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Utilization of fish bone charcoal in feed on growth and physiological responses of catfish fry *Clarias gariepinus*

Pemanfaatan charcoal tulang ikan pada pakan terhadap pertumbuhan dan respons fisiologis benih ikan lele *Clarias gariepinus*

Rizqiyatul Fardila Putri, Mia Setiawati*, Dedi Jusadi, Julie Ekasari

Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia

*Corresponding author: miasetia@apps.ipb.ac.id

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ABSTRACT

This study aimed to assess the impact of charcoal inclusion in feed on the physiological responses and growth of catfish. The experiment employed a completely randomized design (CRD) with five treatments and four replicates. Charcoal doses in the feed were set at 0% (C0, control), 0.5% (C0.5), 1% (C1), 2% (C2), and 3% (C3). The catfish used weighed 3.95 ± 0.03 g and were kept in 20 hapa units (2×1×1 m^3) with a stocking density of 70 fish/hapa for 60 days. The results indicated significant differences ($p<0.05$) in the hepatosomatic index between treatments, with the highest values recorded in C1 (2.15 ± 0.10b) and C0.5 (1.91 ± 0.19b). However, liver glycogen levels did not significantly vary across treatments ($p>0.05$). Charcoal supplementation enhanced blood mineral levels (calcium, phosphorus, manganese, and zinc) in the C2 and C3 groups. The highest blood glucose level was observed in C1 ($p<0.05$). Intestinal histological analysis showed that the highest villi height and surface area were recorded in the C2 group, with values of 540.0 ± 10.2 µm and 34.122 ± 1.311 $µm^2$, respectively. Additionally, 2% charcoal supplementation improved final weight, daily growth rate, and protein retention, while reducing feed consumption and the feed conversion ratio compared to the control. Overall, the inclusion of 2% charcoal positively influenced the intestinal histology of catfish, contributing to enhanced growth performance. The study also demonstrated that charcoal addition affected the hepatosomatic index, blood glucose, and blood mineral levels in catfish.

Keywords: catfish, charcoal, growth, physiological response

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian charcoal dalam pakan terhadap respons fisiologis dan pertumbuhan ikan lele. Penelitian dirancang dengan Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan empat ulangan. Perlakuan dosis charcoal yang yang berbeda yaitu 0 (C0, kontrol), 0.5 (C0,5), 1 (C1), 2 (C2), dan 3% (C3). Ikan lele yang digunakan berbobot 3,95 ± 0,03 g/ekor dipelihara di hapa berukuran 2×1×1 m^3 sebanyak 20 unit dengan kepadatan 70 ekor/hapa selama 60 hari. Hasil penelitian menunjukkan hepatosomatik indeks beda nyata antar perlakuan ($p<0,05$) nilai tertinggi terdapat pada perlakuan C1 2,15 ± 0,10b dan C0,5 1,91 ± 0,19b. Penambahan charcoal pada pakan tidak berbeda nyata terhadap glikogen hati ($P>0,05$). Penambahan charcoal dalam pakan dapat meningkatkan kandungan mineral darah ikan (kalsium, fosfor, mangan, dan zinc) pada perlakuan C2 dan C3. Nilai glukosa darah tertinggi pada pakan yang diberi pakan C1 ($p<0,05$). Hasil histologi usus menunjukkan nilai tertinggi pada tinggi vili dan luas permukaan vili usus terdapat pada perlakuan C2 (2% charcoal) dengan tinggi vili (540,0 ± 10,2 µm) dan luas permukaan vili (34122 ± 1311 µm). Penambahan charcoal 2% pada pakan dapat meningkatkan bobot akhir, laju pertumbuhan harian, retensi protein, menurunkan konsumsi pakan dan feed convertion ratio dibandingkan kontrol. Pemberian charcoal 2% dalam pakan berpengaruh baik terhadap histologi usus ikan lele, sehingga dapat meningkatkan pertumbuhan pada perlakuan C2. Pada penelitian ini juga di temukan bahwa penambahan charcoal dalam pakan berpengaruh terhadap indeks hepatosomatik, glukosa darah, serta mineral darah ikan lele.

Kata kunci: *charcoal*, ikan lele, pertumbuhan, respons fisiologis

INTRODUCTION

The cultivation of catfish (*Clarias* sp.) is one of the most popular freshwater aquaculture practices among the community due to its relatively simple process, wide market demand, and high protein content (Rihi, 2019). Over a four-year period (2018–2022), catfish production experienced an average annual growth rate of 10.90% (DJPB, 2022). Catfish farming typically involves juvenile fish with an initial size of 4–5 cm, which reach harvest size after a maintenance period of two to three months (Manik *et al*., 2022). During the rearing period, proper management practices are essential to enhance production efficiency and optimize feed absorption. Effective feed management is necessary to minimize losses caused by inefficient or excessive feed usage (Kurniawan, 2019).

Feed plays a significant role in fish growth. Insufficient feeding can make fish more susceptible to diseases and may fail to meet their basic metabolic needs, leading to growth inhibition, reduced development, and even mortality (Tangguda *et al*., 2022). Aminullah (2019) explained that larger fish require less feed, while smaller fish require more. This is because smaller fish are in a rapid growth phase, necessitating a higher feed intake compared to larger fish. Hence, understanding the relationship between fish size and feed requirements is crucial for achieving optimal growth and ensuring sustainable aquaculture practices.

Feeding habits and the nutrient content of feed are very critical factors that need to be considered in aquaculture practices. In addition to nutrient composition, the ability of fish to digest and absorb feed must also be taken into account, as these factors significantly influence growth and reduce the feed conversion ratio. Impaired nutrient absorption often results from the suboptimal physiological functioning of the digestive system in fish, such as in catfish (*Clarias* sp.). The digestive system is closely related to nutrient absorption within the fish's body, and efficient intestinal performance enhances nutrient uptake (Risna *et al*., 2020). Increased intestinal length and villi surface area facilitate greater nutrient absorption, leading to higher nutrient assimilation and faster growth (Yu *et al*., 2016).

The larger the surface area of intestinal villi, the greater the potential for nutrient absorption in the digestive tract. In order to improve feed quality and enhance nutrient absorption, the use of feed additives is a viable strategy. One such additive is fishbone charcoal derived from striped catfish (*Pangasius* sp.), which can be incorporated into fish feed to improve its digestibility and nutrient uptake efficiency. Charcoal acts as an adsorbent with high absorption capacity, making it effective in enhancing nutrient uptake in fish. Striped catfish (*Pangasius* sp.) bones, a byproduct of fillet processing plants, contain carbon, making them a suitable material for charcoal production. Charcoal can be produced from both plant-based materials and animal bones (Siregar *et al*., 2015).

The high availability of striped catfish bones at fillet processing plants in Karawang offers a significant opportunity to add economic value to this waste by converting it into charcoal. Several studies have explored the use of fish bones for charcoal production. For instance, Siswati *et al*. (2015) produced charcoal from tuna bones, while Nurhadti *et al*. (2018) utilized belida fish (*Chitala* sp.) bone waste for the same purpose. Thu *et al*. (2010) reported that charcoal enhances feed utilization by normalizing cell membranes in the intestine and reducing intestinal surface tension, thereby improving nutrient absorption. This innovative approach not only optimizes waste management but also contributes to sustainable aquaculture practices by increasing feed efficiency and promoting fish growth. According to Pirarat *et al.* (2015), the addition of 2% activated charcoal to Nile tilapia feed enhances growth, reduces the feed conversion ratio, and positively influences intestinal morphology.

Similarly, Aderolu *et al*. (2016) reported that incorporating 2.5% rice husk charcoal into catfish feed improves weight gain and growth rate. Charcoal's adsorption of harmful organic compounds can enhance fish growth, immunity, and meat quality (Boonanuntanasarn *et al*., 2014). Previous study by Mabe *et al*. (2017) demonstrated that supplementation with 4% bamboo charcoal in common carp feed has the potential to improve blood serum parameters, intestinal function, and fatty acid composition. Additionally, Boonanuntanasarn *et al*. (2014) found that adding charcoal to feed increases mineral levels (calcium, chloride, and iron) in the blood of Nile tilapia. Based on the studies above, nutrient absorption in fish is heavily influenced by the physiological function of the digestive system, particularly in catfish (*Clarias* sp.). Efficient and effective nutrient absorption can reduce production costs, including feed and maintenance expenses. Therefore, incorporating fishbone charcoal into feed is expected to improve the physiological response and growth performance of catfish juveniles.

MATERIALS AND METHOD

Time and location of research

This study was conducted from December 2022 to February 2023 at the Fish Nutrition Laboratory, Department of Aquatic Cultivation, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University.

Research design

This study employed an experimental method, with a Completely Randomized Design (CRD) consisting of five treatments and four replications. The supplementation levels were selected based on the study by Quaiyum *et al*. (2014), with treatments as follows: C0 (control), C0.5 (0.5% charcoal/kg), C1 (1% charcoal/kg), C2 (2% charcoal/kg), and C3 (3%).

Research procedures

Preparation of fishbone charcoal

Striped catfish (*Pangasius* sp.) bone waste was boiled in water for 30 minutes to remove any remaining soft tissue from the bones. The fish bones were then burned in a furnace at 600°C for four hours. Afterward, the charcoal was ground to a size of 74µm. The charcoal was then activated using an acid solution, with 10 g of carbonized fish bones mixed with 100 mL of 0.1 N acetic acid (CH3COOH). The mixture was left to stand for 12 hours at room temperature, followed by filtration to separate the filtrate from the residue using filter paper. The charcoal was then dried in an oven for five hours at 150°C. Finally, the charcoal was analyzed for its ash content and mineral composition.

Preparation of feed

The feed used in this study was a formulated feed to which charcoal was added at varying concentrations of 0%, 0.5%, 1%, 2%, and 3%, as shown in Table 1. The charcoal was mixed with all

Table 1. Formulation of the test feed containing charcoal at different doses.

Raw materials	**Charcoal doses(C) %**				
	C0	**C0.5**	**C1**	**C2**	**C3**
Fishmeal	5	5	5	5	5
Soybean meal	25	25	25	25	25
Meat bone meal	5	5	5	5	5
Poultry by-product meal	13	13	13	13	13
Wheat pollard	20	20	20	20	20
Wheat flour	20	20	20	20	20
Tapioca flour	8	7.5	7	6	5
Fish oil	2	2	2	2	2
Premix	2	2	2	2	2
Charcoal	0	0.5	1	2	3
Proximat analysis results (%)					
Protein	30.51	30.36	30.94	30.42	30.59
Fat	5.08	5.84	6.07	6.09	6.54
Moisture content	10.00	10.50	9.40	9.40	9.50
Ash content	10.00	10.61	11.37	12.57	13.26
Crude fiber	4.04	4.06	4.05	4.08	4.11

[1]NFE = Nitrogen-free extract; [2] GE = *Gross energy* 1 g protein = 5.6 kkal GE, 1 g carbohydrate/NFE = 4.1 kkal GE, 1 g fat = 9.4 kkal GE (Watanabe, 1988); [3] C/P: refers to the ratio of energy (in kcal GE) to the protein content of the feed.

other raw materials and thoroughly blended until homogeneous. The mixture was then pelletized and dried in an oven for 8 hours at 50°C. The test feed was subsequently analyzed for proximate composition to determine its nutrient content. The feed was then stored at room temperature.

Preparation of fish container

The fish used in this study were catfish (*Clarias* sp.) obtained from the Balai Besar Perikanan Budidaya Air Tawar (BBPBAT) Sukabumi. The fish were maintained in hapa nets with dimensions of 2×1×1 m³, which were placed in a concrete pond lined with HDPE (High-Density Polyethylene) with dimensions of 20×10×1.5 m³, located at the Experimental Pond of the Department of Aquatic Cultivation, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The stocking density was 70 fish per hapa, with an initial size of 7-8 cm, and they were maintained for 60 days. The fish were fed twice a day at 08:00 and 17:00 WIB ad libitum. The maintenance system used was stagnant water without aeration.

Water quality measurements

The water quality parameters measured during the maintenance period included total ammonia nitrogen (TAN), temperature, and pH. Temperature measurements were taken three times a day (morning, noon, and evening). pH and TAN were measured once a week. The results showed that during the 60-day maintenance period, the water temperature ranged from 27-30°C, pH ranged from 6.8-7.4, and TAN ranged from 0.05-0.15.

Tested parameters

The parameters evaluated in this study including feed consumption, feed conversion ratio (Huisman, 1987), survival rate (SR), daily growth rate (Huisman, 1987), protein retention (Takeuchi, 1988), hepatosomatic index, liver glycogen, blood glucose, blood chemical analysis, histological analysis of the intestine using hematoxylin and eosin staining.

Data analysis

The obtained data were tabulated using Microsoft Excel 2013 software. Data analysis was performed using analysis of variance (ANOVA) with SPSS version 20. If the results were found significantly differences, further analysis was conducted using Duncan's multiple range test with 95% confidence interval. The mineral blood parameters and organoleptic analysis were descriptively analyzed and tabulated using software, to be presented in narrative form.

RESULTS AND DISCUSSION

Results

Hepatosomatic index and liver glycogen

After 60 days of feeding with different charcoal doses, the highest HSI values were found in the C1 and C0.5 treatments, which were significantly different ($p<0.05$) from the other treatments. The liver glycogen values did not show significant differences ($p<0.05$) across the treatments, as shown in Table 2.

Blood biochemistry

The results of blood mineral analysis (calcium, phosphorus, zinc, manganese) with the addition of fish bone charcoal in the feed showed significant differences between treatments. The analysis of calcium, phosphorus, zinc, and manganese in the blood indicated the highest values in the 3% treatment, while the lowest values were found in the control group. Table 3 shows that the blood glucose analysis yielded significant differences ($p<0.05$), with the highest value in the C1 treatment (106.39 ± 2.47 mg/dL) and the lowest in the C0.5 treatment (76.16 ± 12.68 mg/dL).

Table 2. Hepatosomatic index and liver glycogen in catfish after being fed with treatments containing different charcoal doses for 60 days.

Parameters	Charcoal doses (%)				
	C0	C0.5	C1	C2	C3
HSI (%)	1.70 ± 0.15[a]	1.91 ± 0.19[b]	2.15 ± 0.10[b]	1.75 ± 0.07[ab]	1.81 ± 0.13[ab]
Liver glycogen (mg/100 mL)	0.62 ± 0.9	0.54 ± 0.04	0.62 ± 0.11	0.60 ± 0.07	0.63 ± 0.04

Note: The mean values ± standard deviation (n=3) followed by different superscript letters on the same row indicate significant differences ($P<0.05$). HSI: Hepatosomatic index.

Histology of the instestines

The addition of fish bone charcoal in the feed had a significant effect on the increase in the height and width of the villi in the intestines of catfish (p<0.05). The histological parameters of the catfish intestine are presented in Table 4. The highest values for villus height and surface area were found in the C2 treatment (2% charcoal), with a villus height of 540.0 ± 10.2 µm and a villus surface area of 34.122 ± 1.311 µm.

Table 3. Glucose and blood mineral concentrations in catfish after being fed with different feed added different charcoal doses for 60 days.

Parameters	**Charcoal doses (C) %**				
	C0	**C0.5**	**C1**	**C2**	**C3**
Calsium (Ca) mg/dL	12.89	14.35	14.93	15.22	15.57
Phosphorus (P) mg/dL	3.37	7.12	7.26	7.46	7.53
Zinc (Zn) mg/dL	0.58	0.73	0.75	0.78	0.81
Manganese (Mn) mg/dL	0.06	0.09	0.10	0.10	0.10
Blood glucose (mg/dL)	80.27 ± 1.19^{a}	76.16 ± 12.68^{a}	106.39 ± 2.47^{b}	100.37 ± 5.89^{b}	83.65 ± 2.55^{a}

Note: The mean values ± standard deviation (n=3) followed by different superscript letters on the same row indicate a significant difference (P<0.05).

Table 4. Villus height and surface area of the catfish intestine after being fed with different doses of fish bone charcoal for 60 days.

Treatments	Villus height (µm)	Surface area (µm)
C0	435.8 ± 23.7^{a}	22052 ± 2391^{a}
C0.5	444.0 ± 20.0^{a}	25354 ± 2106^{ab}
C1	453.0 ± 11.9^{a}	27088 ± 6001^{bc}
C2	540.0 ± 10.2^{c}	34122 ± 1311^{c}
C3	497.7 ± 18.8^{b}	31866 ± 598^{bc}

Note: The mean values ± standard deviation (n=3) followed by different superscript letters on the same row indicate a significant difference (P<0.05) in the increase of villus height and surface area; C: charcoal (0, 0.5, 1, 2, 3%).

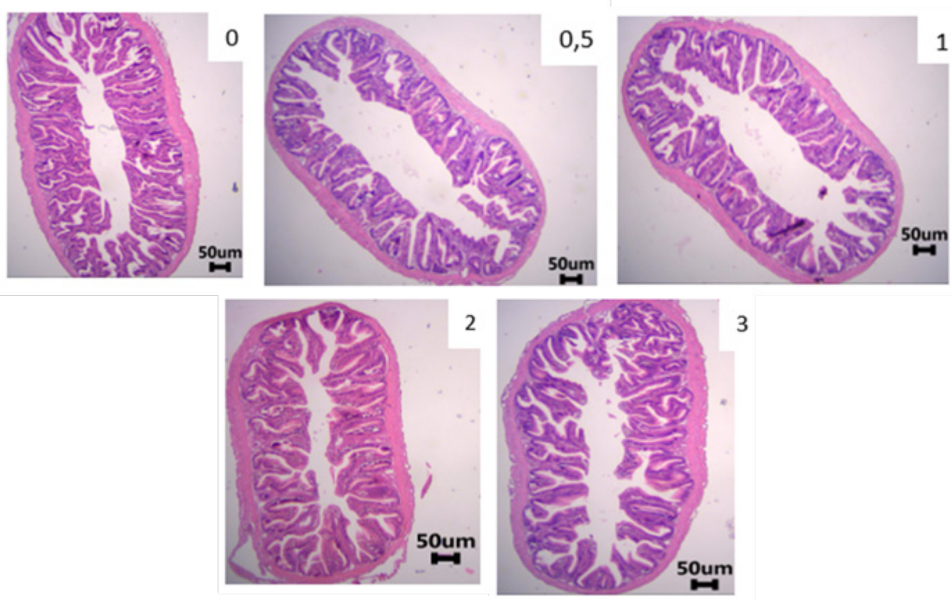

Figure 1. Histology of the intestine, with the following labels: 0: 0% control, 0.5: 0.5% charcoal, 1: 1% charcoal, 2: 2% charcoal, 3: 3% charcoal (hematoxylin and eosin staining).

Fish growth

After 60 days of feeding with treatments containing different levels of charcoal (C0: 0%, C0.5: 0.5%, C1: 1%, C2: 2%, C3: 3%), the results showed no significant differences in survival rate (SR) among treatments, with values ranging from 85.71% to 90.48%. The amount of feed consumption (FC) decreased with the addition of charcoal in treatments C2 and C3 ($p<0.05$), while treatments C0.5 and C1 showed no significant difference compared to the control. There was no significant difference between C2 and C3 in terms of feed consumption, but both were significantly different from the control. Daily growth rate (DGR) showed significant differences ($p<0.05$), with the highest value observed in the C2 treatment, followed by C3. The feed conversion ratio (FCR) was lowest in the C2 treatment ($p<0.05$) compared to other treatments. Retention values showed significant differences between treatments ($p<0.05$), with the highest value observed in C2, while the lowest was in the control treatment.

Discussion

The addition of charcoal to fish feed can significantly affect the hepatosomatic index (HSI), as shown in Table 2. The highest value was observed in the C1 treatment (2.15 ± 0.10%), while the lowest value was in the control treatment (1.70 ± 0.15%). Charcoal can influence the hepatosomatic index, which is the ratio between liver weight and body weight. HSI is commonly used as an indicator of liver health, as the liver plays a vital role in metabolism and detoxification. It is suspected that the addition of charcoal in the feed may absorb gases in the digestive tract, thereby reducing pressure on the liver organ of the fish. Elhetawy *et al.* (2023) stated that adding charcoal to the feed positively affects the digestive system and liver function in fish. The addition of charcoal in the feed impacts HSI, where higher HSI values indicate that the liver is absorbing more nutrients. HSI is often used as a growth indicator, as the liver is the center for nutrient metabolism in the body (Setiawati *et al.*, 2016).

The relationship between the hepatosomatic index and blood minerals plays an essential role in maintaining health and bodily functions. Minerals are inorganic elements that fish require as structural components of body tissues and fluids, contributing to metabolism and growth. Although the amount of minerals needed is minimal, they perform critical functions. Minerals also help regulate pH, osmotic pressure, signal transmission, and the active transport of glucose and amino acids (Jacoeb *et al.*, 2015). Calcium and phosphorus are macro minerals necessary for skeletal development, physiological function, and fish growth (Zimmer *et al.*, 2019).

Boonanuntanasarn *et al.* (2014) stated that the addition of bamboo charcoal to the feed affects blood mineral contents such as calcium, chloride, and iron. Phosphorus (P) is a high-energy component in the form of adenosine triphosphate (ATP), required for energy transduction and essential for all cellular activities. The analysis of calcium and phosphorus minerals in the blood of catfish, presented in Table 3, shows that as the dose of fishbone charcoal increases, the Ca/P ratio in the blood of the catfish also increases. This indicates that the absorption properties of charcoal with minerals can influence the electrolyte balance in the fish's plasma (Olson, 2010). During the growth phase, fish require more calcium and phosphorus for the formation of bones, teeth, and scales, for

Table 5. Growth performance of catfish after being fed with feed containing different doses of charcoal for 60 days.

Parameters	Charcoal doses (C) %				
	C (0)	C (0.5)	C (1)	C (2)	C (3)
W0 (gr)	3.95 ± 0.03[a]	4.00 ± 0.00[a]	3.98 ± 0.03[a]	3.98 ± 0.02[a]	3.97 ± 0.01[a]
Wt (gr)	56.56 ± 0.61[a]	54.35 ± 1.80[a]	53.96 ± 2.57[a]	65.96 ± 5.30[b]	56.12 ± 4.85[a]
JKP (gr)	5059.49 ± 38.54[b]	5125.68 ± 12.86[b]	5089.87 ± 35.39[b]	4955.02 ± 55.54[a]	4964.59 ± 44.25[a]
TKH (%)	85.71 ± 4.67[a]	90.48 ± 1.78[a]	89.52 ± 0.67[a]	88.10 ± 1.78[a]	89.52 ± 2.43[a]
LPH (%)	4.17 ± 0.10[a]	4.18 ± 0.03[a]	4.16 ± 0.07[a]	4.46 ± 0.11[b]	4.22 ± 0.19[ab]
FCR	1.53 ± 0.05[b]	1.57 ± 0.01[b]	1.53 ± 0.06[b]	1.26 ± 0.07[a]	1.46 ± 0.13[b]
RP (%)	33.10 ± 4.75[a]	34.47 ± 4.77[a]	35.84 ± 1.91[a]	47.53 ± 0.22[b]	37.65 ± 2.11[a]

Note: The mean values ± standard deviation (n=3) followed by different superscript letters on the same row indicate a significant difference (P<0.05); P: Charcoal, W0: initial fish weight, Wt: final fish weight, FC: feed consumption, DGR: daily growth rate, SGR: specific growth rate, FCR: feed conversion ratio, and PR: protein retention.

energy breakdown and production, and for body movement. A deficiency in phosphorus is marked by slow growth, low feed efficiency, and inhibited bone mineralization (Lall, 2021).

Furthermore, the increase in manganese and zinc levels in the blood of catfish can be observed in Table 3. Manganese helps activate enzymes that support the synthesis of glycosaminoglycans, which are essential for bone matrix formation, and supports protection against oxidative damage through antioxidant functions (Taskozhina *et al*., 2024). Manganese is also important in the regulation of enzymes that influence bone metabolism. This study indicates that manganese deficiency can hinder bone development, while its supplementation may potentially enhance bone strength. The zinc content in the blood of catfish can vary depending on the fish species and the aquaculture environment. High levels of zinc can stimulate growth and maintain the resilience of catfish juveniles as an essential metalloenzyme (Broto *et al*., 2017).

The high zinc content in animals is likely due to high zinc levels in the surrounding water. Zinc is an essential micronutrient that is crucial for various physiological processes, including bone homeostasis. Most zinc in the human body is stored in bones. Zinc is not only a component of bones but also a vital cofactor for many proteins involved in the stability of microstructures and bone remodeling. Two types of zinc transporter proteins in the membrane have been identified in mammals: the Zrt and Irt-like protein (ZIP) family and the zinc transporter (ZnT) family, which regulate zinc intake and export, responsible for the transport of zinc across cellular membranes and intracellular compartments to maintain zinc homeostasis in the cytoplasm and intracellular compartments (Huang *et al*., 2020). Zinc absorption in the body is influenced by body zinc status, feed type, excess copper, and low albumin and transferrin levels. Zinc deficiency in fish is characterized by cataracts, fin damage, skin disease, and stress, which can inhibit growth.

Glucose is found in the blood of fish, and its primary function is to be metabolized to generate calories or energy. The absorption of glucose from the intestines and the breakdown of glucose reserves in tissues occurs through glycogenolysis. After food consumption, carbohydrates are broken down into glucose in the intestines, then absorbed by transporters such as SGLT1 on the epithelial cells of the intestines. This transporter moves glucose from the lumen of the intestines into the bloodstream, resulting in increased blood glucose that provides energy for the body (Gromova *et al*., 2021). This result aligns with the findings of Amigo *et al.* (2020), who reported that consuming charcoal can increase blood glucose, but this increase does not have negative effects on the body. Under normal conditions, the body maintains glucose levels between 97.13–103.36 mg/dL (Purwanti *et al*., 2014).

All treatments resulted in average blood glucose values within the normal range, indicating that the fish remained healthy. Normally, glucose that is formed will enter cells, stimulating glycogenesis and lipogenesis. Increased blood glucose levels in fish often occur as a response to stress, particularly in conditions where fish require more energy to maintain osmoregulatory and homeostatic processes during exposure to environmental stress. Osmoregulatory activity requires energy to maintain ion and water balance, especially when fish adapt to fluctuations in salinity or exposure to toxic pollutants, which often increase energy demand and affect glycogen reserves as a short-term energy source (Kultz, 2015). This increase in glucose levels plays an important role in meeting the energy demands for activities such as osmoregulation, involving various energy processes that take place in the gills, liver, and muscles.

Charcoal is a universal adsorbent that affects physiology, digestive metabolism, and increases the surface area of intestinal villi in fish (Boonanuntanasarn *et al*., 2014). This study indicates that the addition of charcoal in the feed improved the histology of catfish intestines, particularly in the length and surface area of the intestinal villi. The histological results of the catfish intestines with the addition of fish bone charcoal in the feed are presented in Table 4. The highest villus height and surface area of the intestinal villi were found in the C2 treatment with a length of 540.0 ± 10.2 μm and a surface area of 34.122 ± 1.311 μm.

The addition of charcoal in the feed can enhance nutrient absorption by improving intestinal morphology, such as increasing the length and cross-sectional area of the villi, which is directly related to the efficiency of nutrient absorption. This mechanism is supported by charcoal's ability to act as an absorbent, capturing gases and toxins in the digestive tract, reducing the risk of harmful substance absorption, and thus improving nutrient assimilation. Nasir (2002) stated that the longer the villi in the intestines, the greater the surface area, thus allowing more efficient nutrient absorption. The use of charcoal in feed has gained attention due to its ability to absorb gases in the digestive tract, preventing their absorption into the body.

The gases formed in the fish's intestines result from biological and chemical processes in the digestive system. Charcoal can bind to toxins and anti-nutritional metabolites in the digestive tract, which can interfere with the absorption of nutrients essential for fish. Jiang *et al.* (2021) stated that the addition of bamboo charcoal to feed improves growth rates and nutrient utilization efficiency in Blunt Snout Bream fish. This is due to charcoal's ability to detoxify toxins in the intestines, which, in turn, improves nutrient absorption and supports better metabolism. Based on statistical tests, the amount of feed consumption differed significantly ($P<0.05$), suggesting that the addition of charcoal influenced feed palatability.

According to Subandiyono and Hastuti (2016) the senses of sight, smell and taste as well as the shape and smell of food are important for fish in finding the location and identifying the type of food. Additionally, the shape and size of feed tailored to a particular fish species can influence how the feed is swallowed and broken down, supporting a more efficient digestive process. The addition of 2% charcoal to the feed increased the daily growth rate of catfish, as shown in Table 5. The results indicate that the addition of charcoal to the feed significantly differed ($P<0.05$) from the control group. Growth enhancement was observed in the C2 (2%) treatment with an average of 4.46 ± 0.11 g. The higher daily growth rate in the C2 treatment is suspected to be due to the greater number of villi in the intestines compared to the control and other treatments. Histological examination revealed that the number of villi in the C2 treatment was higher.

In addition, the surface area of the intestines is related to nutrient absorption efficiency. The larger the intestinal villi surface area, the higher the potential for absorption in the digestive tract. Growth mechanisms are closely linked to the fish's digestive organs. The addition of charcoal in the feed can activate the function of the intestines in absorbing nutrients. Nurhayati *et al.* (2021) stated that charcoal has the potential to condition the intestinal cell membranes, reducing tension by removing gases and other harmful substances in the intestines, thus improving nutrient utilization and absorption. In the feed conversion ratio (FCR), the lowest value was found in the C2 treatment ($P<0.05$) compared to other treatments, and this corresponds with the highest daily growth rate (DGR) observed in the C2 treatment.

Tahapari and Darmawan (2018) stated that DGR is determined by the digestible and absorbable nutrient content in the feed, which contributes to the increase in fish length and weight. Pirarat *et al.* (2015) stated that the supplementation of 2% bamboo charcoal can improve growth and histomorphology of Nile tilapia intestines. The addition of charcoal in the feed is suspected to increase protein retention. Based on this study, the highest protein retention value was found in the C2 treatment, with a value of 47.53 ± 0.22%, while the lowest protein retention was observed in the control treatment at 33.10 ± 4.75%.

Michael *et al.* (2017) showed that the addition of 30g/kg charcoal to the feed improves survival, growth, feed conversion ratio, protein efficiency, and energy retention. Protein retention values can indicate the fish's ability to utilize feed nutrients as an energy source for activity or store them in the body for growth (Poernomo *et al*., 2015). High protein content in fish indicates that the feed's nutritional value is good, and the fish can digest the feed effectively, which leads to growth, as indicated by changes in body biomass and length during the rearing period. Thu *et al.* (2010) stated that charcoal supplementation in fish feed can improve digestibility, which affects the increase in protein content in the fish's body. Charcoal acts as a detoxifying agent in the digestive tract by absorbing toxins and reducing metabolites that hinder nutrient absorption. This allows nutrients, especially proteins, to be absorbed and utilized more efficiently, ultimately enhancing growth and the quality of the fish meat.

CONCLUSION

The addition of 2% charcoal in the feed positively influenced the histology of the catfish's intestines, leading to improved growth in the C2 treatment. This study also found that the inclusion of charcoal in the feed had an effect on the hepatosomatic index, blood glucose levels, and blood minerals of catfish.

SUGGESTIONS

It is recommended that further research be conducted on the use of fish bone charcoal in feed to assess its impact on the health status of catfish.

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