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IDENTIFICATION OF DIGESTIVE ENZYME OF *Anguilla bicolor bicolor* DURING SEED EEL PHASE IN CONTROLLED CONTAINER

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Abstract :- The main constraint for Indonesian shortfin eel aquaculture is high mortality rate for glass eel to elver phase. This aim of study was to determine the activity pattern of digestive enzymes (protease, amylase and lipase) during seed eel to elver phase, i.e. day 1-71 of rearing period. Indonesian shortfin eel (Anguilla bicolor bicolor) during seed eel phase was reared in aquarium with aeration and filtration systems and fed with natural feeds (Artemia nauplii, Daphnia and silk worms). Enzyme activity samplings were carried out at day 1, 14, 28, 42, 56 and 71 of rearing period in aquarium. Measurement data were descriptive-quantitatively analyzed and presented in graphs. The study results indicated that 1) activities of protease, lipase and amylase were starting to be detected since day 1 with small-scaled activity for protease and large-scaled activity for lipase and amylase and 2) protease activity increased for 1-71 days-seed eel, amylase activity decreased at day 42 of rearing and re-increased at day 56 to 71 and lipase activity continuously decreased since day 1 to 71. The highest protease was detected at day 71, while the highest amylase and lipase activities were at day *1*.

Keywords – *amylase, digestive enzymes, seed eel, lipase, protease*

I. INTRODUCTION

Indonesian shortfin eel (*Anguilla bicolor bicolor*) is one of fish species that has sale value in international market (Japan, Hong Kong, Netherlands, Germany, Italia, and many other countries), making it potential as export commodity. Unlike in other countries (Japan and European countries), eel resources in Indonesia has yet to be widely utilized although this fish is abundant in both seed number and consumption [1]. Eel is of high nutrient as its meat contains 1,337 mg 100 gr^{-1} Docosahexaenoic Acid (DHA) good for children growth, while salmon and mackerel only have 820 mg 100 gr^{-1} and 748 mg 100 gr^{-1}, respectively. Fresh meat of eel contains 742 mg 100 gr^{-1} Eicosapentaenoic Acid (EPA), while salmon and mackerel only have 492 mg 100 gr^{-1} and 409 mg 100 gr^{-1}, respectively [2]. Having such high nutritient content, it is not surprising that eels become featured products in international market.

Eel's high nutrient content and delicious flavor make demand for eel commodity continues to increase over years. Such demand is met with catch from nature and aquaculture. Recently, eel supply for aquaculture activity tends to increase and eventually increase in such activity leads to increasing demand on seed eel. This, of course, leads to exploitation on seed eel from nature because up to now eel aquaculture activity still depends on seed eel catch from nature. Continuing catch activity on seed eel from nature can bring harm on the sustainability of seed eel in nature [1]. Increasing demand from local and international markets on eel makes the fish continues to be exploited. Massive exploitation on eel for both trade and consumption has been occurring since long ago [3]. As the consequence, today the population of which in several countries is decreasing, including the population of *A. bicolor* [4]. High exploitation on seed eel in nature is because of efforts to produce seed eel is yet to succeed, in addition to low survival rate (SR) during seed eel rearing. Up to today, aquaculture techniques related to seed eel are still limited to maintenance of the magnification and for such activity fish farmer catches fish seeds from nature [5]. Efforts to massively produce seed eels are continued to be carried out although low survival rate remains the constraint [6].

More than 50% failure in seed production is due to death during larval stadium [7]. Imperfect growth of seed eel, particularly on its digestive system, is one of the causes of the death. In eel aquaculture, the critical phase is during seed eel to elver (fingerling) rearing period. Death during this phase is because of inaccurate feeding management. Accurate feeding on fish seed rearing requires several basic information, among others is development of digestive tract and enzyme. Digestive enzymes are protein in digestive system serve to hydrolyze feed to make it in simpler shape and eventually can be absorbed by body [8]. The presence of digestive enzyme is a biological indicator for the capability of a fish to digest its feed. At the time high enzyme activity, it can be assumed that physiologically fish body is already capable of well-processing its feed [9]. The

development of digestive system is followed with the development of digestive enzyme, system and enzymatic function during larval stadium that are still very simple and yet to be developed perfectly [10].

This study aimed to determine the activity pattern of digestive enzymes of seed eel captured from nature starting from day 1 to day 71 of rearing period. Information on such activity pattern is expected can be used to determine feeding time and feed type in line with digestive enzyme activity that eventually can improve the effectiveness of feed utilization and increase survival rate on seed eel rearing.

II. MATERIALS AND METHODS

2.1 Material and Tools

Materials used in this study were 0.3 gram eel digestive tract for protease and amylase activity observations, 0.5 g eel digestive tract for lipase activity observation, 1% starch, phosphate buffer of pH 6.9, DNS reagent (3.5 dinitrosalicylic acid + KaNa-Tartrate), glucose, casein substrate buffer, pH 7.5 phosphate buffer, trichloroacetic acid (TCA), NaOH, Folin's reagent, tyrosine, vegetable oil, pH 7.0 phosphate buffer, acetone:ethanol solution and phenolphthalein (PP).

Tools used in this study were aquarium for seed rearing, DO meter, thermometer, vortex, 10 ml serological pipette, micro pipette, waterbath, spectrophotometer, 2.0 ml micro tube, thermal incubator, centrifuge, 50 ml burette and shaker incubator.

2.2 Maintenance Procedures Seed Eel In Laboratory

It began with seed eel rearing in laboratory to obtain seed eels of various sizes for digestive enzyme analyses. A total of 300-400 seed eels were prepared for each aquarium. Seed eels were reared in three aquariums of 60 x 40 x 30 cm^3 in size, equipped with aeration and internal filter systems. The rearing media used 5 ppt water salinity with the water was first previously precipitated in water container for 2-3 days and then aerated to maintain oxygen supply in the media. Water of 50 liters was put into each aquarium, allowed to stand and aerated for 2-3 hours. The seed eels were fed with *Artemia nauplii* at morning and evening since day 1 to 7 of rearing, with *Daphnia* for day 8 to 14 and with silk worm at morning and evening for day 15-71. To maintain the quality, the water of the rearing media was siphoned every morning and evening and a total of 20% aquarium's total water volume was replaced periodically every day. Temperature, pH and DO in this study were measured as physico-chemical parameters of water quality with temperature and pH were measured every morning and evening while DO was once a week for 10 rearing weeks.

2.3 Determination of Protease Activity

Protease activity was determined by following modified method from Bergmeyer *et al.* (1983), i.e. 50 µl digestive tract sample extract was mixed with 350 µl casein substrate prior to addition of 150 µl pH 7.5 phosphate buffer. The mix was then incubated at 30 °C for 30 minutes prior to addition of 1 ml TCA and allowed to stand 10 minutes. The sample was then centrifuged and the supernatant of which was added with 1.7 ml NaOH and 0.5 ml Folin's reagent. After being allowed to stand for 15 minutes, the mix's absorbance was read at λ 578 nm.

2.4 Determination of Lipase Activity

Lipase activity was determined by following modified method from Linfield *et al.* (1955), i.e. 3 gram vegetable oil and 1 gram polyvinyl alcohol were dissolved in pH 5.0 phosphate buffer prior to addition of the sample and incubation at 30 °C for 30 minutes. After added with 20 ml acetone:ethanol, the mix was titrated using 1N NaOH.

2.5 Determination of Amylase Activity

Amylase activity was determined by following modified method from Bernfield *et al.* (1984), i.e. 1 ml digestive tract sample extract was added with 1 ml starch prior to incubation at room temperature for 3 minutes. 1 ml DNS reagent was then added and the mix was heated in boiling water for 15 minutes. After added with 9 ml distilled water, the mix's absorbance was read at λ 540 nm.

2.3 Data Analysis

Data analysis in this study was seed eel's digestive enzyme activity analysis for a set time period, i.e. 10 rearing weeks. Data acquired from the measurement were analyzed quantitatively and presented in histogram graphs describing rearing period and observation variables. Water quality's physico-chemical parameters of eel rearing media were analyzed, including temperature, pH and DO; all of which were presented in a table that was descriptively analyzed.

Activity calculation:

$$\text{Enzyme activity (U/g)} = \frac{\text{Enzyme x Fp x V Buffer}}{\text{T x W Enzyme x BM}}$$

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Note: V Buffer = Buffer Volume (ml)
T = Incubation Time (minute)

W Enzyme = Enzyme Weight (ml)
Fp = Dilution factor
BM = Molecular Weight (ml)

III. RESULTS AND DISCUSSION

3.1 Water Quality during Rearing Period

See Table 1 for measurement result of water quality's physico-chemical parameters during seed eel rearing period. Based on Table 1, the value of the three parameters were still in optimal range to support the life of seed eels.

3.2 Activity Pattern of Protease, Amylase and Lipase

Figure 1 shows that the pattern of protease activity continued to increase. Protease activity at early rearing period was first found in small quantity and continued to increase along rearing period. Albeit decreases at day 14 and 42, they were not that significant compared to increases at day 28, 56 and 71 of the rearing period. Mature Japanese eel has high protease activity because the structure of mature eel digestive tract is already of perfect shape [11]. Detected protease activity during early rearing period indicated that seed eel is already capable of hydrolyzing its feed containing protein at early rearing age although the enzyme performance is yet to be optimum because the digestive tract system of which is yet to perfect. The statement was also supported by explaining that in nature seed eel consumes phytoplankton [12]. It is as natural feed containing high plant protein that is more difficult to digest because such protein is enveloped in cellulose, hence detected protease activity in small quantity during early rearing period [13-14]. High protease activity relates to pancreas role in secreting enzymes that work during mature age [15].

Because starting from being captured until reaching 1 gram in weight is critical phase for seed eel, not to mention low protease activity during such phase, the seed should be fed with natural feed containing enzymes, i.e. living feed. Protease activity of seed eel continued to increase from early rearing period until day 71 of rearing because during the period the seed eel's digestive tract was starting to develop and the body tissue was starting into perfect shape. This is in line with study previously who explained that gastric muscle of 6 cm-seed eel is still very simple, different to that of 14 cm-seed eel with more complex structure and circular and longitudinal muscles of which can be differentiated one another [12]. More complex seed eel digestive organs make it easier for feed to be digested, in addition to and higher enzyme activity of which.

Lipase activity pattern during early rearing period was seen high and tended to decrease along rearing age. The decrease might be due to more complex feed structure consumed by the seed eel, making it difficult for it to digest the feed. This is in line with study previously who stated that decrease in enzyme activity may be due to feed structure that is different with fish larval body structure, hence slow hydrolysis process that lead to low detected enzyme activity [16]. Low lipid content in the feed can also lead to low lipase activity in seed eel. Lipid content in *Tubifex* is around 13.77%, lower than protein and carbohydrate of which, i.e. around 54.72% and 22.25%, respectively [17].

See Figure 3 for data acquired from amylase activity measurement. Amylase activity pattern of seed eel showed decrease, although it tended to increase at day 56 and 71. The lowest activity of the enzyme was at day 42 of rearing period, while the highest was at day 1, indicating high body response on consuming phytoplankton as feed [12]. The structure of natural feed consumed by seed eel prior to rearing makes the feed easy to hydrolyze by seed eel's digestive tract. The structure was also the main factor for high enzyme activity during early observation, similar with silver catfish larva that has high amylase activity during early observation [18].

Study on Senegal fish (*Solea senegalensis*) [19] and red drum fish (*Sciaenops ocellatus*) [20] explained that the peak of amylase activity is during young larval period. Amylase activity on Atlantic halibut larva (*Hippoglossus hippoglossus*) and silver catfish larva *(Pangasius hypophthalmus*) that consuming of artemia is higher than protease and lipase activities [9,18]. Fish consuming phytoplankton and *Artemia* during early rearing period indicates that younger rearing age has higher response for amylase activity on *Artemia*.

Detected enzymes' activities at day 1 of rearing period indicated that very simple hydrolysis process took place in seed eel digestive tract. Such process also occurred in brown-marbled grouper fish at day 1 of lipase and amylase activity observation [16]. In general, activity pattern of amylase and lipase tended to decrease at day 42 of rearing period because the feed structure continued to be more different to the seed eel structure, making digestive organ affected, particularly the intestines. The growth of carnivorous fish's intestinal length can be influenced by its feed type and size [21].

Activity pattern of protease and amylase in seed eel was found decreased at day 14 of rearing period. It is assumed because the feed given was unable to be well digested by the seed eel, in addition to different feed structure that slowed down hydrolysis process in digestive tract of seed eel and eventually led to low identified enzyme activity. In contrast, lipase activity of seed eel increased at day 14 of rearing period, indicating higher lipid hydrolysis process compared to protein and carbohydrate hydrolyses. Activity pattern for the three

enzymes detected at day 56 of rearing period was found tended to increase, may be due to more complex body tissue structure, including enzyme-producing tissue [22].

3.3 Change in Weight of Seed Eel

See Figure 4 for data of change in weight of seed eel during rearing period. Seed eel weight continued to significantly increase during rearing period, particularly at day 42 of rearing age. The increase soared after the seed eel reached day 60 of rearing age with average weight of more than 1 gram.

IV. FIGURES AND TABLES

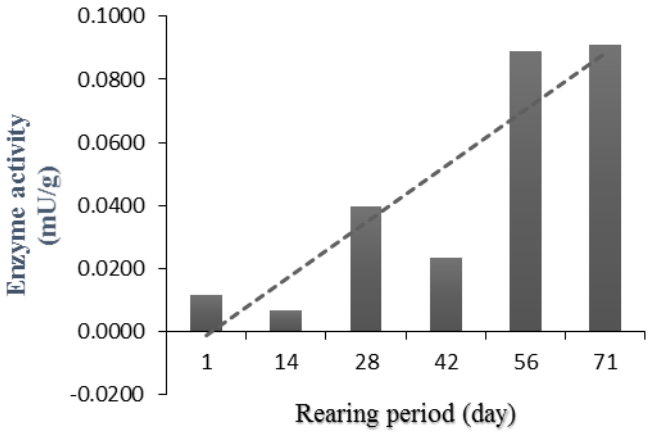

Fig 1: Protease activity of seed eel digestive tract (seed eel to elver)

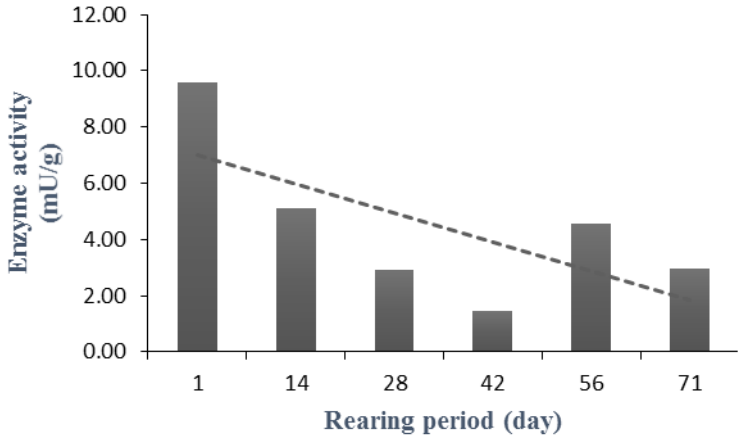

Fig 2: Lipase activity of seed eel digestive tract(seed eel to elver)

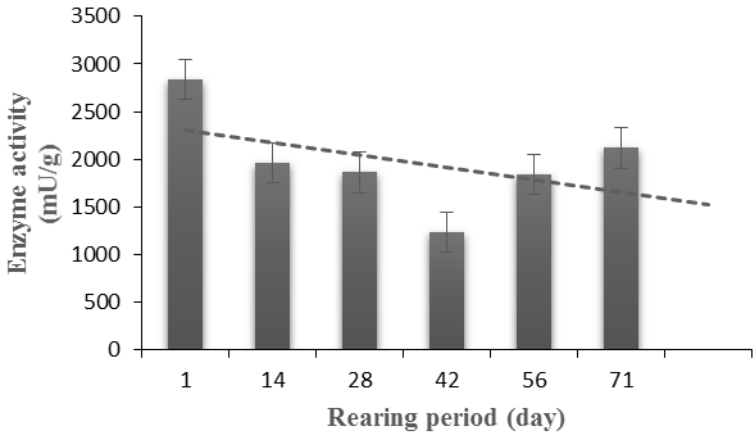

Fig 3: Amylase activity of seed eel digestive tract

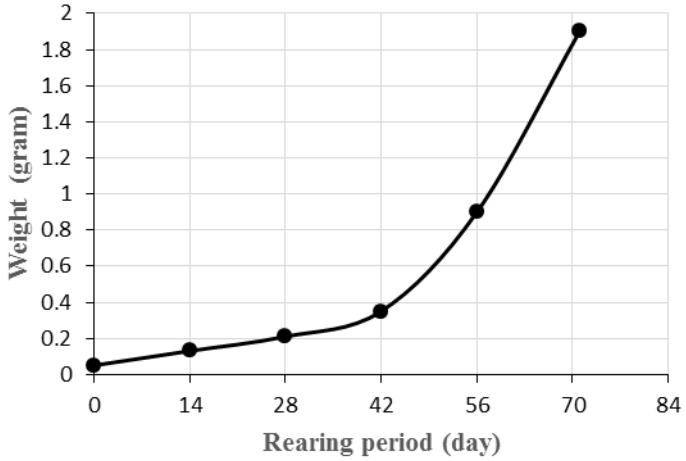

Fig 4: Growth of seed eel

Table 1: Water Quality Parameters

No.	Parameter	Unit	Measurement Tool	Value	References
1	Temperature	^{o}C	Thermometer	27–30	26–30[23]
2	Dissolved Oxygen	mg L^{-1}	DO meter	5.3–6.2	3.5–5.8[24]
3	pH	-	pH meter	6–7	6–8[25]

V. CONCLUSION

Protease, lipase and amylase activities were starting to be detected since seed eel phase, i.e. day 1 of rearing period. Protease activity increased along rearing age, while lipase and amylase activities tended to be high at early rearing period and eventually decreased until the end of rearing period.

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