A COMPARATIVE STUDY ON THE ACTIVITY OF HEPATIC AND MUSCULAR CATALASE IN FRESHWATER FISH SPECIES

ELENA CIORNEA [1*], GABRIELA VASILE [1], DUMITRU COJOCARU [1], SABINA IOANA COJOCARU [1]

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Abstract: The paper performs a comparative determination of the hepatic and muscular catalase activity in three 2 summer-old cyprinids species, namely common carp (*Cyprinus carpio*), crucian (*Carassius auratus gibelio*) and bighead carp (*Aristichthys nobilis*), all coming from an intensive growth system. The results obtained evidence higher values (3.58 times higher in common carp, 6.55 higher in bighead carp and 2.07 higher in crucian, respectively) recorded by this marker-enzyme of oxidative stress, for all species under investigation, at the level of liver - known as the main center of substances' metabolism.

INTRODUCTION

As generally known, all aerobic organisms possess catalase - a biocomponent enzyme belonging to the class of oxidoreductases, with hemine or feroporphyrin IX as prosthetic group. The literature of the field suggests that the proteic part is not absolutely necessary for life, once its absence - possibly resulting from a genetic accident, may be compensated, at least partially, through destruction of the hydrogen peroxide catalyzed by peroxidases and - especially in animals - by glutation peroxidase. By the presence of the functional thiolic group of cysteine, the latter one protects the thiolic enzymes against the detrimental action of oxygen while, by its oxidated and reduced forms, it forms an important redox system (BODEA *et al*., 1964).

According to some authors (RUDNICK, 1967; BRAUNBECK *et al*., 1987; ORBEA *et al.*, 2000; SÓLE *et al*., 2004; FERNANDEZ - DIAZ *et al*., 2006), in the case of fish, catalase is an *adaptation enzyme*. Thus, a study on its activity in the rainbow trout grown in floatable cages showed that the activity of the hepatic and muscular enzyme gets modified as a function of water temperature, density of fish batches, quality of the administered food and age of the individuals (BATTES *et al*., 1974 - 1975).

As to the percent distribution of catalase in the formation of the hepatic cell, the following values were recorded: 66% in hyaloplasma, 18% in microsomes and 16% in the nucleus (RADHAKRISHNAN and SARMA, 1966).

The literature of the field also shows that, in the case of fish, environmental factors such as: temperature, salinity, season, as well as the feeding habitat induce modifications in the peroxisomal enzymatic activity, seen as also depending on the species (FAHIMI and CAJARAVILLE, 1995; ROCHA *et al*., 2003), catalase being well-known as an enzymatic peroxizomal marker (AEBI, 1984). It has been also demonstrated that the season, age and sex influence the morphology of peroxizomes from the hepatic tissue in fish. Thus, in the *Mugil cephalus* species, the hepatic peroxizomes tend to increase, both along the summer and in aged individuals (ORBEA *et al.*, 1999). More than that, in *Salmo trutta*, the individual sizes of the peroxizomes and their total volume per hepatocyte, but not their number, get modified during the annual reproduction cycle, in both genera (ROCHA *et al*., 1999).

Several studies have been devoted to the influence of some chemical substances (aluminium, cadmium, uranium, phenanthrene, endosulfan, phenyl-carboxylic acids, petroleum, etc.) on the catalasic activity in various tissues: sanguine, hepatic, renal and branchial (AINY *et al*., 1996; OTTO and MOON, 1996; MCFARLAND *et al*., 1999; VARANKA *et al*., 1999; SÓLE *et al*., 2000; IKIĆ *et al*., 2001; PANDEY *et al*., 2001; JENA *et al*., 2002; ACHUBA and OSAKWE, 2003; BUET *et al*., 2005; GULCIN *et al*., 2005; LIMA *et al*., 2006; SUN *et al*., 2006).

The present paper systematizes the results of the investigations on the activity of catalase, an enzyme actively involved in the oxidative stress, from the hepatic and muscular tissue of some autochtonous and allochtonous cyprinids (common carp, crucian and bighead carp) from the second growth summer, coming from an intensive growing system.

MATERIALS AND METHOD

The experiments were performed on two summer-old representatives of common carp (*Cyprinus carpio*), crucian (*Carassius auratus gibelio*) and bighead carp (*Aristichthys nobilis*) from the Piscicultural Farm of Vlădeni - Iaşi district. Fresh samples of hepatic and muscular tissue have been taken over, on which the activity of catalase was determined titrimetrically, with potassium permanganate, the results obtained being expressed in mg oxygenated water/ml/30 min. (COJOCARU, 2008).

In the end, the results were statistically interpreted by the Anova test, the unifactorial pattern (FOWLER *et al*., 2000; ZAMFIRESCU and ZAMFIRESCU, 2008).

RESULTS AND DISCUSSION

Catalase, universally occurring in nature, evidences its activity in all aerobic microorganisms, as well as in the cells of plants and animals. At cellular level, the enzyme occurs almost exclusively in peroxizomes, reducing the level of the hydrogen peroxide, while it is absent in chloroplasts. Hydrogen peroxide is the most stable of all active species of oxygen, being a very strong nucleophylic oxidative agent, responsible for the inhibition of the enzymes from the Calvin cycle. Besides other enzymes, it is one of the most efficient catalysts known up to now, the reactions it catalyzes being essential for life.

The enzyme acts as a regulator of the H_2O_2 level, on also acting as a detoxification agent, as the oxygenated water has a toxic effect upon the tissues, thus protecting the cell through catalysis of the oxygenated water formed in the cells under the action of aerobic dehydrogenases.

A first objective of the present investigation involved determination of the catalase activity in the hepatic and muscular tissue of the two year-old common carp. Thus, as also graphically evidenced (Fig.1), the activity of catalase in the muscle (4.69 mg oxygenated water/ml/30 min.) represents but 27.86% of the one present in the liver (16.83 mg oxygenated water/ml/30 min.), which might be explained through the enzyme's involvement in processes producing considerable amounts of oxygenated water.

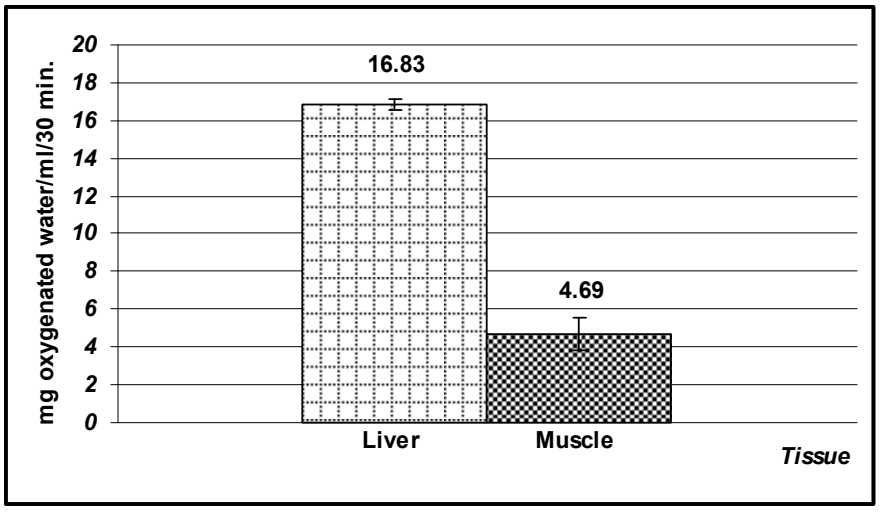

Fig.1. Hepatic and muscular catalase activity in two summer-old *Cyprinus carpio*

In the bighead carp (Fig.2), mention should be made of the fact that, on one side, the catalasic activity records much lower (about 7 times) values in the muscular tissue, comparatively with the hepatic one while, on the other, this is diminished in both tissues, comparatively with the *Cyprinus carpio* representatives, significant differences being evidenced in the muscular tissue, where the catalasic activity is two times higher in common carp.

The distinctly higher values recorded for the hepatic tissue agree with the literature data, which evidence - each time - maximum activities in the hepatic tissue (BATTES *et al*., 1974 - 1975).

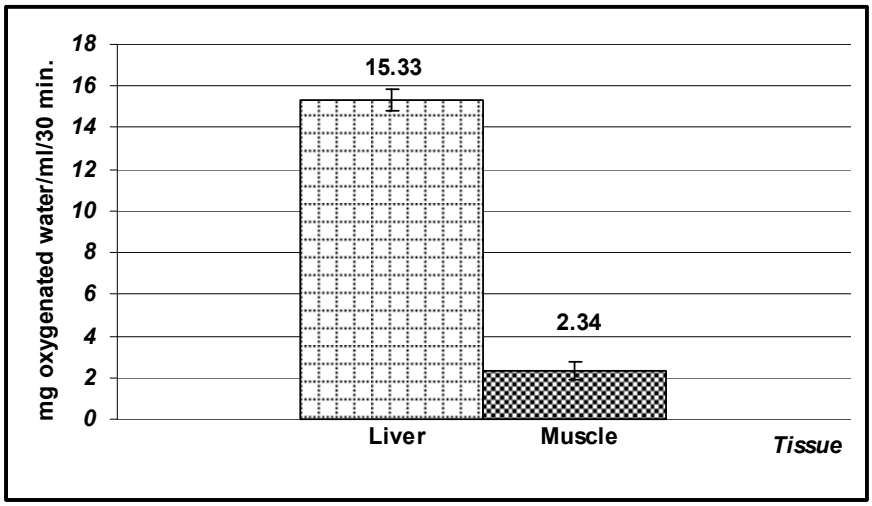

Fig.2. Hepatic and muscular catalase activity in two summer-old *Aristichthys nobilis*

The latest species taken into study was the crucian, a case in which the catalase activity values recorded were comparable with those of the previously-investigated species, mention being made of the fact that maximum values are registered at the level of the muscular tissue (7.85 mg oxygenated water/ml/30 min.). Another observation refers to the moderate difference observed between the muscular and the hepatic activity, comparatively with the previously-analyzed species, the enzymatic activity at muscular level being about 48% lower (Fig.3).

The present results agree with the literature data, according to which, in the same organism, the maximum activity of catalase should be registered in the liver, followed by erythrocytes, brain, spinal marrow, gonads, muscle and liver (PANIKER and IYER, 1972).

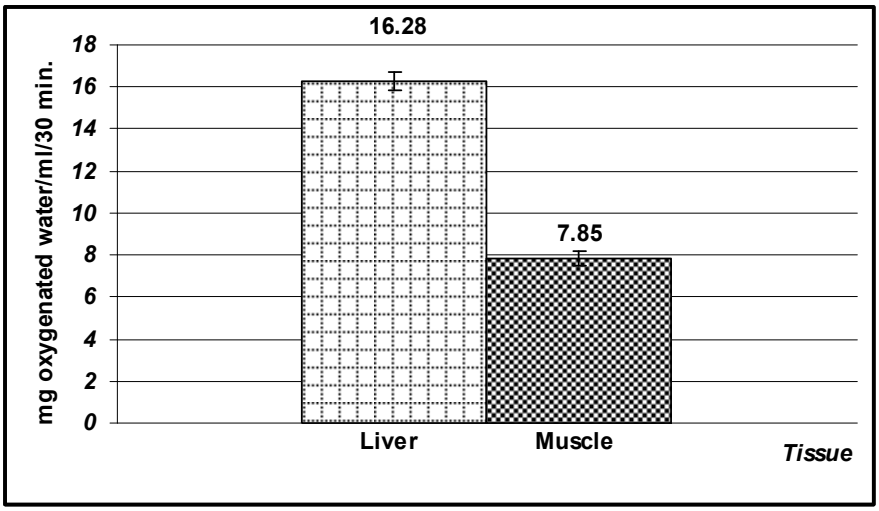

Fig.3. Hepatic and muscular catalase activity in two summer-old *Carassius auratus gibelio*

The last objective of our researches was to test the statistical significance of the obtained data, on the basis of the Anova test, the unifactorial pattern with an equal number of observations

in the cell. Consequently, the null and alternative hypotheses of the test could be established, while a comparison between the critical and the calculated factors (*calculated F* and *critical F*) led to the acceptance of one of them (whether significant differences on the catalasic activity in the cyprinids species under study are observed or not).

As to the hepatic tissue, the results obtained are listed in Tables I - II, *calculated F* (15.3402) being higher than *critical F* (3.8852), which suggests the existence of certain differences from one species to another (Fig.4).

Table I. Summary of the Anova test unifactorial pattern of catalase activity from the hepatic tissue in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

Species	Count	Sum	Mean	Variance
Cyprinus carpio	5	84.15	16.83	0.1011
Aristichthys nobilis	5	76.67	15.33	0.2658
Carassius auratus gibelio	5	81.43	16.28	0.1936

Table II. Calculated and critical values of the factors of catalase activity from the hepatic tissue in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

Source of variation	SS	g. l.	$\overline{SS}$	Calculated F	p	Critical F
Internal	5.733	2	2.866	15.3402	0.0004	3.8852
External	2.242	12	0.186			
Total	7.976	14				

SS = squares sum, g. l. = degree of freedom, $\overline{SS}$ = mean squares sum
p = probability

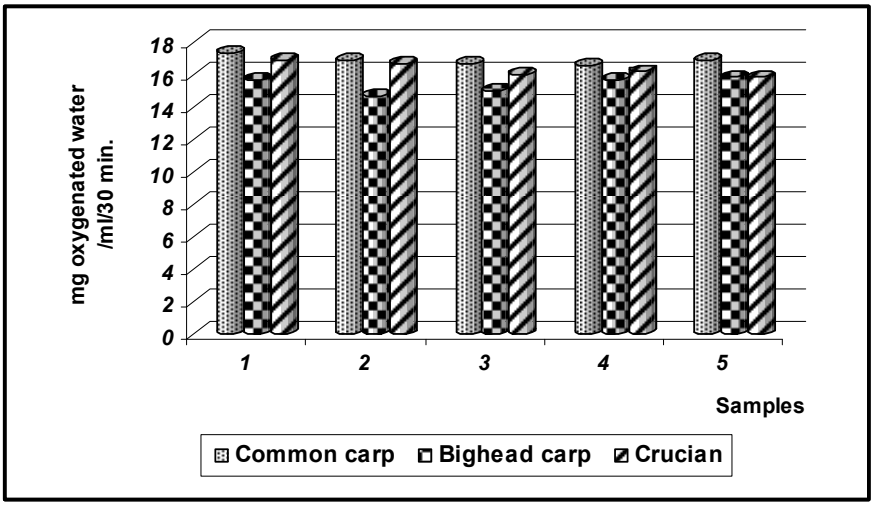

Fig.4. Comparative representation of hepatic catalase activity in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

Equally, the data on the muscular tissue were statistically processed (Tables III - IV), the observation being made that the calculated value of the factor is significantly higher than its critical value (110.6139 *versus* 3.8852), which supports the observation that, in the muscle, catalase records oscillating values from one species to another, representing 59.74% in common carp and 29.8% in bighead carp, respectively, comparatively with the values registered in crucian (Fig.5).

Tabel III. Summary of the Anova test unifactorial pattern of catalase activity from the muscular tissue in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

Species	Count	Sum	Mean	Variance
Cyprinus carpio	5	23.48	4.69	0.7353
Aristichthys nobilis	5	11.73	2.34	0.1936
Carassius auratus gibelio	5	39.27	7.85	0.1069

Tabel IV. Calculated and critical values of the factors of catalase activity from the muscular tissue in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

Source of variation	SS	g. l.	$\overline{SS}$	Calculated F	p	Critical F
Internal	76.389	2	38.194	110.6139	1.8552	3.8852
External	4.143	12	0.345			
Total	80.532	14				

SS = squares sum, g. l. = degree of freedom, $\overline{SS}$ = mean squares sum
p = probability

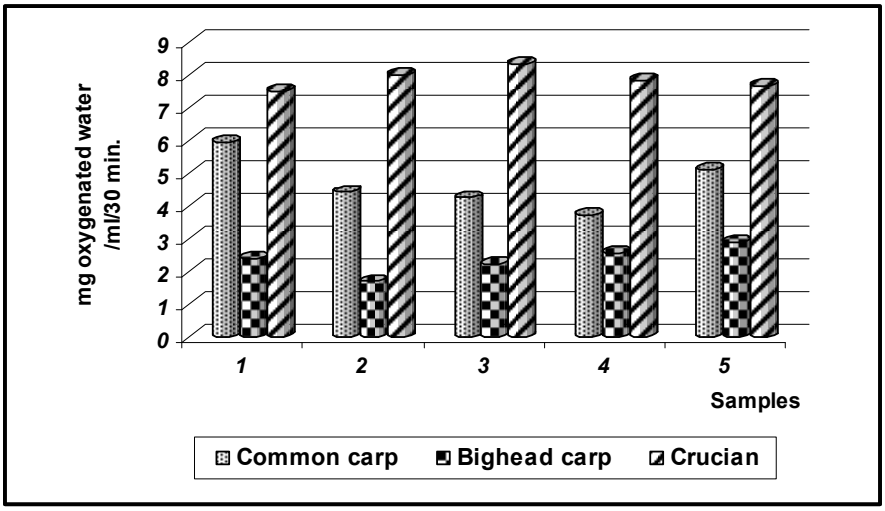

Fig.5. Comparative representation of muscular catalase activity in two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio*

CONCLUSIONS

A comparative analysis on the activity of catalase in the hepatic and muscular tissues of two summer-old *Cyprinus carpio*, *Aristichthys nobilis* and *Carassius auratus gibelio* representatives permits the conclusion that, on one side, significant difference are recorded, in the investigated tissues, as to their enzymatic activity - much higher values being found out, each time, in the liver - while, on the other, as to the different behavior of the enzyme from one species to another, the minimum activity being observed, in both tissues, in bighead carp.

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1) "Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology, Bd. Carol I, Nr. 20 A, 700506, Iaşi, România
*) ciornea@uaic.ro