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Supplementation of Different Types of Biochar in Diets of *Catla catla*: Effects on Carcass Composition, Hematology, and Mineral Status

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***C. catla*, Biochar, Hematology, Mineral status, Carcass composition, Nutrients**

ABSTRACT

This study investigates the impact of different biochar based diets on the carcass composition, hematological indices, and mineral status of *Catla catla*, a significant species in aquaculture. Biochar, a carbon-rich product derived from pyrolysis of organic matter. Recently, its use in aquaculture has gained interest due to its ability to improve water quality, function as a feed additive, and manage fish waste. In this study, *C. catla* (average weight; 6.29±0.04 g) were fed with six different diets, including a control diet without biochar and five experimental diets each containing 2 g/kg of different types of biochar such as farmyard manure biochar (FMB), parthenium biochar (PB), vegetable waste biochar (VWB), poultry waste biochar (PWB) and corncob waste biochar (CWB). *Moringa oleifera* seed meal was used a basal diet. The results revealed significant variations (p<0.05) in carcass composition, hematological parameters, and mineral content based on the type of biochar used in the diets. Notably, the diet containing PWB led to the highest protein content and most beneficial hematological impacts, suggesting its potential utility in enhancing the nutritional profile and health status of *C. catla* fingerlings whereas PB negatively impacted all parameters These findings highlight the potential of using biochar, particularly PWB, as a dietary supplement in sustainable aquaculture management. However, further research is needed to understand the long-term implications of these dietary changes on fish health and productivity.

INTRODUCTION

Aquaculture has long been recognized as a sustainable solution to meet the increasing demand for nutritious and protein-rich food (FAO, 2018). Among the diverse species utilized in fish farming, *Catla catla*, commonly known as thaila, is of prime importance due to its fast growth rate, adaptability, and substantial nutritional contributions (Hossain *et al*., 2013). Previous studies have highlighted the potential of *C. catla* in terms of high protein and essential fatty acids content, contributing to human health and well-being (Das *et al*., 2010).

There is a huge demand of fishmeal (FM) for aquafeeds (Oliva-Teles *et al*., 2022). Nevertheless, due to its scarcity and expensive cost, it is challenging to meet the fish requirements (Ahmad *et al*., 2021). Plant by products, which have a high protein content and all the necessary amino acids, are an affordable and ecologically friendly substitute for FM. *Moringa oleifera* seed meal (MOSM) is used as a viable substitute for FM (Hussain *et al*., 2018). It contains minerals, fatty acids and carbohydrates. According to Padayachee and Baijnath (2020), this tree is also known as the horseradish tree or Lam. Its many pharmacological properties have given it the term miracle tree (Daghaghele *et al*., 2021). However, it also has certain anti-nutritional factors such as phytates and glucosinolates, that negatively affects the fish health. Fish feed needs additives to satisfy the dietary requirements of fish (Ogunkalu, 2019).

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Recently, the use of biochar in aquaculture has gained considerable interest due to its ability to improve water quality, function as a feed additive, and act as a potential means of managing waste materials (Mahari *et al*., 2022). Biochar production from biomass is a cost-effective and environmentally beneficial form of biomass treatment (Zhou *et al*., 2021). Biochar is a carbon-rich product derived from the pyrolysis (at 700 ºC) of organic matter under limited oxygen conditions, widely known for its potential in enhancing soil fertility and sequestering carbon (Lehmann and Joseph, 2009).

The promising aspect of biochar lies in its highly porous nature and the presence of functional groups on its surface, which can adsorb nutrients, thereby potentially enhancing feed efficiency and fish growth (Park *et al*., 2011). Biochar may be produced from almost as numerous different feed stocks as there are different forms of biomass, such as animal manures, food scraps, paper products, rice husks and even urban green trash. Utilization of real wastes is prioritized for the management of different kinds of waste. Previous studies have shown that dietary biochar can have a significant impact on the growth performance, intestinal health, and feed utilization in fish (Liu *et al*., 2016).

However, the influence of different types of biochar on the carcass composition, hematological parameters, and mineral status of *C. catla* is yet to be thoroughly investigated. Given the beneficial properties of biochar and the need for sustainable fish farming practices, this study aims to elucidate the effects of different biochar diets on *C. catla*. Particularly, their impacts on carcass composition, hematological indices, and mineral status, thereby contributing the sustainable aquaculture management.

MATERIALS AND METHODS

Fish and experimental setup

A total of 270 *C. catla* fingerlings were taken from a Government Fish Hatchery Satiana Road, Faisalabad. The fish were acclimatized to laboratory conditions for a period of 2 weeks prior to the start of the experiment. Fifteen fingerlings (average weight; 6.29±0.04 g) per tank (triplicate; 3×3) were put in V-shaped tanks with continuous aeration. Water quality parameters such as water temperature (25-28℃), pH (6.5-8.2) and dissolved oxygen (DO, 5.5-6.4 mg/L) were monitored through thermometer, pH meter, and DO meter, respectively, on daily basis.

Experimental diets

All types of biochars were bought from Department of Environmental Sciences, GCUF, Faisalabad. Six different diets were prepared, comprising a control diet without biochar, and five experimental diets each containing 2 gkg of a different types of biochar i.e, parthenium biochar (PB), farmyard manure biochar (FMB), poultry waste biochar (PWB), vegetable waste biochar (VWB) and corncob waste biochar (CWB). The ingredients of the diets were analyzed following the procedures of AOAC (2016) and purchased from commercial market (Table I). After purchasing ingredients, they were crushed and to ensure that all the ingredients were evenly distributed throughout the mixture, fish oil was added to the blender at a slow pace. Chromic oxide (1%) was incorporated as inert marker. The prepared dough required an additional 10–15% water, which was added in the mixer. An automated pelletizer was then used to transform the diet into pellets.

Table I. Composition of ingredients in experimental diets (%).

Ingredients	Control	PB	FMB	PWB	VWB	CWB
Biochar (g/kg)	0	2	2	2	2	2
MOSM*	52	52	52	52	52	52
Fish meal	16	16	16	16	16	16
Rice polish	9	9	9	9	9	9
Fish oil	7	7	7	7	7	7
Wheat flour	12	10	10	10	10	10
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, *M. oleifera* seed meal; **Vitamin (Vit.) premix /kg: Vit. A: 15,000,000 IU, Vit. B6: 4000 mg, Vit. K3: 8000 mg, Vit. C: 15,000 mg, Vit. B12: 40 mg, Vit. D3: 3,000,000 IU, B2: 7000 mg, Ca pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg, Folic acid: 1500 mg. ***Mineral premix /kg: Mn: 2000 mg, Zn:3000mg, Fe: 1000 mg, Cu: 600 mg, Ca: 155 g, P: 135 g, Mg: 55 g, Na: 45 g, Co: 40 mg, I: 40 mg, Se: 3 mg. PB, parthenium biochar; FMB, farmyard manure biochar; PWB, poultry waste biochar, VWB, vegetable waste biochar, CWB, corncob waste biochar.

Feeding and sampling

Triplicate tanks were used to evaluate the impact of different diets on overall performance of fingerlings. Moreover, fingerlings were replicated in tanks to ensure robustness and reliability of the results. Each group was given diets for a period of 90 days. The feed was offered at 5% of the body weight of fish twice daily (Khalid *et al*., 2022). At the end of the feeding trial, 5 fish from each group were randomly selected, starved for 24 h, and then weighed and sampled for carcass composition, hematological analysis, and mineral status determination.

Carcass composition analysis

The proximate composition of the fish carcass was determined following the procedures outlined by AOAC (2016). The moisture, crude protein, crude lipid, and ash content were calculated.

Hematological analysis

Blood samples were collected from the caudal vein of the fish for hematological analysis. Parameters such as RBC count, Hb concentration, PCV, WBC count, and differential leukocyte count were determined following the methodology by Hussain *et al*. (2022). Following formulae were used to calculate blood related indices.

Mean corpuscular hemoglobin concentration (MCHC) = Hb/PCV × 100

Mean cell volume (MCV) = PCV/RBC × 10

Mean corpuscular hemoglobin (MCH)= Hb/RBC×10

Whereas, white blood cells (WBCs) and red blood cells (RBCs) were counted using a hemocytometer with a Neubauer chamber (Blaxhall and Daisley, 1973). The Wedemeyer and Yastuke (1977) technique was used to determine haemoglobin (Hb).

Mineral analysis

The mineral status of the fish was determined using an Atomic Absorption Spectrophotometer following the method described by Ahmad *et al*. (2023).

Statistical analysis

The data were subjected to One-way Analysis of Variance (ANOVA) (Steel and Torrie, 1960) using Co-stat software. Differences among means were compared using Tukey's test (Snedecor and Cochran, 1989) and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Carcass composition

The effect of different types of biochar on the carcass composition of *C. catla* was evaluated (Table II). The obtained results significantly varied across different diets. *C. catla* fed on test diet-IV (PWB) recorded the highest protein content (19.63±0.13%), followed by test diet-III (FMB) (18.88±0.09%) and test diet-V (VWB) (18.20±0.20%). The protein content was found to be least in test diet-II group (PB) (16.35±0.12%). Test diet-II (PB) showed the highest fat content (5.72±0.10%), whereas the lowest was observed in test diet-IV (PWB) (3.86±0.06%). Ash content was highest in the test diet-II group (PB) (2.16±0.06%), and lowest in the test diet-IV group (PWB) (1.87±0.05%). Moisture content was found to be maximum in test diet-II group (PB) (75.77±0.07%), whereas the minimum was observed in the test diet-IV group (PWB) (74.64±0.13%). In conclusion, the carcass composition of *C. catla* significantly varied based on the type of biochar incorporated into the diets.

Hematology

The impacts of different biochar diets on the hematological parameters of *C. catla* were examined (Table III) and found to exhibit significant differences. The RBC count was the highest in fish fed test diet-IV group (PWB) (2.77±0.07 $\times 10^6 mm^{-3}$), followed by test diet-III (FMB) (2.61±0.01 $\times 10^6 mm^{-3}$) and test diet-V (VWB) (2.56±0.27 $\times 10^6 mm^{-3}$). The lowest RBC count was observed in test diet-II (PB) (1.44±0.43 $\times 10^6 mm^{-3}$). Fish fed on test diet-IV (PWB) exhibited the highest WBC count (8.04±0.10 $\times 10^3 mm^{-3}$), while those fed on test diet-II (PB) showed the lowest (6.23±0.24 $\times 10^3 mm^{-3}$). The highest platelet count was found in fish from the test diet-IV group (PWB) (68.17±0.43), and the lowest in the test diet-II group (PB) (57.55±0.23). The Hb concentration was highest in test diet-IV (PWB) (8.65±0.25 g/100ml), while it was lowest in test diet-II (PB) (6.75±0.46 g/100ml). The highest PCV was observed in test diet-IV (PWB) (26.61±0.14%), and the lowest in test diet-I (Control) (19.60±0.08%). Fish from the test diet-IV group (PWB)

Table II. Carcass composition (%) of *C. catla* fingerlings fed on different types of biochar supplemented MOSM based test diets.

Diets	Biochar types	Protein	Fat	Ash	Moisture
Test diet-I	Control	$17.27±0.14^{d}$	$5.32±0.22^{ab}$	$2.06±0.05^{ab}$	$75.35±0.13^{b}$
Test diet-II	PB	$16.35±0.12^{e}$	$5.72±0.10^{a}$	$2.16±0.06^{a}$	$75.77±0.07^{a}$
Test diet-III	FMB	$18.88±0.09^{b}$	$4.37±0.15^{d}$	$1.91±0.03^{cd}$	$74.84±0.05^{cd}$
Test diet-IV	PWB	$19.63±0.13^{a}$	$3.86±0.06^{e}$	$1.87±0.05^{d}$	$74.64±0.13^{d}$
Test diet-V	VWB	$18.20±0.20^{c}$	$4.74±0.14^{cd}$	$1.95±0.03^{bcd}$	$75.11±0.07^{bc}$
Test diet-VI	CWB	$17.62±0.09^{d}$	$5.10±0.18^{bc}$	$2.00±0.03^{bc}$	$75.28±0.15^{b}$

At ($P<0.05$), the means of rows with different superscripts vary substantially. The data are the mean of three replicates. For abbreviations see Table I.

Table III. Hematological parameters of *C. catla* fingerlings fed on MOSM based test diets supplemented with different types of biochar.

Diets	Biochar types	RBC (10^6mm^{-3})	WBC (10^3mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
Test diet-I	Control	1.50±0.14^{b}	6.71±0.08^{cd}	60.25±0.35^{c}	6.87±0.40^{b}	19.60±0.08^{f}	29.45±0.28^{e}	30.52±0.16^{e}	97.28±0.44^{f}
Test diet-II	PB	1.44±0.43^{b}	6.23±0.24^{d}	57.55±0.23^{d}	6.75±0.46^{b}	21.30±0.29^{e}	28.36±0.26^{f}	31.00±0.29^{e}	101.32±0.49^{e}
Test diet-III	FMB	2.61±0.01^{a}	7.83±0.06^{ab}	64.34±0.56^{b}	8.50±0.19^{a}	25.00±0.04^{b}	33.45±0.21^{b}	42.55±0.09^{c}	172.56±0.19^{b}
Test diet-IV	PWB	2.77±0.07^{a}	8.04±0.10^{a}	68.17±0.43^{a}	8.65±0.25^{a}	26.61±0.14^{a}	34.42±0.08^{a}	44.36±0.19^{b}	181.64±0.21^{a}
Test diet-V	VWB	2.56±0.27^{a}	7.51±0.55^{ab}	63.39±0.44^{b}	8.47±0.21^{a}	23.51±0.32^{c}	32.80±0.14^{c}	41.61±0.28^{d}	162.85±0.13^{c}
Test diet-VI	CWB	2.44±0.08^{a}	7.32±0.08^{bc}	61.21±0.40^{c}	7.36±0.12^{b}	22.51±0.37^{d}	31.35±0.33^{d}	49.04±0.35^{a}	159.74±0.15^{d}

For statistical details and abbreviation, see Table II.

Table IV. Mineral status in *C. catla* fingerlings fed different types of biochar supplemented MOSM based test diets.

Minerals	Control diet	PB II	FMB III	PWB IV	VWB V	CWB VI
Ca (%)	0.79±0.02^{de}	0.72±0.05^{e}	0.96±0.04^{b}	1.08±0.02^{a}	0.90±0.03^{bc}	0.84±0.06^{cd}
Na (mg/g)	4.81±0.01^{e}	4.60±0.03^{f}	5.72±0.02^{b}	5.95±0.04^{a}	5.39±0.01^{c}	5.04±0.02^{d}
K (%)	6.50±0.03^{e}	6.20±0.04^{f}	8.11±0.03^{b}	8.95±0.06^{a}	7.70±0.02^{c}	7.04±0.03^{d}
P (%)	1.22±0.04^{c}	1.10±0.02^{d}	1.66±0.07^{a}	1.70±0.03^{a}	1.41±0.04^{b}	1.34±0.01^{bc}
Fe (µg/g)	45.72±0.18^{e}	43.10±0.41^{f}	51.82±0.35^{b}	53.04±0.22^{a}	48.96±0.40^{c}	46.61±0.36^{d}
Cu (µg/g)	2.98±0.01^{e}	2.45±0.03^{f}	4.12±0.02^{b}	4.98±0.04^{a}	3.84±0.01^{c}	3.52±0.02^{d}
Mg (%)	2.91±0.01^{d}	2.64±0.01^{e}	3.50±0.04^{b}	3.84±0.05^{a}	3.32±0.03^{c}	3.04±0.01^{d}
Zn (µg/g)	2.65±0.04^{e}	2.24±0.06^{f}	3.98±0.01^{b}	4.85±0.03^{a}	3.03±0.05^{c}	2.94±0.07^{d}
Mn (µg/g)	9.48±0.17^{e}	8.85±0.28^{f}	11.98±0.46^{b}	12.54±0.38^{a}	11.05±0.19^{c}	10.45±0.41^{d}
Se (mg/g)	0.61±0.03^{a}	0.68±0.04^{a}	0.31±0.05^{cd}	0.22±0.04^{d}	0.42±0.02^{bc}	0.54±0.07^{ab}

For statistical details and abbreviation used, see Table II.

exhibited the highest values for MCHC (34.42±0.08%), MCH (44.36±0.19 pg), and MCV (181.64±0.21 fl). The lowest values were recorded in the test diet-II group (PB) for MCHC (28.36±0.26%), and in the test diet-I group (Control) for MCH (30.52±0.16 pg) and MCV (97.28±0.44 fl). Overall, the results suggest that the type of biochar has a significant influence (p<0.05) on the hematological parameters of *C. catla*, with diets containing poultry waste biochar demonstrating the most beneficial impacts.

Mineral status

The influence of different biochar types on the mineral composition of *C. catla* was assessed (Table IV) and results significantly varied across the diets. *C. catla* fed on test diet-IV (PWB) exhibited the highest calcium (Ca), sodium (Na), potassium (K), iron (Fe), magnesium (Mg), zinc (Zn), manganese (Mn) and copper (Cu) content (1.08±0.02%), (5.95±0.04 mg/g), (8.95±0.06%), (53.04±0.22 µg/g), (3.84±0.05%), (4.85±0.03 µg/g), (12.54±0.38 µg/g), and (4.98±0.04 µg/g) whereas the lowest was observed in the test diet-II (PB) group (0.72±0.05%), (4.60±0.03 mg/g), (6.20±0.04%), (43.10±0.41 µg/g), (2.64±0.01%), (2.24±0.06 µg/g), (8.85±0.28 µg/g) and (2.64±0.01%), respectively. Fish from the test diet-IV (PWB) and test diet-III (FMB) groups showed the highest phosphorus (P) content (1.70±0.03% and 1.66±0.07%), respectively and the lowest was observed in the test diet-II group (PB) (1.10±0.02%). Selenium (Se) content was notably higher in the control diet (test diet-I) and in the test diet-II group (PB) (0.61±0.03 mg/g and 0.68±0.04 mg/g, respectively), and lowest in the test diet-IV group (PWB) (0.22±0.04 mg/g). In summary, these findings indicate that the mineral composition of *C. catla* can be significantly influenced by the type of biochar incorporated into their diets.

DISCUSSION

The findings from the current study highlight the profound effects of different biochar types (PWB, PB, FMB, VWB, and CWB) on the carcass composition, hematology, and mineral content of *C. catla,* thereby contributing to sustainable aquaculture production.

The carcass composition analysis showed significant variation in protein, fat, ash, and moisture content among the six dietary treatments. Biochar's porous nature allows nutrients to be absorbed, potentially enhancing feed efficiency and fish growth (Park *et al*., 2011). The fish fed with PWB (test diet-IV) exhibited the highest protein content, an observation that aligns with the study by Santos *et al*. (2019), who reported increased protein levels in fish fed with biochar supplemented diets. The enhancement of protein content could be attributed to the high nitrogen content and the slow-release property of PWB, resulting in more efficient protein synthesis (Novak *et al*., 2019). Furthermore, the least fat content observed in test diet-IV could be due to a better conversion of dietary lipids into energy or lean tissue, which agrees with the findings by Hossain *et al*. (2020). The carcass composition of fish, indicative of its nutritional value, varies depending upon the species, age, diet, and environmental factors (Shearer, 1994; Vásquez-Torres *et al*., 2011). Thu *et al*. (2010) found that the carcass composition of *Paralichthys olivaceus* (Japanese flounder) had increased markedly ($p<0.05$) as compared with control when fed with bamboo charcoal (biochar). They observed that the biochar supplementation improved the nitrogen retention in the body of fish. Thus, boosting the flesh quality and health of fish.

Hematological parameters are critical indicators of fish health status and physiological responses to environmental changes (Hrubec *et al*., 2000; Parrino *et al*., 2018). Therefore, evaluating hematological indices such as RBC count, Hb concentration, PCV, WBC count, and differential leukocyte count is essential in aquaculture management. Our results revealed significant differences in RBC, WBC, Hb, PCV, MCHC, MCH, and MCV among different dietary treatments. The highest values for most parameters were found in fish fed with PWB, indicating improved physiological and immunological responses. This is in accordance with Wang *et al*. (2014), who reported that biochar diets could stimulate fish immunity by enhancing the leukocyte activity. Similarly, Elghalid (2022) observed that different levels of biochar showed significant effect on blood parameters (Hb, RBCs and PCV) as compared with the control in broiler chicks.

The bioaccumulation of trace minerals in aquatic organisms is crucial in understanding the trophic transfer of these minerals in aquatic food webs and their implications on human health (Rainbow, 2002). The analysis of mineral content in *C. catla* revealed that the fish fed on PWB diet had the highest concentrations of essential minerals like Ca, Na, K, P, Fe, Cu, Mg, Zn, and Mn, excluding Se. Biochar's capability to enhance mineral content can be attributed to its high cation-exchange capacity, which increases the availability of these minerals (Lehmann *et al*., 2011). The significantly higher Se content in the control diet and in the test diet-II could be attributed to the geochemical origin of the feed ingredients or the difference in the Se binding and release properties of the used biochars. However, detailed analysis of mineral composition in *C. catla* has been seldom conducted, underlining the importance of the current research. In conclusion, our study substantiates the potential of using different types of biochars, particularly PWB, as dietary supplements to improve the nutritional quality of *C. catla*. However, further studies should be undertaken to understand the underlying mechanisms and the long-term implications of these dietary changes on the health and productivity of the fish.

CONCLUSION

The outcomes of this research suggested that dietary biochar derived from PWB can be supplemented in MOSM based diet at 2 g/kg for *C. catla* fingerlings. This feed additive could optimize the carcass composition, hematological indices, and mineral status of *C. catla* significantly. Other sources of biochar also showed over all positive results, except for PB. Moreover, using biochar as a feed for *C. catla* proved to be an effective way to culture intensively by enhancing nutrient absorption. More study is needed to analyze the influence of various biochar types on fish health in order to better understand the physiological value of biochar.

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Ethical statement

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

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