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APPLICATION OF ENZYME FUNCTIONAL FEED AS A SUPPLEMENT TO THE GROWTH OF SILVER POMPANO (*TRACHINOTUS BLOCHII*) IN FLOATING NET CAGE, PANGANDARAN DISTRICT

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ABSTRACT

The study aims to determine the effect of applying papain enzyme to functional enzyme feed (PFE) with optimal doses to produce the highest growth of pomfret in floating net cages, Pangandaran Regency. This research was conducted from September 2022 to December 2022 with 60 days of fish rearing. The method used in this study was a completely randomized design (CRD) with five treatments and 3 replications. The treatments were A (artificial feed containing 5% papain enzyme in PFE), B (artificial feed containing 3.75% papain enzyme in PFE), C (artificial feed containing 2.5% papain enzyme in PFE), D (artificial feed containing 1.25% papain enzyme in PFE), and E (control). The effect of each treatment was tested by analysis of variance (ANOVA) F test at a 5% test interval, if there was a significant difference it was continued with Duncan's multiple range test. The parameters observed in this study included fish survival (SR), Daily Growth Rate (LPH), Feeding Efficiency (EPP), and water quality at the study site. The results obtained from this study were the use of functional enzyme feeds to increase fish survival (SR), Daily Growth Rate (LPH), Feeding Efficiency (EPP), and water quality at the study site. The highest growth value in this study was in treatment A (artificial feed containing 5% papain enzyme in PFE) with LPH of 3.25 ± 0.112 %, EPP of 80 ± 6.164 %, SR of 100%, and the range of water quality in the location research namely dissolved oxygen (DO) levels ranging from 5.6 to 6.7 mg/L, temperature ranging from 27 to 29 ^{o} C, pH ranging from 7 to 8, and salinity ranging from 32 to 34 ppt.

Keywords: *survival rate, feeding efficiency, papain enzyme, daily growth rate, trachinotus blochii*

INTRODUCTION

The star pomfret (Trachinotus blochii) is a fish cultivated in Indonesia in 2007 and was successfully carried out for the first time at the Riau Marine Aquaculture Fisheries Center (BPBL) (Pranata & Haryanti, 2014). Capture fisheries production and pomfret aquaculture production, especially in West Java, in 2018 produced 50,974.75 tonnes (KKP, 2018). According to the data (KKP, 2020), the demand for and the high price of the star pomfret with a selling price of around Rp. 80,000 to Rp. 95,000/kg. In 2016, the market demand for star pomfret was 3,061 tons, however, pomfret cultivators could only fulfill 74.74% of the demand for star pomfret, which was 2,288 tons (KKP, 2016). Star pomfret is one of the main commodities besides grouper which is cultivated in Pangandaran Regency.

For farmers, feed is the biggest production cost in aquaculture, reaching 60-70% of the total production cost. (KKP, 2014) stated that the selection of the type of feed for pomfret must be based on fish needs, quality, nutrition, and economic value. Commercial feed that is usually given to Pomfret has a protein content of 37% and 9% fat. One feeding strategy that is expected to reduce production costs and increase feed efficiency in aquaculture is by applying papain enzymes to artificial feeds to increase fish growth and increase feed conversion efficiency.

The papain enzyme is a protease that can hydrolyze proteins into peptides or amino acids. In the field of aquaculture, the enzyme papain is used as a catalyst in hydrolysis reactions in the manufacture of fish protein hydrolysis (Fadli, 2013). Research on the papain enzyme has been carried out by many researchers such as eels (Sagita et al., 2017), tilapia (Rostika, Sunarto, et al., 2018), and cantang grouper (Rostika, Rizal, et al., 2018), which can increase growth rates and feed efficiency. In addition, research has been carried out regarding (Halawa et al., 2020) the addition of the enzyme papain and Recombinant Growth Hormone (rHG) to the growth of pomfret seeds. Currently, enzyme functional feeds have been produced, which are pellets containing the enzyme papain. Based on this, it is necessary to research the use of functional enzyme feed on pomfret to determine the effect of growth, feeding efficiency, survival, water quality parameters, and intestinal histological conditions on pomfret.

METHODS

Time and Place of Research

This research was conducted from September 2022 – December 2022. The maintenance of star pomfret was carried out in the Floating Net Cage of FPIK Unpad in Pangandaran Regency. Histology testing of the star pomfret intestine was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Jatinangor Unpad. Meanwhile, the manufacture of functional enzyme feed is carried out at the Tropical Marine Fisheries Laboratory, FPIK Pangandaran.

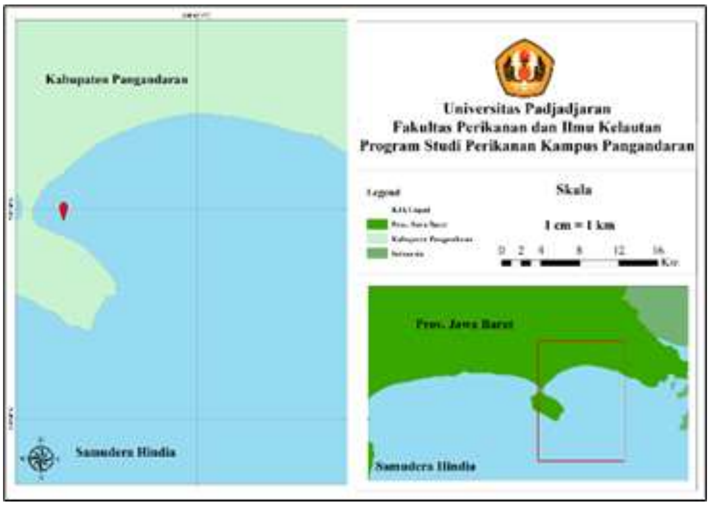

Figure 1. Location of floating net cages in Pangandaran

Research design

The method used in this study was an experimental method with a completely randomized design (CRD) consisting of 5 treatments and 3 replications.

a. Treatment A: Feed containing 5% papain enzyme in PFE
b. Treatment B: Feed containing 3.75 % papain enzyme in PFE
c. Treatment C: Feed containing 2.5 % papain enzyme in PFE
d. Treatment D: Feed containing 1.25% papain enzyme in PFE
e. Treatment E: Without PFE (control)

The effect of each treatment was tested by analysis of variance (ANOVA) F test at a 5% test interval, if there was a significant difference it was continued with Duncan's multiple range test.

Research Tools and Materials

The tools used in this study were floating net cages, digital scales, rulers and millimeter blocks, DO meters, pH meters, refractometers, surgical instruments, ovens, and cameras. The materials used in this study were the Pomfret test, commercial feed, young papaya, and bouin solution.

Research procedure

Manufacture of papain enzymes

The stage of making the papain enzyme begins with preparing the main tools and raw materials, namely unripe papaya fruit. The papaya fruit is weighed and then cut into several pieces and the seeds are removed. Then the papaya fruit that has been cut and cleaned from seeds is then shaved into thin sheets/slices. The thin papaya sheets/slices are then poured and flattened into a container or baking sheet, then put in the oven at a temperature of 50oC ^{for} 24 hours or until dry evenly. After the papaya fruit is dry, then the papaya fruit sheets/slices will then be milled using a grinding machine to become fine powder and stored in a dry and airtight container.

Mixing papain enzymes in commercial feed

The stages of mixing the papain enzyme into the feed and then giving it to the fish include mixing commercial feed using progol solution, tapioca flour, and papain enzyme powder with the appropriate dosage which will then be reprinted using a feed molding machine. After the feed is printed, the feed will then be dried using an oven with a temperature of 50 ^{o}C for 12 hours, which will then be called enzyme functional feed. The last stage is the feed will be given to the test fish.

Preparation of research containers

The maintenance container used is a net measuring 6 mx 6 mx 1.5 m and has been divided into 9 parts with a size of 2 m x 2 m x 1.5 m. The net is placed tied to the supporting poles of the KJA. The net submerged in water is about 0.12 m high. Each net is weighted to keep the net square. Each net is marked with a treatment to make it easier when doing research.

Pisciculture

During the maintenance period for the star pomfret, the test feed that had been given a functional enzyme feed mixture was carried out every day with a frequency of feeding 2 times a day, namely in the morning and evening and the amount of test feed given was 3% of the fish weight for one day. The frequency of feeding and the amount of feed given will increase along with the growth in length and weight of the fish. In addition, the survival value of the star pomfret was also observed by looking at the number of dead star pomfret each day. Then sampling activities are carried out every 10 days to measure fish growth so that the growth rate and feed efficiency can be observed. In addition, water quality measurements were carried out and determine the feasibility of water quality using several parameters including temperature, degree of acidity (pH), and dissolved oxygen (DO).

Parameters observed

Fish Survival (SR)

According to (Effendie, 2002), the value of survival rate (SR) can be calculated using the following formula:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Information:

SR = Survival of fish (%)

N_t = Number of individual test fish at the end of rearing (tails)

N_0 = Number of individual test fish at the start of rearing (tails)

Daily Growth Rate

Calculation of the daily growth rate uses the formula proposed by (Effendie, 1997)the following:

$$G = \frac{\ln Wt - \ln Wo}{t} X\ 100\%$$

Information:

G = Daily growth rate (%)

W_t = Average weight of fish at the end of rearing (tail)

W_o = Average weight of fish at the beginning of rearing (tail)

t = maintenance time (days)

Feeding Efficiency

The efficiency of feed utilization is calculated according to the formula (National Research Council, 1993) in (Iskandar & Elrifadah, 2015) :

$$EPP = \frac{Wt - W0}{F} x\ 100\%$$

Information:

EPP = Feeding Efficiency (%)

F = Amount of feed given during the study (g)

$Wt_{\_}$ = average seed weight at the end of the study (g)

W_o = average seed weight at the start of the study (g)

Intestinal Histology of Fish

examination using the method according to (Iji et al., 2001) to the following formula:

$$LV = \frac{b + c}{c} \times a$$

Information:

LV = surface area of villi (μm²)

a = villi height (μm)

b = apical width of villi (μm)

c = basal width of villi (µm)

Histological preparations for the intestine of star pomfret were made at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Water quality

Measurement of water quality, namely for temperature, degree of acidity (pH), salinity (ppt), and dissolved oxygen (DO) during the study used a pH meter, DO meter, refractometer, and thermometer which refers to water quality standards, namely temperature 28 – 32 °C, degree of acidity (pH) 7.5 – 8.5, and dissolved oxygen (DO) >5 mg/L according to the standard (BSN, 2013).

Data analysis

The results of the data obtained were analyzed descriptively through observational studies with supporting data and related literature. Furthermore, statistical analysis was carried out using a completely randomized design (CRD) with three replications. The data obtained were analyzed using analysis of variance with the F test to determine the effect of each treatment then to see the differences between treatments then continued with Duncan's multiple range test with a confidence level of 95% (Gaspersz, 1994).

RESULTS AND DISCUSSION

Survival/Survival Rate (SR)

Survival is a comparison of the number of living organisms at the end of the study with the number at the beginning of the study expressed in percent. The greater the percentage means the greater the number of organisms that live during the rearing period. The average survival of the star pomfret is presented in Figure 2.

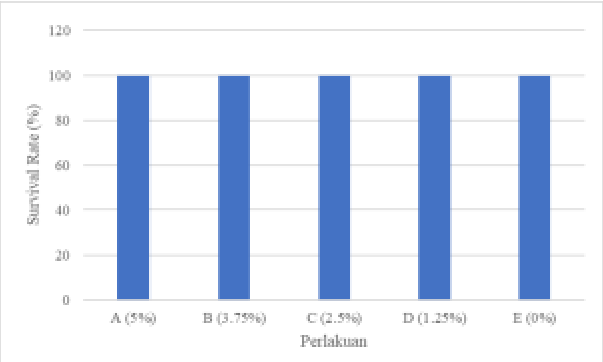

Figure 2. Survival of Star Pomfret for 60 Days

Based on the measurement results, the survival rate between treatments was not significantly different, namely 100%. This high survival value is influenced by the characteristics of the star pomfret which has disease resistance and relatively easy maintenance. Fish survival is usually influenced by feed factors and environmental conditions. This is supported by the statement (Miati, 2019)that good feeding and supportive environmental conditions will increase fish survival. In addition, water quality parameters can also affect the survival of fish in a study conducted by (Arrokhman et al., 2012), the high survival rate in the salinity treatment indicates that the pomfret can adapt well to conditions of high and changing salinity.

However, the provision of functional enzyme feed to the pomfret did not give significantly different results, so it was considered not too influential on the survival of the pomfret. This is in direct proportion to the research conducted by (Pathak et al., 2019) on the growth and survival of the star pomfret at different salinities from inland salty groundwater sources which gave results that were not significantly different, with the survival value of the star pomfret reaching 100%. The high survival of fish can be caused by good and proper fish maintenance supported by good environmental conditions to produce good survival. In addition, there is an assumption that the star pomfret has a high survival rate so that the star pomfret can survive well under any conditions.

Daily Growth Rate (LPH)

Growth is a change in size, weight, length, and volume within a certain time. This growth is specifically expressed by changes in the number or size of cells that make up body tissues over a certain time (Gusrina, 2008). Based on the results of observations, the use of functional enzyme feed can provide various results for the growth of pomfret. The use of this enzyme functional feed provides a good response for the growth of pomfret. This can be seen from the increase in the average weight of pomfret each time sampling is carried out (10 days). The average weight of the star pomfret in each treatment increased as the study progressed, presented in Figure 3.

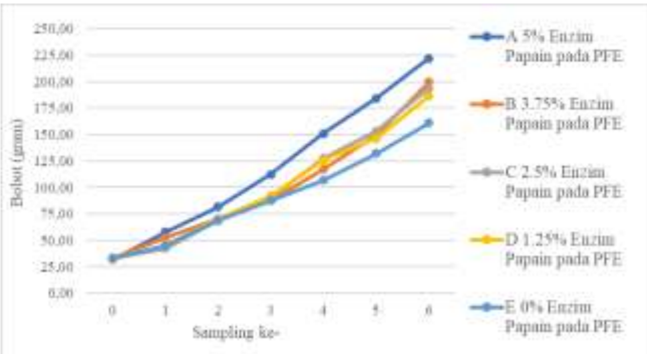

Figure 3. The Growth Pattern of the Star Pomfret for 60 Days

The overall growth pattern of the star pomfret starting from day 1 to day 60 shows a logarithmic phase or phase in which growth progresses rapidly and fish weight increases every day. The highest average weight of fish on the 60th day was in treatment A at 222.33 grams/head, then treatment B at 199.67 grams/head, treatment C at 194.00 grams/head, treatment D at 186.67 gram/head, and the smallest was treatment E of 161.00 gram/head.

Fish growth is influenced by internal factors and external factors. Internal factors include heredity, sex, and age. While external factors include food and water quality (Effendie, 1997). The daily growth rate serves to calculate the percentage of fish growth per day.

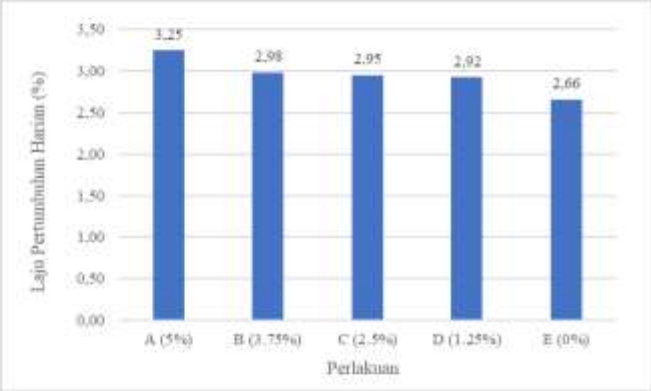

Figure 4. Graph of Daily Growth Rate of Pomfret Fish for 60 Days

Based on the results of observations that have been carried out for 60 days, the value of the resulting daily growth rate is in the range of 2.66 – 3.25%. The highest daily growth rate was in treatment A with the addition of the papain enzyme as much as 5% which was 3.25 ± 0.112% and the lowest daily growth rate was in treatment E without the addition of papain enzyme in the feed of 2.66 ± 0.072%. The results of statistical analysis using ANOVA showed that the treatment gave a significant difference in the weight growth of the pomfret so it was continued with the Duncan test results at a 95% confidence interval which are presented in Table 1.

Table 1. Duncan's Multiple Range Test Daily Growth Rate (LPH)

Treatment	Average Daily Growth Rate
A	3.25 ± 0.112 a
B	2.98 ± 0.068 b
C	2.95 ± 0.051 bc
D	2.92 ± 0.064 bc
E	2.66 ± 0.072 c

Note: Values followed by the same letter are not significantly different according to the range test of Duncan's multiple at a 95% confidence level

In this study, it was suspected that the papain enzyme was able to hydrolyze the protein contained in the feed so that the feed given to fish could be utilized efficiently and increase the daily growth rate. The star pomfret is a carnivore fish. The star pomfret is equipped with a pyloric caeca which is a modification of the fish intestine that functions as a digestive organ. Therefore the digestive process takes place quickly so that it can play a role in optimizing the work of the intestine in the process of digesting food. In this case, the addition of exogenous enzymes is thought to increase the workability of the intestine to accelerate the process of protein hydrolysis in the feed, so that more protein is broken down into amino acids and more and more amino acids are absorbed by the fish's body.

These results are consistent with research conducted (Fadli, 2013)on tiger grouper which is the parent of cantang grouper which gives a daily growth rate of 3.24%/day in the treatment of 5% papain enzyme and has a value that is not much different from this study. According to (Taqwdasbriliani et al., 2013), protein hydrolysis occurs with the help of proteolytic enzymes that act as catalysts in the cell. Protein hydrolysis is carried out by exogenous enzymes, one of which is the papain enzyme. The addition of papain enzymes can help produce more amino acids so that the feed consumed by fish can be utilized more efficiently. In general, the digestibility of fish to feed is influenced by several factors, including the chemical properties of water, water temperature, type of feed, size, and age of fish, the nutritional content of the feed, frequency of feeding, and the amount and type of digestive enzymes present in the digestive tract of feed (National Research Council, 1993).

Feeding Efficiency (EPP)

Feeding efficiency is the ratio between the resulting body weight gain and the total feed used during the rearing period. The greater the feed efficiency value produced, the better the feed utilization process by the fish so that the resulting weight increases. Factors that affect the efficiency of feeding include the amount of feeding, the amount of feed consumption (feed energy content), as well as the completeness of nutrients in the feed (Djajasewaka, 1985).

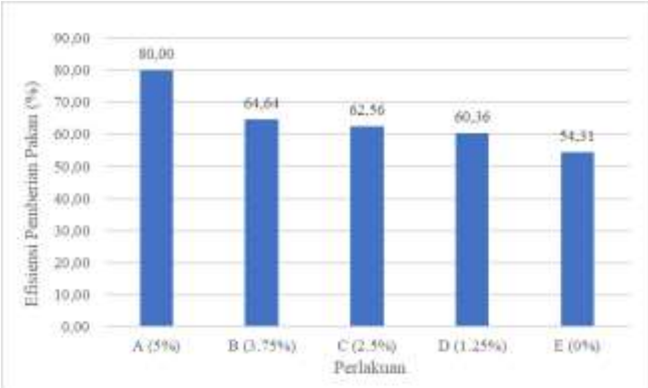

Figure 5. Feeding Efficiency of Pomfret Star Fish for 60 Days

Based on the results of observations that have been carried out for 60 days, the value of feeding efficiency is in the range of 54.31 – 80%. The highest feeding efficiency value was found in treatment A with the addition of the papain enzyme as much as 5% which was 80 ± 6.164% and the lowest feeding efficiency value was found in treatment E without the addition of the papain enzyme of 54.31 ± 1.12%. It is suspected that the papain enzyme functions as an exogenous enzyme in fish feed which can increase the hydrolysis of fish protein so that the digestibility of the feed increases. High levels of digestibility can increase the rate of absorption of amino acids into the body for growth (Amalia & Arini, 2013). In addition, the efficiency of feeding can be influenced by the quality of the feed.

The addition of the papain enzyme to the enzyme functional feed has a significant effect on the efficiency of feeding. The results of statistical analysis using ANOVA showed that the treatment gave a significant difference in the weight growth of the pomfret so it was continued with the Duncan test results at a 95% confidence interval which are presented in Table 2.

Table 2. Duncan's Multiple Spacing Test Feeding Efficiency (EPP)

Treatment	Average Daily Growth Rate
A	80 ± 6.164 a
B	64.64 ± 0.28 ab
C	62.56 ± 058 bc

D	60.36 ± 2.23 bc
E	54.31 ± 1.12 cd

Note: Values followed by the same letter are not significantly different according to the range test of Duncan's multiple at a 95% confidence level

In treatment A, the administration of the papain enzyme to the functional feed of the enzyme as much as 5% had the highest feeding efficiency value of 80%, which was suspected to be able to utilize the protein in the feed properly and efficiently so that the amount of protein that was broken down into amino acids increased and could be used by the body. fish. On the other hand, in treatment E without the administration of the enzyme papain on functional feed, the enzyme gave the lowest feed efficiency value of 54.31%. This can be expected because the amount of protein that is broken down into amino acids is small so it is not utilized optimally by the body of the fish (Marzuqi et al., 2012). If the quality and quantity of protein in the feed is lacking it will result in slow fish growth, so that protein in body tissues will be used to maintain more important body tissue functions, but if protein in excess feed will be excreted as nitrogen in the form of ammonia (Hardy & Kaushik, 2021).

The value of feeding efficiency obtained ranged from 54.31 - 80% where this range of values can be said to be good following the statement (Craig & Helfrich, 2002), then the results of giving papain enzymes to enzyme functional feed on the feeding efficiency of pomfret are said to be good because when seen from the test results from Multiple spacing in Table 2 shows a significant difference in the value of feeding efficiency. The value of good feeding efficiency is thought to be due to good feed quality and optimal water quality around the research site. Giving papain enzymes in artificial feed can increase protein retention, feed efficiency, feed consumption, and daily growth rate.

Water Quality Parameters

Observation of water quality was used as a supporting parameter during the research. Water quality parameters measured during the study were temperature, degree of acidity (pH), and dissolved oxygen (DO) every day. The measurement results show that the water quality during the study still meets eligibility for the maintenance of star pomfret (Table 3).

Table 3. Water Quality Range Value in Floating Net Cage

Parameter	**Unit**	**Value in Floating Net Cages**	***Quality Standards**
Temperature	^{o}C	27–29	28 – 32
DO	mg/L	5.6 – 6.7	> 5
pH	-	7 – 8	7.5 – 8.5
Salinity	ppt	32–34	28 – 34

*Basic standards according to(BSN, 2013)

Dissolved oxygen (DO) levels on the East Coast of Pangandaran have a value range of 5.6 - 6.7 mg/L, this is following the provisions issued by the (DJPB, 2013)dissolved oxygen levels for the maintenance of star pomfret is 3 - 6 mg/L. Temperature is a factor that can affect the level of dissolved oxygen (DO) in a body of water. Low temperatures can increase dissolved oxygen (DO) content which will then increase the metabolic rate of aquatic organisms (Ntengwe & Edema, 2008). Dissolved oxygen is a factor needed by fish for breathing and metabolism in the body which will result in movement, growth, and reproduction.

The rate of oxygen consumption in fish is the amount of oxygen consumed by aquatic biota in a certain time and the size of the fish, then it is closely related to the oxygen levels contained in water. The presence of oxygen is affected by the temperature in these waters, thereby affecting the process of exchanging substances or the metabolism of living things in them (Asnawi, 1993). Oxygen consumption is an indicator of respiration which also shows energetic metabolism. According to (Ezraneti et al., 2019), this metabolic process will produce energy which will then be used by fish to maintain their life including adaptation to the environment such as salinity.

Fish growth can be affected by the initial size of the fish at the time of the study. The adaptability of fish to salinity is also influenced by age and level of development where in general the ability of fish to osmoregulate differs at different age levels (Ezraneti et al., 2019). Besides affecting the growth and oxygen consumption of fish, it also affects the ability of fish to live and tolerate environments with lower salinity. The value of the range of salinity found at the study site ranges from 32-34 ppt which indicates that this range is following the quality standards contained in (BSN, 2013).

The degree of acidity (pH) that is too low or too high can cause fish growth to be stunted due to stress on the fish. According to (DJPB, 2013), the optimal pH for the maintenance of star pomfret is 7 - 8.5. Meanwhile, the pH on the East Coast of Pangandaran is 7 - 8 and can be said to be following the quality standard. In addition, temperature is one of the factors that can affect fish growth because it can affect fish appetite so it will affect the growth rate of fish and feed efficiency. During the maintenance period of the star Pomfret, the temperature range is 27 - 29 ^{o}C so it is still following the issued quality standard figures (BSN, 2013).

CONCLUSION

Functional feeding containing the enzyme papain had a significant effect on increasing daily growth rate (LPH), feeding efficiency (EPP), and intestinal histology of pomfret. The addition of papain enzyme to functional feed with the best dose was in the 5% treatment which resulted in a daily growth rate of 3.25 ± 0.112%, feed efficiency of 80 ± 6.164%, and the surface area of the villi in the intestinal histology of the pomfret was 2049.9 μm^2. While the administration of the papain enzyme in functional feed did not have a significant effect on the survival of the star pomfret with a value of 100%. The condition of water quality parameters on the coast of Pangandaran East Coast can be said to have fairly good conditions following quality standards, namely dissolved oxygen (DO) levels with values ranging from 5.6 - 6.7 mg/L, temperatures with values ranging from 27 - 29^{o} C, pH with values ranging from 7 – 8, and salinity with values ranging from 32 – 34 ppt.

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