The effect of papain enzyme in various doses to the growth and intestinal of cantang grouper (Epinephelus fuscogutattus lanceolotus)

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Abstract. This research was carried out to observe the appearance of the cantang grouper (Epinephelus fuscogutattus lanceolotus) intestine which was fed with exogenous papain enzyme-added, and the fish were raised in the cage on the coast of Pangandaran, West Java Province from January to March 2019. A completely Randomized Design Method in this study was performed with five treatments and three repetitions with enzyme doses of 0%, 1.25%, 2.5%, 3.75%, and 5% for 60 days. The fish used were two months old with an average weight 20 ± 10 g and randomly placed in fifteen cages with the size of 1m x 1m x 1.2m. All fish were fed commercial diets (CP: 40%) at a feeding level of 5% sampling was carried out every 10 days for 60 days. The Average Daily Gain (ADG) calculation was done by calculating the initial and final weights, the fish were dissected and the intestine conditions was carried out by taking the digestive tract from the anterior to the posterior. The parameters observed were ADG and intestine conditions (number of villi, villi length, necrosis, and goblet cells) in the region of anterior, midterior, and posterior. The results showed that the ADG was significantly different in all treatments, the best was the addition of enzymes 3.75% and 5% namely 2.26 and 2.30 gram/day. The number of villi at the anterior intestine was 32, the length of villi was 290.34 μm, necrosis cells was 39 and goblet was 168. Meanwhile, at the midterior the number of villi was 26, the length of villi was 275.18 μm, necrosis cells was 47 and goblet was 177. The number of villi at the posterior was 28, the length of villi was 277.26 μm, Necrosis cells was 56 and goblet was 201. Overall it can be concluded that the feed was added with papain enzyme of 3.75 % shows a significant effect on the growth and intestinal conditions of the Cantang grouper.

Keywords: cantang grouper; papain enzyme; growth; villi length; number of villi

1. Introduction
Cantang grouper (Epinephelus fuscogutattus lanceolotus) is a hybrid grouper fish originated from tiger grouper and kertang grouper, which is widely cultivated in the sea and ponds in Indonesia including the coast of Pangandaran Regency, West Java. Its white flesh and soft taste make this fish popular with consumers. The duration of cultivation from seed to consumption size (600-700 gr) took as long as 7 months, but when they were given an exogenous Papain enzyme, the duration of cultivation became faster [1]. When this enzyme is given to fish, it will start working in the intestinal tract and affect the
metabolism of fish including grouper [2]. Growth performance such as Growth Rate, Average Daily Gain and Feed Conversion Ratio will increase both in marine fish and freshwater fish [3-5]. Cantang grouper is given the enzyme papain as much as 2%-6% which is mixed in the artificial feed to increase its protease content in the intestine.

The intestinal organ is a multifunctional organ with various important functions, including a defense function because the intestine is a physical fence to protect from the pathogens in fish [6]. In the aquaculture industry, this is important because the intestine is one of the main access points for pathogens. The cultured fish are generally fed with commercial diets, so that farmers are able to control the health of the fish, including put a drugs and vaccines in diets. The last reasons, the immune system in teleost gut tolerates the microbes by symbionts to colonized, and these microbial communities is a mechanism to modulate fish pathogens.

The intestine is an organ that functions in the process of digestion and absorption of nutrients. The condition for nutrients absorption, when there is a high number of villi, wide villi and a large number of villi in the anterior intestine, meaning that there is an increase in absorption of nutrients into the bloodstream. For example, the intestinal surface area of broilers has something to do with the rate of absorption. The larger the surface area of the intestinal villi, the greater the chance of absorption from the digestive tract [7].

Based on the research of Mo et al. [8], there is the probability of using soybean meal added with papain to replace half of the fish meal used in wet pellets (49% fish meal and 45% trash fish) for marine aquaculture system. The marine fish used were gold sea bream (Rhabdosargus sarba), brown spotted grouper (Epinephelus bleekeri) and pompano (Trachinotus blochii). The results showed that feed with papain gave a better relative growth rate and feed conversion ratio than feed without papain. Generally, the fish body weight gain determine by the amount of papain added to the feed rather than the composition of the feed. A similar result was also found in Rachmawati et al. [9] for Black Tilapia (Oreochromis niloticus) In local catfish, the surface area of the intestinal villi can affect feed absorption, so that later it can optimize the feeding of cultured fish. The absorption of catfish feed is more optimal if the intestinal villi are healthy and the surface area of the villi is high.

This research was carried out to observe the appearance of the Cantang grouper (Epinephelus fuscoguttatus lanceolotus) intestine which was fed with exogenous enzyme papain-added feed and maintained in a marine cage on the coast of Pangandaran, West Java Province from January to March 2019.

2. Method
Research on grouper enlargement was carried out at Floating Net Cage at Pangandaran Regency, West Java Province in January-March 2019. Analysis of water quality parameters was carried out in-situ, while analysis of intestinal conditions was carried out at the Biology Laboratory of Faculty of mathematics and natural science, Padjadjaran University.

2.1. Research design
The readily kept fish were the seeds of the nursery at the Community Hatchery in Situbondo Regency, East Java Province. This research was conducted for 60 days, with research tools and materials including a set of tools and materials for making papain enzymes, a set of tools and materials for fish seed maintenance, electric scales, a set of tools and materials for proximate analysis, a set of tools and materials for enzyme analysis and intestinal conditions.

2.2. Research procedure
As the test animal, cantang grouper seeds were used with a length of 8 cm. Fish were reared in the floating net cages with a density of 40 fish per tub. Furthermore, the fish were given pellets with a protein content of 40%, which had been spiked with the enzyme papain with various doses. Feeding level 5% is given in the morning, maintenance is carried out for 60 days. Measurement of biomass
weight was carried out every 10 days, while water quality was at the beginning and end of the observation.

2.3. Observation of variables
The variables observed in this stage of the study consisted of growth parameters (Average Daily Gain), and intestinal conditions (number of villi, villi length, necrosis cells, and goblet cells). The formula used is as follows.

Average Daily Gain (ADG) is calculated using the formula:

\[ ADG = \frac{W_t - W_0}{T} \times 100\% \]

Note:
- \( ADG \): Specific growth rate (% / day)
- \( Wo \): Average fish weight at the beginning of the study (g)
- \( Wt \): Average fish weight at the end of the study (g)
- \( T \): Duration of maintenance (days)

The design used in this research is a completely randomized design (CRD) consisting of 5 treatments and 3 repetitions. The treatment of papain enzyme levels used in this study was as follows:

- A: Papain enzyme dose on 0% pellets
- B: Papain enzyme dose in diets 1.25%
- C: Papain enzyme dose in diets 2.50%
- D: Papain enzyme dose in diets 3.75%
- E: Papain enzyme dose in diets 5.0%

The linear model of the completely randomized design used in this study can be seen in the following formula:

\[ Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \]

Note:
- \( Y_{ij} \): observations on the \( i \) treatment and repetitions \( j \)
- \( \mu \): general mean
- \( \tau_i \): effect of \( i \) treatment
- \( \varepsilon_{ij} \): random effect of \( i \) treatment and \( j \) test

2.4. Intestinal conditions
Intestine was observed using the exploratory histological method. The sample was then made into histological preparations by staining with hematoxylin-eosin (HE). The parameters observed were following:

1. The number of villi,
2. The length of the villi,
3. The number of necrosis cells,
4. The number of goblet cells.

To determine the growth performance, we carried out sampling and weighing the feed every 10 days. The analytical method used to process the growth performance data was analyzed using Anova and Duncan, while the intestinal condition parameters were analyzed descriptively.

3. Result and discussion

3.1. Average Daily Gain
Average Daily Gain (ADG) is a representation quantity of how much bodyweight is added (grams) per day. The table below shows the ADG of fresh grouper in various treatments.
Table 1. Average daily gain of cantang grouper with various treatment dose of papain enzyme.

| Treatment (%) | Repetitions | Total | Average | Variance |
|---------------|-------------|-------|---------|----------|
|               | 1           | 2     | 3       |          |
| A (0%)        | 1.81        | 1.86  | 1.85    | 5.52     | 1.84a   | 0.000700 |
| B (1.5%)      | 2.00        | 1.91  | 1.95    | 5.86     | 1.95b   | 0.002033 |
| C (2.50%)     | 2.08        | 2.14  | 2.11    | 6.33     | 2.11c   | 0.000900 |
| D (3.75%)     | 2.23        | 2.28  | 2.26    | 6.77     | 2.26d   | 0.000633 |
| E (5%)        | 2.33        | 2.28  | 2.30    | 6.91     | 2.30d   | 0.000633 |

* The different small letters indicate significant differences between the means (ANOVA followed by Duncan test, p<0.5)

Growth is a process of increasing length and weight of an organism which can be seen from change in length and weight in units of time. Fish growth is influenced by the quality and quantity of feed, age, and water quality. Average Daily Gain on the best Cantang grouper on fish treated with 3.75% and 5%, while other treatments were not as good as these treatments. It is suspected that other treatments cannot metabolize protein by lack of proteases inserted from the outside (exogenous enzymes). The result was not optimal for grouper meat deposition, due to the inappropriate levels of the protease and saponin enzymes from papain [10,11].

The papain enzyme treatment had a good effect on the growth of Cantang grouper respectively, 3.75 and 5%, namely ADG 2.26 and 2.36 grams per day and then 2.5% treatment, namely 2.11 grams per day. According to Hidayat et al. [12], growth is influenced by several internal and external factors. Internal factors such as heredity, resistance to disease, and the ability to use food, while external factors might be influenced by a physical, chemical, and biological characteristics of waters. According to Laining et al. [13] growth is a change in fish size both in weight, length, and volume over a certain period of time which is caused by tissue changes due to the division of muscle and bone cells which are the largest part of the fish's body, causing additional weight or length of the fish.

3.2. Intestine Condition

The following is the condition of the number and length of the villi, in the anterior, mid, and posterior intestine in the intestinal tract grouper after these fish were treated (table 2).

Table 2. The number of villi, villi length in intestine of anterior, mid and posterior cantang grouper based on papain enzyme dosage.

| Treatment (%) | Number of Villi | Length Villi (micrometer) |
|---------------|----------------|---------------------------|
|               | anterior       | mid                       | posterior      | anterior       | mid                       | posterior      |
| A (0%)        | 28a            | 31                        | 24b            | 288.71a        | 317.94a                   | 295.51a        |
| B (1.25%)     | 33a            | 26                        | 28b            | 286.65a        | 287.37b                   | 291.2b         |
| C (2.50%)     | 35a            | 30                        | 34a            | 291.37a        | 259.53d                   | 260.14d        |
| D (3.75%)     | 19b            | 27                        | 25b            | 275.26b        | 284.78b                   | 288.45b        |
| E (5%)        | 32a            | 26                        | 28b            | 290.34a        | 275.18c                   | 277.26c        |

* The different small letters indicate significant differences between the means (ANOVA followed by Duncan test, p<0.5)

The data in table 2 can be illustrated by the bar diagram as follows (figure 1 and figure 2):
The intestine is an organ that functions in the process of digestion and absorption of nutrients, but the intestines in fish can function as a digestive gland [14]. The best nutrient absorption process is found in the proximal/anterior intestine, which has a large villi height, width, and number of villi, which can be associated with increased absorption of nutrients into the bloodstream [15]. This can be seen in table 2 and figure 1, where the number of villi and villi lengths are more numerous and longer in the anterior intestine compared to the posterior ones in all treatments, except for the addition of 3.75% papain enzyme treatment. The number of villi in the anterior is more than the posterior part, this is common in the anterior/front. In the addition of 3.75% enzyme treatment, the conditions of the anterior and posterior intestines were the same as the number of villi, this indicated a better, healthier condition in the cantang grouper intestine when compared to others. The following is the condition of the number of necrosis cells and goblet cells in the villi of cantang grouper after these fish were subjected to treatment (table 3).

**Table 3.** Necrosis cells and goblet cells in the intestine of cantang grouper based on papain enzyme dose.

| Treatment | Anterior | Mid | posterior | anterior | Mid | Posterior |
|-----------|----------|-----|-----------|----------|-----|-----------|
| A (0%)    | 62^a     | 50^b| 58^a      | 167^b    | 152^c| 170^c     |
| B (1.25%) | 47^b     | 63^a| 52^a      | 136^d    | 148^c| 150^c     |
| C (2.50%) | 66^a     | 45^b| 48^a      | 210^a    | 176^a| 189^b     |
| D (3.75%) | 51^b     | 47^b| 43^a      | 146^c    | 169^b| 157^d     |
| E (5%)    | 39^c     | 47^b| 56^a      | 168^b    | 177^c| 201^a     |

*The different small letters indicate significant differences between the means (ANOVA followed by Duncan test, p<0.5)
The anterior intestine is the part that receives food from the stomach of course, there is still the influence of stomach acid (HCl) which will go to the anterior intestinal tissue. Having a lot of Goblet cells is one of the protection of the intestine against its tissue epithelial from the influence of stomach acid that is brought by food from the stomach because acid stomach will damage the intestinal epithelial tissue.

This shows that the greatest absorption of nutrients occurs in the anterior part where there are more and higher villi, while the villi increasingly towards the posterior have shorter and grooved villi [16]. According to Erian et al. [16], villi height varies depending on the region and species. This is consistent with the function of this part of the intestine in the opinion of Erian et al. [16] which states that the anterior part of the intestine serves to 1) transport food material from the stomach to the posterior intestine, 2) for complete digestion by secretion of enzymes from the walls and accessory glands, 3) to absorb the end products of digestion into blood and lymph vessels on its walls, and 4) to secrete certain hormones (namely Secretin, stimulates pancreatic secretions). The functions of the posterior gut include fluid absorption, mucus secretions (more goblet cells), and some digestion achieved by enzymes present in food material, and excretion.

According to Hassaana et al. [15], the intestine is an organ that is often exposed to pathogenic agents and parasites. These agents enter the intestine through food that enters the digestive tract. Some of the damage that is often found in the intestines of fish are goblet cell proliferation, hemorrhage, villi atrophy, metaplasia, epithelial cell necrosis, and bleeding. The most common damage found in the intestines of fish is necrosis and atrophy of the epithelial layer, epithelial desquamation, and necrose and hemorrhage. Necrosis that occurs in the intestinal tissue of the fish samples is characterized by the appearance of damaged tissue, this is known to be caused by acute exposure to toxins, the presence of bacteria, viruses, and parasites [15]. The function of the posterior gut includes fluid absorption, mucus secretions, and some digestion which is accomplished by the enzymes present in the food material, and excretion.

Goblet cells are the dominant type of mucus cells in the intestinal epithelium of fish. The nucleus of the goblet cells is located in the basal part, and, in the central area, the cells expand and then contract to form an apical pore through which mucus is secreted. The main molecule in mucus is mucin, which plays an important role in the maintenance of the epithelial barrier against pathogens. Mucin granules, which predominate in cells, can have variable electron density and have the characteristic of goblet aggregates of thecae cells. The granules are secreted constitutively and, in response to extracellular stimuli, using a highly regulated process that involves transport of the granules via actin remodeling, anchoring of the granules to the membrane, and exocytosis of the granules. The number of goblet cells varies in the intestines of fish infected with parasites, increasing in number around the site of infection [15].

Data in table 3 shows that grouper intestines with additional the papain enzyme were 2.5%, healthier than other grouper intestines seen from the number of villi and villi length which was better than others. This is in accordance with Nafiu et al. [7] that papaya contains chymopapain which functions as an antihepatotoxic, antibacterial, antihypertensive, antioxidant, anti-inflammatory and even papaya can heal wounds and is healthy when given in the form of papaya fruit water extract at a dose of 100 mg/kg/day for 10 days.

4. Conclusion
In conclusion, dose of 3.75 and 5% papain enzyme could give the best result of intestinal condition at anterior, mid anterior, and post anterior with the result of the number of villi were 32, 39, and 47. The length of villi were 290.34 μm, 275.18 μm, 277.26 μm. The results of necrosis cells were 39, 47, and 56. Meanwhile, the goblet was 168, 177, and 201. Briefly, the feed with additional enzyme of papain with dose 3.75% shows a significant effect on the growth and intestinal condition of the Cantang grouper. The papain enzyme can be added up to 5% without negative effect. The study about the utilities of the active compound in papain enzyme should be examined.
Acknowledgment

The research team would like to thank the Directorate General of Higher Education, Ministry of Education, and Culture of the Republic of Indonesia for the Applied Research Grant for the 2019-2021 fiscal year Contract Number: 1827/UN6.3.1/LT/2020.

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