Gut and intestinal biometrics of the giant trevally, *Caranx ignobilis*, fed an experimental diet with difference sources of activated charcoal [version 2; peer review: 2 approved]

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Abstract

**Background:** The giant trevally, *Caranx ignobilis*, is a commercially important marine fish in Indonesia. This species was initially cultured in Aceh Province. Previous reports showed that charcoal has a positive effect on survival and feed utilization of the giant trevally. However, the effects of adding charcoal to the diet on gut and intestine biometrics has, to our knowledge, never been described.

**Methods:** Four activated charcoal sources were tested in this study using a completely randomized experimental design; coconut shell charcoal, mangrove wood charcoal, rice husk charcoal, and kernel palm shell charcoal. All treatments were performed with four replications. Juvenile giant trevally (average body weight, 16.52 ± 3.12 g; and average total length, 10.26 ± 0.64 cm) were stocked into the experimental tank at a density of 15 fish per tank. The fish were fed an experimental diet twice daily at 7 AM and 5 PM *ad satiation* for 42 days.

**Results:** Analysis of variance showed that adding charcoal to the diet had significant effects on the length and width of the foveola gastrica and villous intestine (*P* < 0.05). The greatest length and width of the foveola gastrica was recorded in fish fed an experimental diet of rice husk charcoal with average values of 311.811 ± 9.869 µm and 241.786 ± 10.394 µm, respectively. The greatest length of intestinal villous was found in fish fed the mangrove wood charcoal diet, with a value of 135.012 ± 5.147 µm, but this length was not significantly different to
that in fish fed rice charcoal and kernel palm shell charcoal. However, the greatest width of intestinal villous was recorded in fish fed the control diet (without charcoal; P < 0.05).

**Conclusion:** The optimal sizes of the foveola gastrica and villous intestine were found in fish fed an experimental diet with rice husk charcoal.

**Keywords**
Foveola gastrica, villous intestine, coconut shell, mangrove wood, rice husk, and kernel palm shell

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**Competing interests:** No competing interests were disclosed.

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Organogenesis of the digestive system occurs as fish age, and this process is strongly dependent on the quantity and quality of food, which is related to the development of mucosal cells, amplification of apical plasma membranes, and formation of the foveola gastrica and intestinal villi. It has been hypothesized that adding activated charcoal to the diet triggers the digestive organogenesis system process. In this study, we tested four charcoal sources in the diet to evaluate the morphology of the gut and intestine of giant trevally. Information on the gut and intestinal morphology is important to understand the absorption mechanism of nutrients from the diet.

**Methods**

**Time and site**

The study was conducted at the Center for Brackish Water Aquaculture, Ujung Batee, Aceh, Indonesia from February to July 2018. The activated charcoal was characterized at the Integrated Laboratory of Calibration, Universitas Gajah Mada, Yogyakarta, Indonesia. Histological samples were prepared at the Laboratory of Histology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia.

**Experimental design**

A completely randomized experimental design with five treatments consisting of control and four different charcoal sources was used in this study. The experimental groups were: (A) the experimental diet without charcoal, (B) the experimental diet with 2% charcoal from coconut shell, (C) the experimental diet with 2% charcoal from mangrove wood, (D) the experimental diet with 2% charcoal from rice husk, and (E) the experimental diet with 2% charcoal from kernel palm shell. All treatments were performed with four replications.

**Experimental fish**

A total of 300 giant trevally juveniles of mixed sex (average body weight, 16.52 ± 3.12 g; total length, 10.28 ± 0.64 cm) were purchased from a local farmer in Lancang Barat Village, Aceh Utara District, Aceh, Indonesia. The fish were acclimatized in ponds (ponds size 2 m x 1.8 m and temperature of around 29°C) at the Center for Brackish Water Aquaculture, Ujung Batee for 2 weeks. The fish were fed an experimental diet containing 50% crude protein twice daily at 7 AM and 5 PM at 3% of body weight per day (Table 1).

**Charcoal preparation and activation**

The raw coconut shells, mangrove wood, rice husks, and kernel palm shells were chopped and ground. Approximately 500 g of the ground materials were placed on aluminum foil and heated in a furnace at 400°C for 1 hour. Nitrogen gas was flowed into the furnace to remove the oxygen. Then, the temperature was decreased to 30°C gradually and held for 1 hour. After 1 hour, the charcoal was removed from the furnace, sieved through a No. 40 mesh, and held in a jar before activation.
activating. A total of 100 g of sieved charcoal was taken and mixed with 400 ml of 0.2 M citric acid. The solution was stirred for 24 hours. After 24 hours, the solution was filtered through filter paper. The filtered charcoal was washed with distilled water and dried in an oven at 110°C for 24 hours.

Diet preparation
The experimental diet was formulated from both plant and animal-based protein sources, such as Ebi-shrimp meal, fish meal, blood meal, soybean meal, rice flour, and corn flour. All raw materials were subjected to a proximate analysis before use in the formulation. Three types of amino acids i.e. isoleucine, L-tryptophan, and DL-methionine were also added (Table 1). A total of 2% of the tested charcoal sources was added to the formulation (Table 1). The formulated diets were subjected to a proximate analysis before use in the experiment.

Stocking and feeding
The fish was captured randomly, measured for body weight and total length, and then distributed into 20 plastic containers (48 × 43 × 70 cm) at a stocking density of 15 fish per container. The water volume in the container was 75 L. The fish were fed an experimental diet twice daily at 7 AM and 5 PM to satiation for 42 days.

### Histological sample preparation
Gastric and intestinal samples were collected at the end of the study. Three fish from each treatment were taken randomly from the experimental tanks. The fish were anesthetized with 30 mg L⁻¹ clove oil, and the abdomen of the fish was gently dissected following the procedure of Purushothaman et al. The stomach and intestines were removed with scalpel scissors and preserved in 4% formalin for 1 week. Histological sampling was carried using the paraffin method based on Osman and Caceci. The samples were dehydrated through an alcohol series and cleared in xylol. Subsequently, the gut and intestine samples were embedded in paraffin. The paraffin block was sectioned to 6 µm, and the sections were stained with hematoxylin and eosin. The size (height and width) of villi was determined using a binocular microscope (Zeiss Primo Star, Carl Zeiss Suzhou Co., Ltd., Suzhou, China) which was connected to a CCD camera and computer monitor. All efforts were made to lessen harm to the animals by complying to the guidelines of ethics animal use in research of Syiah Kuala University.

### Data analysis
The qualitative gut and intestinal morphology data were subjected to one-way analysis of variance followed by Duncan’s multiple range test. The analysis was performed using SPSS.
ver. 18.0 software. The qualitative (histological) gut and intestinal data were analyzed descriptively. A $P$-value $< 0.05$ was considered significant.

**Results**

Adding activated charcoal to the diet significantly affected the length and width of the foveola gastrica and intestinal villi ($P < 0.05$). In general, fish fed the activated charcoal diets produced better results than those not fed the charcoal (Figure 1 and Figure 2). The best foveola gastrica morphology was obtained with the rice husk charcoal and the mean length and width of the foveola gastrica were 311.811 µm and 241.786 µm, respectively; followed by coconut shell charcoal (257.040 µm and 183.816 µm), kernel palm charcoal (229.969 µm and 169.131 µm µm), and mangrove wood charcoal (229.595 µm and 166.509 µm).

The greatest length of the villous intestine was recorded in fish fed a diet with activated charcoal than those not fed the activated charcoal (Figure 3). The greatest growth of intestinal villi was determined in the mangrove active charcoal (mean, 135.012 µm) group, but this value was not significantly different from the rice husk or kernel palm shell charcoals (Figure 4). However, the greatest intestinal villi width was obtained in the treatment without activated charcoal (38.341 µm), and this value was significantly different from the other treatments.

The qualitative (histological) gut and intestinal data were analyzed descriptively. A $P$-value $< 0.05$ was considered significant.

**Discussion**

The results show that adding activated charcoal to the diet of *C. ignobilis* significantly affected foveola gastrica and intestinal villi biometrics. According to Pirarat *et al.*19, activated charcoal plays a significant role stimulating the development of epithelial cells of the digestive organs. Activated charcoal in the diet functions as a decontaminating agent to eliminate pathogenic organisms and toxic compounds, such as mycotoxins20. Hence, a longer foveola gastrica and larger intestinal villi were able to provide more nutrients to be absorbed due to a larger surface area of digestive organs43. Optimal development of the alimentary tract was recorded in giant trevally juveniles fed the experimental diet containing rice husk charcoal. This was presumably due to the high hemicellulose, cellulose, and lignin contents in the rice husk charcoal. A previous report indicated that rice husk charcoal contains 29.3% hemicellulose, 34.4% cellulose, and 19.2% lignin44, while mangrove wood charcoal has 30% hemicellulose, 36% cellulose, and 28% lignin45, coconut shell charcoal has 19.27% hemicellulose, 33.61% cellulose, and 36.51% lignin46, and kernel palm charcoal has 26.27% cellulose, 12.61% hemicellulose, and 42.96% lignin47. Maria and Banu48 and Jamilatun *et al.*49 reported that the concentration and quality of charcoal depend on the composition of hemicellulose, cellulose, and lignin. The quality of the activated charcoal is higher

![Figure 1](image-url). **Figure 1.** The average of length and width of the foveola gastrica. (A) Diet without charcoal, (B) diet with coconut shell charcoal, (C) diet with mangrove wood charcoal, (D) diet with rice husk charcoal, (E) diet with kernel palm shell charcoal.
Figure 2. Histology of *foveola gastrica* from a juvenile giant trevally. (A) Diet without charcoal, (B) diet with coconut shell charcoal, (C) diet with mangrove wood charcoal, (D) diet with rice husk charcoal, (E) diet with kernel palm shell charcoal. M, tunica mucosa; SM, tunica submucosa; Mc, tunica muscularis; Le, lamina epithelialis; Lp, lamina propria; m, muscle; Lm, longitudinal muscle fibers; Cm, circular muscle fibers (Cm).

Figure 3. The average length and width of intestine villi from juvenile giant trevally. (A) Diet without charcoal, (B) diet with coconut shell charcoal, (C) diet with mangrove wood charcoal, (D) diet with rice husk charcoal, (E) diet with kernel palm shell charcoal.
when these three components increase. According to Jasman\textsuperscript{50}, rice husk contains 85–95\% activated charcoal, while mangrove wood has 76\% activated charcoal\textsuperscript{51}, kernel palm shell 65\% activated charcoal\textsuperscript{47}, and coconut shell has 60\% activated charcoal\textsuperscript{46}.

The microscopic observations showed that the intestinal villi of the fish fed the diet with activated rice husk charcoal had a more pointed shape compared to other treatments, in which the villi tended to be round and blunt. According to Guo et al.\textsuperscript{52}, blunt or rounded villi probably occur due to inflammation in the intestinal mucosa, which is characterized by infiltration of neutrophils into the lamina propria. An increase of intestinal villus size is related to nutrient absorption capacity. According to Nafis et al.\textsuperscript{53}, long mucosal folds increase nutrient absorption and reduce food flow movement due to reduced peristaltic contractions, which provides sufficient time to optimally absorb nutrients. The increase in intestinal villi size is strongly related to the activities of digestive enzymes, such as lactase, sucrase, alkaline phosphatase, and disaccharidase\textsuperscript{54–57}.

The morphology of the intestinal villi of fish fed a diet without activated charcoal was wider and shorter than that of fish fed the diets with activated charcoal. This was probably due to impaired intestinal mucosal integrity, causing interference in nutrient absorption. According to Choc\textsuperscript{58}, shortening of the intestinal villi is related to the accumulation of intestinal pathogenic bacteria, resulting in increased susceptibility to infection in the intestinal mucosal layer. This causes the digestive organs to form more secretory cells than absorbent cells, which reduces nutrient uptake\textsuperscript{59,60}. The active charcoal likely acts as an adsorbent of metabolic pathogens in the intestine in the form of endotoxins and ammonia, therefore, it was able to improve intestinal function\textsuperscript{61}.

**Conclusions**
The application of activated charcoal in the diet significantly affected the length and width of the foveola gastrica and intestinal villi of giant trevally, *C. ignobilis*. The optimal biometrics of the foveola gastrica and intestinal villi were observed in fish fed the experimental diet with activated rice husk charcoal.

**Data availability**
Figshare: Gut and intestinal biometrics of the giant trevally, *Caranx ignobilis*, fed an experimental diet with difference sources of activated charcoal. https://doi.org/10.6084/m9.figshare.12203525.v2\textsuperscript{40}.

This project contains the following underlying data:
- DATA BIOMETRIC GUT OF GIANT TREVALLY *Caranx ignobilis* Edited (XLSX). (Raw biometric data for the foveola gastrica of all fish examined in this study.)
• DATA BIOMETRIC OF INTESTINE OF GIANT TREVALLY Caranx ignobilis (carc. LXSX). (Raw biometric data for the intestinal villi of all fish examined in this study.)

Figshare: Gut and intestinal biometrics of the giant trevally, Caranx ignobilis, fed an experimental diet with difference sources of activated charcoal. https://doi.org/10.6084/m9.figshare.12269606.v2

This project contains uncropped, unprocessed images of the foveola gastrica of the giant trevally.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Version 2

Reviewer Report 13 October 2020

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✔ Abdus Salam
Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh

Gut and intestinal biometrics of the giant trevally, *Caranx ignorabilis*, fed an experimental diet with different sources of activated charcoal.

The title should be different not difference. Others are fine.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Aquaculture, Aquaponics, fish nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 10 July 2020

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✔ Abdus Salam
Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh,
Bangladesh

- It seems the manuscript has potentials in the scientific world, and work was done and formatted with care. The abstract is concise but well written.

- The introduction has supported with up to date literature and aim and objectives are well described.

- Results supported with details and tables and figures were provided.

- The discussion was also well described and literature supported.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Aquaculture

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 June 2020

https://doi.org/10.5256/f1000research.26249.r63939

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✅ Srikanta Samanta
The paper has communicated a short but interesting study on the beneficial effects of adding different types of charcoal as a dietary component of giant trevally. The addition of charcoal has significantly improved the gastro-intestinal microstructures including foveola gastrica & villous intestine and thereby, has the potentiality to improve the fish nutrition and health. The rice husk was recorded as the best source of charcoal with respect to 3 other sources including coconut shell, mangrove wood, and kernel palm shell. However, some deficiencies have been noticed which needs rectification.

1. In Abstract: This species was---: Better to use The species was---

2. Four activated charcoal sources were tested in this study -----but in Experimental Design mentioned ----A completely randomized experimental design with five different charcoal sources----

3. In Abstract: average total length, 10.26 ± 0.64 cm, In Experimental fish : total length, 10.28 ± 0.64 cm

4. ponds size 2 x 1.8 m. No unit given after 2. It may be 2 m X 1.8 m

5. (see Table 1): see may be deleted

6. Table 1 : CaCo3 : CaCO3

7. In Abstract: and 241.786 μm --- In Figure 1 241.768

8. Figure 2 and 4: Histological sample ---- may be replaced by ---- Histology

9. Sentence to be modified: Hence, a longer foveola gastrica and larger intestinal villi provide a larger surface area to absorb nutrients43.

10. Sentence to be modified: A previous report indicated that rice husk charcoal contains 39% hemicellulose, 44% cellulose, and 30% lignin44: Since the addition of 3 parameters are giving values >100%, you may mention "up to" otherwise people will not understand.

11. Sentence to be modified/changed: The active charcoal likely acts as an adsorbent of metabolic pathogens in the intestine in the form of endotoxins and ammonia to improve intestinal function61. The sentence to be rewritten to increase clarity.

12. It has been noticed that the SE values have gone out of the bar area. Please check about it and reason behind. For the paper, the fish growth parameters may be given as supplements for better clarity.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Aquatic Chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

---

**Author Response 22 Jul 2020**

**Samadi Samadi**, Syiah Kuala University, Banda Aceh, Indonesia

Dear Prof. Srikantha Samanta,
Thank you very much for your comment and we will revise the article based on your suggestions.
Best regards,
Samadi

**Competing Interests:** No competing interests were disclosed.
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