

**ADDITIONAL OF PAPAIN CRUDE ENZYME EXTRACT (PCEE), IN  
FEED ON THE GROWTH RATE OF CANTANG GROUPER (*Epinephelus  
fuscogutattus lanceolatus*) FRY IN FLOATING NET CAGE AT  
PANGANDARAN COASTAL**

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**ABSTRACT**

Cantang grouper (*Epinephelus fuscogutattus lanceolatus*) is one of the most important economical fish species but it's growth is slower than other fish. The addition of enzymes to artificial feed is done to maximize the protein for fish growth. Papain enzymes are exogenous enzymes that aid in the process of protein hydrolysis. This research aims to determine the effect of papain crude enzyme extract (PCEE) on daily growth rate, feed efficiency, number and length of villy. This research was conducted on June - November 2017 at floating net cage Pangandaran Coastal- West Java. The test fish used is cantang grouper with average weight of each 15 gr and length of 7 - 8 cm as much as 900 fishes. The research method used was Completely Randomized Design (CRD) consisting of 5 treatments: A (5% PCEE), B (3,75% PCEE), C (2,5% PCEE), D (1,25% PCEE) and E (without PCEE) with 3 replications. The data analysis with F test is followed by Duncan multiple range test. The results of this research show that the 5 % PCEE can increasing the daily growth rate (3.16 %), feed efficiency ( 71.56 % ), number of villy (53 pieces) and length of villy 523 mm.

**Keywords:** papain crude enzyme extract (PCEE), Cantang Grouper, Daily Growth Rate, Feed Efficiency, Number and Size of Villy.

**INTRODUCTION**

One of highly-demanded fishes is grouper (*Ephinephelus sp*) with its good prospect due to its fairly high selling price. It is true particularly for *Cantang* grouper (*Ephinephelus fuscogutatts lanceolatus*) which is a hybrid between tiger grouper and giant grouper, i.e. around IDR100,000.- up to IDR120,000.- per kg of 500 grams size in March 2017 in the fish-culturist in Pangandaran Regency-West Java Province. This high price is the result of combined seed and feed prices plus

the fairly long period to cultivate it (6 months) due to its relatively low Daily Growth Rate (DGR). However, its culture in the sea has not been commonly done in Pangandaran Regency, therefore the fish needs to be cultured.

According to Halver et al (2011) grouper is a carnivore which prefers animal-based feed hence its protein content is higher (45 %) than its herbivore counterparts (25 %). The time this fish takes to grow is fairly long due to its relatively low Daily Growth Rate. This results in its high production cost. Meanwhile, it has been known that protease enzyme from papain is effective in reducing the energy for activating metabolism process (Howard 2010 and Rostika et al 2015, 2016), so that the Daily Growth Rate can increase. The currently available information on papain enzyme dose is not optimal yet in supporting the fish's growth and survival. Therefore, an experiment will be conducted on the application of papain enzyme in the form of Papain Crude Enzyme Extract (PCEE) which is expected to improve the grouper's growth performance.

Based on the results of research conducted by Rostika, et al (2015) on the use of PCEE in freshwater fish, i.e. tilapia (*Tilapia niloticus*), it is found that it successfully improves the fish growth performance. The application of PCEE can increase the protease content in the tilapia's juvenile intestines, resulting in an increased Protein Efficiency Ratio by 2.13% and Daily Growth Rate (*Oreochromis niloticus*) up to 2.67%. The same improvement also occurs to Nile tilapia (*Osteochilichthys hasselti*) growth performance (Rostika et al, 2016), however only Nile tilapia at juvenile stadia can be influenced by PCEE and in the fish at its seed stadia the influence is insignificant.

Papain enzyme is a protease enzyme capable of hydrolyzing protein complex compound into simple elements (amino acids), hence it can be easily digested optimally by the fish body.

The addition of papain as an exogenous enzyme into the feed can improve the feed's protein hydrolysis. This will result in the increased feed digestibility. This increased digestibility can improve the amino acid absorption into the body for growth. It can be expected based on the feed efficiency to protein efficiency ratio value and specific growth rate and survival rate (Amalia, Subandiyono, Arini 2013). The research conducted by Taqwdasbriliani, Hutabarat, Arini (2013) finds that the combination of papain enzyme and bromelain enzyme in tiger grouper feed shows a feed efficiency of  $45.682 \pm 0.444\%$ , protein efficiency ratio of  $97.579 \pm 0.946\%$  and specific growth rate of  $1.108 \pm 0.131\%/day$  and survival rate of 100%.

The results of research conducted by Mo et al. (2016) who add papain enzyme to paste feed made of fish powder and fishmeal for duskytail grouper (*Epinephelus bleekeri*), seabream (*Rhabdosargus sarba*), and pompano (*Trachinotus blochii*) indicates higher relative weight gain and feed conversion ratio than the feed with no papain.

This research aims at the right dose of Papain Crude Enzyme Extract (PCEE) in Cantang grouper at enlargement stadia which produce the best daily growth rate, feed efficiency, number of villi, and length of villi.

## **RESEARCH METHOD**

This research is conducted from April 2017 to December 2017, in Aquaculture Laboratory of Fisheries and Marine Sciences Faculty. The fish cultivation is done in the Floating Net Cage in Pangandaran Regency. The enzyme activity test is performed in Chemical Organic Laboratory of Mathematics and Natural Sciences Faculty. Finally, the histological test in the grouper intestine organ is done in Biosystem Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Padjadjaran University.

The equipment used to make PCEE include knife, blender, grater, cutting board, scales, roasting pan, lamp oven, sieve, glass bottle, spoon, plastic pack, and so on. The tools for cultivating the fish include boat, floating net cage, scales, landing net, fish feed of Scretting brand, stationery, calculator and so forth. The tools to for histological analysis include digital microscope, microtome, surgical instruments, fixative solution, ringer solution and others.

The method used in this research is the experimental one with Group Random Design (GAD) consisting of 5 treatments and 3 repetitions. Fifteen floating nets are prepared to keep the grouper at a density distribution of 50 fishes/net. The treatments in this research are Treatments A, B, C, D, and E (the feed with 5%, 3.75%, 2.5%, 1.25%, and 0% PCEE).

The influence of each treatment is tested using F test analysis of variance (ANOVA) at a test interval of 5%. When significant difference is found, it is then followed with Duncan's multiple range test.

The observation parameters are Daily Growth Rate (Effendi 1997 in Nugraha 2016), Feed Efficiency (Tacon 1993 in Amalia 2013), analysis of Fish Intestinal Tract (number of villi, length of villi, and necrosis).

### **Daily Growth Rate**

According to Suhendar (2016) growth is changes to size in terms of both length, weight or volume within certain period. Meanwhile, energetically, growth is expressed by the presence of changes in total body energy content in certain period which occurs when there is an excess of free energy remaining from the energy available in the feed minus the energy used for standard metabolism, digestion proses and for activities (Gusrina 2008).

The use of PCEE in grouper's feed gives a good response to the fish growth, as can be seen from the grouper's improved average individual weight in each treatment.

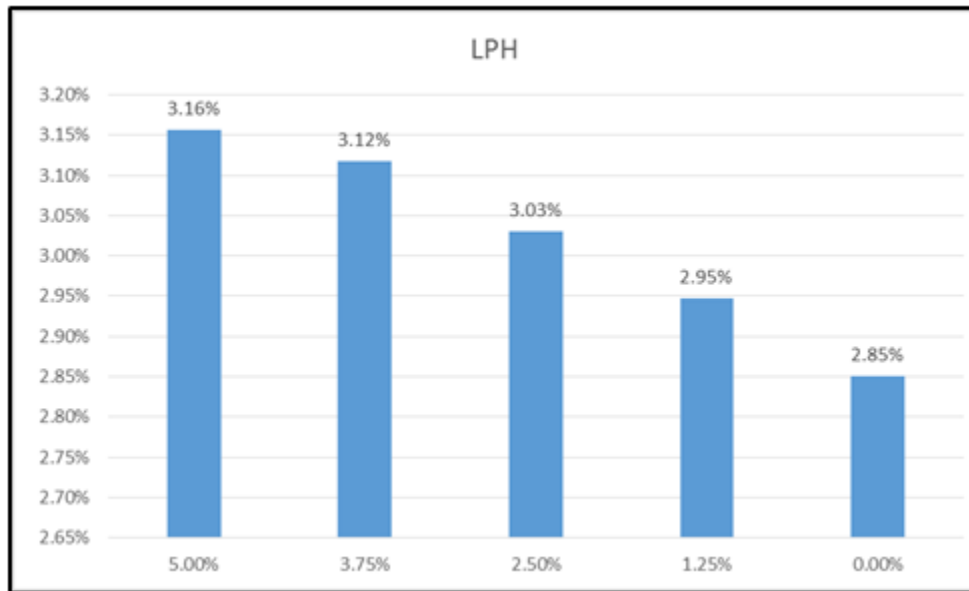
**Table 1: Average Daily Growth Rate of Grouper at Various Doses of PCEE**

Treatment	Average DGR (%)
A (PCEE 5.00%)	3.16 <sup>a</sup>
B (PCEE 3.75%)	3.12 <sup>a</sup>
C (PCEE 2.50%)	3.03 <sup>b</sup>
D (PCEE 1.25%)	2.95 <sup>b</sup>
E (PCEE 0% )	2.85 <sup>c</sup>

The value followed by the same small letters is insignificantly different at a confidence rate of 95%.

The Duncan's test results are as follows: treatments A and B (PCEE 5% and 3.75 %) are 3.16 % and 3.12 %, significantly different from other treatments, yet A and B are insignificantly different. Treatments C and D are significantly different from treatments A, B and E. Treatments C and D are insignificantly different from each other. And finally treatment E (lowest DGR at 2.85%) is significantly different from other treatments. The resulting DGRs reject the outcome of temporary assumption, i.e. the best treatment will only be treatment B (the feed with papain crude enzyme extract of 3.75%), while in reality the best result is obtained from treatment A (the feed with papain crude enzyme extract of 5%).

This value is different from Sugianto (2016) which finds that the best DGR of *Tilapia sp.* is found in the administration of PCEE at 3.75% dose, resulting in DGR value of 2.68%. The differences in this research are not limited to only PCEE, rather its medium is also different, i.e. sea water, combined with the good seed quality and high feeding level (5%). This DGR value is excellent, because it is above the minimum value of 1% (Retnosari 2007). The cultivated grouper has a size of 8 cm, nearly perfect digestive tract. Effendie (1997) explains that the intestine length relatively increases when the fish size gets bigger. Hence, when the fish is given the feed containing enzyme it will grow faster, because its intestines will absorb protein and nutrition more perfectly. The very existence of exogenous enzyme increases the intestinal work power to help accelerate the hydrolysis process of protein in the feed (Mo, 2016), hence the hydrolyzed protein becomes amino acids faster and they are absorbed by the fish body in larger quantity. This is supported by Hopher's (1990) statement which says that the feed digestability is influenced by the presence of enzyme in digestive tract and the time the consumed feed takes to react with the digestive enzyme.



**Figure 1: Bar Diagram of Daily Growth Rate of Grouper Seed During Research**

**Feed Efficiency (FE)**

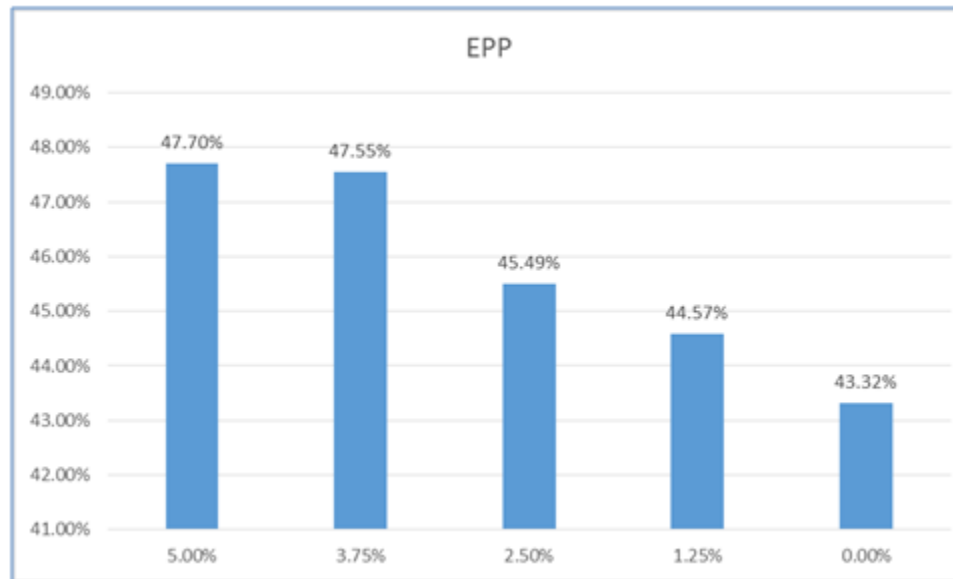
Feed efficiency describes the influence of feed administration on the fish consuming the feed and the utilization of feed administered so that it can improve the fish growth (Gusrina 2008). The higher the FE the better the feed quality and the efficiency at which this feed is transformed into meat, enabling lower production cost required to produce the fish meat (Effendi 2004).

After being kept for 60 days with an addition of PCEE at protease activity of 6.73 Unit/Mg protein in the feed, the grouper produces varied FE values (Table 2). The highest FE value is found in treatment B (feed plus PCEE 3.75% ), i.e. 47,70 %.

**Table 2: Average Feed Efficiency of Grouper During Research**

Treatment	Average FE (%)
A (PCEE 0% )	47.70% <sup>a</sup>
B (PCEE 3.25%)	47.55% <sup>a</sup>
C (PCEE 3.50%)	45.49 % <sup>b</sup>
D (PCEE 3.75%)	44.57 % <sup>b</sup>
E (PCEE 4%)	43.3 % <sup>c</sup>

The results of Duncan-test analysis shows that treatments A and B are significantly different from treatments C, D and E. This finding rejects the hypothesis which says that the best treatment is treatment D (feed plus PCEE 3.75%). It is suspected that this treatment with insignificantly different result, the given dose is less effective. This varied grouper's feed efficiency values also have varied value differences (Figure 2).



**Figure 2: Bar Diagram of Grouper's FE During Research**

The highest FE values are in treatments A and B (47.70% and 47.55%), which are followed by treatments C and D (45.49% and 44.57%), then treatment E (43.3%). This shows that their FE values are classified as excellent. The different FE values are the result of different PCEE doses. The addition of protease enzyme at the right dose to the feed is actually helpful in accelerating the fish growth. It is consistent with the statement of Sari *et al.* (2013) which suggests that fish needs enzyme. This enzyme can take the form of both endogenous and exogenous enzymes to help accelerate the protein digestion and hydrolysis processes into amino acids. The feed is digested optimally with the assistance of enzyme in the feed and inside the fish digestive tract so that the produced energy can be used to trigger the fish growth. The feed efficiency value is directly proportional to its daily growth rate value. This confirms the statement made by Huet (1970) in Sugianto (2016) which says that the high growth rate is related to the high feed efficiency.

The FE value (47.7%) in this research is consistent with Sugianto (2016), i.e. PCEE dose at 3.75% in tilapia shows a value of 48.31%, as well as with Suhendar (2016) who states that the FE value of Nilem carp (*Osteochilus hasselti*) with PCEE 3% is 37.7%. The research by

Taqwdasbriliani *et. al.* (2013) finds that the tiger grouper's (*Epinephelus fuscogutattus*) FE value with an added papain and bromelain enzyme combination at a dose of 75% and 25% is 45.68%.

Based on the observation result in intestine organ, the following data can then be presented.

**Table 3: Number and Length of Villi in Grouper's Intestines During Research**

Treatment (PCEE)	Number of villi	Length of villi (mm)
A (PCEE 5.00%)	53 <sup>a</sup>	524.5 <sup>a</sup>
B (PCEE 3.75%)	52 <sup>a</sup>	390.3 <sup>b</sup>
C (PCEE 2.50%)	31 <sup>c</sup>	353.6 <sup>b</sup>
D (PCEE 1.25%)	36 <sup>c</sup>	539.3 <sup>a</sup>
E (PCEE 0%)	45 <sup>b</sup>	306.6 <sup>c</sup>

The number of villi in intestines of the grouper provided with treatments A and B is the highest, i.e. 53 and 52. These are significantly different from treatments E, C and D. The length of villi is different between A, B and C, D plus E. The size of villi according to the the results of Kuperman and Kuz'mina's (1994) research in Asri (2015) influences the absorption process. Longer microvilli absorb the feed faster than shorter microvilli and the existence of microvilli has been one way to expand the process of absorption of nutrients.

### CONCLUSION

Based on the research results, it can then be concluded that:

The addition of PCEE at 3.75% to the grouper's artificial feed has some influence in accelerating DGR by 3.18, FE by 47.55 %, number of villi in grouper's intestine by 53 and length of villi by 524 mm.

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