Papain Enzyme as Feed Additive for Grouper Cantang (Epinephelus fuscoguttatus-lanceolatus) and Silver Pompano (Triachonus blochii)

Rita Rostika¹, Iskandar Iskandar², Yuli Andriani², Mochammad Ichsan C.Purnama³, Fitrie Meyllianawaty Pratiwy² and Rega Permana¹

¹Fisheries Pangandaran Class Study Program, Faculty of Fisheries and Marine Science, Universitas Padjadjaran
²Fisheries Study Program, Faculty of Fisheries and Marine Science, Universitas Padjadjaran
³Fisheries Study Program Almuni, Faculty of Fisheries and Marine Science, Universitas Padjadjaran

Email: ritarostika_unpad@yahoo.com

Abstract. This research aims to observe growth and intestines condition of mariculture fish species, grouper cantang dan silver pompano which feed had been incorporated with papain enzyme (PE) containing papaya derived protease. The research was conducted in 2018 at The Floating Net Cages at Pangandaran Beach, Indonesia. A complete randomized design with five treatments and three repetitions was performed. The treatments involved were A (control ) B (1, 2.5% of PE added to feed), C (2.50% of PE added to feed), D (3.75% of PE added to feed), E (5.00% of PE added to feed). In addition, the research experiment if silver pompano with the same manner was conducted in 2020. The treatments were V (2% of PE added to feed), W (3% of PE added to feed), X (4% of PE added to feed), Y (5% of PE added to feed) and Z (6% of PE added to feed) for 2 months of observation time. The results showed that additional PE incorporation to feed could increase Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) on fish. The addition of the papain enzyme dose in cantang grouper feed has a positive effect on the growth and intestinal conditions of the fish. At doses of 3.75% and 5% gave the best response to ADG (2.26 grams / day and 2.30 grams / day), FCR (1.41% and 1.40%) and SR (68% and 69%). The cantang grouper intestine which was given the addition of papain enzyme was 3.75% and 5%, the best conditions were the number of villi (52 and 53 pieces), the best length of the cantang grouper villi was in the 125% and 5% treatments, namely 539.3 and 524.5. Meanwhile the papain enzyme at a dose of 5% gave the best response to body weight, ADG (2.46 gram/day), SGR (0.83 %/day) and SR in silver pompano (100%).

1. Introduction
Cantang grouper (Epinephelus fuscoguttatus-lanceolatus) and silver pompano (Triachonus blochii) have a high selling value, around Rp. 100,000 and Rp 60,000 per kg per fish farmers in Pangandaran Regency in January 2020. The duration of fish maintenance is a problem encountered in the cantang grouper and silver pompano cultivation. Low growth is caused by several factors, including genetic factors, poor water quality, and the amount of feed that is not suitable for fish needs. Therefore, in order to provide good growth results, it is necessary to add exogenous enzymes.
The effect of exogenous protease supplementation in fish pellets on growth, nutrient retention, digestibility and serum biochemical index of gibel carp, *Carassius auratus gibelio* was evaluated. The results of this study indicate that adding exogenous proteases to pellet feed containing 30 g / kg of fish meal, can increase growth, digestibility of dry matter and crude protein, and protein and lipid retention for carp [1].

Papain enzyme is a protease enzyme that is able to hydrolyze protein complex compounds into simple elements (amino acids) so that they can be easily digested optimally by the body. Based on the research results[2] regarding the use of the enzyme papain in tilapia (*Tilapia niloticus*), it was successful in increasing its growth performance, as well as for nilem fish (*Osteochillus hasselti*) [3]. The presence of enzymes in feed will increase the digestibility of fish to feed ingredients. Fish need enzymes [4], in the form of endogenous and exogenous enzymes to help speed up the digestion and hydrolysis process. The feed is digested optimally with the help of enzymes in the digestive tract and in the end the feed energy produced can be used to spur fish growth [4].

The effect of papain enzyme on fish growth was shown by several research results. The results of research [5], the treatment of papain enzyme in feed as much as 5% was able to produce the highest average growth, which was 3.24% / day in tiger grouper (*Epinephelus fuscoguttatus*). The dose of the enzyme papain in feed as much as 3.75% can produce optimal growth in nilem (*Osteochillus hasselti*) [6] with a daily growth rate of 0.58% and a daily growth of 0.24 grams per day and feeding efficiency of 43.81%. Furthermore, The application of the papain enzyme was able to increase the protease levels [2] in the juvenile gut of tilapia so that the effect was able to increase the Protein Efficiency Ratio and Growth Rate of *Oreochromis niloticus* by 2.13% and 2.67%.

Papain has well-established pharmacological properties (antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial) than papain is a candidate in the treatment of a wide variety of diseases (eg. dengue fever, gastrointestinal tract infections and periodontitis) and for wound healing. Saponins contained in the papain enzyme have broad pharmacological activities including: immunomodulator, anti-tumor, anti-inflammatory, antiviral, anti-fungal, can kill shellfish, hypoglycemic, and hypocholesterol effects [7].

This research aims to observe growth and intestines condition of mariculture fish species, grouper cantang and silver pompano which feed had been incorporated with Padjadjaran Enzyme (PE) containing papaya derived protease.

2. Materials and Method

2.1 Research Location and Time

Research for grouper cantang was carried out for 70 days starting from February 2018 to May 2018, while silver pompano research was carried out for 60 days from May 2020 to August 2020. All experiments were carried out in floating net cage, Pangandaran District, West Java Province.

2.2 Materials

The materials used during the study were nets measuring 3 mx 3 mx 1.5 m, mesh size 0.75 - 1 inch, divided by 4 waring sizes of 1.5 mx 1.5 mx 1 m, digital scales, DO meters, pH meter, thermometer, a set of tools for making papain enzymes, a set of tools for measuring proximate ingredients, a set of tools for analyzing the intestine, a sprayer, and various plastic bowls of various sizes. The experiments were used 750 for cantang grouper test fish, 100 days old, “M” brand feed, unripped papaya fruit, water as a solvent.

2.3 Research Procedure

As the test animal, cantang grouper fingerlings with the initial size 20 g and silver pompano with the initial size 200 g. Fish were reared in net cage with a density of 40 individuals / net. Furthermore, the fish were fed by the feed wich contain papain enzymes 40% protein with 5% feeding level and 60 days
rearing periods. The measurement of biomass weight was carried out every 10 days, while water quality was at the beginning and at the end of the observation.

2.4 Observation of Variables
The variables observed in this research consisted of growth parameters (Average Daily Gain, Daily Growth Rate, Feed Conversion, Survival Rate), and intestine conditions (Villi Length, Number of Villi).

2.4.1 Average Daily Gain (ADG) is calculated using the formula [8]:

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

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)

2.4.2 Daily Growth Rate (Effendi 1997 in Nugraha 2016):

\[
DGR = \frac{(\ln W_t - \ln W_0)}{t} \times 100\%
\]

Note:
- DGR : 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)

2.4.3. Feed Conversion Ratio (FCR) is calculated using the formula [9]:

\[
FCR = \frac{FI}{(W_t + D) - W_0} \times 100\%
\]

Note:
- FCR : feed conversion ratio
- FI : Weight of feed consumed (g dry weight)
- Wo : Fish biomass weight at the beginning of the study (g)
- Wt : Fish biomass weight at the end of the study (g)
- D : Dead fish weight (g)

2.4.4. Survival Rate (SR) is calculated by using the following formula [10]:

\[
SR = \frac{N_t}{N_0} \times 100\%
\]

Description:
- SR : Fish survival (%)
- Nt : Number of fish alive at the end of the study (tail)
- No : Number of fish at the beginning of the study (tail)

Method used in this study was an experimental method with a completely randomized design (CRD) consisting of 5 treatments and 3 replications. In each experimental unit, 50 groupers were cultured.
• Treatment A: Feed with the addition of 0% EP.  
• Treatment B: Feed with the addition of 1.25% EP.  
• Treatment C: Feed with the addition of 2.50% EP.  
• Treatment D: Feed with the addition of 3.75% EP.  
• Treatment E: Feed with the addition of 5% EP.

In each experimental unit as many as 50 silver pompano cultured.
• Treatment A: Feed with the addition of 2% EP.  
• Treatment B: Feed with the addition of 3% EP.  
• Treatment C: Feed with the addition of 4% EP.  
• Treatment D: Feed with the addition of 5% EP.  
• Treatment E: Feed with the addition of 6% EP.

The effect of each treatment was tested by means of analysis of variance (ANOVA) F test at a 5% confidence interval, if there is a significant difference, it is followed by Duncan's multiple distance test and regression.

3 Results and discussion

3.1 Average Daily Gain

Based on measurements of body weight and ADG of cantang grouper and silver pompano, it can be observed in Table 1 as follows.

| Treatments | Cantang Grouper | Treatment | Silver Pompano |
|------------|----------------|-----------|----------------|
|            | Initial weight (grams) | ADG | Initial weight (grams) | ADG |
| A1         | 10.15           | 1.81     | V1         | 215 | 2.3 |
| A2         | 10.35           | 1.86     | V2         | 218 | 2.27 |
| A3         | 9.95            | 1.85     | V3         | 217 | 2.25 |
| average    | 1.84            |          |            |     | 2.27 |
| B1         | 10.00           | 2.00     | W1         | 215 | 2.32 |
| B2         | 10.00           | 1.91     | W2         | 216 | 2.30 |
| B3         | 9.85            | 1.95     | W3         | 217 | 2.30 |
| average    | 1.95            |          |            |     | 2.31 |
| C1         | 10.05           | 2.08     | X1         | 218 | 2.27 |
| C2         | 10.10           | 2.14     | X2         | 216 | 2.31 |
| C3         | 10.05           | 2.11     | X3         | 214 | 2.31 |
| average    | 2.11            |          |            |     | 2.30 |
| D1         | 9.90            | 2.23     | Y1         | 219 | 2.53 |
| D2         | 10.20           | 2.28     | Y2         | 218 | 2.67 |
| D3         | 10.00           | 2.26     | Y3         | 217 | 2.18 |
| average    | 2.25*           |          |            |     | 2.46* |
| E1         | 10.15           | 2.33     | Z1         | 215 | 2.36 |
| E2         | 10.05           | 2.28     | Z2         | 216 | 2.24 |
| E3         | 9.60            | 2.3      | Z3         | 215 | 2.31 |
| average    | 2.30*           |          |            |     | 2.30 |

Notes: An asterisk behind the number indicates that the number are significantly different
From Table 1 it can be seen the longer the cultivation time, the higher the body weight. The presence of feed at a 5% feeding level makes the fish gain weight, but statistically, the increase is significantly different for each treatment.

Cantang grouper and silver pompano, as carnivorous fish, tend to require feed with high protein concentrations, namely between 45% - 50% [11,12]. According to [13] in order for feed protein to be utilized properly and maximally, it is necessary to have additional proteolytic enzymes from outside. In this research, the exogenous papain enzyme dose of 3.75 and 5% was able to increase the ADG performance of cantang grouper, namely 2.25 grams - 2.3 grams / day. The dose of the 5% papain enzyme in silver pompano produced ADG of 2.46 grams / day.

In addition to nutrient protein degradation, proteases are also required in a number of body biochemical reactions such as pathogenicity mechanisms, blood coagulation processes, sporulation processes, differentiation, a number of post-translational protein processes and extracellular protein expression mechanisms [14].

Exogenous enzymes have a beneficial effect on the intestinal microbial population thereby improving nutrient digestion, absorption and making the nutrients needed by fish for growth [15]. Exogenous enzymes as proteases are useful for increasing the nutritional value of vegetable protein by breaking down protein into free amino acids and releasing some nutrients that are not available [1,16,17].

From the observational data, it can be calculated that the FCR and SR of cantang grouper and silver pompano can be seen in Table 2 as follows.

Table 2. Feed Conversion Ratio and Survival Rate for Cantang Grouper and Silver Pompano at Various Doses of Addition of Papain Enzymes

| Treatments | Cantang Grouper | Silver Pompano |
|------------|----------------|----------------|
|            | FCR | SR (%) | SGR | SR (%) |
| A1         | 1.56 | 68     | V1  | 0.80   |
| A2         | 1.51 | 66     | V2  | 0.80   |
| A3         | 1.55 | 76     | V3  | 0.79   |
| **Average A** | 1.53 | 72     |     | 0.80   |
| B1         | 1.49 | 70     | W1  | 0.80   |
| B2         | 1.49 | 72     | W2  | 0.80   |
| B3         | 1.50 | 72     | W3  | 0.80   |
| **Average B** | 1.49 | 71.3   |     | 0.80   |
| c1         | 1.46 | 72     | X1  | 0.80   |
| c2         | 1.44 | 66     | X2  | 0.80   |
| c3         | 1.50 | 74     | X3  | 0.79   |
| **Average C** | 1.46 | 70.67  |     | 0.80   |
| D1         | 1.41 | 74     | Y1  | 0.85   |
| D2         | 1.41 | 70     | Y2  | 0.90   |
| D3         | 1.38 | 64     | Y3  | 0.76   |
| **Average D** | 1.40 | 69.3   |     | 0.83*  |
| E1         | 1.41 | 72     | Z1  | 0.81   |
| E2         | 1.39 | 68     | Z2  | 0.78   |
| E3         | 1.39 | 64     | Z3  | 0.77   |
| **Average E** | 1.39* | 68      |     | 0.78*  |

Feed conversion ratio (FCR) is a measure of how efficiently an animal converts feed to body mass, and it is determined by dividing the weight of feed fed by live weight gain over some time interval (feed / gain). In this study, it can be seen that the highest FCR is in cantang grouper treated with E or added with the enzyme papain as much as 5%.
Based on previous research [18] on carnivorous groupers, this also happens to the best SGR of silver pompana fish (carnivores) when feed is added with the enzyme papain by 5% namely 0.83 % per day.

Papaya produces papain-rich latex (the main proteolytic enzyme) and contains phenolics, flavonoids and alkaloids as the main phytochemicals. It is explained that papaya and its products are nutritional supplements and therapeutic agents in various disease conditions[19].

Survival rate (SR) is a response to the body's resistance to internal and external stress. Cantang grouper is relatively susceptible to parasite and disease, although during the study this fish was able to survive up to 70%. The survival rate of silver pompano during the study was 100%, meaning that during the study period (70 days) there was no death. This shows that silver pompano fed with the addition of papain enzyme treatment gives good results, it can be seen from the response that makes the fish healthy and not stressed.

3.2 Intestine conditions
According to [20] the anterior part of the intestine functions for 1) transport of foodstuffs, 2) complete digestion by enzyme secretions from the walls and gland accessories, 3) absorbs the end products of digestion into the blood vessels and lymph in their walls, and 4) to secrete certain hormones (namely secretin, stimulates pancreatic secretions). The function of the posterior gut is the absorption of fluid, mucus secretions and some digestion which is achieved by the enzymes present in the food material, and its excretion.

Table 3 shows the condition of the number and length of the grouper’s intestine villi at the end of the study.

| Treatment | Number of Villi | Length Villi |
|-----------|----------------|--------------|
| A         | 45 b           | 306.6 c      |
| B         | 36 c           | 539.3 a      |
| C         | 31 c           | 353.6 b      |
| D         | 52 a           | 390.3 b      |
| E         | 53 a           | 524.5 a      |

Data in Table 3 show that the intestine of grouper cantang with the addition of the enzyme papain. In cantang grouper, the best number and length of villi was the addition of the enzyme papain by 3.75% and 5%. This is in accordance with [19] that papaya contains chymopapain which functions as an antihepatotoxic, antibacterial, antisickling, antioxidant, anti-inflammatory, 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 / days for 10 days.

4 Conclusion
The addition of the papain enzyme dose in cantang grouper feed has a positive effect on the growth and intestinal conditions of the fish. At doses of 3.75% and 5% gave the best response to ADG (2.26 grams / day and 2.30 grams / day), FCR (1.41% and 1.40%) and SR (68% and 69%). The cantang grouper intestine which was given the addition of papain enzyme was 3.75% and 5%, the best conditions were the number of villi (52 and 53 pieces), the best length of the cantang grouper villi was in the 125% and 5% treatments, namely 539.3 and 524.5. Meanwhile the papain enzyme at a dose of 5% gave the best response to body weight, ADG (2.46 gram/day), SGR (0.83 %/day) and SR in silver pompano (100%).
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