

The influence of the food factor on the components of the antioxidant protection system in fish

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Abstract. Functional feeding complexes that correct metabolic processes in animal bodies, increasing their productivity and preventing infectious diseases are a new direction in the development of modern feed production. Such feeds, thanks to a set of components, have a wide range of healing effects. We have developed a feed additive consisting of probiotics, adaptogens, vitamins and amino acids. The aim of the work was to study the effect of a new feed additive on the antioxidant system of fish. During the research, it was found that the content of glutathione (GSH) in male fish raised on the background of biologically active feed additive doubled, and in females by more than 30%, and the activity of glutathione-S-transferase also increased. Therefore, on the background of the use of new biologically active feed additive, the resistance of the fish body to free radical and peroxide processes increases. **Keywords:** functional feeding complexes, probiotics, adaptogens, amino acids, vitamins, antioxidant system.

1 Introduction

The productivity of aquaculture directly depends on the nutritional factor. The most relevant trend in fish feeding is functional feed complexes. They not only provide the nutritional needs of fish, but also manage metabolic processes, revitalizing the body. Modern feed complexes should combine classic and new biologically active functional ingredients that prevent diseases, accelerate growth, level of psychogenic and oxidative stress, improving fish products.

The feed complex must have functional properties so that the fish coming to the consumer gets added utility.

The importance of such feed additives for the development of aquaculture is obvious. In artificially created aquatic fish farming ecosystems, the success of production is completely controlled by anthropogenic factor. It is a person who determines the composition of feed and the quality of hydrochemical parameters of fish habitat. Unbalanced feeding slows down the growth of fish, leads to stress, disruption of metabolic processes, and weakens the immune defense system. [1-3].

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Under stress, peroxidation is activated with a distinct damaging effect on the cell [4-7]. This is evident in the disruption of barrier, receptor, catalytic function, DNA breaks, disorders of fish homeostasis [7, 8].

To eliminate reactive oxygen species formed during peroxidation, an antioxidant protection system is activated [9-11], one of the components of which is glutathione-S-transferase, which uses glutathione (GSH) as a reducing agent [12-14], which is a low-molecular antioxidant capable of binding lipophilic compounds, preventing their penetration into the phospholipid bilayer of the plasmalemma and into the cell.

Glutathione-S-transferases (GSTs) are the enzymes of the second stage of detoxification that catalyze the conjugation of the reduced form of glutathione (GSH) with xenobiotic substrates for the purpose of detoxification, giving them hydrophilic properties that reduce toxicity. Substances obtained at the second stage of detoxification are excreted through the intestines from the body [15-17].

Antioxidants, in particular adaptogens, are necessary to block free radicals. Also among the antioxidants are substances that are produced by intestinal normobiotics and probiotics [18-19].

Antioxidants stabilize the phospholipid layer of the plasmalemma, protecting plasma membranes from destruction. Therefore, we used adaptogens and probiotics in the development of feed additives. The use of adaptogens as part of feed additives is harmless to the body and is able to strengthen the immune defense system and the endurance of the body in relation to environmental factors.

The composition of the feed additive, which we have developed, includes vitamins and amino acids. First of all, we focused on essential amino acids and vitamins that are not synthesized in fish body. The introduction of vitamin-amino acid complexes in the composition of feed additives in quantities corresponding to the requirements for functional ingredients [20-21], provides an intensification of fish growth and at the same time gives them added utility, thereby improving the quality of commercial fish. Vitamins and essential amino acids in the composition of feed additives increase the biological value of fish.

When creating a feed additive, we selected a probiotic which microorganisms are genetically programmed to produce polyene antibiotics in order to protect fish organism from infectious diseases and form healthy microbiocenosis to increase the growth rate of fish and a set of biomass. The use of adaptogen characterized by high antioxidant activity as part of a feed additive was intended to increase endurance, immunity, and survival.

The use of a complex of essential amino acids and vitamins in quantities corresponding to the functional ingredient made it possible to create fish with the desired properties and added utility, and also increased the biological and nutritional value of fish.

The aim of the work was to create a feed additive with antioxidant properties and evaluate its effectiveness.

2 Materials and research methods

For the research, two groups of mature African catfish of their own reproduction were formed, 50 individuals each.

Observations had been continuing for three months: the first group received LimCorm brand Catfish feed with a feed additive containing a probiotic based on *Bacillus subtilis* (1g/kg of feed), adaptogen tricesan 40 mg/kg of feed, vitamin-amino acid complex Chictonic 2 ml/kg of feed.

Catfish of the second group served as a control and received only the main diet – «Catfish» brand feed from LimKorm.

The fish were kept in pools with a volume of 1.9 m³ with a water temperature of 26 C and oxygen content of about 4 mg/l. Water purification in the pools was carried out by filters on quartz sand. Every day, ¼ of the volume of water in the pools was refreshed.

After three months, studies were conducted on the muscle tissue of catfish, in which glutathione (GSH) and the enzymatic activity of glutathione-S-transferase were determined (GST). The determination of GSH and GST was carried out in the laboratory of Ulyanovsk state university using a Hitachi AAA 835 analyzer. Mathematical processing of the obtained results was carried out using the program «STATISTICA-6» [22].

3 Research results

The obtained results showed that in african catfish, the content of glutathione and the activity of glutathione-S-transferase in muscle tissue in fish of the control and experimental groups were different (pic. 1, 2).

During the study of level of glutathione in males and females of the african catfish of the control group, it was established that the content of this component in males was 0.545 mmol/g of protein, and in females 0.497 mmol/g of protein (Fig. 1).

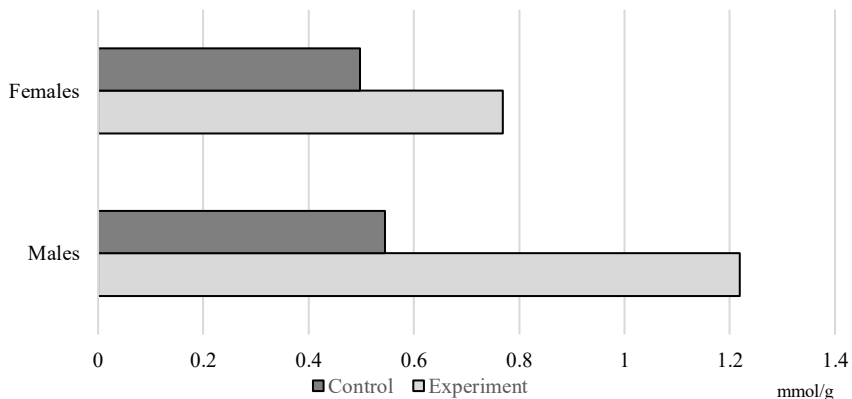


Fig. 1. Glutathione content in fish muscles.

In males of the experimental group, whose cultivation was carried out using a feed additive, the level of glutathione was 1.219 mmol/g of protein, which is twice as high as in males of the control group. In females, the content of glutathione was 0.769 mmol/g of protein (Fig. 1).

The high level of glutathione in the muscle tissue of the catfish of the experimental group, which were grown against the background of a new feed additive, indicates a high resistance of fish to oxidative stress, which can contribute to the intensification of growth and increase productivity.

According to picture 1. a lower level of glutathione in the muscle tissue of catfish was found in the control group. Glutathione provides neutralization of free radicals formed during peroxide processes, acting as a "trap" [15-17], while the level of glutathione decreases.

On the next stage, the activity of glutathione S-transferases was studied. Glutathione S-transferases are a family of enzymes that catalyze the conjugation of reduced form of glutathione (GSH) with xenobiotic substrates for the purpose of detoxification. The results of the study of enzyme activity in the muscle tissue of catfish are shown in figure 2.

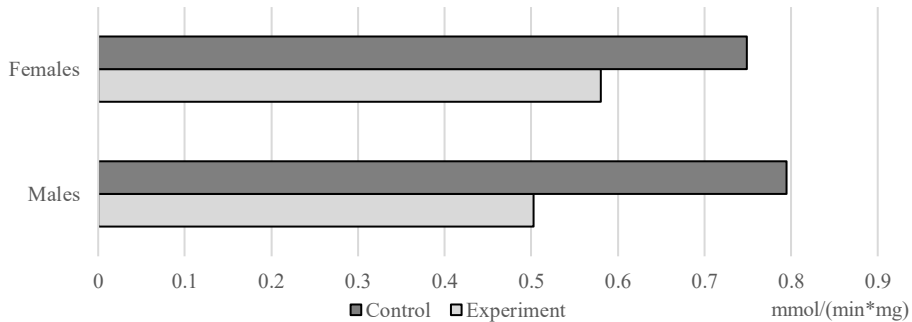


Fig. 2. Glutathione S-transferase activity in fish muscles.

During the study of glutathione S-transferase in the muscle tissue of males and females of african catfish of the control group, it was established that the activity of this enzyme in males was 0.503 mmol/(min*mg) of protein, and in females it was slightly higher - 0.580 mmol/(min*mg) of protein.

The activity of glutathione S-transferase enzyme in muscle tissue was higher in males of african catfish of the experimental group, which were grown against the background of a new feed additive, than in the control group, and amounted to 0.795 mmol/(min*mg) protein, and in females of the experimental group – 0.749 mmol/(min*mg) protein.

An increase in the activity of glutathione S-transferase in the muscle tissue of catfish of the experimental group, which were grown using a feed additive, indicates increase in the detoxification processes of xenobiotic substrates entering the body of fish from the outside, or formed inside the body and over-reactive free radicals.

The use of a feed additive developed by us containing adaptogens, probiotics and vitamin-amino acid complex should provide an intensification of fish growth, as the intestinal microbiota formed under the action of probiotics provides the production of biologically active substances that increase the body's resistance to chronic stress accompanying the process of growing fish in intensive aquaculture. Mostly, this mechanism is realized through the glutathione– glutathione S-transferase system. According to the results of our studies, the level of glutathione and the activity of glutathione S-transferases using them increase in the body of fish against the background of biologically active substances of the feed additive.

It is quite reasonable to believe that the use of feed additive normalizes the natural intestinal microbiocenosis of fish, activates parietal digestion, reduces the level of pathogenic and conditionally pathogenic microbiota, improves intestinal peristalsis. This contributes to a more complete digestion, absorption of nutrients.

We established that a new feed additive in the diet of clary catfish increases resistance to oxidative stress, due to an increase in the content of glutathione and an increase in the activity of glutathione S-transferase in the muscle tissue of fish.

The increased resistance to oxidative stress formed against the background of feed additives contributes to the improvement of the physiological state of fish.

4 Discussion of the results

The cultivation of fish in the conditions of industrial aquaculture is accompanied by a high level of nitrogenous compounds in the water, which are the products of their vital activity [23].

The increased content of ammonium nitrogen, nitrates and nitrites in the conditions of industrial aquaculture leads to the formation of chronic oxidative stress, which activates the processes of lipid peroxidation and reduces the activity of antioxidant enzymes that

contribute to the destruction of peroxides. All this slows down the growth of fish due to body intoxication. As the results of the study showed, the feed additive is able to reduce the negative impact of the fish habitat on the body. Due to the production of antioxidants, it increases the resistance of the fish body to oxidative stress, increasing the level of glutathione and the activity of glutathione S-transferase in the muscle tissue of catfish.

Glutathione, being an intracellular tripeptide, which includes glutamate, cysteine and glycine (γ -glutamylcysteinylglycine), is formed in the cytoplasm of all eukaryotic cells and provides antioxidant protection of cells, being a trap for free radicals.

A reduced level of glutathione is dangerous for the body and leads to disruption of signal transduction in cells, causes gene expression, slows down cell proliferation and cell differentiation, disrupts cell metabolism and vital activity, triggers apoptosis. Therefore, cells without protection die.

The most important role of glutathione is the formation of thioester conjugates with nitrogen oxides present in the aquatic environment during the cultivation of fish in industrial aquaculture. Glutathione, being a donor of intracellular cysteine, participates in the regulation of nitric oxide synthesis, acting as a thiol buffer for intracellular proteins; it is also part of enzymes, being a cofactor in them.

Glutathione S-transferases are a family of enzymes that take part in the mechanism of protecting cells from the effects of oxidative stress, as they catalyze the reactions of chemical interaction of reduced glutathione with electrophilic compounds. Glutathione S-transferases are able to influence cell regeneration and differentiation, cell apoptosis processes [15-17].

Activation of the antioxidant protection system of catfish during the use of a new feed additive occurs mainly due to the highly effective adaptogen trecrezan. Trackresan activates the production of α - and γ interferons, activates the cellular and humoral defense system, stimulates the phagocytic activity of macrophages.

As a result, the new biologically active feed additive had a more noticeable effect on males, increasing their glutathione content and the activity of glutathione S-transferases to a greater extent, compared with females.

5 Conclusion

The use of the feed additive developed by us has demonstrated its high effectiveness in relation to the antioxidant protection system.

The adaptogen trecrezan, a probiotic based on *Bacillus subtilis* and the vitamin-amino acid complex Chiktonic, included in its composition, showed antioxidant properties; they activated the components of the antioxidant defense system glutathione and glutathione S transferases, which provide the absorption of reactive oxygen species formed during lipid peroxidation.

The multicomponent feed additive that we have developed, by virtue of its composition, is able to protect the membrane structures of cells from damage, protect against toxicants coming from the external environment, provide detoxification of endogenous metabolites, heal the body, prevent infectious diseases, activate immunity. The vitamin-amino acid complex, which is part of the feed additive, intensifies the metabolism of fish, providing essential amino acids and vitamins, but also increases the nutritional and biological value of fish, giving it added usefulness.

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