Evaluation of the effect of fish yersiniosis on the course of antioxidant protection processes in trout

A E Droshnev

Federal State Budget Scientific Institution “Federal Scientific Centre VIEV” (FSC VIEV), Rjazanskiy prospect, Moscow, 109428, Russian Federation

E-mail: asdf1961@yandex.ru

Abstract. Indicators that characterize the state of lipid peroxidation and antioxidant protection in rainbow trout grown in the North-West of the country in normal conditions have been determined. It was found that the amount of malondialdehyde, antioxidant enzymes and total phospholipids had no significant changes in the samples taken in the farms of the Leningrad region and the Republic of Karelia, while the concentration level depended on the age of the fish. The maximum content of the final products of lipid peroxidation malondialdehyde was recorded in fish weighing 600-720 g, which is associated with a high fat content in the diet and the inability of the body to metabolize them completely. The developing pathological process in the body of fish when infected with Yersinia accelerated the intensity of lipid peroxidation, as a result, toxic lipid peroxides accumulated in organs and tissues, and the concentration of malondialdehyde increased from 65.4 to 95.2 %. There were also decreases in the activity of antioxidant protection: the value of catalase decreased from 6.7 to 23.7 %, peroxidase 7.1-19.5 %, the maximum drop in the activity of ceruloplasmin 9.5 %.

1. Introduction

In aquaculture, artificial maintenance of hydrobionts in conditions significantly different from natural ones leads to changes in fish behavioral responses and the appearance of new, previously unknown diseases. Crowded maintenance, abundant feeding, technogenic factors and various types of stress have a negative impact on the body of animals and fish, resulting in the development of an adaptive response, suppression of the immune system, and the emergence of various pathological processes [1]. Therefore, the development of the industry should be accompanied by applied research in the field of health protection of cultivated objects.

Many years of experience in the use of medicinal products for bacterial infections has shown that some drugs are ineffective over time, microorganisms become resistant to antibiotics [2]. And if earlier the treatment strategy was aimed at destroying pathogens in the body of fish, now preference is given to the use of drugs that increase the resistance of the macroorganism to infection with pathogenic agents. First of all, these are immunostimulators and vaccines [3, 4].

Another important factor that ensures the stability of any organism from the influence of pathological processes is antioxidant protection (AOP). The study of the mechanism of AOP began in the 50s of the last century in connection with the damaging factors of ionizing radiation. The processes of lipid peroxidation (PLP) and AOP are in dynamic equilibrium, and their violation leads to the development of various pathologies [5].
There is relatively little information about the processes of PLP and AOP in fish that are poikilothermic animals, both in domestic and foreign scientific literature. Previously, we studied the mechanisms of lipid peroxidation and antioxidant protection of carp and some other fish species in normal conditions, under the influence of stress factors and aeromonosis [6, 7]. There is information about biomarkers of oxidative stress in the liver of sea trout suffering from ulcerative necrosis of the skin [8] and some others [9].

The purpose of this study is to study changes in the course of free-radical processes and the system of antioxidant protection in the normal and in the disease of rainbow trout yersiniosis caused by the bacterium Yersinia ruckeri, a member of the Enterobacteriaceae family [10]. Since 2010, yersiniosis has been detected in the Russian Federation, characterized by mass death and damage to commercial products, thereby causing severe damage to fish farms [11]. Currently, a laboratory diagnosis of the disease has been proposed [12, 13], an anti-bacterial vaccine has been developed and validated [14], but various aspects of metabolism during pathogenesis have not been fully studied.

2. Materials and methods
The work was carried out in 2018-2019 in the laboratory of ichthyopathology of the FSC VIEV and in fish farms of the Leningrad, Tver regions and the Republic of Karelia in the framework of task No. 0578-2019-0008 "to Develop and improve systems of diagnostic, preventive and therapeutic measures for particularly dangerous and common diseases of hydrobionts of viral, bacterial and parasitic nature based on modern molecular-biological, serological methods and means of biotechnology".

During the first stage of work—determination of standard values - 500 trout weighing from 30 to 720 g were subjected to research.

To conduct laboratory experiments on fish infection, they were imported to the aquarium laboratory from enterprises that were safe for infectious diseases. After adaptation, for 14 days, they were used in experiments, formed experimental and control groups (at least 10 copies in each). Isolation of the initial strains from infected fish was performed according to a generally accepted method. In total, 200 specimens of rainbow trout (Oncorhynchus mykiss L.) were used in the experiments.

For experimental infection, two strains of Yersinia ruckeri with the highest virulence were used—"7№3" and "RF12", LD50 at 8°C is 100 million m.c., at 15-18°C – 50 million m. c., isolated from rainbow trout during epizootic periods.

Determination of the level of oxidative-antioxidant reactions in the body of fish in normal and yersiniosis was carried out using the methods given in the manuals for the study of the processes of PLP and AOP in animals [15, 16].

Determination of the level of oxidative-antioxidant reactions in the body of fish in normal and yersiniosis was carried out using the methods given in the manuals for the study of the processes of PLP and AOP in animals [15, 16]. Blood for biochemical studies in fish was taken from the caudal artery. To protect whole blood from clotting, a 1% solution of heparin was used. Blood plasma was obtained by centrifugation for 10 minutes at 3000 rpm. Content of catalase, peroxidase, ceruloplasmin, phospholipids, malondialdehyde (MDA) was determined in blood and plasma.

3. Research result
In the course of the work, the course of PLP and AOP processes in fish during pond cultivation in natural reservoirs was studied.

The quantity of malondialdehyde, antioxidant enzymes, and total phospholipids did not significantly change in the samples taken from farms in the Leningrad region and the Republic of Karelia, and the concentration level depends on the age of the fish. The maximum content of the final products of lipid peroxidation MDA was recorded in fish weighing 600-720 g their accumulation is associated with the fact that trout in this size and weight condition receives compound feed with a high fat content, and the body is not able to metabolize them completely. Table data indicate the highest content of dangerous oxidation products in the blood of fish grown at higher (not optimal for the species) temperatures of 19-24°C in reservoirs of the Tver region, indicating an excess intake of nutrients from food that are not
fully used by energy costs. At high temperatures, hydrobionts spend less energy to maintain the normal course of immuno-physiological processes, and excess material is actively oxidized, the products of peroxidation of which cause destructive violations of the lipid layer of cell biomembranes, this process is burdened by the deposition of excess fat in the body cavity, creating a long-term load on the systems and organs, especially the liver and blood cells. Low activity of the enzymatic link of antioxidant protection and the content of total phospholipids designed to suppress and prevent the accumulation of oxidative products were noted. When compared with the values of indicators relative to other groups, a decrease in the level of enzymes up to 30 % of catalase and peroxidase, ceruloplasmin 40 % and a sharp decline in phospholipids 50 % was revealed, which is an indicator of chronic depletion of the body's AOP system due to the impact of negative factors. The results of the study indicate the need for strict selection of the type of compound feed, guided by the use of low-energy recipes during non-optimal temperature ranges of growing rainbow trout and the use of additional regulatory means.

**Table 1.** Indicators of PLP and AOP in rainbow trout are normal (the oxygen content in water is 9-15 mg/l, the temperature is 13-17°C and moderate densities) and in fish grown at higher temperatures that are not optimal for the species is 19-24°C.

| Place of sampling / water temp. °C | Mass of fish (g) | MDA, µm/l | Catalase, µm | Peroxidase, units of optical density/l×sec | Ceruloplasmin, µm | Phospholipids, g/l |
|-----------------------------------|----------------|-----------|-------------|------------------------------------------|----------------|-----------------|
| Fish farm №1 Tver region/19-24   | 30             | 4.5±0.7   | 3.57±0.7    | 3.36±0.6                                 | 21.6±7.8       | 1.54±0.1        |
| Fish farm №2 Leningrad region/13-17 | 45             | 3.0±0.5   | 4.2±0.8     | 4.4±0.5                                  | 30.8±1.5       | 2.2±0.2         |
|                                   | 650            | 5.2±0.5   | 4.0±0.7     | 4.6±0.5                                  | 24.0±4.5       | 1.9±06          |
| Fish farm №3 Leningrad region/13-17 | 40             | 2.2±0.6   | 5.1±0.4     | 4.3±0.4                                  | 31.5±3.0       | 3.3±0.7         |
|                                   | 670            | 5.0±0.7   | 4.0±0.5     | 4.4±0.5                                  | 28.0±4.0       | 2.2±0.5         |
| Fish farm №4 Karelia /13-17       | 45             | 2.7±0.3   | 6.0±0.8     | 4.2±0.9                                  | 37.2±5.8       | 3.1±0.8         |
|                                   | 700            | 3.2±0.2   | 5.8±0.7     | 4.5±0.7                                  | 35.0±4.7       | 2.6±0.6         |
| Fish farm №5 Karelia /13-17       | 45             | 1.9±0.7   | 7.4±0.4     | 4.7±0.7                                  | 35.5±5.5       | 3.0±0.4         |
|                                   | 720            | 2.1±0.2   | 6.2±0.6     | 4.9±0.6                                  | 32.5±2.5       | 2.7±0.6         |

*P< 0.05

The data obtained in samples taken from farms in the Republic of Karelia and the Leningrad region on the content of phospholipids, the activity of anti-oxidative enzymes and the concentration of peroxidation products in the blood of fish grown under optimal conditions (temperature 13-17°C, oxygen content 9-15 mg/l and moderate densities of fish) are accepted as normative values.

At the next stage, we studied the antioxidant protection of fish, in particular the activity of AOP enzymes and the intensity of lipid peroxidation, in experimental trout yersiniosis. Blood for the study was taken from fish that were directly infected with *Yersinia*, intraperitoneal, in a dose of 50 million m. cells, in a volume of 0.1 cm³.
Studies have shown that there is a correlation between the studied indicators of activity of the antioxidant system and the concentration of lipid peroxidation products, depending on the stage of the infectious process. The analysis of the obtained data showed that the chronic course of yersiniosis causes a shift in the AOP system - a decrease in the values of catalase, peroxidase, and ceruloplasmin was noted.

The activity of antioxidant enzymes in fish when infected with *Yersinia* decreases, which may be due to the peculiarities of metabolism and is, in all probability, adaptive. The value of catalase decreased from 6.7 to 23.7 %, peroxidase 7.1 - 19.5 %, and the maximum drop in ceruloplasmin activity was 9.5 %. Against the background of a decrease in the activity of AOP, an increase in the intensity of free radical processes and the accumulation of peroxidation products in the body, in particular MDA, was noted.

The developing pathological process in the body of fish during infection accelerates the intensity of free radical reactions, as a result, toxic lipid peroxides accumulate in organs and tissues, the concentration of malondialdehyde increased from 65.4 to 95.2 %. The capacity of the antioxidant protection system is not enough to stabilize the oxidation processes within the normal level, which was expressed in a decrease in the concentration of phospholipids in the blood of fish, a decrease in the level to 42.3 %.

The results obtained are consistent with the functional role of phospholipids in the cellular system - their content in cell biomembranes is physiologically determined. When the qualitative and quantitative composition of phospholipids in biomembranes is violated, prerequisites for their destructive changes are created. According to the indicators of total phospholipids in the blood serum, it is possible to judge the processes of lipid peroxidation in the fish body.

The intensity of the process of lipid peroxidation and the functioning of the antioxidant defense system in fish are variable and depend on the metabolic response of the body to stress stimuli of one or another nature.

Thus, the infection caused by *Yersinia* is accompanied by a violation of the balance in the PLP-AOP system, which leads to excessive activation of free radical oxidation processes and is one of the factors of the pathogenesis of this disease.

4. Discussion

The health and productivity of all types of hydrobionts is due to the intensive course of metabolic processes in their body and the intense functional activity of all organs and systems. A large number of physical and chemical processes that provide anabolism and catabolism are carried out using free radical oxidation (FRO) reactions, which are initiated by free radicals that are constantly formed in the body's tissues. The regulation of metabolic processes and physiological functions in the body, as well as the influence of drugs, microbial toxins and physical environmental factors on these processes, is carried out by direct or indirect effects on antioxidant enzymes and the reactions catalyzed by them.

It is known that for the transition of infection to disease, it is important not only the virulence of bacteria, but also the physiological state of the fish body, which is in close relationship with the external environment. The influence of stress factors on the body of fish leads to the formation of conditions that contribute to the emergence of disease and the development of the epizootic process.

Changes in the intensity of free-radical reactions during the disease can serve as a diagnostic test, and the study of the dynamics of these changes makes it possible to monitor the effectiveness of treatment methods, thus being a predictive indicator.

Taking into account that the pathogenesis of yersiniosis affects various aspects of metabolism, in our work, we paid attention to the study of the mechanisms of lipid peroxidation and their antioxidant protection. It was found that lipids, being the most important structural and functional components of biological membranes, are actively involved in the interaction of micro - and macroorganisms in the development of infectious diseases. The degree of disintegration of biomembranes can determine the outcome of the interaction of an infectious agent and a macroorganism, the violation of the integrity of which increases the possibility of penetration of microbes through the protective wall barrier of cells.
The results of our research have shown that under extremely unfavorable environmental factors up to critical values of the parameters of the life of the studied species and the use of high-energy compound feeds in these conditions leads to significant violations of the normal physiological flow of biochemistry of free radical oxidation reactions. In experimental yersiniosis, the degree of violations of these reactions was within the limits comparable to the values in fish kept under critical conditions of existence, thereby increasing the severity of the disease while reducing the overall resistance of the body.

The physiological level and participation in the pathological PLP-process and AOP in aquatic hydrobionts have not been studied enough, and therefore the new data are of great theoretical and practical interest. Knowledge of the adaptive capabilities of the fish body and ways to influence them is an urgent task of ichthyopathology. The information obtained will help to select diagnostic criteria for evaluating biochemical disorders and thus conduct targeted therapy and develop promising means of correction both for independent use and in combination with immunoprophylactic and therapeutic drugs.

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