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ACTIVITY OF DIGESTIVE ENZYMES IN SIBERIAN STURGEON JUVENILES (A*cipenser baeri* Brandt) – A PRELIMINARY STUDY

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ABSTRACT. The study was carried out on Siberian sturgeon fry and fingerlings at the age of 9-44 days post hatching (dph). Activities of proteolytic, lipolytic and amylolytic enzymes, and dicaccharidases were measured in the digestive tract extract. Only alkaline proteases were observed in 9 dph fish, and no lipolytic or amylolytic activity were found. After the first week of exogenous feeding (16 dph), high lypolitic and maltase activities were noted, as well as slight activity of acidic proteases, but no pepsin. Pepsin activity was observed for the first time on 44 dph. Amylolytic activity increased gradually between 3 and 6 weeks post hatching. Activity of dicaccharidases: maltase and trehalase reached the maximum on 30 dph. No lactase or saccharase were observed.

Key words: *Acipenser baeri*, FRY FEEDING, BIOCHEMICAL STUDY, DIGESTIVE ENZYMES, ONTOGENESIS.

INTRODUCTION

Siberian sturgeon has become the most popular sturgeon in aquaculture due to its good rearing characteristics (Berdichevskij et al. 1979, Willot, Brun 1983, Steffens et al. 1990, Kolman 1998). There are many data on breeding, feeding, growth rate and food preferences of this species under natural and controlled conditions (Berdichevskij et al. 1979, Willot, Brun 1983, Semekova 1983, Dąbrowski et al. 1985, Ronay et al. 1991, Kolman et al. 1996, 1997, Prokes et al. 1996, 1997a, b). There is little information, however, on digestive physiology of sturgeons. Some ontogenic studies dealt also with enzymatic development of sturgeons – lake sturgeon, *Acipenser fulvescens* (Buddington 1985), and white sturgeon, *A. transmontanus* (Buddington, Doroshov 1986, Hung et al. 1989, Gawlicka et al. 1995, Herold et al. 1995, Lin et al. 1996). No data were found on the development of enzymatic activity in digestive tract of Siberian sturgeon, so it has became an object of the present study. Knowledge of the digestive enzymatic activity is necessary for preparing diets suitable for each developmental stage of the fish digestive tract.

MATERIAL AND METHODS

The study was carried out on Siberian sturgeon fry obtained in the Experimental Fish Hatchery „Dgał" (Inland Fisheries Institute in Olsztyn) in May 1998. The experiment started on day 9 post hatching (dph), which was the last day of endogenous feeding of the larvae. For the next 2 weeks the fish were fed prestarter ASTA-AC, and then trout starter „Kristall – 3700" produced by Aller Mølle (Kolman et al. 1996). The fish were sampled and weighed every 3 days.

From 9 dph until 44 dph, 10 fish were sampled weekly and weighed. The fish were then frozen, and stored at constant temperature (-18^{o}C). After careful unfreezing, digestive tracts were isolated using a preparation needle, weighed and homogenized with 4 cm^3 of physiologic solution in a glass Potter homogenizer. The homogenate was centrifuged for 10 minutes at 2600 rpm. Protein content was measured in the supernatant using Coomassie brillant blue (Spector 1978). Activities of digestive enzymes were also measured: α-amylase, glucoamylase, maltase, saccharase, trehalase, and lactase, total activity of alkaline and acidic proteases, trypsin, chymotrypsin, pepsin, and triacyloglyceric lipase.

ENZYMATIC ANALYSES

Activity of α-amylase was measured using Caraway's method (Karpiak 1974), and expressed in IU. Samples taken from 7-21 dph sturgeons were incubated for 120 minutes, and of 28 and 42 dph fish – 15 and 5 minutes respectively.

Glucoamylolytic activity was measured as a quantity of glucose released from glycogen during 60 minutes of incubation of a mixture containing 0.1 cm^3 of the extract, 0.1 cm^3 of 1% glycogen solution, and 0.8 cm^3 of 0.15 M veronal-acetate buffer, pH 4.9.

Activities of disaccharidases were measured according to Dahlqvist's method (1968), using 25mM aqueous solutions of disaccharides: maltose, lactose, saccharose, and trehalose. Samples were incubated for 2 hours (trehalose), or 1 hour (all other dicaccharides).

The amount of glucose released from carbohydrates at the presence of disaccharases and glucoamylase was measured in an enzymatic reaction with glucooxydase, using reagents produced by Cormay Lublin. One enzymatic activity unit (u) equalled to 1 μg of glucose released in hour.

Total activity of alkaline and acidic proteases was measured using Anson's method (Kłyszejko-Stefanowicz 1982). It was expressed in units equivalent to 1 μM of ty-

rosine released in 10 minutes from haemoglobin. Pepsin activity was measured according to Ryle (1985), trypsin – according to Geiger, Fritz (1985), and chymotrypsin – according to Geiger (1985), using synthetic chromogenic peptides made by Sigma: N-acetyl-DL-phenylalanine-p-nitroanilide for pepsin, N-benzyl-DL-arginine-p-nitroanilide for trypsin, and N-succinyl-L-phenylalanine-p-nitroanilide for chymotrypsin.

Activity of triacyloglyceric lipase was measured using Cherry-Crandall method (Kłyszejko-Stefanowicz 1982). One unit of activity was equivalent to the amount of enzyme releasing 1 μM of carboxylic acid from olive oil emulsion.

All enzymatic activities were calculated for 1 mg of protein of the enzymatic extract. The results are shown as mean values of three replicates.

RESULTS

The fish showed almost a linear growth over the first two weeks of rearing (Fig. 1). Average body weight of the larvae increased from 19.7±8.6 mg on the last day of endogenous feeding (9 dph) to 131.3±32.4 mg on 23 dph. Over the next 3 weeks the

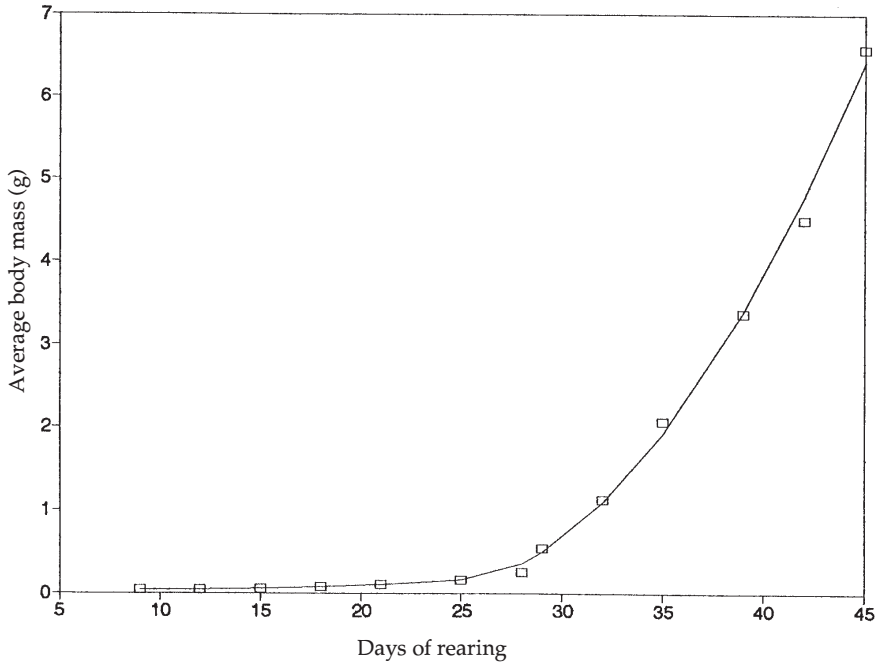

Fig. 1. Dynamics of average body weight of Siberian sturgeon fry

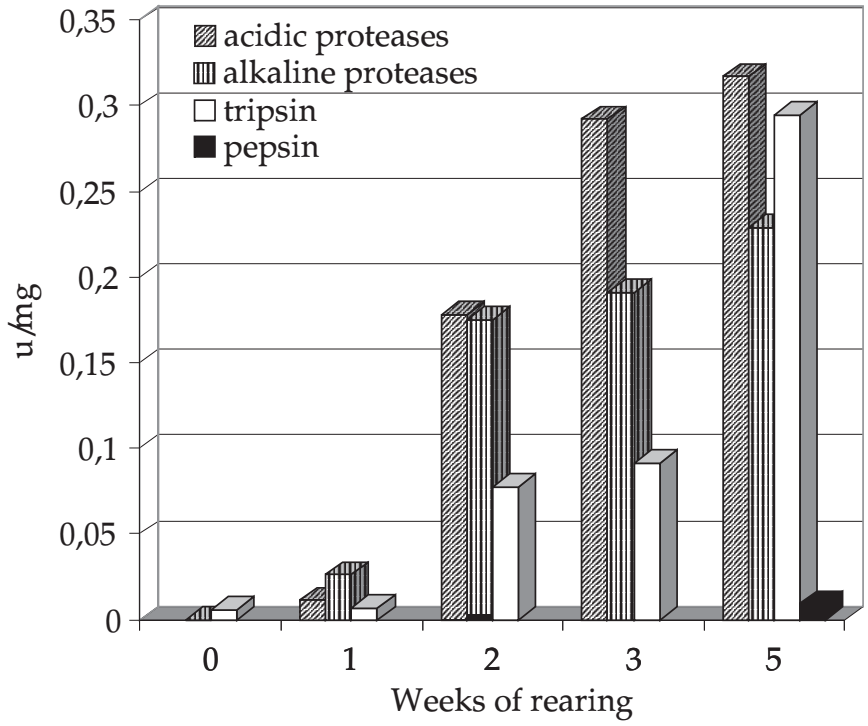

Fig. 2. Activity of proteolytic enzymes in digestive tracts of Siberian sturgeon fry.

fish weight increased considerably, and in the fourth week of rearing a three fold increase was noted (467.8±96.4 mg). On the last day of the experiment (44 dph), average fish body weight was 6353±542 mg.

Incubation conditions were set before measuring the enzymatic activity. Appropriate time of incubation, differing from the original method, was established for each enzyme as described in the Material and Methods. Optimum pH for each reaction was established based on own studies carried out on 1+ fingerlings (unpublished). These pH values were: 3.0 for acidic proteases, 7.8 for alkaline ones, 7.3 for lipase, 7.7 for α-amylase, 7.1 for saccharase. 6.6 for lactase, 4.9 for glucoamylase and maltase, and 4.1 for trehalase.

At the end of the endogenous feeding (9 dph), some yolk was still present in the yolk sac, and very slight enzymatic activities in the sturgeon digestive tracts were observed (Fig. 2, 3). Only alkaline proteases (0.0153 u/mg), including trypsin (0.0057 u/mg), were found. No lipolytic or amylolytic activity was noted in this stage of development.

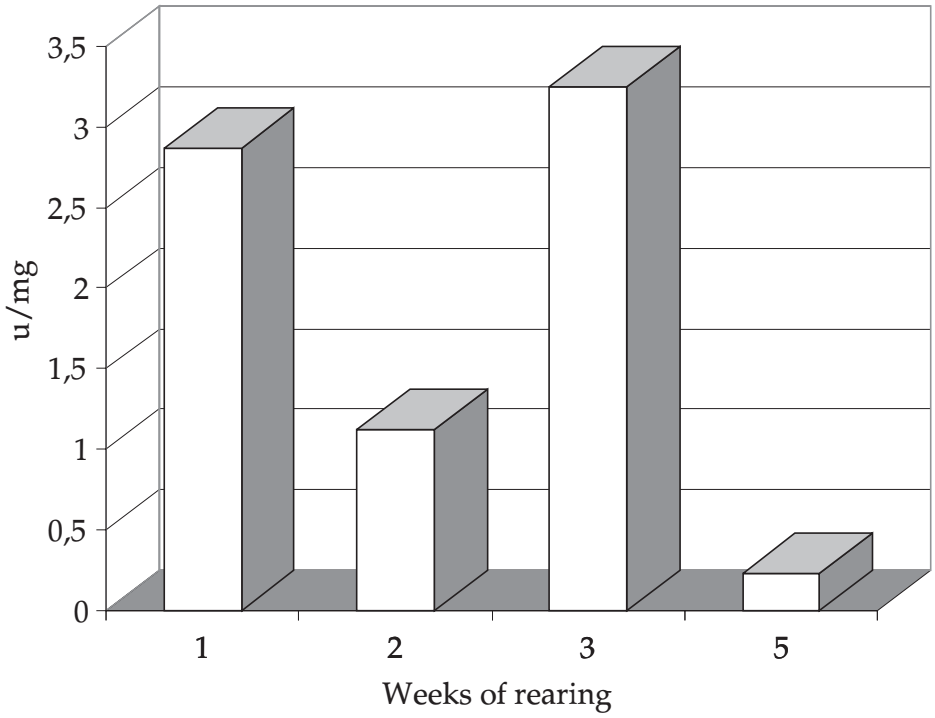

Fig. 3. Activity of lipase in digestive tracts of Siberian sturgeon fry.

Quite high lipolytic activity (2.869 u/mg) occurred after the first week of exogenous feeding of the larvae (16 dph, Fig. 3). At the same time, the first maltase activity was observed (1.2154 u/mg) (Fig. 4), and a slight activity of acidic proteases, but no pepsin which was observed for the first time only on 44 dph (Fig. 2).

Over the next 2 weeks, especially in the third week of rearing, acidic proteases started to predominate over alkaline ones (Fig. 2). Gradual increase of proteolytic activity was observed until the last day of the study (44 dph). Fast increase of trypsin activity from 0.0912 u/mg to 0.2946 u/mg took place between 30 and 44 dph, when the fish were fed trout starter „Kristall – 3700" (Fig. 2).

Lipolytic activity fluctuated considerably over the study period (Fig. 3). It distinctly dropped on 23 dph comparing to the first week of exogenous feeding, and reached a maximum of 3.551 u/mg on 30 dph, but in the next two weeks (44 dph) it decreased again to 0.228 u/mg.

Between weeks 3 and 6 post hatching, amylolytic activity increased rapidly. Activity of α-amylase increased from 0.0069 u/mg on 21 dph to 2.1318 u/mg at the end of

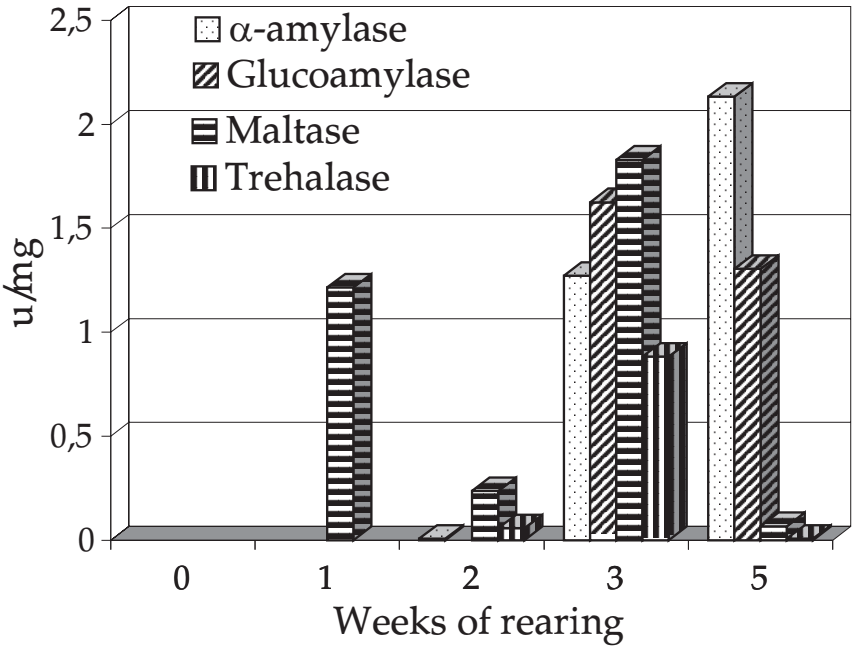

Fig. 4. Changes of activity of glycolytic enzymes in digestive tracts of Siberian sturgeon fry.

the experiment (Fig. 4). Glucoamylolytic activity appeared in the 4th week post hatching (Fig. 4).

Dicaccharidases: maltase and trehalase reached their maximum activity on 30 dph. Dynamics of maltase activity was very similar to that of lipase (Fig. 4). Its activity on 23 and 44 dph was considerably lower than on 16 and 30 dph. Digestive tract in Siberian sturgeon contained no lactase or saccharase, except 30 dph when very slight activities were observed.

DISCUSSION

Biochemical, histological and anatomic similarities in early development of white and lake sturgeons observed by Buddington (1985), Buddington, Doroshev (1986), and Gawlicka et al. (1995) were found also in the present study, at the enzymatic level, for Siberian sturgeon. The results confirm the suggestion by Buddington (1985) that secretion pattern of digestive enzymes is probably genetically determined and common for all sturgeons, being only slightly modified by environmental conditions.

The last author distinguished 3 phases of early development of digestion in sturgeons. The first phase – yolk sac stage, lasts in lake and white sturgeons from 1 to 16 dph, the second – larval feeding, from 16 to 24 dph, and the third – metamorphosis, corresponding to teleost fish development, from 24 to 30 dph. Enzymatic activity and distribution in this phase reach their final pattern for juvenile and mature fish (Buddington 1985).

In Siberian sturgeon development is similar, and similar phases may be distinguished. In the present experiment, however, due to early beginning of artificial feeding (9 dph), all phases appeared about 7 days earlier. In the first phase – endogenous feeding – lasting from 1 to 9 dph, the yolk sac material was utilised. Similarly as in the case of white and lake sturgeons (Buddington 1985, Buddington, Doroshev 1986, Gawlicka et al. 1995), it is hard to explain how Siberian sturgeon uses yolk, because enzymatic activity is very low. Only alkaline proteases are present at this stage. The results concerning these enzymes are similar to those obtained by Buddington (1985) for *Acipenser fulvescens*, and by Savasquete et al. (1993) for *Sparus aurata*.

Histological observations by Gawlicka et al. (1995) indicate that pincytosis and intracellular digestion in yolk sac endodermis plays an important role in this developmental stage of white sturgeon. Similar data were obtained by Buddington (1985) for lake sturgeon. In the present study the whole digestive tracts, together with the content, were used, so intercellular enzymes should also appear in the homogenates. Surprisingly, no lipolytic enzymes or disaccharases were found. The study did not answer the question how yolk material is digested in sturgeons. The question is not very important for the fishery practise, but it is very interesting.

Contrary to white and lake sturgeons (Buddington 1985, Buddington, Doroshev 1986), no acidic proteases were found in Siberian sturgeon during endogenous feeding of larvae. These activities appeared in *Acipenser baeri* after one week of artificial feeding, increased over the next 2 weeks in a linear way, and on 23 dph reached higher level compared to alkaline proteases (Fig. 2). Buddington (1985) and Gawlicka et al. (1995) observed early secretory activity in sturgeon stomach, but results of the present study showed slight pepsin activity in Siberian sturgeon as late as on 42 dph. Our results are similar to those obtained for many teleost fish in which no acid or pepsin secretion occurs until metamorphosis (Tanaka 1971), and do not confirm the observations by Buddington (1985) and Buddington, Doroshev (1986) who found pepsin in gut of endogenously feeding white and lake sturgeons fry. This apparent contradiction most probably resulted from different methods used in the studies. In our study

enzyme activity was evaluated using highly pepsin-specific method, while other authors applied semi-quantitative method, the results of which could be affected by all enzymes that hydrolyzed gelatine in an acidic environment. In our study other acidic proteases, different from pepsin, were found in Acipenser baeri.

The second phase – larval feeding – lasted from 10 to 22 dph. During this phase activity of lipolytic and proteolytic enzymes considerably increased, and alkaline proteases predominated in the first week of artificial feeding. Similar results were obtained by Buddington and Doroshev (1986) for white sturgeon fed pellets, and by Dąbrowski and Głogowski (1977) for common and grass carp, and for whitefish fry. Among glycolytic enzymes, only maltase was observed in Siberian sturgeon.

Taking into consideration Buddington's (1985) enzymatic indices of metamorphosis, we may assume that this phase started in Siberian sturgeon about 23 dph. It was accompanied by considerable drop of lipolytic and maltase activity, but α-amylase and trehalase appeared.

Interesting pattern of enzymatic activity occurred in sturgeons on 30 dph. After 3 weeks of feeding on Kristall, the fish digestive tracts showed maximum activities of triacyloglyceric lipase, and three glycolytic enzymes: glucoamylase, maltase, and trehalase. Also α-amylase was highly active – 1.2714 u/mg. Presumably, protein metabolism that predominated in this period over the previous developmental phases, was efficiently supplemented with highly energetic carbohydrate and lipid digestion products. This was reflected in fast fish growth (Fig. 1). It is noteworthy that on 30 dph, slight activities of other disaccharidases – lactase, saccharase and cellobiase were observed. We suppose that these activities were not connected with specific enzymes, but were rather a side effect of high activities of other glycolytic enzymes which normally show slight affinity to non-specific glycosidic bounds (Karpiak 1974).

Presumably, lactase found using histological methods in white sturgeon by Gawlicka et al. (1995), does not occur in *Acipenser baeri* larvae. Lack of ability to digest lactose, saccharose, and cellulose was also demonstrated by other authors for white sturgeon juveniles (Hung et al. 1989, Fynn-Aikins et al. 1993, Herold et al. 1995). The effects of using starch in sturgeon feeding are not quite clear. The same authors found that starch is less assimilated by white sturgeon juveniles compared to glucose (Hung et al. 1989), but in another experiment contrary results were obtained (Lin et al. 1996). The authors did not explain the contradiction.

The results of the present study concerning amylolytic activity confirm conclusion of Fynn-Aikins et al. (1993) that starch is a valuable and cheap feed supplement

also for Siberian sturgeon. It should be, however, used not earlier than in the 4th week post hatching, when activities of α-amylase and glucoamylase are sufficiently high for successful digestion. It seems that the product of starch hydrolysis – maltose – may be used as feed supplement for younger fish, since in week 2 post hatching its activity was already sufficiently high (Fig. 4). The study, however, should be repeated with increased frequency of sampling, to evaluate more accurately time of appearance and development of activity of all digestive enzymes.

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STRESZCZENIE

AKTYWNOŚĆ ENZYMÓW TRAWIENNYCH U WYLĘGU JESIOTRA SYBERYJSKIEGO (*Acipenser baeri* Brandt) - BADANIA WSTĘPNE

Badania przeprowadzono na wylęgu i narybku jesiotra syberyjskiego w wieku 9 - 44 dni po wylęgu (dph). Ryby od początku aktywnego żerowania tzn. od 9 dph karmione były starterem ASTA-AC, a od 23 dph starterem pstrągowym „Kristall - 3700" firmy Aller Molle.

W wyciągach z przewodów pokarmowych ryb oznaczano zawartość białka oraz aktywność następujących enzymów: alfa-amylazy, glukoamylazy, maltazy, sacharazy, trehalazy, laktazy i celobiazy, ogólną proteaz zasadowych i kwaśnych, trypsyny, chymotrypsyny, pepsyny i lipazy triacyloglicerolowej.

Z badanych enzymów w 9 dph stwierdzano jedynie obecność proteaz działających w środowisku zasadowym, nie udało się zaobserwować aktywności enzymów rozkładających lipidy i cukry. Aktywność lipolityczną na dość wysokim poziomie odnotowano po pierwszym tygodniu egzogennego karmienia larw (16 dph). W tym czasie ujawnia się aktywność maltazy (1,2154 u/mg) oraz niewysoka aktywność proteaz kwaśnych, wśród których brak jest pepsyny, jej aktywność stwierdzono po raz pierwszy dopiero w 44 dph. Między 3 a 6 tygodniem po wylęgu obserwowano stały, szybki przyrost aktywności amylolitycznej. Disacharydazy maltaza i trehalaza wykazywały maksymalną aktywność w 30 dph. Nie stwierdzano natomiast obecności laktazy i sacharazy.

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