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Original article

Biochar as a feed supplement for nutrient digestibility and growth performance of *Catla catla* fingerlings

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A B S T R A C T

The purpose of the current research was to determine the impact of various biochar supplements on nutrient digestibility and growth performance of *Catla catla* fingerlings fed on *Moringa oleifera* seed meal (MOSM) based diet. An experiment with 90 days of feeding was conducted to investigate the efficacy of biochar obtained from different sources such as parthenium, farmyard manure, poultry waste, vegetable waste, and corncob waste at 2 mg/kg. There were15 fingerlings in each tank in the triplicate set of tanks. Fingerlings were fed at the rate of 5 % of their live wet weight. From each tank, feces were collected twice daily and stored to determine nutrient digestibility. Results showed that poultry waste biochar (test diet-IV) at 2 mg/kg was the best source to significantly ($P < 0.05$) improve weight gain % (256.58 %) and feed conversion ratio (1.19) than the other biochar sources and control diet. Furthermore, in terms of nutrient digestibility, optimum fat (81.90 %), protein (75.92 %), and gross energy (74.84 kcalg^{-1}) values were obtained by using the same type of biochar (poultry waste). Conclusively, among all the five biochar sources, poultry waste biochar proved to be the best one, improving fish body performance.

1. Introduction

Aquaculture is one of the largest industries that produces the most significant amount of protein food, which aims to fulfill the dietary needs of consumers around the world (Dawood, 2021). Fish is well recognized as a valuable source of high-quality, readily digestible protein and a variety of vitamins and minerals (Gore et al., 2021). Fish is an essential nutrient in the human diet and is also present in the global aquatic product industry for consumers (Selamoglu, 2021). The development of the related sectors is primarily due to the increasing demand for fish protein and technological advancements (Fazio et al., 2021). Furthermore, the rapid growth of the human population and the decrease in fish production from inland natural lakes have demanded aquaculture development. Fish are the basic contributors of polyunsaturated fatty acids for human diet, with cardioprotective effects. Seafood products have attracted considerable attention as a source of high amounts of significant nutritional components in the human diet (Duran and Talas, 2009). This industry has the fastest growth rate than any food production technique. Its development and contribution promise to fight against hunger and malnutrition problems and meet the world's extensive food demand (El-Saadony et al., 2021).

Indian major carp dominates the freshwater aquaculture system, which is 87 % of the total aquaculture yield. These fish species are the most significant components of the carp polyculture system because they represent a diverse range of trophic levels (Ghosh et al., 2021). *C. catla* produces around 6 % of overall aquaculture production, and it ranks seventh among fish species produced in the world (Yearbook, 2018). There are numerous benefits of its farming are high commercial value, fast growth rate, the texture of meat, consumer demand, and the acceptability of artificial diet (Biswal et al., 2021). It shows higher palatability for a plant-based diet (Mandal and Ghosh, 2019).

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Due to the higher nutritional value of fish, the price of fish feed impacts aquaculture, so the cost of feed determines the fish's cost. Fish meal prices are increasing day by day due to rising demand and low production rates (Wang et al., 2021b). To fulfil the demand of fish meal, plant by-products are used instead of fish meal because these are valuable source of essential amino acids and protein as an alternative, as well as economical and environment friendly. MOSM, having reasonably high protein content, is a suitable protein replacer for fish meals. MOSM is considered more cost-effective as compared to both fish meals and soybean meals (Hussain et al., 2022).

M. oleifera is also known as the horseradish tree or Lam (Padayachee and Baijnath, 2020). The "miracle tree" is another name for of its multiple pharmacological potentials (Daghaghele et al., 2021). Being a drought-resistant tree native to Indian subcontinent, it is a fast-growing member of the Moringaceae family (Islam et al., 2021). It is recognized by its fruit, a tall, woody pod that unlatches into two leaflets when fully developed (Milla et al., 2021). *M. oleifera* seed contains oil which has various possible uses due to its characteristics (Magalhães et al., 2021). This plant is enriched with fats, carbohydrates, proteins, minerals (calcium and potassium), vitamins, phenolic compounds, and amino acids (Daghaghele et al., 2021).

A dark-black carbonaceous material known as biochar or charcoal is generated from biomass which is produced at low oxygen levels and pyrolysis at temperatures as high as 700 °C (Lao and Mbega, 2020). Like charcoal and activated charcoal, biochar is a pyrogenic carbonaceous material produced by pyrolysis from organic carbon-rich sources (Man et al., 2021). Biochar is a carbon-rich substance made from various organic waste feedstocks, such as sewage sludge and agricultural waste (Wang et al., 2021a). Biochar can treat soil remediation, gas storage, wastewater, and separation (Xiang et al., 2020). From the past decade, biochar addition as a dietary ingredient in animal production has grown extensively because it increases digestion and metabolism of nutrients (Chen et al., 2019). Similarly, there are noticeable benefits of biochar in improving growth performance, disease resistance, feed utilization efficiency, and immunological functions in animals such as broilers (Fu et al., 2015), turkeys (Majewska et al., 2009), ducks (Ruttanavut et al., 2009), goats (Villalba et al., 2002), and piglets (Mekbungwan et al., 2004). The aim of the present research was to determine the body parameters of *C. catla* fingerlings by using a biochar-supplemented plant meal diet.

2. Materials and methods

2.1. Fish maintenance and experimental conditions

The fingerlings were acclimatized for two weeks after being bought from the Government Fish Hatchery, Satiyana Road, Faisalabad. Fifteen fingerlings were kept randomly in each tank. To prevent fungal infection, fish were treated with NaCl solution (Rowland, 1991). Diet was provided two times a day.

2.2. Formulation of diets

Feed ingredients were finely grinded and then passed through the mesh. Fish oil was gradually added to the feed mixture and was homogenized with the help of an electric mixer. 10–15 % water was added into the mixer to make the dough ready for pelleting (Lovell, 1989). Then the resulting dough was passed through a pelleting machine in order to make pellets. The ingredients for each test diet, including the control diet, are listed in Table 1..

2.3. Feeding procedure and fecal collection

Fish were fed on their prescribed diet twice a day (dawn and dusk). Fish were given feed at a rate of 5 % of their total live wet weight. The initial weight of the fish was noted. For each diet, three sets of tanks with a total of fifteen fingerlings in each tank were utilized. After two hours of the feeding period, each tank was emptied of the leftover diet. Before being replaced with tap water, the tanks were thoroughly cleaned to remove any remaining diet. Valves were used to collect feces from each tank's fecal tube for lab analysis. The thin fecal threads were kept carefully to prevent the breakage and leaching of nutrients. Each replicate's feces were oven dried and stored for chemical analysis.

2.4. Growth study

To evaluate the growth parameters, the initial and final weights were weighed. Initially, the fish weighed 8.08 g. The formulas (1–2) given below were used to assess the growth performance of fingerlings:

$$\%\ \text{Weight gain} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial Weight}} \times 100 \quad (1)$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}} \quad (2)$$

Uneaten feed was collected from the tanks after feeding session, and the difference in feed quantity resulted in fish feed consumption.

2.5. Chemical investigation of feces and feed

The samples of the ingredients for feed and feces were homogenized with a grinding machine before being examined using standard methods (Cunniff, 1995). The moisture of feed and feces was examined by drying them for 12 h at 105 °C. A micro Kjeldahl instrument was used to determine the protein level. The ash content was determined in an electric furnace. The fiber was estimated as dried residues were lost after igniting. The fat was extracted using the Soxtec HT2 1045 equipment and the petroleum ether extraction technique. Oxygen bomb calorimeter was used to analyze the gross energy.

2.6. Chromic oxide estimation

Acid digestion method was used to determine the content of chromic oxide added as an inert marker (Divakaran et al., 2002) in feces and test diets following spectrophotometer-based oxidation with a perchloric reagent at 350 nm.

2.7. Calculating nutrient digestibility

The following formula (3) was used to calculate the apparent nutrient digestibility coefficient (ADC %) for diets:

$$\text{ADC}(\%) = 100 - 100 \times \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker feces} \times \text{Percent marker in diet}} \quad (3)$$

2.8. Statistical analysis of the data

A one-way analysis of variance (ANOVA) was carried out on data of growth performance and nutrient digestibility coefficients

Table 1
Composition of ingredients in test diets (%).

Ingredients	Test diet I (Control)	Test diet II Parthenium	Test diet III Farmyard manure	Test diet IV Poultry waste	Test diet V Vegetable waste	Test diet VI Corncob waste
Biochar (mg/kg)	0	2	2	2	2	2
MOSM*	52	52	52	52	52	52
Fish meal	16	16	16	16	16	16
Wheat flour	12	10	10	10	10	10
Rice Polish	9	9	9	9	9	9
Fish oil	7	7	7	7	7	7
Chromic oxide	1	1	1	1	1	1
Vitamin Premix	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Mineral premix	1	1	1	1	1	1

* MOSM = Moringa oleifera seed meal.

(Steel and Torrie, 1960). To estimate mean differences, Tukey's honesty significant difference test, and a value of $P < 0.05$ was taken to indicate statistical significance (Snedecor and Cochran 1989). The study of statistical data was conducted using the Co-Stat software.

3. Results

3.1. Growth performance

The growth performance of *Catla catla* fingerlings fed on biochar-supplemented *M. oleifera* seed meal-based diet supplemented with biochar is presented in Table 2. The initial weight of fingerlings was found to be comparable in this study. A considerable ($P < 0.05$) increase in weight gain was observed in the fish fed on poultry waste biochar (test diet IV) in comparison of the other biochars and the control diet. A negative impact on growth was observed in the fish that was fed on parthenium biochar that showed minimum growth than the fish fed on other biochars. Even the fish fed on the control diet showed more increase than the one provided on parthenium biochar. No, fingerlings were died during this study.

The highest weight gain (16.13 g) and weight gain % (256.58 %) of *C. catla* fingerlings were observed at test diet-IV that were fed on poultry waste biochar supplemented MOSM based diet. All other test diets, including the control diet, showed an increase in growth except the fingerlings given test diet-II that were fed on a parthenium biochar-supplemented MOSM-based diet. The lowest weight gain (5.96 g) and weight gain % (93.05 %) were noted in test diet-II.

3.2. Nutrient digestibility

Tables 3, 4, and 5 demonstrate the nutritional content of the control diet, test diets, and feces. All diets, including the control diet, had comparable amounts of nutrients. However, the most significant protein content (22.08 %), fat (3.71 %), and gross energy (2.14 %) in feces were found in the fingerlings fed on a parthenium biochar-supplemented MOSM-based diet (test diet II). The feces of fish given test diet-IV that were provided on poultry waste biochar supplemented MOSM based diet discharged the least amount of fat (1.61 %), protein (9.34 %) and gross energy (1.08 %). Although the Parthenium biochar supplemented diet showed the most excellent discharge in feces than all the other diets, yet the control diet showed lesser discharge in feces than the one fed on the parthenium biochar supplemented diet. All other biochar-supplemented diets except parthenium biochar were comparable.

4. Discussion

Formulation of an ideal fish feed is the basic requirement of intensive aquaculture development that is compatible to the body requirements of cultured fish. Moreover, feed supplementation is considered necessary for healthy animal growth and the sustainability of aquaculture industry. In this study, biochar was used as a feed additive with little implications in fish feed. However, the beneficial effects in other livestock species like poultry, cattle, pigs, and goats are reported by (Kalus et al., 2020; Goiri et al., 2021; Schubert et al., 2021; Al-Azzawi et al., 2021). The findings of current research were compared to that of other farmed animals.

In the present study, the best growth indices values were found using poultry waste biochar at 2 mg/kg. Similarly, (Abdel-Tawwab et al., 2017) demonstrated that *Oreochromis niloticus* fed supplemented diets with activated charcoal level (7.0 g/kg) for 8 weeks showed considerable improvement in growth parameters. The improved growth performance of fish is attributed to several variables, including the ability of activated charcoal to adsorb contaminants and gases from the digestive tract, which improves food digestion. The results of our research agreement with the findings

Table 2
Different types of biochar supplemented MOSM-based test diets as feed for growth of *C. catla*.

Growth Parameters	Control	Biochar Sources				
		Parthenium	Farmyard manure	Poultry waste	Vegetable waste	Corncob waste
Test Diet I	Test Diet II	Test Diet III	Test Diet IV	Test Diet V	Test Diet VI	
Initial weight (g)	6.23 ± 0.03^{a}	6.27 ± 0.05^{a}	6.26 ± 0.03^{a}	6.29 ± 0.04^{a}	6.29 ± 0.04^{a}	6.25 ± 0.03^{a}
Final weight (g)	14.84 ± 0.08^{e}	12.37 ± 0.30^{f}	19.50 ± 0.20^{b}	22.42 ± 0.11^{a}	18.17 ± 0.11^{c}	16.46 ± 0.11^{d}
Weight gain (g)	8.60 ± 0.06^{e}	5.96 ± 0.32^{f}	12.05 ± 0.54^{b}	16.13 ± 0.08^{a}	11.88 ± 0.07^{c}	10.21 ± 0.1^{d}
Weight gain (%)	138.02 ± 0.83^{e}	93.05 ± 5.51^{f}	194.23 ± 9.53^{b}	256.58 ± 1.06^{a}	188.72 ± 0.06^{c}	163.46 ± 3.05 ^{d}
Weight gain/day	0.10 ± 0.00^{e}	0.07 ± 0.00^{f}	0.13 ± 0.00^{b}	0.18 ± 0.00^{a}	0.13 ± 0.00^{c}	0.11 ± 0.00^{d}
Feed intake/day	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}
FCR	2.26 ± 0.0^{b}	3.23 ± 0.10^{a}	1.60 ± 0.0^{e}	1.19 ± 0.03^{f}	1.61 ± 0.06^{d}	1.85 ± 0.03^{c}

At ($P < 0.05$), the means of rows with various superscripts vary substantially.
The data are the averages of three replicates.

Table 3
Analyzed composition (%) of fat, protein and gross energy in diets of *C. catla* fingerlings fed on MOSM based test diets supplemented with different types of biochar.

Diets	Biochar Sources	Protein	Fat	Gross Energy
Test Diet I	Control	31.60 ± 0.06	8.04 ± 0.03	3.53 ± 0.03
Test Diet II	Parthenium	31.64 ± 0.01	8.09 ± 0.02	3.54 ± 0.03
Test Diet III	Farmyard manure	31.62 ± 0.02	8.05 ± 0.01	3.54 ± 0.04
Test Diet IV	Poultry waste	31.62 ± 0.02	8.07 ± 0.04	3.55 ± 0.02
Test Diet V	Vegetable waste	31.63 ± 0.01	8.09 ± 0.04	3.57 ± 0.03
Test Diet VI	Corncob waste	31.62 ± 0.02	8.05 ± 0.04	3.55 ± 0.04

Within each row, all mean values are substantially different ($P < 0.05$).
The data is the average of three replicates (Mean Standard ± Deviation).

Table 4
Analyzed composition (%) of protein, fat and gross energy in feces of *C. catla* fingerlings fed on MOSM based test diets supplemented with different types of biochar.

Diets	Biochar Sources	Protein	Fat	Gross Energy
Test Diet I	Control	17.94 ± 0.06^b	3.19 ± 0.03^b	1.65 ± 0.04^b
Test Diet II	Parthenium	22.08 ± 0.04^a	3.71 ± 0.06^a	2.14 ± 0.02^a
Test Diet III	Farmyard manure	10.92 ± 0.18^e	2.17 ± 0.06^e	1.26 ± 0.04^e
Test Diet IV	Poultry waste	9.34 ± 0.12^f	1.61 ± 0.05^f	1.08 ± 0.05^f
Test Diet V	Vegetable waste	12.10 ± 0.02^d	2.54 ± 0.06^d	1.41 ± 0.03^d
Test Diet VI	Corncob waste	13.97 ± 0.05^c	3.01 ± 0.06^c	1.54 ± 0.04^c

Within each row, all mean values are substantially different ($P < 0.05$).
The data is the average of three replicates (Mean Standard ± Deviation).

Table 5
Apparent Digestibility Coefficient (ADC%) of *C. catla* fingerlings fed on MOSM based test diets supplemented with different types of biochar.

Diets	Biochar Sources	Protein	Fat	Gross Energy
Test Diet I	Control	55.28 ± 0.33^e	67.96 ± 0.58^d	61.03 ± 2.46^d
Test Diet II	Parthenium	47.12 ± 1.12^f	63.39 ± 1.19^e	49.60 ± 0.89^e
Test Diet III	Farmyard manure	72.50 ± 0.85^b	77.94 ± 1.09^b	70.99 ± 2.31^{ab}
Test Diet IV	Poultry waste	75.92 ± 0.37^a	81.90 ± 0.77^a	74.84 ± 1.90^a
Test Diet V	Vegetable waste	69.46 ± 0.32^c	74.46 ± 0.40^c	68.35 ± 1.04^{bc}
Test Diet VI	Corncob waste	64.80 ± 0.39^d	69.50 ± 1.82^d	65.31 ± 2.69^{cd}

Within each row, all mean values are substantially different ($P < 0.05$).
The data is the average of three replicates (Mean Standard ± Deviation).

of (Quaiyum et al., 2014). The researchers observed that adding 2 % bamboo biochar and 0.2/kg biochar to the diet of striped catfish and brown trout (*Salmo trutta*) resulted in improved body weight, FCR and survival rate, respectively. Moreover, (Michael et al., 2017) found the improved growth performance, proximate chemical composition, and nutrient absorption parameters of red tilapia juveniles by using 3 % commercial wood charcoal. Juvenile flounders were fed on a diet containing 5 g of bamboo charcoal each kg of diet resulted in a substantial weight gain of 18 % (Thu et al., 2010). Yoo et al. (2005) research supports the results of the current study, who conducted nutritional supplementation with 5 and 10 g/kg diets of wood vinegar mixture and charcoal that improved the feed efficiency ratio and weight gain of fish. The researchers associated the growth performance of flounder with the availability of macronutrients like protein, leading to a better nutrient build-up in the body. Fish became healthier, their nutritional absorption boosted, and their feed efficiency increased by using charcoal, which has a carbon content of 85–95 %, a large surface area and pores that improved in nutrient absorption and fish growth (Thaib et al., 2021).

In the same sense, the positive effects of biochar are evident in the case of other animals like broilers. Likewise with the findings of Kutlu et al. (2001), we have reached the same conclusion by adding 2.5 % of oak charcoal to the diet of chicken broilers resulted in a 2 % increase in body fat weight. However, the results were not statistically significant. On the other hand, (Boonanuntanasarn et al., 2014) provided Nile tilapia with a diet enriched with graded activated charcoal levels but found no statistically significant differences in its growth. These negative results can be associated with several parameters of charcoal, like its supplementation level, composition or source, experimental conditions (design, raising, and period), and differences in feeding habits and initial body weight of fish.

Regarding apparent digestibility coefficient, poultry waste biochar improved the protein, fat, and gross energy digestibility up to 75.92 %, 81.90 %, and 74.84 $kcalg^{-1,}$ respectively. As far as we know, few facts are available to us at this time on the effect of biochar on the nutrient digestibility parameter of fish. Thu et al., (2010) found a significant increase in the protein digestibility values (89 %) at 4 % of bamboo charcoal in Japanese flounder. The fundamental role of charcoal is to positively affect the functions of digestion and absorption of nutrients (Jahan et al., 2014) explained that better nutrient utilization by using activated charcoal supplemented feed is attributed to the growth-promoting microbes present along the gastro-intestinal tract of animals. In goats, (Van et al., 2006) and (Mirheidari et al., 2019) noticed improved protein digestibility by adding bamboo charcoal and chicken manure biochar (1.5 %) as a dietary additive, respectively. Similarly, lambs also experienced changed digestive and rumen functions by consuming charcoal-based diets (Poage et al., 2000). However, no improvement in the dry matter digestibility was recorded by (Phongpanith, 2013) in goats, even after using 1 % of rice husk biochar in daily feed. The authors supposed that it might be due to a non-suitable experimental design.

5. Conclusion

The outcomes of this research suggested that dietary biochar derived from poultry waste can be supplemented in MOSM based diet at 2 mg/kg for *C. catla* fingerlings. This feed additive could optimize the growth performance and nutrient utilization parameters of *C. catla* significantly. Other sources of biochar also showed overall positive results, except for parthenium. Moreover, using biochar as a feed for *C. catla* proved to be an effective way to culture intensively by enhancing nutrient absorption.

CRediT authorship contribution statement

Muhammad Adnan Khalid: Writing – original draft, Funding acquisition, Formal analysis, Methodology. **Syed Makhdoom Hussain:** Project administration, Funding acquisition, Supervision, Conceptualization, Investigation, Formal analysis, Resources, Writing – review & editing, Project administration. **Shahid Mahboob:** Data curation, Funding acquisition, Resources, Software, Project administration, Writing – review & editing. **K.A. Al-Ghanim:** Formal analysis, Funding acquisition, Methodology, Writing – review & editing, Visualization. **Mian N. Riaz:** Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

The procedures and methods used in the study followed the ethical guidelines of Government College University Faisalabad.

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