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Effects of Bamboo Charcoal Added Feed on Reduction of Ammonia and Growth of *Pangasius hypophthalmus*

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Abstract

A 50-day feeding trial was conducted to determine the effects of dietary bamboo charcoal (BC) on ammonia (NH_3-N) excretion and growth performances of *Pangasius hypophthalmus*. Four levels of BC (0%, 0.5%, 1% and 2%) were supplemented to the diet composition and fed to fish (initial body weight 1.18 ± 0.04 g) twice a day. At the end of the trial, mean of final weight (g), final length (cm), weight gain (g), length gain (cm), percent weight gain, percent length gain, specific growth rate (% per day), feed conversion ratio, survival rates and water quality parameters i.e, ammonia (NH_3-N), pH, and dissolved oxygen were measured and found that fish fed 2% BC diet showed significantly ($P<0.05$) higher growth enhancement than those of fish fed the control diet (0% BC). Ammonia concentration over the experimental period decreased with increasing dietary BC. Moreover, in histological observation it was found that the villus height and villus area in all intestinal segments tended to increase with increasing dietary BC supplementation. The present results indicate stimulating effects of dietary BC on intestinal villi and the diet supplemented with 2% BC was found to have a suitable level to fulfill the maximum growth performances of *P. hypophthalmus* and to decrease the ammonia concentration.

Keywords: Bamboo charcoal; Ammonia; Growth; *Pangasius hypophthalmus*

Introduction

There is a great potential for *Pangasius hypophthalmus* in Bangladesh. It is very much demandable in local markets because of its lower market price. The vast majority of poor people consume *P. hypophthalmus* as this fish is delicious and tasty due to its high fat content. Moreover, the climate, water and soil conditions of Bangladesh have proved totally suitable for *P. hypophthalmus* production and it is one of the most suitable catfishes for rearing in ponds [1]. *Pangasius* culture has proved itself as a profitable enterprise due to year round production, quick growth and high productivity. In addition, it can be stocked at a much higher density in ponds compared to other cultivable species [2].

However, costly feed and low market price has slowed progress in farming of this fish. In addition, due to high accumulation of nitrogenous waste products that is toxic to fish considered as a limiting factor for growth and survival of fish are affecting the culture of this species [3]. One of the sources of this nitrogenous waste (e.g. ammonia) is from the supplemented feed that fed to fish. An effective way to reduce the waste load is to modify aqua feeds with the aim of reducing excretion of nitrogen, phosphorus and total solids relative to fish growth [4,5]. Therefore, several efforts have been made to produce high quality animal products without using medicines and to reduce environmental contamination by efficient utilization of natural substances. Some of these natural substances (e.g. wood charcoal, bamboo charcoal, coconut shell charcoal etc.) are not cited in the scientific literature, but are used locally. Like wood charcoal bamboo charcoal (BC) is also an activated charcoal made by dry distillation of a thick-stemmed bamboo and powder of which is known as a universal adsorbent, because it can bind with variety molecules since it contains a complex network of pores of various shapes and sizes [6]. Now-a-days, BC has been used in animal feed formulation as an additive because they absorb ammonia and nitrogen, and activates the intestinal function through eliminating the poisons and impurities from the gastrointestinal tract of land animals [7,8]. Utilization of charcoal from wood or bamboo may provide an economical way to eliminate noxious substances because of their cheaper cost [9]. Moreover, BC is considered to have a higher adsorption capacity than wood charcoal because it has about 4 times more cavities, 3 times more mineral content and 4 times better absorption rate [6]. Reports have clarified the ammonia adsorption effect of BC in aqueous solution [10], and dietary addition of BC effects on digestion, nitrogen retention and excretion of growing goats [8]. They also found that goats fed a diet containing 0.5 g of BC per kg of body weight grew faster than the controls. Recently it was found that bamboo charcoal boosts tilapia growth [11]. However, very limited studies about BC in aquatic animal nutrition as a feed ingredient have been conducted.

Therefore, the major purpose in the present study was to assess the effectiveness of dietary BC supplementation on growth performance of *P. hypophthalmus* and elimination of ammonia nitrogen excretion from the water during study period.

Materials and Methods

Experimental site

The experiment was carried out in the Backyard Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. The experiment was conducted for a period of 50 days. The research work was undertaken in 13 glass aquaria (average capacity 50L). An adequate level of dissolved oxygen in each aquarium was maintained through artificial aeration during the experimental period.

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Preparation of BC

Bamboo was cut into small pieces and put into a tightly sealed container made of iron. This is then placed on a hot fire for at least an hour. Once the fire was out, the container left to cool down completely before it opened. The BC then pounded into a fine powder and the composition of BC is as follows in Table 1.

Feed formulation

Standard fish feed (Table 2) obtained from commercial feed company (Mega Feed Co. Ltd., Bangladesh) was used as a basal feed supplemented with BC powder at 0, 0.5, 1.0 and 2% in control, T_1, T_2 and T_3, respectively. Diets were prepared by mixing the dry ingredients and water (35% of the dry weight of ingredients) and then pellet-type diets were produced through a meat grinder with a diameter disc (size, 1.9-2.2 mm). The diets were later oven dried (60°C for 2 h) to approximately 11% moisture. After preparation, the diets were stored at refrigerator until used.

Fingerling collection and stocking of fish: Fingerling of *P. hypophthalmus* was collected from the local fish seed retailer and carefully transported to avoid physical injury. One fish per liter stocking density and three aquaria were used for each treatment.

Feeding rates: Fingerlings were fed with experimental diets twice a day in the morning at 9.00 am and in the afternoon at 3.00 pm throughout the study period. Fingerling in each aquarium were fed daily at the rate of 7% of their body weight; the amount was fixed after observing that it was not interested to take more than that amount of feed.

Sampling procedure: Every 10 d, the body weight of fish were measured. For weighing 5 fingerlings were collected from each aquarium. All animals were placed on paper towels to remove excess water and then weighed using an electric balance (Mettler PJ3000). After recording the length and weight of fingerlings were released in the aquarium.

Measurement of water quality parameters

All the following water quality parameters were recorded at 10 days interval.

Item	%
Ash	6.35
Nitrogen	0.57
Phosphate	1.06
Potassium	2.10
Silicon dioxide	1.20
pH	8.50

Table 1: Composition of BC powder.

Item	%
Moisture	10
Protein	28
Crude fat	6
Ash	18
Crude fiber	7
Calcium	1.8
Phosphorus	0.7
Gross energy (Kcal/Kg)	3400

Table 2: Ingredient and chemical composition of the basal commercial diet.

Ammonia: Ammonia (NH_3-N) of the aquarium water was recorded in mg/l with the help of ammonia test kit (Hanna Instrument Ammonia Test Kit for Fresh Water).

Dissolved oxygen: Dissolved oxygen of the aquarium water was recorded in mg/1 with the help of a dissolved oxygen meter (Model Oxi 3150i, Germany).

pH: pH of the water was recorded with the help of a pH meter (pH meter L20 ME 1-1 LER TOLEDO, Switzerland).

Histological observation of intestine

For the histological observation intestines were quickly removed from fish and place in 10% formaldehyde fixative solution. Then the anterior and middle central portions of the intestine of 0.5 cm thickness were put into the cassettes separately for histological examination. They were dehydrated in graded alcohol series, embedded in paraffin, sectioned for 5-7µm in thickness using a microtome (MICROM HM355S, Germany) and stained with Haematoxylin and Eosin, then mounted in DPX mountant and photographed with an OLYMPUS-CX41 microscope which was equipped with the SONY DSC-W220 camera. At least two glass slides were prepared from each portion of the intestine.

Data analysis

The collected data were statistically analyzed by one way ANOVA (analysis of variance) with the help of SPSS (Statistical Package for Social Science) to see whether the influence of different treatments on these parameters were significant or not.

Results

Water quality parameters

Ammonia: The ammonia (NH_3-N) values of water ranged from 0.02 ± 0.01 to 2.06 ± 0.12 mg/l during the study period. Significantly ($P < 0.05$) higher value of ammonia which is not good for fish was found in the control while lowest value which is suitable for fish was found in T_3 (2% BC) (Figure 1).

pH

The pH values of water ranged from 6.89 ± 0.11 to 7.81 ± 0.09 during the study period. The highest level of pH was found in the control while the lowest level of pH was found in T_3 (2% BC). There were significant differences ($P<0.05$) between the control and T_3. A proportional relationship between pH and ammonia (NH_3-N) concentration were also observed during the study period (Figure 2).

Dissolved oxygen

During the study period dissolved oxygen content of water ranged from 4.58 ± 0.32 to 5.97 ± 0.09 mg/l. The highest value 5.97 ± 0.09 mg/l was found in T_3 which was significantly ($P<0.05$) higher than the control. An inverse relationship between dissolved oxygen and ammonia (NH_3-N) concentration were observed during the study period (Figure 3).

Growth parameters

As shown in Table 3 the maximum growth enhancement was noticed at the 2% BC supplementation level. However, fish groups that received dietary BC from 0.5 to 2% level showed higher values of weight gain, SGR and FCR than the control. No significant differences in final length and length gain were observed between fish fed BC containing

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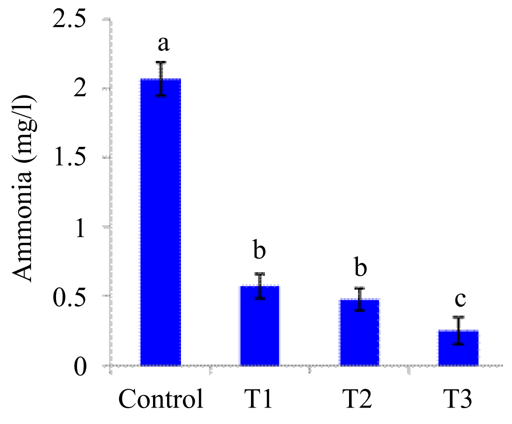

Figure 1: Ammonia (NH_3-N) concentration (mg/l) in different treatments during the study period. a, b, c means with different superscripts are significantly different from each other (P<0.05).

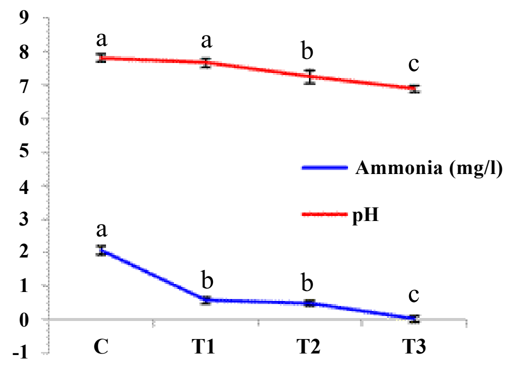

Figure 2: pHin different treatments during the study period. a, b, c means with different superscripts are significantly different from each other (P<0.05).

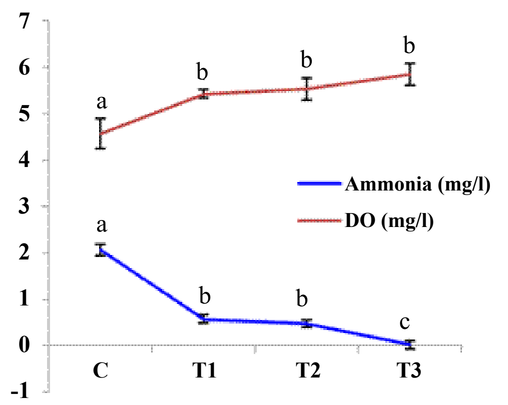

Figure 3: Dissolved oxygen (mg/l)in differenttreatments. Different superscript alphabets in each treatment group are significantly different at P<0.05.

diet. It was also seen that the survival rates of *P. hypophthalmus* under the trail treatments were significantly (P<0.05) higher than the control. In an experiment in the earthen pond our study showed that 1.5% BC supplementation level was suitable for the maximum growth enhancement (data not shown) (Table 3).

Values are presented as mean ± SE. Values in the same row having different superscript letters are significantly different (P<0.05). The lack of superscript letter indicates no significant differences among treatments.

Mean weight gain=mean final weight (g)-mean initial weight (g);

Mean length gain=mean final length (cm)-mean initial length (cm);

% Weight gain=(final body weight−initial body weight)×100/initial body weight;

% Length gain=(final body length−initial body length)×100/initial body length;

Specific growth rate (SGR)=(Log final body weight−Log initial body weight)×100/feeding period;

Feed conversion ratio (FCR) = feed fed/live weight gain;

Survival rate (%)=No. of fish harvested ×100/No. of fish stocked.

Histological observation

Intestine of *P. hypophthalmus* of all treatments had almost normal structure but there was slight change in the villus height and lumen area of those intestines. It was observed that villus height was increased and consequently lumen areas of those intestines were decreased with increasing BC supplementation level (Figure 4).

Parameters	Control	T_1	T_2	T_3
Initial weight (g)	1.17 ± 0.40	1.17 ± 0.40	1.17 ± 0.40	1.17 ± 0.40
Initial length (cm)	3.63 ± 0.15	3.63 ± 0.15	3.63 ± 0.15	3.63 ± 0.15
Final weight (g)	2.88 ± 0.03^{c}	3.02 ± 0.06^{c}	3.21 ± 0.03^{b}	3.67 ± 0.05^{a}
Final length (cm)	6.36 ± 0.29	6.79 ± 0.43	6.82 ± 0.16	7.10 ± 0.34
Weight gain (g)	1.71 ± 0.03^{c}	1.85 ± 0.06^{c}	2.03 ± 0.03^{b}	2.50 ± 0.05^{a}
Length Gain (cm)	2.73 ± 0.29	3.16 ± 0.43	3.19 ± 0.16	3.46 ± 0.34
% Weight gain (%)	145.32 ± 2.70^{c}	157.22 ± 5.58^{c}	172.80 ± 2.73^{b}	212.46 ± 4.71^{a}
% Length gain (%)	75.22 ± 7.99^{c}	87.06 ± 12.07^{b}	87.79 ± 4.63^{b}	95.41 ± 9.53^{a}
SGR (% per day)	0.73 ± 0.01^{c}	0.78 ± 0.02^{c}	0.84 ± 0.01^{b}	0.97 ± 0.01^{a}
FCR	3.44 ± 0.06^{d}	3.18 ± 0.11^{c}	2.89 ± 0.04^{b}	2.35 ± 0.05^{a}
Survival rate (%)	77.33 ± 0.88^{b}	84 ± 1.15^{a}	82 ± 0.57^{a}	84 ± 1.15^{a}

Table 3: Growth parameters of *P. hypophthalmus.*

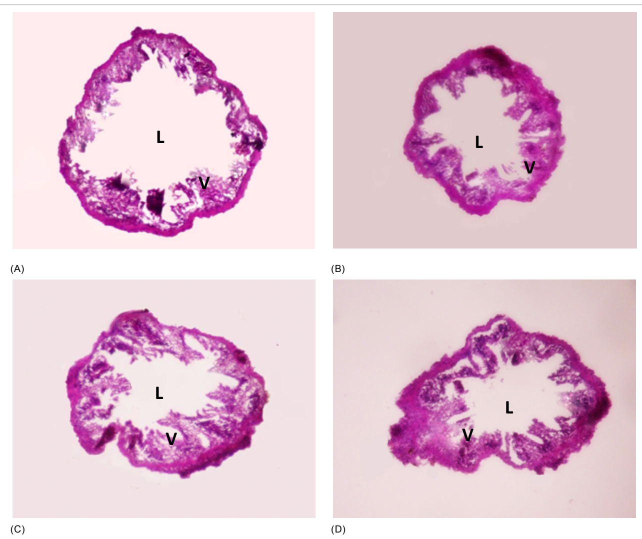

Figure 4: Histological section of the intestine of *P. hypophthalmus*collected from the (A) control; (B)T_1; (C)T_2; and (D) T_3. (V=villus, L=lumen)

Discussion

Water quality parameters

One important objective of this study is to determine whether the ammonia nitrogen could be reduced by dietary BC. Some studies have reported on the ammonia nitrogen excretion of Japanese flounder (*Paralichthys olivaceus*) [12-15]. In the present experiment, the maximum ammonia (NH_3-N) concentration was found in the control while minimum concentration was recorded at T_3 and concentration increased with decreasing BC supplementation level. This result showed the dose-related effect of dietary BC on ammonia concentration.

During the study period a relationship among ammonia, pH, and dissolved oxygen were observed. In the control, ammonia and pH values were high and DO was low. It was found that ammonia and pH were decreased but DO was increased with increasing BC supplementation level and the lowest value of ammonia was found in T_3 where the higher level of BC (2%) was supplied. These results indicated that the dietary BC supplementation could be a potential feed additive to eliminate the ammonia from the culture system.

Growth performances

Based on the growth data, the optimum dietary BC supplementation level for the *P. hypophthalmus* was found to be 2% of the diet. The maximum mean final weight was 3.68g in T_3 where higher level of BC was supplied. The minimum mean final weight was 2.89 g in the control where 0% BC was supplied. Again the maximum mean final length was 7.1 cm observed in T_3 and the minimum mean final length was 6.37 cm observed in the control. However, our experiment in the earthen pond indicated a little bit lower concentration of BC (about 1.5%) than the aquarium was the optimum for the growth of *P. hypophthalmus* and elimination of ammonia (data not shown). The present finding was less than that found in study where the highest weight gain was obtained at 4% BC supplementation in tiger puffer fish [15]. Although it was found 0.5% BC was suitable level for optimum growth of juvenile flounder [16]. These results indicated the species-related effect of dietary BC on growth, and it might be because of the differences in digestion and feeding behaviors of these species. In the Aigamo ducks [17] it was found that the highest mean final weight gain was in 1% SB (mixture of bamboo charcoal powder and bamboo vinegar solutions) group.

In the present experiment the mean weight gain of *P. hypophthalmus* in different treatments varied from 1.71 to 2.50 g. The highest mean weight gain was found in T_3 while the lowest mean weight gain was observed in the control. Again the highest mean length gain (3.46 cm) was found in T_3 and the lowest mean weight gain (2.73 cm) was found in the control which is almost similar to the findings of [16]. In white leghorn hens (*Gallus domesticus*) the highest weight gain was observed in case of 0.5% of BC supplementation [17] whereas in Aigamo ducks the mean weight gain of 1% SB group was 8% heavier than the control group [18].

The higher percent weight gain was found in T_3 and it was 212.46% while the lower percent weight gain was found in the control which was 145.32%. Again the higher percent length gain was 95.41% which was found in T_3 and the lower percent length gain was 75.23% which was found in the control. More or less similar type of percent weight gain was observed in Japanese flounder by [16].

The specific growth rates of our studied fish under different treatments were varied from 0.73 to 0.97%. SGR value was higher in T_3 where BC level was higher (2%) and SGR value was lower in control where fish reared without BC. SGR were observed 0.02 to 0.68% in *P. hypophthalmus* using 35-40% protein containing feed [19]. The result obtained in our study was much lower than that (3.09 to 3.51%) was found by [20] in Thai pangas. The FCR under different treatments ranged from 2.36 to 3.44. The improved performance of FCR was observed at 2% BC containing diet that is similar to the findings of [16,18].

Our findings indicated that the dietary BC supplementation could be a potential feed additive to enhance the growth of *P. hypophthalmus* and supports research in tiger puffer [15] and other studies that reported growth in goats [8], in broiler chicks [21], and in Japanese flounder [22].

Histological studies

During the experiment it was observed that, intestine of *P. hypophthalmus* of all treatments had almost normal structure. The villus height and villus area of intestines of same treatment were almost in similar structure but there was a slight change in the internal configurations of intestines of different treatments. It was also observed that villus height was increased and lumen area was decreased with increasing BC supplementation level. Increased height of intestinal villi means a greater surface area for nutrient absorption [23]. Greater villus height and increased cell mitosis numbers in the intestine are indicators of activation of the function of the intestinal villi [24,25]. Furthermore, increased villus size was also associated with activated cell proliferation in the crypt [26] and provided more surface area for nutrient absorption and thus improved nutrient digestibility [23]. These reports suggest our findings that the increased villus height and decreased lumen area in BC supplemented fish would be multiplicatively stimulated by the influence of BC.

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