchial lamellas extending from them were observed. In the thickness of the gill petals, there was a cartilaginous hyaline arch, surrounded by connective tissue with large blood vessels located in it. The branchial lobe is covered with a stratified squamous respiratory gill epithelium. The main mass consisted of respiratory cells, characterized by small size, located in the center of the nucleus, basophilic cytoplasm.

The primary branchial epithelium contained mucous and rod cells located in the areas between the branchial lobes and along the edge of the petal. Mucous cells were characterized by large size, displaced nucleus, oxyphilic cytoplasm. Rod cells had a regular round shape, a nucleus displaced to the basal part, and a weakly oxyphilic cytoplasm. The lamellae were covered with a two-layer squamous respiratory epithelium located on the basement membrane, under which the vascular layer is located, represented by a row of columnar cells, between the lateral surfaces of which cavities filled with blood are localized. The development of the pathological process in the gills was accompanied by swelling, areas of twisting and fusion, respiratory lamellae with the development of congestion were identified.

An increase in the height of the branchial epithelium and a reduction in the surface relief of epithelial cells, necrosis of the petals of the second order were found. In the presence of a tumor of the branchial lobes, polymorphic multinucleated cells of the fibroplastic row were detected. Large yellow-green pigment granules were observed in the cytoplasm of the cells. In the central part of the tumor, degeneration and necrosis of cartilage tissue, disintegration of tissues at the base of the petals, degeneration of cartilage and deformation of the petals were revealed. Between the petals of the second order, mucus was visualized with an admixture of erythrocytes, leukocytes, macrophages and undifferentiated cells with a large light nucleus and cytoplasm. Areas of growth of the respiratory epithelium of lamellae and filament were identified. Hypertrophy and hyperplasia of the goblet cells were accompanied by dystrophy of the respiratory cells. Deformation of the filaments and atrophy of the lamellae were observed in the form of sloughing of the respiratory epithelium or the absence of lamellae on one or both sides of the filament. Necrotization of respiratory epithelial cells is more pronounced in the upper part of the lamellae. Histological examinations of the gills are markers for monitoring the effects of environmental factors affecting farmed fish in fish farms.

Myocardiocytes and blood vessels were identified in the heart.

In the gastrointestinal tract, the presence of integumentary epithelium of the villi of the mucous membrane was noted, intestinal crypts and goblet cells were detected. Collagen fibers were observed in the loose fibrous connective tissue of the submucosal layer of the mucous membrane of the small and large intestine. With the development of the pathological process in the intestine, hyperemia, edema of the intestinal mucosa were observed, the levels of diamine oxidase and D-lactate in the damaged intestine were increased ($p<0.05$). The activities of protease, lipase, amylase, Na^+/K^+-ATPase and alkaline phosphatase are reduced ($p<0.05$), the permeability of the intestinal mucosa increased. The liver capsule and interlobular connective tissue were clearly distinguishable. In the hepatic lobules, uniform staining of the cytoplasm of hepatocytes was observed, the nuclei had a clear outline and were located in the central part of the cells (fig. 1).

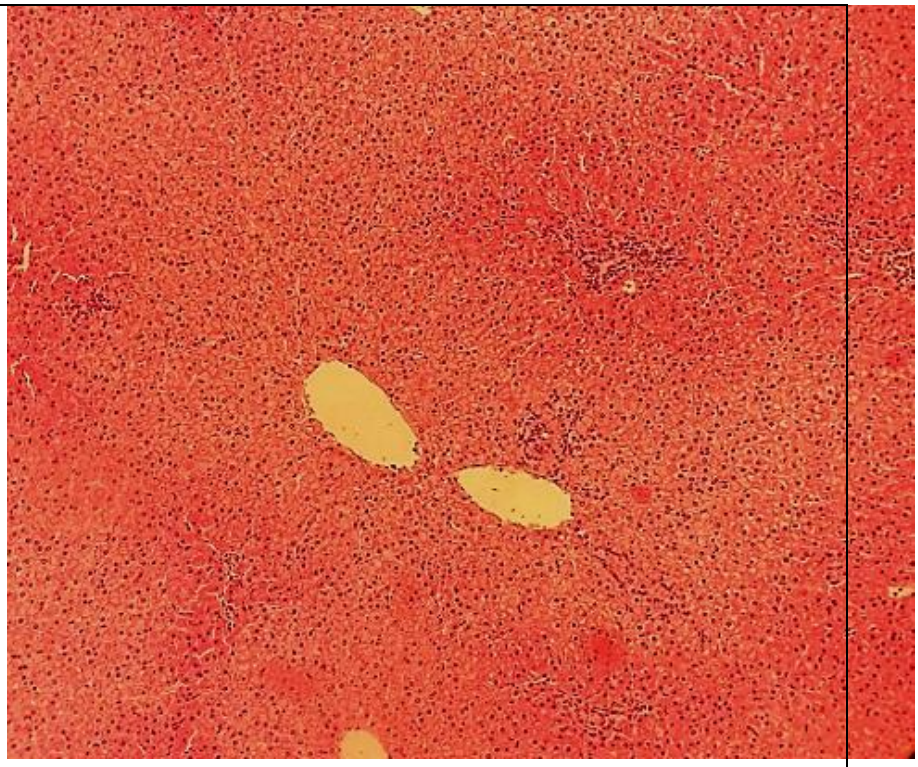

Figure 1 – Liver of *Cyprinus carpio*, 3 years of age, weighing 1.0–1.5 kg. Hematoxylin and eosin. Circ. 10, vol. 10, 40

In the spleen, the interstitial tissue of the stroma was observed, a clear border between the cortical and medulla of the lymphatic follicle. At high magnification of the microscope, plasma cells were found in the cortex of the lymphatic follicles. In the medulla, lymphocytes were visible, which had a uniformly colored appearance.

Discussion

Aquaculture is currently a promising direction for obtaining fish products; the limiting factors for the development of the industry are insufficient knowledge of the physiological and immunological status of aquatic organisms [11].

With the growth in the number of breeding achievements, attention should be paid to morphological variability when growing under conditions that differ from natural ones, in particular under conditions of recirculation, which is reflected in a decrease in the natural resistance of the organism to the effects of external factors that cause the risks of the development of pathology of susceptible species of aquatic organisms [12]. With an increase in jointly reared objects, the risk of the appearance of various pathologies increases, the share of under-received aquaculture production due to various fish diseases accounts for up to 30.0% of the potential "harvest" [13]. The survival rate of the white sea bream *Diplodus sargus* under experimental infection with *S. epidermidis was* 43.3–46.7% [5]. Seasonal changes affect the innate immunity of common carp, so the highest phagocytic and blood plasma complement activity was observed in autumn, decreased in summer and winter, and the lowest

activity was found in spring [14]. The processes of intercellular communication, sorption, aggregation of heterogeneous biofilms and phenotypic plasticity of microorganisms expand the adaptive potential and cause the circulation of antibiotic-resistant strains among the population of susceptible species and environmental objects [15]. The addition of the probiotic *P. pentosaceus* at a *dose* of $1x10^8$ CFU/g allowed to increase (P<0.05) the activity of protease and lysozyme, the number of erythrocytes and leukocytes, the effect of interleukin-1beta (*il1b*) (P=0.016), interleukin 8 (*il8*) (P=0.007), interleukin 6 (*il6*) (P<0.001), catalase (*cat*) (P=0.041) and gene expression of glutathione peroxidase (*gpx*) (P=0.001), as well as superoxide dismutase (SOD) (P<0.001), glutathione peroxidase (GPx) (P<0.001), and malondialdehyde (MDA) levels (P<0.001) on intestinal tumor necrosis factor was established [1]. Dietary essential oil of oregano (15 g/kg) had a positive effect on the growth parameters of carp (*Cyprinus carpio L.*); degenerative changes were not detected in the intestinal villi with associated goblet cells, crypts, and submucosal tissues [16].

Conclusion

Taking into account the quantitative and species composition of microorganisms of microbiocenoses Cyprinus carpio, a total of 27 (71.1%) isolates were identified, including 13 (48.1%) *E. coli*; 4 (10.5%) *K. pneumoniae*; 3 (7.9%) *P.vulgaris*; 2 (5.3%) *E. cloacae*; 1 (2.7%) *P. aeruginosa*; 1 (2.7%) *S. aureus*; 1 (2.7%) *S. epidermidis*; 2 (5.3%) *C. albicans*; 1 (2.7%) C. *parapsilosis*. Gill histology is the preferred method for monitoring the effects of environmental factors affecting farmed fish in fish farms. In the gills, gill petals and branchial lamellae extending from them were identified. The main mass consisted of respiratory cells, characterized by small size, located in the center of the nucleus, basophilic cytoplasm. Myocardiocytes and blood vessels were identified in the heart. In the gastrointestinal tract, the integumentary epithelium and goblet cells of the villi and crypts of the intestinal mucosa were observed. The prescribed treatment had a positive effect on the growth rates of carp (*Cyprinus carpio L.*), which in turn contributed to an increase in fish productivity.

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