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Digestive enzyme profiles from foregut contents of blue swimming crab, *Portunus pelagicus* from Straits of Johor, Malaysia

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Abstract This study describes the digestive enzyme profiles from foregut contents of the blue swimming crab, *Portunus pelagicus* (L.), from Straits of Johor, Malaysia. Adult male (mature and immature) and female (ovarian maturation stages 1–4) blue swimming crabs were assayed for the presence of digestive enzymes (protease, trypsin, amylase and lipase) in the foregut. The results show that in some instances, significant differences in enzyme activities were observed between the sexes of *P. pelagicus*. Protease, trypsin, and amylase were detected in all gonad maturation stages, suggesting that crabs including immature males can readily digest dietary protein and carbohydrates, but not lipids. Trypsin (except for ovarian maturation Stage 4) and lipase (ovarian maturation Stage 4 and mature male only) activities were considerably higher than amylase, indicating that dietary proteins and lipids are more important than carbohydrates and, thus, suggests a carnivorous diet. In conclusion, the changes in enzyme activities between the different maturation stages and sexes reflect natural changes in diet and feeding abilities of *P. pelagicus*.

1. Introduction

The broodstock of blue swimming crab, *Portunus pelagicus*, are hunted extensively in their natural habitat; thus, it is important to develop and maintain a hatchery broodstock to obtain berried females for larval supply. Crab fishery and culture operations are expected to continue to grow in the future (Ikhwanuddin et al., 2012). The limited scientific knowledge of *P. pelagicus* digestive processes, however, is recognized as a major constraint to the future growth of this species. Live crabs are exported to Southeast Asian countries (Sugumar et al., 2013), and have been considered as one of the more valuable and commercially important commodities across many countries (Abol-Munafi et al., 2016; Azra and Ikhwanuddin, 2015). Future development of the *P. pelagicus* industry will require an understanding of the key nutrient requirements of

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this species and the digestive strategies employed to access the appropriate nutrients for broodstock maintenance. The digestive potential of an organism not only relies on its diet but also on its innate capacity to absorb such a diet with certain enzymes. A literature review revealed only a few of research on the digestive physiology of the brachyuran crab (Cui et al., 2016; Shentu et al., 2015), but that their dietary composition is well documented. For example, the dietary nutrition of portunid crabs has been studied in *P. pelagicus* (Ikhwanuddin et al., 2014; deLestang et al., 2000), sentinel crab, *Podopthalmus vigil* (Sudhakar et al., 2011), ornate blue crab, *Callinectus ornatus* (Mantelatto and Christofoletti, 2001), sand crab, *P. segnis* (Pazooki et al., 2012) and mud crab, genus *Scylla* (Azra and Ikhwanuddin, 2016; Viswanathan and Raffi, 2015). This study was designed to determine the digestive enzyme profiles of the *P. pelagicus* foregut as well as differences between sexes. The results from this study will provide information on the suitable diet for improving broodstock health and growth of this crab in aquaculture systems.

2. Materials and methods

2.1. Sampling site, crab morphometrics and sexes

Sixteen samples of *P. pelagicus* were collected from fishing at the Jetty Pendas at Gelang Patah, Straits of Johor coastal waters of Malaysia. Collections were standardized by only taking the adults of females, but taking immature and mature *P. pelagicus* for males. Before dissection, crabs were identified for sex (differentiation between males and females was determined based on Lai et al., 2010) and gonad maturation stage based on Ravi et al. (2013) and Safaie et al. (2013). The ovary of the crab in stage 1 was very thin and transparent, and ovaries changed color to creamy upon reaching stage 2. Ovarian maturation stage 3 was characterized by enlarged ovaries with a color change to yellow, and turned to orange or reddish orange upon reaching stage 4. Samples of foregut were used for enzyme analysis to determine their natural diet. The carapace width and weight of crab samples were measured and recorded along with gonad maturation stages.

2.2. Enzyme bioassay

In this study, four main types of enzymes were studied, including protease, trypsin, amylase and lipase. All enzyme analyses and protein determination were conducted using BioVision Kits.

2.3. Enzyme preparation

2.3.1. Amylase

A total of 100 mg foregut tissue of *P. pelagicus* was extracted with 0.5 ml Assay Buffer and homogenized. Extracts then were centrifuged at 16,000*g* for 10 min using a microcentrifuge. Samples and enzyme materials were stocked at −20 °C until use. An Amylase Positive Control was dissolved into 50 µl Assay Buffer and stored at −20 °C, and 5 µl Amylase Positive Control was added to three wells and the volume adjusted to 50 µl with distilled water.

2.3.2. Lipase

A total of 40 mg foregut tissue of *P. pelagicus* was homogenized with 4 volumes of Assay Buffer. Extracts were then centrifuged at 13,000*g* for 10 min to remove insoluble materials. Following centrifugation, the supernatant was directly diluted in Assay Buffer. Samples and enzyme materials were stored at −20 °C until use.

2.3.3. Trypsin

A total of 100 mg foregut tissue of *P. pelagicus* was extracted with 4 volumes of Trypsin Assay Buffer and homogenized. Samples were then centrifuged in a microcentrifuge at 14,000*g* for 10 min to obtain a clear extract. Samples and enzyme materials were stored at −20 °C until use. Aliquots of 5 µl of supernatant were transferred into wells and Assay Buffer was added to adjust the volume 50 µl/well.

2.3.4. Protease

A total of 40 mg foregut tissue of *P. pelagicus* was homogenized with 4 volumes of Assay Buffer and centrifuged for 10 min at 16,000*g* to remove insoluble material and obtain a clear extract. Samples and enzyme materials were stored at −20 °C until use. Aliquots of 5 µl of serum were transferred into wells, and the volume was adjusted to 50 µl/well by the addition of Assay Buffer.

2.4. Enzyme assay

2.4.1. Amylase

The Amylase Activity Colorimetric Assay Kit (Catalog No.: K711-100) was used with ethylidene-pNP-G7 as the substrate. This substrate has been specifically cleaved by α-amylase, and the smaller fragments produced can be acted upon by α-glucosidase, which causes the release of chromospheres that can then be measured at 405 nm. A nitrophenol standard curve was prepared using several volumes of nitrophenol standard mix added to wells. 0, 2, 4, 6, 8, and 10 µl of 2 mM nitrophenol standard mix were added into a 96-well plate in duplicate to generate 0, 4, 8, 12, 16 and 20 nmol/well nitrophenol standards. For each well, distilled water was added to make a total volume of 50 µl. For reaction mixtures, 50 µl Assay Buffer and 50 µl Substrate Mix were added together to make 100 µl of reaction mix. 100 µl of the reaction mix was added to each well that contains samples and mixed, along with standards and positive control. The reaction was measured immediately to obtain OD_{T0} (T_0 signifies time zero). The reaction system was incubated at 25 °C for 10 min, and absorbance was read at 405 nm. The enzyme activity calculation was based on the nitrophenol standard curve and the Eq. (1);

$$\text{Amylase Activity} = \frac{B}{T \times V} \times \textit{Sample Dilution Factor} \qquad (1)$$

where B is the amount of nitrophenol from the Standard Curve obtained from obtained from ΔOD (ΔOD = ODT_1–ODT_0) (in nmol) with T is the time between T_0 and T_1 (in min) and V is the pretreated sample volume added to the reaction well (in ml). The final unit should be in: $\frac{\frac{\text{nmol}}{\text{min}}}{\text{ml}} = \frac{\text{mU}}{\text{ml}}$.

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