RESEARCHES ON THE ACTIVITY OF OXIDOREDUCTASES FROM TISSUES HARVESTED IN DIFFERENT STAGES OF DEVELOPMENT AT *CYPRINUS CARPIO*

OANA MIHAELA ARTENI[1]*, ZENOVIA OLTEANU[1], LĂCRĂMIOARA OPRICĂ[1], MIHAELA BĂLAN[1]

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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 *Cyprinus carpio* aged one respectively three summers. Data analyses showed that enzyme activities present differences tissue-dependent and also related with the individual age. It was also considered in the study the type of food used to feed fish at different ages.

INTRODUCTION

Oxygen availability is a limiting growth factor and chronic hypoxia or hyperoxia may be an important environmental stressor influencing fish growth (Wilhelm Fihlo et al., 2005). 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 (Miller et al., 1993; Abele et Puntarulo, 2004; Gŭven et al., 2008). If these noxious oxygen derivatives are not controlled by antioxidant defense systems, oxidative stress occurs. The direct effects include peroxidative damage to important macromolecules.

To minimize the negative effects of ROS, fish, like other vertebrates, process an antioxidant defence system, which utilizes enzymatic and non-enzymatic mechanisms Miller et al., 1993 . 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) (Gŭven et al., 2008). 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 (Regoli et al., 2002).

MATERIAL AND METHODS

Research is conducted on two types of tissue, muscle and intestine, collected from four individuals of *Cyprinus carpio* for each stage of development (one and three summers respectively) from Fish Farm Ţigănaşi, Iasi County.

To obtain the enzyme extract animal tissue is ground with a 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 by the method Winterbourne, Hawkins, Brian and Carrell adapted Artenie (Artenie et al., 2008) . The method is, in principle, 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 by the Sinha method (Artenie et al., 2008). 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 by o-dianisidine colorimetric method (Cojocaru, 2005). 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 that enzyme quantity that catalyzes one micromole hydrogen peroxide decomposition in one minutes in reaction optimum conditions.

Total soluble proteins are determined by the Bradford method (Artenie et al., 2008). 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 AND DISCUSSIONS

The data concerning superoxid dismutase activity from both types of tissue are revealing higher values in intestinal tissue than muscle tissue for both developmental stages studied (fig.1). Enzyme activity recorded in muscle tissue from specimens of three summers old crap (2574 ± 0198 U / mg protein) is higher than one summer old individuals (1029 ± 0453 U / mg protein). In intestinal tissue superoxide dismutase activity presents higher values in the first stage of development (7324 ± 1150 U / mg protein) compared with the third stage (2574 ± 0198 U / mg protein).

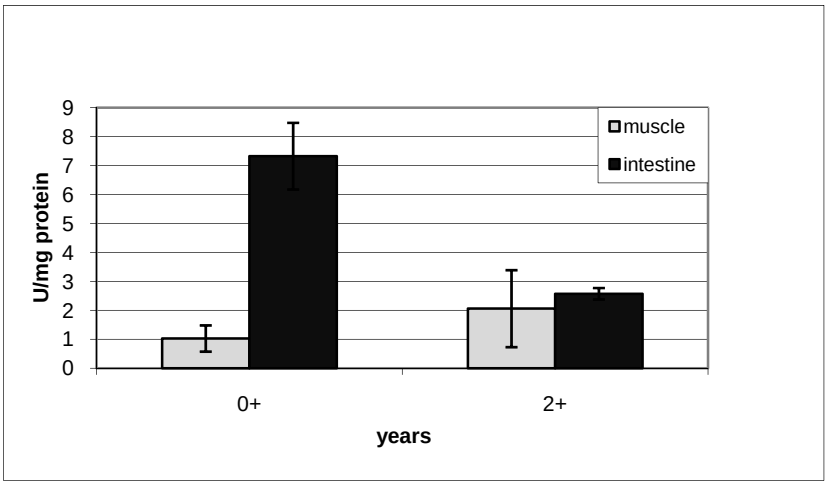

Fig. 1. Superoxide dismutase dynamic activity in muscle and intestine in *Cyprinus carpio* of various ages

Catalase activity is higher in intestinal tissue than in muscle (fig.2), the maximum being recorded in muscle in the third growth summer, and in the intestine of juvenile stage (214,721 ± 70,173 U / mg protein, respectively 808,658 ± 148,656 U / mg protein). In the third stage of development differences between catalase activity in muscle and the intestine are extremely small with a value of 214,721 ± 70,173 U / mg protein in muscle, respectively 223,127 ± 80,046 U / mg protein in intestinal tissue.

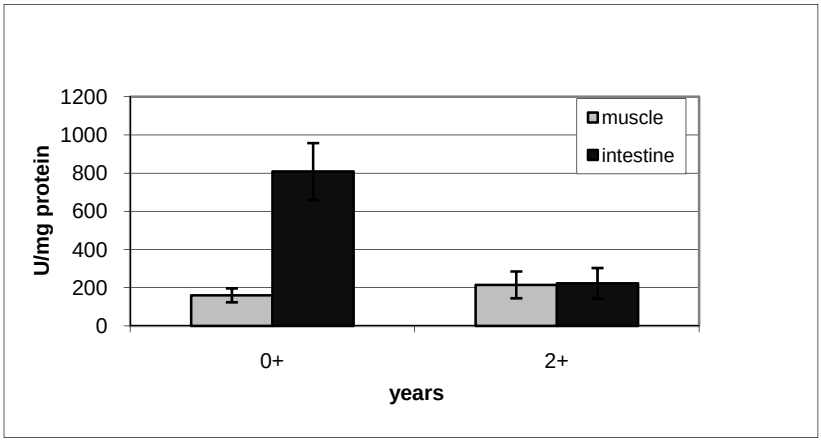

Fig. 2. Catalase dynamic activity in muscle and intestine in *Cyprinus carpio* of various ages

Peroxidase, like superoxid dismutase and catalase, shows higher values in the intestinal tissue than in muscle (fig.3). Peroxidase activity in muscle is higher at the age of three summers (11,412 ± 4155 U / mg protein) compared to the juvenile stage (3527 ± 1262 U/mg protein), situation inversed in intestinal tissue (123,420 ± 9396 U / mg protein for one summer old individuals respectively 64,581 ± 19,301 U / mg protein for three summers).

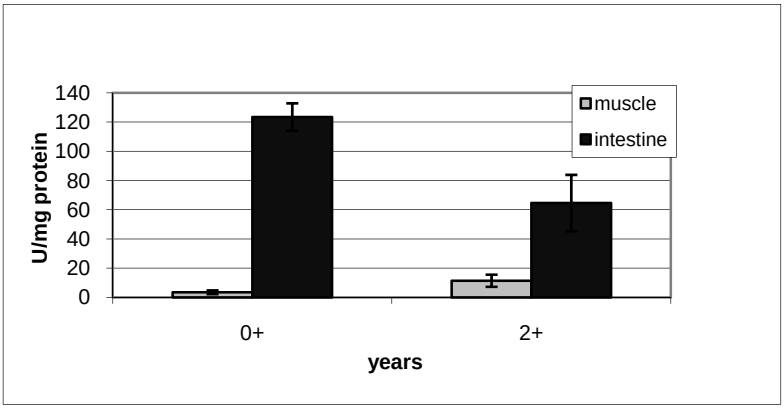

Fig. 3. Peroxidase dynamic activity in muscle and intestine in *Cyprinus carpio* of various ages

Higher values of oxidoreductases studied activity in intestinal tissue to muscle tissue are comparable to those in the literature (Parihar et Dubey, 1995; Fatima et al., 2000;).This event is explained by the gills appear to be susceptible to oxidation, partly because of their defensive phagocytic activity and partly for presenting fewer antioxidant resources in comparison 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.

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 (Rueda et al., 2004). 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 age was used a mixture of flours made from 40% sunflower, 40% corn and 20% grist with a chemical composition 36% protein and 5.86% fat.

CONCLUSIONS

- studied enzymes activities recorded difference in the nature of tissue examined and the age of analyzed individuals;
- studied oxidoreductases activity is higher in the intestinal than in muscle tissue at both stages of development analyzed.

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[1]Alexandru Ioan Cuza "University of Iasi, B-dul Carol I, Nr. 20A, 700506, Iasi-Romania
*oana_artenie@yahoo.com