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THE HISTOLOGICAL DESCRIPTION OF INTESTINE OF THE *Epinephelus lanceolatus fuscogutattus* ADMINISTERED WITH SIMPLICIA PAPAYA**

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Running title: Histological description of grouper

ABSTRACT

Fish intestine and growth histological descriptions constitute the parameters which are a function of internal and external condition. The external factors include water quality, feed quality and feed quantity. Currently, grouper's growth is quite low, when in fact the protein content of its feed is high. The purpose of this study was to determine the effect of simplicia papaya on histological description that affects the growth *Epinephelus lanceolatus fuscogutattus*. Grouper's absolute weight and grouper's protein efficiency ratio which is fed with Simplicia papaya-added feed. The research is conducted using Completely Randomized Design with 5 treatments and 3 repetitions (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 grouper cultivation is done in a Floating Net Cage in Pangandaran Regency. The addition of simplicia papaya at 3.75% and 55 to the grouper's artificial feed has some influence in number of necrosis cell of 169 and 183, and the number of goblet cell too. The addition of simplicia papaya at 5%, 3.75% and 2.5% of the grouper's artificial feed has some increasing absolute gain by 161.36 grams, 152.19 grams, 152.09 grams. The addition of simplicia papaya at 5%, and 3.75% of the grouper's artificial feed has some increasing in protein efficiency ratio by 3.18% and 3.19% respecttively.

Keywords: absolute gain, Epinephelus lanceolatus fuscogutattus, 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 their close contact with the environment, fish homeostatic mechanisms are highly dependent on existing conditions in their immediate surrounding, so even slight variations in water quality can cause a wide variety of biological responses (Authman, 2015; Nussey et al., 1995). For the evaluation of the impact of environmental contaminants on biota, various biochemical, molecular and histocytopathological biomarkers are usually applied. In contrast to commonly used biochemical and molecular biomarkers of pollutant

exposure or effect, which still might be recovered or repaired at the molecular level (Authman, 2015; Gaber et al., 2014; Yeganeh et al., 2016), histopathological alterations represent more reliable indicators of specific influences of pollutants on aquatic organisms (Bernet et al., 1999). Since pollution caused histopathological changes in organs and tissues frequently occur before producing irreversible effects on the biota, histological methods are considered as a sensitive and early warning signs of pollution and therefore, have the advantage to be used in evaluation of potential risk for the species survival, as well as for the environmental protection (Gaber et al., 2014).

The demand for grouper shows a significant increase each year. Currently, demand for grouper in Asian markets, including ASEAN countries, is fairly high particularly in Hong Kong, China, Singapore, Taiwan and Japan. Grouper (*Epinephelus* sp) is a commercial sea fish species many people begin to cultivate, for both its hatchery and enlargement for such reason as its sound promising prospect.

Much like grouper because it tastes good, contains 168 kcal, 32.4% protein, 1.2% fat, mineral calcium, phosphor, iron, vitamins A and B1, and its sales price is fairly high. For Cantang grouper which is a hybrid of tiger (*E. fuscoguttatus*) and giant groupers (*E. lanceolatus*), it sells to around Rp100,000.- to Rp120,000.- per kg from farmers in Pangandaran in March 2017. This high price is a result of its seed price, feed cost and the long period it takes to grow it (6 months) thanks 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 dipeptide and amino acid (Winarno, 1983). Pure papain is highly costly at US$35-US$170. Hence people make simplicia papaya which is made of raw fresh papaya simplicia and apply it to fish feed.

Based on research of Rostika (2017) the application of simplicia papaya can increase the protease content in tilapia's (Oreochromis niloticus) juvenile intestine and as a result it can improve Protein Efficiency Ratio by 2.13% and Growth Rate up to 2.67%. Necrosis cell is a type of irreversible cell death occurring when there is a heavy or elongated injury until at one time the cell can neither adapt nor repair itself (Ariestyawati, 2009). Meanwhile, a goblet (Sugiyanto, 2016) cell is a single cell existing in the intestine epithelial lamina, located in between single-layered, cylindrical epithelial cells which take a cup form, narrowing in its lower part already expanding in its upper part. Sometimes, its form resembles that of a tulip or bell (Andini et. al., 2017). In fish, the distribution of goblet cells is varied. The variations of incoming food stimulate epithelial and goblet cells to give some response to both the types and consistence of these incoming food. This is what cause the difference in goblet cell distribution as well as its type and number in a tissue or organ.

The calculation result of the number of goblet cells in proximal, medial, distal intestines indicates the different number of goblet cells. The number of goblet cells seems to be increasing towards caudal direction (Andini et. al., 2017).

Fadli et. al. (2013) shows that the addition of simplicia papaya to feed increases the protease enzyme content in tilapia's intestine. The addition of simplicia papaya to feed can improve the feed protein deposition into body for fish growth. Sari et. al. (2016) suggests that the addition of simplicia papaya can hydrolyze the protein of soybean meal flour in artificial feed and has significant influences on Feed Efficiency (FE), Protein Efficiency Ratio (PER), and Relative Growth Rate (RGR), and insignificant influence on Survival Rate (SR) of tilapia seeds. The purpose of this study was to determine the effect of simplicia papaya on histological description that affects the growth *Epinephelus lanceolatus fuscogutattus*.

MATERIALS AND METHODS

This research was carried out from April 2017 to December 2017, in Aquaculture Laboratory of Fisheries and Marine Sciences Faculty. Fish (*Epinephelus lanceolatus fuscogutatus*) cultivation is done in the Floating Net Cage in Pangandaran Regency. The enzyme activity test is conducted in Chemical Organic Laboratory of Mathematics and Natural Sciences Faculty. Finally, the histological test in the intestine grouper organ is done in Biosystem Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Universitas Padjadjaran.

The equipment used to make simplicia papaya 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 on Scretting brand, stationery, calculator and so forth. Tools to for histological analysis include digital microscope, microtome, surgical instruments, fixative solution, ringer solution and others. Simplicia papaya is obtained from pulverizing young papaya, drying and smoothing using Disc Mill and Ball Mill, then filtered using steel sieve with mesh number size 60.

The method used in this research is the experimental one with Completely Random Design (CRD) consisting of 5 treatments and 3 repetitions, for 60 days. 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 at 5%, 3.75%, 2.5%, 1.25%, and 0% simplicia papaya). Fish seed from Situbondo Marine Fish Seed Center was placed randomly at 15 floating net, given pellet feed, for 2 weeks with a 3% feeding rate and then gave trash fish 6 %.

Observation of histological intestine was taken in a microscope, with a magnification of 10x10, and 40x10 lenses. Image capture or documentation has to be done using optical lenses.

Histological preparations of intestine grouper were noted. In the intestine, the observed parts are necrosis, and goblet cell. Simplicia papaya was made.

Absolut weight gain calculated with formula as follows (Gause & Trushenski 2014):

$$\text{Absolut weight gain} = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100.$$

Protein Efficiency Ratio (PER) calculated with formula as follows (Gao et. al. 2010):

$$\text{Protein efficiency ratio} = \text{fish wet weight gain/protein intake}$$

The influence of each treatment is tested using F test analysis of variance (ANOVA) at a test interval of 5%. When a significant difference is found, it is then followed by Duncan's multiple range test. The observation parameters are analysis of Fish Intestinal Tract (necrosis and number of goblet cell), absolute weight gain and protein efficiency ratio.

RESULTS AND DISCUSSION

Necrosis is a cell death as a result of acute or trauma cell (such as: oxygen deficiency, extreme temperature change, and mechanical injury), where this cell death occurs in a way that cannot be controlled which may cause damage to cells. In this research, the number of necrosis cell under observation in the grouper intestine preparation is as follows (Table 1).

Table 1 Number of Necrosis Cell in Grouper's Intestine during Research

Dose 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%)	195b

Necrosis cells in the intestines of groupers given with treatments A and B are significantly fewer than those in other treatments. This is because these groupers' health is better than others. It can be said that the exogenous enzyme given to these groupers delivers a good result for the fish health, hence their necrosis 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 will cause the cells to die where the cells can no longer compensate the demand for change. A group of cells undergoing death can be determined from the existence of lysis enzymes which dissolve various elements of cells as well as inflammation. Leukocyte will help digest the dead cells and then morphological changes begin. Necrosis is generally caused by pathological stimulus. In addition to pathological stimulus, cell death can also occur through a

programmed cell death mechanism where after reaching certain lifetime the cell will die (Andini et. al., 2017).

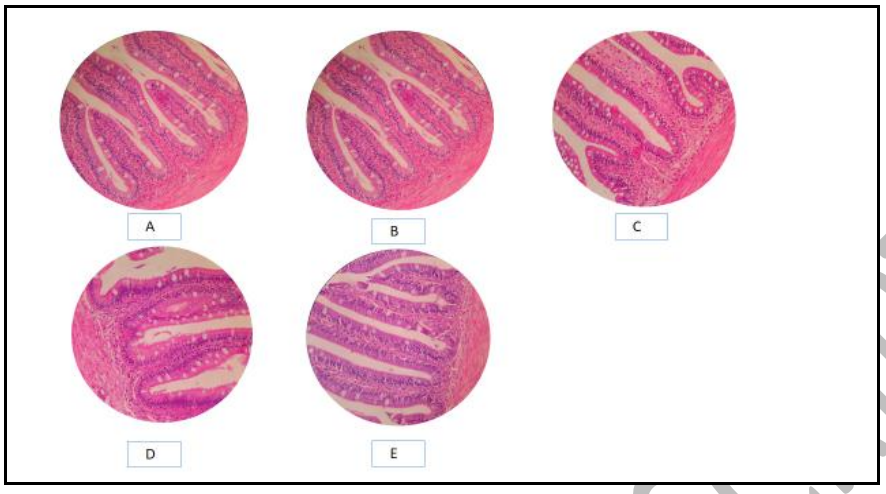

Figure 1 Goblet Cell in Intestine's Grouper

Figure A shows the intestine cell condition with the effect of 5.00% simplicia papaya, B shows the effect of 3.75% simplicia papaya, C shows the effect of 2.50% papaya simplicia, D shows the effect of 1.25% simplicia papaya, E shows the effect of 0% simplicia papaya)

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 depicted in the following figure which shows the histology of grouper intestine in each treatment (Figure 1).

The variation of incoming food gives some stimulus to epithelial and goblet cells to respond to the type and consistency of those incoming food. This can lead to the different distribution, type and number of goblet cells in a tissue or organ. The grouper feed gave has been applied with simplicia papaya at different doses.

Grouper Absolute Weight

Energetically, growth is expressed by the change in total body energy content at 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, digestion process and activities. The absolute weight in this research is listed in the table 2 below.

Table 2 Absolute Weight of Grouper in every treatment during 60 days

Dose of simplicia papaya	Grouper Gain (gram)
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 papaya simplicia as much as 2.5 to 5% gives the best gain that is significantly different (152.09 - 161.36 grams). While addition of simplicia papaya dose is lower than 2.5% the gain is only 129.03 grams - 136.70 grams). Fadli et. al (2015) research finds that the treatment of adding papain enzyme to feed by 5% can produce the highest average Growth Rate, i.e. 3.24%/day in tiger grouper (Epinephelus fuscugutattus). Meanwhile, Ananda's (2013) research finds that the optimum dose for adding papain to artificial feed in shark catfish's specific growth rate is 1.16 gram for 100 grams feed.

Protein Efficiency Ratio

Protein Efficiency Ratio (PER) is used to find out the use of protein by comparing the obtained weight and 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 During 60 days

Dose 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 combination as exogenous enzyme to feeding is done at varied doses and they produce varied PER values. The highest PER values are found in treatments A and B at 3.18% and 3.19% respectively and the lowest PER value is found in treatment E at 3.50% (Table 3).

Based on the results of Sugianto's (2016) research, the PER value when 3.75% simplicia papaya is added is 2.13% in tilapia. Meanwhile, Amalia et al. (2013) report that the PER value when 2.25% papain enzyme is added is 1.97% for African catfish (*Clarias gariepinus*). The PER value difference in each of these test fish is because the fish intestine there has been protease enzyme at varied doses.

Tengjaroenkul et. al. (2000) statement in Sugianto (2016) on digestive enzyme development in tilapia which shows that all digestive enzymes, including a protease enzyme, have been in the fish seed's intestine. The addition of exogenous enzyme papain to feed lead to the increased and faster digestion process in the intestine to break feed protein into amino acid. According to

Muchtadi (1989) for one to determine the protein quality of a feed, he/she can see it from how much of this protein can be digested or absorbed by the body. 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 the amount of amino acid which could be absorbed and used by the fish body would be.

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

CONCLUSION

The addition of simplicia papaya at 3.75% and 55 of the grouper's artificial feed has some influence in number of necrosis cell of 169 and 183. The addition of simplicia papaya at 5%, 3.75% and 2.5% to the *Epinephelus lanceolatus fuscogutattus* artificial feed has some increasing absolute gain by 161.36 grams, 152.19 grams, 152.09 grams. The addition of simplicia papaya at 5%, and 3.75% of the gouper's artificial feed has some increasing in protein efficiency ratio by 3.18% and 3.19%.

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REFERENCES

Ananda, T., D. Rachmawati, and I. Samidjan. 2015. The Effect of Papain on Artificial Feed on Patin Fish Growth. (*Pangasius hypopthalmus*). Journal of Aquaculture Management and Technology. 4 (1): 47-53.

Andini, D., Zainuddin, M Jalaluddin, Fitriani, U. Balqis, N. Asmilia, Hamdan. 2017. Goblet Cell Distribution on Local Catfish Intestine (*Clarias Batrachus*). JIMVET. 01(3): 299-304 (2017) ISSN : 2540-9492

Ariestyawati, P. P. 2009. Effect of Frequency of Feeding on Siamese Patin Fish Seed Growth (*Pangasianodon hypophtalmus*). Thesis. Faculty of Fisheries and Marine Sciences. Universitas Padjadjaran. Jatinangor.

Authman, M.M., 2015. Use of fish as bio-indicator of the effects of heavy metals pollution. J. Aquac. Res. Dev. 6. https://doi.org/10.4172/2155-9546.1000328

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22:25–34. https:// doi.org/10.1046/j.1365-2761.1999.00134.x.

Fadli, J., Sunaryo, and A. Djunaedi. 2013. Giving Papain Enzyme to Commercial Feed on Growth and Efficiency of Tiger Grouper Feed (*Epinephelus fuscogutattus*). Journal of Marine Research, 2 (3): 50-57

Gaber, H.S., Abbas, W.T., Authman, M.M.N., Gaber, S.A., 2014. Histological and biochemical studies on some organs of two fish species in Bardawil Lagoon, North Sinai, Egypt. Glob. Vet. 12:1–11. https://doi.org/10.5829/idosi.gv.2014.12.01.76199.

Gao, W., Liu, Y. J., Tian, L. X., Mai, K. S., Liang, G. Y., Yang, H. J., ... & Luo, W. J. (2010). Effect of dietary carbohydrate to lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition, *16*(3): 327-333.Himonides, A.T., A. K. D. Taylor. A. J. Morris. 2011. A Study of the Enzymatic Hydrolysis of Fish Frames Using Model Systems Food and Nutrition Sciences, http://www.SciRP.org/journal/fns. 575-585. doi:10.4236/fns.2011.26081

Gause, B., & Trushenski, J. (2011). Replacement of fish meal with ethanol yeast in the diets of sunshine bass. North American Journal of Aquaculture, *73*(2): 97-103.

Himonides, A.T., A. K. D. Taylor. A. J. Morris. 2011. A Study of the Enzymatic Hydrolysis of Fish Frames Using Model Systems Food and Nutrition Sciences, http://www.SciRP.org/journal/fns. 575-585. doi:10.4236/fns.2011.26081

Kiernan JA. 1990. Histological & Histochemical Methods : Theory & Practice. Second Edition. Pergamon Press. 330-354

Lucas, J.F., D. Castañeda, D. Hormigo. 2017. New trends for a classical enzyme: Papain, a biotechnological success story in the food industry. Trends in Food Science & Technology. Volume 68, Pages 91-101.

Mo, W.Y. , R.S.S.Lau, A.C.K.Kwok, M.H.Wong. 2016. Use of soybean meal and papain to partially replace animal protein for culturing three marine fish species: Fish growth and water quality. Environmental Pollution Volume 219, Pages 815-820

Muchtadi, D. 1989. Evaluation of Food Nutrition Value. Centre of Food University. IPB. Bogor.

Nussey, G., Van Vuren, J.H.J., Du Preez, H.H., 1995. Effect of copper on the differential white blood cell counts of the Mozambique tilapia (*Oreochromis mossambicus*). Biochwz. Physiol. Biochem. Physiol. IIlC 11:381–388. https://doi.org/10.1016/0742- 8413(95)00064-X

Rostika, R., Sunarto, Sugiyanto, H.N., Dewanti, L.P. 2018. Papain-Crude Enzyme on Nile Feed Efectivity which Cultured at Floating Net Cage Cirata. IOP Conference Series Earth and Environmental Science. The 2nd International Symposium on Marine and Fisheries Research. 139.

Sugiyanto, H. N. 2016. Effectiveness of Papain Rough Enzyme Extract on the Growth of Tilapia (Oreochromis niloticus) in the Floating Net Cages of Cirata Reservoir. Faculty of Fisheries and Marine Sciences. Universitas Padjadjaran. Jatinangor.

Sari, W. A. P., Subandiyono, and Sri Hastuti. 2013. Giving Papain Enzyme to Increase the Utilization of Feed Protein and Growth of Larasati Tilapia Seeds (*Oreochromis niloticus Var.*). Journal of Aquaculture Management and Technology. 2 (1): 1-12.

298 Tengjaroenkul, B., BJ. Smith., T. Caceci and SA. Smith. 2000. Distribution of intestinal enzyme
299 activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L.
300 Aquaculture 182: 317-327.

301 Yeganeh, S., Adel, M., Ahmadvand, S., Ahmadvand, S., Velisek, J., 2016. Toxicity of organic
302 selenium (Selemax) and its effects on haematological and biochemical parameters and
303 histopathological changes of common carp (*Cyprinus carpio* L., 1758). Toxin Rev. 35.
304 https://doi.org/10.1080/15569543.2016.1213749

305 Yuwono, E and Sukardi, P. 2008. Water Animal Physiology. Puwokerto: Unsoed Press.

306 Zhang J. 2016a. Coinfection with type 1 and type 2 PRRSV. Vet Rec 178(12):288-90.

307 Zhang J. 2016b. Porcine deltacoronavirus: Overview of infection dynamics, diagnostic methods,
308 prevalence and genetic evolution. Virus Res 226:71-84.

309 Zimmerli, S., Bernet, D., Burkhardt-Holm, P., Schmidt-Posthaus, H., Vonlanthen, P., Wahli, T.,
310 Segner, H., 2007. Assessment of fish health status in four Swiss rivers showing a decline of
311 brown trout catches. Aquat. Sci. 69:11–25. https://doi.org/10.1007/ s00027-006-0844-3.