The effect of *D. dendriticum* invasiveness on the antioxidant system of omul from the Selenga River population

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Abstract. The article presents data on the influence of the degree of invasion by *D. dendriticum* plerocercoids on the parameters of the antioxidant blood system of omul of the Selenga River population. Infection with helminths affected the haematological parameters of omul blood. The blood of omul infected with *D. dendriticum* has a significant decrease in the content of total protein, haemoglobin, red blood cells and white blood cells. Moreover, the severity of these indicators depended on the level of infection of the omul. The most significant decrease in these indicators is noted in omul with a high degree of invasion. The results of studying the composition of white blood cells show a substantial reduction in the content of lymphocytes and an increase in the number of segmenting clear neutrophils in omul with a high degree of invasion, as well as eosinophilia in all groups of infected fish, which is characteristic for parasitic attacks. The summary antioxidant activity significantly increased in the group of fish with a low degree of invasion (2-3 copies of plerocercoids). However, as the degree of attack increased (average degree – 5-10 copies of plerocercoids; high degree – 18 or more copies of plerocercoids), this indicator does not differ from that in uninfected individuals. Significant differences in blood catalase activity (catalase number) are not found between uninfected and infected omul. Information on changes in the antioxidant system parameters can serve as non-specific markers concerning the state of the fish organism, adaptation to changing environmental conditions and, ultimately, the quality of raw fish foodstuffs.

1. Introduction

Fish is a valuable food product for modern humans, characterized by its high biological and taste properties. In the total balance of animal proteins consumed in the world, fish proteins account for about 20% [1]. The average fish consumption in the Russian Federation is about 20 kg per capita and, in recent years, there has been a tendency for waste to be reduced (recommended rate is 22 kg/year per person). When comparing the per capita consumption of fish in the Russian Federation with the leading world fishing powers, it should be noted that Russia is in fifth place after Japan (65 kg/year), Norway (47 kg/year), China (25 kg/year) and the USA (23 kg/year). At present, the Russian market for fish and fish products is far from saturated. The accelerated development of the domestic fishing industry is needed. In many constituent entities of the Russian Federation, special programs for the development of fishery complexes have been launched [2].

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Fish diseases are one of the main factors hindering the successful development of the fishing industry, reducing food quality and the safety of fish products, in particular, helminth infections of fish. More than 1000 species of fish, including 250 commercial ones, live in natural reservoirs. The level of helminthiases has recently been increasing, especially for freshwater fish. In Russia, many parasitic diseases are recorded, the causative agents of which are transmitted to humans through fish and their processed products. At present, it is difficult to find even single individuals of natural populations who are free of helminths [3].

Baikal omul is a fish of the whitefish genus of the salmon family, endemic to Lake Baikal. The value of this fish is due to omul being a commercial species, a brand of Lake Baikal. It has a high demand not only among the local population but also among tourists. Information on the status of omul stocks in 2016-2017 showed a significant decrease in its commercial catches in the past decade and a half, associated with a sharp reduction in its biomass. On October 1, 2017, a ban on industrial fishing for omul entered into force. The right of limited catch, except for the spawning period, remains with representatives of indigenous minorities who live in two districts on the territory of the Republic of Buryatia [4].

When examining omul, it was found that its infection with parasitic diseases, like other fish species, increases every year. The research results indicate the widespread prevalence of diphyllobothriasis in Baikal omul [3]. Concerning morphological characteristics, in general over the past 30 years, there has been a trend towards an increase in linear weight indicators of omul, and the stabilization, and even improvement, of growth and maturation indicators against the background of a decrease in stocks [4].

The biological value of raw fish foodstuffs largely depends on the state of the organism and the level to which an invasive species have inhabited it. Invasive species can affect the antioxidant status of fish. The identification and analysis of markers of oxidative stress accompanying various pathologies of fish are essential, as the deterioration of the body increases mortality, reduces the production of fish products, or makes it unfit for consumption [5, 6]. Besides, many helminths cause allergic reactions and autoimmune responses [7].

This work aims to study the effect of *D. dendriticum* invasiveness on some indicators of the antioxidant blood system of omul of the Selenga River population.

2. Materials and methods

Biological material was obtained on September 18-19, 2019, from sexually mature forms of Baikal omul under conditions of spawning migration, as part of a quota for scientific research in the Kabansky district of the Republic of Buryatia. Morphobiological and parasitological studies were conducted according to generally accepted methods in ichthyology.

The fish blood was taken at the time of the catch, from the caudal artery using a bloodletting needle. The total number of red blood cells and white blood cells was calculated in a Goryaev’s chamber using vital staining of blood cells. The haemoglobin content was determined by the cyanmethemoglobin method. Blood smears were fixed with methanol and then stained with azure-eosin by the Romanovsky method. Stained blood smears determined the species composition of white blood cells. The total protein content in the blood serum was determined by the refractometric method [8].

The total content of antioxidants (TCA) in the blood serum of fish was determined using a «Tsvet-Yauza-01-AA» liquid chromatography using the amperometric method. The mass concentration of water-soluble antioxidants was measured using a calibration graph of the dependence of the signal output on the strength of quercetin [9, 10]. The determination of catalase activity in the blood was carried out by the permanganate method (according to Bach and Zubkova). Enzyme activity is expressed in terms of catalase number [11].

Statistical processing of the results was carried out using the software package "Biostat-2006." Due to the small sample size, the nonparametric Mann – Whitney test was used for comparing differences between the studied parameters. The results of the study are presented in the form of the median (Me)
and the upper and lower quartiles (Q1 – Q3). The results were considered reliable when reaching the level of significance of differences (p = 0.95).

3. Research results and discussion

The sample of fish (uninfected and infected with *D. dendriticum* plerocercoids) amounted to 17 individuals. The average fish length was 33-37 cm and weight 500-600 g. Fish individuals were divided into the following groups according to the degree of infection:
- omul not infected with helminths (group 1, n = 2),
- omul with a low degree of invasion (group 2, the invasion index (II) was 2-3 copies of plerocercoids, n = 6),
- omul with an average degree of invasion (group 3, II was 5-10 copies of plerocercoids, n = 4),
- omul with a high degree of invasion (group 4, II was 18 or more copies of plerocercoids, n = 5).

Table 1 presents the main haematological parameters of the blood of the Baikal omul, not infected and infected with *D. dendriticum* plerocercoids.

| Indicator/ groups | Uninfected omul (group 1) | Low invasion omul (group 2) | Omul with the average invasion (group 3) | Highly invasive omul (group 4) |
|------------------|--------------------------|-----------------------------|-----------------------------------------|------------------------------|
| Total protein, g% | 10.4 (10.2-10.6)         | 9.6* (9.3-9.9)              | 9.5* (9.3-9.7)                          | 8.5** (8.2-8.8)              |
| Hemoglobin, g%   | 11.2 (10.7-11.8)         | 9.6* (9.4-9.9)              | 9.4* (8.9-9.9)                          | 8.9** (8.7-9.2)              |
| Red blood cells, mln / μl | 1.3 (1.2-1.4) | 1.05* (1.0-1.1)           | 1.0* (0.9-1.1)                          | 0.85** (0.8-0.9)           |
| White blood cells, thousand / μl | 10.5 (10.3-10.7) | 8.3* (8.0-8.7)          | 7.8* (7.5-8.2)                          | 6.9** (6.5-7.3)           |
| Lymphocytes, %   | 77.0 (75.3-78.8)         | 74.5 (70.8-78.3)           | 71.7 (71.7-75.7)                       | 66.9 (66.9-69.1)          |
| Monocytes, %     | 4.2 (3.8-4.6)            | 5.2 (4.6-5.7)              | 4.9 (3.7-6.1)                          | 5.8 (4.7-6.9)             |
| Segmentonuclear neutrophils, % | 10.0 (8.8-11.2) | 11.1 (10.3-11.9)         | 12.0 (9.8-14.3)                       | 15.2* (14.0-16.4)         |
| Stab neutrophils, % | 7.8 (6.6-9.0)            | 8.3 (7.2-9.4)              | 8.4 (7.2-9.7)                          | 9.2 (7.8-10.6)            |
| Eosinophils, %   | 0 (6.0-0.6)              | 0.4* (0.2-0.8)             | 0.5* (0.3-0.8)                         | 0.6* (0.3-0.9)            |
| Basophils, %     | 1.0 (0.9-1.1)            | 0.5 (0.2-0.8)              | 0.5 (0.3-0.8)                          | 1.0 (0.4-1.6)             |

* a,b,c - significant differences relative to groups 1, 2 and 3 (p= 0.95).

As seen in the data from Table 1, in the blood of omul infected with *D. dendriticum*, there was a significant decrease in the content of total protein, haemoglobin, and the number of red and white blood cells. Moreover, the severity of these indicators depended on the level of infection of omul. The most significant decrease in these indicators was noted in omul with a high degree of invasion (group 4).
The results of studying the composition of white blood cells showed a significant decrease in the content of lymphocytes and an increase in the number of segmentonuclear neutrophils in omul with a high degree of invasion (group 4), as well as eosinophilia in all infected fish (groups 2, 3 and 4).

The results obtained are consistent with the data of Mazur O.E. and Tolochko L.V. (2015) in the study of the cytomorphological and biochemical composition of blood of the bottom-deep morphotype of Baikal omul infected with \textit{D. dendriticum} plerocercoids, during spawning migration. The authors found a decrease in haemoglobin synthesis and an increase in the proliferative activity of erythropoietic cells. It was noted that changes in the population composition of white blood cells, the concentration of immunoglobulins and total protein indicated a violation of the proliferation and differentiation of cellular elements and the suppression of the immune response in infected fish [12].

Merchina S.V. et al. (2018) found that almost all fish caught and grown in the Ulyanovsk Region were infected with various parasites by 90-95%. But at the same time, the infection of two-year-old carp with opisthorchosis did not have a significant effect on haematological parameters. The concentration of haemoglobin, the number of red and white blood cells, and the saturation of the red blood cells with haemoglobin did not exceed the conventional norm. The leukocyte formula of the mirror carp affected by opisthorchosis did not differ from the conditional norm. At the same time, the authors observed an increase in the number of monocytes, eosinophils and neutrophils in individuals, which is characteristic of both the summer season and the pathological processes inherent in parasitic diseases [13]. In parasitic diseases, macrophages, neutrophils and eosinophils from the first line of defence [14].

Table 2 shows the summary antioxidant activity of blood serum and the activity of blood catalase of Baikal omul, not infected and infected with \textit{D. dendriticum} plerocercoids.

| Indicator/ groups | Uninfected omul (1 group) | Low invasion omul (2 group) | Omul with the average invasion (3 group) | Highly invasive omul (4 group) |
|-------------------|--------------------------|----------------------------|----------------------------------------|------------------------------|
| Summary antioxidant activity, mg / 100 ml | 36.7 (31.0-41.2) | 45.2\textsuperscript{a} (41.6-48.9) | 38.7 (34.0-43.6) | 37.9 (33.5-42.3) |
| Catalase activity (catalase number) | 12.6 (11.4-13.8) | 12.6 (11.7-13.5) | 11.3 (9.4-13.3) | 12.8 (12.0-13.6) |

\textsuperscript{a} - significant differences relative to group 1 (p = 0.95).

As seen from the data in Table 2, the summary antioxidant activity increased in the 2nd group of animals (with a low degree of invasion). With an increase in the degree of invasion (groups 3 and 4), this indicator did not differ from that of uninfected fish (group 1).

No significant differences in blood catalase activity (catalase number) were found in uninfected and infected omul.

The data available in the literature on the responses of the antioxidant system of fish infected with helminths are scarce and highly controversial. They indicate a change in the activity of antioxidant enzymes in infected fish. The nature and direction of these changes depend on the host species and the parasite, as well as on the stage of the latter life cycle. As a result of invasion in the host’s body, the synthesis of reactive oxygen forms is intensified, causing the elimination of parasites, which can lead to inhibition of the activity of the antioxidant enzymes of the host [15, 16].

Bello A.R.R. et al. (2000) found that infection with trematode \textit{Clinostomum detruncatum} did not bring about the reliable induction of superoxide dismutase and catalase enzymes in the muscles of silver carp but peroxidation in tissues increased sharply. The authors concluded that invasion stimulated the development of oxidative stress in fish and damage to muscle cell membranes [17].

According to Mozhdeeganloo Z. and Heidarpour M. (2014), an increase in the content of thiobarbituric acid reactive products and a decrease in the concentration of free SH-groups, capable of
binding peroxides, were established in the gills of a goldfish infected with *Dactylogyrus* spp monogeny. They indicated not only the presence of an increased level of free radicals but also about inflammatory processes in the tissues [18].

Studies of the activity of antioxidant enzymes in the muscle tissue of the Black Sea sprat invaded by the larvae of the nematode *Hysterothylacium aduncum* by Skuratovskaya E.N. and Zavyalov A.V. (2006 showed that catalase activity was significantly reduced in infected fish compared to healthy fish. Moreover, the minimum values of enzyme activity were established in individuals containing the most significant number of parasites. Peroxidase activity significantly decreased with an increase in the degree of invasion. The results obtained indicate that, with high infection by nematode larvae, the movement of enzymes of the protective antioxidant system is inhibited, which once again confirms the inhibitory effect of parasites on the host's protective molecular networks [19].

Mikryakov V.R. and Silkina N.I obtained similar results (2006) in the study of bream affected by *L. intestinalis.* The levels of lipid peroxidation in the blood serum and the liver of infected fish significantly increased (by 50-90% and 43-92%, respectively) compared to healthy fish. The total antioxidant activity decreased, but to a lesser extent than lipid peroxidation (by 12-22% and 9-17%, respectively). The noted effects were pronounced in fish invaded by more giant worms. The authors suggest that the increase in the parasite negative impact is associated with an increase in the stressful effect on the host and the suppression of its protective systems, including antioxidants [20].

However, Skuratovskaya E. N. et al. (2013) established other patterns in the study of the activity of antioxidant enzymes in the blood of marling affected by the nematode *H. aduncum.* The activity of catalase and superoxide dismutase was 1.5 times higher in infected individuals compared with uninfected ones. For peroxidise and glutathione educates, no significant differences in blood erythrocytes were found, while the activity of glutathione transfers in infected fish was more than four times higher than that in healthy individuals (p = 0.99). Thus, it is clear that the data presented indicate the influence of the degree of invasion on the responses of the antioxidant system of fish, which has pronounced tissue specificity [21]. This conclusion is consistent with the studies of Rudneva I.I. et al. (2004), on the parasitic system of the custodies *Botriocephalus grégarius* and the Black Sea flounder *Psetta maxima maeotica.* These data showed that the activity of most antioxidant enzymes increased significantly in the liver and muscles of infected fish compared with the values of uninfected fish. Moreover, the response of the antioxidant system in the liver was more pronounced than in the muscles and depended on the number of parasites in the fish (r > 0.6) [22]. However, according to Buchmann K. and Lindenstrom T. (2002), there is an opinion that the antioxidant activity of host tissues can be modified during the life cycle of the parasite depending on the stage of its development [23].

Recently, researchers have been trying to use generalized indicators of the state of an organism undergoing various adverse effects. Different integral quantities have been proposed that characterize general trends in the biochemical status of living systems, for example, the comprehensive index of enzymatic antioxidant activity (II EAOA). Skuratovskaya E. N. et al. (2013) found that this indicator was significantly higher in infected fish compared to those uninfected. This process made it possible to prove the effect of parasitic invasion on the state of the enzyme antioxidant system of the blood of merling. The authors noted an increase in the activity of key antioxidant enzymes and II EAOA of the blood of infected fish. It is clear that the introduction of the parasite into the walls of the digestive system and the release of metabolites into the host organism enhance the processes of free radical oxidation and are influential factors stimulating the activity of antioxidant enzymes [21].

4. Conclusion
Infection with helminths affected haematological parameters of omul blood. In the blood of omul infected with *D. dendriticum,* there was a significant decrease in the content of total protein, haemoglobin, red blood cells and white blood cells. Moreover, the severity of these indicators depended on the level of infection of the omul. The most significant decrease in these indicators was noted in omul with a high degree of invasion. The results of studying the composition of white blood
cells showed a substantial reduction in the content of lymphocytes and an increase in the number of segmentonuclear neutrophils in the omul with a high degree of invasion, as well as eosinophilia in all groups of infected fish, which is typical for parasitic diseases.

We found that the degree of invasion of *D. dendriticum* influenced the parameters of the antioxidant blood system of omul of the Selenga population. The summary antioxidant activity significantly increased in the group of fish with a low degree of invasion. With an increase in the degree of aggression, this indicator did not differ from that of uninfected individuals. Significant differences in the activity of blood catalase (catalase number) were not found between uninfected and infected omul.

The data obtained indicate the need for studies of other enzymes of the antioxidant system, including tissue. The results show the need to study the total content of antioxidants in different organs and tissues of fish infected with plerocercoids. Since the reaction of the host's immune system such as the production of antibodies, mediators and cytokinesis wave-like and depends on the time and period of invasion.

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