

## PROTEASE, LIPASE AND AMYLASE ACTIVITIES IN BARRED LOACH, *Nemacheilus fasciatus* C.V.

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**Abstract.** Barred loach is wild fish in which there is no information related to its enzymatic digestive capacity yet. Therefore research on this subject needs to be conducted. The spectrophotometry method was used to study protease, lipase, and amylase activities in barred loach. The study used 89 fish with an average weight of  $3.61 \pm 0.26g$  and  $1.68 \pm 0.21g$ . The results showed that pH 7.0 was the highest protease activity. Similarly, lipase activity found between pH 7.0 and pH 8.1. Still, the amylase activity found to be high at pH 8.1. Protease activity also found to be high in the posterior intestine than in the hepato-pancreas and anterior intestine. Lipase and amylase activities did not found differences between hepato-pancreas, anterior intestine, and posterior intestine. In conclusion, the protease, lipase, and amylase activities of barred loach found along the gastrointestinal tract in both large and small fish, which shows that barred loach can digest protein, fat, and carbohydrates in diets better.

**Keywords:** amylase, barred loach, lipase, protease

### Citation

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### INTRODUCTION

Barred loach, *Nemacheilus fasciatus*, is a wild fish that inhabits streams on a rock bottom. This fish has a good taste so that many people like it as a consumption fish. The maximum length of a barred loach is 10 cm, the body is elongated round, and there are lines on the body, it has a barbell in its mouth, a short dorsal fin and a semicircular mouth (Kottelat et al., 1993; Prakoso et al., 2016). However, its population in public waters had reduced because of declining water quality and overfishing (Risyanto et al., 2012). Domestication of the bared loach is essential to increase their population in nature or meet the needs of the community both for consumption and ornamental fish.

Barred loach has the potential for domestication or ex-situ conservation. The success of conservation or domestication will depend on the knowledge of barred loach biology. Previous studies of barred loach had been carried out including bio-ecology (Tjahjo et al., 2000), prospective commodities (Emmawati et al., 2011), biology (Risyanto et al., 2012), growth (Prakoso et al., 2016), reproduction & growth (Prakoso et al., 2017), gonadosomatic index and gonadal histological structure (Nurhidayat et al., 2017), and genotype and phenotype performance (Aththar et al., 2018). However, further biological studies primarily related to digestive enzyme activity still need to be done.

Assessment of the performance of digestive enzymes from barred loach is one of

the most important aspects to be carried out, and information about this is needed to align the nutritional needs with their digestive capacity. Fish with different trophic levels have a digestive ability that reflected in digestive enzyme activity. *Rasbora lateristriata*, for example, not have a stomach, and hydrolysis of feed protein depends on proteases released by the pancreas (Susilo et al., 2018). In contrast to *Morone saxatilis*, *Chelon labrosus* and *Lutjanus malabaricus*, which have a perfect stomach so that they can utilize pepsin produced for the initial hydrolysis of protein in the feed (Baragi et al., 2013; Pujante et al., 2017; Mazumder et al., 2018). This study was conducted to find out the protease, lipase, and amylase activities in barred loach. This information is useful for the preparation of barred loach feed formula to support its domestication. The discovery of digestibility in barred loaches, as reflected in the activity of digestive enzymes, is also the novelty of this study.

## MATERIALS AND METHODS

### Fish and Preparation Sample

The study was conducted by a survey method through purposive random sampling. The locations for barred loach sampling were on the Pelus River in Purwokerto, Banyumas at coordinates of 07°23'27.57"S and 109°14'50.42"E. Barred loach was captured using fishing tools in the form of a handline and small hand net. There were 89 fish used in this study, which had a total length of between 6.0 - 9.0 cm and a wet weight ranging from 1.51 - 3.78 g (Figure 1). The fish was acclimated in the UNSOED P.S. D3 laboratory for 24 hours before being used for research. The fish were divide into two size groups, namely large size (mean body weight:  $3.61 \pm 0.26$  g) and small size (mean body weight:  $1.68 \pm 0.21$  g). In each group, the fish were regrouped

into four groups, with 11 fish each in a sample pool. In each group, the fish was dissected and isolated digestive organs, namely hepatopancreas, anterior and posterior intestine. A labeled bottle was used to store isolated digestive organs. During surgery and isolation of the digestive organs, fish were placed on ice blocks to keep the digestive organs at cold temperatures.

### Homogenization of Digestive Organs

Hepatopancreas, anterior, and posterior intestine were destroyed using an electric homogenizer in a cold solution of 50 mM buffer Tris-HCl pH 7.5 containing 10.0 mM NaCl in a ratio of 1: 8 w / v (1 g organ: 8 mL buffer). Homogenate was then centrifuged with a refrigerator centrifuge (4°C) at 12,000 rpm for 15 minutes and the supernatant obtained was stored in a refrigerator at -80°C (Thongprajukaew et al., 2010a). The concentration of dissolved protein in the crude extract of the enzyme was determined by the method of Lowry et al. (1951) using albumin as a standard.

### Measurement of Protease Activity

The casein hydrolysis method (Walter, 1984) with modification was used to measure protease activity. The buffer used was 0.1 M phosphate buffer (pH 7.0), 0.1 M Tris-HCl (pH 8.1) and 0.1 M Glycine-NaOH (pH 10.0). The reaction mixture consisting of a substrate (350 µL), buffer (375 µL) and enzyme extract (25 µL) were incubated for 30 minutes at 37°C. At the end of the incubation, 750 µL of 8% TCA reagent was added to stop the reaction. The same procedure was carried out on the blank, except for the enzyme extract that was added after giving 750 µL of 8% TCA reagent. The reaction mixtures were store in the refrigerator for one hour and then centrifuged at 6000 rpm for 10 minutes. The supernatant obtained was then measured for its ab-

sorbance at 280 nm, and the protease specific activity expressed as a unit (U): Abs.h<sup>-1</sup>.mg<sup>-1</sup> protein of crude enzyme extract.

#### Measurement of Lipase Activity

The *p*-Nitrophenylpalmitate (*p*-NPP) hydrolysis method was used to measure lipase activity (Markweg-Hanke et al., 1995). The buffer used was 0.1 M phosphate buffer (pH 7.0) and 0.1 M Tris-HCl (pH 8.1). The reaction mixture consisting of buffer (575 µL), enzyme extract (25 µL) and *p*-NPP substrate (200 µL) was incubated for 30 minutes at 37°C. After incubation, 400 µL of 0.1 M Na<sub>2</sub>CO<sub>3</sub> reagent was added to stop the reaction. The same procedure carried out on the blank, except for the enzyme extract that was added after the 0.1 M Na<sub>2</sub>CO<sub>3</sub> reagent. The reaction mixture was then centrifuged at 10,000 rpm for 15 minutes, and supernatant absorption measured at 410 nm. Lipase specific activity expressed as a unit (U): Abs.h<sup>-1</sup>.mg<sup>-1</sup> protein of crude enzyme extract.

#### Measurement of Amylase Activity

3,5-Dinitrosalicylic acid method (Areekijserree et al., 2004) with the starch sub-

strate was used to measure amylase activity. The buffer used was 0.1 M phosphate buffer (pH 7.0) and 0.1 M Tris-HCl (pH 8.1). The reaction mixture consisted of a substrate (200 µL), buffer (275 µL), and enzyme extract (25 µL) were incubated for 15 minutes at 37°C, and the reaction was stopped by the addition of 500 µL of 1% DNS reagent. The reaction mixture was then placed in boiling water for 5 minutes. The same procedure was carried out on the blank, except for the enzyme extract that was added after administration of a 1% DNS reagent. The reaction mixture was diluted in double-distillate water at 1:3 ratios. The reaction mixture was then measured for absorbance at 540 nm, and the amylase specific activity was expressed as a unit (U): Abs.h<sup>-1</sup>.mg<sup>-1</sup> protein of crude enzyme extract.

#### Data Analysis

Quantitative data in the form of protease, lipase and amylase activities obtained in this study were analyzed by One Way Anova using the SPSS 18 version of the Window software package.



Figure 1. Barred loach, *Nemacheilus fasciatus* C.V.

**RESULTS AND DISCUSSION**

**Protease Activity**

The protease activity showed a significant difference between pH and digestive organs ( $p < 0.05$ ). Protease activity in the hepatopancreas, anterior intestine, and posterior intestine of large and small fish differed significantly between the pH ( $p < 0.05$ ) and

it appeared that protease activity was higher at pH 7.0 than at pH 8.1 and 10.0 (Table 1). The mean of protease activity at the three pH tested showed the difference between the digestive organs and the highest protease activity found in the posterior intestine (Figure 2), both in small and large fish, still there did not see to be the difference between the two fish sizes ( $p > .05$ ).

Table 1. Protease activity ( $\text{Abs}\cdot\text{h}^{-1}\cdot\text{mg}^{-1}$  protein) in hepatopancreas, anterior and posterior intestine of barred loach in different pH and fish sizes

	Large fish ( $3.6\pm 0.5$ )			Small fish ( $1.68\pm 0.2$ )		
	hepatopancreas	anterior intestine	posterior intestine	hepatopancreas	anterior intestine	posterior intestine
pH 7.0	$0.455\pm 0.04^a$	$0.371\pm 0.05^a$	$0.593\pm 0.13^a$	$0.416\pm 0.17^a$	$0.437\pm 0.03^a$	$0.461\pm 0.05^a$
pH 8.1	$0.208\pm 0.05^b$	$0.103\pm 0.06^b$	$0.309\pm 0.08^b$	$0.098\pm 0.04^b$	$0.115\pm 0.01^b$	$0.255\pm 0.06^b$
pH 10.0	$0.350\pm 0.12^c$	$0.233\pm 0.02^c$	$0.454\pm 0.08^c$	$0.210\pm 0.05^b$	$0.209\pm 0.05^c$	$0.340\pm 0.06^b$

Note: with the same letter are not significantly different from each other ( $P > 0.05$  ANOVA)

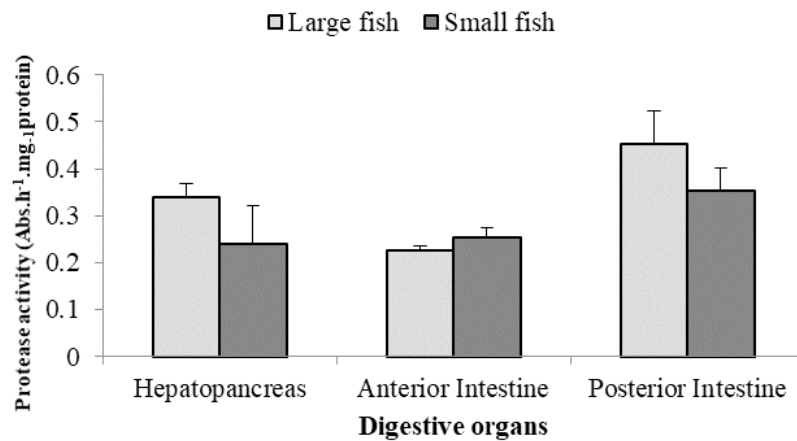


Figure 2. The mean (+sd) protease activity of the barred loach in different digestive organs and fish sizes

The results of this study similar that studied in *Catla catla* that had a maximum protease activity at pH 7.0 (Khangembam et al., 2012) and quite similar to those found in *Betta splendens*, *Sander lucioperca* and *Carrasius auratus gibelo* (Thongprajukaew et al., 2010; Solovyev et al., 2015). However, an optimal pH of protease activity in *Labeo ro-*

*hita* and *Hypthalmichthys molitrix* that was 8-9 (Kumar et al., 2007). In hybrid sturgeon (*Huso dauricus* ♀ x *Acipenser schrenki*) and *Archosargus probatocephalus* high activity of protease was found at pH 8-9 (Ji et al., 2012; Merino-Contreras et al., 2018). The higher protease activity in neutral conditions thought to be related to the intestine environment of

fish, as found in the *Lota lota* and *Cyprinus carpio* that has a pH of intestine around 7.0 (Izvekova et al., 2012; Solovyev et al., 2015).

Protease activity found in all digestive organs, in which the posterior intestine had higher protease activity than the anterior intestine and hepatopancreas. The results of this study were similar to *Tilapia rendalli*, which had higher protease activity in the posterior intestine (Hlophe & Moyo, 2013) and scaleless carp, *Gymnocypris przewalskii*, which had higher trypsin activity in the anterior and posterior intestine (Tian et al., 2019). But the results of this study are different from those found in *Hyporhamphus regularis ardelio* and loach, *Paramisgumus dabryanus* which found protease activity, especially trypsin has decreased in the posterior intestine (Day et al., 2011; Yang et al., 2018). The presence of protease activity in the barred loach digestion tract showed that barred loach could digest proteins in neutral to alkaline conditions both in the anterior and posterior intestine. Protease activity, also found along the digestive tract, shows the potential of barred loach fish can be utilized to be better food protein.

Differences in fish size did not cause variations in protease activity in this study. Still, in contrast to previous studies on *Hyporhamphus regularis ardelio*, *Gambusia punctate* and *Rasbora lateristriata* that was showed differences in protease activity (trypsin) at different fish sizes (Day et al., 2011; Falcon-Hidalgo et al., 2011; Susilo et al., 2018). So it seems that fish with different species exhibit different abilities related to their ability to digest feed containing protein. In this barred loach the difference in fish size does not indicate differences in protease activity, which suggests no difference in digestive capacity and feeding habits.

### Lipase Activity

The results showed that lipase activity occurred in neutral and slightly alkaline conditions, but there was no significant difference ( $P > .05$ ) between the pH, digestive organs and fish size (Table 2). However, in all digestive organs of barred loach showed lipase activity (Figure 3).

Lipase activity occurs at pH 7.0-8.0 in barred loach, and this result was different from previous studies conducted in *Betta splendens* which showed high lipase activity at pH 8.0-11.0 (Thongprajukaew et al., 2010b) and *Carassius auratus gibelio* high lipase activity found at pH 9.0 (Solovyev et al., 2015). The presence of lipase activity along the digestion tract had also investigated in *Tilapia rendalli*. In *Tilapia rendalli*, lipase activity was lower in the posterior than in the anterior intestine (Hlophe & Moyo, 2013). Different results also found in *Polyodon spathula*, which showed lipase activity which only found in the stomach, but not found in the intestine (Ji et al., 2012). The presence of lipase activity along the digestive tract shows that barred loach consumes feed derived from animals and vegetables, still in general, lipase activity in herbivorous fish is lower than omnivores and carnivores. The results of this study also showed that barred loach could better use feed lipids due to lipase activity that suspected to secreted from the pancreas and active in neutral to alkaline conditions.

The ability of barred loach to used dietary fat did not seem to change with a change of the fish size. The results of this study were no different from those found in marine herbivore fish, *Hyporhamphus regularis ardelio* (Day et al., 2011). However, separate from the results of previous studies on seabream, *Diplodus puntazzo* (Savona et al., 2011), catfish, *Horabagrus brachysoma* (Prasad & Sunee-sha, 2013) and yellow rasbora, *Rasbora later-*

*istriata* (Susilo et al., 2018). The results of this study suggest that barred loach had no difference in their ability to digest fat at different sizes. An indication that there was no change in feeding habits in barred loach with differences

in fish size because, in general, herbivore fish have lower lipase activity than omnivorous or carnivorous fish (Langeland et al., 2013).

Table 2. Lipase activity (Abs.h<sup>-1</sup>.mg<sup>-1</sup> protein) in hepatopancreas, anterior and posterior intestine of barred loach in different pH and fish sizes

	Large fish (3.6±0.5 g)			Small fish (1.68±0.2 g)		
	hepatopancreas	anterior intestine	posterior intestine	hepatopancreas	anterior intestine	posterior intestine
pH 7.0	0.285±0.15 <sup>a</sup>	0.192±0.12 <sup>a</sup>	0.431±0.25 <sup>a</sup>	0.295±0.23 <sup>a</sup>	0.277±0.17 <sup>a</sup>	0.223±0.01 <sup>a</sup>
pH 8.1	0.150±0.02 <sup>a</sup>	0.127±0.07 <sup>a</sup>	0.272±0.10 <sup>a</sup>	0.335±0.11 <sup>a</sup>	0.293±0.05 <sup>a</sup>	0.320±0.19 <sup>a</sup>

Note: with the same letter are not significantly different from each other (P>0.05 ANOVA)

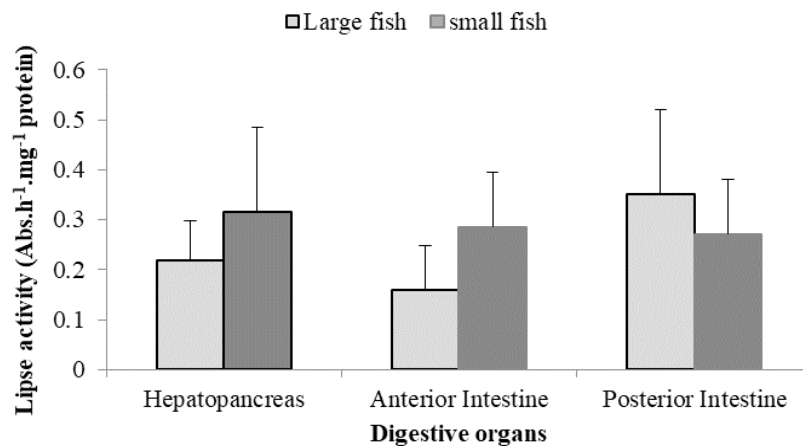


Figure 3. The mean (+sd) lipase activity of the barred loach in different digestive organs and fish sizes

### Amylase Activity

The results showed that amylase found in neutral (pH 7.0) and alkali (pH 8.1), still the pH 8.1 had higher amylase activity (p <0.05; Table 3). However, differences in digestive organs and fish size did not result in significant differences (p> 0.05; Figure 4).

Amylase activity in this study did not differ from the results of previous studies on *Betta splendens* which had higher activity in the pH range of 8-11 (Thongprajukaew et al., 2010b) and also in *Carassius auratus gibelio* which had higher activity at pH 7-9 ( Solovyev et al., 2015). However, the results of this

study were different from those found in *Tilapia rendalli* that showed lower amylase activity in the anterior intestine than in the posterior intestine (Hlophe & Moyo, 2013). *Polyodon spatulas* also had high amylase activity in the anterior intestine and low in the posterior intestine (Ji et al., 2012).

The amylase activity of barred loach found in the digestive tract and also in the hepatopancreas indicates that the barred loach digestion channel is a suitable medium for amylase activity secreted by the pancreas that requires a neutral to an alkaline environment. Amylase activity along the

gastrointestinal tract also showed the potential of barred loach to be able to utilize feed containing carbohydrates. An indication that barred loach can use protein-level feed for omnivorous or herbivorous fish.

Amylase activity in this study did not influence by the difference of fish size and the results are similar to those found in *Hyporhamphus regularis ardelio* (Day et al., 2011), *Gambusia punctata* (Falcon-Hidalgo et al., 2011) and *Rasbora lateristriata* (Susilo et al., 2018), but different from *Limia vittata* (Falcon-Hidalgo et al., 2011), *Diplodus puntazzo* (Savona et al., 2011) and *Horabagrus brachysoma* (Prasad & Suneesha, 2013). The absence of changes in amylase activity with different fish sizes showed no difference in feeding habits in the barred loach. Herbivorous fish usually have higher amylase activity than

omnivores and carnivores (Day et al., 2011).

Based on the discussion described above, it can conclude that protease activity found in the hepatopancreas, anterior and posterior intestine. The activity was higher in neutral conditions (pH 7.0) and the posterior intestine. Lipase found in the hepatopancreas, anterior and posterior intestine, but there was no difference in activity between different organs/digestive segments. Amylase activity found to be higher in alkaline conditions (pH 8.1), but there was no difference between various organs or gastrointestinal parts. Further research is needed to reveal the nutrient requirements of barred loach related to their digestive capacity so that information on nutrient requirements can be obtained and to formulate barred loach feed formulas.

Table 3. Amylase activity (Abs.h<sup>-1</sup>.mg<sup>-1</sup> protein) in hepatopancreas, anterior and posterior intestine of barred loach in different pH and fish sizes

	Large fish (3.6±0.5 g)			Small fish (1.68±0.2 g)		
	hepatopancreas	anterior intestine	posterior intestine	hepatopancreas	anterior intestine	posterior intestine
pH 7.0	0.146±0.03 <sup>a</sup>	0.123±0.01 <sup>a</sup>	0.234±0.07 <sup>a</sup>	0.189±0.09 <sup>a</sup>	0.176±0.01 <sup>a</sup>	0.154±0.01 <sup>a</sup>
pH 8.1	0.386±0.09 <sup>b</sup>	0.470±0.03 <sup>b</sup>	0.495±0.05 <sup>b</sup>	0.328±0.08 <sup>b</sup>	0.402±0.05 <sup>b</sup>	0.486±0.07 <sup>b</sup>

Note: with the same letter are not significantly different from each other (P>0.05 ANOVA)

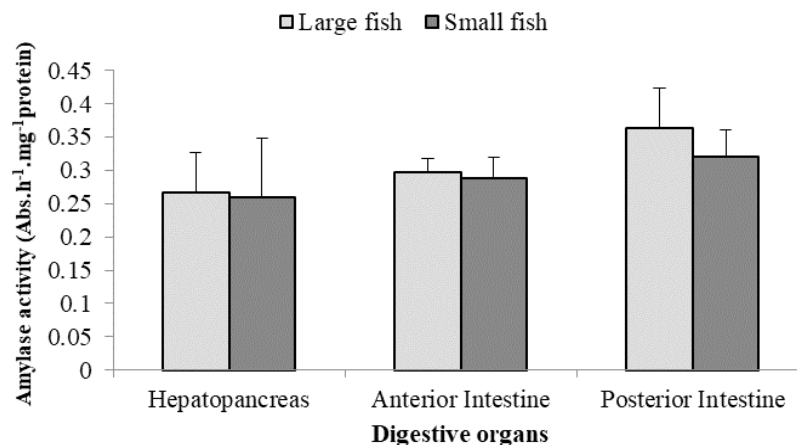


Figure 4. The mean (+sd) amylase activity of the barred loach in different digestive organs and fish sizes

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