



Evaluating the addition of starry triggerfish (*Abalistes stellaris*) bone charcoal as a feed supplement to the growth performance and intestinal villi length of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Nile tilapia is a freshwater fish that is widely cultivated in Indonesia. During its development, tilapia aquaculture has constraints such as low growth rates. Therefore, it is necessary to add a feed additive to the feed in the form of charcoal. Charcoal is an adsorbent that has high absorption so that the addition of charcoal in the feed can increase the absorption of nutrients of tilapia. A 45-days trial was conducted to determine the percentage of addition of triggerfish bone charcoal (*Abalistes stellaris*) as a feed supplement that affect the growth performance and intestines of tilapia. The research design was a non-factorial Completely Randomized Design (CRD) with 4 treatments and 2 replications as treatments including: treatment A (control), B (addition of 1% charcoal), C (addition of 2% charcoal) and D (addition of 3% charcoal). A total of 240 tilapia fingerlings (5-7 cm) were used and placed in 8 aquariums then filled with 72 liters of water. Providing ad libitum (unlimited) feed with a frequency of 2 times a day. The ANOVA test showed that the addition of triggerfish bone charcoal (*A. stellaris*) in the feed was not significantly different ($P > 0.05$) in weight growth, absolute length and daily length growth but it was significantly different ($P < 0.05$) in feed conversion ratio and the efficiency of feed utilization. Based on result, it was concluded that the addition of 3% charcoal was the best treatment which gave the lengthiest of villi which was increasing the length of the intestinal villi to 71.11 μm from the initial length of 162.22 μm . easal at 233.33 μm , showed the highest feed conversion ratio value and feed efficiency (1.12 %, 89.6%).

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the freshwater commodities that is in great demand by various groups of local and international communities (Yanti and Muchlisin, 2013; Fadri *et al.*, 2016). One of the problems in fish farming today is feed (KKP, 2013). Feed is the most important component and determines the success of fish farming and the availability of feed is a major factor in achieving maximum production. The availability of artificial feed is an obstacle in cultivation business because the largest investment from venture capital, which reaches 40-89% of the total production cost, is in feed.

The feed consumed by fish are digested into simple molecules that can be absorbed by the body. Feed is absorbed by the intestinal wall and enters the circulatory system (Fitriyanti, 2011). Not all of the feed given can be digested and absorbed properly by the body of the fish; therefore, it can affect growth and feed conversion. Efforts should be made to optimize the utilization of feed nutrients to improve the quality of feed; thereby, increasing the rate of growth and cultivation time becomes faster. One method to improve feed quality is the addition of supplements to the feed. Supplement is an ingredient that is added to feed in relatively small amounts for a specific purpose. One of the

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supplements that can be given to feed is charcoal. Charcoal is a porous solid containing 85 - 95% carbon. Selection of basic materials for charcoal must meet several criteria which are low inorganic elements, availability of materials (inexpensive and easy to obtain) and have good durability. [Thu *et al.* \(2010\)](#) stated that charcoal plays a role in normalizing cell membranes in the intestine and reducing the surface pressure of the intestine by eliminating and also absorbing gases and toxins that exist throughout the intestine. as a result, increasing nutrient absorption.

Several researchers have conducted research in the form of observations on micro villi which show the charcoal in feed can increase the surface area of the intestinal villi therefore the fish has better nutrients absorption and decreasing the feed conversion ([Mekbungwan *et al.*, 2004](#)). Adding activated charcoal as much as 2% added to the feed can improve the tilapia intestinal villi length of up to 182.22 μm in 45 days ([Risna *et al.*, 2020](#)), but according to ([Pirarat *et al.*, 2015](#)) the addition of 2% and 3% charcoal in feed will increase the length of the intestinal villi up to 107.27 μm and 126.01 μm . According to ([Lan *et al.*, 2016](#)) the additional surface provided by charcoal or biochar provides additional space for the adhesion of biofilms and increases the rate of dissolution of solid feed ingredients through more efficient syntactic microbiota in fish digestive tract.

In addition, charcoal can absorb and remove lipophilic and hydrophilic toxins from the blood as the adsorption power of large biochar surfaces interacts with intestinal permeability properties. Various biochar mechanisms can remove toxins from the body. First, biochar can interfere with the enterohepatic circulation of toxic substances between the intestines, liver and bile thereby preventing compounds such as esters and progestogens, digitoxins, organic mercury, arsenic and indomethacin compounds from being extracted from the bile. Second, compounds such as digoxin will be absorbed in the intestine. Third, compounds such as pethidine can be absorbed into biochar passively into the intestine. Fourth, biochar can take compounds that diffuse between blood and urine. Biochar or charcoal can absorb toxins in the digestive tract (series connected organs). Each charcoal adsorbent capacity on mycotoxins is different, such as the adsorption of ochratoxin A from 0.80 to 99.86% and 98.93% deoxynivalenol ([Galvano *et al.*, 1998](#)).

The longer the villi in the intestine, the larger the cross-sectional area of the villi; therefore, nutrient

absorption is maximized. The charcoal is able to absorb ammonia and also removes toxins and impurities from the digestive tract that interfere with fish health. Charcoal generally refers to the carbon residue from wood, bamboo, cellulose, coconut shells or various industrial wastes that remain after heating organic matter in the absence of oxygen ([Firdus *et al.*, 2020a](#); [Firdus *et al.*, 2020b](#)). Charcoal is an odorless, tasteless black powder that works to absorb various toxins, gases without any specific action. Because it has a large surface area, charcoal has high absorption capacity.

One of the materials that can be used as charcoal is fish bones and the triggerfish (*A. stellaris*) bone is not utilized much. This is because the bone texture is very hard, different from other fish bones, so it is usually ended up as waste. Using it as charcoal for feed can add the economic value of the bone. Fish bones generally contain the element carbon so they are very good for charcoal.

Several studies that have utilized fish bone charcoal, among others are the use of biochar in feed provided a significant difference in the growth of tilapia in terms of weight and length during the cultivation period in the control treatment increased body gain by 1% while the one given biochar was able to increase growth. 1.25 times the contribution of coal ([Najmudeen *et al.*, 2019](#)). And also, the research that has been done on conjoined catfish (*Pangasius hypophthalmus*) ([Lan *et al.*, 2016](#)). The fish growth rate was increased by 36% by adding biochar to feed and by 44% by adding charcoal. Increasing the specific growth rate in this study with 1% biochar feed converted to 55%, it is assumed that biochar has a very large surface area depending on the production method ([Lehmann and Joseph, 2009](#)). The bamboo charcoal supplements at 5 g / kg feed significantly improve the growth performance of flatfish (*Paralichthys olivaceus*) in the juvenile stage that affects the level of digestibility characterized by an increase in fish body protein ([Thu *et al.*, 2010](#)). The purpose of this research is to study the effect of giving charcoal on the intestinal villi, the growth of tilapia and to determine the best concentration of adding charcoal in feed.

Materials and Methods

Tools and materials

The main raw material used as charcoal is triggerfish bones waste obtained from Fishing port Lampulo, Banda Aceh City. Meanwhile, the juvenile tilapia, used as test animals measuring 5-7 cm, were obtained from the Jantho Fish Juvenile Center (BBI), Aceh Besar. Other ingredients used are

commercial fish feed and fish oil. The tools used during the study were a furnace, scale, gauge, an aquarium equipped with an aerator installation and a Surface Area Analyzer (NOVA Quantachrome Version 11.0)

Making charcoal

The fish bones are washed clean and then boiled for 30 minutes. Next, it is dried in the sun for 3-4 days until it's dry and brittle so it's easy to crush. The dried fish bones were then heated using a furnace at 600° C for 2 hours. then the charcoal was characterized using the method de BET (Brunauer Emmett Teller). The method used in this study is a modification of Handayani et al. (2020) and Husna et al. (2020).

Preparation of containers and test animals

The containers used in this study were 8 aquariums (60x40x40 cm). Before being used the container is cleaned, fitted with aeration to maintain the water then filled with 72 liters of water. The water is from the well nearby. Prior to be using, the water is deposited for 7 days. The fish divided, 30 fish per aquarium after acclimated for 4 days and fasted for 24 hours to eliminate the food remains in the body.

Preparation of feed

The feed is commercial feed F999. Charcoal is added to the feed with different concentrations: 0%, 1%, 2%, and 3% of 500 grams of commercial feed and the feed is re-pelleted with the addition of 50% water, 1% CMC and 0.2% fish oil as an attractant then it was air dried for 2-3 days so the quality of the feed is maintained. Providing ad libitum (unlimited) feed with a frequency of 2 times a day, at 08.00 WIB and at 17.00 WIB. Fish samples were taken for histological tests in the beginning and the end of study. The test was carried out in the Veterinary Medicine laboratory of Syiah Kuala University while the fish sampling on weight and length gain was carried out at the beginning, middle and end of the study.

Research design

This study used the Completely Randomized Design method (non-factorial) with 4 treatments and 2 replications. The treatment design used in this study was the addition of triggerfish bone charcoal (*A. stellaris*) as a feed supplement in feed which can divided into A (without the addition of charcoal), B (addition of 1% charcoal), C (addition of 2% charcoal), D (additional of 3% charcoal). Charcoal is added to the feed with the re-pelleting method (modified from Handayani and Syahputra, 2018). The study was conducted for 45 days. Fish samples were taken for histological tests in the beginning

and the end of study but the fish sampling on weight and length gain was carried out at the beginning, middle and end of the study. While water quality data were measured on the first day of the study and the last day of the study.

Observation

Observation of the weight and length of tilapia was carried out at the beginning of research, middle and at the end of the study. Meanwhile, intestinal villi observations were carried out at the beginning and end of the study. The data can be obtained in the form of an overview of the intestinal villi of tilapia, growth in absolute weight and length, daily growth rate, feed conversion ratio and feed utilization efficiency.

Research parameters

Absolute weight gain (AWG)

The growth of the tested fish biomass can be calculated using a formula (Effendie, 1997; Putra et al., 2019), namely: $AWG = W_t - W_o$, where: AWG = Increase in fish biomass (g); W_t = Average weight of test fish at the end of the study (g); W_o = Average weight of test fish at the start study (g).

Absolute length gain (ALG)

Absolute length gain is the increase in length (difference end length and start length) during the rearing period. Absolute length gain can be calculated (Safriani et al., 2019; Putra et al., 2020): $ALG = L_t - L_o$, where: ALG = absolute length gain (cm); L_t = average length of fish at the end of maintenance (cm); L_o = average length of fish at the beginning of rearing (cm).

Specific growth rate (SGR)

The specific growth rate is calculated by the formula (Tacon, 1993) as follows: $SGR = (\ln W_t - \ln W_o) / t \times 100 \%$, where: SGR = specific growth rate (%); W_t = average fish weight at the end of study (g); W_o = average fish weight at the beginning of the study (g); t = time experiment (days).

Feed conversion ratio (FCR)

FCR is the value of the given feed efficiency. Feed conversion ratio using the formula (Tacon, 1993) as follows: $FCR = F / (W_t + D) - W_o$, where: FCR = feed conversion ratio; F = amount of feed given (g); W_t = weight of fish biomass at time t (g); W_o = weight of fish biomass at the beginning of maintenance (g); D = weight of dead fish biomass during maintenance (g).

Efficiency of feed utilization

The efficiency of feed utilization is calculated using the formula (Tacon, 1993) as follows: $EFU = ((W_t - W_o) / F) \times 100\%$, where: EFU = efficiency of feed utilization (%); W_t = final biomass at the end of the study (g); W_o = initial biomass at the

beginning of the study (g); F = total feed consumed during the study.

Data analysis

The data obtained in the study were analysed using ANOVA (Analysis of Variance) at a 95% confidence interval to test whether there is an effect between treatments. The data analysed using ANOVA were data on absolute weight growth (AWG), absolute length growth (ALG), specific growth rate (SGR), feed conversion ratio (FCR) and efficiency of feed utilization (EFU), while water quality data and intestinal villi length data were displayed in tabular form and picture then described descriptively. Then the data is presented in tabular and picture form.

Results

Charcoal characteristics

To find out the characteristics of the charcoal, the surface area and the pore characteristics, that will be used as a feed supplement BET analysis was carried out. The surface area of charcoal greatly affects the absorption rate; it will increase along with the loss of impurities that clog the pores of charcoal, through high temperatures heating. Heating at 600 °C causes the loss of impurities in the form of organic compounds present in fish bones such as fat and protein thus opening the pores of a charcoal. Data on surface characteristics and pores of charcoal are presented in Table 1.

Length of Intestinal Villi

The results of the study which was carried out for 45 days found that the length of the intestinal villi is as presented in Table 2.

Growth Parameters

The results of the study found the value of absolute weight growth (AWG), absolute length growth (ALG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Efficiency of Feed Utilization (EFU) as presented in Table 3.

Table 1. Charcoal Characteristics.

No.	Test Type	Result
1.	Surface area	35.841 m ² /g
2.	Pore radius	40.749 Å
3.	Micropore volume	0.202 cc/g
4.	Micropore surface are	48.839 m ² /g

Water quality parameters

The value of water quality parameters during fish cultivation is presented in table 4. The data were obtained at the beginning of the study and at the end of the study.

Table 2. Intestinal villi length.

Treatment	Intestinal villi length	
	Initial (µm)	End (µm)
A (0%)	162.22 µm	77.80 µm
B (1%)	162.22 µm	177.78 µm
C (2%)	162.22 µm	102.22 µm
D (3%)	162.22 µm	233.33 µm

Table 3. absolute weight growth (AWG), absolute length growth (ALG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Efficiency of Feed Utilization (EFU).

Parameter	Treatment			
	A (0%)	B (1%)	C (2%)	D (3%)
AWG (gram)	10.4±2.40 ^a	10.0±1.53 ^a	10.5±2.05 ^a	13.6±1.19 ^a
ALG (cm)	2.95±0.57 ^a	3.1±0.39 ^a	3.0±1.20 ^a	3.8±0.64 ^a
SGR(%)	3.6±0.67 ^a	3.5±0.26 ^a	3.2±1.42 ^a	4.1±0.18 ^a
FCR	1.98±0.63 ^a	1.92±0.25 ^a	3.36±0.73 ^a	1.12±0.009 ^b
EFU (%)	49.8±1.6 ^a	52.6±3.8 ^a	30.8±2.3 ^a	89.6±0.8 ^b

Description: The value listed is the average value -rata ± standard deviation. Different superscript letters on the same line show significantly different values (p < 0.05).

Table 4. Water quality parameters.

Treatment	NH ₃ (mg/l)		Temperature (°C)		pH	
	early	end	early	end	early	end
	A	0.045	0.40	27	26	6
B	0.045	0.24	27	27	6	5
C	0.045	0.51	27	27	6	5
D	0.045	0.15	27	26	6	6

Discussion

Based on the results of the analysis of the length of the intestinal villi in the Table 2 and Figure 1, it shows that the highest length of intestinal villi is in treatment D 233.33 µm followed by treatment B of 177.78 µm then treatment C is 102.22 µm. The shortest is in treatment A, which is 77.80 µm. The charcoal addition in the feed can optimize the digestive tract (Pirarat et al., 2015).

Treatment D resulted in the intestinal villi length of 233.33 µm. The length of the intestinal villi in this treatment indicated that the addition of triggerfish bone charcoal in the feed could improve growth performance and improve intestinal morphology of tilapia. In addition, the intestines also play a role in regulating water and electrolyte balance as well as immunity which can be seen from the picture of the blood. This is in accordance with the results of previous research, the addition of activated charcoal to feed has an effect on the blood, leukocytes, erythrocytes, hemoglobin, platelets and hematocrit of tilapia (Azhari et al., 2020).

Charcoal contains 85-95% carbon which has a large surface area and has pores that function to

maximize nutrient absorption and increase fish growth; therefore, it can make fish healthier, increase nutrient absorption and increase feed efficiency. Based on observations on the intestinal epithelial tissue, it shows that the intestinal microvillies of fish fed with charcoal supplemented feed develop more than the intestines of fish that are not fed with charcoal supplements. This is supported by the results of other similar research by (Risna et al., 2020) which stated that added activated charcoal to fish feed and succeeded in increasing the length of the intestinal villi to 182.22 μm from the initial length of 162.22 μm . easel at 344.44 μm . And also, the study by (Pirarat et al., 2015) which stated the addition of charcoal as much as 2% and 3% on feed

will increase the length of the intestinal villi to 107.27 μm and 126.01 μm .

In other treatments the intestine is not developing properly, this can occur due to the unoptimized provision of charcoal so the area of nutrient absorption capacity is smaller which results in a decrease in growth performance. The low growth of intestinal villi can affect the absorption rate of food which results in decreased water quality. This, also supported by the levels of ammonia (NH_3) in water at treatment C 0.51 ppm. Ammonia, temperature and pH are important factors in water quality. If the three of them are less supportive, it will affect the health of the fish which also affects the morphology/ intestinal performance of the fish.

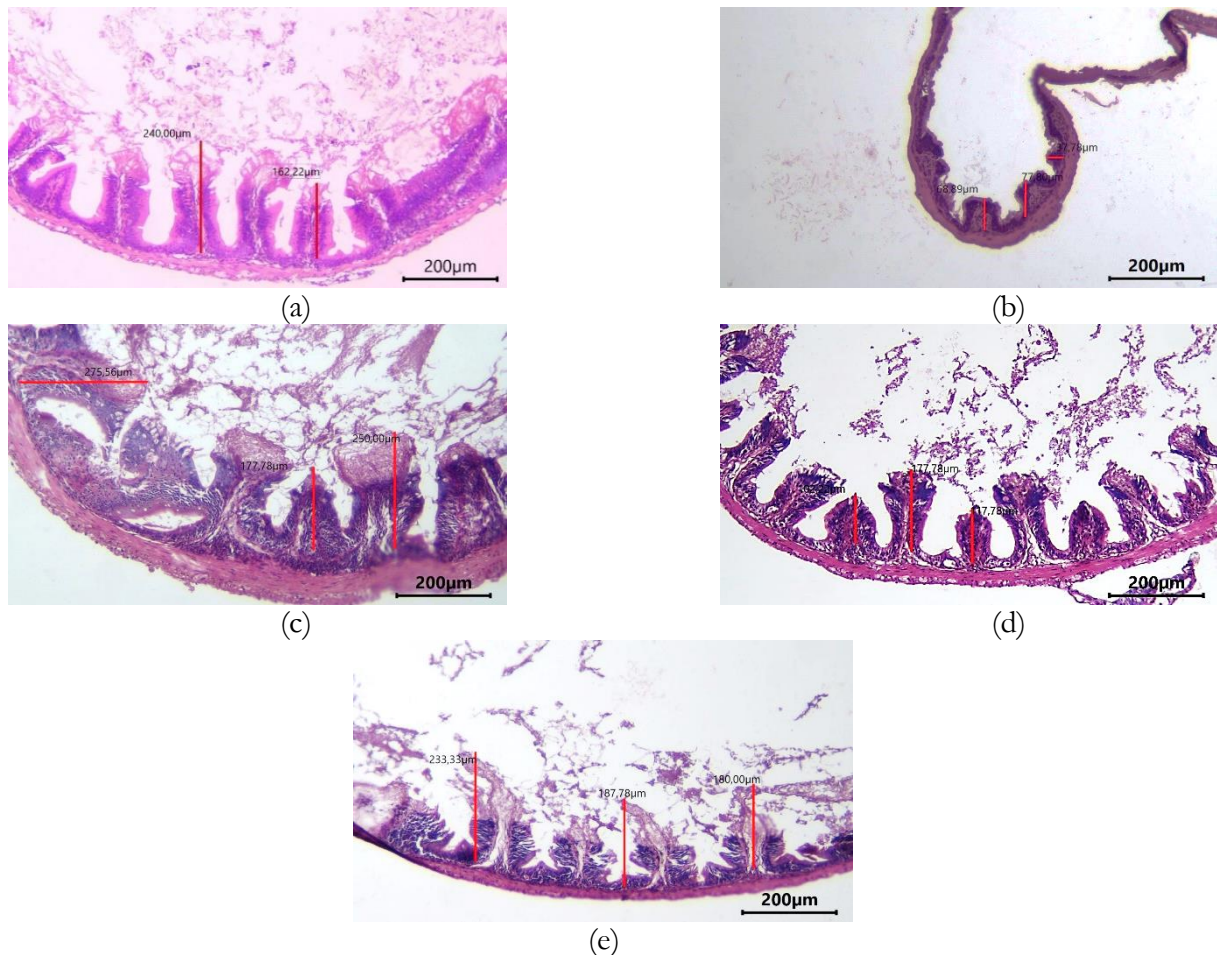


Figure 1. (a) Initial (b) Treatment A with different cutting preparation (c) Treatment B (d) Treatment C (e) Treatment D. Bar scale 200 μm . magnified 40x.

Whereas in treatment A, without the addition of charcoal, there was no additional growth in the length of the intestinal villi. This was due to the absence of the addition of charcoal into the feed so that it did not have an effect on changes in the length of the intestinal villi of tilapia. The length of the intestinal villi in treatment A after 45 days

period was 77.80 μm . The length of the intestinal villi is shortened due to several factors: the gut health itself which greatly impacts the adsorption (absorption) of food, quality of feed (quality of nutrition / nutrient content, density of food). Therefore, the intestine can shrink because of the density of the food making it difficult to digest.

Based on the observation result made during the 45 days, the growth of the absolute weight the highest found in treatment D amounted to 13.6 ± 1.19 grams. The height growth in treatment D caused by the presence of charcoal that can absorb mucus in the formation of biofilm surface epithelium; thus, the nutrients is not inhibited which reduce the use of feed by bacteria based on research by (Mekbungwan et al., 2004). The feed containing charcoal makes the intestinal villi elongate compared to control. Nasir (2002) states that the longer the villi in the intestine, the larger the cross-sectional area of the villi. Therefore, the nutrient absorption will be maximized. Charcoal able to absorb the metabolism residue that damage the health of the fish so toxins were not absorbed in the intestine that affects the growth. The values of absolute weight growth in other treatment are very low which is caused by the fish deficiency in digesting and the feed.

Based on the results of ANOVA test and Duncan test, shows that the evaluation of the addition of charcoal triggerfish (*A. stellaris*) bone as a feed supplement to histology intestine of tilapia (*O. niloticus*) was not significantly different in weight growth absolute length growth and absolute growth rate daily ($p > 0.05$). However, similar to the results of absolute weight growth, absolute length growth and daily growth rates in treatment D also had higher values than other treatments. This is also supported by the feed conversion ratio in treatment D which has the best value compared to other treatments.

The low feed conversion ratio in treatment D was due to the good absorption feed by the fish. In addition, charcoal also be able to control and clean up ammonia, and nitrogen, absorb toxins, the toxins released by microbes such as *Aspergillus* spp, and activate bowel function so that the absorption will feed can have an effect on decreasing feed conversion (Mekbungwan et al., 2004). Based on the ANOVA test results and Duncan's continued test, it was shown that the evaluation of the triggerfish (*A. stellaris*) bone charcoal addition as a feed supplement to the intestinal histology of tilapia (*O. niloticus*) was significantly different in the feed conversion ratio ($p < 0.05$). The feed conversion ratio of Treatment D significantly different compare to treatments A, B and C. This is also in sync with the value of feed utilization efficiency which shows that D treatment has the best results compared to other treatments.

Table 4 shows the lowest ammonia value was in treatment D, 0.15 mg / l. The high and low level of ammonia presence occurs due to the influence of

the charcoal used. In treatment D, a high growth rate indicates that the feed given can be digested properly; therefore, no leftover undigested feed is released by the fish. The feed given contains high protein so when the fish intestines cannot properly absorb it, it will be re-secreted. The secreted feed has decomposed into NH_3 , CH_4 , CO_2 , and H_2S . This is what happened in other treatments, high NH_3 values adversely affects growth values. This is because the intestines are not able to absorb the feed properly. High ammonia will be toxic to biota which will cause death or inhibit growth.

Temperature is a factor that plays a role in the cultivation environment because temperature is very influential for the growth of organisms. Based on Table 4, the temperature during the study ranged between $26-27^\circ\text{C}$, which means that the temperature fluctuation was not too high and still within normal limits $25-30^\circ\text{C}$ (BSNI, 2009). The high and low temperature of the maintenance medium will affect the metabolic process so that it can affect the ammonia concentration. The temperature of the maintenance medium was between $26-27^\circ\text{C}$ which indicates that the metabolism process was going well. The ideal pH value for the growth of aquatic biota is between 6.8- 8.5 (BSNI, 2009). A low pH will cause the solubility of metals in water to be higher which will be toxic to aquatic organisms, while a high pH will cause ammonia levels in the water to be higher and will also be toxic to organisms in the water.

Conclusion

The addition of triggerfish bone charcoal as a feed supplement can increase the length of the intestinal villi. The addition of 3% charcoal was the best treatment which gave the lengthiest of villi which was $233.33 \mu\text{m}$ and significantly increased efficiency of feed utilization (89.6%), feed conversion ratio (1.12%).

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