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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Effects of Starter Diets on Pancreatic Enzyme Activity in Juvenile Sterlet (*Acipenser ruthenus*)

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(Received 23.12.08, Accepted 18.3.09)

Key words: *Acipenser ruthenus*, digestive enzymes, pancreas, trypsin, amylase, lipase

Abstract

Nine-day-old sterlet (*Acipenser ruthenus*) larvae were reared for 21 days on one of three commercial diets with different protein and fat contents: Bio Kyowa, Aglo Norse, or Perla Larva Proactive. At the end of the experiment, the sterlet juveniles were sampled for histological analysis of the pancreas and evaluation of lipase, trypsin, and amylase activity. Fish fed the Bio Kyowa and Aglo Norse diets were the largest. Survival was highest in the Aglo Norse group. There were no differences between groups in histological analysis of the pancreas, and no histological anomalies. The highest lipase activity was observed in fish fed the diet with the highest lipid content (21%) - the Aglo Norse diet. Trypsin activity was higher in fish fed Bio Kyowa with a protein content of 55% than in fish fed Aglo Norse with a protein content of 59%.

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Introduction

All sturgeon species are endangered due to overfishing, river damming, regulation, and deepening, and strengthening of river banks. Sturgeons can be reared in aquaculture (Dettlaff and Goncharof, 2002), and a considerable amount of data concerning rearing, breeding, and feeding of sturgeons is available (Williot et al., 2001; Furne et al., 2005). Production of market size sturgeon depends mainly on artificial feeding, thus detailed studies of digestive processes and digestive enzymes are of great importance. Composition and secretion of digestive enzymes in white sturgeon (*Acipenser transmontanus* Richardson) have been described (Buddington and Doroshov, 1986a,b) and epithelial and brush border digestive enzymes were analyzed (Gawlicka et al., 2002). However, digestion physiology and digestive enzymes of sturgeon are still insufficiently known. Such knowledge would help in the formulation of appropriate diets and nutritional requirements of all developmental stages of sturgeon. It would help to reduce mortality and enhance growth rate, feed utilization, and health.

The composition of digestive enzymes in sturgeons depends on the fish age, diet composition, and genetic and other factors (Buddington and Doroshov, 1986a). The stomach, exocrine pancreas, and intestine produce various types of digestive enzymes. Gastric, pancreatic, and intestinal enzymes participate in food digestion. The pancreas synthesizes and secretes lipases, amylases, and proteases (trypsin and chymotrypsin), which are active under alkaline conditions, into the intestinal lumen. Enzyme action is complementary, and results in complete digestion of food components, allowing their absorption and transport by enterocytes (Zambonino Infante and Cahu, 2001).

The early development of sturgeons includes three phases: first - the yolk sac stage, second - actively feeding larva, and third - metamorphosis during which enzymatic activity reaches the level typical for juveniles and adults (Buddington, 1985; Zoltowska et al., 1999). When sturgeon larvae begin exogenous feeding, their digestive tract is completely developed (Wegner et al., 2008) and the digestive enzyme composition is similar to that of adults (Buddington and Doroshov, 1986b). Thus, their ability to digest food seems higher than in other fish. Carbohydrates and lipids are better assimilated by sturgeon larvae than by older individuals (Buddington and Doroshov, 1986b). Adult sturgeons have lower lipid and protein requirements than young individuals (Garling and Wilson, 1976). At the same time, protein utilization for energy production increases (Kaushik et al., 1982) and lipase activity decreases (Buddington and Doroshov, 1986b).

The endogenous feeding period in sterlet lasts up to the ninth day after hatching (Wegner, 2008). Then, larvae begin eating exogenous food. Larvae metamorphose after pyloric caeca differentiate on the 12th day post hatching. Development of pyloric caeca is the last main morphological change in the digestive tract and confirms transfer to the juvenile stage (Bisbal and Bengtson, 1995).

The aim of present study was to evaluate the activity of the digestive enzymes, lipase, α-amylase, and trypsin, accompanied by histological analysis of the pancreas in sterlet (*Acipenser ruthenus* L.) juveniles fed commercial diets with different fat and protein contents.

Materials and Methods

Experimental design. The experiment was carried out in the Department of Ichthyobiology and Fisheries of the Faculty of Animal Sciences, Warsaw University of Life Sciences. Nine-day sterlet larvae were stocked into 20-l aquaria placed in the water recirculation system with a biological filter and UV lamps. Temperature was maintained at 21.5±0.7ºC and pH at 7.98-8.09. Initial fish density was 7.5 ind/l.

The larvae were reared for 21 days (to 30 days post hatching). The larvae were fed one of three diets, Bio Kyowa (Kyowa Hakko Koygo, Tokyo, Japan), Aglo Norse (Larvae Feed Ewos,

Bergen, Norway), or Perla Larva Proactive (Trouvit, Nutresco Aquaculture, Holland), five times a day (every two hours) from the first day of the experiment (Table 1). In the first week the amount of feed equaled 100% of their biomass, in the second - 50%, and in the third week - 10%.

Table 1. Protein and fat contents (%) in tested feeds.

Feed	*Protein*	*Fat*
Bio Kyowa	55	10
Aglo Norse	59	21
Perla Larva Proactive	62	11

Histological and biochemical analyses. Every week, twelve larvae or juveniles were sampled from each experimental group and preserved for histological analysis. The fish were anesthetized with MS-222, weighed to an accuracy of 0.01 mg, measured (LT) to an accuracy of 0.02 mm, preserved in Bouin's solution embedded in paraffin, and longitudinally cut into 5-μm sections using a microtome (Leica RM2265, Leica Microsystems, Nussloch, Germany). The preparations were stained with alcian blue/Schiff's reagent (Pearse, 1985).

Microscopic analyses were performed with a Nikon Eclipse 90i microscope connected to a Nikon Digital Sight DS-U1 camera (Nikon Corporation, Tokyo, Japan). Morphometric measurements were made using NIS-Elements AR 2.10 software (Nikon Corporation, Tokyo, Japan). For each fish, ten measurements of the nucleus, nucleolus, and cell volume of the exocrine pancreas were taken (n = 10 x 12).

At the end of experiment, eight fish were sampled from each group for measurement of digestive enzyme activity. The fish were frozen in liquid nitrogen and stored at -80ºC before analysis. Samples were homogenized in appropriate buffers, according to procedures described below, then centrifuged at 4ºC for 15 min at 15,000 x *g*. Enzyme samples were analyzed in triplicate. Spectrophotometric measurements were taken using an M501 Camspec spectrophotometer (Camspec Ltd., Sawston, UK).

Lipase activity (EC 3.1.1.3) was measured at 25°C using a specific substrate p-nitrophenyl palmitate (p-NPP) according to the method developed by Winkler and Stuckman (1979). Activity was expressed as μmoles of p-nitrophenol (p-NP) released over 1 min by a 1-g extract of the fish (U/g fish). α-amylase (EC 3.2.1.1) activity was measured using 3.5-dinitrosalicylic acid (DNS) at 30°C (Bernfeld, 1955). Activity was given as μmoles of maltose released over 1 min by a 1-g extract of fish (U/g fish). Trypsin (EC 3.4.4.4) activity was measured at 25°C using specific substrate α-N-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA), according to Erlanger et al. (1961). Activity was expressed as μmoles of p-nitroaniline released over 1 min by a 1-g extract of fish (U/g fish).

Statistical analysis. Results were subjected to statistical analysis using Statgraphics Plus 4.1 and Statistica 5 software. Arithmetic means and standard deviations were calculated for survival, total body length, wet body mass, morphometric parameters, and enzyme activity. Differences among groups were tested using one-way ANOVA and LSD test and considered significant at $p \leq 0.05$.

Results

Body mass, length, and survival are shown in Table 2. At the end of the experiment, the pancreas was visible as a small compact organ, enveloped by serosa, located behind the liver, between the anterior intestine and the glandular stomach. There were no differences in pancreas histology among treatments. Pancreatic cells showed centrally located large nuclei with distinct nucleoli (Fig. 1). Proenzyme granules were detected in the cytoplasm. The pancreatic ducts and blood vessel network were well developed. No adipose cells were found. There were no significant differences in cell or nucleolus volume (Table 3). Enzyme activity on the last day of the experiment is shown in Table 4.

Table 2. Total body length, wet body mass, and survival of fish fed different kinds of feed (mean±SD, n = 12).

	Wet body mass (g) on day...			*Total body length (mm) on day...*			*Survival (%)*
Feed	*7*	*14*	*21*	*7*	*14*	*21*	
Bio Kyowa	0.027±0.010^{ab}	0.069±0.027^{bc}	0.201±0.076^{b}	18.469±1.018^{a}	23.359±2.967^{a}	33.38±4.809^{b}	59.11±7.373^{b}
Aglo Norse	0.035±0.012^{c}	0.085±0.032^{a}	0.197±0.087^{b}	19.441±1.555^{b}	25.268±3.364^{a}	33.026±5.21^{b}	71.06±2.772^{a}
Perla	0.0201±0.004^{a}	0.072±0.029^{b}	0.086±0.034^{a}	17.984±0.609^{a}	23.700±2.936^{a}	25.548±3.104^{a}	57.2±4.038^{b}

Values in a column with different superscripts significantly differ ($p \leq 0.05$).

Discussion

Feeding is one of the most important issues in fish rearing. Larvae of some freshwater species, including bighead carp (*Aristchthys nobilis,* Richardson; Carlos, 1988) and some salmonids (Dabrowski, 1984), can be fed commercial starter diets without supplementation of live food. In other species, poor results in larvae fed artificial diets may be due to inappropriate nutrients in the diet, poor appetite, or defective digestion and absorption (Kolkovski and Dabrowski, 1999). Often, they result from incomplete development of the digestive tract (Ostaszewska, 2005). The histological picture of the exocrine pancreas can indicate the condition of larvae since there is distinct degeneration of pancreatic tissue and damage to the acinus structure in starved fish (Crespo et al., 2001). In our experiment, there were no such histological anomalies in the exocrine pancreas.

Differences in growth were related to the feed composition. Although deposition of fat in the pancreas indicates an excessive level of lipids in the feed (Ostaszewska et al., 2005), the group fed the Aglo Norse diet (with the highest fat level) showed no pancreas fattening and had the highest survival rate.

Levels of enzymatic activity obtained by different authors are difficult to compare due to different methods applied. Compared to other fish, lipase activity in juveniles of sterlet and other sturgeon species was low (German et al., 2004; Furne et al., 2005). Lipase reached its highest value in the group fed Aglo Norse, which contained the highest lipid level. Lipase activity significantly differed between the groups fed Bio Kyowa and Perla, although the difference between lipid contents in these diets was negligible (10 and 11%, respectively). These results show that the lipid content of the feed does not significantly affect lipase activity in juvenile sterlet, similar to results obtained for white sturgeon, *Acipenser transmontanus* Richardson (Buddington and Doroshov, 1986b).

Together with survival and growth rate measurements, monitoring of trypsin activity may be useful for evaluating feed quality and the nutritional status of fish (Drossou et al., 2006). In starved Japanese flounder (*Paralichthys olivaceus* Temminck and Schlegel) larvae and juveniles, there were drops in trypsin and amylase activity accompanied by a reduction of pancreas volume and partial necrosis of exocrine secretory cells (Gwak et al., 1999). Similarly, there was a decrease in amylolytic activity, followed by a drop of protease and lipase activities in starved Adriatic sturgeon, *Acipenser naccari* Bonaparte (Furne et al., 2008). Trypsin activity decreased in starved Nile tilapia (*Oreochromis niloticus*. L.) larvae (Drossou et al., 2006).

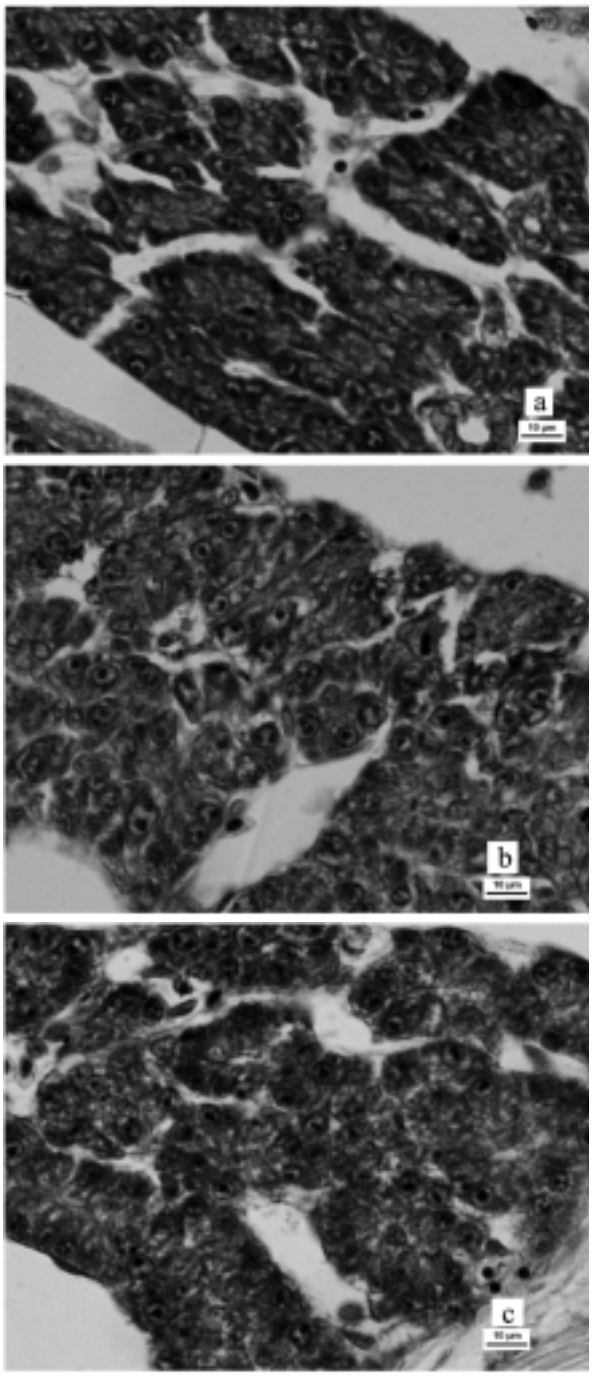

Fig. 1. Pancreas of sterlet juveniles fed diets with different fat and protein contents: (a) Bio Kyowa, (b) Aglo Norse, (c) Perla. Staining = alcian blue/Schiff's reagent (AB/PAS).

Table 3. Average volumes (mean±SD, n = 120) of nuclei, nucleoli, and exocrine pancreas cells in sterlet juveniles fed one of three commercial diets.

	Feed		
Avg volume (µm) of...	*Bio Kyowa*	*Aglo Norse*	*Perla*
Nucleus	70.32±21.12[b]	86.37±25.51[a]	84.74±22.34[a]
Nucleolus	5.09±1.99	5.28±2.51	5.57±2.41
Cell	569.65±225.18	575.61±247.35	524.02±202.42

Values in a row with different superscripts significantly differ ($p \leq 0.05$).

Table 4. Average enzyme activity (average±SD, n = 8) in sterlet juveniles fed different diets.

Enzyme activity (U/g fish)	*Feed*		
	Bio Kyowa	*Aglo Norse*	*Perla*
Lipase	0.042±0.015[a]	0.046±0.018[ab]	0.028±0.009[b]
Amylase	0.349±0.045[ab]	0.439±0.009[b]	0.247±0.043[a]
Trypsin	0.207±0.028[b]	0.127±0.047[a]	0.153±0.043[ab]

Values in a row with different superscripts significantly differ ($p \leq 0.05$).

Trypsin is a pancreatic enzyme, more important than chymotrypsin in the white sturgeon (Buddington and Doroshov, 1986c). The highest trypsin activity obtained in the present study was similar to that reported for carnivorous pricklebacks (*Stichaeidae*; German et al., 2004), herring larvae (*Clupea harengus* L.; Koven et al., 2002), and Atlantic sturgeon (*Salmo salar* L.; Sajjadi and Carter, 2004). The highest trypsin activity in our study was in fish fed Bio Kyowa, which had the lowest protein content. The protein content in feed did not affect the activity of proteolytic enzymes in spotted sorubim *Pseudoplatystoma corruscans* Spix and Agassiz 1829 (Lundstedt et al., 2004). A much lower trypsin activity occurred in fish fed Aglo Norse, which contains twice as much fat as Bio Kyowa. A high dietary fat level may reduce protein availability and proteolytic enzyme activity (Gawlicka et al., 2002; Schulz et al., 2007).

The pancreatic enzyme, α-amylase, catalyzes hydrolysis of starch and other polysaccharides. Low amylase activity suggests potential inability to digest carbohydrates in white sturgeon (Buddington and Doroshov, 1986a). Carnivorous fish such as sturgeons fed low-carbohydrate diets did not develop an effective carbohydrate digestion system (Hidalgo et al., 1999). Digestive enzyme activity was much higher in various species of pricklebacks (*Stichaeidae*) than in sterlet (German et al., 2004).

In summary, the Aglo Norse feed was the best of the three commercial diets used in the experiment. Fish fed Aglo Norse had the highest growth and survival rates.

Acknowledgement

The present study was financed from Scientific Research Committee funds, grant N 31103032/2256

References

Bernfeld P., 1955. Enzymes of carbohydrate metabolism. pp. 149-541. In: S.P. Colowick, N.O. Kapalan (eds.). *Methods in Enzymology*, vol. 1. Academic Press, New York.

Bisbal G.A. and D.A. Bengtson, 1995. Development of the digestive tract in larval summer flounder. *J. Fish Bio.*, 47:277-291.

Buddington R.K., 1985. Digestive secretions of lake sturgeon, *Acipenser fulvescens*, during early development. *J. Fish Biol*., 26:715-723.

Buddington R.K. and S.I. Doroshov, 1986a. Digestive enzyme complement of white sturgeon (*Acipenser transmontanus)*. *Comp. Biochem. Physiol*., 83A:561-567.

Buddington R.K. and S.I. Doroshov, 1986b. Development of digestive secretions in white sturgeon juveniles *Acipenser transmontanus*. *Comp. Biochem. Physiol*., 83A:233-238.

Buddington R.K. and S.I. Doroshov, 1986c. Structural and functional relations of the white sturgeon alimentary canal (*Acipenser transmontanus). J. Morph*., 190:201-213.

Carlos M.H., 1988. Growth and survival of bighead carp *Aristichthys nobilis* fry fed at different intake levels and feeding frequencies. *Aquaculture*, 68:267-276.

Crespo S., Marin de Mateo M., Santamaria C.A., Sala R., Grau A. and E. Pastor, 2001. Histopathological observations during larval rearing of common dentex *Dentex dentex* L. (Sparidae). *Aquaculture*, 192:121-132.

Dabrowski K., 1984. The feeding of fish larvae: present "state of the art" and perspectives. *Reprod. Nutr. Dev*., 24:807-833.

Dettlaff T.A. and B.F. Goncharof, 2002. Contribution of developmental biology to artificial propagation of sturgeon in Russia. *J. Appl. Ichthyol*., 18:266-270.

Drossou A., Ueberschar B., Rosenthal H. and K.H. Herzig, 2006. Ontogenic development of the proteolytic digestion activities in larvae of *Oreochromis niloticus* fed with different dirts. *Aquaculture*, 256:479-488.

Erlanger B., Kokowsky N. and W. Cohen, 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys*., 95:271-278.

Furne M., Hidalgo M.C., Lopez A., Garcia-Gallego M., Morales A.E., Domezain A., Domezaine J. and A. Sanz, 2005. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture*, 250:391-398.

Furne M., Garcia-Gallego M., Hidalgo M.C., Morales A.E., Domezain A., Domezain J. and A. Sanz, 2008. Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccari*) and trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol*., 149A:420-425.

Garling D.L. and R.P. Wilson, 1976. Optimum dietary protein to energy ratios for channel catfish fingerlings, *Ictalurus punctatus*. *J. Nutr*., 106:1368-1375.

Gawlicka A., Herold M.A., Barrows F.T., de le Noue J. and S.S.O. Hung, 2002. Effect of dietary lipids on growth, fatty acid composition, intestinal absorption and hepatic storage in white sturgeon (*Acipenser transmontanus* R.) larvae. *J. Appl. Ichthyol*., 18:673-681.

German D.P., Horn M.H. and A. Gawlicka, 2004. Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: *Sticchaeidae*): ontogenetic, dietary, and phylogenetic effects. *Physiol. Bioch. Zool*., 77:789-804.

Gwak W.S., Seikai T. and M. Tanaka, 1999. Evaluation of starvation status of laboratory reared Japanese flounder *Paralichthys olivaceus* larvae and juveniles based on morphological and histological characteristics. *Fish. Sci*., 65:339-346.

Hidalgo M.C., Urea E. and A. Sanz, 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, 170:267-283.

Kaushik S.J., Dabrowski K. and P. Luquet, 1982. Patterns of nitrogen excretion and oxygen consumption during during ontogenesis of common carp (*Cyprinus carpio*). *Can. J. Fish. Aquatic Sci*., 39:1095-1105.

Kolkovski S. and K. Dabrowski, 1999. Diets for fish larvae - present state of art. pp. 406. In: *World Aquac. Symp.*, World Aquac. Soc., Sydney, Australia.
Koven W., Rojas-Garcia C.R., Finn R.N., Tandler A. and I. Roonestad, 2002. Stimulatory effect of ingested protein and/or free amino acids on the secretion of the gastro-endocrine hormone cholecystokinin and tryptic activity, in early-feeding herring larvae, *Clupea harengus*. *Mar. Biol*., 140:1241-1247.
Lundstedt L.M., Melo J.F.B. and G. Moraes, 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. *Comp. Biochem. Physiol*., 137B:331-339.
Ostaszewska T., 2005. The morphological and histological development of digestive tract and swim bladder in early organogenesis of pike-perch larvae (*Stizostedion lucioperca* L.) in different rearing environments. *Treatises and Monographs*, Warsaw Agriculture Univ. (in Polish).
Ostaszewska T., Dabrowski K., Palacios M.E., Olejniczak M. and M. Wieczorek, 2005. Growth and morphological changes in digestive tract of rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins. *Aquaculture*, 245:273-286.
Pearse A.G.E., 1985. *Histochemistry. Theoretical and Applied, vol. 2. Analytic Technology*. Churchill Livingstone, New York.
Sajjadi M. and C.G. Carter, 2004. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L.). *Aquac. Nutr*., 10:135-142.
Schulz C., Bohm M., Wirth M. and B. Rennert, 2007. Effect of dietary protein on growth, feed conversion, body composition and survival of pike perch fingerlings (*Sander lucioperca*). *Aquac. Nutr*., 13:373-380.
Wegner A., 2008. *The Morphological Changes in Digestive Tract of Sterlet Acipenser Ruthenus (L.) Larvae during Endogenous and Exogenous Stage of Feeding*. Ph.D. Thesis, Warsaw Univ. Life Sciences, Warsaw (in Polish).
Wegner A., Ostaszewska T. and M. Kamaszewski, 2008. Morphological change in digestive tract of sterlet (*Acipenser ruthenus*, L.) larvae during yolk feeding. pp. 685-686. In: *Aquaculture Europe 2008*. Sep. 15-18, Krakow.
Williot P., Sabeau L., Gessner J., Arlati G., Bronzi P., Gulyas T. and P. Berni, 2001. Sturgeon farming in Western Europe: recent developments and perspectives. *Aquat. Living Resour*., 14:367-374.
Winkler U.K. and M. Stuckman, 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bacteriol*., 138:663-670.
Zambonino Infante J.L. and C.L. Cahu, 2001. Review. Ontogeny of the gastrointestinal tract of marine fish larvae. *Comp. Biochem. Physiol*., 130C:477-487.
Zoltowska K., Kolman R., Lopienska E. and H. Kolman, 1999. Activity of digestive enzymes in Siberian sturgeon juveniles (*Acipenser baeri* Brandt) - a preliminary study. *Arch. Pol. Fish*., 7(1):201-211.