EXPERIMENTAL STUDIES ON THE α-AMYLASE ACTIVITY AND STRUCTURE OF THE MEDIAN SEGMENT OF THE DIGESTIVE TRACTUS IN *HYPOPHTHALMICHTHYS MOLITRIX* OF VARIOUS AGES

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Key words: α-amylase, digestive tractus, age, morphostructure, *Hypophthalmichthys molitrix* **Abstract**: The main objective of the present study was to determine the amylolytic activity in the median portion of the digestive tractus in individuals of *Hypophthalmichthys molitrix* (silver carp) occurring in the first and, respectively, fourth summer of growth, as well as to investigate some morphostructural aspects occurring at this level.

INTRODUCTION

Starting from the general truth that fish represents an aliment with a high dietetic and nutritive value, special attention has been recently paid, from the part of the specialists in the field, to the analysis of some of its biochemical and physiological parameters.

Amylases are largely occurring in nature, both in the vegetal and animal kingdom; up to now, only α -amylases have been identified in animals. In superior animals they are secreted by the pancreas and in some species by the salivary glands (in humans, monkey, guinea pig, squirrel, rat, mouse). Low amounts of α -amylase may be also traced in the saliva of herbivorous animals, while it is absent in the saliva of carnivora. In blood, muscles, liver and urine, negligible amounts of α -amylase should be also mentioned (COJOCARU, 1997).

In the case of fish, α -amylases are present along the whole length of the digestive tractus, once known that the relative activity of such enzymes is closely correlated with the nature of the alimentary regime (GHOSH *et al.*, 2005). In the predominantly herbaceous *Tylapia* genus, the activity of α -amylase is distributed along the whole length of the gastro-intestinal tract while, in the case of pearch (*Perca fluviatilis*), which is a carnivorous species, the pancreas is the only source of amylolytic activity (OPREA and GEORGESCU, 2000).

MATERIALS AND METHOD

The experiments involved taking over of samples from the median segment of the digestive tube from 10 individuals of silver carp (*Hypophthalmichthys molitrix*), for each age in part.

The activity of α -amylase has been determined by the Métais - Bieth method; for each individual in part, the intestinal content has been removed through scraping, three parallel dosings being performed each time, the data provided representing the average values of these repetitions (COJOCARU, 2005). In a final step, the data obtained have been processed statistically, on calculating the standard error of the average value, the standard deviation, variation and precision coefficient of the average, as well as the (lower and upper) limits of the confidence intervals between which the intestinal amylasic activity of the silver carp is oscillating (VARVARA *et al.*, 2001).

As to the structural aspects of the median part of the digestive tube, the usual methods of histological techniques have been applied (MUREŞAN *et al.*, 1974). Consequently, three individuals were dissected for each age in part, the digestive tract being unrolled along its whole length, after which rectangular fragments were taken over.

RESULTS AND DISCUSSION

A first stage of the present investigations was devoted to the analysis of some morphostructural aspects from the median portion of the digestive tube, in *Hypophthalmichthys molitrix* individuals of various ages (one and, respectively, four summers), for putting into evidence some possible intraspecific differences induced by age, the more so that the researches devoted to such problems are quite scarce (BREMER, 1978; TREVISAN, 1979; ATHIKESAVAN *at al.*, 2006).

In one summer-old individuals of *Hypophthalmichthys molitrix*, the height of the mucous tunic of the medium intestine is of 375 μ m, the epithelium height being of 100 μ m; instead, the muscular tunic is thin, with a thickness of only 187.5 μ m (Fig. 1).

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Fig.1. Cross-section through the medium intestine of one summer-old *Hypophthalmichthys molitrix* (assembly; 100x) (original photo)

In four summer-old individuals, the part following the posterior region of the so-called "stomach" (the medium intestine) has a similar structure to that of the thin intestine, its mucous tunic evidencing almost cylindrical plaits, a very tall epithelium, rich in calix-shaped cells, both the epithelium and the corione of the mucous tunic evidencing only few lymphoidal infiltrations. In the medium intestine of the four summer-old silver carp, the height of both the mucous and submucous tunic is of 500 μ m, while the muscular tunic is slightly thinner, with a thickness of 225 μ m, *versus* 187.5 μ m, the value measured in one summer-old fry (Figs. 2 - 3).



Fig.2. Cross-section through the medium intestine of four summer-old *Hypophthalmichthys molitrix* (assembly; 100x) (original photo)



Fig.3. Cross-section through the medium intestine of four summer-old *Hypophthalmichthys molitrix* (detail: epithelium with calix-shaped cells; 400x) (original photo)

As to the micrometric aspects of the thickness of the mucous and muscular tunic, higher values are recorded in four summer-old adults, which eat preponderantly phytoplankton, once known that, in herbivorous organisms, the musculature of the digestive tube is much more developed than in those consuming food of animal origin (Fig. 4).





The digestive tractus of fish contains a large spectrum of hydrolases, which facilitates scission of the various nutrients and integration of their decomposition products into the cellular and tissular metabolism. In various fish species, these enzymes are differentiated, firstly by the level of their catalytic activity (VASILE and CIORNEA, 2006).

The food entering the gastro-intestinal tractus is subjected to some complex biochemical transformations, realized by the specific enzymes of the digestive organs. In the digestive tractus

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of cyprinids, there prevail the enzymes involved in the hydrolysis of polyglucides, among which a special part is played by α -amylase (VASILE *et al.*, 2006 a; 2006 b; 2006 c).

Study of the amylolytic activity in the digestive tube of one summer-old *Hypophthalmichthys molitrix* representatives shows extremely uniform values over the 2.286 - 2.547 mg starch/ml x 30 min. interval (Table I, Fig. 5).

Samples	Average activity (mg starch/ ml x 30 min.)	$s\bar{x}$	S (σ)	CV%	m%	LS	LI
1.	2.404	0.027	0.048	2.002	1.156	2.524	2.284
2.	2.561	0.017	0.030	1.181	0.682	2.636	2.485
3.	2.547	0.015	0.027	1.071	0.618	2.617	2.481
4.	2.456	0.013	0.023	0.962	0.555	2.515	2.397
5.	2.332	0.012	0.020	0.894	0.516	2.384	2.280
6.	2.335	0.019	0.033	1.429	0.825	2.418	2.252
7.	2.286	0.005	0.008	0.391	0.225	2.308	2.264
8.	2.417	0.010	0.018	0.775	0.436	2.463	2.372
9.	2.453	0.027	0.048	1.967	1.135	2.573	2.333
10.	2.367	0.006	0.012	0.511	0.295	2.397	2.337

Table I. α-Amylase activity in the median part of the digestive tube in one summer-old *Hypophthalmichthys molitrix*

 $S \overline{x}$ = standard error, $S (\sigma)$ = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = upper limit of confidence interval, LI = lower limit of confidence interval



Fig.5. α-Amylase activity in the median part of the digestive tube in one summer-old *Hypophthalmichthys molitrix*

Analysis of the experimental results, listed in Table II, obtained for the median segment of the digestive tube, in four summer-old individuals of silver carp has shown that, on one side,

the enzymatic activity is slightly higher than in the one summer-old ones and, on the other, it is quite homogenous, varying between 2.267 - 2.667 mg starch/ml x 30 min. (Figs. 6 - 7).

Samples	Average activity (mg starch/ ml x 30 min.)	$s\bar{x}$	S (σ)	CV%	m%	LS	LI
1.	2.656	0.004	0.007	0.288	0.166	2.675	2.637
2.	2.511	0.004	0.007	0.305	0.176	2.530	2.492
3.	2.396	0.004	0.007	0.329	0.190	2.415	2.376
4.	2.448	0.001	0.002	0.092	0.053	2.453	2.443
5.	2.433	0.004	0.007	0.316	0.182	2.452	2.414
6.	2.438	0.006	0.011	0.473	0.273	2.467	2.409
7.	2.267	0.001	0.002	0.097	0.056	2.273	2.262
8.	2.306	0.005	0.010	0.449	0.259	2.332	2.281
9.	2.667	0.008	0.014	0.529	0.305	2.702	2.632
10.	2.473	0.005	0.009	0.373	0.215	2.496	2.450

Table II. α-Amylase activity in the median part of the digestive tube in four summer-old *Hypophthalmichthys molitrix*

 $S \overline{x}$ = standard error, $S (\sigma)$ = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = upper limit of confidence interval, LI = lower limit of confidence interval



Fig. 6. α-Amylase activity in the median part of the digestive tube in four summer-old *Hypophthalmichthys molitrix*

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Fig.7. Graphical comparative representation of α -amylase activity from the median part of the digestive tube in silver carp of various ages

CONCLUSIONS

Similarly with other fish species, the morphology and structure of the digestive tube in *Hypophthalmichthys molitrix* individuals are adapted mainly to the type of consumed food, as well as to their age.

The data obtained on the amylolytic activity at the level of the median portion of the digestive tube indicate the existence of some homogeneity among the representatives of the same age, as well as higher values in four summer-old individuals.

REFERENCES

Athikesavan, S., Vincent, S., Ambrose, T., Velmurugan, B., 2006. J. Environ. Biol., 27 (2): 391 - 395.

Bremer, H., 1978. Gegenbaurus Morphol. Jahrb., 124 (5): 727 - 735.

Cojocaru, D. C., 1997. Enzimologie, Ed. Gama, Iași.

Cojocaru, D.C., 2005. Enzimologie practică, Ed. Tehnopress, Iași.

Cojocaru, D.C., Olteanu, Zenovia, Ciornea, Elena, Oprică, Lăcrămioara, Cojocaru, Sabina - Ioana, 2007. Enzimologie generală, Ed. Tehnopress, Iași.

Ghosh, K., Sen, S. K., Ray, A. K., 2005. Acta Ichthyologica et Piscatoria, 35 (2): 111 - 117.

Mureșan, E., Gaboreanu, M., Bogdan, A. T., Baba, A. I., 1974. Tehnici de histologie normală și patologică, Ed. Ceres, București.

Oprea, L., Georgescu, Rodica, 2000. Nutriția și alimentația peștilor, Ed. Tehnică, București.

Trevisan, P., 1979. Anat. Anz., 145 (3): 237 - 248.

Varvara, M., Zamfirescu, Ş., Neacşu, P., 2001. Lucrări practice de ecologie, Ed. Univ. "Alexandru Ioan Cuza" Iași.

Vasile, Gabriela, Ciornea, Elena, 2006. Studii și Comunicări, Nr. 21, Complexul Muzeal de Științele Naturii "Ioan Borcea" Bacău, 411 - 415.

Vasile, Gabriela, Ciornea, Elena, Cojocaru, D. C., 2006 a. Lucr. St., Seria Zootehnie, Iași, 49: 1006 - 1013.

Vasile, Gabriela, Ciornea, Elena, Cojocaru, D. C., 2006 b. An. Şt. Univ. "Alexandru Ioan Cuza" Iași, s. II a. Genetică și Biologie Moleculară, Tom VII, Fasc. 1, 79 - 82.

Vasile, Gabriela, Ciornea, Elena, Cojocaru, D. C., 2006 c. An. Şt. Univ. "Alexandru Ioan Cuza" Iași, s. II a Genetică și Biologie Moleculară, Tom VII, Fasc. 1, 75 - 78.

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