INTESTINAL HISTOLOGY OF THE GROUPER *Epinephelus lanceolatus fuscoguttatus* ADMINISTERED WITH SIMPLICIA PAPAYA**

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ABSTRACT

The grouper (*Epinephelus* sp) is a marine fish species that many people commercially cultivate, for hatchery and rearing, and showing such promising prospect. Fish intestine and growth histology are a function of internal and external conditions, namely water quality, feed quality and feed quantity. However, even though the protein content of its feed is high, the growth of the grouper, *Epinephelus lanceolatus fuscoguttatus* is quite low. The purpose of this study was to determine the effect of simplicia papaya on the growth of the grouper particularly, absolute weight and the protein efficiency ratio. The research was conducted using Completely Randomized Design with 5 treatments and 3 replications (A: simplicia papaya 5% application, B: simplicia papaya 3.75% application, C: simplicia papaya 2.5% application, D: simplicia papaya 1.25% application, and E: 0% simplicia papaya). The groupers were cultivated in a floating net cage in Pangandaran Regency, Indonesia. The addition of simplicia papaya at 3.75% and 55% to the grouper's artificial feed had influenced the number of necrotic cells at 169 and 183 cells, respectively, and so were the number of goblet cells. The addition of simplicia papaya at 5%, 3.75% and 2.5% of the grouper's artificial feed had increased the absolute weight gain by 161.36, 152.19 and 152.09 g, respectively. The addition of simplicia papaya at 5%, and 3.75% of the grouper's artificial feed increased the protein efficiency ratio by 3.18% and 3.19%, respectively.

Keywords: absolute gain, *Epinephelus lanceolatus fuscoguttatus*, histological intestine, papain crude enzyme extract, protein efficiency ratio

INTRODUCTION

The health of aquatic organisms and water quality are interconnected and directly proportional (Zimmerli *et al.* 2007). Due to close contact with their environment, the homeostatic mechanisms of fishes are so highly depended on the existing conditions in their immediate surroundings, that even a slight variations in water quality can cause a wide variety of biological responses (Authman 2015; Nussey *et al.* 1995). In evaluating the impact of environmental contaminants in biota, the various biochemical, molecular and histo-cytopathological biomarkers are used. In contrast to the commonly used biochemical and molecular biomarkers of pollutant exposure or effect, which can still be recovered or repaired at the molecular level (Authman 2015; Gaber *et al.* 2014; Yeganeh *et al.* 2016), the histo-pathological alterations represent more reliable indicators of specific influences of pollutants on aquatic organisms (Bernet *et al.* 1999). Since pollution-induced histo-pathological changes in organs and tissues frequently occur before producing irreversible effects on the biota, the histological methods are considered to address

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those sensitive and early warning signs of pollution. These changes are then used in evaluating the potential risk for the species survival, as well as the environmental hazards (Gaber *et al.* 2014).

The demand for grouper significantly increases each year. Currently, demand from among Asian markets, including the ASEAN countries, is fairly high particularly in Hong Kong, China, Singapore, Taiwan and Japan. The grouper (*Epinephelus* sp.) is a marine fish species that many people commercially cultivate, for hatchery and rearing, and showing such promising prospect.

Grouper is a much desired part of the menu because it tastes good, contains 168 kcal, 32.4% protein, 1.2% fat, mineral calcium, phosphor, iron, vitamins A and B1, per kilograms and it commands a fairly high price. As of March 2017, the Cantang grouper, which is a hybrid of tiger grouper (*E. fuscoguttatus*) and giant groupers (*E. lanceolatus*), sells at around Rp 100,000 to Rp 120,000 per kg from farmers in Pangandaran, Indonesia. This high price accounts for the seed price, feed cost and the long growing period of 6 months due to its relatively low daily growth rate (DGR).

Papain contains a lot of proteolytic enzyme (protein decomposer). Hence, the dried papaya powder (papain) is widely used by industrial entrepreneurs to many kinds of product for its ability to catalyze polypeptide chain breakdown reaction by hydrolyzing its peptide bond into simpler compounds, such as dipeptide and amino acid (Winarno 1983). Pure papain is highly priced at US$35-US$170 per kilograms. Thus, people make papain from fresh raw simplicia papaya and apply it to fish feed.

The application of simplicia papaya can increase the protease content in the juvenile tilapia's (*Oreochromis niloticus*) intestine and as a result it can improve the Protein Efficiency Ratio (PER) by 2.13% and growth rate up to 2.67% (Rostika 2017). Cell necrosis is a type of irreversible cell death occurring when there is a heavy or elongated injury until such time that the cell can neither adapt nor repair itself (Ariestyawati 2009). A goblet cell is a single cell existing in the intestine epithelial lamina, located in between the cup-shaped single-layered, cylindrical epithelial cells which, narrows down in its lower part but expands in the upper part (Sugiyanto 2016). Sometimes, its form resembles that of a tulip or bell (Andini *et al.* 2017). In fish, the distribution of goblet cells varies according to the variety of incoming food stimulants. The epithelial and goblet cells respond to both the types and consistency of these incoming food causing the difference in goblet cell distribution as well as its type and number in a tissue or organ. The calculated result of the number of goblet cells in proximal, medial, distal intestines indicates the different number of goblet cells that tends to increase towards the caudal direction (Andini *et al.* 2017).

The addition of simplicia papaya to feeds increased the protease enzyme content in tilapia's intestine. Thus, improving the feed protein absorption/assimilation for fish growth (Fadli *et al.* 2013). Moreover, the addition of simplicia papaya had hydrolyzed the protein of soybean meal flour in artificial feed and has significantly influenced the Feed Efficiency (FE), Protein Efficiency Ratio (PER), Relative Growth Rate (RGR), and the Survival Rate (SR) of tilapia seeds (Agustian *et al.* 2013). The purpose of this study was to determine the effect of simplicia papaya on the histological growth of the grouper *Epinephelus lanceolatus fuscoguttatus*.

MATERIALS AND METHODS

This research was carried out in April to December 2017, at the Aquaculture Laboratory of Fisheries and Marine Sciences Faculty. The fishes (*Epinephelus lanceolatus fuscoguttatus*) were cultivated at the floating net cage in Pangandaran Regency, Indonesia. The enzyme activity test was conducted at the Chemical Organic Laboratory of Mathematics and Natural Sciences Faculty and the histological test of the groupers' intestines was done at the Biosystem Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Universitas Padjadjaran, Indonesia.

The simplicia papaya extract was prepared using the blender and grater. The young papaya was pulverized, dried and smoothened using Disc Mill and Ball Mill, then filtered using a steel sieve with mesh size 60. The fishes were cultivated in a floating net cage.

For the histological analysis, the equipment used included digital microscope, microtomes, and surgical instruments. The intestine was observed under a microscope with a magnification of 10x10, and 40x10 lenses and the necrotic and goblet cells were recorded. Image capture or documentation was done using optical lenses.

The experiment was done for 60 days using the Completely Random Design (CRD) consisting of 5 treatments and 3 replications. Fifteen floating nets were prepared to keep the groupers at a density distribution of 50 fishes/net. The treatments included Treatments A, B, C, D, and E (the feed at 5%, 3.75%, 2.5%, 1.25% and 0% simplicia papaya). The fish seeds from Situbondo Marine Fish Seed Center were randomly poured into the 15 floating nets, were given pellet feed for 2 weeks with a 3% feeding rate and 6% trash fish as feed additive.

The absolute weight gain was calculated using the formula of Gause and Trushenski (2011):

Absolute weight gain = [(final weight − initial weight)/initial weight] × 100

Protein Efficiency Ratio (PER) was calculated as follows (Gao *et al.* 2010):

Protein efficiency ratio = fish wet weight gain/ protein intake

The data were analyzed using F test analysis of variance (ANOVA) at a test interval of 5%. Followed by Duncan's multiple range test when a significant difference was found. The number of necrotic and goblet cells were recorded and the absolute weight gain and protein efficiency ratio were computed.

RESULTS AND DISCUSSION

Necrosis is a cell death that results from an acute or traumatic cell due to oxygen deficiency, extreme temperature changes, and mechanical injury. This cell death occurs in a way that cannot be controlled and may cause damage to tissues. The number of necrotic cells observed inside the grouper intestine are presented in Table 1.

Table 1 Number of necrotic cells in the grouper's intestine

Dosage of simplicia papaya	Number of necrosis
A (simplicia papaya 5.00%)	183c
B (simplicia papaya 3.75%)	169c
C (simplicia papaya 2.50%)	204a
D (simplicia papaya 1.25%)	217a
E (simplicia papaya 0.00%)	195b

The necrotic cells in the intestines of groupers applied with treatments A and B are significantly fewer than those in other treatments, indicating that these groupers' health is better than others. It can be said that the exogenous enzyme given to these groupers improved the fish's health, hence their necrotic cells are fewer than those in other treatments.

Too intense stimulus (low oxygen etc.) lasting for a long time and exceeding the cell's adaptive capacity had caused the cells to die as the cells could no longer compensate for the demand for change. The group of cells undergoing death can be determined from the existence of lysis enzymes that dissolved the various cell elements and from the presence of inflamed cells. Leukocyte will help digest the dead cells and then morphological changes begin. Necrosis is generally caused by pathological stimulus. However, cell death can also occur through a programmed cell death mechanism where after reaching a certain lifetime the cell will naturally die (Andini *et al.* 2017).

The mucus substance is a carbohydrate component found in the form of polysaccharides, glycoprotein and proteoglycan, and glycolipid (Kiernan 1990). In fish, the distribution and number of goblet cells are varied (Andini *et. al.* 2017) as shown in the histology of the grouper's intestine in each treatment (Fig. 1).

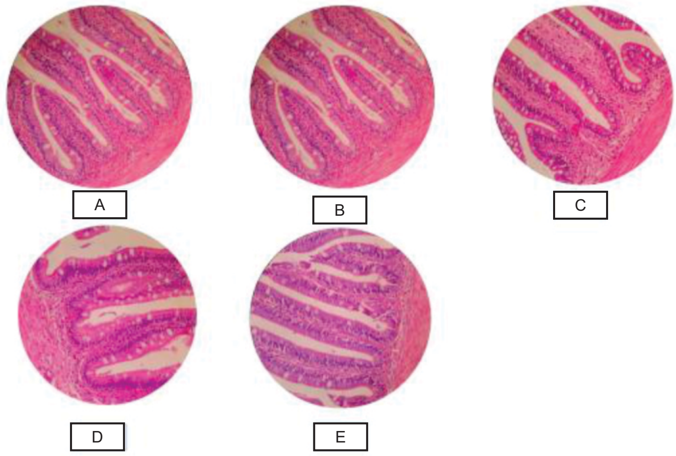

Figure 1 Goblet cells inside the grouper's intestines (the intestinal cell condition with: A. the effect of 5.00% simplicia papaya, B. the effect of 3.75% simplicia papaya, C. the effect of 2.50% simplicia papaya, D. the effect of 1.25% simplicia papaya, E. the effect of 0% simplicia papaya)

The variation of incoming food stimulates the epithelial and goblet cells to respond to the type and consistency of the incoming food resulting in the differences in distribution, type and number of goblet cells in a tissue or organ. The grouper feed was given with simplicia papaya at different dosages.

Grouper Absolute Weight

Growth is expressed by the change in total body energy content at a certain period of time. Growth occurs when there is a remaining free energy after the energy available in the feed is used for standard metabolism, digestive processes and activities.

Table 2 Absolute weight of grouper per treatment after 60 days

Dosage of simplicia papaya	Grouper gain (g)
A (simplicia papaya 5.00%)	161.36a
B (simplicia papaya 3.75%)	152.19a
C (simplicia papaya 2.50%)	152.09a
D (simplicia papaya 1.25%)	136.70b
E (simplicia papaya 0%)	129.03b

The addition of 2.5% to 5% simplicia papaya produced the best gain that is significantly higher than the rest of the treatment (152.09-161.36 g) (Table 2). While the addition of simplicia papaya dosage that is lower than 2.5% has gained only 129.03-136.70 g. In other studies (Fadli *et al.* 2013), the addition of papain enzyme to the feed by 5% has produced the highest average growth rate, i.e. 3.24%/day in tiger grouper (*Epinephelus fuscoguttatus*). Moreover, the optimum dose for adding papain to artificial feed in shark catfish's specific growth rate is 1.16 g for 100 g feed (Ananda *et al.* 2015).

Protein Efficiency Ratio

Protein Efficiency Ratio (PER) is used to determine the use of protein by comparing the obtained weight with the protein consumption; the higher the ratio value the more efficient the feed protein use is (Yuwono & Sukardi 2008).

Tabel 3 Protein Efficiency Ratio of Grouper after 60 days

Dosage of simplicia papaya	Protein Efficiency Ratio (%)
A (simplicia papaya 5.00%)	3.18a
B (simplicia papaya 3.75%)	3.19a
C (simplicia papaya 2.50%)	3.33b
D (simplicia papaya 1.25%)	3.40b
E (simplicia papaya 0%)	3.50c

The addition of papain crude enzyme extracts as exogenous enzyme to feeds done at various dosages had consequently produced various PER values. The highest PER values were observed in treatments A and B at 3.18% and 3.19%, respectively and the lowest was observed in treatment E at 3.50% (Table 3).

In Sugiyanto's (2016) research on tilapia, added with a 3.75% simplicia papaya, the PER value was 2.13%. Moreover, Amalia *et al.* (2013) reported that when 2.25% papain enzyme was added, the PER value was 1.97% for African catfish (*Clarias gariepinus*). The PER difference in each of these test fish is probably due to the protease enzyme at various doses inside the fish's intestine.

A study on the digestive enzyme in tilapia showed that all digestive enzymes, including a protease enzyme, have been developed in the fish seed's intestine (Tengjaroenkul *et al.* 2000 in Sugiyanto 2016). The addition of exogenous enzyme papain to the feed has led to the increased and faster digestion in the intestine to break the feed protein into amino acid. The protein quality of a feed is determined from how much of this protein can be digested or absorbed by the body (Muchtadi 1989). An easily digestible protein indicates the great amount of an amino acid which can be absorbed and used by the body, because most will be disposed of by the body together with feces. In other words, the greater the amount of protein which can be hydrolyzed into amino acid, the greater would be the amount of amino acid which could be absorbed and used by the fish's body.

The hydrolysis of papain in fish protein powder using the G-50 bed, works in a way that most of the hydrolyzed peptides are in the fractionation of various columns (1500-30000 Dalton) (Himonides *et al.* 2011). After 20 minutes of hydrolysis only a small portion of the solute is completely removed from the column, and after further hydrolysis (120 minutes), what remains is a smaller molecule with complete elimination.

CONCLUSION

The addition of simplicia papaya at 3.75% and 5% of the grouper's artificial feed has influenced cell necrosis resulting in a population of 169 and 183, respectively. The addition of simplicia papaya at 5%, 3.75% and 2.5% to the *Epinephelus lanceolatus fuscoguttatus* artificial feed has increased the absolute gain by 161.36, 152.19 and 152.09 grams, respectively. The addition of simplicia papaya at 5% and 3.75% of the grouper's artificial feed has increased the protein efficiency ratio by 3.18% and 3.19%.

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