Effects of the addition of activated charcoal in feed on the morphology of intestinal villi of Giant Travelly juveniles (*Caranx ignobilis*)

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Abstract. This study aims to determine the effect of administration of different concentration of activated charcoal in feed on the morphology of intestinal villi of Giant Travelly juveniles (*Caranx ignobilis*). This research was conducted in February to March 2018 which took place at the Brackishwater Aquaculture Center (BPBAP) in Ujong Batee, Aceh, and a histology laboratory at the Faculty of Veterinary, Syiah Kuala University. The method used in this study was an experimental method using a completely randomized design (CRD) with five treatments and four replications. The treatment with different concentrations of active charcoal was treatment A (0%), B (1%), C (2%), D (3%), and E (4%). Results of the study found that treatment C showed the significant difference with other treatments (p <0.05) by morphological values of height (123.6 µm), basal width (63.8 µm), and apical width (42 µm), respectively. Thus it can be concluded that the addition of activated charcoal in feed at 2% can improve the absorption of nutrition in the intestine.

1. Introduction

The diversity of fish cultivation is needed so that it not only produces fish meat, but also other production. Indonesia’s biodiversity on fish and shrimp is well-known over the world [1,2,3]. Giant Travelly (*Caranx ignobilis*) has begun to be maintained in the waters of the sea and ponds on the east coast of Aceh, Indonesia. This fish is quite popular because the taste of the meat is good, chewy, savory, and not much compared to other fish. Giant Travelly fish is a marine fishery commodity Indonesian origin which has a potential value to be cultivated because it has advantages, including cultivation techniques that are relatively easy. Muhammadar et al [4] stated that aquaculture would be comfortable if the balance between external factors (such as salinity, temperature, DO) and internal (such as nutrition) was achieved. The improvement of aquaculture commodity using immunostimulant has also been established [5,6,7,8]. In intensive aquaculture commercial feed was used 60-70% of production costs, and it is expected that low prices with high quality. The use of live feed in aquaculture may also improved its production [9,10]. It stated that in the process of making these feeds many use additive materials which become inhibitor factors in the digestive process [11]. These materials such as plant...
seeds, legumes and feather flour. Inhibitor materials can reduce the quality of feed and interfere with the digestion process of protein to amino acids [12]. The other inhibitor factors in digestion can occur by improper storage of feed, resulting in changes in temperature, pH and an increase in negative microbes in the feed to the digestive tract. The digestive organ is an intermediary organ between the internal and external environment which has the main function as digestion and absorption of nutrients. Disorders of the digestive tract are an important problem that requires an immediate solution.

Many detailed studies of land animals such as ruminants [13] and poultry [14] which through adding activated charcoal to feed will help better digestion. However, there are a number of studies that show that adding activated charcoal to aquatic animal feed can improve survival, digestibility and reduce food conversion [15, 16, 17, 18]. However, studies of these aquatic animals have not described the intestinal morphological character in the digestive process. Therefore, the morphological characteristics of the digestive organs, especially the small intestine in Giant Travally need to be studied so that they can function properly in their cultivation.

2. Materials and Methods

2.1 Place of study
This research was conducted at the Brackish Water Cultivation Center (BBAP) at Ujong Batee Aceh Besar and the Histology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University.

Table 1. Tools and materials used in this study include the following:

| No | Tool and Materials | Function |
|----|--------------------|----------|
| 1  | Sample Bottle      | for the place of fish soaked with 10% BNF solution |
| 2  | Cutting board      | for cutting fish digestion |
| 3  | Surgical instruments | to dissect sample fish |
| 4  | Glass preparations | for sample sites |
| 5  | Cover glass        | to close the sample on the glass preparation |
| 6  | Microscope         | to see damage to intestinal cells in fish |
| 7  | Refrigerator       | for storage of samples that have been preserved |
| 8  | 10% BNF            | solution to preserve fish |
| 9  | Kuwe fish biota sample | |
| 10 | DO meter           | to measure dissolved oxygen |
| 11 | pH meter           | to measure the acidity of water |
| 12 | Thermometer        | to measure water temperature |
| 13 | Masks              | as a nose and mouth protector |
| 14 | Hands glove        | to keep it sterile |

2.2 Experimental design
The study was carried out experimentally for 15 days using a completely randomized design (CRD) with 5 treatments and 3 replications. The treatment in the study was the provision of activated charcoal with different concentrations, namely:
Treatment: A as much as 0% activated charcoal (control)
Treatment: B as much as 1% activated charcoal
Treatment: C as much as 2% activated charcoal
Treatment: D as much as 3% activated charcoal  
Treatment: E as much as 4% activated charcoal

2.3 Observation of Intestinal Histology
Surgery was carried out by doing an incision on the abdomen starting from the anus perpendicular to the linea alba, then cutting along the line up to the back of the operculum and then slicing it towards the ventral fin. The incision is evaporated, and the intestinal organs are collected and cleaned with physiological NaCl.

To see changes in the intestine microscopically, preparations were made by first fixing the intestine using BNF 10% (Neutral Buffered Formalin) for 1x24 hours, dehydrating with multilevel alcohol and waiting 2 hours ago from alcohol 70%, 80%, 96%, absolute I, and absolute II (100% alcohol). The intestine is then cleared using xylol I, and xilol II for 2 hours. The infiltration process using paraffin I, II, for 2 hours was inserted into the waterbath, into the process of embedding the network. The tissue was slashed with a thickness of 5µm and the preparation was stained with haematoxylin-eosin (HE) staining.

Histological preparations were carried out sequentially according to [19] starting from xylol I, xilol II for 2 minutes each. Alcohol (absolute I), alcohol (absolute II) each for 2 minutes, alcohol 96% I, alcohol 96% II, alcohol 96% for 2 minutes. Next clean with running water until clean, once to acid alcohol, and water. The preparations were soaked in 96% alcohol, 96% alcohol for 1 minute each, then soaked in xylol I for 2 minutes and xilol II for 2 minutes. After that an observation was made using a microscope with 10x40 and 10x100 magnifications.

2.4 Data analysis
The effect of active charcoal between treatments was analyzed using table, image, and variance analysis (ANOVA) and continued with Duncan’s multiple test [20].

3. Results and Discussions
Histological monitoring results in Giant Travellly intestine (Caranx ignobilis) were given activated charcoal at a dose of 0%, 1%, 2%, 3%, and 4%. Intestine histology in the control and maintenance groups of 2% can be found in high or normal villi where lamina epithelium, tunica muscularis and lamina propria can be found, at 1%, and 4% can be displayed lamina epithelium, tunica muscularis, and lamina propria.

The worst damage seen by Giant Travellly histology occurred in the regulation provided by 3% activated charcoal where the lamina propria could not be seen again, even though the tunic muscularis and lamina epithelium were intact.
Figure 1. Histological results of cellulose bowel organisms on HE 10 x 40 staining with a 50µm scale, and (lamina epithelium, b. Lamina propria, c. Tunica muscularis).

Table 2. Results of height of villi, width of basal, apical width of intestine in Giant Travelly juveniles for a maintenance period of 35 days.

| Treatment | Villi Height (µm) | Basal Width (µm) | Apical width (µm) |
|-----------|------------------|-----------------|------------------|
| A         | 55.2 ± 15.88<sup>a</sup> | 52.6 ± 3.28<sup>a</sup> | 39.2 ± 5.21<sup>bc</sup> |
| B         | 90.2 ± 23.79<sup>b</sup> | 54.2 ± 5.06<sup>ab</sup> | 33.8 ± 3.11<sup>ab</sup> |
| C         | 123.6 ± 32.69<sup>c</sup> | 63.8 ± 20.82<sup>b</sup> | 42.0 ± 8.54<sup>bc</sup> |
| D         | 43.2 ± 7.85<sup>a</sup> | 44.8 ± 8.31<sup>a</sup> | 22.6 ± 4.29<sup>a</sup> |
| E         | 68.4 ± 7.40<sup>ab</sup> | 66.2 ± 11.88<sup>b</sup> | 44.4 ± 8.01<sup>c</sup> |

Arrow note: a = Lamina epithelium, b = Lamina propria, c = Tunica muscularis

Description: If the same superscript letters in the same column show no significant effect (P> 0.05) and vice versa if the different superscript letters in the same column show significantly different (P <0.05).

Based on the data in the table 2 showed the height of villi ranges from 43.20 - 123.60 mm, the width of the basalis ranges from 44.80 - 66.20 mm. Apical widths range from 22.60 - 44.40 mm. The ANOVA test results showed that the active charcoal addition in fish feed significantly
affected villi height (P < 0.05) but did not significantly affect the basal width and apical width (P > 0.05). In treatment C (123.6 µm) it was found that the average value of villi height was more maximal than the other treatments and the values were significantly different from all treatments (p < 0.5). The highest basal width value was found in treatment E (66.2 µm), and this value was not significantly different from treatment A, B and C (p > 0.05). The value of the apical width tended to be the highest in treatment E (44.4 µm) and the value was significantly different from treatment B and D (p < 0.05).

The use of activated charcoal in the fisheries sector has been widely developed, as reported by [15], the addition of activated charcoal in artificial feed was able to increase protein retention, feed efficiency, feed consumption and daily growth rate. The optimum fish growth is depend on several aspects such as feed quality and proportion [21, 22, 23]. The concentration of activated charcoal given at each treatment can determine the amount of protein hydrolysis in artificial feed. The results of this study found that the intestinal villi structure for treatment C (2% activated charcoal content) was higher than the other treatments and the value was significantly different (p < 0.05). In the intestine that has a high villous size has a wider area of microvilli so that the number of microvilli will be large. It is stated that the size and number of microvilli affects the process of absorption of nutrients in the intestine [24].

Increased height and surface width of villi is thought to be due to the active ingredients in activated charcoal which can increase the proliferation of epithelial cells in the small intestine villi resulting in an increase in the height and width of villi. The increase and width of villi correlates closely with the increase in the number of epithelial cells on the surface of the small intestinal villi [26], besides that villous height can be associated with active epithelial cell division in the villi [27].

According to [28] states that the higher the small intestine villi, the greater the effectiveness of absorption of food extracts through the small intestine epithelium. However, for the high absorption of Giant Travelly food (Caranx ignobilis) it must be followed by the width of the basal and high apical width. Therefore the best treatment is found in treatment C. Generally, dense villi are found in the duodenum and jejunum and the amount decreases in the ileum. On peripheral villi surrounds there are many columnar epithelial cells which have the capacity of small intestinal absorption. In this area it contains hundreds of microvilli in the periphery of the cavity epithelial enteric, which is about 1-1.5 µm length, and is 0.1 µm width, so that the absorption area can increase hundreds of times. After the food is absorbed into the villi, then it will go to the circulation system in the spleen and blood.

**Conclusion**

Based on the results of observations and studies of histology in the Giant Travelly intestine (Caranx ignobilis) It can be concluded that the administration of 2% activated charcoal in feed was histologically elevated in Villi, basal width and apical width so that the most optimal absorption was possible.
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