ON THE ACTIVITY OF SOME INTESTINAL ENZYMES IN THE *CTENOPHARYNGODON IDELLA* SPECIES

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Key words: alanine-aminotransferase, aspartate-aminotransferase, pepsin, trypsin, digestive tube, grass carp
Abstract: The present paper systematizes the results attained on the activity of some transferases and proteases from the median part of the digestive tube, in three summer-old grass carp from the Piscicultural Farm of Ţigănaşi - Iaşi district, the data obtained being statistically processed for each parameter in part. The values recorded support the idea that the digestive tube of *Ctenopharyngodon idella* might be regarded as a potential source of proteolytic and aminotransferasic enzymes.

INTRODUCTION

A correct fish feeding with food containing all necessary nutritive substances, in the amounts required by the organism, for assuring maximum growth, without affecting its general physiological condition, represents one of the essential conditions of a sound growth, under profitable economic conditions.

Digestion is a complex biochemical and mechanical process occurring at the level of the digestive tube, during which the ingested raw aliments are transformed into simpler, easily assimilable substances.

Once swallowed, the food goes - without significant modifications - towards the esophagus. In the mouth of fish, the food suffers no chemical transformation once the salivary glands are absent, chemical digestion beginning only in the intestine (in the case of species without stomach), that is the nutritive substances are completely digested, prior to their absorption (OPREA and GEORGESCU, 2000).

The paper analyzes the activity of some intestinal enzymes, starting from the idea that the digestive tube of cyprinids might represent a possible source of proteolytic and transaminasic enzymes.

MATERIALS AND METHOD

The investigations were performed on three summer-old representatives of the *Ctenopharyngodon idella* species, from the Piscicultural Farm of Ţigănaşi - Iaşi district. Under laboratory conditions, fresh tissue samples have been taken over from the living fish while, after its death, fragments were cut from the median part of the digestive tube (between the esophagus and the duodenum) - from which the nutritive rests had been previously eliminated.

The alanine- and aspartate-aminotransferase activity was determined by the colorimetric method, with 2, 4 - dinitrophenylhydrazyne, while that of pepsin and trypsin - with the Folin - Ciocâlteu reactive, on using caseine and denaturated hemoglobin, respectively, as a substrate (ARTENIE and TĂNASE, 1981; COJOCARU, 2008).

For each parameter and individual in part, parallel determinations have been performed, in view of subsequent statistical processing, involving calculation of the mean standard error and deviation, variance, confidence level, superior and inferior limit, as well as of the variation and precision coefficient of the mean value (ZAMFIRESCU and ZAMFIRESCU, 2008).

RESULTS AND DISCUSSION

Ctenopharyngodon idella, also known as the grass carp or Cteno, performs its active feeding at 20°C, the digestive tube being 2 - 3.5 times longer than the body. Initially, the fry consumes phyto- and zooplankton, after which it gradually passes to the macrophytophagous nutrition. As this species consumes submerse and - especially - emerse vegetation, it came to be called “living mower”. When the aquatic plants are absent, the grass carp consumes Spanish trefoil, clover, maize etc., possibly “attacking” the additional food of the common carp (*Cyprinus carpio*) (GROZEA and BURA, 2002). The grass carp preferably consumes *Potamogeton angustifolius, Ceratophyllum demersum, Ceratophyllum submersum, Elodea canadensis, Lemna sp., Phragmites communis, Phararis arundinacea, Scirpus lacustris, Carex vulpina, Typha sp*., out of the existing aquatic vegetation, and *Trifolium repens, Medicago sativa, Sailix sp.,* leaves of

acacia, vetch as green mass, distributed in certain places of the piscicultural basin, respectively, out of the terrestrial plants (MACOVEI, 2008).

The economic interest manifested for the extended growth of such fish is justified by several reasons: first, it consumes and up grades the macrophyte vegetal biomass, which other fish species do not use; it combats the aquatic vegetation without either technical means or fuel consumption; it contributes to improving the living conditions for the other fish species grown, together with it, in polyculture, especially by improving the oxygen regime and by increasing the biogenic substances, as well as the planktonic and bentonic biomass.

At worldwide level, *Ctenopharyngodon idella* is grown in numerous countries (China, Austria, Germany, Taiwan, Malaysia, Hong Kong, Sweden, Canada, USA, France, Romania, Israel, Egypt, Finland, Great Britain), which is another proof of its economic value (BUD *et al*., 2004).

A first objective of the present study was to determine the activity of alanine-aminotransferase, an enzyme also known as glutamate-piruvate-transaminase (GPT or TGP), which catalyzes the conversion reaction of alanine and α-ketoglutarate into piruvate and glutamate (COJOCARU, 1997).

The results obtained show that the activity of alanine-aminotransferase varies between 18.9 and 24.4 conventional extinction units, the highest coefficient of mean variation - of 1.224 - being registered in individual number two. The data listed in Table I indicate narrow values for all statistical indices.

Table I. The values of the activity of intestinal alanine-aminotransferase and of the main statistical indices in three summer-old *Ctenopharyngodon idella*

Samples	Individual activity *(CEU)	Mean *(CEU)	Standard error	Standard deviation	Variance	Confidence level (95%)	VC%	m%
1	18.9	18.9	0.115	0.2	0.04	0.496	1.058	0.61
	18.7							
	19.1							
2	21.5	21.6	0.152	0.264	0.07	0.657	1.224	0.707
	21.4							
	21.9							
3	22.8	22.7	0.088	0.152	0.023	0.379	0.67	0.387
	22.6							
	22.9							
4	21.2	21.3	0.145	0.251	0.063	0.625	1.185	0.684
	21							
	21.5							
5	24.5	24.4	0.13	0.225	0.05	0.56	0.92	0.531
	24.25							
	24.7							

*(CEU) = conventional extinction units, VC% = mean variation coefficient, m% = mean precision coefficient

The values of mean and standard deviation permitted the subsequent calculation of the (inferior and superior) limits of the confidence intervals characterizing the activity of alanine-aminotransferase in the digestive tube, on using a critical value $t(\alpha, n-1)$, given by $\alpha = 0.05$ (*i.e*., a 95% probability) and $n-1$ degrees of freedom (where n represents the number of values from each sample), *i.e*. $t(0.05, 4) = 2.132$.

As graphically illustrated in Figure 1, the limits of the confidence intervals are extremely narrow for all samples under investigation, the smallest interval varying between 22.61 and 22.91 conventional extinction units.

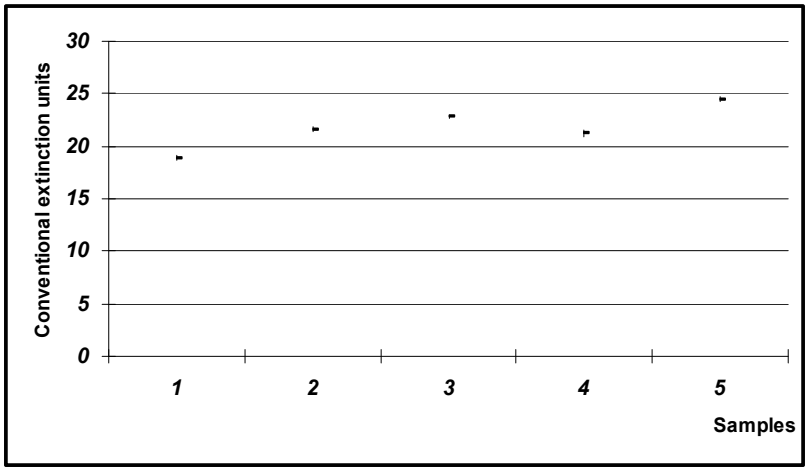

Fig.1. Confidence intervals of the alanine-aminotransferase activity in three summer-old *Ctenopharyngodon idella*

Aspartate-aminotransferase, also known as glutamate-oxaloacetate-transaminase (GOT or TGO), catalyzes the transamination reaction between aspartate and α-ketoglutarate, with formation of glutamic acid and oxalyl-acetic acid.

Table II provides an overall image on the activity of this intestinal aminotransferase, somehow lower values, comparatively with alanine-aminotransferase (representing only 72.81% of its activity) being observed, on one hand while, on the other, somehow more ample variations (oscillating between 12.9 and 18.03 conventional extinction units) were recorded.

Table II. The values of the activity of intestinal aspartate-aminotransferase and of the main statistical indices in three summer-old *Ctenopharyngodon idella*

Samples	Individual activity *(CEU)	Mean *(CEU)	Standard error	Standard deviation	Variance	Confidence level (95%)	VC%	m%
1	14.3	14.3	0.115	0.2	0.04	0.496	1.398	0.807
	14.1							
	14.5							
2	18	18.03	0.317	0.55	0.3	1.368	3.054	1.763
	17.5							
	18.6							
3	12.9	12.9	0.145	0.251	0.06	0.625	1.945	1.123
	12.7							
	13.2							
4	17.9	17.9	0.115	0.2	0.04	0.496	1.117	0.645
	17.7							
	18.1							
5	16.2	16.1	0.158	0.275	0.07	0.684	1.701	0.982
	15.9							
	16.45							

*(CEU) = conventional extinction units, VC% = mean variation coefficient, m% = mean precision coefficient

On analyzing the confidence intervals of the aspartate-aminotransferase activity in the digestive tube of *Ctenopharyngodon idella* representatives, mention should be made of the fact that they are somehow larger, the largest one being registered in individual number two (17.48 - 18.58 conventional extinction units).

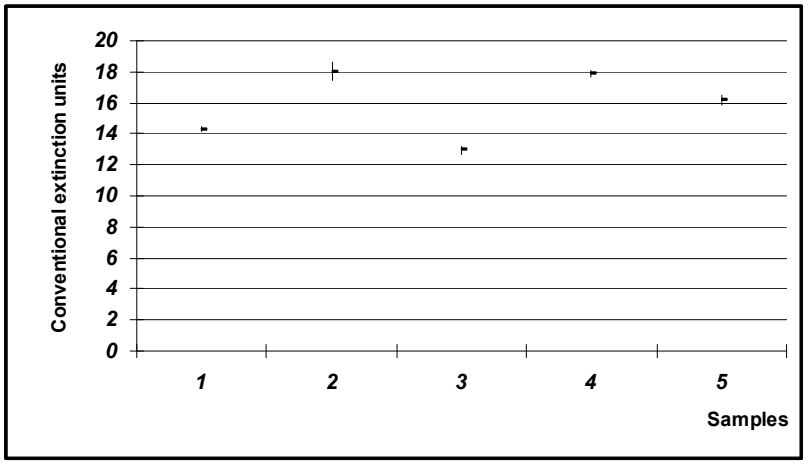

Fig.2. Confidence intervals of the aspartate-aminotransferase activity in three summer-old *Ctenopharyngodon idella*

A comparative graphical representation of the mean activity of the two intestinal transaminases (Fig.3) permits the conclusion that alanine-aminotransferase evidences higher values, comparable with those given in the literature, which suggests that alanine transamination occurs more intensely than that of the aspartic acid (KONOVALOV, 1980).

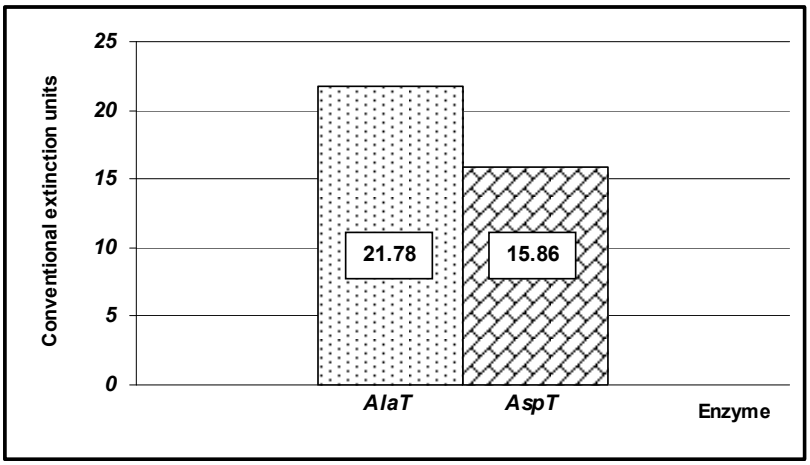

Fig.3. Comparative representation of the alanine- and aspartate-aminotransferase activity in three summer-old *Ctenopharyngodon idella*

The following stage of the present investigation involved the determination of the activity of intestinal pepsin, an endopeptidase which splits preferentially the peptidic links from the substrate, created by the aminic groups of the aromatic aminoacids - phenylalanine and tyrosine.

In fish, the proteasic activity either increases or decreases proportionally with the amount of proteins present in food, as well as with the quality and quantity of the available food,

which actually represents an adaptation of the secretory function of the intestinal tractus to the type of food (VASILE *et al*., 2005).

In the case of 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 (GUILLAUME *et al*., 1999).

In grass carp, the activity of intestinal pepsin varies between 0.26 and 0.65 nmoles Tyr/min. x ml, the highest variation and precision coefficient of the mean being of 5.844 and 3.374, respectively (Table III). The extremely low values recorded for intestinal pepsin agree with the literature data, according to which, in cyprinids, fish having no stomach and, consequently, with a reduced gastric function, gastric digestion, maintained especially through the pepsin, disappears. Such fish species use the buccal cavity and the pharynx for breaking up the food, a mechanical function replacing the stomachal one, the absence of gastric digestion being compensated by a more abundant presence of trypsin in the intestine.

Table III. The values of the activity of intestinal pepsin and of the main statistical indices in three summer-old *Ctenopharyngodon idella*

Samples	Individual activity (nmoles Tyr/ min. x ml)	Mean (nmoles Tyr/ min. x ml)	Standard error	Standard deviation	Variance	Confidence level (95%)	VC%
1	0.3046	0.3	0.005	0.008	0.021	2.907	1.678
	0.2945						
	0.3121						
2	0.3593	0.35	0.002	0.003	0.008	1.01	0.583
	0.3542						
	0.3612						
3	0.2656	0.26	0.001	0.003	0.007	1.13	0.652
	0.2689						
	0.2629						
4	0.6562	0.65	0.012	0.021	0.052	3.253	1.878
	0.6312						
	0.6735						
5	0.3359	0.33	0.011	0.019	0.048	5.844	3.374
	0.3156						
	0.3548						

VC% = mean variation coefficient, m% = mean precision coefficient

Here again, the limits of the confidence intervals of the activity of intestinal pepsin have been calculated and plotted graphically (Fig.4), a more ample variation domain being observed in the last two samples subjected to analysis (0.63 - 0.67 and 0.31 - 0.35 nmoles Tyr/min. x ml).

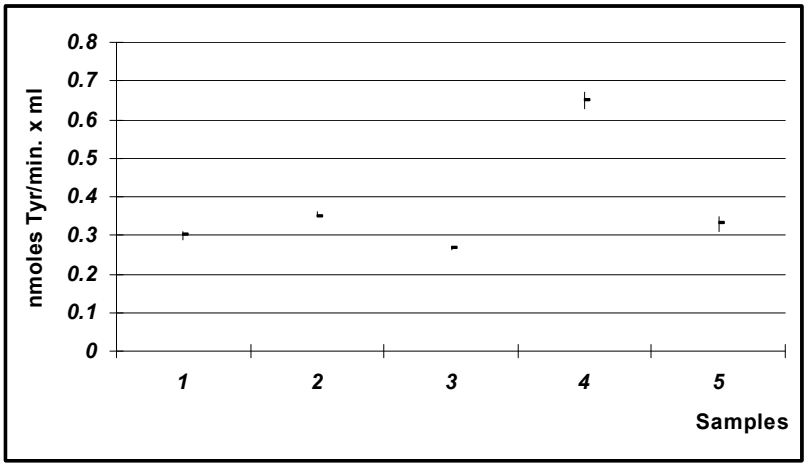

Fig.4. Confidence intervals of the intestinal pepsin activity in three summer-old *Ctenopharyngodon idella*

Trypsin is also an endopeptidase present in fish, synthesized in the pancreas, as its inactive zymogene (trypsinogene), the activation of which occurs, nevertheless, either at intestinal level, under the action of enterokinase, or autocatalitically, which causes a subsequent chain of reactions - through the action of the newly-formed trypsin on chemotrypsinogene, followed by the successive activation of other enzymes, namely elastase, colagenase, carboxypeptidase and phospholypase. Trypsin splits preferentially the peptidic links formed by arginine and lysine, being present and active both in the fish of prey and in the peaceful species (OPREA and GEORGESCU, 2000; COJOCARU, 2008).

In fish, trypsin evidences an optimum activity at pH = 7.5 - 8.5 (APETROAEI, 1995), the existing literature data showing differences in the enzymatic spectrum, in close correlation with the nutrition type, the fish of prey having a higher content of proteolytic enzymes while, in peaceful fish, the glycosidases are prevailing (MĂRGĂRINT, 1982).

In the case of cyprinids, trypsin acts directly upon the albumins, thus finalizing their simplification into aminoacids, which is also the case of the species possessing a stomach; consequently, trypsin finalizes the digestion of proteins.

The results obtained on the activity of intestinal trypsin in three summer-old grass carp evidence somehow more ample oscillations from one individual to another, the minimum value being of 0.356 nmoles Tyr/min. x ml, while the maximum one is of 0.802 nmoles Tyr/min. x ml (Table IV).

As evidenced in Figure 5, the limits of the confidence intervals plotted for the activity of intestinal trypsin are extremely narrow, the values obtained during parallel determinations being only slightly different from the mean value.

Table IV. The values of the activity of intestinal trypsin and of the main statistical indices in three summer-old *Ctenopharyngodon idella*

Samples	Individual activity (nmoles Tyr/ min. x ml)	Mean (nmoles Tyr/ min. x ml)	Standard error	Standard deviation	Variance	Confidence level (95%)	VC%
1	0.4531	0.453	0.002	0.004	0.011	1.014	0.585
	0.4589						

Samples	Individual activity (nmoles Tyr/ min. x ml)	Mean (nmoles Tyr/ min. x ml)	Standard error	Standard deviation	Variance	Confidence level (95%)	VC%
	0.4498						
2	0.6406	0.642	0.004	0.008	0.020	1.294	0.747
	0.6512						
	0.6348						
3	0.3593	0.356	0.004	0.006	0.017	1.949	1.125
	0.3489						
	0.3621						
4	0.7968	0.794	0.004	0.008	0.019	1.012	0.584
	0.8012						
	0.7856						
5	0.8	0.802	0.01	0.017	0.43	2.181	1.259
	0.8215						
	0.7868						

VC% = mean variation coefficient, m% = mean precision coefficient

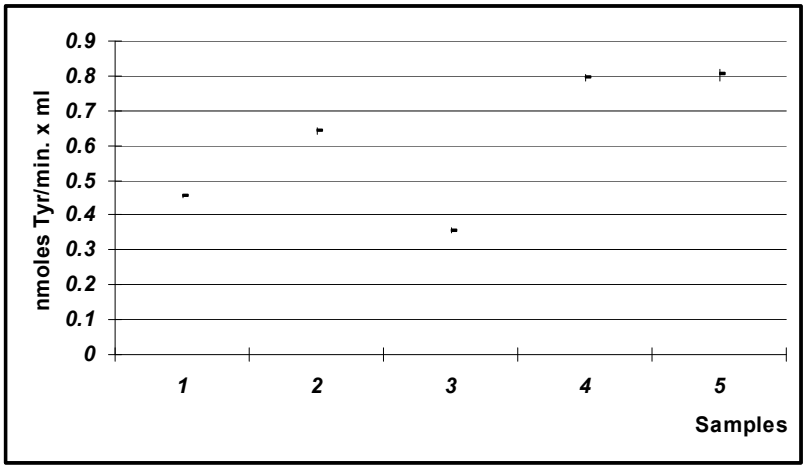

Fig.5. Confidence intervals of the intestinal trypsin activity in three summer-old *Ctenopharyngodon idella*

A comparative analysis of the mean values recorded by the activity of intestinal pepsin and trypsin (Fig. 6) shows that pepsin represents only 63.33% of the triptic activity, which might be explained by the fact that, generally, pepsin acts at stomachal level, in the presence of a slightly acid pH, while trypsin usually acts at intestinal level, where it is present in higher amounts.

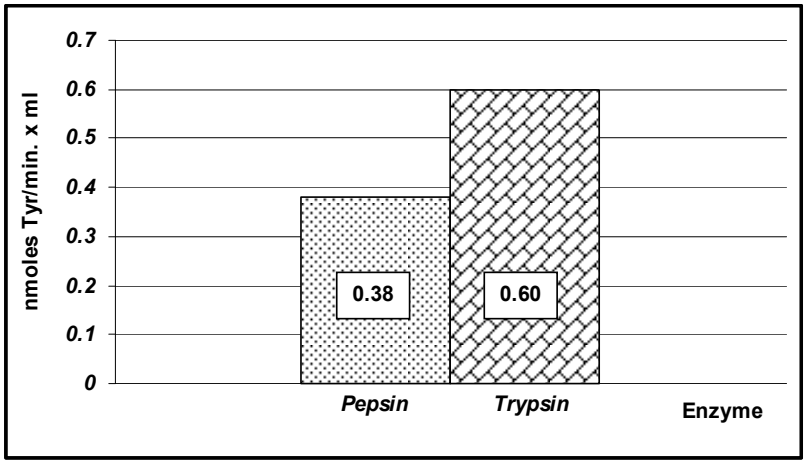

Fig.6. Comparative representation of pepsin and trypsin activity in three summer-old *Ctenopharyngodon idella*

CONCLUSIONS

The results of the present investigation permit the following conclusions:

1. At the level of the median part of the digestive tube, one may observe a quite significant proteolytic and transaminasic activity, which might be correlated with the absence of the stomach in this species, proteins degradation occurring mainly at intestinal level.

2. The enzymatic activity observed was more pronounced in the case of alanine-aminotransferase and, respectively, trypsin, the limits of the confidence intervals being quite narrow for all biochemical parameters taken into study.

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