

STUDIES ON THE ACTIVITY OF SOME PEPTID-HYDROLASES AT SILVER CARP, BIGHEAD CARP AND GRASS CARP

Elena Ciornea, Gabriela Dumitru

„Alexandru Ioan Cuza” University, Faculty of Biology, Iași, Romania
e-mail: ciornea@uaic.ro

Abstract

The digestion is a biochemical and mechanical complex process which takes place at digestive tube level, by way of which the ingested brute nourishment are transformed in substances more simple, easily to absorb. Within our study we've proposed to determinate the activity of pepsin and trypsin from the medium intestine's level at five representatives belonging to *Hypophthalmichthys molitrix* (silver carp), *Aristichthys nobilis* (bighead carp) and *Ctenopharyngodon idella* (grass carp) of two summer-old. The proteinasic activity was evaluated through the dosage of hydrolysis products of substratum under the action of these enzymes (hemoglobin for pepsin and casein in the case of trypsin) with the help of Folin-Ciocalteu reactive through colorimetric method. The analysis of obtained results underlines, on the one hand, major differences in what concerns the activity of the two enzymes implicated in the proteins metabolism, the medium value of pepsin's activity being, in general, more inferior comparatively with the trypsin's level activity at all three culture cyprinid species taken into study, and on the other hand exist differences between species, the grass carp presenting an activity more diminishing in the case of both peptid-hydrolases.

Key words: pepsin, trypsin, culture cyprinids

INTRODUCTION

The alive organisms represent the center of multiple biochemical reactions that issue with very big speeds in mild conditions of temperature pressure, all these being impossibly to realize if the alive organisms wouldn't dispose of a diversified list of biochemical compounds with biocatalizatory role, known as enzymes, the enzymatic activity being a trusty indicator of metabolic transformations in the supervised growth of fish [1].

It is known that, in the case of fish, the peptid-hydrolasic activity increases or decreases proportionally with the food proteins content, but also in function of the quality and quantity of available food, so taking place an adaptation of secretory function of intestinal tractus at the type of food [18].

MATERIALS AND METHOD

To obtain the samples were sacrificed each five exemplars belonging to each cyprinid species in part (*Hypophthalmichthys molitrix*, *Aristichthys nobilis* and

Ctenopharyngodon idella) of two summer-old, clearing out the intestinal content through scraping.

After the digestive tractus was unfolded along its whole length, followed the identification of medium portion, mojarig and analyzing well determined quantities of biological material, in obtained extracts determining the pepsin and trypsin activity, through the degradation productions dosage by colorimetrical method [2, 5, 7].

For each sample in part were realized each three parallel determination, all obtained results being processed statistically, on the base of mean values and standard deviation drawing subsequently confidence interval limits [11, 17, 19].

RESULTS AND DISCUSSION

A first objective of the present study was the underlining of the influence that exerts the incubation medium temperature and the enzyme concentration in medium on the peptid-hydrolasic activity.

The temperature is a factor that can influence decisively enzymatic catalyzed reactions through stability modification and enzyme affinity, respectively, of the split's speed of the enzyme-substratum complex. In the most of cases, the enzymes are biomolecules with a reduced thermal stability, and superior catalytic activity are registering in domains relatively narrows of temperature, especially in the case of animal origin enzymes [6].

As we can observe also from the graphical representations (Figs.1 - 2), both

the pepsinic activity as well as the trypsinic one from the digestive tube median part, present the maximum values in interval between 35 - 40°C, values comparable with those cited from the literature data, the enzyme being active on an enough interval temperature (20 - 50°C). In this sense, some authors [14] remark a highly stability and activity of proteolytical enzymes exposed for 30 min. at 50°C, while, after the maintenance for 30 min. at 60°C the enzymes would reduce the activity at half.

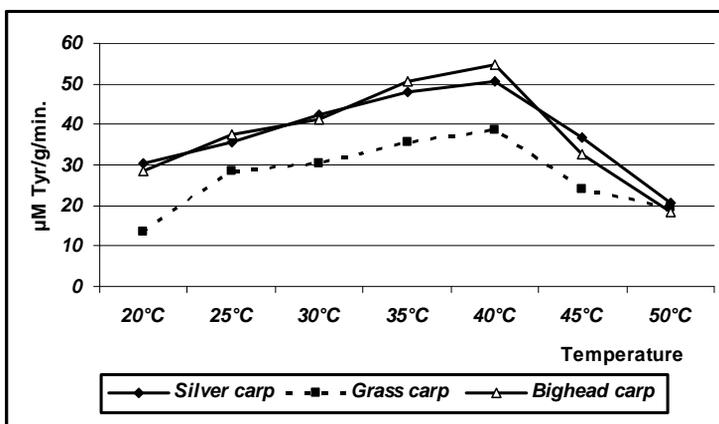


Fig.1. Influence of the temperature medium incubation on the activity of intestinal pepsin

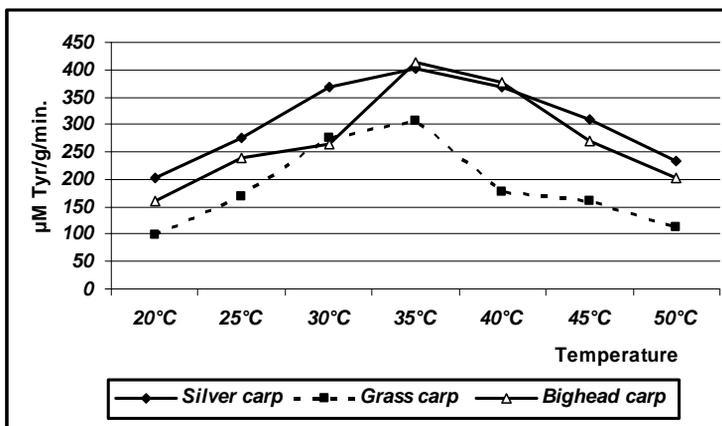


Fig.2. Influence of the temperature medium incubation on the activity of intestinal trypsin

Determining the influence of enzyme concentration on the proteolytical reaction speed, it was ascertained that the highest value was obtained in the case of initial

extract (the maximum concentration enzyme), decreasing then progressively once with the increase of the dilution degree (Figs. 3 - 4).

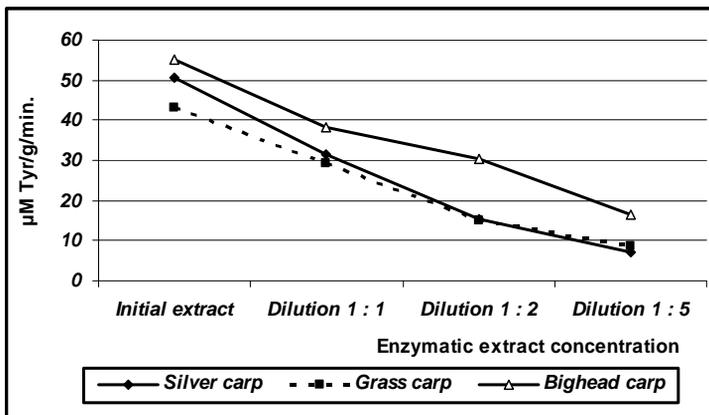


Fig.3. Influence of the concentration on the activity of intestinal pepsin

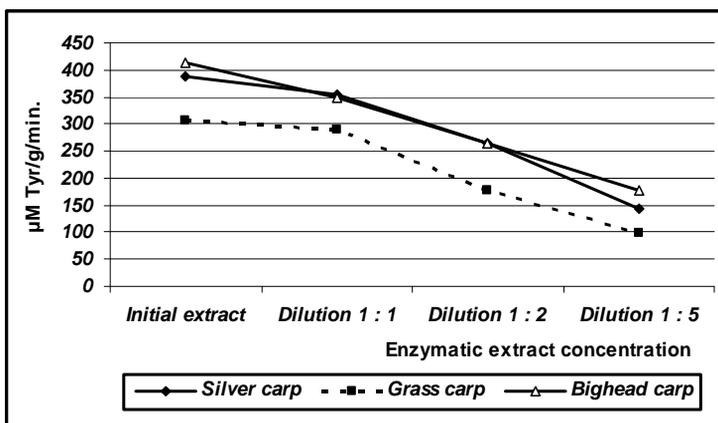


Fig.4. Influence of the concentration on the activity of intestinal trypsin

An another stage of our researches was the evaluation of the mean pepsinic and trypsinic activity, using an extract that contained the enzyme in the maximum concentration and incubating the samples at 35, respectively, 40°C, temperatures in which the enzymatic activity was maximum.

The literature data shows that the alimentary diet of silver carp is very various, not being a one rigorous phytoplanktonophagus and varying in the same time in function of the population density, the presence of other fish species and phytoplankton structure [4, 8, 15], existing, however, numerous authors that cite this species as being preponderantly phytoplanktonophagus [9, 13, 16].

In what concerns the bighead carp, the bigger part of the literature data underlines that this species is prevailing zooplanktonophagus, especially when the zooplanktonic biomass is abounding [4, 9, 10].

As it can be noticed from Figure 5, the pepsinic activity from the median intestine portion is extremely low, existing however a sort of difference from a species to another, with values a little higher in the case of *Aristichthys nobilis* exemplars (36.7 - 54.333 µM Tyr/g/min.), this thing could be explain, according to literature data, through the existence in the early development levels of this species, of a specialized portion, called "proto-stomach", but with a lack of enough details concerning the operation itself of this

„organ” or of his capacity to secrete hydrochloric acid or pepsin [3].

By comparison, in the silver carp and grass carp, the pepsin’s activity represents in average only 83.5%, respectively, 61.5%, knowing that, in vegetarian fish the

stomachal region is not clearly individualized, while the mucous membrane of the anterior intestine, between the oesophagus and the choledoc, may evidence or not a weak peptidic activity [12].

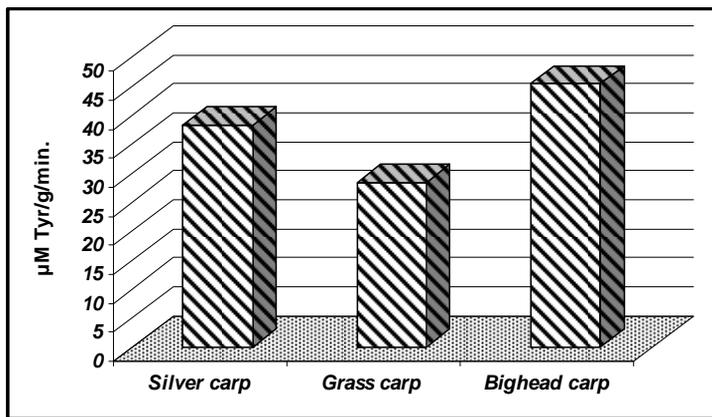


Fig.5. Intestinal pepsin activity in culture cyprinid species

On the base of the mean and standard deviation values were subsequently calculated the confidence intervals limits (upper and lower) of the pepsin’s activity from the digestive tube, using a critical value $t(\alpha, n-1)$, given by $\alpha=0.05$ (namely, a probability of 95%) and $n-1$ degrees of freedom (where n represents the number of values from each sample).

From Figure 6, it can be seen that the confidence intervals limits are a litter larger in the case of silver carp (21.034 - 31.832 µM Tyr/g/min.) and grass carp (35.986 - 51.747 µM Tyr/g/min.), the narrow interval highlighting at the bighead carp (53.058 - 55.608 µM Tyr/g/min.).

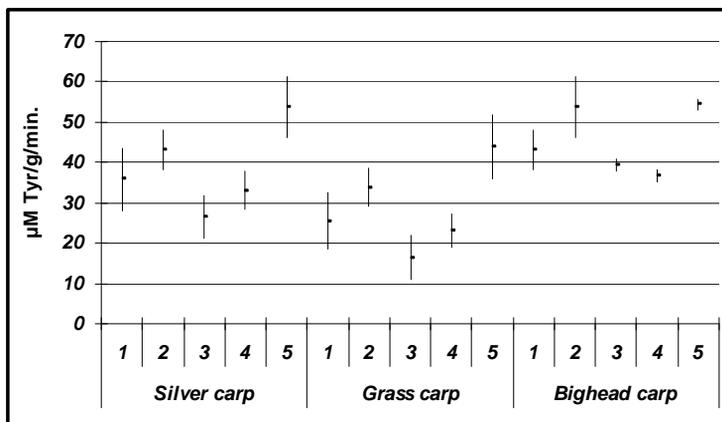


Fig.6. Confidence interval limits of intestinal pepsin activity in culture cyprinid species

Unlike the pepsin, the trypsin registers values significantly higher, the *Aristichthys nobilis* representative species, coming out top again probably, due to the fact that the bighead carp has an alimentary spectrum

rather various done mainly from zooplankton, the silver carp being preponderantly phyto-planktonophagus, and the grass carp macro-phytophagus (Fig. 7).

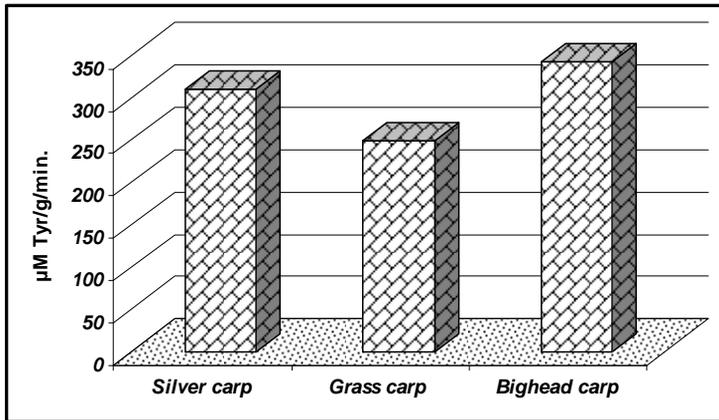


Fig.7. Intestinal trypsin activity in culture cyprinid species

The confidence interval limits of trypsin's activity (Fig. 8), traced in the case of effected parallel determinations, are more narrow comparatively with those evidenced for intestinal pepsin, with the mention that also

in this case, the smaller values were noticed in *Aristichthys nobilis* exemplars (319.851 - 326.348 µM Tyr/g/min., 371.669 - 385.864 µM Tyr/g/min.).

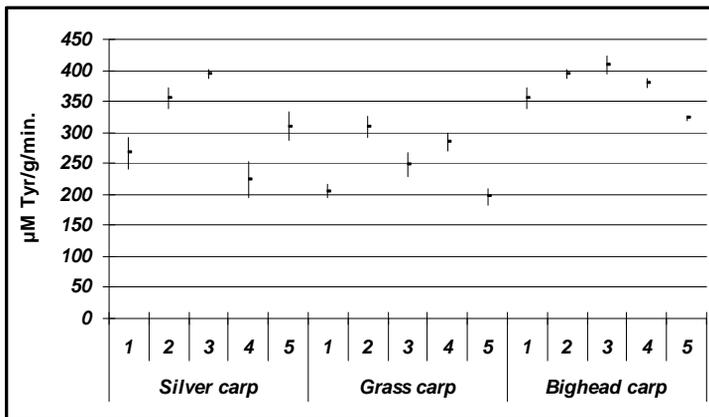


Fig.8. Confidence interval limits of intestinal trypsin activity in culture cyprinid species

CONCLUSIONS

After the determination of incubation medium temperature influence, but also of the enzymes concentration on the proteolytic reaction speed, it was ascertain that as well for pepsin as for trypsin, the maximum speed

of reaction, was obtained at the brute extract variant, this activity diminishing progressively with the dilution, maximum values registering in interval 35 - 40°C.

The comparative analyze of the two peptid-hydrolases activity evidences

remarkable differences between the activity of the two studied enzymes, the species - the second variable factor, influencing insignificantly the proteolytic activity.

REFERENCES

- [1] Arteni V.I.: Activitatea enzimelor - indicator al transformărilor metabolice în creșterea intensivă a peștilor, Piscicultura Moldovei, Lucr. Șt., Iași, 1990, 1: 357 - 364.
- [2] Arteni V.I., Tănase Elvira: Practicum de biochimie generală, Ed. Univ. „Alexandru Ioan Cuza”, Iași, 1981.
- [3] Bremer H.: Histological and histochemical studies on the detection of the esogaster in the agastric cyprinidae *Aristichthys nobilis* Rich. (Marble carp), Gegenbaurus Morphol. Jahrb., 1978, 124 (5): 727 - 735.
- [4] Burke J. S., Bayne D. R., Rea H.: Impact of silver carp and bighead carp on plankton communities of channel catfish ponds, Aquaculture, 1986, 55: 59 - 68.
- [5] Cojocaru D. C.: Enzimologie practică, Ed. Tehnpress, Iași, 2005.
- [6] Cojocaru D. C., Olteanu Zenovia, Ciornea Elena, Oprică Lăcrămioara, Cojocaru Sabina Ioana: Enzimologie generală, Ed. Tehnpress, Iași, 2007.
- [7] Cojocaru D. C., Toma O., Cojocaru Sabina Ioana, Ciornea Elena: Practicum de biochimia proteinelor și acizilor nucleici, Ed. Tehnpress, Iași, 2009.
- [8] Costa - Pierce B. A.: Review of the spawning requirements and feeding ecology of silver carp (*Hypophthalmichthys molitrix*) and reevaluation of its use in fisheries and aquaculture, Reviews in Aquatic Sciences, 1992, 6 (3 - 4): 257 - 273.
- [9] Cremer M. C., Smitherman R. O.: Food habits and growth of silver carp and bighead carp in cages and ponds, Aquaculture, 1980, 20: 57 - 64.
- [10] Dong S., Li D.: Comparative studies on the feeding selectivity of silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*, Journal of Fish Biology, 1994, 44: 621 - 626.
- [11] Gomoiu T. M., Skolka M.: Ecologie. Metodologii pentru studii ecologice, Ed. Univ. „Ovidius” Constanța, 2001.
- [12] Guillaume J., Kaushik S., Bergot P., Metailler R.: Nutrition et alimentation des poissons et crustacés, INRA, Paris, 1999.
- [13] Maheshwari U. K., Roy B., Bhattacharya S. K., Singh I., Yadavin A. K.: Observations on relative length of gut, gastronomic index and food spectrum of silver carp *Hypophthalmichthys molitrix*, Indian Journal of Ecology, 1992, 19: 112 - 114.
- [14] Pavlisko Andrea, Rial Analia, De Vecchi Silvia, Coppes Zulema: Properties of pepsin and trypsin isolated from the digestive tract of *Parona signata*, Journal of Food Biochemistry, 1997, 21 (3): 289 - 308.
- [15] Spătaru P., Gophen M.: Feeding behavior of silver carp *Hypophthalmichthys molitrix* Val. and its impact on the food web in Lake Kinneret, Israel, Hydrobiologia, 1985, 120: 53 - 61.
- [16] Spătaru P., Wohlfarth G. W., Hulata G.: Studies on the natural food of different fish species in intensively manured polyculture ponds, Aquaculture, 1983, 35: 283 - 298.
- [17] Varvara M., Zamfirescu Șt., Neacșu P.: Lucrări practice de ecologie, Ed. Univ. „Alexandru Ioan Cuza” Iași, 2001.
- [18] Vasile Gabriela, Cojocaru D., Misăilă C., Ciornea Elena: Study on the activity of some enzymes involved in protein metabolism of *Carassius auratus gibelio* Bloch., International Symposium Euro-Aliment, Ed. Academica, Galați, 2005, 222 - 225.
- [19] Zamfirescu Șt., Zamfirescu Oana: Elemente de statistică aplicate în ecologie, Ed. Univ. „Alexandru Ioan Cuza” Iași, 2008.