The Effectiveness Of Addition Papain Enzyme In Artificial Diets On Growth And Survival, White Snapper Larva (*Lates calcarifer*, Bloch 1790)

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Abstract: Rearing barramundi larvae using natural food is considered ineffective, so it needs to be limited as early as possible by using artificial feed injected with exogenous enzymes (papain enzymes). This study aims to determine the dose of papain enzyme and the period of predigest feeding which results in the best growth and survival of barramundi larvae. Factorial pattern with completely randomized design was used to determine the appropriate dose of papain enzyme and larval age on growth and survival. The first factor was the dose of papain enzyme (0, 1.5, 3 and 4.5%), the second factor was the age of the larvae (14, 17 and 20 days). The results showed that the difference in the dose of the papain enzyme had a significant effect ($P<0.05$) on the daily growth rate with the highest yield at a dose of 3%, namely 3.25%, but the age treatment did not show results that had a significant effect. Similarly, survival in the treatment of different doses of papain enzyme had a significant effect ($P<0.05$) on survival with the highest yield at a dose of 3%, namely 69.28, while in the age treatment there was no significant effect between treatments. Based on the results of the study it can be interpreted that a dose of 3% is sufficient for artificial predigest feed and can be given to 14 day old larvae.

Keywords: *papain enzyme, barramundi larvae, daily growth rate, survival*

I. INTRODUCTION

The success of the nursery in producing quality seeds is influenced by various aspects, one of which is the feed used. Until now, barramundi hatcheries still rely on natural feed, both from phytoplankton (Nanochloropsis and Tetraselmis) and zooplankton (Rotifera and Artemia), while the use of artificial feed is used when the larvae are approaching harvest, which is at the age of 20-25 days (BPBL AMBON, 2019). However, giving natural food for a long time, namely from larvae entering the age of 2-25 days in hatchery activities, is somewhat less effective due to several factors, namely, the natural feed culture process is very sensitive to the environment, especially to climate change and is susceptible to contamination by pathogenic bacteria which can harm substances. who consume it (Haryati, 2017). In addition, its use is said to be less effective because natural feed requires a large container in the culture process so that it will affect the productivity of the hatchery, requires a lot of labor which has an impact on operational costs and additional costs in the culture process. Therefore, the nursery needs to limit the use of natural feed as much as possible by using artificial feed according to the needs of barramundi larvae.

Substitution of natural feed with artificial feed in rearing barramundi larvae is still ineffective because its use is still limited and combined with natural feed until the larvae enter the seed phase and use it at the age before harvest. Hardianti et al, (2016) reviewed the provision of artificial feed with different compositions to barramundi fry that were 30 days old and showed positive results on growth and maintaining seed life. The potential for using artificial feed in the larval stage of barramundi is quite potential, the artificial feed nutrition is complete and can be adjusted to the needs of barramundi larvae. The main obstacle faced in trying to substitute natural food with artificial food is the incomplete function of the digestive system in the early stages of larvae so that enzyme production is still limited and not sufficient to digest nutrients in the form of complex molecules contained in artificial feed (Rimandi, 2015).

One of the efforts that can be made to maximize the absorption of feed nutrients by barramundi larvae that have immature digestive organs is to limit the complex nutrients contained in the feed before it is given to the larvae. It is expected that artificial feeding with simpler nutrients can be maximally absorbed by barramundi larvae, although the production of digestive enzymes is still limited. The application of artificial feed in the maintenance of barramundi larvae requires special treatment that can ignore the nutrients in the feed, namely predigest.

Predigest is a simplification of feed nutrients from complex compounds to simpler ones, so that they can be absorbed optimally by barramundi larvae. Predigest artificial feed can be done using enzymes. Enzymes are proteins produced by living cells and used by cells to catalyze specific chemical reactions. Enzymes have tremendous catalytic power and are usually greater than synthetic catalysts. The specificity of the enzyme is very high for the substrate. Without the formation of by-products, enzymes are the functional units for metabolism in cells, working according to an orderly sequence. A well-coordinated enzyme system produces a harmonious relationship between a number of different metabolic activities (Shahib, 1992).

One of the enzymes that can be used for predigest artificial feed is the papain enzyme. Papain enzyme is a proteolytic enzyme contained in the papaya plant. Papaya latex contains 10% papain, 45% kimopapain and 20% lysozyme (Winarno, 1983). Papaya sap is found in almost all parts of the papaya plant, except for the roots and seeds. Papain content is most abundant in papaya fruit that is still young (Warisno, 2003). The application of the papain enzyme in artificial predigest feed has been extensively studied by previous researchers. Amalia et al (2013) studied the best dose of papain enzyme in African catfish (C. gariepinus) seeds. From their research, the best dose of papain enzyme was 2.25%. Hamzah (2015) conducted research on the effectiveness of adding the papain enzyme to artificial feed on the recovery of life and growth of pomfret starfish larvae (Trachinotus blochii, Lacepede 1801), in his research showing that the addition of the papain enzyme was 4% at the age of 12 and 15 days on artificial feed for fish larvae. pomfret pomfret has been shown to influence the activity of the protease enzyme, maintaining its life and daily growth. Haryati (2018) studied the effect of artificial feed predigest using the papain enzyme on the degree of protein hydrolysis and protease enzyme activity of mud crab larvae (Scylla olivacea) in zoea 2 and 3 and found that by using the papain enzyme 4.5% mud crab larvae could be identified. feed on zoea 2. However, the use of predigest feed with papain enzymes in rearing barramundi larvae has never been done. Based on the description above, the use of papain enzymes in feed is considered to increase the digestibility of artificial feed that will be given to barramundi larvae. Therefore this research is considered important to do.

II. METHODS

Time and Place of Research

This research was conducted from August to September 2020 at the Takalar Brackish Water Aquaculture Fisheries Center (BPBAP). Analysis of Protein Hydrolysis Degree and Dissolved Protein Was Conducted at the Animal Feed Chemistry Laboratory, Department of Nutrition and Animal Feed, Faculty of Animal Husbandry, Hasanuddin University, Makassar. while the Analysis of Protease Enzyme Activity was carried out at the Nutrition Laboratory of the Research Institute for Brackish Water Aquaculture and Fisheries Extension (BRPBAP3) Maros.

Test Animals

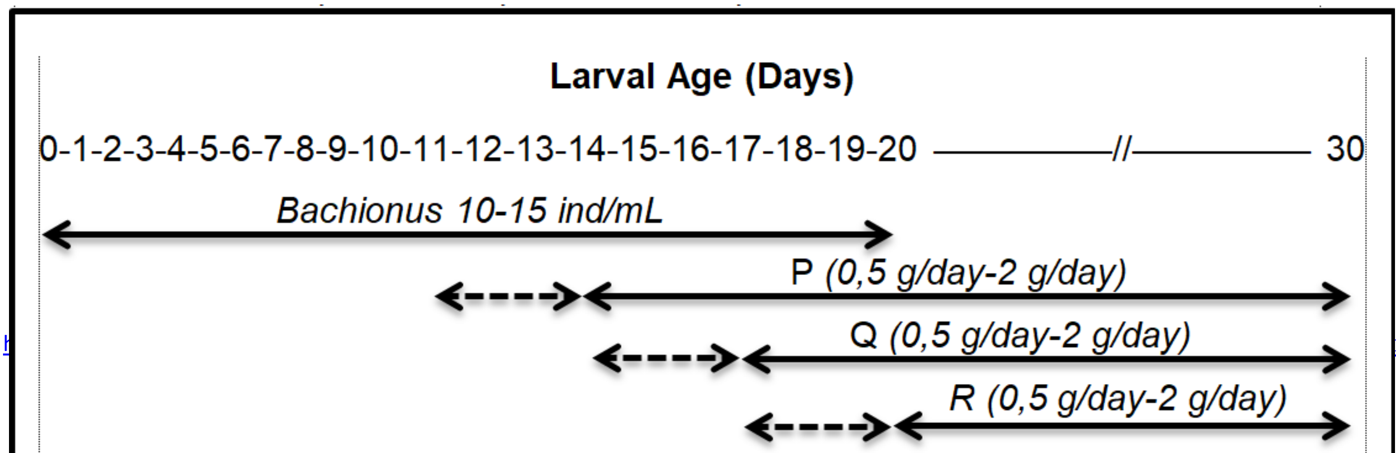
The test animals used in this study were barramundi larvae that were 1 day old which were spread into research containers and reared until the 30th day using the test feed. The containers used in this study were 36 green plastic basins with a capacity of 45 L and filled with 20 L of water. Barramundi larvae used in this study came from hatching eggs at BPBAP Takalar which were hatched in research containers.

Papain Enzyme

The enzyme used in this study was the papain enzyme with the brand Newzime which was produced by the Jepara Brackish Water Cultivation Center (BBBAP). The containers used in this study were 36 green plastic basins with a capacity of 45 L and filled with 20 L of water. The method for preparing and adding the papain enzyme to the test feed was: first of all, papain enzyme powder (according to the treatment dose) which was dissolved in 10 ml of distilled water, the solution was vortexed and allowed to stand for 10-15 minutes, then the enzyme was sprayed into 100 g of artificial feed using a 50 mL sprayer. then the feed is incubated for 60 minutes (Hasan, 2000). The feed that has been incubated is ready to be given to the test animals.

Feed Larvae

The test feed used in this study was the commercial feed used by BPBAP Takalar. The feeding scheme during the study can be seen in Figure 1 below:



Description: ⟷ Treatment feed
⇠---⇢ Overlap
P (12 day), Q (17 day), and R(20 day)

Research Design

The study designed a factorial pattern with a completely randomized design (CRD). There were 2 factors, the first factor was the dose of the papain enzyme (A) and the second factor was the time the feed was predigested with the papain enzyme (D) each given 3 repetitions.

1. The dose of papain enzyme (A) is:
A(0) = (0%)
B(1) = (1.5%)
C(2) = (3%)
D(3) = (4.5%)
2. Predigest feeding time (D) is:
D14 = giving predigest feed to 14 day old larvae
D17 = giving predigest feed to larvae aged 17 days
D20 = giving predigest feed to 20 days old larvae

Parameters

Parameters observed in this study include daily growth rate and survival rate:

Daily Growth Rate

The daily growth rate was observed by taking samples of barramundi larvae at the beginning and at the end of the study. The increase in length during the study was measured using Kamier's formula (1992) as follows:

$$G= (Ln.W_t-Ln.W_o)/t \times 100$$

Description:

Wt = final average individual length (g)
Wo = initial average individual length (g)
G = Daily growth rate (%/day)
t = maintenance time (days)

Survival

Larval survival (%) was determined by counting live fish at the end of the study and compared with the number of fish at the start of rearing, calculated using the following equation:

$$SR= N_t/N_o \times 100$$

Description:

SR = Survival (%)
Nt = Number of larvae at the end (tail)
No = Number of larvae at the start (tail)

Data Analysis

To determine the effect of papain enzyme doses and feeding of predigest barramundi larvae on daily growth rate and survival rate was analyzed using analysis of variance (ANOVA), if the results had a significant effect ($P<0.05$) then proceed with the W-Tukey test to determine the dose the best and the right age for giving predigest feed to barramundi larvae.

III. RESULT

Daily Growth Rate

The average daily growth rate of barramundi larvae for 30 days of rearing is presented in Table 6:

Table 6. The average daily growth rate of barramundi larvae fed with papain enzyme doses and different larval ages.

Treatment	Daily Growth Rate (%)
Dose (%):	
0	1,34±0,09 ^{b}
1,5	1,62±1,13 ^{b}
3	^{3,25} ±0,23a
4,5	^{3,50} ±0,14a
Age (days) :	
14	^{2,39} ±0,98a
17	^{2,36} ±0,96a
20	^{2,53} ±1,08a

Description: Different letters in the same column indicate the real one between the treatment at a level of 5% (p <0.05).

In the daily growth rate parameter, the results of interactions do not significantly affect the results that have no effect. So that data that is disguised in Table 6 is data single. Based on the average value of the daily growth rate of white snapper larvae in Table 6 in the treatment of the Papain enzyme dose ranges from 1.34% - 3.50%. The results of the analysis of the variety (ANOVA) show that the administration of the Papain enzyme dose has a very significant effect (p <0.01) on the daily growth rate of white snapper larvae (Appendix 10). Furthermore, the W-Tuckey test results show that many treatments with the highest dose (D = 4.5%) show the best results, but not significantly different (P> 0.05) (Appendix 13) with treatment C = 3%. Similarly, the lowest dose of dose is in treatment A = 0% statistically not significantly different (P> 0.05) with B = 1.5%. But the confession of C and D was significantly different (P> 0.05) with treatment A and B.

Table 6 also revealed the average value of the daily growth rate of white snapper larvae in the treatment range between 2.36% - 2.53%. The results of the analysis of the variety (ANOVA) show that the age treatment of white snapper larvae does not have a significant effect (P> 0.05) on the daily growth rate (Appendix 11). The highest average daily growth rate is obtained in the treatment r = 20 days, which is 2.53%, while the lowest value is obtained in the treatment Q = 17 days with an average value of 2.36%.

Survival

White Snapper Larvae Larvae can be seen in Table 7:

Table 7. The average survival in white snapper larvae is feed with a dose of papain enzymes and different larvae ages

Tretment	Survival(%)
Dose (%):	
0	49,94±9,86 ^{b}
1,5	51,94±7,10 ^{b}
3	^{69,28} ±5,94a
4,5	^{71,44} ±3,92a
Age (days) :	
14	^{58,17} ±12,41a
17	^{61,46} ±13,45a
20	^{62,33} ±10,52a

Description: Different letters in the same pool indicate the real one between the treatment at a level of 5% (p <0.05).

The data presented in Table 7 is a single analysis, because the interaction between the dose and age treatment does not show results that have a significant effect. Based on Table 7 the average value of the white snapper larvae on the above can be seen that the average value of the dosage of the dose of the Papain enzyme ranges from 49.94%-71.44%. The results of the analysis of the variety (ANOVA) show that the administration of the Papain enzyme dose has a very significant effect (p <0.01) on the face of white snapper larvae (Appendix 15). Furthermore, the results of the further test W-Tuckey show that the synthesis in treatment D is not significantly different (P> 0.05) with treatment C, while treatment C and D are significantly different (P> 0.05) with treatment A and B (Appendix 19) .

In the age treatment of white snapper larvae from Table 7 the average survivor can be seen that the value of the diligence ranges from 58%-62%. The highest results are obtained in treatment R with an average value of 62%, while the highest value is obtained in treatment P with an average value of 58%. Statistically the results of various analysis (ANOVA) show that the face of white snapper larvae is not significantly different (P> 0.01) (Appendix 16) between all treatments.

IV. DISCUSSION

Daily Growth Rate

The administration of papain enzymes in feed shows that artificial feed can increase the growth rate of the daily larvae of white snapper with the right dose. From the results of the analysis of the degree of hydrolysis of dissolved protein and protein indicates the presence of positive results with the addition of papain enzymes into feed, in the growth rate test it is proven that the addition of the enzyme papain with the right dose has a positive impact on the growth rate of larvae and can accelerate natural feed substitution by artificial feed . Giving credigest feed with the enzyme papain in white snapper larvae is able to hydrolyze artificial feed proteins from complex compounds to simpler compounds, so that it can increase the daily growth rate of white snapper larvae. This is supported by the results of research conducted by several previous researchers: Hasan (2000) in carp, Ananda et al. (2015) in catfish (P. hypotpthalmus), Patil and Singh (2014) in giant prawns (macrobrachium rosenbergii), Amalia et al. (2013) in catfish dumbo (C. Gariepenus) and Harisa Hamzah (2015) in Bawal Star (T. Blochii) fish.

The growth rate of the daily white snapper larvae obtained in this study is relatively higher than those obtained by previous researchers, namely Patil and Singh (2014) which reviews the effect of the addition of the papain enzymes on artificial feed on the growth of post giant prawn larvae (macrobrachium rosengergii), within His research obtained a specific growth rate of 1.41% with the addition of 0.1% papain papain enzyme. Similarly, what was obtained by Amalia et al (2013) in catfish dumbo (C. Gariepenus) with the addition of 0.75% papain enzyme can increase the relative growth rate of 2.80%. The high rate of daily growth obtained in this study is caused by a good degree of hydrolysis of feed and protease enzyme activity that suits the needs of the larvae. This shows that the administration of 3% -4.5% papain enzyme dosage into artificial feed is sufficient in meeting the needs of white snapper larvae, the daily growth rate in white snapper larvae is influenced by the presence of energy supply contained in the given feed. It can be said that the amount of energy in the feed is sufficient for the maintenance of the body of the larvae and other activities, so that there are excess energy that can be used for growth. As stated by Lovel (1988), that before growth, energy needs for body maintenance and other activities must be met first.

The feed used in the maintenance of white snapper larvae during the study basically has the same nutritional content, but after predigest using papain enzymes, the digestion of the feed is different caused by the difference in the difference in the dose of the papain enzyme used. This causes the daily growth rate value to be different from each other. The addition of papain enzymes does not add to the protein content, but after being credited the polypeptide molecule becomes simpler which results in easily digested by larvae. From the results of the analysis of the degree of hydrolysis and dissolved protein in feed shows that with the addition of papain enzymes into the feed, the degree of hydrolysis and dissolved protein is significantly different from the feed without the enzyme papain. This is in accordance with what Reed (1975) said, that the enzyme dose is one of the factors that affect the hydrolysis process.

Survival

The synthesis obtained in this treatment is relatively lower when compared to those obtained by previous research in different commodities, namely taqwdasbriliani et al. (2013) on the tiger grouper seed (epinephelus fuscogutattus) which gets a 100% face using 5 ml of papain enzymes. Hutabarat et al. (2015) in freshwater lobster seeds (cherax quadricarinatus) show that the addition of the papain enzyme at a dose of 3.375% can produce a 96.67% face. This indicates the enzyme papain is sufficient to have an influence in the use of artificial feed for the maintenance of white snapper larvae. In this study the dose of the papain enzyme used is considered to be sufficient in supporting the basic needs of the larvae as evidenced by a face that is able to compete and even exceed the maintenance of larvae using natural feed, as evidenced in the analysis of the degree of hydrolysis and dissolved protein, the feed is seen that feed without using the Papain enzyme has a value The degree of hydrolysis of feed and low dissolved protein on the contrary with the addition of papain enzymes in hydrolyzing artificial feed, the degree of hydrolysis and dissolved protein is also high. Because of the use of papain enzymes into artificial feed so that artificial feed protein can be simplified first before being given to so that the larvae in the digestive process of feed larvae are immediately absorbed and do not require a lot of energy in digesting feed.

Signasis is one of the important tor that needs to be measured in the fish maintenance process. The high value of the synthesis obtained can have an impact on the success of a hatchery and enlargement business. Hardyani (2013) states that the value of fish synthesis is the main key in fish farming activities because the number of living fish can affect a value of aquaculture production. White snapper larvae are sea fish larvae whose survival value is very low. Generally sea fish larvae are only able to reach ± 30%. In Nurmasyita's report (2018) stated that the white snapper seeds at the end of maintenance reached 39.4% for 30 days of maintenance.

V. CONCLUSION

The conclusion of this study is that the enzyme papain is effectively used in artificial predigest feed with a dose of 3% and can be given to the larvae of white snapper aged 14 days.

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