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# The effect of feeding on aminopeptidase and non-specific esterase activity in the digestive system of pike-perch (*Sander lucioperca* L.)

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**Abstract:** *The effect of feeding on aminopeptidase and non-specific esterase activity in the digestive system of the pike-perch (Sander lucioperca L.).* The pike-perch (*Sander lucioperca* L.) at the age of 18 days were fed for 21 days using three different diets: Aglo Norse (An), casein-gelatin (Cas), cod meal with gelatin (Mac) and nauplius *Artemia salina* (Art – control diet). On the last day of the experiment, fish fed Art and An diets had the statistically significant highest body mass, length, and survival. On the last day, the highest aminopeptidase activity in the anterior intestine and posterior intestine was registered in fish fed nauplius *Artemia salina*. The lowest activity of this enzyme in the anterior intestine was to be found in fish fed with Cas diet, while there was no difference between among groups in the posterior intestine. The non-specific esterase activity was registered in the stomach, liver, anterior intestine as well as in the posterior intestine. The lowest activity of this enzyme in the stomach was observed in the pike-perch fed with the Cas diet. In the anterior intestine, the highest activity was registered in fish fed with Art, and the lowest – on the Cas and Mac diet. The results of the current research prove that feeding the pike-perch An diet has a positive effect of the survival of the pike-perch, their growth rate and the activity of the enzymes. By contrast, feeding pike-perch Cas and Mac diets did not satisfy nutritional needs of fish, resulting in their low survival, growth rate and low activity of the enzymes examined.

*Key words*: pike-perch, *Sander lucioperca*, aminopeptidase, non-specific esterase, histochemistry

## INTRODUCTION

Considering a dynamic development of aquaculture in the world, it becomes necessary to increase the production of fish fodder, to improve its quality and to look for cheaper, alternative protein sources (Hardy 1996). At present, the most common and the best source of protein for fodder production is fish meal. However, the prices getting higher and interest growing bigger makes us search for alternative protein sources to substitute fish meal in fodder (Hardy 1996). The most frequent sources are vegetable products and various food industry by-products. This is why research is done on introducing different protein sources to fodder, sources like soybean meal, casein, gluten and others (Ostaszewska et al. 2005a, Kamaszewski et al. 2010, Kamaszewski et al. 2013). However, alternative protein sources often have an unbalanced amino acid pattern and have endogenous dietary factors, which may lead to a decrease in growth rates as well as lesions in the digestive system of fish (Ostaszewska et al. 2005a, Ostaszewska et al. 2010).

The Percidae, among them the pike-perch (*Sander lucioperca*) are popular in aquaculture. Notwithstanding this, cannibalism, high death rate or poor uptake of artificial diets constitute a serious problem in breeding the pike-perch in recirculating aquafarming systems (Ostaszewska et al. 2005b, Szczepkowski et al. 2011). Therefore research is done to optimize feeding especially the earliest life stages of the pike-perch and to examine the physiology of the fish digestive system. To this end, studies are ongoing where digestive enzymes during ontogeny are observed, their occurrence, location and activity. This information can be helpful when time indications are made when to launch a diet feeding the species and whether the diet meets nutritional requirements of the species given (Kamaszewski et al. 2010).

The aim of this research was to define the influence of various diets on the survival, growth rate and distribution as well as the activity of aminopeptidase M, exopeptidase from intestine, and non-specific esterase, enzyme related in lipid metabolism, in the digestive system of juvenile pike-perch.

## MATERIAL AND METHODS

The experiment was conducted in the Department of Ichthyobiology and Fisheries, Warsaw University of Life Sciences. Juvenile stages of pike-perch, at the age of 18-day post hatching (total body lenght 18.59 ±1.68 mm; body weight 0.05 ±0.01 g) were breeding for three weeks (from 18$^{th}$ to 39$^{th}$ day after hatching). Fish were stocked at a density of 5 individuals per litre in water recirculation system equipped with a biological filter and UV lamps. The water temperature was 20.6 ±0.9°C, pH 7.5–8.1, and the content of total ammonia nitrogen did not exceed the level of 0.1 mg/L, and the nitrite – 0.01 mg/L. The 14 h light: 10 h dark photoperiod was applied. Aquariums were illuminated with poor light (100 lx). The experiment was conducted in four nutritional groups, five repetitions each. Fish were fed every two hours using following diets: commercial diet Aglo Norse – An (Larvae Feed Ewos – Bergen, Norway), two experimental Casein-gelatin diets – Cas (Ostaszewska et al. 2005a) and cod meal-gelatin diet – Mac (Kamaszewski and Ostaszewska 2013). The control group was fed *Artemia salina* nauplii *ad libidum*. The protein and lipid content is given in the Table 1. In the first week of the experiment, fish were given a feed ration representing 50% of their biomass, in the second week 15% and in the third – 10%.

TABLE 1. The content of protein (%) and lipids (%) in diets used in the experiment (according to the manufacturer's data)

| Diet | Protein (%) | Lipid (%) |
|---|---|---|
| *Artemia salina* (Art) | 50 | 10 |
| Aglo Norse (An) | 59 | 21 |
| Casein-gelatin (Cas) | 48 | 11 |
| Cod meal-gelatin (Mac) | 64 | 8 |

To conduct histochemical examinations, 10 fish were sampled from each group on the first and on the last day of the examination. Fish were anesthetized using MS-222 preparation (tricaine methanesulphonate, Sigma-Aldrich, Munich, Germany), weighed to the nearest 0.01 g (body weight, BW), measured

in total length (LT) to 0.02 cm and frozen with liquid nitrogen. The research material was stored at –80°C until it was analyzed. Samples were cut to a thickness of 10 μm using a cryostat (Leica CH 1900, Leica Microsystems, Nussloch, Germany). The activity of aminopeptidase M (membrane alanyl aminopeptidase, EC 3.4.11.2) was detected using a method according to Nachlas et al. using a substrate L-LeucineβNaphthyl-amide (Sigma) in 0.1 M phosphate buffer of pH 7.0 (Lojda et al. 1979). The activity of non-specific esterase (EC 3.1.1) was detected using a method according to Gomori using a substrate 1-Naphthyl acetate (Sigma) in 0.1 M phosphate buffer of pH 7.4 (Lojda et al. 1979). The activity of enzymes on histochemical preparations was marked on the grounds of the intesity of coloration. In research was using scale, where (+++) means a very strong histochemical reaction (90–100% area of cell with a positive reaction), (++) means a strong histochemical reaction (60–90% area of cell with a positive reaction), (+) means a moderate histochemical reaction (40–60% area of cell with a positive reaction), (+/-) means a weak reaction (0–40% area of cell with a positive reaction) and (-) means no reaction on preparations examined. Microscopic examinations and photographs were made using a microscope Nikon Eclipse 90i and a co-operating camera Nikon Digital Sight DS-U1 (Nikon Corporation, Tokyo, Japan).

The average as well as the standard deviation for the survival, total length and weight of the fish were calculated using a program Statistica ver. 10.0. Differences between the groups were tested using one-way ANOVA and Tukey's (HSD) post hoc test ($P \leq 0.05$).

## RESULTS AND DISCUSSION

The highest survival rate in the experiment was observed in fish fed with nauplii *Artemia salina* (65.5 ±14.8%), while the lowest rate was shown in the fish fed with Mac (35.4 ±16.4%), and the differences were statistically significant (Table 2). On the twenty first day of the experiment fish fed with *Artemia salina* and An diet had a statistically significant higher total body length and body weight in comparison to fish fed experimental diets Cas and Mac (Table 2).

When breeding a pike-perch in aquaculture, it is a common practice to feed the earliest stages of this species with nauplii *Artemia salina*, and only 17–19 days after hatching feed them artificial diets. It is a period when the digestive system of a pike-perch is fully

TABLE 2. Survival (%), total length, and body weight of pike-perch fed with experimental diets on twenty first day of the experiment (mean ±SD, *n* = 10)

| Growth parameters | Diets | | | |
|---|---|---|---|---|
| | Art | An | Cas | Mac |
| Survival (%) | 65.5 ±14.8$^{a}$ | 62.3 ±7.7$^{a}$ | 43.9 ±18.1$^{ab}$ | 35.4 ±16.4$^{b}$ |
| Total body length (mm) | 43.2 ±1.9$^{a}$ | 42.6 ±2.7$^{a}$ | 26.2 ±2.5$^{b}$ | 28.5 ±2.6$^{b}$ |
| Body weight (g) | 0.61 ±0.08$^{a}$ | 0.58 ±0.11$^{a}$ | 0.11 ±0.03$^{b}$ | 0.14 ±0.04$^{b}$ |

Means with different letter superscripts in the same row are significantly different ($P \leq 0.05$).

differentiated and becomes capable of digesting and absorbing nutrient contents from diets (Ostaszewska 2005). According to Ljunggren et al. (2003) and Ostaszewska et al. (2005b), a fodder that meets the nutritional requirements of juvenile pike-perch stages is the Aglo Norse diet (An). It was also proven in the current research that fish fed the An diet had the quickest growth rate as well as the greatest survival rate among fish fed on experimental diets.

Histochemical analysis revealed the presence of aminopeptidase M in the brush border and in the supranuclear enterocyte area of the anterior and posterior intestine in the pike-perch of all nutritional groups on the first and the last day of the experiment (Fig. 1). As with the examined fish, the presence of aminopeptidase in the anterior and posterior intestine was observed in other species of fish (Segner et al. 1989, Tengjaroenkul et al. 2000). On the last day of the experiment an elevated aminopeptidase M grade was reported in all the nutritional groups in comparison to the first day of the experiment (Table 3), as in Segner et al. (1989) and Gisbert et al. (1999). On the last day of the experiment there was a very strong reaction of aminopeptidase M in the brush border of the anterior intestine in fish fed with nauplii *Artemia salina*, while the weakest reaction was registered in fish fed Cas diet (Table 3).

In the posterior intestine the activity of aminopeptidase M was weaker comparing to the anterior intestine (Table 3). On the last day of the experiment a strong reaction of aminopeptidase M in the brush border of the anterior intestine in fish fed with nauplii *Artemia salina* was observed, while in other groups the activity of the enzyme experienced no difference and showed the same level as on day one (Table 3). Influence the feeding had on the aminopeptidase M activity in juvenile pike-perch stages was observed in the brush border of the anterior intestine. High activity of this enzyme was present in specimen fed with nauplii *Artemia salina*. As in Segner et al. (1989) it was proven that the larvae of European whitefish (*Coregonus lavaretus*) fed with zooplankton showed higher aminopeptidase M activity than fish fed with commercial fodder. The lowest activity of aminopeptidase M in the brush border of the anterior intestine was to be observed in the pike-perch fed with Cas diet. The low activity of the enzyme can be a proof that the Cas diet does not meet nutrititional requirements of the developing pike-perch (Kamaszewski and Ostaszewska 2013).

The presence of non-specific esterase was reported in the gastric epithelium, gastric glands, anterior intestine, posterior intestine and liver of the pike-perch. A similar location of this enzyme in various fish species was described by many authors (Hirji and Courtney 1983, Gawlicka et al. 1995, Gisbert et al. 1999, Kozarić et al. 2006).

On the first day of the experiment the gastric epithelium was reported to show a non-specific esterase activity on a modest level, and the gastric glands – on a weak level (Fig. 1; Table 3). The activity expression of non-specific esterase in gastric epithelium maintained a steady level in all the nutritional groups excluding the Cas group, where the activity of the enzyme declined on the last day of the experiment (Table 3). On the last day, the activity level of the enzyme

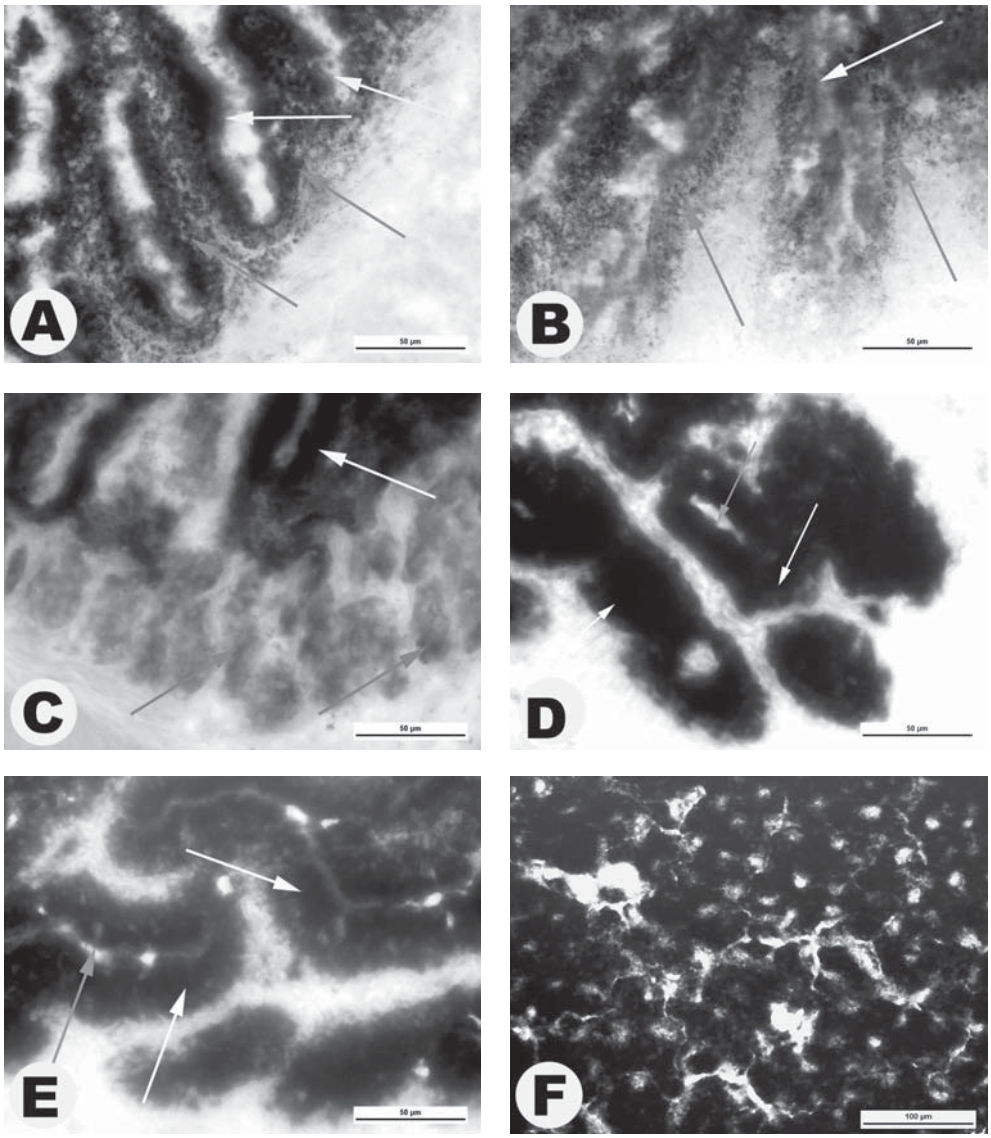


FIGURE 1. Localization of the digestive enzymes in the cross-section of pike-perch gastrointestinal tract. Aminopeptidase M location: in the striated border (white arrows) and in the supranuclear area enterocyte (grey arrows) of the anterior intestine (A) and posterior intestine (B). Non-specific esterase location: in the gastric epithelium (white arrows) and in gastric glands (grey arrows) (C), in the enterocyte cytoplasm (white arrows) and in the striated border (grey arrows) of the anterior intestine (D) and posterior intestine (E). Location of non-specific esterase in hepatocyte cytoplasm (F)

TABLE 3. Location and activity of aminopeptidase M and non-specific esterase in gastric epithelium and gastric glands, anterior and posterior intestines and liver hepatocydes of the pike-perch fed with: nauplii *Artemia salina* (Art), Aglo Norse (An), casein-gelatin (Cas), cod meal with gelatin (Mac) on the first and the last day of the experiment

| Enzyme | Distribution of enzymes | | 1st day of experiment | 21st day of experiment | | | |
|---|---|---|---|---|---|---|---|
| | | | | Art | An | Cas | Mac |
| Aminopeptidase M | Anterior intestine | Brush border | + | +++ | ++ | + | ++ |
| | | Supranuclear area of enterocyte | +/- | ++ | + | + | + |
| | Posterior intestine | Brush border | +/- | ++ | +/- | +/- | +/- |
| | | Supranuclear area of enterocyte | +/- | + | + | + | + |
| Non-specific esterase | Stomach | Epithelium | + | + | + | +/- | + |
| | | Gastric glands | +/- | + | + | + | + |
| | Anterior intestine | Brush border | ++ | ++ | + | +/- | +/- |
| | | Supranuclear area of enterocyte | +++ | ++ | ++ | ++ | ++ |
| | Posterior intestine | Brush border | + | + | ++ | + | + |
| | | Supranuclear area of enterocyte | ++ | ++ | ++ | ++ | ++ |
| | Liver | | +++ | +++ | +++ | +++ | +++ |

was higher in gastric glands in all the nutritional groups in comparison to the first day (Table 3). The non-specific esterase activity increase in gastric glands can be a sign of the stomach being properly developed and carrying out all physiological functions (Gawlicka et al. 1995).

In the anterior and posterior intestine, the non-specific esterase was located in the brush border of the anterior intestine and in the supranuclear area of enterocyte (Fig. 1). On the first day of the experiment a weaker reaction of non-specific esterase in the brush border was reported, while the activity was higher in the supranuclear area of enterocyte of the anterior and posterior intestine (Table 3). In comparison to the first day of the experiment, there was a moderate activity of the enzyme in the brush border in the pike-perch fed with An diet, and a low activity in the specimen fed with Cas and Mac (Table 3). On the last day of the experiment, the non-specific esterase activity in the supranuclear area of enterocyte declined slightly in all nutritional groups (Table 3). The non-specific esterase activity in the enterocyte brush border of the anterior intestine in fish fed with nauplii *Artemia salina* stayed high from the first to the last day of the experiment (Table 3). In all the other nutritional groups the activity decreased (Table 3). In the brush border of the posterior intestine, the non-specific esterase activity on the last day of the experiment was low, as it was on the first day except for fish fed with An diet, where the activity was slightly higher (Table 3). On the last day of the experiment the

non-specific esterase activity in the supranuclear area of enterocyte of the posterior intestine was on a similar level in all the nutritional groups and did not change compared to the first day of the experiment (Table 3). The non-specific esterase in the pike-perch showed a higher activity in the supranuclear area of enterocyte compared to other fish species (Segner et al. 1989, Baglole et al. 1998, Tengjaroenkul et al. 2000, Kozarić et al. 2006). The non-specific esterase induces and supports the process of pinocytosis (Ribeiro et al. 1999), a mechanism of digesting and absorbing nutrients in the intestine. The non-specific esterase showed signs of lower activity in the anterior and midgut intestine of starving fish (Baglole et al. 1998). The pike-perch fed with Cas and Mac diets were reported to experience a lower non-specific esterase activity in the brush border of the anterior intestine compared to specimen fed with An and Art. The decrease in the activity was probably due to the fact that Cas and Mac diets do not meet the nutritional requirements of the developing fish.

The non-specific esterase activity in liver was on a similar level on the first and on the last day of the experiment (Table 3). High enzyme expression occured in hepatocyde cytoplasm (Fig. 1). The high activity of the enzyme is associated with intensive lipid and carbohydrate metabolism ongoing in the hepatocyde cytoplasm (Gawlicka et al. 1995, Kozarić et al. 2006).

## CONCLUSIONS

The results of present studies prove that feeding the pike-perch An diet has positive effects on their survival, growth rates and enzyme activity. By contrast, casein-gelatin and cod meal-gelatin diets did not meet the nutritional requirements of the pike-perch, resulting in low survival rate, slow growth rates and low activity of the enzymes examined.

## REFERENCES

BAGLOLE C.J., GOFF G.P., WRIGHT G.M., 1998: Distribution and ontogeny of digestive enzymes in larval yellowtail and winter flounder. J. Fish Biol. 53, 4, 767–784.

GAWLICKA A., TEH S.J., HUNG S.S.O., HINTON D.E., de la NOÜE J., 1995: Histological and histochemical changes in digestive tract of white sturgeon larvae during ontogeny. Fish Physiol. Bioch. 14, 5, 357–371.

GISBERT E., SARASQUETE M.C., WILLIOT P., CASTELLÓ-ORVAY F., 1999: Histochemistry of the development of the digestive system of Siberian sturgeon during early ontogeny. J. Fish Biol. 55, 3, 595–616.

HARDY R.W., 1996: Alternate protein sources for salmon and trout diets. Anim. Feed Technol. 59, 1, 71–80.

HIRJI K.N., COURTNEY W.A.M., 1983: Non-specific carboxylic esterase activity in the digestive tract of the perch, *Perca fluviatilis* L. J. Fish Biol. 22, 1, 1–7.

KAMASZEWSKI M., OSTASZEWSKA T., 2013: The effect of feeding on morphological changes in intestine of pike-perch (*Sander lucioperca* L.). Aquacult. Int. DOI 10.1007/s10499-013-9693-y.

KAMASZEWSKI M., NAPORA-RUTKOWSKI Ł., OSTASZEWSKA T., 2010: Effect of feeding on digestive enzyme activity and morphological changes in the liver and pancreas of pike-perch (*Sander lucioperca*). Isr. J. Aquacult. – Bamidgeh. 62, 4, 225–236.

KAMASZEWSKI M., PRASEK M., OSTASZEWSKA T., DABROWSKI K., 2013: The influence of feeding diet containing wheat gluten supplemented with dipeptides or free amino acids on structure and development of the skeletal muscle of carp (*Cyprinus carpio*). Aquacult. Int. DOI 10.1007/s10499-013-9683-0.

KOZARIĆ Z., KUŽIR S., PETRINEC Z., GJURČENIĆ E., OPAČAK A., 2006: Histochemical distribution of digestive enzymes in intestine of goldline, *Sarpa salpa* L. 1758. J. Appl. Ichthyol. 22, 1, 43–48.

LJUNGGREN L., STAFFAN F., FALK S., LINDEN B., MENDERS J., 2003: Weaning of juvenile pikeperch, *Stizostedion lucioperca* L., and perch, *Perca fluviatilis* L., to formulated feed. Aquacult. Res. 34, 4, 281–287.

LOJDA Z., GOSSRAN R., SCHEBLER T.H., 1979: Enzyme histochemistry. A laboratory manual. Springer-Verlag, Berlin, Heidelberg, New York.

OSTASZEWSKA T., 2005: Developmental changes of digestive system structure in pike-perch (*Sander lucioperca* L.). Electr. J. Ichthyol. 2, 65–78.

OSTASZEWSKA T., DABROWSKI K., PALACIOS M.E., OLEJNICZAK M., WIECZOREK M., 2005a: Growth and morphological changes in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins. Aquaculture 245, 1–4, 273–286.

OSTASZEWSKA T., DABROWSKI K., CZUMIŃSKA K., OLECH W., OLEJNICZAK M., 2005b: Rearing of pike-perch larvae using formulated diets – first success with starter feeds. Aquacult. Res. 36, 12, 1167–1176.

OSTASZEWSKA T., DABROWSKI K., KAMASZEWSKI M., GROCHOWSKI P., VERRI T., RZEPKOWSKA M., WOLNICKI J., 2010: The effect of plant protein based diet, supplemented with dipeptide or free amino acids on PepT1, PepT2 expression and digestive tract morphology in common carp (*Cyprinus carpio* L.). Comp. Bioch. Physiol. Part A. 157, 2, 158–169.

RIBEIRO L., SARASQUETE C., DINIS M.T., 1999: Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup, 1858) larvae. Aquaculture 171, 3–4, 293–308.

SEGNER H., RÖSCH R., SCHMIDT H., von POEPPINGHAUSEN K.J., 1989: Digestive enzymes in larval *Coregonus lavaretus* L. J. Fish Biol. 35, 2, 249–263.

SZCZEPKOWSKI M., ZAKĘŚ Z., SZCZEPKOWSKA B., PIOTROWSKA I., 2011: Effect of size sorting on the survival, growth and cannibalism in pikeperch (*Sander lucioperca* L.) larvae during intensive culture in RAS. Czech J. Anim. Sc. 56, 11, 483–489.

TENGJAROENKUL B., SMITH B.J., CACECI T., SMITH S.A., 2000: Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. Aquaculture 182, 3–4, 317–327.

**Streszczenie:** *Wpływ żywienia na aktywność aminopeptydazy i niespecyficznej esterazy w układzie pokarmowym sandacza (Sander lucioperca L.).* Sandacze (*Sander lucioperca* L.) w wieku 18 dni były żywione przez 21 dni trzema dietami: Aglo Norse (An), kazeina-żelatyna (Cas), mączka z dorsza z żelatyną (Mac) i naupliusami *Artemia salina* (Art – dieta kontrolna). Ostatniego dnia doświadczenia ryby żywione Art i An miały statystycznie istotnie większą masę i długość ciała oraz przeżywalność. Ostatniego dnia doświadczenia najwyższą aktywność aminopetydazy w jelicie przednim i tylnym stwierdzono u ryb żywionych naupliusami *Artemia salina*. Najniższą aktywność tego enzymu w jelicie przednim stwierdzono u ryb żywionych dietą Cas, w jelicie tylnym zaś nie stwierdzono różnic między grupami doświadczalnymi. Aktywność niespecyficznej esterazy stwierdzono w żołądku, wątrobie, jelicie przednim i tylnym. Najniższą aktywność tego enzymu w żołądku obserwowano u sandaczy żywionych dietą Cas. W jelicie przednim najwyż-

szą aktywność stwierdzono u ryb żywionych Art, natomiast najniższą u ryb żywionych Cas i Mac. Wyniki obecnych badań potwierdzają, że żywienie sandaczy dietą An korzystnie wpływa na przeżywalność, tempo wzrostu ryb i aktywność enzymów. Żywienie sandaczy dietami Cas i Mac powodowało natomiast niską przeżywalność ryb oraz tempo wzrostu, a także niską aktywność badanych enzymów.

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