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BIOMETRIC AND ENZYMATIC INVESTIGATIONS AT THE LEVEL OF THE DIGESTIVE TUBE OF THE SILVER CARP OF VARIOUS AGES

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INTRODUCTION

In Romania, fish breeding has been considerably extended by the valorization of the waters of the accumulation lakes, which permitted an intensive aquaculture of some fish species with doubt economic importance.

Special attention is paid by the investigators to the process of fish biochemical adaptation to the new environmental and food conditions, the researches being devoted, on one side, to the influence of food on the enzymatic digestive systems - once known that, in the gastro-intestinal tractus there occurs hydrolytic scission, assimilation and integration of the various food components in the metabolism of substances - and, on the other, the dynamics of the various enzymes which catalyze in the tissues the transformation of the products entering the blood from the digestive tube is being manifested (ARTENIE, 1990).

In the living organisms, the aminotransferases catalyze the transamination process, of special importance being - among aminotransferases - aspartate-aminotranferase or glutamico-oxaloacetate-aminotransferase and alanin-aminotransferase or glutamico-piruvate-aminotranferase.

The two aminotransferases, evidencing a pronounced catalytic activity, are largely occurring in various animal organs, such as: liver, myocardium, skeletal muscles, kidneys, etc., while their activity is more reduced in the sanguine serum (COJOCARU, 2005; COJOCARU *et al*., 2007).

Literature data on the influence of certain parameters on the activity of transaminases from various tissues evidence a decreased activity of theirs, resulting from the partial or total substitution of proteins from the standard allowance, by semi-liquid foul dejections (MĂRGĂRINT *et al*., 1980), a rhythmical enzymatic oscillation over a 24 hour period, in the case of huck (ARTENIE *et al*., 1998), along with a pronounced influence of food on the activity of alanine- and aspartate-aminotranferase from the liver and blood tissue (FERNANDEZ *et al*., 1999; REHULKA, 2000; LEE *et al*., 2003; ZHAO *et al*., 2006), while other studies do not view the diet as a stimulating or activating factor of amino-transferasic enzymatic activity (GOMEZ - REQUENI, 2004; PERES and TELES, 2005).On the other side, numerous investigations put into evidence a transaminazic activity differentiated as a function of growth density (HRUBEC *et al*., 2000), of the state of health (GRIZZLE and KIRYU, 1993; REHULKA, 2003), of the influence of certain toxic agents (ADHAM *et al*., 2002; PEPELJNJAK *et al*., 2003; YANG and CHEN, 2003) and of the presence of some biostimulating antibiotics in food (ARTENIE, 1990) and, equally, as a function of species (ÇELIK, 2004).

The present study develops a comparative analysis of the digestive tube length, *versus* the total bodily length, on one hand, and standard length, on the other, the aspartate- and alanine-aminotransferasic activity from the median part of the digestive tube in one and, respectively, four summer-old silver carp individuals (*Hypophthalmichthys molitrix*).

MATERIAL AND METHOD

The experiments were performed on silver carp representatives in various development stages (one - 0_+ and, respectively, four summer-old - 3_+), from the Ezăreni Accumuation, district of Iaşi, to which no additional food had been administered, the samples being taken over from the median part of the digestive tube, the rests of the intestinal content being removed through scraping.

The digestive tube was measured by biometry techniques (GROZEA and BURA, 2002), while the activity of aminotranferases was determined by the colorimetrical method, with 2,4-dinitrophenyl-hydrazine, the results being expressed in UE/g/min (COJOCARU, 2005).

Mention is made of the fact that three parallel dosings have been performed for each sample, the data provided representing the average values of these repetitions; in a final step, the results obtained have been processed statistically, the standard error, the standard deviation, the meanand precision coefficient variation being calculated, as well as the lower and upper limits of the confidence intervals over which each parameter in part oscillates (VARVARA *et al*., 2001).

RESULTS AND DISCUSSION

A first objective of the present investigation considered some biometric aspects characteristic for one and, respectively, four summer-old *Hypophthalmichthys molitrix* representatives.

To this end, 30 individuals from each development stage in part were sacrificed, after which the digestive tractus was separated along its whole length.

The measurements performed after a previous removal of the adipose tissue permitted calculation of the main statistical indices (DRAGOMIRESCU, 1998; GOMOIU and SKOLKA, 2001).

Literature data confirm that, in the case of fish, the length of the digestive tube varies as a function of species, age and - especially - food regime (STĂNCIOIU *et al*., 2006; APETROAEI, 2007).

Consequently, in the silver carp fry, the average length of the digestive tube is of 51.116 cm, while the adults register a value of 494.85 cm, which indicates a 9.68 times increase of the digestive tractus (Tables 1, 2).

Table 1. Values of the main statistical indices of the analyzed variables in one summer-old *Hypophthalmichthys molitrix*

Statistical indices	Analyzed variable		
	Ltd (cm)	L (cm)	ls (cm)
Mean	51.116	12.366	10.366
Standard error	1.263	0.131	0.139
Median	52.5	12.25	10.25
Mode	55	11.5	10
Standard deviation	69.19	0.718	0.764
Variance	47.873	0.516	0.585
Range	18	2	2.5
Minimum	41	11.5	9
Maximum	59	13.5	11.5
Confidence level (95%)	2.583	0.268	0.285
Upper limit	53.7	12.634	10.652
Lower limit	48.533	12.098	10.081
CV%	13.535	5.809	7.378
m%	2.471	1.06	1.347

Ltd = digestive tube length,
L = total length of the body,
ls = standard length of the body,
CV% = mean variation coefficient,
m% = mean precision coefficient

Table 2. Values of the main statistical indices of the analyzed variables in four summer-old *Hypophthalmichthys molitrix*

Statistical indices	Analyzed variable		
	Ltd (cm)	L (cm)	ls (cm)
Mean	494.85	65.183	56.8
Standard error	7.66	0.933	1.032
Median	490	63.5	55.5
Mode	510	63	58
Standard deviation	41.959	5.113	5.657
Variance	1760.58	26.146	32.01
Range	157.5	18.5	23
Minimum	415	55.5	43
Maximum	572.5	74	66
Confidence level (95%)	15.667	1.909	2.112
Upper limit	510.517	67.092	58.912
Lower limit	479.182	63.273	54.687
CV%	8.479	7.844	9.96
m%	1.548	1.432	1.818

Ltd = digestive tube length,
L = total length of the body,
ls = standard length of the body,
CV% = mean variation coefficient,
m% = mean precision coefficient

On the basis of the average values and of standard deviation, the (upper and lower) limits of the confidence intervals of the digestive tube length have been subsequently calculated as a function of a critical value $t\ (\alpha,\ n\text{-}1)$, given by $\alpha = 0.05$ and $n\text{-}1$ degree of freedom, that is $t\ (0.05,\ 29) = 2.045$.

In silver carp fry, the average length of the digestive tube oscillates between 48.533 - 53.7 cm while, in adults, it ranges between 479.182 - 510.517 cm, once known that, in the peaceful species with a vegetarian food regime, the pronounced elongation of the digestive tube may be explained by the fact that the food is more difficultly digested, a larger digestion surface being therefore created (STĂNCIOIU *et al.*, 2006) (Fig. 1).

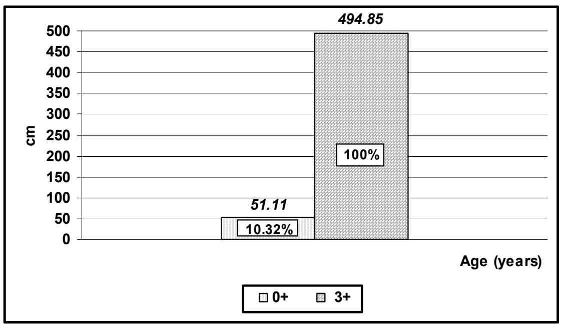

Fig.1. Digestive tube average length in silver carp of various ages

If we consider the ratios between digestive tube length and total bodily length, as well as that between digestive tube length and standard bodily length in one summer-old *Hypophthlamichthys molitrix* representatives, it can be observed that these attaining values of 4.133 and 4.930, respectively.

With the increase in bodily length, the ratio between the length of the digestive tube and that of the body increases, too, so that in the case of silver carp adults the ratio between the length of the digestive tube and the total length of the body is of 7.591, while that between the length of the digestive tube and the standard bodily length is of 8.712, this modification being, probably, the result of their growing tamed, as well as of their food regime.

If, after hatching, in the first stages of fish development, the food is prevailingly proteic, being composed of small zooplanktonic organisms, it gets modified during the growing process, inducing certain structural and functional modifications of the digestive tractus. A typical example in this respect is provided by the *Hypophthalmichthys molitrix* species which, as mentioned in the literature (ANTALFI and TÖLG, 1972, cited by GROZEA and BURA, 2002; STĂNCIOIU *et al*., 2006), consumes zooplankton exclusively in the fingerling stages, until reaches a length of 5 - 10 cm, the digestive tractus representing less than 100% of the bodily length; along this interval, the filtering surface of the branchiae is developing, while the digestive tube reaches a 6 - 7 times higher length than the bodily length, permitting to the fingerling to be fed with phytoplankton while, in the case of adult fish, by consuming numerous vegetal plankton organisms (especially diatomees), its length becomes, in some cases, even 15 times longer than the bodily length (MAHESHWARI *et al*., 1992; ZHAO *et al*., 2001; JANG *et al*., 2004).In a subsequent stage of the experiment, the activity of alanine- and aspartate-aminotransferases from the median part of the digestive tube was determined, once known that, at this level, the digestive enzymes manifest their highest catalytic potential, the medium intestine being thus defined - although improperly - as “stomach”.

To this end, 20 individuals from each species in part were employed, the rests of intestinal content being removed through scarping.

The experimental data obtained for alanine-aminotransferase in silver carp fry evidence a quite ample variation in the enzymatic activity from one individual to another, the minimum value recorded being of 0.361 UE/g/min., the maximum one - of 1.016 UE/g/min., while the mean value of the 60 parallel determination was of 0.737 UE/g/min. Mention should be made of the fact that alanine-aminotransferase shows an activity quite close to the maximum value in half of the individuals under analysis, ranging between 0.958 - 1.016 UE/g/min, while the values recorded in the others are between 0.361 - 0.641 UE/g/min.

As to the intestinal aspartate-aminotransferase in one summer-old representatives of the *Hypophthalmichthys molitrix* species, mention should be made of the fact that, comparatively with the other transaminases under investigation, its activity registers much lower and much more homogenous values, between 0.207 - 0.480 UE/g/min. (Fig. 2).

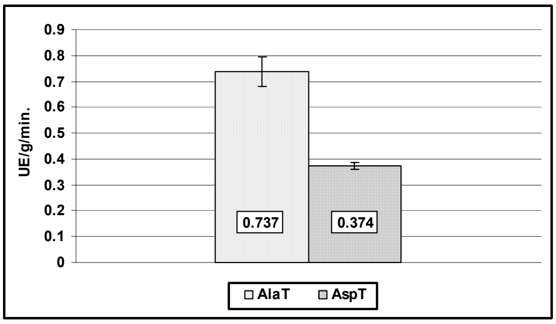

Fig.2. Alanine- and aspartate-aminotranspherase activity in fry silver carp

The activity of alanine-aminotransferase in the digestive tube of four summer-old *Hypophthalmichthys molitrix* representatives records slightly higher values than in summer-old individuals of the same species, the mean value being of 0.744 UE/g/min. In exchange, the aspartate-aminotransferase activity in silver carp representatives of the same age represents only about half of the alanine-aminotransferase activity, the average value recorded, of 0.390 UE/g/min, being, simultaneously, of almost the same order of magnitude as the one evidenced in the first summer of growth (0.374 UE/g/min.) (Fig. 3).

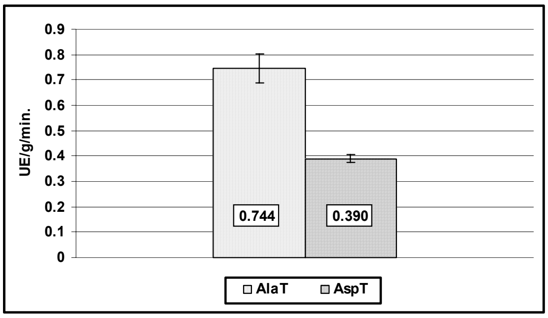

Fig.3. Alanine- and aspartate-aminotranspherase activity in four summer-old silver carp

To check the possible differences or similarities between the alanine- and aspartate-aminotransferase activity from the median part of the digestive tube, the ANOVA test with only one factor and equal number of observations in the cell was applied, followed by the calculation of the square sums, factor value, as well as critical value, which permitted settlement of the null (H_0) and alternative (H_1) hypotheses of the test (Tables 3 - 6).

Table 3. Summary of the Anova test unifactorial pattern of alanine-aminotransferase activity in silver carp of various ages

Age (years)	Count		Sum	Mean	Variance
0_+	20		14.751	0.73755	0.066187
3_+	20	14.882	0.7441	0.065225	

Table 4. Calculated and critical values of the factors of alanine-aminotransferase in silver carp of various ages

Source of variation	SS	g. l.	$\overline{SS}$	Calculated F	Critical F
Internal	0.000429	1	0.000429	0.006529	4.098172
External	2.496835	38	0.065706		
Total	2.497264	39			

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

Table 5. Summary of the Anova test unifactorial pattern of the aspartate-aminotransferase activity in silver carp of various ages

Age (years)	Count	Sum	Mean	Variance
0_+	20	7.487	0.37435	0.004264
3_+	20	7.815	0.39075	0.004815

Table 6. Calculated and critical values of the aspartate-aminotransferase in silver carp of various ages

Source of variation	SS	g. l.	$\overline{SS}$	Calculated F	Critical F
Internal	0.00269	1	0.00269	0.592539	4.098172
External	0.172486	38	0.004539		
Total	0.175176	39			

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

As also illustrated by the graphical representations, minor differences may be noticed in the activity of transaminases, as a function of the evolution stage in which the individuals of the species under study occur, with the observation that - within one and the same population - the alanine-aminotransferase has a more fluctuating evolution (Figs. 4 - 5).

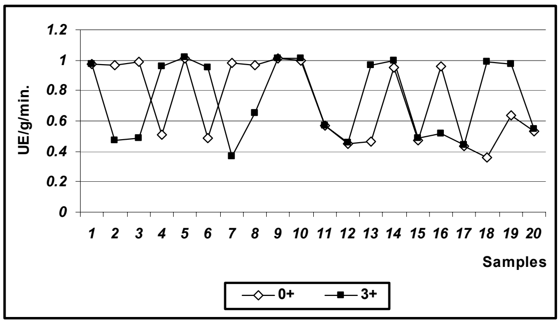

Fig.4. Graphical comparative representation of alanine-aminotranspherase activity in silver carp of various ages

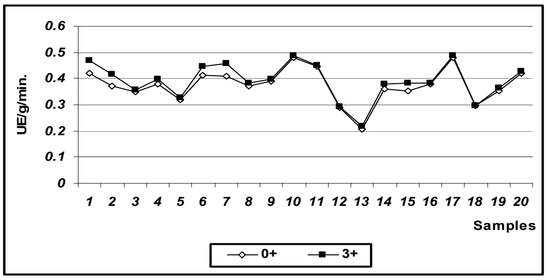

Fig.5. Graphical comparative representation of aspartate-aminotranspherase activity in silver carp of various ages

ABSTRACT

The paper presents in parallel, a comparative study of length of digestive tube reported to the total length of body and to the standard one, as well as the activity of aspartat- and alanin-aminotransferazic from the median portion of the digestive tube at *Hypophthalmichthys molitrix* exemplars of one summer old, respectively four summers old.

At the sapling an elongation of the digestive tube take place, this elongation being of 9.7 times (51,116 cm at one summer exemplars and 494,85 cm at the four summer ones). In early stages, the rapport between the length of the digestive tube and the total length of body is of 4,133, and the ratio between it and the standard length is 4,930. At the adult exemplars, the ratio between the length of the digestive tube and the total length of body is of 7,591, and the ratio between it and the standard length is 8,712.

In what concern the intestinal transaminazic activity on remark, that the activity is higher at the exemplars of four summers. We can also conclude that the values of alanin-aminotransferase are much higher and not uniform at the individuals of both ages.

CONCLUSIONS

1. In silver carp fry, the mean value of the digestive tube length is of 51.116 cm, which represents only 10.32% of the length evidenced in four summer-old individuals (494.85 cm), this elongation being correlated with both age and modification of the trophic spectrum.
2. The activity of the transaminases under analysis evidence, on one side, oscillations from one individual to another, more reduced - yet statistically insignificant - in the case of fry, while, on the other hand, considerable differences should be mentioned between the two enzymes taken into study, the alanine-aminotransferase showing about 50% higher values in both developmental stages in part.

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