Pharmacoprophylaxis of experimental aeromonosis of carp in the conditions of farms in the Krasnodar region

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Abstract. The article presents the results of studies on the preventive efficiency of the drug DON-1 in experimental carp aeromonosis. As a result of the research, it was determined that the addition of the DON-1 drug to the aquatic environment, in which the fish is kept, on the background of optimal water temperature and balanced diet helps to reduce the proportion of death in the experimental group in the acute course of aeromonosis, as well as weakening the clinical manifestation of the chronic process of aeromonosis and accelerating recovery. The mentioned effect has been proven both during intraperitoneal infection of test samples of carp and osmotic infection of individuals in a separate container.

1 Introduction

Russian researchers point out that the epizootic situation of infectious and invasive fish diseases in the Russian Federation is very diverse. The causative agents of the trigger mechanism of the epizootic process are very different from each other, and mainly it depends on the place where the microorganism lives [1, 2].

The most common diseases in fish farming with a wide variety of pathogens are parasitic diseases. They are followed by diseases of bacterial, virological and mycotic etiology. Such bacterial diseases of fish as aeromonosis and pseudomonosis of carps, myxobacteriosis of trout and sturgeon fish, bacterial hemorrhagic septicemia of fish, despite the whole range of measures taken in fisheries, are serious adverse factors hindering the development of aquaculture [3-6].

Both in natural ecosystems and in aquaculture conditions, fish are often exposed to mass infections with aeromonosis, which can cause significant economic damage to fish farms [7, 8].

In natural reservoirs in the beginning of the spring-summer period, as well as when growing fish in a closed water supply (at a constant high water temperature), aeromonosis...
occurs with pronounced clinical signs [9]. The isolated pathogens at the same time have a high virulent activity [10]. This was confirmed in laboratory studies of virulence by an indirect method on a DNA medium with a depolymerization zone from 5 to 14 mm. During the parallel determination of the pathogenicity of isolated cultures by using a biological test on white mice, it was found that opportunistic aeromonads in these cases are highly toxic. The death of animals occurs in the interval from 4 to 18 hours [11].

Because of the development of chemotherapeutic drugs resistance and the insufficient level of ongoing health measures, there is a need for further improvement and development of effective means and measures of therapeutic and preventive measures in order to improve the epizootic situation of modern fish farming [12-15].

Raw crotonolactone (a group of unsaturated lactones), which is an intermediate in the production of crotonolactones, obtained by oxidation of furfural with hydrogen peroxide, is among the active preparations against bacterial pathogens of fish. The pharmacodynamic effects of raw crotonolactone consist in its participation in the oxidation of carbohydrate metabolism products due to succinic, formic and maleic acids, which are able to enhance oxygen uptake by muscle tissue, being a kind of tissue respiration catalysts. Formic acid is an intermediate product of the oxidation of not only carbohydrates, but also fats and proteins. The use of crotonolactone improves the provision of various substrates for tissue respiration, especially when exposed to exogen and endotoxins.

An analysis of the literature data showed that at the present time the therapeutic and prophylactic efficiency of raw crotonolactone in the conditions of pond and warm-water fish farms for bacterial diseases of fish of various species has not been studied enough, which makes further research in this sphere a promising direction.

In this regard, the aim of the work was to study the preventive efficiency of raw crotonolactone in experimental carp aeromonosis (with different methods of its introduction into the body of carp) and to determine the prospects for its further study.

2 Materials and methods

As an object of study, the dosage form of the pharmaceutical substance crotonolactone (raw) – the drug DON-1. This drug is a liquid composition mixture consisting of 35% of 2-butenolide (crotonolactones), 35% of maleic and fumaric acids and 30% of Aqua destillata.

Evaluation of the effectiveness of the pharmacoprophylactic action of the drug DON-1 was carried out with two methods of infection of fish with aeromonads: intraperitoneal and osmotic (when bacteria were introduced into the water) on the 5th day after the fish was treated with the drug.

The studies were conducted on carp yearlings in the amount of 40 in the conditions of the farm of the Krasnodar region, formed into two groups (experimental and control, n=20) and then placed in 4 different aquariums (n=10, depending on the method of infection).

Previously, in 7 days, all fish were subjected to quarantine in order to adapt to aquatic conditions to identify and cull the affected fish. Intergroup differences consisted in the fact that on the first and second days of the experiment, the test sample of the DON-1 drug was added to the experimental aquariums at a rate of 0.01 mg/l. At the time of the introduction of the drug, the flow stopped and the optimal oxygen content was maintained using a microcompressor. Water exchange and feeding were restored 2 days after the introduction of the drug.

Then, on the 5th day after the first introduction of the DON-1 drug, experimental infection of fish with aeromonads was carried out, both in experimental and control aquariums in two ways:...
Method 1 – 10 fish from the group were intraperitoneally injected with 0.125 ml of physiological solution containing 1x10^10 CFU of aeromonads in 1 ml;

Method 2 – 10 other fish were infected by the method of baths in a separate container with a volume of 3 liters, having previously scarified 1 cm^2 of the lateral surface of the body. The infection exposure was 4 hours, the concentration of aeromonads in water was 1x10^10 CFU/ml.

A strain of *Aeromonas veronii* biovar sobria with high virulence (DNA activity – 9 mm, LD_{100} with intraperitoneal injection of 1.25x10^9 CFU/fish) was used for the bioassay.

Reisolation of the pathogen and evaluation of the effectiveness of the drug was carried out according to generally accepted methods in ichthyopathology and bacteriology.

The conditions of keeping and feeding in both aquariums were identical: in flow aquariums at a temperature of +18–20°C and an oxygen content of 4.0–6.0 mg/l. Throughout the experimental period, the fish were fed with RGM-5M granular feed in the amount of 5% of body weight.

The prophylactic efficiency of the DON-1 drug under conditions of experimental infection was determined by the degree of manifestation of clinical signs, the survival of fish and also on the basis of bacteriological studies (determination of contamination of parenchymal organs with aeromonads on the 5th and 10th days after treatment of the fish with the drug). For this purpose, 3 specimens of fish were caught from each group.

### 3 Results and discussion

The results of the research in the acute experiment with intraperitoneal injection showed that the death of the fish lasted 96 hours. On the first day of the experiment, the death of fish without clinical signs (fulminant form) was revealed, then both in the experimental and control groups typical signs of the acute form of aeromonosis were noted – ruffling of the scales, exophthalmia, ascites, the presence of hemorrhages and an inflammatory reaction at the site of scarification.

In the control group with this infection, the mortality rate was 100%, in the experimental group it was 80%. In 20% of the experimental fish, there were no signs of an acute manifestation of the disease, although bacterial culture from the parenchymal organs of the surviving fish on the 12th day after infection revealed their rather high contamination (from 2x10^5 to 1.6x10^7 CFU/g).

In the second series of experiments, when aeromonads were introduced into the water (osmotic method of infection), clinical signs of the disease appeared on the 3rd day. On the 5th day in the control group the maximum number of fish with disease was 70%, and in the experimental group it was 50%, and the clinical manifestations of the disease were not so pronounced (Fig. 1).

By the 12th day of the experiment, the disease reached its peak. By this time, fish with an acute form of aeromonosis (ruffling of the scales) appeared in the control group (30%), while in the experiment only fish with 3 degrees of damage appeared (the presence of hemorrhages at the site of scarification). From the 16th day, a gradual recovery was noted in both groups of fish with the priority of the experimental fish. Complete recovery in this group occurred on the 27th day, and in the control group – on the 35th day from the moment of infection.
Experimental group

Days of observations

Control group

Days of observations

Fig 1. Influence of prophylactic treatment with DON-1 on the dynamics and structure of the clinical picture of aeromonosis (experimental and control groups)

Legend:
- Without clinical signs
- The presence of hemorrhages at the site of scarification
- Ruffling of the scales on other parts of the body
- Ruffling of the scales, ascites, exophthalmia
Bacteriological studies carried out during the experiment showed that on the 5th day there were no significant differences between the fish of the experimental and control groups (Table 1).

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**Table 1:** Contamination of the internal organs of fish with aeromonads (CFU/g) during the prophylactic use of the drug DON

The control reisolation on the 10th day after infection showed that the contamination of the parenchymal organs of the fish of the control group remained at a high level (1.2 \times 10^7 - 8.4 \times 10^7), while in the experimental group a pronounced decrease in contamination was noted (2.0 \times 10^5 - 5.2 \times 10^6) or even absence of increase compared to the first reisolation (1.9 \times 10^7).

**4 Conclusion**

Thus, on the basis of the conducted studies, the preventive efficiency of the drug DON-1 in carp aeromonosis at a concentration of 0.01 g/m^3 with a double treatment was determined, which manifests itself in a 20% reduction in mortality in the acute course of the disease and in weakening the clinical manifestation of the chronic process, accelerating recovery. The low cost of the drug, the availability and manufacturability of production volumes, as well as its positive effect based on the results of industrial studies, allow us to recommend the drug DON-1 as a prophylactic mean against carp aeromonosis in pond farms and for further clinical trials in order to expand the range of indications.

**References**
