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PROLINE AS METABOLIC FACTOR OF RECONSTRUCTION IN SPAWNING AND FACTOR OF EFFICIENCY IN FERTILIZATION PROCESSES FOR DIFFERENT SPECIES OF FISH

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The activities of PO and P5CD enzymes of proline catabolism have been studied in different organs of trout (*Parasalmo mikiss*) before spawning and during spawning, as well as the effect of citruline on the activity of these enzymes again before spawning and during spawning. PO and P5CD activities have been determined according to the generated amount of glutamate. The obtained data were compared with different organs of carp fish (*Cyrpinus carpio*) with the activities of these enzymes up to spawn and during spawn. The studies have shown that the activities of enzymes of proline catabolism of both trout and carp fish significantly increase, and citruline has a stimulating effect on the activities of these enzymes in both organisms.

***Keywords*:** catabolism of proline, proline-oxidase, piroline-5-carboxilat-dehydrogenase.

Introduction. Proline's transmutation into glutamate in different biological systems mainly occurs by formation of pyrroline-5-carbonation acid. Proline oxidase (PO) and pyrroline-5-carboxsilat-dehydrogenase (P5CD) enzymes provide proline's catabolism. Both PO and P5CD have been detected in bacteria, plants and animals and are considered mitochondrial enzymes. Citokrom-c also takes part in oxidation process of proline as an electron acceptor [1]. Proline has its own metabolic system with his peculiarities, it is a multi-functional amino acid, in particular, it is an activator of Krebs cycle, energetic role of proline in insects is proved, is a higher molecular substratum for oxidation processes, is a component of stress proteins and receptors, a regulator of redox potential, carbon source, an inactivator of reactive radicals, as well as physicochemical modulator for a number of metabolic processes. In addition, studies of recent years have shown that proline catabolism regulating enzymesr espond in cases of genotoxic, inflammatory and fertilizer stresses. In particular, during fertilizer stress proline is a source of energy and provides three-carbonate cycle with carbon. Relatively recent research leads to the conclusion that proline is also stress substratum in the micro-environment of neoplasms [2]. The most recent studies have shown that the role of proline metabolism regulator is in apoptosis during cancer and in deprivation of oxygen in

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cancer cells [3]. One of the important features of proline is also the fact that it has a unique role in stressful situations of organisms, in particular, in many plants the degree of accumulation of proline increases in response to various stressful situations [4]. With proline involvement this bio chemical spectrum of fairly large influence allows to assume that it must have a pretty big role in such physiological state of organisms, as spawn and fertilization. So, it was interesting for us the role of proline, specifications of enzymes of proline catabolism and the impact of such a multifunctional modulator of citruline during fish spawning, which can also be considered a special stressful situation. These studies were especially interesting in the sense that in literature during fish spawning period there are almost no data on the biochemical changes, there are also no data of similarities and differences of activities of proline enzymes catabolism in a variety of fish.

It is known that in each period of Fish life biochemical substrates of certain amount are typical in tissues, by which their vitality, paces of development and maturation of the body is determined. Fats and proteins are the most important storage nutrients, which are spent to meet the energetic needs of cells and tissues and for the formation of sexual products. The effectiveness of fish spawning can be assumed by the amount of giraud- and water-soluble proteins. At the Scientific Research Institute of Caspian fish economy, researches have been performed in the organisms of Caspian kilkis and the connection of the amount of lipids and water-soluble proteins of fish of different regions with the efficiency of spawning process has been explored and revealed that the higher the quantity of these substances are in the muscles of fish, the bigger is the productivity of the fish [5]. Trout fish spawning usually begins in the 3rd year of the life and for spawning the most notable is deep autumn, but sometimes it also takes place in winter. The spawning period depends on the type of fish, water temperature and climatic zone and can last about a month. During this period a number of changes take place about the fish: colour, behaviour, which allows us to assume that these changes are also due to the specific biochemical changes.

Citruline is an amino acid, which is not included in the composition of building proteins, but it has a large number of physiological effects, in particular, participates in urea metabolism, arginine is synthesized from citruline: citruline is an intermediate metabolite in the process of neutralization of toxic ammonia, is widely used in sports, including bodybuilding as a way to improve muscle blood flow and other processes, so, it is interesting how it influences on the activity of enzymes of proline catabolism of fish and especially during spawning period how it may affect the activity of these enzymes. Studies have shown that sitrulin-malate in case of 6 *g* daily dose reduces muscle fatigue, increases ATF production for about 34%, as well as increases the concentration of fosfocreatine for about 20% after exercises [6]. In 2015 scientists of Mayo Clinic (MN, USA) published an article, according to which the adoption of citruline regulates the synthesis of muscle protein in case of a short-term deficiency of the muscle protein in healthy people, moreover, the emergence of citruline does not cause any hormonal change [7].

Materials and Methods. The activity of PO and P5KD has been studied in different organs of trout (*Parasalmo mikiss*) grown in lakes and compared with the activity of the same enzymes of carp fish (*Cyrpinus carpio*) in different organs. 10% homogenat has been prepared for determination of enzymes activity of proline

catabolism. Potassium-sodium-phosphate buffer (pH 8.0) has served as an environment for homogenizing. Homogenisation has been performed by glass Potter-Elvejiem homogenator.

Determination of Enzymes Activity of Proline Catabolism. An incubation mixture has been prepared, which contained 53 *mM* potassium-sodium-phosphate buffer (pH 8.0), 0.2 *mM* L-proline, 1.6 *mkM* citocrom-c, 4 *mkM* NAD+0.5 *mL* homogenate and citruline of corresponding number in corresponding version. The incubation was made at temperature 37°*C* during 1 *h*. After incubation the reaction was stopped with 96% ethyl alcohol. Samples were centrifuged by 8000 *g* acceleration for 10 *min*. The activities of PO and P5CD were determined by the amount generated by glutamate. Glutamate was determined by chromatographic method [8]. For determination of glutamate quantity 0.16% of CdC_{12} with 60% alcoholic solution was performed elution during 1 *h*. Calorimeter was performed by photoelectric colorimeter.

Statistical Analysis. The statistical reliability of received data has been calculated by the method of variation statistics by separating the reliabily criterion due to Student's *t*-test.

Results and Discussion. We compared the activity of enzymes of proline catabolism in different organs of trout fish before and during spawning with the similar studies previously carried out in our laboratory in different organs of carp fish [9] and we can state that the activities of enzymes of proline catabolism obviously increase in all the organs of both trout fish and carp fish in the spawning period (Tab. 1).

Table 1

The activity of PO and P5CD in different organs of trout fish and carp fish before spawning and during spawning (mkmol glutamate in 1 g fresh tissue, n = 5, M ± m)

Organs	Trout fish before spawning	Trout fish during spawning		Carp fish before spawning	Carp fish during spawning
		male	female		
Kidneys	1.19 ± 0.08	4.02 ± 0.29	16.98 ± 0.98	0.81 ± 0.1	6.5 ± 0.8
Liver	2.12 ± 0.14	4.42 ± 0.30	8.47 ± 0.61	1.41 ± 0.3	27.9 ± 1.4
Heart	5.21 ± 0.037	6.2 ± 0.40	11.30 ± 0.76	2.82 ± 0.5	4.2 ± 0.6
Branchia	2.74 ± 0.11	5.32 ± 0.38	28.47 ± 1.05	–	–
Brain	2.67 ± 0.19	4.64 ± 0.31	5.19 ± 0.37	–	–
Caviar	1.26 ± 0.32	–	7.79 ± 0.55	–	–

Tab. 1 shows, that before spawn the activity of enzymes in the heart of trout fish is 2 times higher compared with the activity of enzymes in the heart of carp fish, and the activities of enzymes of fish are almost the same in the kidneys and the liver. During the spawning in the kidneys, liver and brain of the females of trout fish the activity increases about 4 times, in the heart about 2 times, and caviar about 6 times whereas in the spawning period the activity of enzymes of kidneys of carp fish increases about 8 times, in the liver, about 20 times, in the heart about 1.5 times. That is, the growth of activity of enzymes is higher in the spawning period in carp fish in comparison with trout fish, which may be caused by the living conditions of inhabiting of organisms, by the peculiarities of fish spawning and the degree of productivity, for carp it is more productive than for trout and, of course, by the difference in physicochemical properties of enzymes. And already in the spawning period the obvious increase of the enzymes of proline catabolism in the

organs of fish is due to the fact that during physiological changes of the organisms and stressful situations a reconstruction of metabolic processes occurs and mobilizing proline is actively involved in exchange processes, through glutamate filling the cell's energy requirements and ensuring the effectiveness of the fertilization process. The survey also shows that the activities of enzymes of organs of males and females of trout fish are various, which is explained by sexual dimorphism.

In other part of the researches the impact of citruline on the activity of proline catabolism enzymes in different organs of trout fish females were studied in the spawning period and the results of the studies were again compared with the impact of citruline on the activity of enzymes in various organs of carp fish (Tab. 2). Citruline was taken in $3 \cdot 10^{-5}$ *M* concentration.

Table 2

The impact of citruline on the activity of PO and P5CD in different organs of trout fish and carp fish (μmol glutamate in 1 g fresh tissue, n = 5, M ± m)

Organs	Citruline concetration, $3 \cdot 10^{-5}$ *M*	Activity of PO and P5CD in different organs of trout fish	Activity of PO and P5CD in different organs of carp fish
Kidneys	without effector	16.85 ± 0.89	0.94 ± 0.1
	citruline	23.71 ± 1.02	25.2 ± 2.3
Liver	without effector	8.54 ± 0.59	1.96 ± 0.3
	citruline	38.9 ± 1.23	29.6 ± 2.9
Heart	without effector	11.43 ± 0.75	2.9 ± 0.6
	citruline	15.61 ± 0.85	23.8 ± 2.4
Branchia	without effector	28.37 ± 1.07	–
	citruline	31.3 ± 1.15	–
Brain	without effector	5.21 ± 0.37	–
	citruline	20.89 ± 0.98	–

The data of Tab. 2 show, that both in different organs of trout fish and in different organs of carp fish citruline leaves quite a stimulating effect on the activity of enzymes of proline catabolism. For example, in the kidneys of trout fish the activity of enzymes increases on the impact of citruline about 1.5 times, and in the kidneys of carp fish about 27 times. In the liver of trout fish on the impact of citruline the activity of enzymes increases about 4.5 times, and in the liver of carp fish about 15 times. In the heart of trout fish the activity of enzymes increases about 1.5 times, and in the heart of carp fish about 8 times. The table also shows that by the impact of citruline in the branchia of trout fish the activity of enzymes increases very little, and in the brain it grows about 4 times. Data proving the citruline activating effect on proline catabolism enzyme activity are present also in some native works. For example, the citruline has a certain activating influence on the activity of these enzymes of bean granivorous beetles, which allows to get PO and P5KD preparation from these beetles [10]. Thus, we can say that in different organs of carp fish citruline leaves more stimulating effect on the activity of enzymes of proline catabolism, than in different organs of trout fish.

Conclusion. Summing up the results of the study and their comparison with the activity of enzymes of proline catabolism in different organs of carp fish, we can say that these 2 organisms differ not only in size, accommodation of living conditions and productivity, but also in their enzymatic features. In addition, this

research once again confirms the role of proline in physiological changes of organisms, in non-standard and stressful situations as stress metabolite, and we can affirm that proline is a restructuring biochemical factor of metabolic processes in the fish spawning process and a biochemical factor providing the efficiency of fertilization process.

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