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The Effect of Different First Feeds on Proteolytic Activity of the Northern Pike, *Esox lucius* Linneaus 1758, Post-Larvae

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Abstract

The Northern Pike, *Esox lucius*, post-larvae were fed with trout micro diet, *Artemia* nauplii and plankton at the first ten days after yolk - sac absorption and the proteolytic activity of the post-larvae were measured. According to the results, the proteolytic activity was significantly affected by the different type of first feed ($P<0.05$). The highest protease activities were obtained on 2nd day in *Artemia* (228.22±15.44 U/mg) and plankton (302.78±45.85 U/mg) groups, and on 3rd day in micro diet (202.05±19.79 U/mg) group. After this point, the enzymatic activity started to decrease in all groups. Thereafter, the conspicuous increase in protease activities of larvae was determined on the day 8th in *Artemia* and plankton group, but on the day 9th in micro diet group ($P<0.05$). In contrast to other feeding groups, it is observed that feeding pike post-larvae with plankton caused more positive effect on proteolytic activity than the other groups.

Keywords: Northern pike, ontogeny, post-larvae, first feeding, proteolytic activity.

Turna Balığı, *Esox lucius* Linneaus 1758, Post-Larvalarının Proteolitik Aktivitesi Üzerine Farklı İlk Beslemelerin Etkisi

Özet

Turna, *Esox lucius*, balığı post-larvaları besin keselerinin tükenmesi takiben ilk on günlük dönemde alabalık mikro diet yemi, *Artemia* nauplii ve plankton ile beslenmiş ve post-larvaların proteolitik aktivitesi ölçülmüştür. Çalışmanın sonucunda, farklı tip besinlerin proteolitik aktivite üzerinde istatistiki açıdan önemli bir etkisinin olduğu tespit edilmiştir ($P<0,05$). Proteaz aktivitesinde ölçülen en yüksek değerler *Artemia* (228,22±15,44 U/mg) ve plankton (302,78±45,85 U/mg) gruplarında 2. günde, mikro diyet (202,05 ± 19,79 U/mg) grubunda ise 3. günde elde edilmiştir. Bu noktadan sonra, tüm gruplardaki proteaz aktivitesi düşmeye başlamıştır. Daha sonra, proteaz aktivitesinin 8. günde *Artemia* ve plankton gruplarında, 9. günde ise mikro diyet grubunda bariz bir şekilde arttığı belirlenmiştir. Diğer besinlerle karşılaştırıldığında, turna balığı post-larvalarını plankton ile beslemenin proteolitik aktivite üzerine daha olumlu etki yaptığı gözlenmiştir.

Anahtar Kelimeler: Turna balığı, larval gelişim, post-larva, ilk besleme, proteolitik aktivite.

Introduction

Pike is one of highly valuable carnivorous freshwater fish species in all over the world. It is used as a very effective bio-meliorator in the commercial freshwater polyculture systems. On the other hand, pike is very popular fish species for anglers. Although it's artificial propagation technics are well known (Szabo, 2008; Gokcek *et al*., 2012a), larval nutritional requirements are still obscure. On the other hand, its intensive culture is still hindered by the high rate of cannibalism which starts at early larval stage (Kucska *et al*., 2006).

Identification of digestive enzyme activity of an alternative fish species is very important to understand the nutritional requirement of larvae. After hatching, the intestine looks like a simple tube. Thus, digestive capacity is directly related with available enzymes in digestive tract during larval stage. Studies on enzyme activities can explain nutritional physiology and help resolve nutritional problems, such as digestibility of an artificial diet. One of the most important problems is to establish of the nutritional requirement of a candidate species for commercial production. Hofer and Köck (1989) suggested that, from the profile of the digestive enzymes, it is possible to predict the ability of a species to use different nutrients (Noori *et al*., 2012).

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As known, after absorption endogenous energy sources, larvae need adequate exogenous energy sources. After hatching of pike larvae in commercial hatcheries, fish are fed with plankton (especially with *Rotatoria* species and *Copepod*s) for one week and then they are stocked to the earthen fertilized ponds for nursery in Hungary (Szabo, 2008). In advance of stocking them to the earthen ponds, the hatchery owners have limited time to sell their products in market. It is clear that to have healthier product means easy to sell them with good price. Although proteolytic enzyme activity of starved pike larvae were already presented (Gökçek *et al*., 2012b), the effect of different first feeds on digestive enzyme activity is still unknown. Thus, the aim of this study was to determine whether pike larvae can alter their digestive capacity in response to dietary changes during early developmental stages and to reveal whether live *Artemia* nauplii or micro diet starter feed provide any significant contribution in proteolytic activity.

Material and Methods

Source of Larvae and Experimental Design

Pike larvae were produced from broodstock in captivity in Dinnyés Fish Farm, Hungary. Routine procedure of artificial propagation was applied for fertilization (Szábo, 2008). After hatching, larvae were transferred to the research unit of the Department of Fish Culture at Szent Istvan University, Gödöllö, Hungary. Post-larvae (50 ind/L) were stocked into 20 L aquarium with aeration and the water temperature was 10±1°C during experiment. Larvae were fed with *Artemia* nauplii (Utah, Salt Lake), plankton (collected vertically from an earthen fish pond in Isaszeg, Hungary) and trout micro diet (Coppens, Nederland) feed 8 times per day. Half an hour later of feeding activity, about 500 mg larvae were sampled into 3 ml eppendorf tubes without water and freezed in -80°C refrigerator for ten days after yolk sac absorption. Until protease analysis, samples were kept into -80°C refrigerator.

Sampling and Analytical Procedure

Pike larvae were randomly collected for 10 times, and samples were rinsed in distilled water and stored at −80°C until protease analyses. Extracts of pike larvae were prepared by homogenization followed by centrifugation (16000 g, 30 min, 4°C).

Total protease activities of pike larvae was measured as described by Walter (1984), using casein (10 mg ml^{-1}) as the substrate in 50 mM Tris-HCl buffer at pH 8. The mixtures including extracts of larvae were incubated with the substrate and then the reaction was stopped by addition of 500 µl trichloro acetic acid (TCA) (120 g L^{-1}). The absorbance was recorded at 280 nm. One unit of enzyme activity was defined as 1 µg of tyrosine released per minute. All measurements were carried out in triplicate. The soluble protein concentrations of pike larvae were determined according to Bradford (1976).

Statistical Methods

Data were analyzed by using SPSS statistical software (SPSS, 17.0). Data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Bartlett's test) prior to their comparison. Comparisons were made using a one-way ANOVA test. Then, Post-Hoc Duncan multiple-comparison test was used for significant differences at the $P<0.05$ level.

Results

Protease activities of pike post-larvae are summarized in Table 1 and the chemical composition of first feeds were given in Table 2. The daily differences observed among protease activities of larvae fed different first feed were statistically significant ($P<0.05$). The protease activity of larvae suddenly increased to the maximum value on 2nd day in Artemia (228.22±15.44 U/mg) and plankton (302.78±45.85 U/mg) group, whereas the same situation was observed on 3rd day in micro diet (202.05±19.79 U/mg) group. Then, the enzyme activity slightly started to decrease in all groups. Moreover, the conspicuous increase in protease activities of larvae was determined on the day 8th in Artemia and plankton group, but on the day 9th in micro diet group ($P<0.05$).

Discussion

The digestive enzyme activity is generally used as an indicator of larval food acceptance and to some extent can serve as an indicator for digestive capacity in relation to the type of feed offered (Nolting *et al*., 1999; Zambonino Infante and Cahu, 2001; Suzer *et al*., 2007). Gökçek *et al*. (2012b), measured the proteolytic activity of starved pike larvae from yolk-sac absorption to the death point at relatively high incubation temperature (13°C). The maximum proteolytic activity was measured on the 2nd day after yolk-sac absorption (595.22±9.07 U/mg protein) and then enzyme activity started to decrease. Similarly, the protease activity reached to maximum values on 2nd day in Artemia and plankton group and on 3rd day in the larvae fed with micro diet group in this study. Ribeiro *et al*. (1999), reported an increase in the enzymatic activity during the first ten days after hatching of *Solea senegalensis*, followed by a decrease. Then, a strong increase was observed probably due to developing of brush border membranes enterocytes, which occurred concurrently with a decrease in cytosolic enzyme, leucine-alanine peptidase (Kolkovski, 2001). In the present study, this

Table 1. Protease activity of pike post-larvae fed different feed

	Protease (U/mg)		
Day	*Artemia nauplii*	Plancton	Micro diet
1	158.36±10.88[a]	148.21±9.86[a]	96.97±9.13[b]
2	228.22±15.44[a]	302.78±45.85[b]	168.82±21.90[c]
3	170.71±36.67[a]	233.21±24.96[b]	202.05±19.79[a]
4	142.00±19.18[a]	219.9±55.94[b]	154.91±1.73[a]
5	97.36±2.02[a]	150.6±47.07[b]	161.83±59.80[b]
6	81.30±3.43[a]	115.58±12.21[b]	47.17±2.85[c]
7	79.81±3.72[a]	88.10±8.89[ab]	90.17±1.31[b]
8	117.81±19.51[a]	105.26±18.20[a]	92.21±52.36[a]
9	91.71±18.24[a]	81.91±23.44[a]	120.31±6.39[b]
10	76.17±20.95[a]	105.81±6.02[b]	72.91±3.41[a]

In all lines, means with different superscripts are significantly different from each other (P<0.05).

Table 2. The chemical composition of first feeds

Feed	Crude Protein (%DW)	Crude Lipid (%DW)	Carbonhydrite (%DW)	Ash (%)	Energy (MJ. kg^{-1})
Microdiet	66	11	6	9.6	19.5
Artemia	62	12	20	6.8	20.9
Plankton	n/a	n/a	n/a	n/a	n/a

Mikrodiet (Trout starter, Coppens, Holland), *Artemia* nauplii (Salt Lake, Utah) n/a: not available.

expected increase was occurred on 8th day in the Artemia and plankton group, whereas on 9th day in the micro diet group.

Although weaning can be achieved in several species, introduction of micro diet to post-larvae had limited success (Kolkovski, 1995; Kolkovski and Tandler, 1995; Kolkovski, 2001). The feeding behavior of pike is well known that this fish attacks to the moving objects, so the reduced enzymatic activity of enzymes in larvae may mainly be a consequence of starvation (Noori *et al*., 2012). On other hand, the poor performance of micro diet in the rearing of pike larvae may be due to incomplete functioning of the digestive tract at the starting of exogenous feeding. Moreover, micro diets contain 60–90% dry matter compared to only 10% in zooplankton (Kolkovski, 2001). This may lead to insufficient digestibility since it is much harder to break down dry hard particles than live organisms.

According to the results, the larvae fed with plankton had statistically higher protease activity than the other groups (P<0.05). Several authors pointed out that fish larva have limited enzymatic activity after hatching and exogenous sources supports digestive process (Lauff and Hofer, 1984; Walford and Lam, 1993). However, the cysteine proteases from *Artemia* which is over 90% related to use as hatching enzyme, in yolk utilization and as a digestive enzyme may not be recommended for fish larvae if the pH in the intestine is around 8 (Garcia- Ortega *et al*., 1998). In this context, pike larvae may not able to use these enzyme in early stage of ontogeny. Also, Artemia was less digested than the plankton, either as a result of indigestible exoskeleton (Finn and Kapoor, 2008). On the other hand, Munila- Moran *et al*. (1990) measured very high enzymatic activity in adult copepods compared to *Artemia* and these may be the reasons of the statistical differences between two groups.

In conclusion, the micro diet was rejected when offered as a sole diet at the onset of exogenous feeding due to feeding behavior of pike larvae. Furthermore, the better digestive performance was obtained from plankton group due to fact that plankton (especially copepods) rather than *Artemia* are the natural prey of pike larvae. Finally, further research on other enzymes like lipase and amylase secretion in early larval stage of pike by combination of different first feed has to be conducted to be able to formulate an appropriate artificial diet for early weaning of this species.

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