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The Effectiveness of *Papain Enzyme* Supplementation in Artificial Feed on Growth and Survival Rate of Mangrove Crab Larvae (*Scylla Olivacea*)

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Abstract*—The ability of mud crab (*Scylla olivacea*) larvae to digest artificial feed depends on the availability of digestive enzymes. To improve the ability of the larvae to digest artificial feed can be done by adding exogenous enzymes. Papain is a protease enzyme capable of hydrolyzing proteins into simpler molecules so that the larvae can utilize artificial feed optimally. This study aimed to determine the appropriate dosage of papain enzyme and stadia of artificial feeding on growth and survival rate of mud crab (*Scylla olivacea*) larvae. The experimental design used was a factorial pattern with complete randomized design, the first factor was the dose of papain enzyme (0%, 1.5%, 3.0% and 4.5%), while the second factor was the different stadia of artificial feeding that started, at stadium Zoea II and Zoea III. The results showed that the difference of papain enzyme dose, different stadia of artificial feeding and the interaction between the dosage of papain enzyme and the artificial feeding stage had a significant effect (P <0,05) to the growth of carapid width and length and specific growth of individual weight. The artificial feeding larvae from Zoea II stadium with 3.0% and 4.5% papain enzyme dose were not significantly different (P> 0.05) compared to those administered with artificial feeds starting Zoea III stadia, but higher and significantly different (P <0.05) than those fed artificial feeds starting Zoea II stadia with a dose of 0% and 1.5% papain enzyme. The survival rate of larvae from zoea I to megalopae at a dose of 3.0% and 4.5% papain enzyme was not significantly different (P> 0.05) but higher and significantly different with 0% and 1.5% dose (P < 0.05). In the rearing of mud crabs larvae, artificial feed that has been predigesting with a dose of enzyme papain between 3.0% to 4.5% can be given starting stadia Zoea II.

***Keywords*— artificial diet; growth; papain enzyme dose; stadia larvae; survival rate.**

I. INTRODUCTION

The prolonged use of live food is not practical, nor is it economical [1], [2]. Live food culture depends on the weather [3], [4]. Food is one of the main sensitivities for society to climate. Rainy seasons, long droughts or extreme weather, such as floods and storms, can have a significant effect on crop yields and livestock production. The modern agricultural technology was born to help reduce this vulnerability and increase production. Drought or long drought in a country will have an impact on global production. Climate change has a measurable impact on the quality and quantity of globally produced foods. This is unlikely when compared with the significant increase in global food production that has been achieved over the last few decades

In the large-scale hatchery, the use of live feeding needs to be limited in time, and its role needs to be replaced with artificial feeds where nutritional composition can be tailored to the larval needs, lower production costs and higher flexibility [5]. Prices of feed ingredients and farming species fluctuate for various reasons. Cost and income are significant for customers to choose products that deliver satisfactory results. Using a proportional composition, feeding options and feeding regime can offer a unique support system that is used as the best economic performance model [1]. Customers and critical sources to adapt to this desire.

With annual aquaculture production reaching hundreds of thousands of metric tons in 2003, China cultivated more mangrove crabs than any other country [6]. Total production can be higher because the culture of mangrove crabs is traditionally a small scale business but massively run the family. The production of some of these small farms may not be included in official statistics. Mud crabs that live in warm temperatures, most of its production are in southern China. Indonesia and other Asian countries, such as Vietnam, the Philippines, and India also produce large-scale farming mud crabs with fast-growing industries. Crabs that live on shore and shallow estuaries and soft mud or sandy bottom.

They are also generally mangrove forest dwellers, hence their common name, "mangrove crab." Mud crabs often hide during the day and eat for the night. They are mostly omnivorous eaters with diets, including *mollusc*s, small *crustaceans*, *polychaetes*, and *detritus*. Mangrove crabs have a tolerance to salinity and explore the estuary and flow to forage. S. serrate [7], [8]. Mangrove crabs typically have strong osmoregulation ability and can survive well in brackish water at salinity as low as 3-5 parts per thousand [7].

In captivity, mud crabs can reach sexual maturity within a year. Carapace width of sexual maturity averages 9 and 14 cm according to the species [9]. Male and female crabs are still in soft-shell condition before copulation. Male crabs transfer *spermatophores* to seminal female containers during sexual intercourse that can survive for long periods of time and are used to fertilize eggs during subsequent spawning. After the intercourse, the newly smeared female crabs are often under the protection of male crabs for several days until the new shell turns to hard. The female crabs' ovab begin to develop and can take several months to mature. In nature, female serrates migrate offshore to spawn so are rarely found in estuaries and coastal waters. Very fertile mangrove crabs that can produce one million to eight million eggs per spawning according to the species and size of the female. Females attach the eggs to their *pleopods* and treat them until they hatch after spawning. This process can take ten days to more than one month according to water temperature.

Mud crabs are very popular throughout the Indo-Pacific region [10], especially in countries where they naturally multiply. They can live out of water for long periods of time. They can survive for more than 1 week when kept moist after being captured and marketed alive traditionally. However, they are also sold as fast food. There are also frozen for the soft-shell crab market. The price may vary from country to country. Mud crabs are generally expensive seafood. The size and type of crab can affect the market price. Female crabs with well-developed ovaries can reach prices several times more expensive than male crabs.

The increased fishing effort by market demand and mangrove habitat destruction during the second half of the twentieth century led to a decrease in mud crabs in many Asian countries [8]. However, this trend does not occur in Australia, where mangrove crab fisheries are strictly regulated, and supplies remain relatively healthy. Increasing demand and high market prices, coupled with reduced stocks have generated interest in the cultivation of mangrove crabs in many countries. Mud crabs have been booming in southern China for over 100 years and in other Southeast Asian countries for decades. In fact, the rapid expansion of this industry has occurred since centuries ago. The further expansion of the industry depends primarily on whether new hatcheries can replace wildly caught crab seeds.

Mangrove crabs are often found in estuaries and along the coast of tropical, subtropical and warm climates from east Africa to the southern tip of Japan and northeast Australia [6]. They also breed in many Pacific island countries. Large size with excellent meat makes it targeted even though the market price is high. In the past, there was much debate about their taxonomy, but it is now widely accepted that there are four species of mud crabs: *Scylla serrata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain*. By different species can live in the same location.

The ability of mud crab larvae (*Scylla olivacea*) to digest the artificial feed depends on the availability of digestive enzymes. Larva is able to utilize artificial feed at the time the enzyme has been produced. The results of Haryati *et al*. [11] study showed that the greatest relative changes in the activity of trypsin, lipase and α-amylase enzyme were the earliest in mud crab (*Scylla olivaacea*) larvae from Zoea II stadium to Zoea III. Based on these studies, artificial feed is thought to be given starting Zoea III. Gawlicka *et al*. [12] suggests that the activity of digestive enzymes is a good indicator to know the digestive capacity, if there is a high enough activity increase then physiologically can be shown that the larvae ready to consume artificial feed.

Furthermore, the results of the Haryati *et al*. [11] study showed that the survival rates of mud crab larvae (*Scylla olivacea*) from Zoea I stadium to megalopa fed artificial feed started Zoea II stadia was lowest compared to those still fed with natural and artificial feed which begin at Zoea III and IV. Efforts to accelerate the use of artificial feed can be done by predigest feed using exogenous enzymes. One of the enzymes that can be used is the enzyme papain. Papain is a protease enzyme that hydrolyzes proteins into simpler molecules.

Some researchers have done research on increasing the utilization of protein feed with papain enzyme. The result of Hasan [13] study in goldfish showed that the addition of 1,3 - 1,7% papain enzyme in artificial feed could increase the growth rate. According to Amalia *et al*. [14], the best dose of papain on the seeds of dumbo catfish (*C. Gariepinus*) was 2.25%, while Hamzah [15] research show that artificial feed that is predicted using 4% papain enzyme can be given to starfish pomfret larvae (*Trachinotus blochhii*) starting at 12 days.

The results of the Hutabarat *et al.* [16] study on freshwater lobster seed (*Cherax quadri carinatus*) showed that the addition of papain enzyme with a dose of 3.375% in feed gave the best response to feed utilization efficiency, protein ratio and relative growth rate, compared with 0% 1.125% and 2.25%, but the response was similar to the survival rate. This study aimed to determine the appropriate dosage of papain enzyme and stadia of artificial feeding on growth and survival rate of mud crab (*Scylla olivacea*) larvae.

II. Materials and Methods

A. Materials

1) Source of Larvae: The mud crab larvae that were used in this study came from the egg hatching. The mud crab brood stock was fed with squid and trash fish as much as 15% of biomass daily with the frequency of feeding twice times daily at 06.00 and 18.00. The container used in larval culture were a black plastic bucket volume of 30 litres, each container filled with water medium with salinity ranges from 30 - 32 ppt.

2) Papain enzyme: The papain enzyme used in this experiment was the Newzime brand produced by the

Brackish Water Cultivation Center (Jepara) of Jepara. The method of preparation and addition of papain enzyme into feed was as follows: first papain enzyme powder (according to treatment dosage) dissolved in 10 ml aquadest. The solution was then vortex and allowed to stand for 10-15 minutes. The enzyme is then sprayed into 100 g of the artificial feed and incubated for 60 minutes [13]. The incubated feed was ready to be administered to the larvae.

3) Larva feed: Live *foods used* in this experiment *was Brachionus* sp, while artificial diets a comercial *feed (*JP 0 dan JP1*). In* the treatment of artificial feeding from Zoea II stage, the combination of *Brachionus* and artificial feed was started from Zoea I to Zoea II, *Brachionus* density was 15 individuals mL^{-1}, and artificial feed 2.5 mg L^{-1}.

In the treatment of artificial feeding from Zoea III, *Brachionus* was given from Zoea II stage to Zoea II, and a combination of Brachionus and artificial feed was given from Zoea II to zoea III. Brachionus density was 30 individuals mL-1. Starting stadia Zoea II to Zoea III were fed artificial feed 5,0 mg L-1 daily, starting stadia Zoea III to megalopa 10 mg L-1 daily as in Figure 1 below.

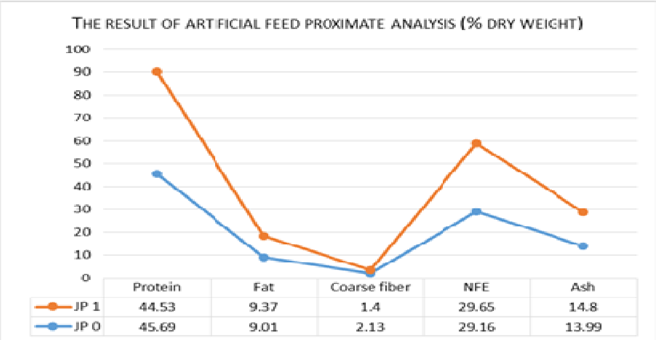

Fig. 1. Artificial feed proximate analysis

Figure 1 above indicates the artificial feed proximate analysis. It is the nutritional composition of the artificial feed. Artificial feed was given six times daily at 06.00, 09.00, 12.00, 15.00, 18.00 and 21.00.

B. Methods

The experimental design used was a factorial pattern with an entirely randomized design, three replications. The first factor was the dose of papain enzyme 0%, 1.5%, 3.0% and 4.5%, while the second factor was stadia where the artificial feed was started, i.e. *Zoea* II and III stadia. The following section is the variables of this research.

1) Growth: The growth of larvae was evaluated based on measuring the length and wide of carapace larvae, as well as the average growth rate of individual weight. Measurements of length and width of the carapace were performed at the beginning and end of the study ie at Zoea I stadium and *megalopa* using an ocular micrometre observed by microscope.

The width growth of the carapace (▲CW) = CWt - CWo. Where CWt = average carapace width at megalopa stage (mm), CWo = average carapace width at the Zoea I stadia (mm). The length growth of carapace (▲CL) = CLt - CLO. Where: CLt = average length of the carapace in stadia megalopa (mm), CLO = average length of the carapace in Zoea-1 stadia. The specific growth rate (SGR) = (Wt – W0)/t x 100. Where: Wt = average individual weight at megalopa stage (mg), Wo= average individual weight at the stadia Zoea I (mg) t = length of culture (days).

2) Survival Rate (%): The survival rate (SR) = Nt / N0 x 100. Where Nt = number of larvae of mangrove crab living on *stadia megalopa*. No = some larvae of mud crab in Zoea I stadia. To evaluate the effect of treatment on growth and survival rate of larvae was used analysis of variance. If the analysis proved that significant treatment followed by *W-Tukey* test to determine which treatment produced the best response

III. RESULTS AND DISCUSSIONS

A. Results

1) Growth: The width growth of carapace was presented in Table 1 whereas the interaction between papain enzyme dosage and stages of artificial feeding was presented in Figure 2. The result of variance analysis showed that the difference of enzyme dose, the difference of artificial feeding stadium and the interaction between the two had a significant effect (p<0,05) to the growth of carapace larvae of mud crabs from Zoea I stadia to megalopa as in Table 1 below.

TABLE I
MEAN GROWTH OF CARAPACE WIDTH (MM) OF MUD CRAB LARVAE FROM ZOEA I UNTIL MEGALOPAE STAGES

Treatment	The wide growth of the carapace (mm)
$P_{.0.0}$ZII	0.988 ± 0.0528^{a}
$P_{1.5}$ZII	1.021 ± 0.0050^{ab}
$P_{3.0}$ZII	1.024 ± 0.0060^{abc}
$P_{4.5}$ZII	1.054 ± 0.0056^{bc}
$P_{0.0}$ZIII	1.072 ± 0.0412^{bc}
$P_{1.5}$ZIII	1.089 ± 0.0400^{c}
$P_{3.0}$ZIII	1.061 ± 0.0079^{c}
$P_{4.5}$ZIII	1.083 ± 0.0042^{c}

P: Papain, Z II: Zoea II, Z III : Zoea III
Different letters in the same row indicate treatments significantly different (P<0.05).

Table 1 above indicates that the Tukey W test results that the growth of carapace width at the dosage of enzyme papain 0% was not significantly different (P> 0.05) compared to 1.5% and 3.0% in artificial feeding larvae starting zoea II stadia, but lower and significantly different than the dose of 4.5% and in the larvae were fed artificial feed predigest starting zoea III stadia. The growth of carapace width which was fed artificial feeds that were predigest using papain enzymes 3% and 4.5% starting Zoea II stadia was not significantly different compared to the predigest-artificial feeding larvae starting at Zoea III for all doses of papain enzyme. The interaction between the doses of papain enzyme and predigest feeding stages to the growth carapace width of Scylla olivacea larvae from *Zoea* I to *megalopa* is presetend in Figure 2 below.

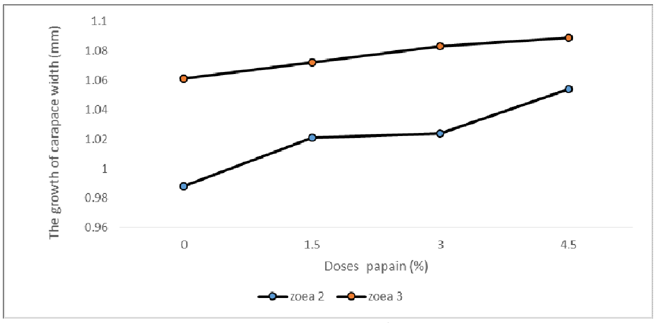

Fig 2. The interaction between the doses of papain enzyme and predigest feeding stages to the growth carapace width of Scylla olivacea larvae from *Zoea* I to *megalopa*

Figure 2 above shows the interaction between different doses of papain enzyme with different stadia of predigestion feeding. It indicates the increased dose of papain enzyme increased the width of the carapace. Based on data of broad growth of carapace, predigest artificial feeding can be given starting Zoea II stadia with dose enzyme papain 3%. Figure 2 above indicates that the growth length of carapace Scylla olivacea larvae from Zoea I to megalopa stages. The difference of papain enzyme dose and the different stadia of artificial feeding is presented in Table 2 below.

TABLE II
MEAN GROWTH OF CARAPACE LENGTH (MM) OF SCYLLA OLIVACEA LARVAE FROM ZOEA I TO MEGALOPAE STAGES

Dose papain (%)	The long growth of carapace (mm)
0.0	3.0445±0.1187[a]
1.5	3.0910±0.0934[ab]
3.0	3.0925±0.0436[ab]
4.5	3.2465±0.0827[b]

Different letters in the same row indicate treatments significantly different (P<0.05).

The result of variance analysis showed that the difference of papain enzyme dose and the different stadia of artificial feeding had a significant effect (p <0.05) on length growth carapace, the interaction between the two has no significant effect. Length carapace growth in predigested feeding larvae using the 0% enzyme papain lowest and significantly different from the doses of 1.5%, 3%, and 4.5%. The growth of carapace length in these three doses was not significantly different. Table 3 below reveals that the specific growth of average individual weights.

TABLE III
AVERAGE OF SPECIFIC INDIVIDUAL WEIGHT GROWTHS (%) OF MUD CRAB LARVAE FROM ZOEA I TO MEGALOPA

Treatment	Specific average individual weight growth (%)
$P_{0.0}$ZII	22.630 ±1.272 [a]
$P_{1.5}$ZII	30.220 ±1.429 [ac]
$P_{3.0}$ZII	30.537 ±2.916 [ac]
$P_{4.5}$ZII	41.463 ±4.475 [bc]
$P_{0.0}$ZIII	32.444 ±1.618 [ac]
$P_{1.5}$ZIII	33.500 ±6.175 [ac]
$P_{3.0}$ZIII	34.982 ±4.327[bc]
$P_{4.5}$ZIII	35.352 ±1.458 [bc]

P: papain, Z II: Zoea II, ZIII: Zoea III
Different letters in the same row indicate treatments significantly differen (P<0.05).

Table 3 above shows the result of the variance analysis including the difference in papain enzyme dosage, different stadia of artificial feeding, and the interaction between different doses of papain enzyme and different stadia of artificial feeding significantly effect (p <0.05) to the specific individual weight growth. The results of W Tukey test showed that artificial feeding started Zoea II stadia at 0% papain doses was similar with 1.5% and 3% and artificial feeding started Zoea III with enzyme concentration of papain 0 % and 1.5% but lower and significantly different (P<0,05) than other treatments. The growth rate of specific individual weights of larvae fed with predigest artificial feeds is presented in Figure 3 below.

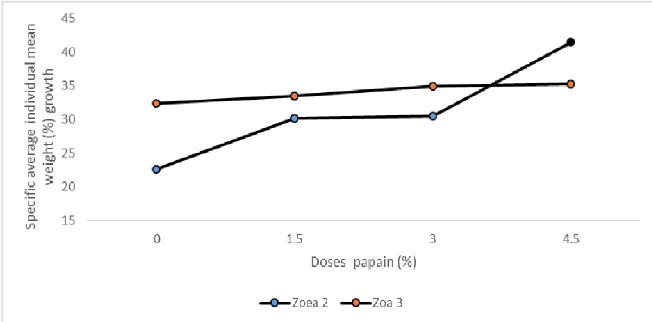

Fig. 3.Interaction between the papain enzyme dose and predigest feeding stages to the average specific individual growth weights of mud crab larvae (Scylla olivacea) from Zoea-1 to *megalopa*

Figure 3 above indicates the growth rate of specific individual weights of larvae fed with predigest artificial feeds started Zoea II stages in doses of 1.5%, 3.0%, and 4.5%. The predigest artificial feeding enzyme starting Zoea III at dosage of 0% papain enzyme, 1.5%, 3.0% and 4.5% were not significantly different (P>0,05). Figure 3 above comprises the interaction between the different doses of papain enzyme and the difference of predigesting feeding stages.

2) Survival Rate: the survival rate of mangrove crab larvae at various doses of papain enzyme from zoea-1 to megalopa stadia was presented in Table 4. The results of variance analysis showed that the difference of dose of papain enzyme and the difference of feeding stadia that had been hydrolyzed using papain enzyme had a significant effect (p <0.05) to the survival rate of mangrove crab larvae from zoea-1 stadia to megalopa.

TABLE IV
THE AVERAGE SURVIVAL RATE OF MANGROVE CRAB LARVAE, SCYLLA OLIVACEA BEGINS STADIA ZOEA-1 TO MEGALOPA AT VARIOUS DOSES OF PAPAIN ENZYME

Dose papain (%)	Survival rate (%)
0.0	11.3330±3.599[a]
1.5	12.7920±2.316[a]
3.0	15.7485±1.461[b]
4.5	17.4985±4.516[b]

Different letters in the same row indicate treatments significantly different (P<0.05)

Table 4 above shows the interaction between different doses of papain enzyme and the difference of feeding stadia that had been hydrolyzed with papain enzyme had no significant effect (p> 0.05). The results of the Tukey W test differing doses of papain enzyme showed that survival rates of artificial feeding larvae which were not hydrolyzed with papain enzyme or at the lowest 0% and 1.5% papain enzyme dose insignificantly difference but lowest and significantly different than those of papain enzymes 3.0% and 4.5%. The results of Hamzah [15] study on starfish pomfret larvae *(Trachinotus blochii*) showed that survival rate of larvae was fed artificial feed predigest with enzyme papain 4% was highest and significantly different from 0%, 1%, 2% and 3%.

B. Discussions

Based on the growth of width and length of carapace and the specific growth rate of individual weight, in the rearing of mud crab larvae, predigest feed can be given starting Zoea II stadia with doses of papain enzyme ranging from 3% to 4.5%. The result of analysis soluble protein content and protein hydrolysis levels showed that in the artificial diet which hydrolysis with enzyme papain 0% and 1,5% significant different but was lowest and insignificantly different compared to a dose of enzyme papain 3.0% and 4,5%. The content of soluble protein at a dose of enzyme papain of 3.0% and 4.5% was not different [11]. The low content of dissolved protein at the dose of papain enzyme was thought to be due to the limited concentration of enzymes that cannot hydrolyse the protein well

The lowest of total amino acid content was also at a dose of enzyme papain 0.0%, i.e. only 29.92%, whereas, at a dose of papain enzyme 1.5%, 3.0% and 4.5% were 36.47%, 37.26%, and 36.04% respectively [11]. The results of Kurniawan et al. [17] also showed the same pattern, the amino acid content of the squid ink sample without the addition of papain enzyme was also lower than the amino acid content of the hydrolysed sample with the addition of 2%. All hydrolysed proteins will produce amino acids, but some proteins also produce amino acids also produce binding protein molecules [18].

Papain is a protease enzyme capable of hydrolysing proteins contained in feed into simpler compounds that are easily digested and absorbed by mud crab larvae, which will have an impact on growth [19]. An easily digestible feed will produce energy supplies, energy will be used for maintenance and activity, and excess energy will be used for growth. Four percent of supplementation of papain to the artificial diet at the age of 12 and 15 days Silver Pompano larvae resulted in better protease enzyme activity [11]. Supplements of bovine trypsin resulted in better growth for *P. Japonicus*. It may be due to the activation of endogenous protease zymogen (s) in the digestive system and resulted in an increased digestive capacity of hepatopancreas [20].

The results of Hamzah [15] study on starfish pomfret larvae (*Trachinotus blochhii*) showed that artificial feeds that have been hydrolysed by papain enzyme at 4% dosage may be given to larvae from 12 days of age, without the addition of papain artificial feed enzymes to be given to fish age larvae 15 days [21]. The results of Patil and Singh [22] study showed that a diet supplemented with 0.1% papain enzyme, LOBA CHEMIE PVT, Bombay is suitable for the better growth and maximum utilization from feed for the rearing of post-larvae of *M. Rosenbergii* was due to the increase in protein digestion. The low survival rate of larvae fed artificial feeds that are not predigested by papain enzymes because the larvae cannot utilize artificial feed so that no energy available for growth and survival. Papain is a protease enzyme that hydrolyses proteins to short peptides in diet, which is the key factor to increase protein digestibility and helps to increase growth factors [19]. The digestible feed will produce energy supply; the energy will be used for maintenance and activity

IV. Conclusion

Based on the results of this study it can be concluded that in the rearing of mud crab larvae (*Scylla olivacea*) starting zoea stadia-1 to *megalopa*, artificial feed that has been predigesting by using enzyme papain dose of 3.0% to 4.5% can be given starting stadia Zoea II

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