RESEARCHES ON THE ACTIVITY OF OXIDOREDUCTASES FROM TISSUES SAMPLED IN DIFFERENT STAGES OF DEVELOPMENT AT *SILURUS GLANIS*

Mihaela Oana Arteni, Z. Olteanu, L. Oprică, M. Bălan

University „A.I.Cuza", Iaşi, Faculty of Biology
e-mail: oana_artenie@yahoo.com

Abstract

The aim of our research is to study the dynamics of the enzymes activity involved in oxidative stress (superoxide dismutase, catalase and peroxidase) performed on two types of tissues, from muscle and intestine, collected from Silurus glanis aged one, two, respectively three summers. Research has been conducted on four individuals for each age; fish were collected from Fish Farm Ţigănaşi, Iasi County. Superoxide dismutase activity was determined using Winterbourne, Hawkins, Brian and Carrell methods adapted by Artenie, catalase activity was determined using Sinha method, peroxidase was determined using colorimetric method with o-dianisidine and soluble proteins were determined using Bradford method. Data analyses showed that enzyme activities present differences tissue-dependent and also related with the individual age. Oxidoreductases activity is higher in intestinal tissue than in muscle tissue for all three stages of development taken into study. It was also considered in sthe study the type of food used to feed fish at different ages.

Key words: *Silurus glanis*, superoxide dismutase, catalase, peroxidase, age

INTRODUCTION

The oxidative/antioxidant status and the consequences of hypoxic/hyperoxic conditions are crucial for aquaculture [12]

Oxygen availability is a limiting growth factor and chronic hypoxia or hyperoxia may be an important environmental stressor influencing fish growth [12]. Fish are frequently exposed to frequent episodes of environmental and physiological hypoxia, and are likely to produce elevated levels of reactive oxygen species (ROS) such as super oxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) as a result of oxidative metabolism during or on recovery of any physiological stress [7], [1], [5]. If these noxious oxygen derivatives are not controlled by antioxidant defense systems, oxidative stress occurs. The direct effects include peroxidative damage to important macromolecules.

Indirectly,changes induced by reactive oxygen metabolites in cellular membranes and components can modify metabolic pathways, resulting in altered physiology and possible pathology [7].

To minimize the negative effects of ROS, fish, like other vertebrates, process an antioxidant defence system, which utilizes enzymatic and non-enzymatic mechanisms [[7]. The enzymes that provide the first line of defence against O^{2-} and H_2O_2 include SOD (EC 1.15.1.1), CAT (EC1.11.1.6) and PER (EC 1.11.1.7) [5].

Antioxidant enzymes act as scavengers of the highly reactive intermediates produced in hydrocarbon metabolism to maintain cell homeostasis. Generally, these enzymes respond differently to different chemical compounds. An individual antioxidant enzyme is unable to provide a general marker of oxidative stress because of the complexity of interactions between provident factors and antioxidants. Therefore multiple antioxidant enzyme values are often measured together to indicate the total oxyradical scavenging capacity, which has a greater indicating value [9].

MATERIAL AND METHODS

Research is conducted on two types of tissue, muscle and intestine, collected from

four individuals of *Silurus glanis* for each stage of development (one, two and three summers old) from Fish Farm Ţigănaşi, Iasi County.

To obtain the enzyme extract the animal tissue is ground with mortar and pistil in an ice water bath with the same amount of glass quartz sand; then is extracted with 0.1 M disodium phosphate. Homogenate obtained is centrifuged for 15 minutes in a cooling centrifuge and the supernatant is used as source of enzyme and total soluble proteins.

Superoxide dismutase activity is determined using Winterbourne, Hawkins, Brian and Carrell adapted Artenie method [2]. The method consists in assessing the ability to inhibit the enzyme reducing nitro blue tetrazoliu salt (NBT) by superoxide radicals in the environment generate response. Results are expressed in U / mg protein as the mean ± SE (standard error). One unit of enzyme activity is that enzyme quantity that causes an 50% inhibition of NBT reduction maximum inhibition.

Catalase activity is measured using Sinha method [2]. In principle, the method consist in hydrogen peroxide determination decomposed remaining after stopping the enzyme action on substrate with a mixture of potassium dichromate and glacial acetic acid. Results are expressed in U / mg protein as the mean ± SE (standard error). One unit of enzyme activity is the enzyme quantity that decompose one hydrogen peroxide micromole (0.034 mg) for one minute at 20^0C temperature and pH=7.

Peroxidase activity is determined using o-dianisidine colorimetric method [3]. The principle of the method is based on measuring the intensity of o-dianisidine color product oxidation with hydrogen peroxide under the peroxidase action. Results are expressed in mU / mg protein as the mean ± SE (standard error). One unit of enzyme activity is the enzyme quantity that catalyzes one micromole hydrogen peroxide decomposition in one minutes in optimum conditions of reaction.

Total soluble proteins are determined using the Bradford method [2]. The principle of the method is based on the observation that the acidic environment dye Coomassie Brilliant Blue G-250 protein forms a complex with maximum absorption at 595 nm.

RESULTS

The data concerning superoxid dismutase activity from both types of tissue are revealing higher values in intestinal tissue than in muscle tissue for all developmental stages studied (table 1 and fig.1). In muscle tissue the higher activity is obtained for one summer old wels catfish samples (1.865±0.237 U/mg protein) while for two summer old individuals is the lowest (1.119±0.248U/mg protein). In intestinal tissue is obseved a maximum superoxid dismutase activity in second development stage and a minimum activity in three summer old wels catfish.

Table 1.

Superoxide dismutase activity in muscle and intestine harvested for *Silurus glanis* of various ages

AGE (YEARS)	ANALYZED TISSUE	SUPEROXID DISMUTASE ACTIVITY [U/mg protein]
0+	muscle	1.865±0.237
	intestine	1.962±0.271
1+	muscle	1.119±0.248
	intestine	5.202±2.129
2+	muscle	1.182±0.062
	intestine	1.636±0.081

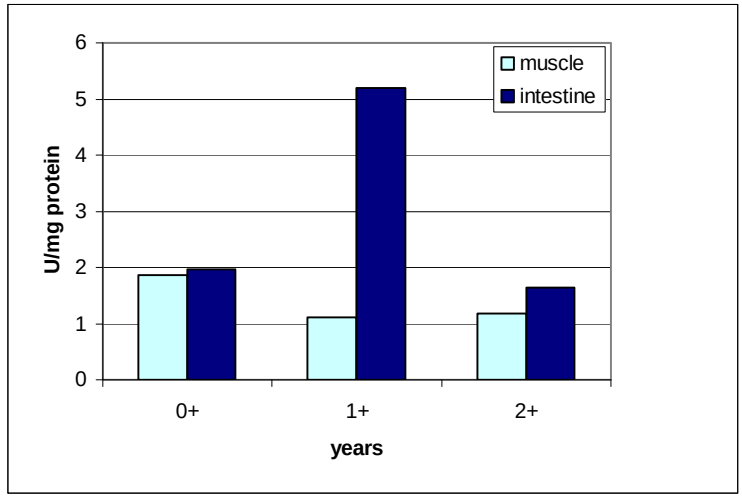

Fig. 1. Superoxide dismutase dynamic activity in muscle and intestine for *Silurus glanis* of various ages

The catalase activity is higher in the intestinal tissue than in muscle tissues, maximum values are recorded in muscle and intestinal tissue for the second summer (233.842±18.728 U/mg protein, respectively 802.707±113.077 U/mg protein). For wels catfish fry between muscle and intestine are very small differences regarding catalase activity (118.762±39.896 U/mg protein, respecively 126.178 ± 58.687 U /mg protein. For wels catfish samples is observed the lowest catalase activity at three summer old individuals in muscle while in intestine at fry stage.

Table 2. Catalase activity in muscle and intestine harvested for *Silurus glanis* of various ages

AGE (YEARS)	ANALYZED TISSUE	CATALASE ACTIVITY [U/mg protein]
0+	muscle	118.762±39.896
	intestine	126.178±58.687
1+	muscle	233.842±18.728
	intestine	802.707±113.077
2+	muscle	82.582±44.504
	intestine	237.88168.105

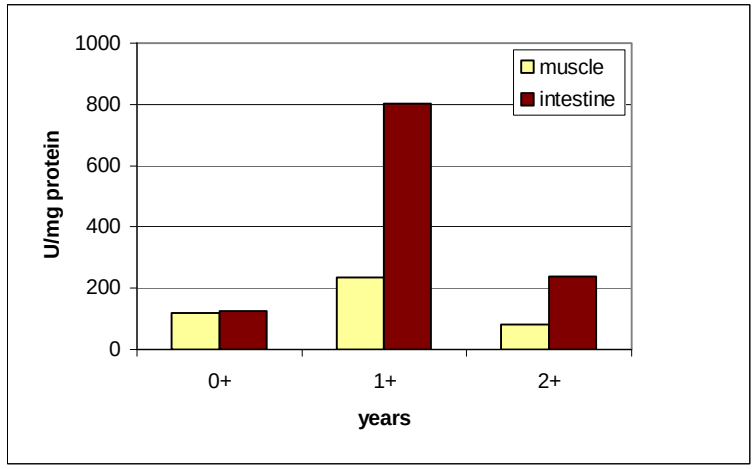

Fig. 2. Catalase dynamic activity graphical in muscle and intestine for *Silurus glanis* of various ages

Like superoxid dismutase and catalase, peroxidase shows higher values in intestinal than in muscle tissue. A similar peroxidase activity is observed in muscle as wel as in the intestine, with a maximum activity in the second developmental stage (8.288±2,531 mU/mg protein for muscle, respectively 40±11.974 mU/mg protein for intestin) and minimum at an age of three summer (229±0.898 mU/mg protein, respectively 5.271±1.311 mU/mg protein).

Table 3.
Peroxidase activity in muscle and intestine harvested for *Silurus glanis* of various ages

AGE (YEARS)	ANALYZED TISSUE	PEROXIDASE ACTIVITY [U/mg protein]
0+	muscle	6.363±4.167
	intestine	12.302±4.675
1+	muscle	8.288±2.531
	intestine	40±11.974
2+	muscle	2.29±0.898
	intestine	5.271±1.311

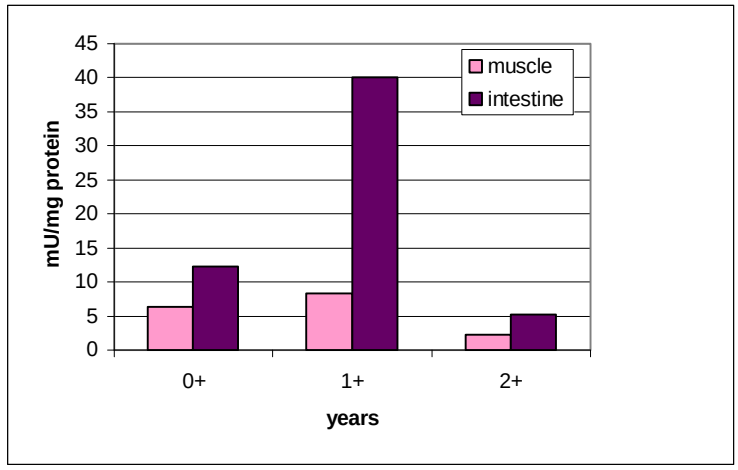

Fig. 3. Peroxidase dynamic activity in muscle and intestine for *Silurus glanis* of varoius ages

DISCUSSIONS

Higher values of oxidoreductases studied activity for intestinal tissue and muscle tissue are comparable to those of the literature [8], [4]. This event is explained by the gills who appear to be susceptible to oxidation, partly because of their defensive phagocytic activity and partly for presenting fewer antioxidant resources compared with other tissues, such as liver.

The functional deterioration associated with ageing is derived from an accumulation of oxidative damage inflicted by non-scavenged ROS on lipids, proteins and nucleic acids. Wdzieczak et al. (1982), in a comparative study of the SOD and CAT activities and lipid peroxidation in erythrocytes and liver of different fish species, reported that younger fish showed high antioxidant activity [11].

Antioxidant defenses in fish are also dependent on feeding behavior and nutritional factors. Dietary levels of lipids and some vitamins have been reported to influence antioxidant defenses and oxidative status of fish. Diets containing low levels of lipid and digestible starch reduce the susceptibility of the fish to oxidation and may enhance the growth rate [10]. One summer-old fish used in our experiments were further fed with pelleted feed with a chemical composition of approximately 48% protein and 11% fat. For the other two ages it was used a mixture of flours made from 40% sunflower, 40% corn and 20% grist with a chemical composition of 36% protein and 5.86% fat.

CONCLUSIONS

- the enzymes activities recorded differences related to the nature of the examined tissue and with the age of the analyzed individuals;

- the oxidoreductases activity is higher in the intestinal tissue than in the muscle tissue for all the analyzed development stages;

- for all enzymes studied the higher activity is recorded for wels catfish individuals aged two summers.

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