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Technology engineering of rearing red tilapia saline (*oreochromis niloticus*) fed on artificial diet enriched with protease enzymes in an eroded brackish water pond

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Abstract. Technology engineering of red tilapia saline (*O niloticus*) was reared in brackish water pond fed on artificial diet enriched with protease enzymes in an eroded brackish water pond. The purpose of this study was to know of technology engineering of red tilapia saline reared in brackish pond that is exposed to effect eroded brackish water pond and the effect of adding protease enzymes in artificial feed to accelerate the growth and survival of red tilapia saline fish. The fish samples in this study used red tilapia saline with and average weight of 1.25 ± 0.09 g / fish with stocking 10 fish / m^2. This research used experimental method with Completely Randomized Design, 4 treatments and 3 replications. The treatments in this study were the addition of papain protease enzyme in the artificial feed with different dose ie A (0 mg / kg of feed), B (350 mg / kg of feed), C (700 mg / kg feed), D (1050 mg / kg feed). The parameters to be determined include relative growth rate (RGR), feed utilization efficiency (EPP), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR), and water quality.

Keywords: Growth, survival, red tilapia saline, protease enzyme

1. Introduction

Red tilapia fish farming (*O. niloticus*) intensively in ponds in hilink program using artificial feed enriched with protease papain enzymes to accelerate growth and survival. The protease papain enzyme added in the diet can hydrolyze the protein so as to improve the efficiency of feed utilization and growth.

The fish Red tilapia was one of the cultivars that have good prospect to be developed because much favored by society [1,2]. Chervinski [3] adds that red tilapia was also tolerant of low or high temperatures and was euryhalin. According to Hassan et al. [4] and Chervinski [3] red tilapia which is nourished in the sea has advantages such as faster growth, more compact meat, smell and taste more tasty.

The most prodedlems faced in the cultivation of red tilapia intensively include slow growth of fish resulting from the use of vegetable materials as a source of protein in commercial food, such as soybean meal meal containing anti-nutritional substances or phytic acid. The high content of phytic acid in soybean meal resulted in the utilization of vegetable protein in the feed less than optimal. According to Cao et al. [5], Hossain and Jauncey [6] phytic acid content in soybean flour reached 3.88% or 59.9% of total phosphorus. According to Kumar et al. [7], phytic acid can bind minerals such as calcium, magnesium, zinc, copper, iron and potassium, thus interfering with the absorption and digestion of fish

Eroded is a condition of sea water in tidal conditions that inundate parts of the river and land. Eroded phenomenon that occurred in approximately the last 10 years was a very high eroded condition, the impact was not only the river that overflows the water, but some of the land area in the coastal areas included settlements, yard, rice fields, maritime industry, community activity space, offices, also participated. Many human activities are disturbed by this eroded phenomenon, even the most apprehensive impact of this eroded phenomenon was the changing socio-cultural conditions as well as the very basic pattern of environmental adaptation [8, 9].

Environmental changes due to the phenomenon of eroded was very visible on the coast, the pattern of geographic changes in a region was a very real impact, one of which was a productive pond area that can no longer diopersionalkan, pond land has been turned into a stretch of submerged sea water , so that changes in the physical properties of the camps and biological pond water also experienced a very significant change. Biota that usually live in pond waters have a habitat shift except that have strong physiological adaptation capability.

Eroded phenomenon not only affects biological biological changes, but also affects the damage to the construction of ponds as well as the design of the pond before. The missing ponds of the embankment due to high tides become a common sight in the coastal areas, so the ponds have been very difficult to strive for Eroded phenomenon. The disappeared ponds of the embankment due to high tides became a common sight in the coastal areas, so the pond has been very difficult to the cultivation area, this incident is very influential on the decreased of fish land production (pond).

This high eroded is one of the consequences of world climate change. Behavioral changes that tend to violate environmental moral ethics have been carried out by humans on earth, some of which are the burning of earth fossils that exceed the natural absorption capacity, the cutting of vegetation as a carbon sink absorbs more than the naturally balancing capacity, resulting in decreased decline buffer capacity in various ecosystems on earth. Increased geothermal was one of the negative impacts of a series of events. The increase in sea water volume was a reflection of the increase in geothermal that melts icebergs in the polar regions [8].

One attempt to overcome the above perodedlem was to add protease enzyme papain in the feed ingredients. Chung [10], argues that papain protease enzyme is one of the exogenous enzymes that are expected to inhibit anti-nutrients, especially phytic acid, so as to improve the efficiency of feed utilization and growth. Papain protease enzymes added in the diet can increase nutrient absorption and regulate nutrient excretion (such as phosphorus, nitrogen, and minerals) and can hydrolyze phytic acid (phosphate elemental reserves) in fish feed into inositol and phosphoric acid.

Research on the addition of papain protease enzyme to the feed has been performed on several species, and has been shown to have an effect on the increase of protein digestibility, feed utilization, and growth in rainbow trout (Oncorhynchus myksiss Walbaum) [11], in fish Salmon salar L) [12], on vaname shrimp (Lipopenaeus vannamei) [13], in tiger grouper *(Epinephelus fuscoguttatus*) [23] and on tilapia (O.niloticus)[31]. Research on the addition of papain protease enzyme in red tilapia fish meal (*O. niloticus*) on pendederan I has not existed. Therefore, this study aims to evaluate the effect of the addition of papain protease enzyme to the efficiency of feed utilization, growth and survival of red saline tilapia on I pendederan.

The main problem in this research was the cultivation of red tilapia fish experiencing constraints resulting from the use of feed ingredients. One of them was the use of vegetable source of raw materials containing anti-nutrients in the form of phytic acid. This causes important minerals and proteins in the feed can not be absorbed properly by the body, so that the efficiency of feed utilization was not maximal in fish body. Efforts that can be made to reduce the content of phytic acid was the addition of protease enzyme papain in artificial feed that serves to hydrolyze phytic acid in vegetable standards, such as soybean meal meal. According to Rachmawati and Hutabarat [23], grain plants such as soybeans are one source of vegetable protein containing anti-nutritional substances such as phytic acid that can inhibit the absorption of nutrients by the body so that the efficiency level of nutrient utilization of feed is less than optimal. Efforts made to improve the utilization of nutrients is to add protease enzyme papain in the feed. Papain protease enzymes in the diet can increase nutrient uptake and regulate nutrient excretion (such as phosphorus, nitrogen, and minerals) and can hydrolyse phytic acid (phosphate elemental reserves) in fish feed into inositol and phosphoric acid. The destruction of these phytic acid anti-physique substances, then the metabolic processes such as breakdown of proteins and minerals complex in the body can run well. The scheme of this perodedlem solving approach can be seen in the intensive cultivation of red tilapia fish in ponds in hilink program using artificial feed enriched with protease papain enzymes to accelerate growth and survival. The protease papain enzyme added in the diet can hydrolyse phytic acid in fish feed to inositol and phosphoric acid, thereby increasing the efficiency of feed utilization and growth.

The purpose of this study is to examine the effect of adding papain protease enzyme in artificial feed and to know the best dose of papain protease enzyme in artificial feed on the efficiency of feed utilization, growth, and survival of red tilapia saline (*O. niloticus*). As well as finding the best dose of papain protease enzyme in artificial feed on the efficiency of feed utilization, growth and survival of red salt tilapia.

2. Materilas and Methods

2.1. All Time and place

This research was conducted on April-Agustus 2018 at Pond culture Marine and Fishery goverment of Pekalongan City.

2.2. Research Materials

The materials used in this study were test animals, test feed, container maintenance and protease papain enzymes. The research materials used were as follows.
Animal test

The test animals used in this study were red saline tilapia (*O. niloticus*) at stalk stage I which had an average weight of 1.07 ± 0.09 g / fish. Red tilapia fish used amounted to 120 fish derived from red tilapia fish saline marine and fishery goverment in Pekalongan City.
The test feed used in this study was pellet-shaped artificial feed with 30% protein content and minimum energy 260 kcal DE / kg referred to SNI [25], plus protease papain enzyme with dose A (0 mg / kg of feed), B (350 mg / kg of feed), C (700 mg / kg of feed), D (1050 mg / kg of feed.

2.3. Maintenance container

The container used during maintenance is a 1x1x1 size penculture in a 1200 m² pond area which was sown by the Marine and Fisheries Office of Pekalongan City. maintenance using seaweed biofilter system to keep water quality to stay good, then stocked red tilapia saline seed density 10 fish / m². referring to Rachmawati and Samidjan's research [9].
Papain protease enzyme

The protease papain enzyme used in this research plan is the papain protease enzyme of Natuphos 5000® brand in powder form manufactured by PT. BASF Indonesia. Papain protease enzyme used in the study.

2.4. Research methods

The method used in this research is an experimental method conducted at Pond culture Marine Department fisheries and Marine science used Randomized Complete Design (RCD) which was a design where the treatment was involved entirely randomly on the experimental units. This study used 4 treatments with 3 repetitions, namely:

The parameters to be determed include relative growth rate (RGR), feed utilization efficiency (EPP), protein efficiency ratio (PER), feed conversion ratio (FCR), survival (SR) and water quality. The treatments in this study modify Primary (2015), which states that the addition of a dose of the papain protease enzyme 500 mg / kg of feed is the best dose for the red saline tilapia.

2.5. Research procedure

The procedures in this study include the preparation stage and the implementation stage. The stages are as follows:

2.5.1. Preparation phase

Preparation of test animals

The test fish to be used are first selected based on size and weight, completeness of body organs and physical health, then red tilapia adapted to the media saline. Based on the research from Setiawati and Suprayudi [24], red tilapia grow optimally in 20 ppt. The process of adaptation on red tilapia fish seed is done for 4 days. Adaptation was done at 1 day interval from low salinity (fresh water) to high salinity (brackish water). The salinity on the media was gradually increased from 5ppt, 10ppt, 15ppt, to 20ppt. This method of adaptation of red tilapia refers to the method used in the Laboratorium Pekalongan University at Samaran, Pekalongan red tilapia fish and then inserted into penculture for 1 week for adaptation to feed and new environment.

The test feed used in this study uses the ingredients such as fish meal as a source of animal protein, soybean flour as a source of vegetable protein, corn flour, bran flour and wheat flour as a source of carbohydrate, fish oil and corn oil as a source of fat, minerals and vitamin mix as a source of vitamins, CMC as binder or adhesive and protease papain enzyme as input material to break up phytic acid molecule. Preparation of test feed conducted in this study, among others, to test proksimat feed ingredients, preparing formulations and making feed. Proximate test results can be seen in Table 1.

Table 1. Proximate Analysis of Raw Materials for Composers Used in Research (in% Dry Weight)

Material	ash	lipid	fiber	Protein	BETN	Total
Fish meal (*)	31,97	12,15	7,14	50,40	3,93	100,00
Soybean meal (**)	8,94	1,47	2,53	55,55	31,52	100,00
Corn meal (*)	0,27	0,68	0,03	0,45	98,57	100,00
Ricemeal (*)	10,83	1,22	12,04	14,71	61,21	100,00
Wheat flour (*)	1,31	12,76	0,22	12,88	72,82	100,00

Source: * Animal Feed Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2015)
** Laboratory of Physics and Environmental Chemistry, Center for Brackishwater Aquaculture (BBPBAP), Jepara

Based on the results of proximate analysis in Table 1. it can be seen the value of each raw material. The nutritional content of the proximate analysis results are then used to calculate the feed formulation. The feed feeding formulation can be seen in Table 2.

Table 2. Formulation and Proximate Analysis of Test Feed Used in the Study

Types of Feeding Materials	Treatment*)				
	A	B	C	D	
Papain Enzime	0	0,035	0,070	0,105	
Fish meal	25,00	25,00	25,00	25,00	
Soybean meal	25,00	25,00	25,00	25,00	
Corn meal	13,30	13,50	13,60	13,77	
Rice meal	13,80	13,69	13,57	13,47	
Wheat flour	13,90	13,80	13,80	13,71	
Fish oil	1,50	1,50	1,50	1,50	
Corn oil	1,50	1,50	1,50	1,50	
Vit Min Mix	4,00	4,00	4,00	4,00	
CMC	2,00	2,00	2,00	2,00	
Total (g)	100	100	100	100	
Proximate analysis					
Protein (%)*	30,37	30,34	30,32	30,29	30,31
Lipid (%)*	8,44	8,43	8,43	8,41	8,44
BETN (%)*	40,54	40,60	40,62	40,66	40,63
Energy (kcal)	275,99	275,92	275,93	275,84	275,99
Rasio E/P	9,09	9,10	9,10	9,11	9,11

Information:
a. Calculated based on Digestible Energy according to Wilson [31] for 1 g of protein is 3.5 kcal / g, 1 g of fat is 8.1 kcal / g, and 1 g of carbohydrate is 2.5 kcal / g.
b. According to De Silva [14], the E / P value for optimal growth of fish ranges from 8-9 kcal / g.
c. (*): Animal Food Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2015)

dose ie A (0 mg / kg of feed), B (350 mg / kg of feed), C (700 mg / kg feed), D (1050 mg / kg feed).

The feeding process begins by adding the protease papain enzymes according to the treatment of soy flour. The papain protease enzyme to be added to the soybean mixture beforehand was dissolved with 50 ml of water at 45°C. This refers to Fox et al. [17] in which the papain protease enzyme was dissolved at 45-60 °C, then added to soybean meal, stirred evenly and allowed to stand for ± 24 hours in an airtight container.
Feed making was done by weighing all the necessary materials. After that, mix all ingredients starting from the smallest amount of ingredients to the largest amount of ingredients. Pellet-shaped feed dough is dried by aerated air by putting it in the room. A complete feeding procedure is presented in Appendix 3.

Implementation of research

The red saline tilapia (*O. nilotius*) to be used in the research was first weighed to determine the initial weight of the fish. Red tilapia salt (*O. nilotius*) then incorporated into penculture in ponds penculture size 1x1x1 in the area of 1200 m² ponds were souvenirs belonging to the Office of Marine and Fisheries Pekalongan City. maintenance using seaweed biofilter system to keep water quality to stay good, then stocked red tilapia seed saline density 10 fish / m². referring to Rachmawati and Samidjan's research [9] were used dose 300 to 600 mg/kg adding the protease papain enzymes.

Data collection

Relative Growth Rate (RGR)

The relative growth rates in this study can be calculated using the Steffens [27] formula as follows:

$$RGR = \frac{Wt - Wo}{Wo \times t} \times 100\%$$

Information :

RGR = Relative growth rate (RGR)
W0 = Saline tilapia weight at the beginning of the study (g)
Wt = Saline tilapia weight at the end of the study (g)
t = Duration of study (days)

Efficiency of Feed Utilization (EPP)

The Value of Feed Utilization Efficiency (EPP) can be determined by Tacon [28] formula, as follows

$$EPP = \frac{Wt - Wo}{F} \times 100\%$$

Information :

EPP = Feed Utilization Efficiency (%)
W0 = Saline red tilapia biomass weight at the beginning of the study (g)
Wt = Saline red tilapia biomass weight at the end of the study (g)
F = Number of feed red tilapia consumed during the study (g)

Protein Eficiency Ratio (PER)

The value of the protein eficiency ratio (PER) was calculated using the Tacon [28] formula, as follows:

$$PER = \frac{(Wt-Wo)}{Pi} \times 100\%$$

Information :

PER = Protein Efficiency Ratio
W0 = Biomass weight of the test animal at the beginning of the study (g)
Wt = The weight of animal biomass test at the end of the study (g)

Pi = The amount of test feed consumed multiplied by the protein content of the test feed

Food Convention Ratio (FCR)

According to Steffens [27], FCR is calculated based on the following formula:

$$FCR = \frac{F}{(Wt + D) - Wo}$$

Information:

FCR = (Food Convertion Ratio) Feed conversion ratio;
F = Weight of food eaten (g)
Wt = The biomass weight of the salt tilapia fish at the end of maintenance (g);
D = The weight of dead tilapia fish (g);
Wo = The biomass weight of the red tilapia copy at the beginning of maintenance (g)

Survival Rate (SR)

The survival rate or Survival Rate (SR) was calculated to determine the survival rates of test animals during the study, the survival can be calculated based on the Effendi [15] formula:

$$SR = \frac{Nt \times 100\%}{No}$$

Information :

SR = Survival rate
N0 = Number of test animals at the beginning of the study (tail)
Nt = Number of test animals at the end of the study (tail)
D =% protein in food

Data analysis

Data analysis was done after the research was done, after got the data of SGR, RGR, PER, FCR, EPP, and SR at the end of the research. Data obtained then analyzed using analysis of variance (ANOVA). Prior to the ANOVA, the data were first tested for normality, homogeneity test, and adivitas test to know that the data are normal, homogenous and additive to be tested further ie the analysis of variance. After analysis of variance, if found a highly significantly difference (P <0.01) or significantly difference (P <0.05) then Tukey> S test to be able to know the differences between treatments [26].

3. Results and Discussion

Results

Relative growth rate (RGR)

Based on the weight measurement done at the beginning and end of research conducted for 42 days, obtained relative growth rate data on red tilapia fish (*O. niloticus*). The average calculation of the relative growth rate was presented Fr Based on Table 3 above, the average value of relative growth rates at each treatment from highest was C treatment of 7.75 ± 0.10^{a}% / day and lowest was treatment A of 4.19 ± 0.01^{c}% / day. Based on data relative growth rate of red tilapia saline (*O. niloticus*). The daily growth rate data have been tested the distribution of normality test, homogeneity test, and aditivity test indicating that the data spread normal, homogeneous, and additive so that has fulfilled the requirement of analysis of variance. Result of analysis of variance to relative growth rate of red saline tilapia gave significant effect (P <0,01) with F count> F table (0,01) to relative growth rate on red tilapia fish in the medium of hygiene.

Differences in the effect of treatment with other treatments can be determined by Tukey'S test for relative growth rates of red tilapia significantly different From Tukey'S test results showed that the mean value of C-A, C-B treatment was very different, but not significantly different from C-D.

Efficiency of protein feed utilization (EPP)

Efficiency of feed utilization showed how much feed was used by fish. Based on the research indicated that the highest EPP (%) value was at C (55.25 ± 0.33^{a}) and lowest A (36.25 ± 0.24^{c}) (Table 3).

The result of EPP data variety analysis on red saline tilapia fish showed the addition of papain enzyme in artificial feed with different percentage gave highly significantly effect (P <0,01) with F count> F table (0,01) against EPP on red tilapia saline. Furthermore, to find out the mean of intermediate values between treatments, Tukey'S test on red tilapia fish showed that there was a significant difference between the C-B, C-A treatment and did not differ significantly with C-D.

The protein efficiency ratio (PER)

The ratio of protein efficiency was value that showed the amount of fish weight resulting from the weight of the test feed protein consumed. Based on the research results, the value of protein efficiency ratio calculation presented in Table 3. Very significantly effect on Protein Efficiency Ratio (PER) Ratio (%) red tilapia saline (P <0.01 Furthermore, to determine the mean of intermediate values between treatments Tukey'S test on red tilapia fish showed that there was a marked difference between the treatment of CB, CA and did not different significantly with the CD.

The result of the analysis of various data of protein efficiency ratio on red tilapia saline fish showed the addition of protease papain enzyme in artificial feed with different dose gave a very highly significantly effect (P <0,01) with F count> F table (0 , 01) to the protein efficiency ratio of saline red tilapia. Furthermore, to find out the mean of intermediate values between treatments, Tukey'S test on red tilapia fish (O. niloticus) showed that there was a significant difference between the C-B, C-A treatment and did not differ significantly with C-D.\

Feed conversion rate (FCR)

The feed conversion ratio is an index of total feed utilization for growth or the number of grams of feed required by fish to produce 1 g of wet weight of fish (Stickney, 1979 in 9). Based on the result of research, showed that the lowest value of FCR at C (1.25 ± 0.02^{a}) and highest A (1.52 ± 0.03^{c}), the value of feed conversion ratio presented in Table 3.

Very significantly effect (P <0.01) on the Feed Conversion Rate Ratio (FCR) of Red Tilapia saline. Furthermore, to find out the mean of intermediate values between treatments Tukey'S test on red tilapia fish that there are significant differences between CB, CA and CD treatments.

Based on the above table, the average feed conversion ratio in each treatment A (1.52 ± 0.03^{c}), B (1.47 ± 0.17^{b}), C (1.25 ± 0.02^{a}), and D (1.34 ± 0.09^{b}).

The result of data analysis of feed conversion ratio on red tilapia fish showed the addition of protease papain enzyme in soybean meal on artificial feed with different dose gave a highly significantly effect (P <0,01) with F value> F table (0,01) to feed conversion ratio on red tilapia fish. From Tukey'S test results on saline red tilapia showed that there were significant differences between the treatments of C-B, C-A and C-D.

Survival rate

Based on the results of the study, it was found that the survival value of red saline *O. niloticus* showed the highest yield on C treatment (93.33 ± 5.77^{a}). The results of the calculation of survival are presented in Table 3. The same supercript in the column showed no significant difference.

The result of variance analysis has significant effected on the survival of red tilapia saline (P <0,05). Furthermore, to find out the mean of intermediate values between treatments, Tukey'S test on red tilapia fish showed that there were significant differences between the treatments of C-B, C-A and C-D. Based on Table 3 above, the analyzed variance showed a significantly effect (P <0.05), whereas the highest survival outcome was C treatment of 93.33 ± 5.77^{a}%, then the lowest was D (83.33 ± 5.77^{b}%), B (76.67 ± 5.77^{b})%, and lowest A (70 ± 10^{c}%). The results of the analysis of multiplication data on survival red tilapia showed that the addition of papain protease enzyme in the artificial feed with different doses gave a significant effect (P< 0,05) with F count ≤ F table (0,05) on the survival of red tilapia saline fish. in Table 3.

Table.3. Absolute growth of weight. Survival Rate , Conversion ratio on the cultivation of red tilapia fish in ponds on the program Agromina politan Pekalongan City.

	Treatment*)			
	A (0 mg/kg feed)	B (350 mg/kg feed)	C(700 mg/kg feed)	D(1050 mg/kg feed)
1.Relative growth rate (RGR) (%/day)	4.19±0.01c	5.97±0.15b	7.75±0.10a	6.50±0.07b
2. EPP value (%)	36.25±0.24c	43±1.13b	55.25±0.33a	46.31±0.38b
3.Protein efficiency ratio (PER) (%)	1.23±0.01c	1.43±0.05b	2.76±0.01a	1.63±0.04b
4. Food convertion ratio (FCR)	1.52±0.03c	1.47±0.17b	1.25±0.02a	1.34±0.09b
5. Survival (%)	70±10c	76.67±5.77b	93.33±5.77a	83.33±5.77b

Description :: The same sign of the superkrip shows no significantly difference
Treatment with the addition of protease papain enzyme in artificial feed with different dose ie
Treatment protease papain enzyme used dose A (0 mg / kg of feed), B (350 mg / kg of feed), C (700 mg / kg feed), D (1050 mg / kg feed).

Table 4. Results of Water Quality Parameters on Red Tilapia During in the research.

Treatment	temperate (°C)	pH	DO (mg/L)	NH_3 (mg/L)	Salinity (‰)
A	25-31.5	7,5-7,8	5,97 6,75	<0,001-0,006	18-20,5
B	25-31.5	7,4-7,5	5,87-6,39	<0,001-0,005	18-20
C	25-31	7,5-7,9	5,89-6,59	<0,001-0,002	18-9,37
D	25-32	7,5-7,7	5,83-6,49	<0,001-0,005	19-20
literature	25 – 32*	6,5 – 8,5*	≥3*	<0,20*	10-20**

Description: *: SNI [25]
**: Setiawati and Suprayudi [24]

The result of measurement of water quality parameter showed that the water quality parameter value during the research was still in proper condition to be used as a medium of red tilapia saline fish culture in the medium of hybridity, it is based from the library about the optimum water quality condition for red tilapia saline (*O. niloticus*) in the medium of salinity.

Discussion

Relative growth rate (RGR)

Variation analysis results (Table 3) showed that the addition of protease papain enzymes with different doses had a very significant effect (P <0.01) on the relative growth rate of red salt tilapia (*O. niloticus*), it was suspected that protease papain enzymes could reduce anti-nutrients in the feed ingredients used. These results are in accordance with the opinion of [1,2,15] that papain protease enzymes added to artificial feeds can function well to reduce the phytic acid content present in plant-based foods and promote relative growth and feed efficiency.

The highest relative growth rate was found in C treatment with dose of protease papain enzyme 600 mg / kg of feed that is equal to 7.75 ± 0.10% / day, presumably the dose is the best dose to hydrolyze phytic acid which can inhibit the decomposition of nutrients and minerals in feed, nutrients can be well absorbed by fish. According to Chung [10], Hunter [19] papain protease enzymes function to increase the absorption of nutrients and regulate the excretion of nutrients (phosphorus, nitrogen and minerals) by hydrolyzing phytic acid found in artificial feed into inositol and phosphoric acid. The lowest relative growth result was obtained at treatment A of 4.09 ± 0.04% / day, suspected in the treatment of phytic acid contained in the feed still not decompose. Based on the research of Rachmawati and Hutabarat [23], the treatment which is not added by papain protease enzyme still contain phytic acid which still not hydrolyzed so protein and mineral complex that can not be utilized by body for growth.

The results of the research on the relative growth rate were rated higher when compared to Revindran (2000), Olusola and Nwanna [22], Rachmawati and Samidjan [9] studies, on freshwater tilapia *(O. niloticus*) of 2.50 ± 0.09% / day, Rachmawati and Hutabarat [23] research on fish tiger grouper (*Epinephelus fuscoguttatus)* of 3.39 ± 0.08% / day, Amin et al. (2010), 4.13% / day in catfish (Pangasius pangasius), Amin et al. (2011), in catfish (*Clarias* sp.) Of 4.18 ± 0.14% / day, and Hassaan et al. (2013), 2.11% / day in tilapia tilapia. The difference in value of the relative growth rate can be due to the age, type and size of the test fish used. The amount of minerals and nutrients needed for each type of fish to support growth is also different.

Growth on red salted tilapia fish can be seen that fish are able to utilize nutrient feed well. Utilization of nutrients in saline red tilapia was suspected because the bonds of anti-nutritional substances (phytic acid) with proteins and mineral complexes in the feed has been interrupted by the hydrolysis of protease papain enzymes. Hydrolyzed phytic acid will be inositol and phosphoric acid, so nutrients in the feed can be absorbed more leverage. According to Kim et al.[20], Rachmawati and Hutabarat (2006), Kumar et al. [7] the hydrolysis reaction is suspected to decrease anti physiological substance of phytic acid so that there will be termination of bond between phytic acid with protein and mineral complex become inositol and phosphoric acid. The breaking of the bonds according to Hossain and Jauncey (1993) will have a positive effect on the activity of trypsinogen into the enzyme trypsin solver protein to amino acid pengusunya. The complex bonds formed between phytate and multivalent cations can also decompose. The breakdown of these substances can cause the protein and minerals to not settle so that it can dissolve and can be utilized by the body [28, 13, 29].

Efficiency of feed utilization (EPP)

The results of the variance analysis (Table 3) showed that the addition of protease enzyme papain artificial feed was very significant (P <0.01) to the efficiency of feed use of red tilapia.salin fish. High feed efficiency indicates that the feed can be well utilized by fish, it is suspected that protease papain enzymes can reduce phytic acid in feed, so that feed can be absorbed and digested properly by fish. The statement is in accordance with the opinion of Huet [18], Lovell [21], Watanabe [30], Olusola and Nwanna [22], improving the efficiency of feed utilization in fish can be attributed to the release of phytate feed by protease papain enzyme. The feed can be utilized by fish for better performance. High digestibility performance can also derive nutrient feed that is suspected due to the addition of protease papain enzymes.

The highest efficiency of feed utilization was obtained at the C treatment of the best dose of protease papain enzyme of 700 mg / kg (treatment C) of feed capable of producing EPP, 55.25 ± 0.33[a]%, for red tilapia saline fish, it was suspected that the dose of the papain protease enzyme added to the C treatment of 700 mg / kg of feed was the best dose for the red salt tilapi. The dose was allegedly capable of over hauling phytic acid contained in the feed ingredients, thus improving the nutritional quality [29], that the protease papain enzyme added to the artificial feed works well to reduce the phytic acid content present in the plant-based diet as well as to increase relative growth and feed efficiency.

The efficiency of feed utilization is closely related to growth. The better the utilization efficiency of feed by the fish, then the growth of the fish is also getting better. High feed utilization shows that fish can utilize nutrient feed to be stored in the body and convert it into energy. the statement is in accordance with the opinion of Huet [18], that the high utilization efficiency of feed indicates that a small amount of food is overhauled to meet energy needs and the rest is used for growth.

The relative growth rate of the results of this study is higher, when compared with the results of Olusola and Nwanna [22], of 25.65 ± 0.01% in tilapia tilapia. The high efficiency of feed utilization in this study indicates that the amount of feed given, as well as the feed ingredients used are suitable to meet the needs of the fish, resulting in growth. According to Olusola Nwanna [22], feed efficiency indicates how much feed can be utilized by fish. Low feed efficiency values indicate that fish need more feed to increase their weight because only a fraction of the energy from the feed given is used by fish for growth. Bulbul et al., [1], adds that the addition of fitase in soy flour can improve the efficiency of feed compared with fish meal.

The protein efficiency ratio (PER)

Variation analysis results (Table 3) showed that the addition of protease papain enzyme in the artificial feed had significant effect (P <0,05) to protein salivary protein efficiency ratio of red saline. According to Olusula and Nwanna [22], the value of high protein efficiency ratio is due to the protein can decompose into amino acid and its constituents, so that the absorption of protein in the fish body will be easier.

The results of this study indicate that the highest protein-efficiency ratio value was obtained in treatment C. The best dose of protease papain enzyme of 700 mg / kg (treatment of C) of feed was able to produce PER, respectively of 2.76 ± 0.01a%, for red tilapia (*O.niloticus*) while the lowest protein efficiency ratio value was obtained at treatment A of 0.95 ± 0.03%. These results suggest that the administration of papain protease enzymes in the diet was thought to be able to hydrolyze phytic acid and break the bond between phytic acid and protein and mineral complexes, resulting in more protein being absorbed by the body used for growth. The statement was in accordance with the opinion of Rachmawati and Samidjan [9], the high ratio of protein efficiency can be caused by protease papain enzyme contained in the feed that can reduce and decompose phytic acid and break the bond between phytic acid with protein and mineral complex, digestive enzymes, especially protein-breaking enzymes in breaking down proteins into their constituent amino acids. Winarno (1995) adds that perfect protein hydrolysis will produce high amino acids, so the more that the body absorbs.

This study shows the results of higher protein efficiency ratios from Rachmawati and Samidjan [9] studies, on freshwater tilapia of 0.64 ± 0.09%, Olusula and Nwanna [22], on tilapia tilapia (*O. niloticus*) of 0.17 ± 0.02% and Rachmawati and Hutabarat [23], in tiger grouper of 0.74 ± 0.04%. PER value is influenced by the quality of feed nutrition. The protease papain enzyme added in the diet is thought to break down proteins and mineral complexes bound to phytic acid. The decomposition of protein and mineral complexes will improve feed quality. Protein contained in pakanpun can be well absorbed for energy availability, and then will be used for growth. Kim et al. [20], adding that the protein absorbed from the feed will be used as energy and if there is excess protein then the protein will be used for growth. This ability is influenced by several factors namely the quality of the feed, where the higher the protein dimaanfaat by the body then the use of protein will be more efficient.

Feed conversion rate (FCR)

The result of variance analysis (Table 3) showed that the addition of protease papain enzyme in feeding had significant effect (P <0,05) to conversion ratio of red tolapia saline *(O. niloticus*) feed. Optimal feed utilization will provide good feed conversion ratio value. The well-expended feed will produce energy for growth. Energy is produced from proteins that decomposed into amino acids that can be absorbed properly by the cultivation so that the nutrients in the feed will be utilized optimally. Maximum protein absorption due to phytic acid content in the feed can be hydrolyzed by protease papain enzyme [29].

The results of this study indicated that the value of feed conversion ratio the lowest value obtained at the C treatment of 1.25 ± 0.02ª. for red tilapia fish. The results show that the dose of protease enzyme papain 700 mg / kg of feed on treatment C is the best dose, because it has the lowest FCR value FCR value is related to feed quality, the lowest FCR value is supposed that nutrient (2012), the value of feed conversion ratio is closely related to the quality of feed, so the lower the value the better the quality of feed and the more efficient the fish in using the feed consumed for growth.

The value of feed conversion ratio in this study is lower than Hassaan et al [4] , Tawwab [29] with the lowest value of feed conversion ratio of 3 and Rachmawati and Hutabarat [23] studies on *E. fuscoguttatus* of 2.09 ± 0.19, but higher than research Tawwab [29] of 1.1. These results indicate that the absorption of nutrients and minerals vary in each species, age and size of fish. According to Debnath et al. [15], the addition of papain protease enzyme is able to produce better feed conversion value compared to feeds that are not given protease papain enzymes in catfish (Pangasius pangasius).

Based on the results obtained in this study can be seen that the ratio of feed conversion on red tilapia fish less good. Feed conversion ratio ratio of 1.99 is still said high, because based on research Mirea et al [8], a good feed conversion ratio value for tilapia ranged from 1.43 to 1.7. The Olusola and Nwanna studies [22], on tilapia indigo fish also showed a low feed conversion value of 0.89 ± 0.01. Based on the results can be seen that the role of papain protease enzyme in the conversion ratio of red saline tilapia fish in this study is considered not maximal.

Survival Rate

The result of variance analysis (Table 3) showed that the addition of papain protease enzyme in artificial feed with different doses did not give significant effect (P> 0,05) to survival on saline red tilapia. The results of this study showed the highest survival value obtained by treatment C of 93.33 ± 5.77a%
The obtained survival value is high for tilapia maintained on the hygiene medium. Factors affecting the high survival rate of tilapia are abiotic and biotic factors, among others, competitors, population density, age and ability of organisms to adapt to the environment (Simanjutak, 2007).

The rate of survival of red tilapia in this hybrid medium showed the same value compared to Rachmawati and Samidjan [9], which was 93.33% and higher than Rachmawati and Hutabarat [23], on *E. fuscoguttatus* of 86.66%. Deaths that occur are suspected due to stress fish due to handling at the time of maintenance. The addition of papain protease enzyme did not significantly affect the survival of red saline tilapia, but the survival obtained in this study was high. The high of this life can also be affected by feeding. The amount of feed and the frequency of feeding according to need, will reduce mortality rate in fish. According to Tawwab [29], Hassaan et al.[4] reported the success of the cultivation business is intrinsically influenced by the growth rate and high livelihood. Growth rates and livelihoods are influenced by the availability of environmental quality feeds, pests, and diseases. The availability of feed is a very important factor, because it will determine the growth and survival rate of fish. Feeding will also suitably affect the survival that may reduce fish mortality.

4. Conclusions and recommendations

Conclusion

The results showed that the addition of papain protease enzyme in artificial feed gave a very highly significantly effect (P <0.01) to RGR, EPP, PER, FCR, SR red saline tilapia (*O. niloticus*). The best dose of protease papain enzyme of 700 mg / kg (treatment of C) of feed was able to produce EPP, RGR, PER, FCR, and SR respectively 55.25 ± 0.33^{a}%, 7.75 ± 0.10% / day, 2.76 ± 0.01^{a}% 1.25 ± 0.02a 1.25 ± 0.02^{a} and 93.33 ± 5.77^{a}% for red salted tilapia. Water quality on maintenance media is within a reasonable range for red saline tilapia.

Suggestion

Suggestions that can be given from the as follows:

1. It is advisable to use a dose of papain protease enzyme greater than 700 mg / kg of feed on a red saline tilapia.
2. It is recommended to conduct further research with different species, weights, and stadia of fish.

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