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INTESTINAL CONDITIONS OF SILVER POMPANO (*TRACHINOTUS BLOCHII*) GIVEN WITH FUNCTIONAL ENZYME FEED (PFE) AT FLOATING NET CAGE IN PANGANDARAN REGENCY

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East coast of Pangandaran, fish's intestinal histology, growth rate, papain enzyme, silver pompano, *Trachinotus blochii*.

ABSTRACT

This research aimed to examine the influence of papain enzyme in functional enzyme feed (PFE) at optimal dose for the highest intestinal histological conditions and growth rate of silver pompano (Trachinotus blochii) di Floating Net Cage, Pangandaran Regency. This research was conducted from September 2022 to December 2022 with 60-day raising period. This research used a Completely Randomized Design (RAL) with five treatments and 3 repetitions. The treatments were A (PFE with 5% papain enzyme content), B (PFE with 3.75% papain enzyme content), C (PFE with artificial feed with 2.5% papain enzyme content in PFE), D (artificial feed with 1.25% papain enzyme content in PFE), and E (control). The effects of each treatment were tested using an analysis of variance (ANOVA) F test at test interval 5%, and in case of significant difference this would be followed with Duncan's multiple range test. The parameters observed in this research were intestinal histological conditions (Length of villi (µm), Base Width of Villi (µm), Apical Width of Villi (µm) and Surface Size (µm2)) and Daily Growth Rate (LPH) of the fish. The intestinal histological conditions were: Length of Villi 588.2 µm, Base Width of Villi 220.2 µm, Apical Width of Villi 90.7 µm, and Surface Size 2049.9 μm^2, while the highest growth parameters in this research were with treatment A (PFE with 5% papain enzyme content) with LPH 3.25±0.112%.

Introduction

Functional feed means feed with special ingredient or content which may benefit the growth, health, environment and economy more than traditional feed, leading to big chance of assuring future aquaculture. Functional feed serves to improve fish's performance, productivity and health. Besides containing its composing feed, functional feed should also be equipped with specially chosen high level vegetable protein, complex carbohydrate and harmless probiotic bacteria such as Bacillus subtilis, probiotics and enzyme. This enzyme serves to strengthen digestive system and help organism assimilate nutrients.

Functional feed for fish is expected to give a better effect than common feed for it to support optimal growth. This research examined the extent of silver pompano's (*Trachinotus blochii*) growth rate fed with functional enzyme feed (PFE) at floating net cage in Pangandaran Regency. The enzyme added was derived from papaya which contained papain. It is commonly known that papain has the ability to break protein molecules. Papain enzyme's activities are sufficiently specific that it can only catalyse hydrolysis process well at certain pH and temperature range. Papain is endolytic plant cysteine containing isolated protease enzyme in papaya (*Carica pepaya* L.).

Papain enzyme as proteolytic enzyme is very important in many vital biological processes in all living organisms [1]. Papain shows broad proteolytic activities on protein, short-chain peptide, amino acid esters and amide links and is applied widely in the field of foods and drugs [2]. It specially cuts peptide bonds involving basic amino acids, particularly arginine, lysine and residue after phenylalanine [3]. As a parameter, fish's intestinal histology would be observed for information regarding fish's absorption efficiency and

effectiveness [4].

Research objective

This research aimed to examine the effects of papain enzyme application to functional enzyme feed (PFE) at optimal doze in order to produce the highest intestinal histological conditions and growth rate of silver pompano (Trachinotus blochii) at Floating Net Cage, Pangandaran Regency.

Parameters observed

Daily Growth Rate

Calculation of the daily growth rate uses the formula proposed by [5] the following:

$$G = \frac{lnWt - lnWo}{t} X\ 100\%$$

Information:

G = Daily growth rate (%)
Wt = Average weight of fish at the end of rearing (tail)
Wo = Average weight of fish at the beginning of rearing (tail)
t = maintenance time (days)

Intestinal Histology of Fish

Examination using the method according to [6] to the following formula:

$$LV = \frac{b + c}{c} \times a$$

Information:

LV = surface area of villi (µm²)
a = villi height (µm)
b = apical width of villi (µm)
c = basal width of villi (µm)

Histological preparations for the intestine of star silver pompano were made at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Result and discussion

Based on the observation results, using functional enzyme feed presented varied outcomes of silver pompano's growth. This use of functional enzyme feed gave good response for silver pompano's growth, as observable in the increase in silver pompano's average daily growth rate in each sampling (10 days). The silver pompano's average daily growth rate in each treatment increased along with the research as presented in Table 1.

Treatment	Average Daily Growth Rate
A	3,25±0,112 a
B	2,98±0,068 b
C	2,95±0,051 bc
D	2,92±0,064 bc
E	2,66±0,072 c

Note : Values followed by the same letter are not significantly different according to the range test of Duncan's multiple at a 95% confidence test

The fish's growth was influenced by internal and external factors. The internal factors included heredity, sex, and age, while the external factors included feed and water quality [5]. The daily growth rate served to calculate fish's growth percentage per day.

In this research, papain enzyme was expected to hydrolyze proteins contained in the feed, thus the feed given to the fish would be used efficiently and increase the daily growth rate. Silver pompano is carnivorous. They are equipped with pyloric caeca that are modification of fish intestine serving as a digestive organ. Therefore, the digestive process occurs quickly, contributing to optimizing intestinal work in food digestion process. In this case, adding exogenous enzyme is expected to improve intestine's working capacity to quicken the protein-in-feed hydrolysis process, thus the proteins broken down into amino acids will be more and also the more the amino acids absorbed by fish body.

This result conform to the research conducted by [7] on brown-marbled grouper that is a parent of cantang grouper, presenting daily growth rate 3.24%/day with treatment of 5% papain enzyme addition and with value not far different from this research. According

to [8], protein hydrolysis occurs with the help of proteolytic enzymes serving as catalyzer in cells. This protein hydrolysis is conducted by exogenous enzymes, one of which is papain enzyme. Added papain enzyme helps produce more amino acids, making the feed consumed by fish used more efficiently. In general, fish's digesting feed ability is influenced by some factors, including water's chemical properties, water temperature, type of feed, fish's size and age, nutritional content in feed, feeding frequency, and amount and types of digestive enzymes in digestive tract of food [9].

Fish's Intestinal Histology. Fish's digestive system is slightly differently from mammals, that the digestive process has started from the first line: mouth, oral cavity, and pharynx. The feed swallowed will enter the correct digestive tract through esophagus into stomach and then intestines [10]. Fish intestines mainly serve to do complete digestive process from stomach and to absorb nutrition from food [11]. Fish intestines also serve as the first line barrier against infection and, therefore, the mucosal layer of intestines creates physical, chemical and cellular protection against pathogenic invasion [12]. The silver pompano's histology test in each treatment increased along with the research as presented in Table 2.

Treatment	Length of Villi (µm)	Base Width of Villi (µm)	Apical Width of Villi (µm)	Surface Size (µm^2)
A	588,2	220,2	90,7	2049,9
B	567,7	151,8	72,7	1782,5
C	483,3	150,6	89,4	1370,4
D	475,3	172,4	99,4	1314,7
E	440,8	131,1	93,5	1091,1

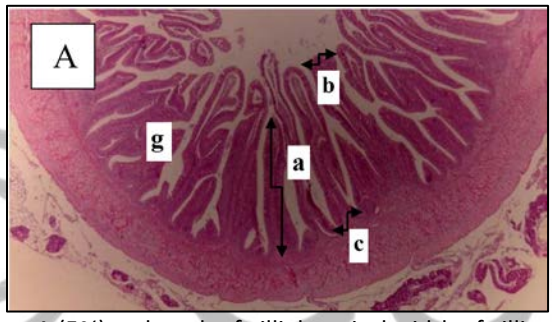

Figure 1. Histology of Fish Intestines A (5%). a: length of villi, b: apical width of villi, c: basal width of villi, d: goblet cell

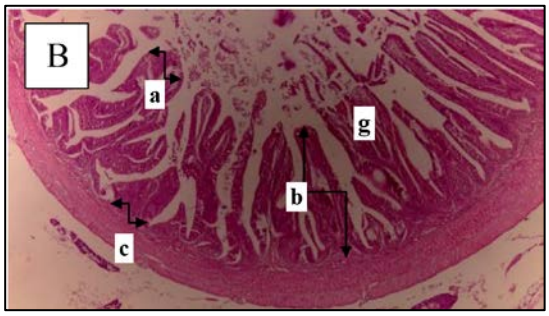

Figure 2. Histology of Fish Intestines B (3.75%). a: length of villi, b: apical width of villi, c: basal width of villi, d: goblet cell

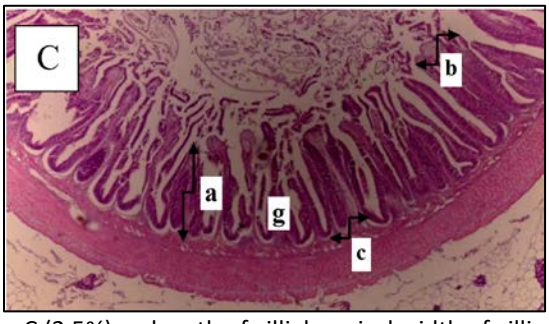

Figure 3. Histology of Fish Intestines C (2.5%). a: length of villi, b: apical width of villi, c: basal width of villi, d: goblet cell

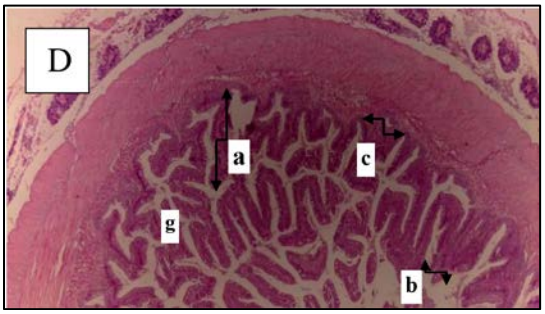

Figure 4. Histology of Fish Intestines D (1.25%). a: length of villi, b: apical width of villi, c: basal width of villi, d: goblet cell

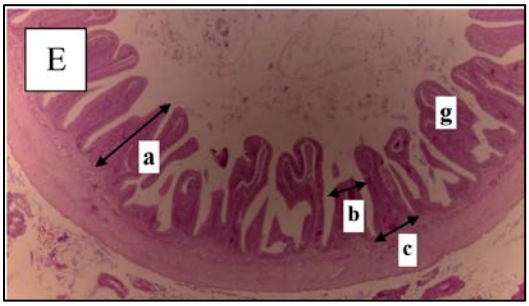

Figure 5. Histology of Fish Intestines E (0%). a: length of villi, b: apical width of villi, c: basal width of villi, d: goblet cell

The results of the 60-day observation show that the surface size of villi of silver pompano ranged from 1091.1 – 2049.9 µm². The biggest surface size of villi was 2049.9 µm² with treatment A with functional enzyme feed. Histologically, it is found that the intestine organ of fish given with functional enzyme feed with 5% papain enzyme had intestinal lamelas and top of villi that were seemingly well-arranged. In addition, the goblet cells in in food digestion seemed to fill intestinal lamelas. This is different from fish's intestinal histology with treatments D and E where the intestinal lamelas seemed irregular and of irregular lengths. Decreasing length of villi is correlated with the replacement of fishmeal-proteins with vegetable proteins, affecting fish growth [13].

Based on the results of research conducted by [14] on increase in goldfish's weight and intestinal villi morphometrics, the more the length, width, and depth of crypt in villi, the more the nutritional absorption area is, that will affect fish growth. Papain enzyme is a protease enzyme commonly used in animal feed industry serving to break proteins down into amino acids, facilitating absorption by the digestive system [15]. Analysis on fish's intestinal histology is considered a good indicator to assess fish's nutritional status [16].

In the research conducted by [17], the histology of hybrid grouper with single cell proteins and "spent grains" application with fishmeal-less formulation presents good results since fish treated without fishmeal addition in the feed has well-arranged intestinal histology. The histology of fish's intestines informs of the efficiency and effectiveness of absorption by fish [4].

According to [18], length of villi varies depending on locality and species. This conforms to the function of the intestinal part according to [19] that intestinal anterior part serves 1) to transport food materials from stomach to posterior intestine, 2) for complete digestion by enzyme secretion from gland walls and accessories, 3) to absorb final products of digestion into blood vessel and lymph on the walls, and 4) to secrete certain hormones (i.e. Secretin, stimulating pancreatic secretion). The functions of posterior intestines including fluid absorption, mucus secretion (excess of goblet cells) and some digestions achieved by enzymes present in food materials, and release. Villi in each part of intestines seemed separated. The surface size of intestinal villi can be influenced by the length, width, body weight gain, increased intestinal cavity and feed consumed. This conforms to [20] that the shape of villi in each part of intestines is different, the amount and length of intestinal villi decreases towards posterior intestines, while goblet cells increase. Two or more villi coming into contact with each other, which should be separated from each other, will have food digestion inhibited.

Result and discussion

Functional feeding containing the enzyme papain had a significant effect on increasing daily growth rate (LPH), and intestinal histology of silver pompano. The addition of papain enzyme to functional feed with the best dose was in the 5% treatment which resulted in a daily growth rate of 3.25 ± 0.112%, and the surface area of the villi in the intestinal histology of the silver pompano was 2049.9 µm² with length of villi 588.2 µm, base width of villi 220.2 µm, and apical width of villi 90.7 µm.

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