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RESEARCH ARTICLE

REVISED 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 2; peer review: 2 approved, 1 approved with reservations]

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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 giant trevally *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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Reviewer Status ? ✓ ✓

	Invited Reviewers		
	1	**2**	**3**
version 2 (revision) 15 Feb 2021		✓ report	✓ report
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Any reports and responses or comments on the

the best results for all parameters.

article can be found at the end of the article.

Keywords
foveola gastrica, villi, ratio, biometrics

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REVISED **Amendments from Version 1**

We have made revisions according to reviewer recommendations, especially on common/scientific names of fish species, error sentences, re-categorized of the methodology section, inserting animal code of ethics, and improving the writing of results.

Any further responses from the reviewers can be found at the end of the article

Introduction

Giant trevally (*Caranx ignobilis*) is a commercially valuable fish species widely distributed in Indonesian waters[1–3]. 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 giant trevally (*C. ignobilis*) the largest species in the Carangidae family[4]. However, fishing continues to increase without conservation efforts, causing the fish population to decline in the last few decades[5–7]. 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 giant trevally (*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, giant trevally (*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 tract[8]. Activated charcoal can be produced with a variety of ingredients, such as coconut shells[9,10], pine wood powder[11], banana peels[12], corn stalks[13,14], peanut shells[14], rice straw and rice husk[14,15], oil palm stems and shells[16,17], wine stalks[18], bamboo[19], almond stems and bark[20], and durian peel[21]. 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. Jasman[22] 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 charcoal[23]. Furthermore, rice husks contain 13%–39% ash, 34%–44% cellulose, and 23%–30% lignin[24], where the higher the content of hemicellulose, cellulose, and lignin, the higher the amount of activated carbon and the better the quality of the charcoal[25].

Several studies have reported applying activated charcoal in fish, such as supplementing activated charcoal in feed on the growth of fugu rubripes (*Takifugu rubripes*)[26], olive flounder (*Paralichthys olivaceus*)[27], Striped catfish (*Pangasiaodon* sp.)[28], nile tilapia (*Oreochormis niloticus*)[8], african catfish (*Clarias gariepinus*)[29], gilthead seabream (*Sparus aurata*)[30], and beluga sturgeon (*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 giant trevally (*C. ignobilis*) indicated that 2% activated rice husk charcoal had an optimal effect on growth of intestinal tissues[32]. However, the appropriate concentration to supplement rice husk activated charcoal in feed for giant trevally (*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.

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 meal, rebon shrimp meal, tapioca flour, coconut oil, $CaCO_3$, 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).

Feeding trial

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%)

Table 1. Feed formulations (g kg^{-1}) with 50% protein content used in the research.

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

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

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

A total of 360 giant trevally (*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 giant trevally (*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 × 43 × 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.

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.*[33]. DGR and SGR were analyzed according to the formula by Muchlisin *et al.*[34,35]. AGR was analyzed based on the formula of Jones[36]:

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

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

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

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

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

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

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 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.

Histological investigation and data analysis

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.*[37]. 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 Caceci[38]. 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 (Ethic Code No. 958/2015).

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, followed by 1.5% rice husk activated charcoal, the control treatment, 1% rice husk activated charcoal, 3% rice husk activated charcoal, and 2.5% rice husk activated charcoal. Furthermore, the highest average SGR value was detected in the 2% rice husk activated charcoal treatment, followed by 1.5% rice husk activated charcoal, the control treatment, 3% rice husk activated charcoal, 1% rice husk activated charcoal, and 2.5% rice husk activated charcoal. The highest average AGR value was observed in the 2% rice husk activated charcoal treatment, followed by the 1.5% rice husk activated charcoal, the control treatment, 3% rice husk activated charcoal, 1% rice husk activated charcoal, and 2.5% rice husk activated charcoal.

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 giant trevally (*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 of 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).

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 giant trevally (*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 and the shortest foveola gastrica width was observed in the 1.5% rice husk activated charcoal treatment (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 giant trevally (*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, while the shortest mean width of intestinal villi was observed in the 1% rice husk activated charcoal treatment (Table 5 and Figure 2).

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

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

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

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

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 giant trevally (*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 Wibisono[40] 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.

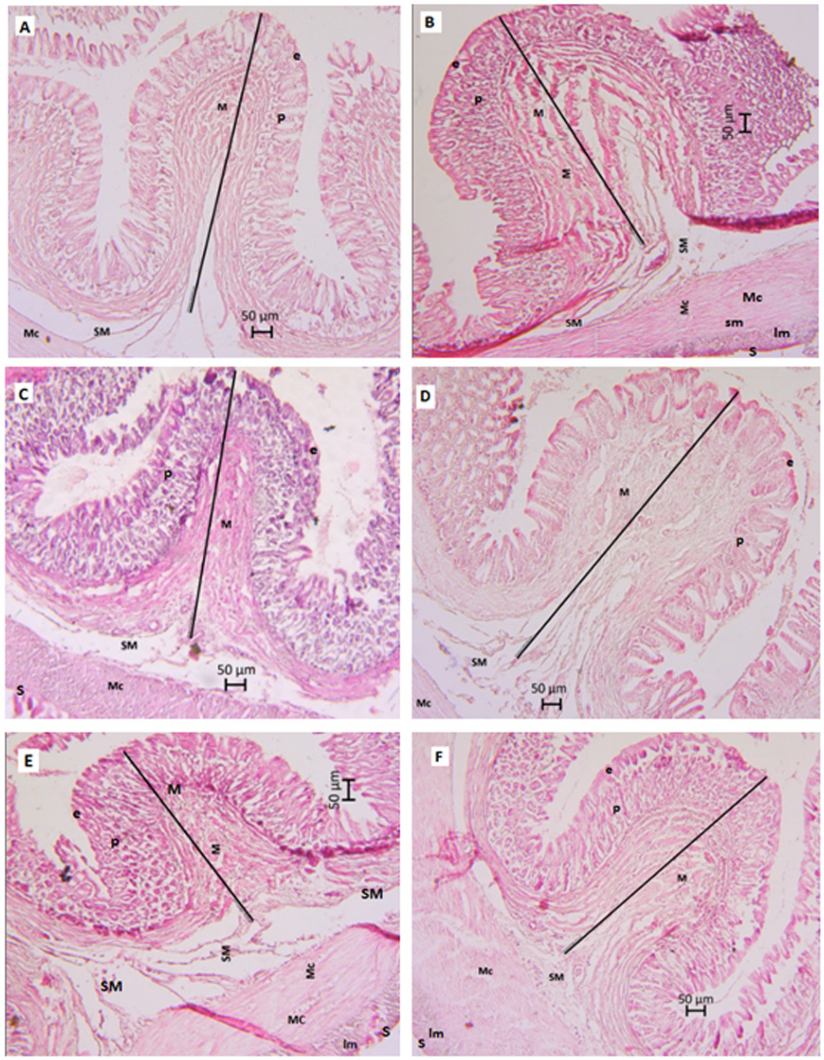

Figure 1. Histological sample of the giant trevally (*Caranx ignobilis*) foveola gastrica. (**A**) Feed without added activated charcoal (control). (**B**) Feed with 1% rice husk activated charcoal. (**C**) Feed with 1.5% rice husk activated charcoal. (**D**) Feed with 2% rice husk activated charcoal. (**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 giant trevally (*Caranx ignobilis*) juvenile intestinal villi.

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

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

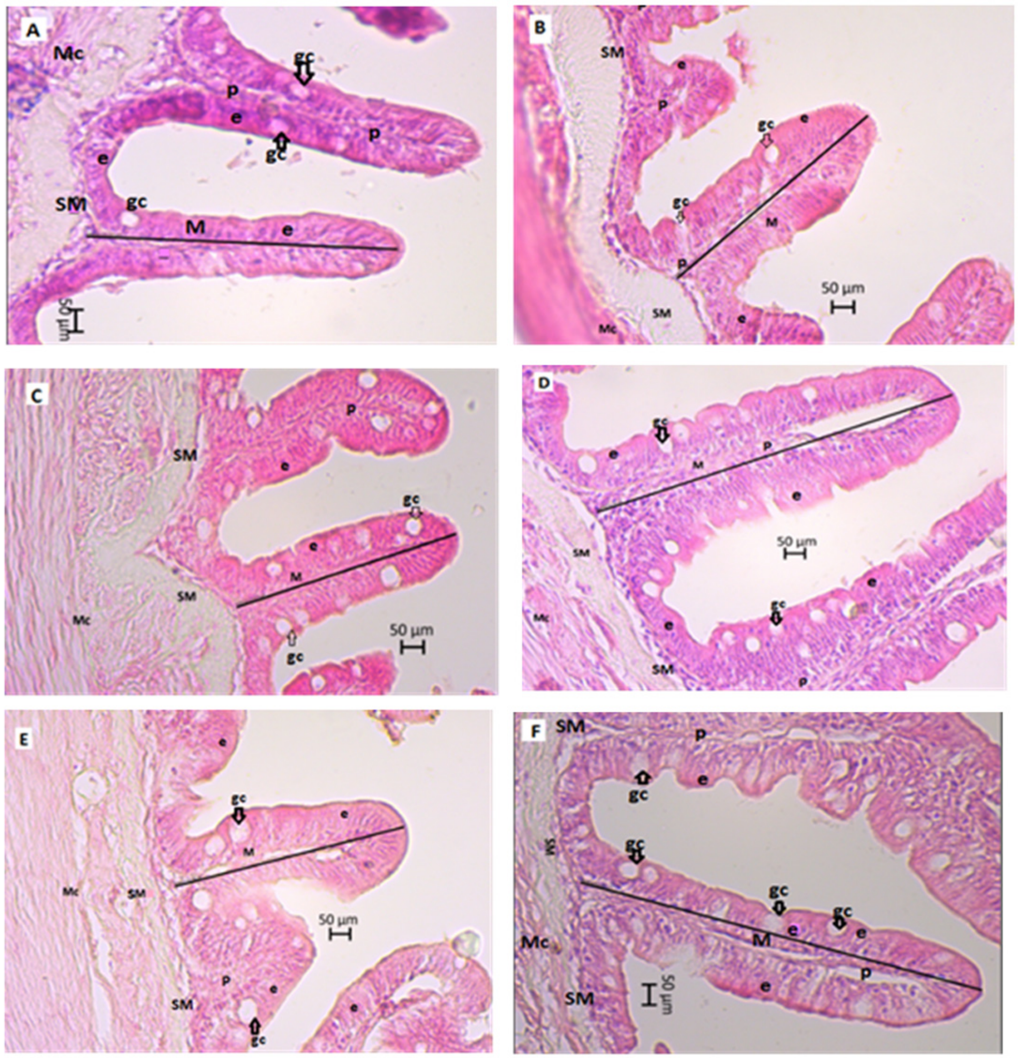

Figure 2. Histological samples of giant trevally (*Caranx ignobilis*) intestinal villi. (**A**) Feed without added activated charcoal (control). (**B**) Feed with 1% rice husk activated charcoal. (**C**) Feed with 1.5% rice husk activated charcoal. (**D**) Feed with 2% rice husk activated charcoal. (**E**) 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.

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 giant trevally (*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 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 giant trevally (*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'mina[43] 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 giant trevally (*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 gastica 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.

Acknowledgments

Thanks to all of the staff at the Ujung Batee Brackish Aquaculture Fisheries Center who assisted with the research. Special thanks go to Mr. Marbawi, Yessi Iman Sari, Amalia, and Humaira Armi for their participation during the research.

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Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 2

Reviewer Report 25 May 2021

https://doi.org/10.5256/f1000research.54560.r85907

✓

Siti Azizah Mohd Nor
Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Terengganu, Malaysia

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*" has contributed useful information for improvement of giant trevally culture through the development of a potential sustainable fish feed product. I am of the opinion that the manuscript is already worthy of indexing in its current form. Authors have generally addressed issues highlighted in previous review reports. However, having said that there are three points that I would like to suggest for authors to consider – also commented in previous review:

Page 3. Please include at the end of the Introduction "Thus, this study aims to identify the appropriate concentration of rice husk activated charcoal supplement in fish feed which will produce optimal effect on growth parameters. We hypothesise that optimal growth can be achieved at a specific concentration".

Page 4. Please state the management regime to monitor (measuring pH etc) and ensure satisfactory water quality (eg waste removal) after the following sentence on this page " The fish were fed the experimental food twice daily at 7 am and 5 pm".

Page 5. It would also be appropriate to add that the data was tested for homogeneity in the data analysis although it appears that they were homogeneous, based on the tabulated results.

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: Aquacuture and Ecological Genetics

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 05 May 2021

https://doi.org/10.5256/f1000research.54560.r81267

Reiny A. Tumbol
Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado, Indonesia

The citations used on this works were closely related and were within the last ten years.

This research was carried out accurately and meets scientific principles. The methods and data analysis used were appropriate to the type of research.

The details of the methods and analysis used in this work were replicable, however there were some details missing. For example, the composition of the feed was not fully explained.

The data was analysed using appropriate statistical analysis that produced reliable and accurate results.

The conclusions were drawn from accurate data produced through appropriate methods and analysis.

Overall, this research has been carried out in accordance with scientific principles and produced results that can be scientifically justified.

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?
Yes

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

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquaculture, fish health includes immunostimulants feed

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 25 January 2021

https://doi.org/10.5256/f1000research.29862.r77124

?

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.