

## ON THE STRUCTURE OF THE MEDIAN SEGMENT OF THE DIGESTIVE TUBE AND ON THE ACTIVITY OF ALPHA-AMYLASE AT *ARISTICHTHYS NOBILIS*, IN VARIOUS STAGES OF DEVELOPMENT

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**Key words:**  $\alpha$ -amylase, digestive tube, *Aristichthys nobilis*

**Abstract:** The investigations have been dedicated to the structure of the median segment of the digestive tube, on *Aristichthys nobilis* individuals of various ages (*i.e.*, one summer- and, respectively, four summer-old), correlated, on one side, with the main feeding regime and, on the other, with the amylasic activity present in this part of the digestive tract. As to the structural aspects, higher values of the thickness of the mucous tunic have been recorded in the four summer-old bighead carp individuals, to be possibly associated with the high values of the  $\alpha$ -amylase activity in this segment of the digestive tube.

### INTRODUCTION

In the case of fish,  $\alpha$ -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  $\alpha$ -amylase is distributed along the whole length of the gastrointestinal tract while, in the case of perch (*Perca fluviatilis*), which is a carnivorous species, the pancreas is the only source of amylolytic activity (OPREA and GEORGESCU, 2000).

Most authors suggest that, in flesh - eating fish, the level of the amylolytic activity is lower than in the omnivorous ones, which is the result of a reduced secretion of amylase (KITAMIDADO and TACHINO, 1960; SHIMEMO *et al.*, 1977; PĂLTĂNEA and GEORGESCU, 1995). More than that, the amylasic activity differs with the structure of the digestive tube, development stages and temperature of the environment (KITAMIDADO and TACHINO, 1960; VASILE *et al.*, 2006).

In the case of the one summer-old carp, the amylasic activity was seen to begin in the esophagus, so that the enzyme - substrate contact is quite short and thus the secretion of amylase - quite reduced (PĂLTĂNEA and GEORGESCU, 1991).

The present paper discusses the results of the activity of  $\alpha$ -amylase from the median segment of the digestive tractus in representatives of the *Aristichthys nobilis* species (bighead carp), occurring in different stages of development (one and, respectively, four summers), in parallels with some structural aspects of this part of the digestive tube.

### MATERIALS AND METHOD

The experiments involved taking over of samples from the median segment of the digestive tube from 10 individuals of bighead carp (*Aristichthys nobilis*), from the Research and Development Station for Aquaculture and Aquatic Ecology of Jassy, for each stage of development in part.

The activity of  $\alpha$ -amylase has been determined by the Métais - Bieth method, the results being expressed in mg of starch/ml x 30 min. (COJOCARU, 2005). 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. In a final step, the data obtained have been processed statistically on calculating the standard error of the average value, the standard deviation, the variation coefficient of the average, as well as the limits of the confidence intervals between which the intestinal amylasic activity of the bighead 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 (DIACONIȚĂ *et al.*, 1953; HUMASON, 1962; MARTOJA and MARTOJA, 1967; MUREȘAN *et al.*, 1974). Consequently, three individuals were dissected for each development stage in part, the digestive tract being unrolled along its whole length, after which rectangular fragments were taken over.

### RESULTS AND DISCUSSION

A first objective of the present study was to determine the activity of  $\alpha$ -amylase in the median part of the digestive tube, once known that the representatives of the *Aristichthys nobilis* species have no stomach.

Analysis of the experimental results obtained on one summer-old individuals (Table I) evidenced that the average values of the amylasic activity vary within quite narrow values, namely between 2.01 - 2.259 mg starch/ml x 30 min.

Table I.  $\alpha$ -Amylase activity in the median part of the digestive tube in one summer-old *Aristichthys nobilis*

Samples	Individual activity (mg starch/ml x 30 min.)	Average activity (mg starch/ml x 30 min.)	Standard error	Standard deviation	CV%
1	2.002	2.010	0.004	0.007	0.375
	2.011				
	2.017				
2	2.125	2.154	0.021	0.036	1.694
	2.195				
	2.142				
3	2.053	2.077	0.012	0.021	1.029
	2.091				
	2.089				
4	2.155	2.152	0.013	0.024	1.120
	2.175				
	2.127				
5	2.248	2.259	0.008	0.014	0.620
	2.255				
	2.275				
6	2.111	2.147	0.019	0.034	1.594
	2.152				
	2.179				
7	2.188	2.170	0.023	0.041	1.892
	2.199				
	2.123				
8	2.005	2.058	0.027	0.046	2.277
	2.075				
	2.094				
9	2.154	2.165	0.008	0.013	0.640
	2.181				
	2.162				
10	2.233	2.261	0.018	0.032	1.418
	2.254				
	2.296				

For a complete image of the enzymatic activity, the average values obtained have been plotted graphically. As shown in Figure 1, the intestinal  $\alpha$ -amylase in one summer-old representatives follows the same curve, for all samples under investigation.

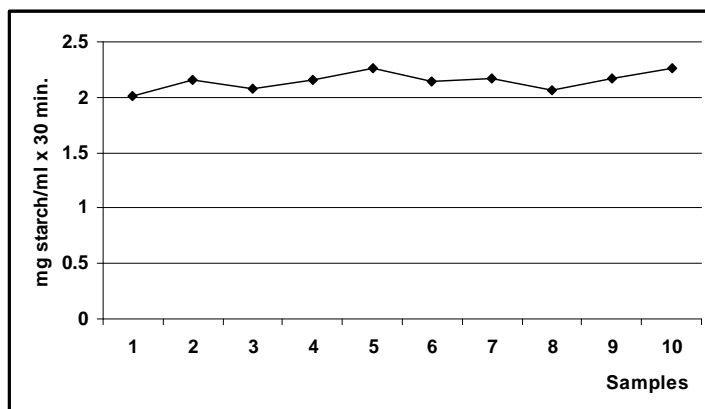


Fig.1. Graphical representation of the  $\alpha$ -amylase activity from the median part of the digestive tube in one summer-old *Aristichthys nobilis*

However, in four summer-old individuals, the activity of  $\alpha$ -amylase record higher values, ranging between 2.155 mg starch/ml x 30 min. (no 5) and, respectively, 2.823 mg starch/ml x 30 min. (no 9) (Table II).

Table II.  $\alpha$ -Amylase activity in the median part of the digestive tube in four summer-old *Aristichthys nobilis*

Samples	Individual activity (mg starch/ml x 30 min.)	Average activity (mg starch/ml x 30 min.)	Standard error	Standard deviation	CV%
1	2.519	2.518	0.0009	0.001	0.067
	2.520				
	2.516				
2	2.481	2.482	0.002	0.003	0.156
	2.479				
	2.486				
3	2.204	2.208	0.002	0.003	0.160
	2.209				
	2.210				
4	2.573	2.575	0.002	0.004	0.160
	2.579				
	2.572				
5	2.155	2.155	0.003	0.005	0.264
	2.161				
	2.149				
6	2.499	2.490	0.004	0.007	0.310
	2.485				
	2.486				
7	2.529	2.529	0.001	0.002	0.114

	2.526				
	2.532				
8	2.694	2.692	0.001	0.002	0.088
	2.693				
	2.689				
9	2.826	2.823	0.005	0.009	0.347
	2.812				
	2.831				
10	2.457	2.455	0.004	0.007	0.306
	2.461				
	2.446				

Figure 2 illustrates the fact that, on one side, the enzymatic activity is higher than that of the one summer-old individuals while, on the other, it oscillates between somewhat larger limits.

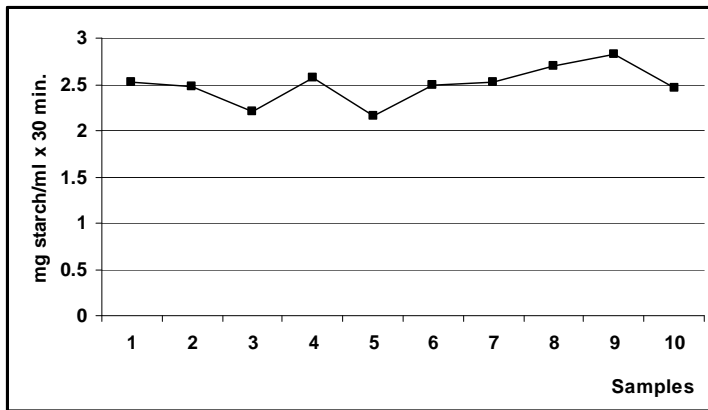


Fig.2. Graphical representation of the  $\alpha$ -amylase activity from the median part of the digestive tube in four summer-old *Aristichthys nobilis*

On the basis of the average values and of standard deviation, the (upper and lower) limits of the confidence intervals of  $\alpha$ -amylase activity have been subsequently calculated as a function of a critical value  $t(\alpha, n-1)$ , given by  $\alpha = 0.05$  (i.e., a probability ratio of 95%), and  $n$  degrees of freedom (where  $n$  represents the number of values within each sample), that is  $t(0.05, 9) = 2.262$  (VARVARA *et al.*, 2001). Consequently, as evidenced by the graphical representation, too, in one summer-old individuals, the limits of the confidence intervals of the intestinal  $\alpha$ -amylase activity are larger than those recorded in four summer-old adults (Figs. 3 - 4).

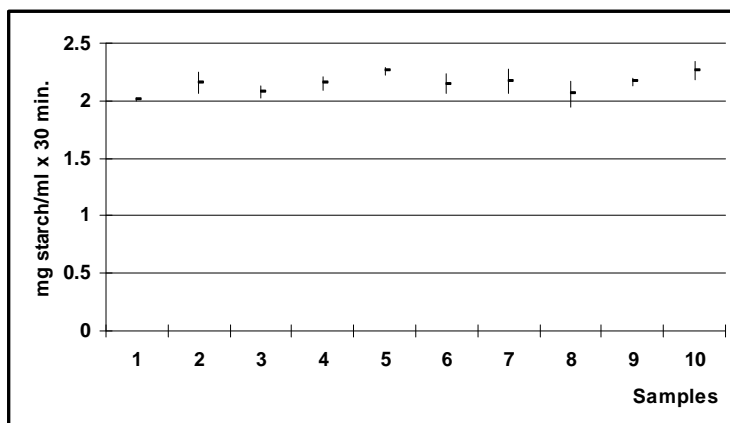


Fig.3. Limits of the confidence intervals of the intestinal  $\alpha$ -amylase activity in one summer-old *Aristichthys nobilis*

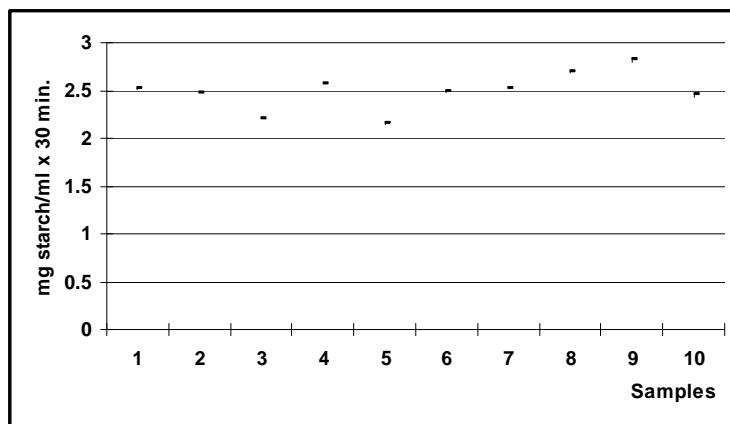


Fig.4. Limits of the confidence intervals of the intestinal  $\alpha$ -amylase activity in four summer-old *Aristichthys nobilis*

In a following step, the average value of the enzymatic activity has been calculated for all individuals subjected to investigation, for each category of age in part. Thus, the comparative graphical representation, on ages, of the average values of the  $\alpha$ -amylase activity in the median part of the digestive tube showed that, in one summer-old *Aristichthys nobilis* representatives, the enzyme exhibits an average value of 2.145 while, in the four summer-old ones, it attains a threshold of 2.492 mg starch/ml x 30 min. In the former case, the medium  $\alpha$ -amylase represents 96.91% of the enzymatic activity manifested in the latter one (Fig.5).

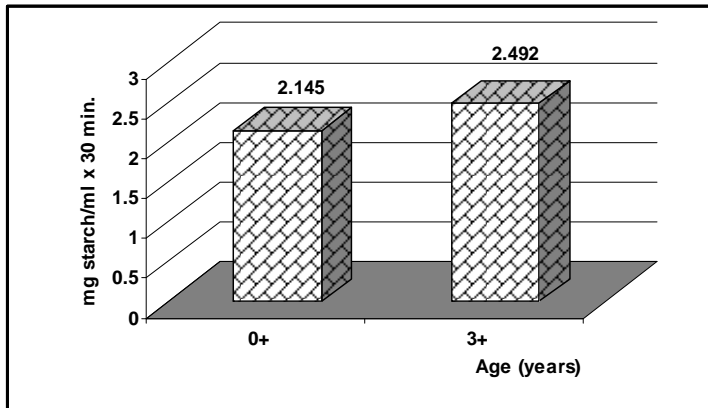


Fig.5. Comparative representation of intestinal  $\alpha$ -amylase activity in *Aristichthys nobilis* in different stages of development

A second objective involved the study of some structural aspects of the median part from the digestive tract for each development stage under analysis in part.

In the one summer - old bighead carp individuals, in the area of the median intestine, the mucous tunic forms either conical or bifurcated folds, with a high epithelium, the caliciformed cells at this level being weakly represented; the corione is flax and weakly impregnated with lymphocytes (Fig.6). The height of the mucous membrane in this segment of the digestive tract is of 250  $\mu$ m, while the muscular tunic is 137.5  $\mu$ m thick.

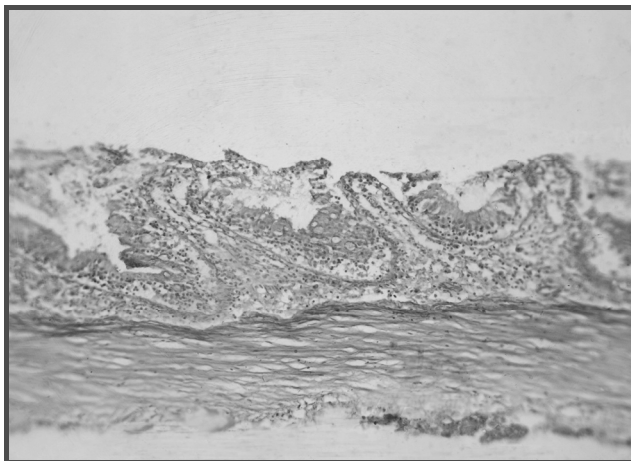


Fig.6. Cross - section through the median intestine of one summer-old *Aristichthys nobilis* (assembly; 200x) (original photo)

In four summer-old individuals, the median intestinal segment evidences a well - developed mucous membrane, with plaits of various shapes, some of them high, cylindrical, others amply trapezoidal, covered by a prismatic epithelium with a striated plateau, very rich in caliciformed cells in full activity. Both the corione and the epithelium show intense

lymphocytary infiltrations, the trapezoidal folds well evidencing very large, some of them 262.5  $\mu\text{m}$  in size, lymphatic follicles (Fig. 7).

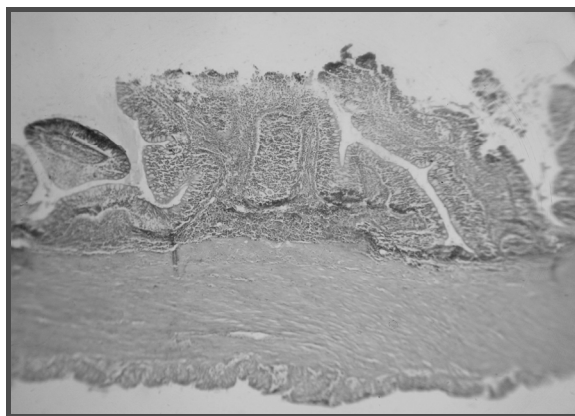


Fig. 7. Cross - section through the median intestine of four summer-old *Aristichthys nobilis* (assembly; 100x) (original photo)

A thorough analysis of the median segment of the digestive tube evidences an intense erosion of the mucous membrane's epithelium, concomitantly with a migration of the lymphocytes towards the intestinal lumen (Fig.8). On the whole, the thickness of the mucous membrane is of 625  $\mu\text{m}$ , the muscular one being also relatively well - developed, with a thickness value of 375  $\mu\text{m}$ .

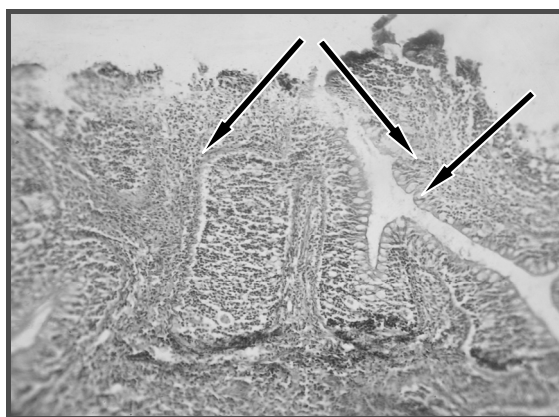


Fig. 8. Cross - section through the median intestine of four summer-old *Aristichthys nobilis* (detail: erosion of mucous and migration of the lymphocytes to the intestinal lumen; 200x) (original photo)

The higher thickness values of the mucous tunic in four-summer old representatives of *Aristichthys nobilis* might be associated with the higher values of  $\alpha$ -amylase in this segment of

the digestive tube (2.492 mg starch/ml x 30 min.), which attests the fact that, in the absence of the zooplankton, this species may consume, instead, phytoplankton.

### CONCLUSIONS

Analysis of the experimental results, attained in the study of the  $\alpha$ -amylase activity from the median segment of the digestive tube of *Aristichthys nobilis* individuals occurring in various stages of development - as correlated with the structural aspects from this level - permitted drawing of the following general conclusions:

The activity of  $\alpha$ -amylase in the median part of the digestive tube in one summer-old *Aristichthys nobilis* individuals ranges between 2.01 - 2.259 mg starch/ml x 30 min while, in the four summer-old ones, the enzymatic activity is higher (2.155 - 2.823 mg starch/ml x 30 min.), oscillating between somehow larger limits.

In one summer old bighead carp, the limits of the confidence intervals are larger than those recorded in four summer-old ones.

As to the microscopic structure of the median segment of the digestive tract, and also as to its thickness (in  $\mu\text{m}$ ), the observation to be made is that the aspect of the mucous and, respectively, of the muscular tunic, gets modified with the age.

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