

Engineering of Papaya *Simplicia* Powder in different size for Grouper Fish (*Epinephelus fuscoguttatus lanceolatus*) feed

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Abstract: Fish growth descriptions constitute the parameters which are influenced by internal and external condition. The external factors include water quality, feed quality and feed quantity. Feed quality including the simplicia papaya particle size which contains protease enzyme and saponin as additional ingredients for grouper feed. Currently, grouper's growth is relatively low, when in fact the protein content of its feed is high. This research aims at obtaining data on character particle of papaya simplicia powder and the grouper's absolute weight and grouper's protein efficiency ratio which is fed with simplicia papaya powder. The research is conducted using Completely Randomized Design with 5 treatments and 3 repetitions (A: 60 μ m simplicia papaya 3.5% application, B: 100 nm simplicia papaya 3.5% application, C: 60 μ m simplicia papaya 2% application, D: 100 nm simplicia papaya 2% application, and E: 0% without simplicia papaya). SPLICIA papaya powder was made from dried papaya, then mashed using disc mill and filtered with sieves measuring 60 μ m and 100nm and the grouper cultivation is done in a Floating Net Cage in Pangandaran Regency. The addition of papaya simplicia at A and B to the grouper's feed has some highest average daily gain by 3.70 % and 3.73 %. The characteristics of simplisia papaya powder size 60 μ m and 100 nm do not have a different effect on the growth performance of grouper seeds.

Keywords: absolute gain, average daily gain, grouper, particle size, simplicia papaya powder

1. Introduction

The demand for grouper shows a significant increase each year. Currently, the demand for grouper in Asia markets, including ASEAN countries, is fairly high particularly in Hong Kong, China, Singapore, Taiwan and Japan. Grouper (*Epinephelus sp*) is a commercial marine fish species many people begin to cultivate, for both its hatchery and enlargement for such reason as its sound promising prospect. Cantang grouper which is a hybrid of tiger and giant groupers, it sells at 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 [1].

Fish growth descriptions constitute the parameters which are influenced by internal and external condition. The internal factors are kind of fish, feed and feeding habit of fish, age of fish, sexual of fish. The external factors include water quality, feed quality and feed quantity. Feed quality including the simplicia papaya particle size which contains protease enzyme and saponin as additional ingredients for grouper feed. Currently, grouper's growth is relatively low, when in fact the protein content of its feed is high.

Papain contains a lot of proteolytic enzyme (protein decomposer), hence the dried unripe 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 [2]. The pure papain is highly costly at US\$ 35-US\$ 170, hence people make SP which is made of raw fresh papaya and apply it to fish feed.

Based on the research of Rostika [1] 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%. The use of nanoparticles as medicine carriers and medicine delivery system has developed in the last few years. The tiny size of nanoparticles allows extract to be easily soluble and to have high absorbance efficiency in the intestines [3]. Micro-sized papaya simplicia has been experimented in *Cantang* grouper fish (*Epinephelus fuscoguttatus lanceolatus*) [1]. The addition of SP at 3.75% to the grouper's artificial feed has some influence on the number of necrosis cells by 169, increasing its absolute gain by 152.19 grams and increasing the protein efficiency ratio by 3.19%.

According to Poulain [3] the porosity measurement shows that the surface area of powder increases with the milling time and it reaches its peak when it is milled for 18 hours. For this reason, this research tries to experiment this powder at 60 μm and 100 nm for *Cantang* grouper fish in sea KJA and its growth response is observed. This research aims at obtaining data on powder character particle of simplicia papaya and the grouper's absolute weight, protein efficiency ratio which is fed with simplicia papaya powder.

2. Materials and Methods

This research is conducted from April 2018 to November 2018, 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.

2.1. Material and method for Making Simplicia papaya powder (SP).

Unripe papaya (*Carica papaya*), mashed with crusher machine then dried. Used disc mill, dried papaya crumbled and then filtered with sieves measuring 60 μm and 100nm. To measure dispersion distribution, we use tornado dry system.

2.2. Material for Culturing The Fish

The tools for cultivating the fish include boat, floating net cage, scales, landing net, fish feed of Japanese brand, trash fish, 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 Completely Random Design (CRD) 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 size of the fish used is grouper with a size of 64-66 grams which is given 4% of trash fish feed from the weight of the fish. which feed every 2 times a day. The treatments in this research are Treatments A, B, C, D, and E (A: 60 μm simplicia papaya 3.5% application, B: 100 nm simplicia papaya 3.5% application, C: 60 μm simplicia papaya 2% application, D: 100 nm simplicia papaya 2% application, and E: 0% without simplicia papaya). The descriptive observation is made on Mean (μm), Median (μm), Mean/Median Ratio, Mode (μm), Deviation Standard (μm), Variance (μm^{-2}), C.V (%) and skewness of simplicia papaya powder. The observation parameters are the fish absolute weight gain and Average Daily Gain. 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.

3. Results and Discussion

Based on the research results on SP particles which are observed physically, the data in Table 1 shows their characters, the mean size of microparticles is 265.7 micron and the that of nano particles is 95 micron, with each having a standard deviation of 1.034 and 1.115 microns. According to Mohanraj & Chen [4] nanoparticles have four strengths in their use as a means of medicine delivery. One of these strengths is that many body circulatory paths can use nanoparticle system. The method used to prepare SP has significant influence on the formation of SP nanoparticles.

Table 1. Physical Parameters of Micro and Nano Simplicia Papaya

	Mesh 60 (micro)	Mesh 100 (nano)
Volume (%)	100	100
Mean (μm)	265.7	95.21
Median (μm)	256.9	85.40
Mean/Median Ratio	1.034	1.115
Mode (μm)	269.2	127.7
Standard of Deviation (μm)	117.9	64.11
Varians(μm^2)	13893	4110
C.V (%)	44.4	67.3
Skewness	0,447 Right Skewed	0,637 Right Skewed
Kurtosis	1,024 Leptokurtic	-0,236 Platykurtic

According to Tiyafoonchai [5] the main objective of making nanoparticles as a medicine delivery system is to regulate the the particle size, surface nature, and release of active substances in specific place inside the body as the right medication target. In this research, the observation is made on the grouper's response to various dosage treatments and SP particle sizes. The dispersion distribution of SP micro- and nano-particles can be seen in Figures 1 and 2.

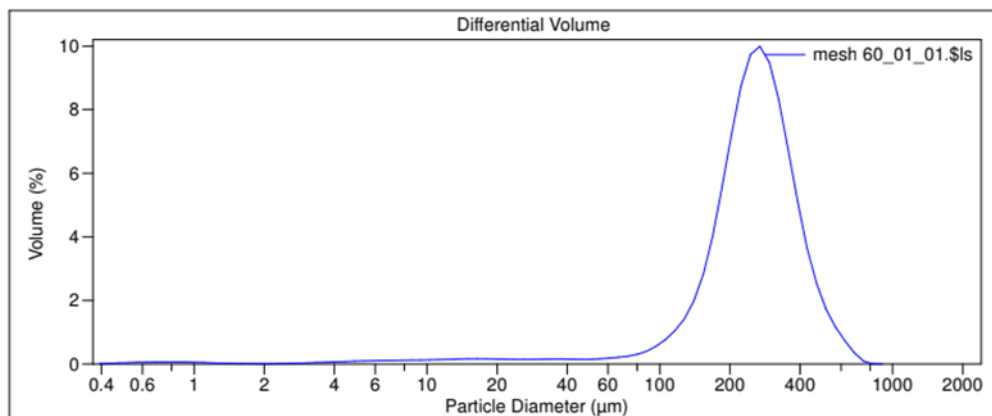


Figure 1. Dispersion distribution of papaya simplicia products that pass-through mesh size 60.

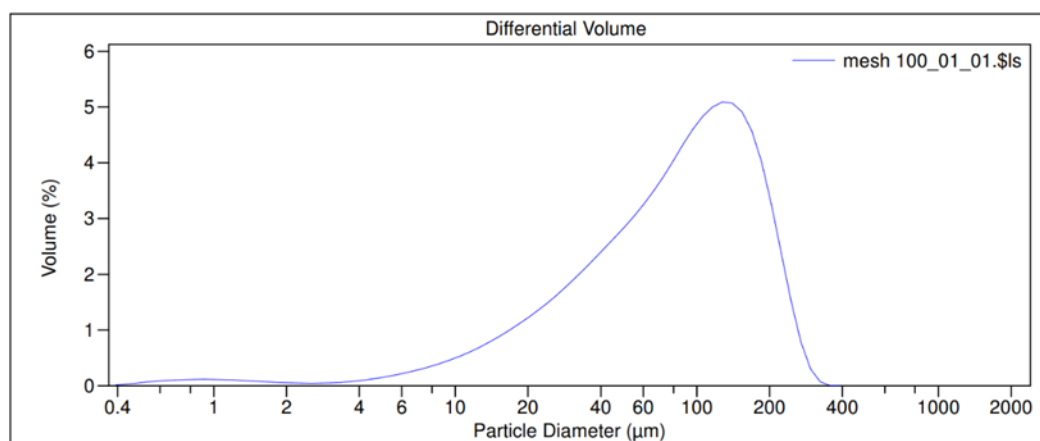


Figure 2. Dispersion distribution of papaya simplicia products that pass-through mesh size 100.

Grouper Fish Growth

The absolute weight gain is calculated from subtracting the final weight with initial weight during maintenance. The greater the body weight gain value, the better the fish in utilizing the feed given, thus the heavier the meat weight it produces [5]. The Average Daily Gain is calculated from dividing the Weight gain with the number of research days. The absolute requirement to be fulfilled for a good feeding is evenly distributed, in a sense that it can be procured that one individual fish receives the same portion of feed as other individuals, thus it can be expected that this even distribution of feeding will generate a uniform and optimal fish growth [6]. In the following table, the Average Absolut Gain and Average Daily Gain values of grouper fish during research are presented.

Table 2. Growth Performance of Grouper Fish Fry Culture

Treatment	Av. Initial weight (gr)	Av. Final weight (gr)	Av. Absolut Gain (gr)	Av. Daily Gain (gr/day)
A	66.67	148.11	81.44	2.04
B	64.22	149.00	84.78	2.12
C	65.56	134.33	68.77	1.72
D	64.11	128.67	64.56	1.61
E	65.56	120.66	55.10	1.38

From this table, it can be seen that at 3.5% Papaya simplicia dose, for both micro and nano sizes, the best outcome is obtained as compared to other treatments. This means that the particle size factor does not affect the growth of fish. This is possible because simplicia papaya contains natural enzyme, i.e. Papain, which is a protease which plays a role in digesting feed particularly protein. The protein from the pellet used contains 50% protein (*dry base*), and wet trash fish contains 76% water, 12.14% protein, and 1.9% fat (wet base). Fish protein consists of essential fatty acids lysine and methionine, as well as fatty acid omega 3 and 6 which are highly needed for fish growth.

This high protein level will be digested by grouper fish aided with digestive fluid and enzymes, including protease enzyme. The additional protease from SP which exists in the initial part of intestine [1] will help increase the feed's protein digestibility, thus the amino acid produced will be greater, as indicated by the increased body protein deposition.

The proteolytic activity of papain has been well described in the literature, including the degradation of elastin and proteoglycans [7]. According to Paul et al [8] papain possesses a very powerful digestive action, superior to pepsin and pancreatin. Papain is a single-chained polypeptide with three disulfide bridges and a sulfhydryl group that are highly essential for the activity of the enzyme. Papain is expressed as an inactive precursor, prepropapain.

Rostika et al's [10] research results indicate that adding SP at 3.75% dose gives a significant influence on Daily Growth Rate, Feed Efficiency of Cantang grouper fish. Fadli's [11] research on Tiger grouper fish gives a result of Daily Growth Rate at 3.24%/day in the administration of SP 5%. This condition shows that the Absolute Weight Gain and Average Daily Gain are better in grouper fish subjected to 3.5% dose treatment as found in the previous research [1]. The following figure is the bar chart.

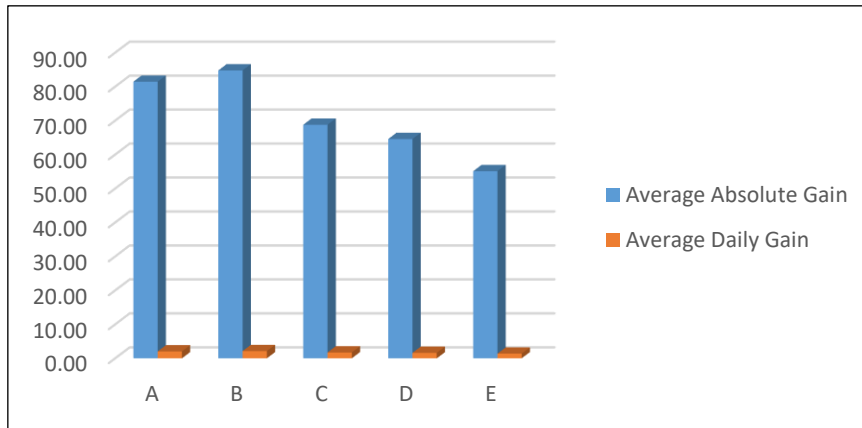


Figure 2. Average Absolute Gain (gr) and Average Daily Gain (gr/day) every treatment.

4. Conclusions

The addition of papaya simplicia at A and B to the grouper's feed has some highest average daily gain by 3.70 % and 3.73 %. The characteristics of simplisia papaya powder size 60 μm and 100 nm do not have a different effect on the growth performance of grouper seeds.

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