

The Efficiency of Feed Utilization, Growth, and Survival Rate Performance of Saline Tilapia (*Oreochromis niloticus*) by the Additional of Papain Enzyme in Artificial Feed

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ABSTRACT

Saline tilapia (*Oreochromis niloticus*) is one of the brackishwater commodities that began to be widely cultivated in fishery sector of Indonesia and has economic value. Lack of efficiency of feed utilization was one of the problems in saline tilapia culture. This can be overcome by the addition of exogenous enzymes that can play important role in feed digestion. One example of exogenous enzymes is the papain enzyme. Papain enzyme is a proteolytic enzyme derived from papaya that was able to break down proteins into amino acids that are easier to digest. The aims of this study were to determine the effect of papain enzyme in artificial feed and to determine the optimal dose of papain enzyme to efficiency of feed utilization, growth, and survival rate performance of saline tilapia (*O. niloticus*). This research was carried out at Coastal Development Laboratory, Jepara, Jawa Tengah on January – March 2018. Test fish used was an average weight of $2,35 \pm 0,05$ g/ind. The research used experimental method by completely randomized design with 4 treatments and 3 replications. The treatments were A, B, C, D with 0g/kg pakan, 3g/kg pakan, 6g/kg pakan, and 9g/kg pakan. The result showed that the addition of papain enzyme was significantly ($P < 0,05$) to efficiency of feed utilization, protein efficiency ratio (PER) and relative growth rate (RGR). However, the papain enzyme had no significant effect ($P > 0,05$) on survival rate (SR). The optimal dose of papain enzyme obtained was 5,5 g/kg feed; 5,4 g/kg feed; 5 g/kg feed capable to produce the maximum of efficiency of feed utilization, protein efficiency ratio, and relative growth rate 63,48%, 1,75%, and 4,61%/day.

Keywords: Saline Tilapia; Papain Enzyme; Feed Utilization; Growth

INTRODUCTION

Saline tilapia (*Oreochromis niloticus*) is one of the brackish water commodities that began to be widely cultivated in fishery sector of Indonesia because it is able to live at a high salinity tolerance in the range 0-45 ppt. Saline tilapia also an important type of economical fish and having great demand by the local and international market because it has a taste of meat that is tasty, savory, and high protein content. According to Perwito stated that saline tilapia is able to live in both brackish water and sea water with a salt content 0-45 ppt, besides that the saline tilapia is a consumption fish that is in great demand by the public because of its delicious meat taste and high protein nutritional content. The development of saline tilapia is

currently to spur fish production as a source of animal protein food for the community and at the same time increase exports in the international market [1].

The production development of saline tilapia has a problem. The problem that is often faced by saline tilapia farmers is the efficiency of the utilization of feed that has not been maximized. Economically high feed efficiency will affect the cost of feed so that it affects the cost of production. Feeding efficiency can reduce production cost, but still having the nutritional value needed by fish is an alternative that need to be pursued. Several ways are carried out to improve feed efficiency including optimizing digestion and absorption of feed and increasing the value of protein efficiency by adding enzymes to digestion. There are two types of enzymes in digestion, namely endogenous

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enzymes and exogeneous enzymes to help accelerate the process of digestion and hydrolysis. In accordance with Mo, that exogeneous enzymes could hydrolyze protein and shorter the chain of peptide in feed so that enhance protein digestion and make feed demolition better and more efficient. Papain enzyme can reduce the amount of nitrogen in water caused by the dismantling of fish feed and excretion, because papain can help the digestion process of protein that is good and efficient [2].

The growth of saline tilapia is closely related to the protein content in feed. The protein content in the feed given must be in sufficient quantities. Papain enzyme have function to help digest protein in the body of saline tilapia so that it can be used for growth. According to Dawood that exogeneous enzyme can increase nutrient absorption of feed. Exogenous enzyme is now often used as fish additives to increase the nutritional value of feed, especially those made from vegetable protein. The use of exogenous enzyme (pepsin, papain, an amylase) is highly recommended to increase the rate of growth. Amylase is an enzyme needed to break down carbohydrates, this enzyme is found in many herbivorous and omnivorous fish. So that the use of exogenous enzyme can help break down vegetable protein.

Several studies have been conducted on the use of the papain enzyme in new artificial feed used for goldfish (*Crypinuscarpio*) with the best concentration of 2%, and Keureling fish (*Tor tambra*) the best concentration of 27,5 mg/gr feed [1]. This study refers to the research of [2], in tilapia (*O. niloticus*) the best concentration of papain is 6g/kg of feed so that papain enzyme in artificial feed can affect the efficiency of feed utilization, growth and survival of saline tilapia (*O. niloticus*).

This study aims to examine the effect and determine the optimum dose of the addition of papain enzyme to artificial feed on the efficiency of feed utilization, growth, and survival of saline tilapia (*O. niloticus*). This research was conducted in January 2018 - March 2018 at the Coastal Area Development Laboratory, Jepara.

RESEARCH MATERIAL AND METHODS

The fish used in this study were saline tilapia (*O. niloticus*) with an average weight of $2.35 \pm 0,07$ gram from the Brackish Water Aquaculture Center, Jepara with stocking density of 1 fish / L (SNI,1999). The basic feed used in the study is pellet-shaped commercial feed with the trademark "Feed FF-999 Floating Fish Seed" with a protein content of 33%. The test feed used is a basic feed supplemented with papain enzyme in accordance with the treatment by spray (sprayed). The maintenance container used in this study is a plastic bucket with a capacity of 25 L. The number of buckets used is 12 pieces. The placement of buckets in each treatment and repetition is drawn randomly.

The procedure in this study consist of a preliminary test, and the implementation stage. Preliminary test was conducted to determine the dose of papain enzyme to be used in the study. The dose of papain enzyme used in the preliminary test is 0g/kg of feed, 2g/kg of feed, 4g/kg of feed, and 6g/kg of feed. The dosage refers to the research of Farraget about the addition of papain enzyme to artificial feed for tilapia and the best

treatment is at a dose of 6g/kg of feed. The best results of preliminary tests that have been carried out on saline tilapia are 6g/kg of feed.

The implementation of this research was carried out with several preparations, such as preparation of containers, test animals, test feed, maintenance media, and water quality management. The container preparation carried out in this study used 12 buckets, then sterilized. Sterilization of tools and materials includes washing the container with detergent, rinsing, then giving chlorine for 24-hours, rinsing and drying. The maintenance container is equipped with aeration using a 100-watt blower so that the oxygen demand for saline tilapia (*O. niloticus*) is fulfilled. Preparation of test fish by first adapting it with maintenance media and artificial feed that has not been mixed with enzymes. Before adaptation, the test fish is selected first to get a uniform weight. This adaptation is done until the fish can adjust to the new feed and environment for one week. Fish retrieval can be done using "seser" and to find out the weight can use an electric scale, then granting is carried out for 1 day before the treatment which aims to remove the metabolic waste from the feed that has been given previously. After selection of test fish, measurements of body weight and length of saline tilapia (*O. niloticus*) were measured using a digital scale with an accuracy of 0,01 g. Body weight measurements were carried out at the beginning and the end of maintenance to determine the growth rate of saline tilapia (*O. niloticus*).

Feeding using the Fix Feeding method with the frequency of feeding three times a day [3]. The test feed was then added with papain enzyme according to treatment, namely feed A (0 g/kg of feed), feed B (3 g/kg of feed), feed C (6 g/kg of feed), and feed D (9 g/kg of feed). The papain enzyme used in this study was papain enzyme obtained from the Brackish Water Aquaculture Center, Jepara under the trademark "NEWZIME". The papain enzyme used is obtained in powder form. The active ingredient of the papain enzyme used was protease 0,16 mU/g, lipase 2,40 mU/g, and amylase 0,73 mU/g.

The media preparation of fish rearing in this study consisted of a mixture of sea water from the 30 ppt salinity brackish water aquaculture center (BBPBAP) which had been deposited for 3 days in a reservoir. The process of mixing between sea water and fresh water uses a ratio of 2:3 to reach 20 ppt salinity [4]. The test media is filtered using Dacron foam to prevent sediment from entering the test container. Maintenance of water quality is carried out by staining the feces and remaining feed 2 times a day, in the morning and evening. Management of water quality in maintenance media is carried out by measuring the water quality parameters observed during the study such as temperature (OC) measured every day. The degree of acidity (pH), dissolve oxygen (DO), and salinity are measured every week. Ammonia (NH₃) measured at the beginning, middle, and the end of maintenance. Water quality in this study was measured using water quality checker, pH meter, and refractometer. The addition of aeration in this study is an effort that is used to increase dissolved oxygen during the fish rearing period.

The experimental design used in this study was a completely randomized design (CRD) using 4 treatment and 3 repetitions. The treatments carried out in this study are as follows :

- Treatment A : test feed with papain enzyme 0g/kg of feed
- Treatment B : test feed with papain enzyme 3g/kg of feed
- Treatment C : test feed with papain enzyme 6g/kg of feed
- Treatment D : test feed with papain enzyme 9g/kg of feed

Data collection

Efficiency of Feed Utilization (EPP)

Based on Tacon (1987), calculation of efficiency of feed utilization using the formula:

$$EPP = \frac{W_t - W_o}{F} \times 100\%$$

Information:

EPP = Efficiency of Feed Utilization (%)

W_o = Weight of fish at the beginning of study (g)

W_t = Weight of fish at the end of study (g)

F = Total weight of feed given during the study (g)

Figure 1 : EPP

Relative Growth Rate (RGR)

According to Zonneveld *et al.* (1991) relative growth rate (RGR) of fish calculate using formula:

$$RGR = \frac{W_t - W_o}{W_o \times t} \times 100\%$$

Information:

RGR = Relative growth rate (% per day)

W_t = Total weight of fish at the end of maintenance (g)

W_o = Total weight of fish at the beginning of maintenance (g)

Figure 2: RGR

Water quality

Water quality parameters that is observed such as temperature (OC), degree of acidity (pH), dissolve oxygen (DO), salinity (ppt) and ammonia (NH3). Observation of water quality of ammonia (NH3) content was carried out at the beginning, middle and the end of the study, to observe the parameters of water quality pH, DO, and salinity carried out every 1 week, while observations of temperature water quality were carried out every day. Measurement of ammonia content was analyzed using a spectrophotometer while pH measurements used a pH meter by dipping the pH meter in the maintenance container and seeing the results on screen. Salinity measurement using a refractometer. Dissolved oxygen (DO) measurements using DO meters[4].

Data analysis

The results of the research data include EPP, and RGR data analyzed by variance analysis (ANNOVA). The data obtained were first tested in the form of normality test, homogeneity test, additivity test before variance analysis (ANNOVA) of the observed variables. If the analysis of variance was found to have a significant effect (P<0,05) or had a very significant effect (P<0,01), Duncan's multiple region test was conducted to determine the difference in middle values between treatment. Tests to estimate the optimum dose of papain enzyme in the

feed tested using Orthogonal Polynomial using the Mapple version 16,0 application, and the water quality analysis was carried out descriptively.

RESULTS

Results of the research shows that the addition of papain enzyme into artificial feed give significant effect (P,0,05) for EPP and RGR does not affect water quality of maintenance container. The results of EPP and RGR values through the addition of exogenous papain enzyme in artificial feed of saline tilapia (*O.niloticus*) can be seen in Table 1, and in the histogram shown in Figure 1, for water quality results can be seen in Table 1.

Treatment	Observed Variables	
	Efficiency of Feed Utilization (%)	Relative Growth Rate (%/day)
A	45,03 ± 2,97b	3,53 ± 0,23b
B	58,92 ± 2,18a	4,19 ± 0,27b
C	64,03 ± 5,67a	4,81 ± 0,24a
D	55,66 ± 3,90a	3,79 ± 0,08b

Table 1: Mean Value of EPP and RGR in Saline Tilapia (*O. niloticus*) during Maintenance.

Information: Different superscript shows significant differences (P<0,05)

Based on the table above, mean value of efficiency of feed utilization (EPP) from the highest to the lowest is treatment C which is 64,03 ± 5,67%, treatment B amounted to 58,92 ± 2,18%, treatment D is 55,66 ± 3,90%, and treatment A resulted to 45,03±2,97%. Mean value of relative growth rate from the highest to the lowest is treatment C amounted to 4,81%/day, treatment B is 4,19%/day, treatment D is 3,79% and treatment A is 3,53%/day.

The result of orthogonal polynomial graphic efficiency of utilization feed (EPP), protein efficiency ratio (PER), and relative growth rate (RGR) in saline tilapia (*O.niloticus*) during research shows in Figure 2.

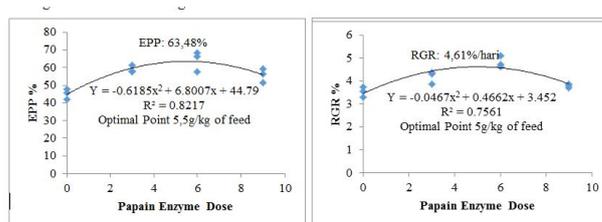


Figure 2: Orthogonal Polynomial Graphic Efficiency of Utilization Feed and Relative Growth Rate

Based on the orthogonal polynomial test on EPP obtained a quadratic patterned relationship (Y= -61.852x² + 68.007x + 44.79) and R² = 0,8217 and optimum point on treatment C

(addition of papain enzyme as much as 6g/kg of feed) obtained optimal dose of papain enzyme which is 0,55% capable of producing a maximum EPP of 63,48%. The R2 value indicates that 82% of EPP is affected by the addition of papain in artificial feed and 18% is influenced by other factors. Orthogonal polynomial test on RGR obtained a quadratic pattern relationship ($Y = -4.6667x^2 + 4.6622x + 3.452$) and $R^2 = 0,7561$ and optimum point in treatment C (addition of papain 6g/kg of feed) obtained the optimal dose of papain enzyme which is 0,50% can produce a maximum RGR of 4,61%/day. The R2 value shows that 75% of RGR is affected by the addition of papain enzyme in artificial feed and 25% is influenced by other factors.

Treat ment	Range of Water Qualit y Param eter Value				
	Tempe rature (OC)	pH	DO (mg/L)	Salinit y	NH3 (mg/L)
A	25,0-2 8,8	6,8	3,7-4,4	20	0,002- 0,003
B	25,0-2 8,3	6,8	3,5-4,3	20	0,003- 0,004
C	25,0-2 8,7	6,7	3,4-4,3	20	0,003- 0,004
D	25,0-2 8,7	6,7	3,5-4,2	20	0,003- 0,005
Library (Feasib ility)	24 - 30a	6,5 - 8,0a	3-5b	10-20c	<0,1d

Table 2: The results of measurement of water quality parameters in the maintenance medium of saline tilapia (*O. niloticus*) during maintenance are presented

The result of measurements of water quality parameter during research shows that water quality parameter value during research still in the decent condition for cultivation medium of saline tilapia (*O. niloticus*). This is based on the library about optimum water quality condition for Saline Tilapia seed (*O. niloticus*).

DISCUSSION

Efficiency of feed utilization

According to the result of variance analysis that have been done, known that the addition of papain enzyme in the artificial feed gave significant effect ($P < 0.05$) to the efficiency of feed

utilization (EPP) saline tilapia (*O. niloticus*). This is showed us that the addition of papain enzyme in the artificial feed for saline tilapia (*O. niloticus*) could help the process of food digestion that were given so that the energy in the feed could fulfill the repair of body tissue needs, fish activity and the growth. The more nutrients in the food that are easily absorbed in the body of the fish, the higher value of the efficiency of the feed utilization in fish. The protein contained in feed is able to hydrolyzed by the papain enzyme into amino acid, so that the feed becomes more easily digested and absorbed by the fish's body. Papain enzyme can increase the decomposition and digestion of feed in the body of the fish. This is reinforced, which states the addition of papain enzyme to feed ingredients, especially those containing vegetable protein, can improve the decomposition and digestion of feed [5].

The highest value of feed utilization efficiency in in treatment C of $64,03 \pm 5,67\%$, treatment B is $58,92 \pm 2,18\%$, treatment D is $55,66 \pm 3,90\%$ and followed by treatment A resulted to $45,03 \pm 2,97\%$. These results suggest that the addition of papain enzyme can increase the digestibility of feed and can affect the efficiency of fish feed utilization. In accordance with Patil and Singh, proteolytic enzyme plays an important role in the process of protein digestion. Proteolytic enzyme converts proteins in feed that enter the body into simple peptides and amino acids so that they can be absorbed by the body. Increased digestibility of feed containing in the papain enzyme due to the availability of proteolytic enzyme in feed which is able to help the process of protein hydrolysis in the digestion of fish.

The results of this study indicate that feed can be consumed properly by saline tilapia because of the high value of the efficiency of feed utilization, so that feed can be digested for growth. Feed efficiency is a comparison of body weight produced with the amount of feed given during maintenance. Feed can be consumed properly by saline tilapia because of high efficiency of feed utilization, so that food can be digested for the growth process. According to Muchlisinget, that high feed utilization efficiency shows that feed has been digested and absorbed optimally by fish for growth. The efficiency of high feed utilization also shows that the application of papain enzyme successfully increases feed utilization.

Papain enzyme is a protease enzyme that is able to hydrolyze protein into simple elements so that the body of saline tilapia can digest protein in feed more efficiently so as to increase the efficiency of feed utilization in saline tilapia. According to Rostikaet, stated that the papain enzyme is a protease enzyme that is able to hydrolyze complex protein compounds into simple elements (amino acids), so that it can be easily digested optimally by the fish's body. Addition of papain hydrolysis. This will result in increasing feed digestibility. The increase in digestion can increase the absorption of amino acids into the body for growth.

Papain enzyme contains protease enzyme which are still mixed with lipase, amylase, protein and fat. The addition of the papain enzyme to saline tilapia feed can cause these ingredients to break down before being eaten by saline tilapia. In accordance with Ihsan and Mahsul, that papain enzyme contains protease enzymes. However, it is still mixed with other compounds such

as lipase, amylase, protein and fat. Protease enzyme play a role in breaking down protein macromolecules into simple's constituent compound. While lipase and amylase play a role in deciphering lipids and carbohydrates. The addition of coarse papain enzyme to the substrate in this case protein lipids, and carbohydrates in fish pallets will cause these macromolecules to beak down before being eaten by fish.

Relative growth rate

Based on the results of variance analysis showed that artificial feed with the addition of papain enzyme had a significant effect ($P < 0,05$) on the relative growth rate of saline tilapia (*O. niloticus*). It is thought that the higher protein value in the feed can cause an increase in growth. Papain enzyme have the ability to break down proteins quickly. According to Isnawatiet, the high growth rate is influenced by the increase in protein content and body fat content which functions as a builder of muscles, cells, and tissues and as an energy source. High growth rates related to high feed efficiency indicate that efficient feed use, so that it can meet energy needs and the rest for growth.

The highest value of the relative growth rate in a row is treatment C of $4.81 \pm 0.24\%$, treatment B of $4.19 \pm 0.27\%$, treatment D of $3.79 \pm 0.08\%$ and treatment A of $3, 53 \pm 0.23\%$. The value of the relative growth rate in saline tilapia which was given in artificial feed added with papain enzyme was higher than saline tilapia which was given artificial feed without the addition of papain enzyme. The addition of papain enzyme can increase the value of the relative growth rate in saline tilapia. This is reinforced by Singh, stated that papain is a protease enzyme that hydrolyze feed protein, which is a key factor to increase protein digestibility, speed up absorption, and help increase growth factors.

The addition of the papain enzyme as an exogenous enzyme in artificial feed aims to increase the digestibility of feed in the body of saline tilapia (*O. niloticus*) and increase feed quality so that fish can digest food efficiently. In accordance with Almeideet, the addition of exogenous enzyme has the potential to optimize the digestibility of plant-based feeds. Furthermore, it is said that if the composition of the ingredients and protein, fat and carbohydrate content in feed in accordance with enzyme activity, it will increase feed digestibility. If feed protein digestibility increase, it will increase essential amino acid supply for fish growth. According to Dawood, enzymes can help fish to break down large organic molecules such as starch, cellulose and proteins into simple substances. Other than that, the exogenous enzyme in fish feed can increase nutrient utilization, thereby reducing nutrient loss. Exogenous enzyme has been shown to increase the nutritional value of feed and reduce environmental pollution. Nowadays, exogenous enzyme is widely used throughout the world as additive in fish feed to increase the nutritional value of fish feed [5-7].

CONCLUSIONS

The conclusions from this research are as follows:

The use of papain enzyme in artificial feed of saline tilapia has a significant effect on the efficiency of feed utilization (EPP) and relative growth rate (RGR) but does not have significant effect on survival (SR).

The optimum dose of papain enzyme addition on efficiency of feed utilization is 5,5 g/kg of feed capable in producing a maximum EPP by 63,48% and the relative growth rate is 5 g/kg of feed capable in producing a maximum RGR by 4,61%/day.

RECOMMENDATIONS

The suggestion that can be given in this study is that the addition of papain enzyme with a concentration of 5 - 5,5 g/kg of feed can be applied to different cultures or larger size of saline tilapia in order to achieve efficient utilization of feed, protein efficiency ratio and increase optimal growth.

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