RESEARCH ARTICLE

Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestines from giant trevally, *Caranx ignobilis* [version 1; peer review: 1 approved with reservations]

Firdus Firdus^{1,2}, Samadi Samadi^{id}³, Abdullah A. Muhammadar^{id}⁴, Muhammad A. Sarong⁵, Zainal A. Muchlisin^{id}⁴, Widya Sari¹, Siska Mellisa⁴, Satria Satria⁶, Boihaqi Boihaqi⁴, Agung Setia Batubara⁴

¹Department of Biology, Universitas Syiah Kuala, Banda Aceh, Aceh Province, 23111, Indonesia

²Graduate School of Mathematics and Applied Science, Universitas Syiah Kuala, Banda Aceh, Aceh Province, 23111, Indonesia

³Animal Husbandry, Universitas Syiah Kuala, Banda Aceh, Aceh Province, 23111, Indonesia

⁴Department of Aquaculture, Faculty of Marine and Fishery, Universitas Syiah Kuala, Banda Aceh, Aceh Province, 23111, Indonesia

⁵Department of Biology Education, Faculty of Teacher Training and Education, Universitas Syiah Kuala, Banda Aceh, Aceh Province, 23111, Indonesia

⁶Center Brackiswater Aquaculture Development, Ujung Batee, Center Brackiswater Aquaculture Development, Ujung Batee, Aceh Besar, Aceh Province, 23361, Indonesia

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Abstract

Background: Research on supplementing feed with rice husk activated charcoal was carried out to determine the effect of variations in the concentration of rice husk activated charcoal on the growth and histological features of the *Caranx ignobilis* intestine.

Methods: This study used an experimental method with a completely randomized design consisting of six treatments and four replications, including adding activated charcoal to feed at concentrations of 0%, 1%, 1.5%, 2%, 2.5%, and 3% for 42 days. The measured parameters included daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency (FE), survival rate (SR), length of foveola gastrica, width of foveola gastrica, length of intestinal villi, and width of intestinal villi. Data were analyzed statistically using one-way analysis of variance and Duncan's test.

Results: The results showed that supplementing fish feed with rice husk activated charcoal at different concentrations significantly affected the values of DGR, AGR, FCR, FE, SR, length of the foveola gastrica, length of the villous intestine, and width of the villous intestine, but did not significantly affect SGR or foveola gastrica width.

Conclusion: The 2% rice husk activated charcoal treatment showed

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1. Karun Thongprajukaew , Prince of Songkla University, Songkhla, Thailand 2. Reiny A. Tumbol , Sam Ratulangi University, Manado, Indonesia 3. Siti Azizah Mohd Nor ^{id} , Universiti Malaysia Terengganu, Terengganu, Malaysia Any reports and responses or comments on the			

the best results for all parameters.

Keywords

foveola gastrica, villi, ratio, biometrics

article can be found at the end of the article.

Corresponding author: Samadi Samadi (samadi177@unsyiah.ac.id)

Author roles: **Firdus F:** Conceptualization, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Samadi S:** Conceptualization, Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; **Muhammadar AA:** Formal Analysis, Validation; **Sarong MA:** Formal Analysis, Validation; **Muchlisin ZA:** Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Sari W:** Methodology, Validation; **Mellisa S:** Data Curation, Software; **Satria S:** Investigation, Visualization; **Boihaqi B:** Investigation, Resources, Software; **Batubara AS:** Methodology, Project Administration, Validation

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Introduction

Giant trevally (*Caranx ignobilis*) is a commercially valuable fish species widely distributed in Indonesian waters¹⁻³. This fish is a top predator in coral reef ecosystems with a relatively long predicted age of 25 years and can grow to a size of 165 cm and 87 kg, making *C. ignobilis* the largest species in the Carangidae family⁴. However, fishing continues to increase without conservation efforts, causing the fish population to decline in the last few decades⁵⁻⁷. Therefore, efforts towards fish domestication are needed to reduce wild fishing. However, several obstacles have been encountered during domestication, such as slow growth of fish due to underdeveloped feed technology in this species.

The development of *C. ignobilis* culture is dependent on trash fish (fish considered to have little value and therefore typically discarded whenever caught) because the fish grow faster than when using commercial feed. However, the costs incurred to provide trash fish are relatively expensive and not balanced with the selling price of fish at harvest. In addition, trash fish are not always available and can carry diseases. Therefore, *C. ignobilis* feed technology needs to be assessed immediately, through the compilation of appropriate feed ingredients so that it can support the nutritional needs of the fish. One of the technologies that has the potential to be applied is supplementing the feed with activated charcoal.

Activated charcoal is a non-nutritional ingredient that binds toxic substances during digestion to increase intestinal absorption of food that enters the digestive tract⁸. Activated charcoal can be produced with a variety of ingredients, such as coconut shells^{9,10}, pine wood powder¹¹, banana peels¹², corn stalks^{13,14}, peanut shells¹⁴, rice straw and rice husk^{14,15}, oil palm stems and shells^{16,17}, wine stalks¹⁸, bamboo¹⁹, almond stems and bark²⁰, and durian peel²¹. Therefore, activated charcoal has good application potential because the resource is easily found and the feed is supplemented with a particular concentration according to the needs of the cultured fish.

High-carbon activated charcoal has the best function, which includes rice husk. Jasman²² reported that rice husk activated charcoal contains 85–95% carbon. In addition, rice husk activated charcoal has an Iod value of 527.8 mg/g, indicating a good quality of absorbent activated charcoal²³. Furthermore, rice husks contain 13%–39% ash, 34%–44% cellulose, and 23%–30% lignin²⁴, where the higher the content of hemicellulose, cellulose, and lignin, the higher the amount of activated carbon and the better the quality of the charcoal²⁵.

Several studies have reported applying activated charcoal in fish, such as supplementing activated charcoal in feed on the growth of *Takifugu rubripes*²⁶, *Paralichthys olivaceus*²⁷, *Pangasiodon*²⁸, *Oreochromis niloticus*⁸, *Clarias gariepinus*²⁹, *Sparus aurata*³⁰, and *Huso huso*³¹. A study on supplementing coconut shell activated charcoal, mangrove wood, rice husk, and oil palm shell in feed on intestinal growth of *C. ignobilis* indicated that 2% activated rice husk charcoal had an optimal effect on growth of intestinal tissues³². However, the appropriate

concentration to supplement rice husk activated charcoal in feed for *C. ignobilis* has not been reported.

Methods

Time and site

This study was conducted from April to September 2019 at the Ujung Batee Brackish Aquaculture Fisheries Center, Ministry of Maritime Affairs and Fisheries, Aceh Besar. The activated charcoal was characterized at the Laboratory and Integrated Testing of Gadjah Mada University, Yogyakarta. The gastric and intestinal histological samples were evaluated at the histology laboratory of the Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh Indonesia.

Experimental design

This study used a completely randomized design method with six treatments and four replications. The fish were fed experimental food containing 50% protein twice a day, at 7 am and 5 pm, at 3% of body weight.

A = Treated feed without activated charcoal (control/0%)

B = Feed treated to contain 1% rice husk activated charcoal

C = Feed treated to contain 1.5% rice husk activated charcoal

D = Feed treated to contain 2% rice husk activated charcoal

E = Feed treated to contain 2.5% rice husk activated charcoal

E = Feed treated to contain 3% rice husk activated charcoal

Experimental fish

A total of 360 *C. ignobilis* juveniles (average weight, 16.47 ± 4.69 g; average length, 9.61 ± 0.71 cm) were collected from local fishermen in Ulee Lheue Village, Banda Aceh City, Indonesia. The fish was acclimatized in the maintenance pond at Ujung Batee Brackish Aquaculture Fisheries Center for 2 weeks.

Juvenile *C. ignobilis* were chosen randomly, and the total weight and length of the fish were measured. The fish were distributed into 24 plastic containers ($48 \times 43 \times 70$ cm) with a water volume of 75 liters (15 fish/container). The fish were fed the experimental food twice daily at 7 am and 5 pm for 42 days.

Charcoal preparation and activation

Rice husk was ground into flour until smooth, and about 500 g was placed in an iron container that was coated with aluminum foil, and burned in a furnace at 400°C for 1 hour. Nitrogen gas was flowed into the furnace to remove oxygen. The temperature was gradually reduced to 30°C for 1 hour. The charcoal was removed from the furnace and filtered through a 40-mesh sieve, and stored in a bottle before activating.

A total of 100 g of charcoal was mixed with 400 ml of 0.2 M citric acid. The solution was stirred for 24 hours and filtered through filter paper. The filtered charcoal was washed with distilled water and dried in an oven at 110°C for 24 hours. The activated charcoal was stored in a desiccator before use.

Feed preparation

The treated feed was prepared from fish flour, rebon shrimp flour, tapioca flour, coconut oil, CaCO₃, isoleucine, L-tryptophan, and DL-methionine, and premixed with 50% protein feed content. All ingredients were mixed and analyzed for protein content. Subsequently the feed was added to the rice husk activated charcoal at 1%, 1.5%, 2%, 2.5%, and 3% (see Table 1 for feed makeup).

Histological sample preparation

Gastric and intestinal samples were excised at the end of the study. Four fish were taken randomly for each replication of the treatments. Fish were anesthetized in 30 ppm clove oil, and the belly of the fish was dissected following the procedure of Purushothaman *et al.*³³. The stomach and intestines were removed with tweezers, and placed in a bottle containing 4% formalin for 1 week. Histological sampling was carried out using the paraffin method based on Osman and Caceci³⁴. Samples were dehydrated through a gradient alcohol series (ethanol solutions of increasing concentration in 70%, 80% and 90%) and dehydrated in xylol. The stomach and intestinal samples were embedded in paraffin blocks. The paraffin blocks were cut 6-mm thick and stained with hematoxylin and eosin. The length and width of the histological preparations were

measured using a binocular microscope (Zeiss Primo Star, Carl Zeiss Suzhou Co., Ltd., Beijing, China) which was projected onto a screen. Treatment of the experimental animals followed the guidelines for the use of animals in research at Syiah Kuala University.

Research parameters

The parameters measured in this study were the daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency (FE), survival rate (SR), length of the foveola gastrica, width of the foveola gastrica, and length of the intestinal villi. The biometric measurements, including the length and width of the foveola gastrica and intestinal villi, were made following the methods of Amiri *et al.*³⁵. DGR and SGR were analyzed according to the formula by Muchlisin *et al.*^{36,37}. AGR was analyzed based on the formula of Jones³⁸:

$$\text{DGR (g day}^{-1}\text{)} = (\text{Wt} - \text{Wo})/t,$$

$$\text{SGR (\% day}^{-1}\text{)} = [(\text{Ln Wt} - \text{Ln Wo})]/t \times 100,$$

$$\text{AGR (cm day}^{-1}\text{)} = (\text{L2} - \text{L1})/\Delta t,$$

$$\text{FCR} = \text{F}/(\text{Wt} - \text{Wo}),$$

$$\text{FE} = \frac{1}{\text{FCR}} \times 100 \%$$

$$\text{Survival rate (\%)} = \frac{(\text{Nt})}{\text{No}} \times 100$$

Where Wo is initial fish weight (g); Wt is fish weight at the end of the study (g); t is the duration of the study (days). L1 is

Table 1. Feed formulations (g kg⁻¹) with 50% protein content used in the research.

Feed Ingredients	Protein Content Ingredients (%)	Feed Formulation (g)					
		Treatment					
		A	B	C	D	E	F
Rebon shrimp flour	58.80	430	430	430	430	430	430
Fish flour	59.00	350	350	350	350	350	350
Tapioca flour	0.5	160	160	160	160	160	160
Coconut oil	0	5	5	5	5	5	5
CaCo3	0	5	5	5	5	5	5
Isoleucine	100	10	10	10	10	10	10
L-Tryptophan	100	17.5	17.5	17.5	17.5	17.5	17.5
DL-Methionine	100	17.5	17.5	17.5	17.5	17.5	17.5
Premix	0	5	5	5	5	5	5
Total (g)		1000	1000	1000	1000	1000	1000
Rice husk activated charcoal (%)	0	0	1	1.5	2	2.5	3

the initial length of the fish (cm), L2 is the final length of the fish (cm), Δt is the rearing period (days). Nt is the number of fish at the end of maintenance, No is the number of fish at the beginning of maintenance.

Data analysis

The research parameters were analyzed statistically using one-way analysis of variance to detect differences followed by Duncan's multiple range test. $P < 0.05$ was considered significant. All data were analyzed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Qualitative (histological) data of the stomach and intestine were analyzed descriptively.

Results

Fish growth parameters

The active rice husk charcoal supplement had a significant effect on DGR and AGR, but did not significantly affect SGR (Table 2). The highest average DGR value was observed in the 2% rice husk activated charcoal treatment (0.359 g/day), followed by 1.5% rice husk activated charcoal (0.289 g/day), the control treatment (0.235 g/day), 1% rice husk activated charcoal (0.221 g/day), 3% rice husk activated charcoal (0.220 g/day), and 2.5% rice husk activated charcoal (0.210 g/day). Furthermore, the highest average SGR value was detected in the 2% rice husk activated charcoal treatment

(1.492%/day), followed by 1.5% (1.408%/day) rice husk activated charcoal, the control treatment (1.122%/day), 3% rice husk activated charcoal (1.060%/day), 1% rice husk activated charcoal (1.046%/day), and 2.5% rice husk activated charcoal (1.009%/day). The highest average AGR value was observed in the 2% rice husk activated charcoal treatment (0.092 cm/day), followed by the 1.5% rice husk activated charcoal (0.081 cm/day), the control treatment (0.068 cm/day), 3% rice husk activated charcoal (0.058 cm/day), 1% rice husk activated charcoal (0.054 cm/day), and 2.5% rice husk activated charcoal (0.043 cm/day).

FCR, FE, and SR

The results showed that adding activated rice husk charcoal to feed significantly affected the FCR ($P < 0.001$), FE ($P < 0.001$), and SR ($P = 0.002$) of *C. ignobilis* juveniles. The best FCR was observed in the 2% rice husk activated charcoal treatment with a value of 1.257, indicating that 1.257 kg of feed is required to increase fish weight 1 kg. The highest FCR value was detected in the 2.5% rice husk activated charcoal treatment at 1.922. Furthermore, the best FE and SR values were also observed in the 2% rice husk activated charcoal treatment with 80.264% and 88.33%, respectively, while the lowest FE and SR values were observed in the 2.5% rice husk activated charcoal treatment at 52.114% and 65%, respectively (Table 3).

Table 2. Daily growth rate (DGR) specific growth rate (SGR), and absolute growth rate (AGR) of juvenile of *Caranx ignobilis*.

Treatment	DGR Value (g day ⁻¹)	SGR Value (% day ⁻¹)	AGR Value (cm day ⁻¹)
A. Without supplementation of activated rice husk (0%)	0.235 ± 0.058 ^{ab}	1.122 ± 0.283 ^{ab}	0.068 ± 0.012
B. Supplementation of 1% rice husk activated charcoal	0.221 ± 0.031 ^a	1.046 ± 0.163 ^a	0.054 ± 0.020
C. Supplementation of 1.5% rice husk activated charcoal	0.289 ± 0.033 ^b	1.408 ± 0.255 ^{bc}	0.081 ± 0.041
D. Supplementation of 2% rice husk activated charcoal	0.359 ± 0.065 ^c	1.492 ± 0.325 ^c	0.092 ± 0.018
E. Supplementation of 2.5% rice husk activated charcoal	0.210 ± 0.018 ^a	1.009 ± 0.126 ^a	0.043 ± 0.009
F. Supplementation of 3% rice husk activated charcoal	0.220 ± 0.021 ^a	1.060 ± 0.109 ^{ab}	0.058 ± 0.017

Note: Numbers followed by different letter superscripts are significantly different ($P < 0.05$).

Table 3. Feed conversion ratio (FCR), feed efficiency (FE), and survival rate (SR) of *Caranx ignobilis* juveniles.

Treatment	FCR	FE (%)	SR (%)
A. Without supplementation of rice husk activated charcoal (0%)	1.638 ± 0.138 ^c	61.381 ± 4.940 ^b	80.00 ± 5.443 ^{bcd}
B. Supplementation of 1% rice husk activated charcoal	1.581 ± 0.174 ^{bc}	63.807 ± 6.932 ^{bc}	70.00 ± 8.607 ^{ab}
C. Supplementation of 1.5% rice husk activated charcoal	1.419 ± 0.099 ^{ab}	70.734 ± 4.771 ^c	83.33 ± 6.667 ^{cd}
D. Supplementation of 2% rice husk activated charcoal	1.257 ± 0.134 ^a	80.264 ± 9.130 ^d	88.33 ± 3.333 ^d
E. Supplementation of 2.5% rice husk activated charcoal	1.922 ± 0.089 ^d	52.114 ± 2.460 ^a	65.00 ± 6.383 ^a
F. Supplementation of 3% rice husk activated charcoal	1.757 ± 0.141 ^{cd}	57.198 ± 4.544 ^{ab}	75.00 ± 10.000 ^{abc}

Note: Numbers followed by different letter superscripts are significantly different ($P < 0.05$).

Biometrics and histology of the foveola gastrica

Adding activated rice husk charcoal to the feed had a significant effect ($P < 0.05$) on the length of the foveola gastrica in *C. ignobilis* juveniles. The highest average length and width of the foveola gastrica were observed in the 2% rice husk activated charcoal treatment with values of 171.574 μm and 120.409 μm , respectively, while the shortest average length of the foveola gastrica was detected in the control treatment (122.287 μm) and the shortest foveola gastrica width was observed in the 1.5% rice husk activated charcoal treatment (89.134 μm) (Table 4 and Figure 1).

Biometrics and histological intestinal villi

Supplementing with rice husk activated charcoal significantly affected ($P < 0.05$) the length and width of the *C. ignobilis* intestinal villi. The longest mean length and width of intestinal villi were observed in the 2% rice husk activated charcoal treatment at 64.027 μm and 16.672 μm , respectively. The shortest mean length of intestinal villi was detected in the 2.5% rice husk activated charcoal (47.204 μm), while the shortest mean width of intestinal villi was observed in the 1% rice husk activated charcoal treatment (11.668 μm) (Table 5 and Figure 2).

Discussion

Supplementing fish feed with different concentrations of rice husk activated charcoal had significant effects on DGR, AGR, FCR, FE, SR, length of the foveola gastrica, length of the villous intestine, and width of intestinal villi, but had no significant effect on SGR or the width of the foveola gastrica. The 2% activated charcoal treatment revealed the best results for all parameters, including DGR (0.359 g/day), SGR (1.492%/day), AGR (0.092 cm/day), FCR (1.257), FE (80.264%), SR (88.33%), foveola gastrica length (171.574 μm), foveola gastrica width (120.409 μm), villous bowel length (64.027 μm), and intestinal villous width (16.672 μm). These results indicate that supplementing with 2% rice husk activated charcoal was a catalyst for absorption of feed nutrients by *C. ignobilis* juveniles, which enhanced the immune system, leading to a high SR. According to Prescott *et al.*³⁹, activated

charcoal functions as a bacterial endotoxin absorbent, which inhibits absorption of nutrients. In addition, Mulyono and Wibisono⁴⁰ reported that activated charcoal absorbs ammonia (NH_3), which is a toxic substance. Kutlu *et al.*⁴¹ stated that adding activated charcoal to feed accelerates the healing process of the mucosa by eliminating intestinal pathogenic bacteria. Furthermore, Thu *et al.*²⁷ reported that activated charcoal plays a role reducing intestinal surface pressure by removing and absorbing gases and poisons that occur along the intestine, so that nutrient absorption is optimal.

Adding more or less than 2% rice husk activated charcoal did not change the DGR, SGR or AGR values from the control treatment, indicating that increasing the rice husk activated charcoal concentration more or less than 2% is no better than control feed. However, the 1.5%–2% rice husk activated charcoal treatment increased feed protein absorption (FE) better than the other treatments, thereby reducing the quantity of feed (FCR) given to the fish. This shows that the 1.5%–2% rice husk activated charcoal treatment functioned effectively and efficiently, resulting in higher fish weights with less feed compared to the other treatments. In addition, the SR was maximum in this treatment, with a value of 83.33% in the 1.5% rice husk activated charcoal treatment and 88.33% in the 2% rice husk activated charcoal treatment. However, different results were reported by Ademola *et al.*²⁹ who found that adding 2.5% rice husk activated charcoal increases the growth and survival of catfish. This difference was likely due to the different test species, which are physiologically different.

Adding activated rice husk charcoal to the feed significantly affected the length of foveola gastrica, but did not affect the width of the foveola gastrica in *C. ignobilis* juveniles. The length of the foveola gastrica was positively correlated with increasing concentrations of rice husk activated charcoal in feed from 0%–2%, but the size decreased at concentrations of 2.5% and 3%. Although there was a decrease in the length of the foveola gastrica in the 2.5% and 3% activated rice husk charcoal treatments, the decrease was not significantly different from the longest foveola gastrica value in the 2% rice husk

Table 4. Average length and width of the foveola gastrica.

Treatment	The average length and width of foveola gastrica $\pm$ SD (μm)	
	Length	width
A. Without supplementation of rice husk activated charcoal (0%)	122.287 $\pm$ 10.649 ^a	103.875 $\pm$ 16.725
B. Supplementation of 1% rice husk activated charcoal	131.583 $\pm$ 3.529 ^{ab}	98.113 $\pm$ 11.229
C. Supplementation of 1,5 % rice husk activated charcoal	137.638 $\pm$ 9.665 ^{ab}	89.134 $\pm$ 11.976
D. Supplementation of 2% rice husk activated charcoal	171.574 $\pm$ 27.023 ^c	120.409 $\pm$ 10.238
E. Supplementation of 2,5% rice husk activated charcoal	147.105 $\pm$ 26.865 ^{abc}	107.075 $\pm$ 28.836
F. Supplementation of 3% rice husk activated charcoal	152.787 $\pm$ 6.226 ^{bc}	107.565 $\pm$ 15.729

Note: Numbers followed by different letter superscripts are significantly different ($P < 0.05$).

Figure 1. Histological sample of the *Caranx ignobilis foveola gastrica*. (A) Feed without added activated charcoal (control). (B) Feed with 1% rice husk activated charcoal. (C) Feed with 1.5% rice husk activated charcoal. (D) Feed with 2% rice husk activated charcoal. (E) Feed with 2.5% rice husk activated charcoal. (F) Feed with 3% rice husk activated charcoal. M, Tunica mucosa; e, epithelium lamina; p, lamina propria; SM, submucosal tunica; Mc, muscular tunica; S, serous tunica; lm, longitudinal muscles.

Table 5. Average length and width of *Caranx ignobilis* juvenile intestinal villi.

	Treatment	Average length and width of intestinal villi $\pm$ SD (μ m)	
		Length	width
A.	Without supplementation of rice husk activated charcoal (0%)	56.831 $\pm$ 5.099 ^b	13.747 $\pm$ 0.225 ^{bc}
B.	Supplementation of 1% rice husk activated charcoal	55.147 $\pm$ 3.920 ^b	11.685 $\pm$ 0.376 ^a
C.	Supplementation of 1,5% rice husk activated charcoal	59.549 $\pm$ 0.298 ^b	14.048 $\pm$ 0.218 ^c
D.	Supplementation of 2% rice husk activated charcoal	64.027 $\pm$ 0.876 ^c	16.672 $\pm$ 0.676 ^d
E.	Supplementation of 2,5% rice husk activated charcoal	47.204 $\pm$ 0.808 ^a	13.666 $\pm$ 0.466 ^{bc}
F.	Supplementation of 3% rice husk activated charcoal	55.182 $\pm$ 3.104 ^b	13.136 $\pm$ 0.607 ^b

Note: Numbers followed by different letter superscripts are significantly different ($P < 0.05$).

Figure 2. Histological samples of *Caranx ignobilis* intestinal villi. (A) Feed without added activated charcoal (control). (B) Feed with 1% rice husk activated charcoal. (C) Feed with 1.5% rice husk activated charcoal. (D) Feed with 2% rice husk activated charcoal. (E) Feed with 2.5% rice husk activated charcoal. (F) Feed with 3% rice husk activated charcoal. M, Tunica mucosa; SM, tunica submucosa; Mc, tunica muscularis; S, serous tunica; e, epithelial lamina; p, lamina propria; gc, goblet cell.

activated charcoal treatment. Pirarat *et al.*⁸ reported that exceeding the optimum concentration of activated charcoal in feed does not have a positive effect on the development of digestive organs and interferes with absorption of nutrients from feed.

Figure 1 shows that the tunic muscularis forms two thin layers of muscle called the circular and the longitudinal muscles. Latania *et al.*⁴² explained the presence and degree of muscular cooperation between the circular and longitudinal muscles indicated whether the type of feed consumed by the fishes was relatively good. This results in better absorption by the lamina epithelium and facilitates the channeling of nutrients by the lamina propria so that the foveola gastrica reacts positively by increasing in size. Furthermore, the results of histological

analysis showed that *C. ignobilis* fed 2% activated charcoal developed an epithelial surface layer covering the entire foveola gastrica. This reinforces the conclusion that growth of the foveola gastrica occurred optimally with the 2% active rice husk charcoal supplement.

Table 5 and Figure 2 show that the average length and width of intestinal villi were optimum in the 2% rice husk activated charcoal treatment, indicating that activated charcoal in feed contributes to increase the length and width of the intestinal villi. Kuperman and Kuz'mina⁴³ and Mekbungwan *et al.*⁴⁴ reported that adding activated charcoal to feed increases the growth and intestinal function of land and aquatic animals and improves FE. Thus, growth of intestinal villi is one of the determinants of nutrient uptake during fish digestion.

Conclusion

The results showed that supplementing the feed with different concentrations of activated rice husk charcoal significantly affected the values of DGR, AGR, FCR, FE, SR, length of foveola gastrica, length of villous intestine, and width of the intestinal villi, but had no significant effect on SGR or width of the foveola gastrica in *C. ignobilis*. The 2% active rice husk charcoal treatment revealed the best results for all research parameters, including DGR (0.359 g/day), SGR (1.492%/day), AGR (0.092 cm/day), FCR (1.257), FE (FE) 80.264%, SR (88.33%), foveola gastrica length (171.574 µm), foveola gastrica width (120.409 µm), villous bowel length (64.027 µm), and villous bowel width (16.672 µm).

Data availability

Underlying data

Figshare: Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestines from giant trevally *Caranx ignobilis*, <https://doi.org/10.6084/m9.figshare.12859973.v1>⁴⁵.

This project contains the raw data for the faveola gastrica and villi of all fish examined in this study.

Figshare: Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestines from giant trevally *Caranx ignobilis*, <https://doi.org/10.6084/m9.figshare.12860033.v1>⁴⁶.

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

Figshare: Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach

and intestines from giant trevally *Caranx ignobilis*, <https://doi.org/10.6084/m9.figshare.12860054.v1>⁴⁷.

This project contains uncropped, unprocessed images of the intestinal villi of giant trevally.

Extended data

Figshare: Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestines from giant trevally *Caranx ignobilis*, <https://doi.org/10.6084/m9.figshare.12901784.v1>⁴⁸.

This project contains the daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), and feed efficiency (FE) of *Caranx ignobilis*.

Figshare: Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestines from giant trevally *Caranx ignobilis*, <https://doi.org/10.6084/m9.figshare.13094930.v2>⁴⁹.

This project contains the feed conversion ratio (FCR) and survival rate (SR) of *Caranx ignobilis*.

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

Acknowledgments

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Karun Thongprajukaew

Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla, 90112, Thailand

Based on my opinion, the manuscript entitled "Supplementation of rice husk activated charcoal in feed and its effects on growth and histology of the stomach and intestine from giant trevally, *Caranx ignobilis*" provides basic information for feed development and aquaculture of giant trevally.

However, the manuscript writing and the presentation of data are not enough to index in its current form. I suggest the authors to use the broken-line analysis for predicting the optimal level of rice husk activated charcoal. More details and considering comments of this manuscript are listed below.

General comments:

1. Please provide common name of fish species, followed by its scientific name in parenthesis. The scientific name of species must be used the full name in the first mention and then use the abbreviations of generic name in the other places.
2. There are several errors relating to the use of capital/lowercase letters, italics, units, symbol, brackets and scientific names. Please carefully check the consistency of writing throughout the manuscript.
3. Some sections contained errors from the use of abbreviations. If all parameters were already abbreviated, so that the subsequent part must be explained using abbreviations. Please carefully check throughout the manuscript.
4. Please replace "%" by "g/kg" for feed ingredients.

Specific comments:

1. Introduction: lack of hypothesis and objective.
2. Methodology section should be re-categorized as time and site, charcoal preparation and

activation, feed preparation, feeding trial (grouped experimental design, experimental fish and research parameters together), histological investigation and data analysis. In addition, it is suitable to replace the treatment titles from "A-F" to "0-3%".

3. Please replace fish flour and shrimp flour by "fish meal" and "shrimp meal", respectively.
4. Methods to collect uneaten feed and water management (as well as water quality) should be given.
5. How to prepare the fish before sampling? Were they starved?
6. Please provide ID or reference number for animal ethics.
7. For parameters indicating growth and feed utilization, it is necessary to provide the references for each equation; these equations are fact and all literatures are not original.
8. Table 1: 1) delete the column "protein content ingredients (%)"; 2) provide detail for the premix composition; and 3) provide proximate chemical composition of basal diet. In addition, this table must be re-written since only one feed formulation was used for all six experimental feeds. The authors should explain the formulation of "basal feed" and then included with varying levels of rice husk activated charcoal (0-3%).
9. For data analysis: Did you test the normality and homogeneity of each of your datasets? Were the data transformed before conducting the statistical analysis? In addition, I suggest the authors to use the broken-line analysis for predicting the optimal level of rice husk activated charcoal.
10. Tables 2 and 3 should be grouped as only one table, as well as for Tables 4 and 5.
11. The results section should be re-written and shortened. It is not necessary to add any value in text again since the readers can access all the values directly from tables or figures. Moreover, some result explanations were not supported by the data in the tables; please carefully check.
12. The references were not well checked and contain many errors, in both content and format. Please carefully check the references section.

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?

Partly

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

Partly

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

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquatic animal nutrition, digestive enzymes in aquatic animals, aquafeed 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, however I have significant reservations, as outlined above.

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