Pathology of cells and tissues of the gastrointestinal tract of African catfish in high-tech industrial aquaculture

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Abstract. There is a possibility of developing a pathological process in the body of fish in the conditions of high-tech industrial aquaculture and at high planting densities, creating chronic stress. Histological studies allow early detection of the initial stages of possible pathology at the cellular level. The studies have shown that apparently healthy individuals of African catfish grown in recirculating aquaculture systems (RAS) revealed pathology of cells and tissues of the gastrointestinal tract. They had edema of the internal muscle layer of the stomach and intestines, detachment of the epithelial layer from the gastric mucosa, violation of the structural organization of gastric fields on histological sections. Thinning of intestinal villus, reduction of their length and branching, violation of their structure up to destruction were also revealed. The study of the tissues of the stomach and intestines was carried out using a research motorized universal microscope Axio Imager.M2 (Carl Zeiss, Germany). To correct the pathological process, the experimental group of fish received a “sporothermin” probiotic with feed, which belongs to the probiotics of the last generation. It is an antagonist of a wide range of pathogenic and opportunistic microbiota and is used as an alternative to antibiotics. The composition of “sporothermin” includes spore forms of bacteria Bacillus subtilis and Bacillus licheniformis, which in the gastrointestinal tract of fish turn into vegetative forms. Bacteria Bacillus subtilis and Bacillus licheniformis are not representatives of the intestinal microbiocenosis of the African catfish, but they regulate its structure by reducing the level of pathogenic and opportunistic microbiota. Pathology of cells and tissues of the gastrointestinal tract have been identified in the fish of the experimental group treated with the sporothermin probiotic.

1. Introduction
African catfish (Clarias gariepinus), a common object of industrial aquaculture, this species of fish is characterized by rapid growth, has a delicious meat rich in essential amino acids and polyunsaturated fatty acids, including omega-3 [1].

Cultivation of fish in conditions of industrial aquaculture is characterized by high density planting, leading to water contamination by the metabolites of the fish. High level of organic substances - products of vital activity of fishes promotes development of conditionally-pathogenic and pathogenic microorganisms which infect fishes, reduce the general resistance and cause development of pathological conditions.

Probiotics are used to normalize intestinal microbiocenosis and prevent the development of pathogenic and opportunistic microbiota [2]. In our studies, we used the probiotic latest generation of...
“sporothermin”. It is created on the basis of spore forms of bacteria Bacillus subtilis and Bacillus licheniformis, which in the gastrointestinal tract turn into vegetative.

Probiotics stimulate the growth of normal intestinal microbiota, suppressing pathogenic. They also strengthen the body’s natural defense mechanisms [3,4]. Probiotic microbiota synthesizes a complex of biologically active substances that regulate metabolism, increase resistance to infectious diseases [5], stimulate intestinal motility and improve its excretory functions. At the background of probiotics, the digestibility of feed and its digestibility improves, the assimilation of nutrients increases, feed costs decrease due to a more complete breakdown of food components and the production of vitamins by microorganisms [6].

The aim of the work was to assess the effect of the “sporothermin” probiotic on the cells and tissues of the stomach and intestines of fish when grown in high-density planting in high-tech industrial aquaculture.

2. Materials and methods
The object of the study was sexually mature females of African catfish grown in the installation of recirculating aquaculture systems (RAS) - “SOM 1100” at high planting density. The research was carried out in the Laboratory of experimental biology and aquaculture of Ulyanovsk state agrarian University.

The water temperature was maintained at 26ºC, the oxygen content in the water was not lower than 4 mg/l.

Two groups of sexually mature females of the same age were formed, 350 individuals each. The first group received traditional feed that did not contain probiotic, the second – experimental was grown on feed containing probiotic. The fish of the experimental group were fed with the probiotic “Sporothermin” at the rate of 4 g per 1 kg of feed. The experiment lasted 3 months.

Production of histological preparations and their microscopy was carried out in the research center of fundamental and applied problems of bioecology and biotechnology of the Ulyanovsk state pedagogical University.

Recording of the material was carried out with 10% neutral buffered formalin. The production of sections was carried out by the classical method [7], the preparations were stained with Mayer’s hematoxylin-eosin and coated with a mounting medium Sub-X Mounting medium (Leica, USA). Histological structure analysis and documentation were carried out using the research motorized universal microscope Axio Imager.M2 (Carl Zeiss, Germany) included: color digital camera AxioCam high resolution HRc; software for analysis and processing of information ZEN pro.

3. Results
Received histological preparations of the stomach wall of female African catfish (figure 1) grown using the probiotic “Sporothermin”, demonstrate that in the area of the body of the stomach are determined gastric folds (plicae gastricae), represented by the mucous membrane and submucosal basis. Gastric fields (akae gastricae) are visible in the photo (figure 2). They are separated from each other by grooves of the mucous membrane. Gastric dimples (foveolae gastricae) (figure 2) are visible in the gastric wall mucosa of African catfish grown using the probiotic “Sporothermin” in the form of recesses of a single-layer multi-row epithelium in its own plate of the mucous membrane (figure 1).

The gastric mucosa of African clary catfish (Clarias gariepinus), grown using the probiotic “Sporothermin”, is covered with a single-layer multi-row epithelium, its own plate (l. propria mucosae). From the muscle plate (l. muscularis mucosae), individual muscle cells depart into the connective tissue of the mucous membrane’s own plate.

The submucosal basis of the stomach of African catfish grown using the probiotic “Sporothermin” consists of loose connective tissue containing a large number of elastic fibers, in which the arterial and venous plexus, a network of lymphatic vessels are located (figure 1).

The muscular membrane of the stomach of African clary catfish (Clarias gariepinus), grown using the probiotic “Sporothermin” is relatively poorly developed in the area of its bottom, is well expressed
in the body and reaches the greatest development in the pyloric department. There are three layers in the muscle membrane, formed by smooth muscle cells - inner and outer circular and middle-longitudinal. There are the plexuses of lymphatic vessels between the layers of the muscular membrane (figure 1).

The serous membrane of stomach of African clary catfish (Clarias gariepinus), grown using the probiotic “Sporothermin” consists of loose connective tissue, which is covered with mesothelium on top.

Female of African clary catfish (Clarias gariepinus) raised without probiotic showed marked swelling of the submucosa and the inner muscle layer of the stomach (figure 3), there was a thickening due to edema of the blood vessel wall (figure 4).

Female of African clary catfish (Clarias gariepinus) raised without the use of probiotic has a detachment of the epithelial layer from the mucosa (figure 3), the structural organization of the gastric fields is disturbed (figure 3), food is present in the lumen of the stomach (figure 3). There is a mosaic in the change of the structure of gastric fields (figures 3, 4). This pattern was observed on all preparations in fish grown without probiotic.
In the study of histological preparations of the intestines of African clary catfish (Clarias gariepinus) grown using the probiotic “Sporothermin” (figure 5, 6), the inner mucous membrane (lamina propria mucosae) is determined, which is represented by loose connective tissue with capillary-type blood vessels. Outside (figure 5) the membrane is covered with a cover epithelium having a single-row multilayer structure. The composition of the epithelium includes highly prismatic main cells-enterocytes (figure 6), glandular goblet cells (figure 6), as well as basal cells belonging to the cambial cell type, capable of active proliferation. They are the source of a highly differentiated population (differon) of both basic and specialized villus and crypt cytotypes.

The muscular layer is clearly revealed (figure 5), separating the mucous membrane itself and the submucosal layer. The submucous layer (tela submucosa) (figure 5) is also represented by loose unformed connective tissue with a large number of blood and lymph vessels of different sizes.

There are two muscle layers under the submucosa (tunica muscularis) - one layer is located circularly (inner), the other outer, in which the smooth muscle fibers lie longitudinally (figure 5). Outside, the intestinal wall is covered with a serous membrane (tunica serosa) (figure 5).

The structural and functional unit of the system “villus-crypt” is represented by villus of great length, branched and filling a significant part of the intestinal lumen, most of the villus are connected to each other. The “villus-crypt” ratio is 1: 6.

Along the entire length of the villus, the polarity in the location of epithelial cells with different degrees of differentiation is clearly traced. There are basal (cambial) epithelial cells at the base, then...
poorly differentiated, closer to the top of the villus there are highly differentiated enterocytes with a central location of the nucleus in the cytoplasm. There is a layer of microvillus on the apical surface, there are glandular cells (mucous, bacaloid) among the enterocytes of highly prismatic morphology. At the bottom of the crypt, a small number the glandular cells with granules in the cytoplasm are located, as well as prismatic epithelial cells. In addition, rare lymphocytes are observed within the epithelial layer.

Figure 5. A fragment of the intestinal wall of African catfish fed probiotic. – Painting hematoxylin-eosin. The zoom of eyepiece x10, lens x 10. 1-serous, 2-blood vessel, 3-submucosa, 4-inner circular muscle layer, 5-mucous membrane, 6-intestinal lumen, 7-outer longitudinal muscle layer.

In control individuals of African clary catfish (*Clarias gariepinus*) grown without probiotic, there are changes in the intestinal mucosa, manifested in the thinning of the villus, reducing their length and branching, reducing the number of contacts between the villus. The destruction of the structure of the villus themselves is characteristic. In some cases, the mucosa is thinned, and in other cases, swelling of the mucosa itself is manifested. There is swelling of the muscular layer of the intestinal wall and serous membrane (figures 7, 8, 9, 10).
Figure 7. Structure of the intestinal wall of African catfish grown without probiotic. Painting-hematoxylin-eosin. The zoom of eyepiece x10, lens x10. Thinning and destruction of the villus, reduction of branching.

Figure 8. The structure of the intestinal wall of African catfish, not receiving probiotic. Painting-hematoxylin-eosin. The zoom of eyepiece x10, lens x10. Shortening and reduction of branching of villus, destruction of villus. Edema of the muscle layer, serosis.
It should be noted that some individuals on the preparation revealed areas with a relatively intact structure of the intestinal mucosa (figure 11).

**Figure 9.** The structure of the intestinal wall of African catfish not receiving probiotic. Painting-hematoxylin-eosin. The zoom of eyepiece x10, lens x20. Shortening and reduction of branching of villus, destruction of villus.

**Figure 10.** The structure of the intestinal wall of African catfish (*Clarias gariepinus*) not receiving probiotic. Painting-hematoxylin-eosin. The zoom of eyepiece x10, lens x20. Shortening and destruction of villus. Swelling of the mucous membrane proper, reducing the branching of the villus.
4. Discussion

In recent years, in industrial aquaculture, probiotics have been used in the cultivation of fish. They are mostly used for the normalization of the gastrointestinal microbiota of fish. Probiotics of the latest generation are antagonists of a wide range of pathogenic and opportunistic microorganisms and are used as an alternative to antibiotics. It is important to have a clear understanding of the nature of their effect on the cells and tissues of the gastrointestinal tract of fish.

The use of probiotics, biologically active substances, or toxicants inevitably causes changes in the cells and tissues of the stomach, intestines, liver; this is convincingly evidenced by the results of a large number of studies [8-12].

Histological studies are used as an indicator of the state of the organism at the cellular level [13].

The results of histological microarchitecture of the stomach and intestines of African clary catfish when grown in high-tech industrial aquaculture are new. The analysis of available literature sources on the studied problem has not revealed similar studies. Therefore, we were not able to compare our own results with those obtained earlier by other researchers. Also, the histological microarchitecture of the stomach and intestines of African catfish when grown with probiotics has not been studied to date. The results obtained on the positive effect of the probiotic “Sporothermin” on the cells and tissues of the stomach and intestines of African catfish are new.

Strains of bacteria Bacillus subtilis in Russia are widely used for the prevention and treatment of gastrointestinal diseases of farm animals and fish [14].

When assessing the histological characteristics of the cells of the gastrointestinal tract of African clary catfish (Clarias gariepinus), the results were obtained, indicating that the probiotic “Sporothermin” leads to a decrease in histological disorders in the gastrointestinal tract of African clary catfish (Clarias gariepinus).

The edema of the submucosa and the inner muscle layer of the stomach was revealed in fish grown without probiotic, the epithelial layer was detached from the mucosa, the structural and functional organization of the gastric fields was violated. Changes of intestinal mucosa are revealed, thinning of villus and decrease of their extension, branching are observed, the number of contacts between them decreases, destruction of villus is observed. There is swelling of the muscle layer of the intestinal wall and serous membrane. In individuals receiving additive probiotic “Sporothermin” as a feed, pathological changes in the tissues of the gastrointestinal tract were absent.
5. Conclusions
Our research suggests that the use of the probiotic “Sporothermin” in the cultivation of African clary catfish (*Clarias gariepinus*) in industrial aquaculture opens up prospects for correcting the negative effects generated by high-tech fish farming systems.

Bacteria *Bacillus subtilis* and *Bacillus licheniformis*, which are part of the probiotic “Sporothermin”, make adjustments to the quantitative and qualitative composition and optimize the intestinal microbiocenosis. It plays an important role in parietal digestion. Due to the restoration of equilibrium in the intestinal microbiocenosis, the number of opportunistic and pathogenic microorganisms decreases. There is a decrease in pathological changes in the tissues of the gastrointestinal tract of fish, which allows for full digestion and absorption of nutrients. Such fish grows faster, gains weight and consumes food well, which corresponds to the norms and requirements for growing fish in high-tech industrial aquaculture.

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References
[1] Romanova E, Lyubomirova V, Romanov V, Mukhitova M, Shlenkina T 2018 *Egyptian Journal of Aquatic Research* **44** 4 pp 315-319
[2] Hai N 2015 *Journal of applied microbiology* **119** 4 pp 917-935
[3] M Zorriezhzahra, Adel M, Delshad S, Tiwari R, Karthik K, Dhama K, Lazado C 2016 *Veterinary quarterly* **36** 4 pp 228-241
[4] Soltani M, Lymbery A, Ghosh K, Hoseinifar S, Kumar V, Roy S, Ringo E 2019 *Reviews in fisheries science and aquaculture* **27** 3 pp 331-379
[5] Glencross B, Booth M, Allan G 2007 *Aquaculture Nutrition* **13** pp 17-34
[6] Kesarcodi-Watson A, Kaspar H, Lategan M, Gibson L 2008 *Aquaculture* **274** 1 pp 1-14
[7] Suvarna K, Layton C, Bancroft's J 2019 *Theory and Practice of Histological Techniques* p 672
[8] Honorato C, da Cruz C, Carneiro D, Márcia R 2011 *Brazilian Journal of Veterinary Research and Animal Science* **48** 4 pp 281-288
[9] Perozo Z, Leones T, Allison A, Rivero A, Rojas H 2009 *Revista Científica de la Facultad de Ciencias Veterinarias de la Universidad del Zulia* **19** 6 pp 607-618
[10] Saraiva A, Cruz C, Eiras J, Costa J, Serrão J 2015 *Aquaculture* **448** pp 375-381
[11] Kaptaner B, Dogan A, Durmuş A, Kankaya E 2016 *Environmental Monitoring and Assessment* **188** 8 p 474
[12] Iheanacho S, Ogueji E, Ikwo N, Igweze N, Chukwuidha C, Onyeneke R 2018 *Turkish Journal of Fisheries and Aquatic Sciences* **18** 3 pp 377-384
[13] Saraiva A, Cruz C, Eiras J, Costa J, Serrão J 2015 *Aquaculture* **448** pp 375-381
[14] Zuenko V, Laktionov K, Pravdin I, Kravtsova L, Ushakova N 2017 *Journal of Ichthyology* **57** 1 pp 152-157