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The Effect of Different Diets on Proteolytic Enzymes Activity of Early Marble Goby *(Oxyeleotris marmoratus)* Larvae

[1]M.V. Van., [1]A.B. Abol-Munafi., [2]A.W.M. Effendy and [3]M. Awang Soh
[1]Institute of Tropical Aquaculture (AKUATROP). University College of Science and Technology, Malaysia (KUSTEM), 21030 Kuala Terengganu, Malaysia
[2]Central of Marine Biotechnology, KUSTEM, 21030 Kuala Terengganu, Malaysia
[3]Faculty of Agrotechnology and Food Science, KUSTEM, 21030 Kuala Terengganu, Malaysia

Abstract: The aim of the present study was to study the effect of different diets on proteolytic enzymes activity during early larval marble goby. The trypsin and chymotrypsin levels of the larvae fed on the various types of food using clear water were very low: 3.635-3.916Umg^{-1} tissue and 1.034-1.204Umg^{-1} tissue, while the trypsin and chymotrypsin levels of the larvae fed with the same types food using green water were higher: 5.274-5.873Umg^{-1} tissue and 1.556-2.236 Umg^{-1} tissue. Among the treatments using green water, the highest trypsin and chymotrypsin levels were observed in the larvae fed on live food (nauplii of copepods mixed nauplii of rotifer). However, there were no significant difference of trypsin and chymotrypsin levels ($p>0.05$) among the larvae fed with different types of food using clear water group and those using green water group.

Key words: Oxyeleotris marmoratus, marble goby, trypsin, chymotrypsin, different diets

INTRODUCTION

Nutrient requirements of all animals vary throughout their life cycle. The changes that occur in the morphology and physiology of aquatic animals between hatching and maturity lead to a number of important variations in feeding and nutritional requirements though the larval, fingerling and adult stages. These variations occur in the morphology of the digestive organs, the digestive processes, the nutrient requirements and the feeding behaviour[1].

The seedling production of larval fish, bivalves and crustaceans for mass production relies still on live diets such as algae, rotifers *(Brachionus plicatilis)* and planktonic Crustacea, e.g., *Artemia*[2]. For examples, microalgae are utilized in aquaculture as live feeds for all growth stages of bivalve molluscs (oysters, scallops, clams and mussels), for the larval/early juvenile stages of abalone, crustaceans and some fish species and for zooplankton used in aquaculture food chains[3]. Microalgae have been believed to play a role in stabilising the water quality, nutrition of the larvae and microbial control. The green water technique, now, has been widely applied for the rearing of shrimp and fish larvae[4]; besides microalgae, rotifer and moina, daphnia, artemia end so on are also used as a suitable live food organism for the early larval stages of marine and freshwater fish[5-7].

In marble goby *(O. marmoratus)*, the gut content analysis during larval stage was done by Liem[8]. On Two Days After Hatching (DAH), the larvae commenced feeding on phytoplankton and the frequency occurrence of phytoplankton increased from 95% on 2 DAH to 100% on 3 DAH. From 5 DAH, the frequency occurrence of algae decreased to 20% then it was totally replaced by zooplankton on 7 DAH. While feeding on phytoplankton, the larvae started to feed on *Brachionus* sp. and nauplii of copepods on 3 DAH with frequency occurrences of 100 and 95%, respectively. The larvae commenced feeding on *Cyclops* 5 DAH and it was found in most of the gut (100%) after 10 DAH. Abol-Munafi *et al.*[9] studied on the effect of different diets and different water range on the early larval stage of marble goby *Oxyeleotris marmoratus*. Growth and survival rate were highest for the larvae fed with the combination of green water and nauplii of copepods (0.14 mm day^{-1} and 43.20%, respectively). Larvae fed with single feed of *Spirulina*, rotifer, artificial diet or infusoria showed least growth and high mortality. The survival rate of larvae improved significantly when green water was given in combination with other feeds.

The aim of the present study was to study the effect of different diets on proteolytic enzymes activity during early larval marble goby.

MATERIALS AND METHODS

Experimental Design: The larvae were reared in 1000 L fibreglass tanks. The treatments was carried out with 2 types of food namely life food (rotifer-*Brachionus* sp. nauplii of copepods and moina)

Table 1: Larval feeding treatments

Treatments	Type of food	Density of food
1	Green water-Life food	0.6 x 10^6 uni.-5 ind.mL^{-1}
2	Green water-Artificial Diet (AD)	0.6 x 10^6 uni.-30 par. mL^{-1} (*)
3	Green water	0.6 x 10^6 uni.
4	Clear water-Life food	5 ind.mL^{-1}
5	Clear water-Artificial Diet (AD)	30 par. mL^{-1}*
6	Clear water-Non-feeding	–

*: 0.4 g/day per each tanks

and artificial food. The artificial diet was artificial plankton B.P, a product of Nippon Formula Food Mfg. Co., Ltd., Japan. Each gram consists of 7.8x10^6 particles, 30-160 μm in size. Live foods was given to larvae at the density of 5 ind/mL. The density of live food was checked using the Sedgwick-Rafter counting cell and the amount needed for feeding was calculated daily. The treatments were designed as listed in Table 1.

Water temperature was maintained at 29-30°C by aquarium heaters. DO and pH during the larvae rearing ranged from 4.3-7.2 mg L^{-1} and 7.0-7.9.

Sampling: The samples were collected at 0, 2, 4 and 6 days old larvae. Thirty larvae from the original sample were fixed before the start of the experiment to compare their histological structure and to establish their initial nutritional state before and after feeding with different diets. Then, 200 larvae from each of treatment were taken for enzyme analysis. All the samples were immediately kept at -70°C until analysis. Fresh samples were used as it necessary for isozymic analysis, because of an importance of enzymatic activity[9].

Preparation of samples for crude enzymes extract: Samples of larvae were collected every 2 days from new hatching until 6 DAH and were pooled and homogenized in cold Tris-HCl 50 mM buffer (pH 7.5). The homogenate was the centrifuged at 4°C at 15,000xRPM for 15 min. The supernatant containing the enzymes was stored at –70°C before analysis.

Enzymes assays: Trypsin and chymotrypsin activities of the enzyme extract was evaluated as described by Garcýa-Carreno *et al.*,[10] using synthetic substrates.

For trypsin activity, 10 μL of enzyme preparation was mixed with 750 μL of 0.1 mM benzoyl-DL-arginin-p-nitroanilide (BAPNA) in 50 mM Tris-HCl at pH 7.5 and 20 mM $CaCl_2$ buffer. The reaction was monitored for 10min at 30°C at 410 nm. Before adding 30% acetic acid to stop the reaction.

Chymotrypsin activity, 10 μL of the enzyme extract was mixed with 750 μL of 0.1 mM succinyl $(Ala)_2$-pro-Phe-p-nitroanilide (SAPNA) in Tris-HCl at pH 7.5 and 20 mM $CaCl_2$ buffer. The reaction was monitored for 10 min at 30°C at 410 nm.

Trypsin and chymotrypsin activity units were calculated by the equation:

Activity units = [$(Abs_{410nm}$/min)x1000xVolume of reaction mixture]/8800 xmg

Where 8800 is the molar extinction coefficient of para-nitroaniline liberated from chromgens BAPNA and SAPNA and mg is the tissue content in the reaction mixture.

Statistical analysis: The collected data were analysis by one-way ANOVA followed by student's test when significant differences were found at a 0.05 level.

RESULTS

The trypsin levels in the larvae were shown in Table 2. Results showed that the treatments of clear water were significant difference ($p<0.05$) with those of green water. Meanwhile, there were no significant difference ($p>0.05$) within the treatments of clear water and green water as well.

The results also indicated that the trypsin levels of larvae fed on the various types of food using clear water were very low (3.635 - 3.916 U mg^{-1} tissue), while the trypsin levels of the larvae fed with the same types food using green water were higher (5.274 -5.873U mg^{-1} tissue). Among the treatments using green water, the highest trypsin level was observed in the larvae fed on live food (nauplii of copepods mixed nauplii of rotifer).

The chymotrypsin levels of larvae were presented in Table 3. Results showed that the chymotrypsin levels in larvae fed with various types of food using clear water were lower than those using green water.

The highest chymotrypsin level was observed in larvae fed on live food using green water (2.236 ± 0.999 Umg^{-1} tissue), the next level belong to the larvae fed on artificial food-green water (1.652 ± 0.792 Umg^{-1} tissue), and the lowest level was detected in non -feeding larvae (1.034 ± 0.589 Umg^{-1} tissue). However, there were no significant difference ($p>0.05$) among the larvae fed with different types of food using clear water group and those using green water.

DISCUSSION

The trypsin and chymotrypsin levels in larvae fed with different types of food using clear water were lower ($p<0.05$) than those using green water. It is suggested that microalgae play an important role in digestion of early larvae stage. This result agreed with Senoo *et al.*[11] that at

Table 2: Trypsin activity (Umg^{-1} Tissue) of larval marble goby cultures with different food treatments

Treatments	Sample size (n)	Trypsin activity
Live food-Clear water	8	3.635 ± 0.910^{a}
Artificial food-Clear water	8	3.916 ± 0.735^{a}
Non feeding-Clear water	8	3.640 ± 0.888^{a}
Live food-Green water	8	5.873 ± 1.548^{b}
Non feeding- Green water	8	5.274 ± 0.815^{b}
Artificial food-Green water	8	5.428 ± 1.023^{b}

Means±SD with different superscripts are significant different ($p<0.05$)

Table 3: Chymotrypsin activity (Umg^{-1} Tissue) of larval marble goby cultures with different food treatments

Treatments	Sample size (n)	Chymotrypsin activity
Live food-Clear water	8	1.092 ± 0.564^{a}
Artificial food-Clear water	8	1.204 ± 0.719^{a}
Non feeding-Clear water	8	1.034 ± 0.589^{a}
Non feeding- Green water	8	1.556 ± 0.485^{ab}
Artificial food-Green water	8	1.652 ± 0.792^{ab}
Live food-Green water	8	2.236 ± 0.999^{b}

Means ± SD with different superscripts are significant different ($p<0.05$)

the postlarvae of marble goby *O. marmoratus* first consume phytoplankton, then they commence feeding on microplankton such as ciliates and rotifers.

There are several present in phytoplankton could potentially influence digestive enzyme activity in fish larvae. Most formulated diets for marine fish larvae contain large amounts of fish meal, which is low in the polyamide spermine[12]. The addition of spermine to the diets of European sea bass larvae has been shown to affect pancreatic enzyme secretion and to induce earlier intestinal maturation[13]. Cahu *et al.*[14,15] observed increased trypsin secretion in European sea bass larvae fed a mixture of free amino acids in their diets. Lazo *et al.*[16] reported that the presence of algae in water used to rear red drum larvae was effected on the activity of trypsin and aminopeptidase. This experiment shows that the presence of algae in rearing water can influence the activity of digestive enzymes in developing larvae of marble goby.

Among the treatments using green water, the highest trypsin and chymotrypsin levels were observed in the larvae fed on live food (5.873 ± 1.548 Umg^{-1} tissue and 2.236 ± 0.999 Umg^{-1} tissue). However, there were no significant difference ($p>0.05$) among the larvae fed with different types of food using clear water group and those using green water group. It can be suggested that the live food and artificial food does not influence to the increase of trypsin and chymotrypsin activities. This result agreed with those reported by various authors. For example, the studies of Zambonino-Infante *et al.*[17] and Kolkovski *et al.*[18] in the sea bass *Dicentrarchus labrax* showed that the live food contribution to direct digestive enzymes may be negligible. Kurokawa *et al.*[19] used a combination of protease assays to determine the total protease activity of pancreatic and intestinal segments of Japanese sardine *Sardinops melanotictus* larvae and ELISA system to determine rotifer protein content in the larval intestine. They concluded that less than 1% of the protease activity was derived from rotifers. It seems that the enzymes of ingested live prey are not a substantial contribution to the digestion in young larvae. Recently, the similar results of Lazo *et al.*[16] in red drum *Sciaenops ocellatus* showed that the specific activity of trypsin, lipase and amylase were not significantly affected by feeding the larvae with zooplankton or the same microparticulate diet.

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