Effectiveness of the antibacterial action of the complex drug "VITAROL-E" in comparison with chloramphenicol and correction of non-specific factors of fish body protection

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Abstract. At adaptation of fishes to new conditions of habitation, in an organism there is an activization of processes with participation of free radicals. The application in combined medication – «Vitarol-E» – in the treatment with aeromonose enables to maintain the essential level of antioxidants in the organism of fish, by that increasing non-specific resistance. Antimicrobial effect of «Vitarol-E» in tested quantities leads to the formation of the concentration in tissues, which provides germicidal activity.

1. Introduction
The most common disease of carp fishes in many regions of the Russian Federation is aeromonosis, and in most cases, the causative agents of the disease belong to mobile forms (pathogenic variants). Aeromonads are constantly present in the aquatic microbiocenosis, and their epizootic significance is determined by virulence and quantity [1].

In warm-water aquaculture, aeromonads can cause significant economic losses, but it is difficult to determine the direct losses that occur with secondary infections [2, 3]. Environmental factors such as crowding, threshold concentrations of oxygen in water, high organic matter content, industrial pollution, sudden temperature fluctuations, injuries and hormonal changes during puberty contribute to the defeat of the fish body by bacteria. Many virulence factors, when combined, can significantly increase the pathogenic effect of microorganisms.

Extracellular products (ECP5): toxins, hemolysins, proteases and acetylcholinesterases increase the negative effect of aeromonads in fish. Virulent determinants, such as the presence of S-layer and serum resistance, are also involved in the mechanisms of bacterial resistance, and non-specific immune and physiological-biochemical reactivity occurs at the macroorganism level [4, 5].

At the same time, the ability of fish to adapt and resist pathogens largely depends on its physiological and biochemical status. In ensuring the normal functioning of various physiological systems and in the pathogenesis of diseases, the intensity of the processes of free radical lipid oxidation is of great importance.

Metabolic disorders in the body of infected hydrobionts may be accompanied by a shift in the pro- and antioxidant balance existing under normal conditions towards free-radical pathologies. In this connection, there is a need to use substances that can inhibit the increasing activity of oxidative processes.
Based on the multifactorial nature of fish aeromonosis, which includes both the biological properties of the pathogen and the susceptibility of hydrobionts, depending on the activity of metabolic reactions in the body and the level of bioantioxidants, it is necessary to screen drugs with antibacterial and antioxidant properties.

The aim of the research was to study the effect of aeromonad infection on the state of the oxidative-antioxidant system of the fish body. Comparative analysis of the effectiveness of chloramphenicol and the complex drug "Vitarol-E" in aeromonosis, in conditions of commercial carp farming.

The level of activity of processes involving free radicals and the state of the antioxidant defense system in the fish body were evaluated using generally accepted methods, including determination of the content of malondialdehyde-MDA, vitamin E, catalase, peroxidase, ceruloplasmin, and superoxide dismutase – SOD [6].

The effectiveness of the drug "Vitarol-E" developed in the laboratory of ichthyopathology of FSC VIEV in production conditions was determined by the clinical condition of fish (2-3-year-old carp) and the results of microbiological and biochemical studies.

Therapeutic feed was prepared on-site in the form of wet bags at the rate of 1500 ml per 1000 kg of feed. The course of feeding was 7 days, the daily rate was 5% of the fish weight.

Bacteriological seeding and blood sampling were performed on the 7th day of feeding, on the 7th and 14th days after the end of giving medical compound feeds.

The antibacterial activity of preparations with Aeromonas hydrophila and Aeromonas sobria cultures isolated from fish before the experiment was studied using serial dilutions in a liquid nutrient medium (MBC, MBC).

To compare the effectiveness of the action according to the above schemes, chloramphenicol (levomycetin) was chosen, which was used in a feeding pond in dosages of 600g/1000 kg of feed, a course of 10 days according to the instructions.

2. Research results and discussion

Biochemical studies of blood samples taken from carp with symptoms of aeromonosis revealed a significant decrease in the activity of enzymes and vitamin E. At the same time, there was a tendency to accelerate lipoperoxidation and, as a result, the accumulation of one of the final toxic substances, malondialdehyde (table 1).

**Table 1.** The level of activity of the antioxidant defense system and the intensity of free radical oxidation in the body of carp during aeromonosis (P<0,05).

| Physiological state | Catalase, mkm H$_2$O$_2$/l x min x 10$^3$ | Peroxidase, Opt. den. un./l x sec | Ceruloplasmin, Un.A/ml plasma | Superoxide dismutase, Un/ml plasma | Vitamin E, mcg/l | MDA mkm/l |
|---------------------|---------------------------------|----------------------------------|-------------------------------|----------------------------------|-----------------|-----------|
| Clinically healthy fish | 3.50±0.73 | 4.60±0.33 | 37.45±4.20 | 28.40±3.10 | 13.95±2.20 | 4.30±0.20 |
| Sick fish | 2.80±0.32 | 3.70±0.40 | 31.20±2.90 | 41.50±2.40 | 8.40±1.90 | 7.60±0.60 |

Analysis of the data obtained revealed that in the blood of infected fish, an increase in the activity of superoxide dismutase, which is the main enzyme of antioxidant protection (AOP), is not sufficient to fully compensate and protect cell structures from the damaging effects of toxic metabolites of lipid peroxidation. The reason is the primary antioxidant deficiency of vitamin E, the concentration of...
which in sick fish decreased by 40.0%, indicating a lack of non-enzyme antioxidants that leads to a weakening of the mechanisms of physiological and biochemical adaptation of the body.

Evaluation of the antimicrobial action of the drugs revealed a high activity of vitarol-E, the minimum concentrations of which delayed the growth of Aeromonas sobria at 0.25 mcg/ml, Aeromonas hydrophila at 0.50 mcg/ml, and bactericidal concentrations for both cultures were 0.75 mcg/ml.

The sensitivity of aeromonads to chloramphenicol was lower, amounting to mbsc-1.25 mcg / ml, MBC-2.00 mcg/ml for Aeromonas sobria and 1.50 mcg/ml, 2.25 mcg / ml for Aeromonas hydrophila.

The results of laboratory testing of the preparations were consistent with the effectiveness of their use in fish farming.

In carp that received seven days of mixed feed with "Vitarol-E", the transition of acute and subacute forms of aeromonosis to chronic was noted. On the seventh day after the end of therapeutic feeding, 40% of the examined fish had non-bleeding ulcers on the body surface, and the rest had the beginning of the tissue scarring process.

At the control catch after 14 days, the absence of symptoms of the disease and complete epithelization of the skin was established.

Conducted microbiological studies of blood samples and parenchymal organs gave a negative result. Stabilization of oxidative-antioxidant values of indicators occurred on the seventh day after the end of therapeutic feeding.

The results obtained are a consequence of the intake of the necessary amount of natural bioantioxidants into the fish body and the powerful bactericidal effect of the drug.

Studies of the intensity of the course of lipid peroxidation reactions and the antioxidant status at the time of chloramphenicol use and after, did not reveal the fact of regulation of biochemical processes. The registered changes were characterized by accelerated development of lipid peroxidation of cell membranes and, as a result, accumulation of a toxic metabolite-malondialdehyde. These changes took place against the backdrop of low capacity of antioxidant defense enzymatic link and biancolilla. The disadvantage of antibacterial therapy with chloramphenicol was that the drug showed more bacteriostatic properties.

When studying the bacobsemination of the internal organs of carp after a course of chloramphenicol application, it was found that a seven-day delay in bacterial growth, unlike the usual 1-3 days, does not affect the change in the cultural and morphological, tinctorial properties of microorganisms, which are restored within 5-10 passages. Maintaining the viability of the pathogen provoked the development of the disease 25 days after therapeutic feeding with a repeated outbreak of epizootics on 35-40 days.

3. Conclusion
Given the data obtained, it should be noted that the development and severity of the disease largely depends on the physiological ability of the fish's body to adapt to new conditions. Infection of fish with aeromonads initiates the processes of oxidation of cell membrane lipids. Toxic peroxides accumulate in the blood of infected carp due to a lack of antioxidants of non-enzymatic origin. The use of antibiotics, in particular chloramphenicol, in the treatment of aeromonosis, leads to a decrease in the capacity of bioantioxidifiers, along with the manifestation of only bacteriostatic properties. A combination of factors contributes to the occurrence of relapses of the disease.

The use of a complex drug – "Vitarol-E" in the treatment of aeromonosis allows maintaining the necessary level of antioxidants in the fish body, thereby increasing non-specific resistance. The antimicrobial action of Vitarol-E in the tested dosage (1500 ml per 1000 kg of feed, 7 days course) leads to the creation of concentrations in the tissues that provide bactericidal activity.

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