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ORIGINAL PAPER

Agro-Waste Biochar Conversion into a Fish Feed Additive: Assessing its Effects on the Health and Performance of *Cyprinus carpio*

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Abstract

Managing agro-waste effectively and sustainably is a significant challenge today. In this study, various waste-derived biochar (BC) derived from agricultural origins such as cotton, wheat, corn, grass, household and green wastes, were used as supplements in the various fish meal formulation. *Cyprinus carpio* fishes were fed with the different diets to investigate their effects on growth, nutrient and mineral digestibility, hematology and body composition. A basal diet of sunflower meal was fed for a period of 60 days. Seven experimental diets were prepared, consisting of a control diet and six test diets, each containing 2% of a distinct biochar sources: household waste (HW), cotton stick (CS), green waste (Gw) wheat straw (WS), corn cob (CC), and grass waste (GW). There were 15 fingerlings in each tank; with three replicas of each test diet and they were fed at 5% with respect to the body weight. The findings demonstrated that supplementing with CCBC substantially ($p < 0.05$) increased the growth performance, digestibility and carcass of Common carp, *C. carpio,* while HWBC showed negative results. The highest efficiency in mineral absorption was observed in the test fishes when with the supplementation of 2% corn cob biochar (CCBC) in the diet. Moreover, the fish blood profiles showed significant improvements ($p < 0.05$) when fed with CCBC. Conclusively, CCBC was found to be the most effective supplementation for improving growth, hematology, carcass, digestibility, and mineral status of *C. carpio*.

Keywords Agro waste · Biochar · *Cyprinus carpio* · Growth performance · Nutrient digestibility

Introduction

The sustainable management of agricultural waste is a significant environmental challenge to contemporary society, as the process of open burning releases harmful pollutants. To mitigate this issue, effective environmental solutions are crucial. One promising strategy is the formation of biochar (BC) from agro residues, as it enhances soil health, adsorbs pollutants, and promotes carbon sequestration, making it a climate-resilient material [1–5]. BC is a carbon rich, pyrolyzed substance, produced in a closed system in which reduction processes take place [6, 3, 4]. In this system, selected carbonaceous materials are burnt in the limited supply of oxygen where the temperature ranges from 300°C or more [7, 3]. These BCs produced from arious organic resources such as leaves, wood, compost, and manure; can later be utilized in all forms of cultivation to improve soil quality and general plant growth [1, 4, 8, 9].

Aquaculture is the fastest-growing food industry providing about 50% of animal protein to meet the nutritional

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needs of consumers globally [10–12]. To meet the rising demand for food and to manage nutritional inadequacies, this industry serves as an essential source of food globally [13]. To keep aquaculture production rates high around the world, a high-quality aqua feed supply is necessary. Therefore, innovative and sustainable production systems are required that can optimize aquaculture productivity while minimizing ecological impacts (e.g. utilizing wild-caught fishes) through the implementation of circular economy principles [14–18]. Supplementation of aquaculture feeds with BC additives has the potential to enhance feed quality and utilization efficiency, leading to improved growth rates and increased overall production yields. This innovative approach can significantly boost the sustainability and productivity of aquaculture industry, while minimizing environmental impacts [2, 19, 20]. Numerous studies have shown that supplementation of BC in animal feed improved animal health by increasing feed intake and weight gain (WG) [21, 22], enhancing nutrient absorption, helping in contaminants and toxins detoxification, reducing antibiotics residuals, and lowering gastric methane emissions [23].

The *Cyprinus carpio* (common carp) is a widely cultivated species, with a significant global presence in Europe, Asia, and beyond, accounting for an annual production of approximately 4 million metric tons [24]. Besides good taste, it is easily available and affordable. Due to its hardy nature, it is highly adaptable and can be easily maintained being an ideal species for freshwater aquaculture [25]. It thrives in a wide range of climatic conditions and withstands a broad variety of salinity and pH levels. Moreover, it is the fastest-growing species and a significant protein contributor [26]. Owing to their omnivorous nature, they have better adaptations and physiological tolerance for consuming plant by-products-based diets. Therefore, this species is an excellent candidate to be evaluated in consuming plant by-products based aqua feed (SFM) supplemented with BC. The effects of BC have not been yet investigated on *C. carpio*. Thus, this study was designated to determine the effectiveness of different BCs such as house waste (HW), cotton stick (CS), corn cob (CC), wheat straw (WS), grass waste (GW), and green waste (Gw) supplementation on the growth, hematology, carcass, digestibility, and mineral status of *C. carpio*. Moreover, the conversion of agro-waste biochar into a valuable fish feed supplement was performed to improve the growth and health of *C. carpio*.

Materials and Methods

Trial Setup

Healthy *Cyprinus carpio* fingerlings (6.11 ± 0.02 g) were obtained from the local Fish Seed Hatchery and then brought to the Laboratory, where they were acclimatized for 15 days and fed a basal diet once daily. Before the experiment began, the fish were immersed in a NaCl solution for 5 s to remove ectoparasites and minimize the risk of future inflammation [27]. A 60-day experimental trial was conducted, comprising 315 fish distributed into seven groups, each with a replicate, and placed in 21 V-shaped tanks (15 fish per tank). Furthermore, water quality parameters such as dissolved oxygen (5.7–7.3 mg/L), pH (7.4–8.5), and temperature (27–31 °C), were closely monitored on regular basis throughout the trial. Twice daily, all groups received test feeds equivalent to 5% of their biomass, and the tank water was simultaneously replaced to maintain optimal conditions. Following a one-hour feeding period, residual feed was retrieved, and the tank was meticulously cleaned to remove residual feed particles. The tank was then replenished with fresh water. Subsequent to this, fecal samples were carefully collected from each tank via the fecal collection tubes, with precautions taken to minimize nutrient loss within the aquatic system. Fecal samples were collected twice a day from the fish for digestibility analysis, using fecal collection tubes. The collected samples were then dried daily in a cool, shaded area, ground into a fine powder, and stored for subsequent chemical analysis [28].

Production of Biochar

A variety of biomass materials, including cotton stick, wheat straw, corncob waste, grass waste, green waste, and house waste, were collected after being crushed and dried, and then used to produce BC. BCs were pyrolyzed individually in a “top lit-up-draft gasifier (TLUD)” at 300 °C for one hour thirty minutes. After cooling, the materials were sieved through a 2 mm mesh to produce fine particles. [29]. The powdered BC samples were subsequently stored in sealed containers and subjected to chemical analysis (Table 1) prior to their incorporation into the experimental feed formulation.

Feed Ingredients and Processing

Seven test diets (TD) were formulated, each supplemented with 2% of a different BCs such as CSBC, WSBC, CCBC, HWBC, GWBC, and GwBC, whereas the control diet (CON) contained no BC. All feed components were pulverized to a uniform size by passing them through a 0.5 mm

Table 1 Physico-chemical properties and elemental composition of different biochars (BCs)

Biochar	Test diets	EC (dS m^{-1})	Ash (%)	Volatile matter (%)	pH	Elemental composition (%)					
						P	C	K	H	*N*	Na
CSBC	I	1.7	19.8	20.0	9.6	0.4	44.2	1.6	3.6	1.7	1.1
WSBC	II	2.8	20.3	23.0	10.1	0.2	42.0	2.2	1.2	1.7	0.7
CCBC	III	1.1	22.0	23.0	10.3	0.2	46.3	1.6	2.8	1.6	0.7
HWBC	IV	0.9	15.2	28.0	10.0	0.5	45.6	1.2	3.4	1.8	0.7
GWBC	V	2.1	16.6	13.0	9.1	0.8	44.7	1.3	1.1	1.6	1.5
GwBC	VI	3.3	18.2	30.0	11.3	0.8	43.5	3.4	1.0	1.5	1.3

Abbreviations: CS = cotton stick, WS = wheat straw, CC = corn cob, HW = house waste, GW = grass waste, and Gw = green waste

Table 2 Composition of dietary ingredients (g)

Ingredients	(TD-I) (CON)	(TD-II) CSBC	(TD-III) WSBC	(TD-IV) CCBC	(TD-V) HWBC	(TD-VI) GWBC	(TD-VII) GwBC
Biochar (g/kg)	0	20	20	20	20	20	20
Sunflower meal	520	520	520	520	520	520	520
Fish meal	160	160	160	160	160	160	160
Wheat flour	120	100	100	100	100	100	100
Rice Polish	90	90	90	90	90	90	90
Vitamin Premix*	10	10	10	10	10	10	10
Fish oil	70	70	70	70	70	70	70
Mineral premix**	10	10	10	10	10	10	10
Chromic oxide	10	10	10	10	10	10	10
Ascorbic acid	10	10	10	10	10	10	10

*Vitamin (Vit.) premix kg^{-1}: Vit. C: 15,000 mg, Vit. B_6: 4000 mg, Vit. A: 15,000,000 IU, Vit. B_2: 7000 mg, Vit. E:30,000 IU, Vit. B_{12}: 40 mg, Vit. D_3: 3,000,000 IU, Folic acid: 1500 mg, Vit. K_3: 8000 mg, Nicotinic acid: 60,000 mg, Ca pantothenate: 12,000 mg

**Mineral premix kg^{-1}: Ca: 155 g, Co: 40 mg, Na: 45 g, Cu: 600 mg, Mg: 55 g, P: 135 g, Fe: 1000 mg, Mn: 2000 mg, Zn:3000 mg, I: 40 mg, Se: 3 m

Abbreviations: BC = Biochar, CON = Control; TD- I, CSBC = cotton stick BC; TD-II, WSBC = wheat straw BC; TD-III, CCBC = corn cob BC; TD-IV, HWBC = house waste BC; TD-V, GWBC = grass waste BC; TD-VI, and GwBC = green waste BC; TD-VII

Table 3 Percent chemical makeup (%) of different feed components

Feed components	Fish meal	Wheat flour	Rice polish	Sun-flower
Dry matter (%)	93.26	91.4	95.07	94.73
Crude Fat (%)	6.95	2.54	11.78	3.23
Crude Protein (%)	50.68	9.71	11.86	41.61
Crude Fiber (%)	1.53	2.85	12.90	4.12
Ash (%)	22.17	2.67	12.21	8.74
Carbohydrates	18.67	82.23	51.25	42.3
Gross Energy (GE) (kcal/g)	2.55	3.00	3.21	2.41

mesh, followed by thorough mixing for a duration of five minutes, during which fish oil was gradually incorporated. Adequate water (10–15%) was added to mixture, resulting in a suitable dough [30]. Finally, the dough was passed via pelleting machine for pellet formation (2 mm). Following oven-drying, pellets were frozen at -20°C until usage. The feed ingredients and composition are shown in Tables 2 and 3.

Growth Study

The weight of *C. carpio* in each tank was recorded at the start and completion of the growth trial. The WG percentage (WG %), feed conversion ratio (FCR), and specific growth rate (SGR) were determined by Khalid et al. [31].

Nutrient Analysis of Feed, Feces and Muscle

The experimental diet, fish body, and excrement samples (1 g each) were homogenized. Conventional analytical techniques [32] were employed to examine these samples. To determine moisture content, samples were subjected to a 12-hour oven drying process at 105°C. A micro Kjeldahl apparatus was used to estimate crude protein (CP; N*6.25), a Soxtec HT2 1045 system was used to find crude fat (CF) by the petroleum ether extraction process, and an electric furnace was used to ignite ash (650 °C) during a 12-hour period. The oxygen bomb calorimeter was used to calculate the gross energy (GE). Following appropriate dilution, the concentrations of minerals were assessed using an Atomic Absorption Spectrophotometer. Following the commercial protocols established by Ahmad et al., [33], calibrated standards were developed for mineral estimation. Using a flame photometer, the amounts of potassium and sodium were determined. The phosphorus concentration was determined using a colorimetric method with an absorbance at 720 nm and ammonium molybdate as the reagent [32]. Chromic oxide was quantified using the acid digestion method, with absorbance measured at 350 nm using a UV/VIS 2001 Spectrophotometer [34].

Nutrient Digestibility

Using the Standard formula, the apparent nutrient digestibility of all experimental diets was determined [35].

Hematological Study

For hematology, 150 mg^{-1} tricane methane sulfonate was used to anesthetize three fish from each tank, after the feeding study had been completed for 60 days. A heparinized syringe was then used to collect blood samples. A blood sample was taken from the caudal vein. Then were sent to the Molecular Laboratory for the purpose of analyzing hematological indices. The hematocrit was calculated by centrifuging capillary tubes in a micro-hematocrit centrifuge, according to standard methods [36]. Blood cell counts, including RBCs, PLTs and WBCs counts, were performed in an approved Neubauer counting chamber [37]. Haemoglobin (Hb) was tested by using the Wedemeyer and Yastuk [38] technique. In order to find the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and corpuscular hemoglobin concentration (MCHC) formulae were used as identified by [31].

Statistical Analysis

One-way ANOVA was used to evaluate the parameters of all data [39]. To compare the differences between means, Tukey's Honest Significant Difference Test was used and considered significant at $p < 0.05$ [40]. The CoStat Computer Package (version 6.303) was employed for statistical data analysis.

Results

Growth Performance

The growth performance of *C. carpio* fed on different BCs-supplemented SFM-based diets are shown in Fig. 1. Current findings indicate that addition of CCBC with an SFM-based diet, substantially enhanced ($p < 0.05$) the growth performance while HWBC significantly decreased the growth of

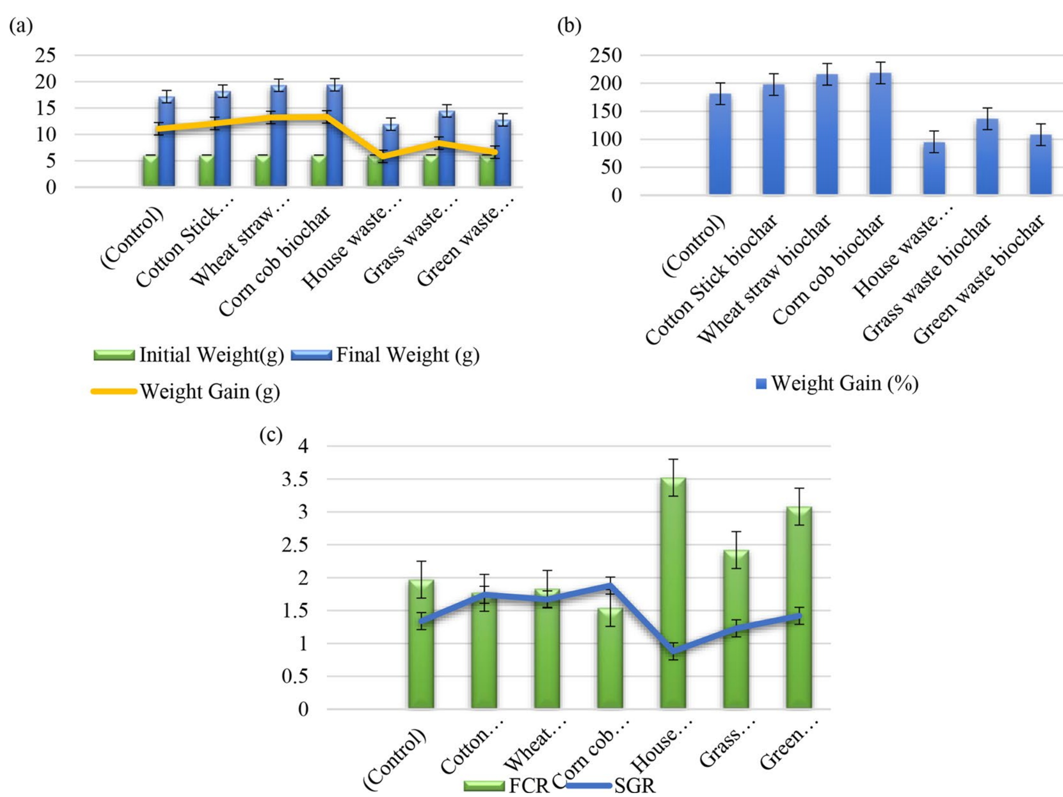

Fig. 1 Growth performance (**a**) Initial weight, final weight and weight gain, (**b**) Weight gain %, (**c**) FCR and SGR of *C. carpio* fed on different BCs supplemented diets

C. carpio. Diets having different types of BC supplementation showed significant results. The fish fed on (TD-IV) supplemented with CCBC had the maximum values of WG (13.32 g), WG% (218.53%), and SGR (1.88%) than other diets including control. In terms of FCR, the lowest value (1.54) was observed in fish fed on (TD-IV) as compared to other diets. It was recorded that the fish fed (TD-V) with HWBC supplementation showed the lowest values of WG (5.83 g), WG% (95.48%) and SGR (0.88%). These findings demonstrated that the addition of CCBC to *C. carpio* diet had a positive impact on the growth of *C. carpio*. However, growth was negatively influenced by HWBC supplemented SFM-based diet.

Nutrient Digestibility

Nutrient digestibility parameters of *C. carpio* fed on various types of BC supplemention are shown in Fig. 2. According to the findings of this research, fingerlings fed on (TD-IV) with CCBC gave significantly ($p<0.05$) different and highest digestibility values (CF (77.94%), CP (77.68%) and GE (75.42 kcal/g)) than all other diets including control. While, lower values of CP (57.13%), CF (61.69%), and GE (37.20 kcal/g) were recorded in fish fed on (TD-V) with HWBC supplementation as compared to all other test diets. The current study concluded that HWBC had a negative impact on *C. carpio* nutrient digestibility. The nutrient digestibility parameters were highest when 2% CCBC supplement was added to *C. carpio* diet.

Body Composition

Figure 3 shows the structural composition of *C. carpio* fed on SFM-based diets supplemented 2% of BCs. The results of whole-body composition, were significantly affected ($p<0.05$) by different types of BCs. Fish fed (TD-IV) with CCBC revealed considerably ($p<0.05$) higher amount of protein (17.87%), ash (2.90%), and moisture (72.97%) whereas fat content (6.27%) was lower in this group. The minimum amount of protein (13.73%), ash (2.28%), highest amount of moisture (74.74%), and fat (9.32%) were observed in (TD-V) containing HWBC. All test diets were notably different from others having SFM-based diets supplemented with different types of BC.

Hematology

Hematological results presented in Table 4 reveal a significant ($p<0.05$) positive impact on RBCs, WBCs, PLT, Hb, and other relevant parameters of hematology. 2% CCBC supplementation showed significant results. The highest values of RBCs (3.83×10^{-6} mm^{-3}), PLT (68.34), Hb (8.85 g/100 ml), PCV% (30.74) and WBCs (8.33×10^{-6} mm^{-3}) were observed in *C. carpio* fed a 2% CCBC diet. The lowest values of RBCs (2.14×10^{-6} mm^{-3}), PLT (47.04), WBCs (6.14×10^{-6} mm^{-3}), MCHC% (20.12), Hb (5.36 g/100 ml), PCV% (18.04), MCH (18.34) and MCV (85.15) were observed on 2% HWBC (TD-V).

Mineral Status

The mineral composition of feces demonstrated that a minimum minerals were discharged through feces in the TD-IV diet (Tables 5, 6 and 7). All minerals in each test diets were substantially different ($p>0.05$) from other test diet. The findings of the mineral status indicated that TD-IV exhibited higher digestibility coefficients than the other test diets. The optimum digestibility values of Ca (65.77%), Na (63.23%),

Fig. 2 ADC (%) of *C. carpio* fed on different BCs supplemented diets

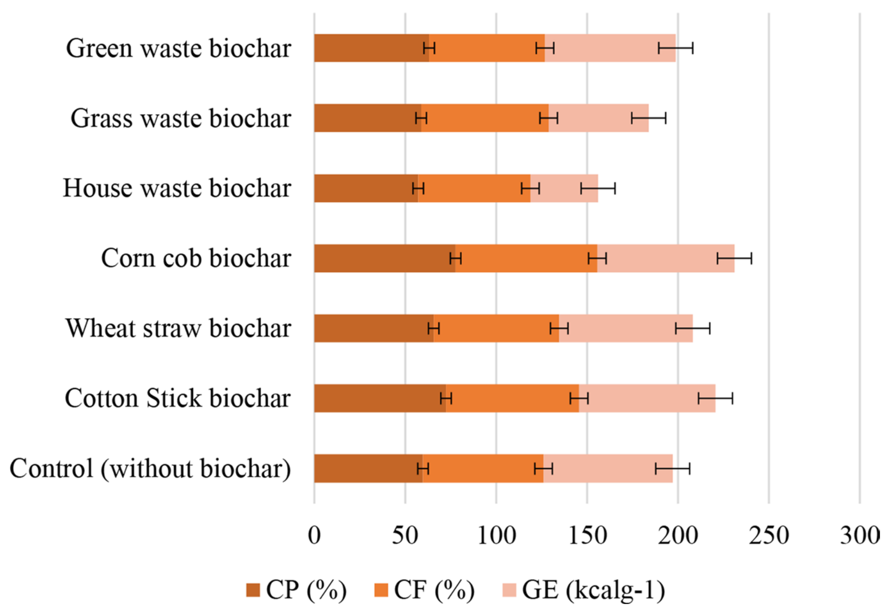

Fig. 3 The body composition of *C. carpio* fed on different BCs supplemented diets

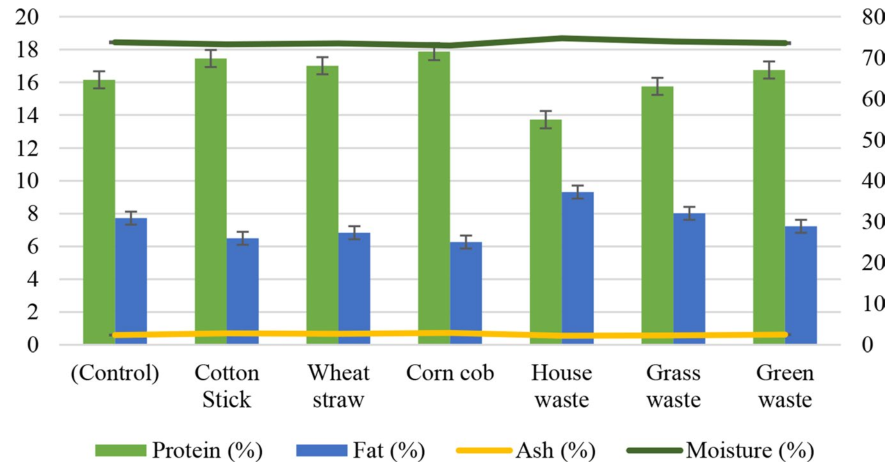

Table 4 The hematological profile of *C. carpio* fed on different BCs supplementation

Test diets	RBCs (10^{-6} mm^{-3})	Hb (g/100 ml)	WBCs (10^{-6} mm^{-3})	MCHC (%)	PLT	PCV (%)	MCV (fl.)	MCH (pg)
I (Control)	2.74 ± 0.04^{e}	6.45 ± 0.01^{de}	7.24 ± 0.03^{d}	25.02 ± 0.01^{e}	55.34 ± 0.036^{e}	24.04 ± 0.04^{e}	95.18 ± 0.07^{c}	23.34 ± 0.57^{c}
II (CSBC)	3.52 ± 0.01^{b}	7.99 ± 0.00^{b}	8.23 ± 0.02^{a}	30.64 ± 0.03^{b}	77.30 ± 0.18^{b}	29.87 ± 0.02^{b}	97.49 ± 0.99^{b}	28.34 ± 0.05^{a}
III (WSBC)	3.12 ± 0.01^{c}	7.15 ± 0.01^{cd}	8.04 ± 0.03^{b}	28.02 ± 0.01^{c}	68.34 ± 0.36^{c}	27.34 ± 0.56^{c}	101.85 ± 1.22^{a}	27.34 ± 0.57^{a}
IV (CCBC)	3.83 ± 0.03^{a}	8.85 ± 0.05^{a}	8.33 ± 0.12^{a}	33.86 ± 0.04^{a}	81.36 ± 0.15^{a}	30.74 ± 0.05^{a}	87.19 ± 0.62^{e}	27.36 ± 0.49^{a}
V (HWBC)	2.14 ± 0.04^{g}	5.36 ± 0.02^{f}	6.14 ± 0.0^{f}	20.12 ± 0.01^{g}	47.04 ± 0.01^{g}	18.04 ± 0.04^{g}	85.15 ± 0.04^{e}	18.34 ± 0.54^{e}
VI (GWBC)	2.44 ± 0.04^{f}	6.15 ± 0.01^{ef}	7.04 ± 0.03^{e}	23.12 ± 0.02^{d}	57.04 ± 0.01^{f}	21.04 ± 0.04^{f}	92.15 ± 0.04^{d}	21.34 ± 0.57^{d}
VII (GwBC)	2.94 ± 0.04^{d}	7.28 ± 0.74^{bc}	7.54 ± 0.03^{c}	27.02 ± 0.01^{d}	61.34 ± 0.36^{d}	26.04 ± 0.04^{d}	98.18 ± 1.04^{b}	25.34 ± 0.5^{7e}

Abbreviations: BC = Biochar, CON = Control; TD- I, CS = cotton stick; TD-II, WS = wheat straw; TD-III, CC = corn cob; TD-IV, HW = house waste; TD-V, GW = grass waste; TD-VI, and Gw = green waste; TD-VII

Table 5 The mineral status (%) in diets of *C. carpio* fed on BC supplemented diets

Test diets	Ca	Fe	K	Na	*P*	Zn	Cu
I (Control)	3.19 ± 0.03	0.0127 ± 0.0002	0.25 ± 0.03	0.20 ± 0.03	0.581 ± 0.005	0.0291 ± 0.0004	0.0112 ± 0.0003
II (CSBC)	3.13 ± 0.05	0.0129 ± 0.0001	0.24 ± 0.03	0.22 ± 0.03	0.580 ± 0.001	0.0293 ± 0.0002	0.0114 ± 0.0002
III (WSBC)	3.14 ± 0.03	0.0128 ± 0.0002	0.22 ± 0.05	0.21 ± 0.02	0.583 ± 0.002	0.0295 ± 0.0003	0.0113 ± 0.0002
IV (CCBC)	3.16 ± 0.04	0.0124 ± 0.0003	0.26 ± 0.02	0.23 ± 0.02	0.585 ± 0.001	0.0296 ± 0.0001	0.0116 ± 0.0001
V (HWBC)	3.13 ± 0.03	0.0125 ± 0.0002	0.23 ± 0.03	0.20 ± 0.04	0.584 ± 0.002	0.0290 ± 0.0004	0.0112 ± 0.0002
VI (GWBC)	3.14 ± 0.04	0.0126 ± 0.0001	0.25 ± 0.03	0.23 ± 0.03	0.587 ± 0.002	0.0291 ± 0.0002	0.0113 ± 0.0002
VII (GwBC)	3.15 ± 0.04	0.0127 ± 0.0003	0.24 ± 0.02	0.26 ± 0.02	0.583 ± 0.003	0.0294 ± 0.0003	0.0114 ± 0.0003

Abbreviations: BC = Biochar, CON = Control; TD- I, CS = cotton stick; TD-II, WS = wheat straw; TD-III, CC = corn cob; TD-IV, HW = house waste; TD-V, GW = grass waste; TD-VI, and Gw = green waste; TD-VII

K (73.30%), P (71.25%), Fe (71.95%), Cu (70.99%), and Zn (69.80%) were obtained in *C. carpio* fed the diet TD-IV.

Discussion

BC produced from waste and biomass materials has been widely used in studies to treat and remediate aquaculture wastewater [12]. However, research on the application of BC as a feed supplement is limited, particularly in the context of aquaculture [31, 41, 20]. This study investigated the utilization of agro-waste BC to enhance the health and growth of *C. carpio* as a strategy for sustainable fish feed production. The beneficial effects of BC supplementation include enhanced growth by elevating wight gain (WG), feed intake and nutrient utilization in different fish species [42]. This research reveals that CCBC is a valuable supplement for fish feed since it increases both the growth rate of fish and their ability to assimilate nutrients. Research conducted by Kim et al. [43] demonstrated that biochar rich in nutrients, such as CCBC, can enhance feed efficiency and promote faster growth in fish, highlighting its potential as a beneficial feed supplement. Our results resembled with that of Khalid et al. [44] who found maximum growth rate with lowest FCR value in *Catla catla* when fed with 2 mg/kg poultry waste biochar (PWBC) added to moringa based diet. Quaiyum et al. [45] also reported that 2% supplementation of bamboo charcoal (BC) is an optimal dosage for improved growth of

Table 6 The mineral status (%) in feces of *C. carpio* fed on BC supplemented diets

Test diets	Ca	Fe	Na	K	Zn	*P*	Cu
I (Control)	1.94 ± 0.02^{c}	0.0064 ± 0.0001^{a}	0.13 ± 0.01^{abc}	0.13 ± 0.01^{ab}	0.0146 ± 0.0006^{b}	0.33 ± 0.01^{c}	0.0055 ± 0.0002^{c}
II (CSBC)	1.32 ± 0.03^{f}	0.0045 ± 0.0001^{b}	0.09 ± 0.01^{cd}	0.08 ± 0.01^{de}	0.0108 ± 0.0003^{b}	0.22 ± 0.00^{f}	0.0039 ± 0.00001^{f}
III (WSBC)	1.46 ± 0.03^{e}	0.0050 ± 0.00001^{b}	0.10 ± 0.01^{bcd}	0.10 ± 0.01^{cd}	0.0122 ± 0.0003^{b}	0.24 ± 0.00^{e}	0.0043 ± 0.00001^{e}
IV (CCBC)	1.15 ± 0.04^{g}	0.0038 ± 0.0001^{b}	0.08 ± 0.01^{d}	0.07 ± 0.01^{e}	0.0092 ± 0.00001^{b}	0.18 ± 0.00^{g}	0.0034 ± 0.00001^{g}
V (HWBC)	2.54 ± 0.02^{a}	0.0074 ± 0.0001^{a}	0.15 ± 0.00^{a}	0.15 ± 0.00^{a}	0.0183 ± 0.0002^{a}	0.39 ± 0.00^{a}	0.0065 ± 0.0002^{a}
VI (GWBC)	2.20 ± 0.08^{b}	0.0071 ± 0.0001^{a}	0.14 ± 0.02^{ab}	0.15 ± 0.02^{a}	0.0162 ± 0.0003^{a}	0.36 ± 0.01^{b}	0.0061 ± 0.0001^{b}
VII (GwBC)	1.81 ± 0.05^{d}	0.0056 ± 0.00001^{a}	0.11 ± 0.01^{bcd}	0.11 ± 0.01^{bc}	0.0135 ± 0.0003^{b}	0.20 ± 0.00^{d}	0.0049 ± 0.00001^{d}

Abbreviations: BC = Biochar, CON = Control; TD- I, CS = cotton stick; TD-II, WS = wheat straw; TD-III, CC = corn cob; TD-IV, HW = house waste; TD-V, GW = grass waste; TD-VI, and Gw = green waste; TD-VII

Table 7 The mineral digestibility (%) of *C. carpio* fed on BC supplemented diets

Test diets	Ca	Fe	Na	K	Zn	*P*	Cu
I (Control)	42.12 ± 2.85^{c}	52.96 ± 0.74^{e}	45.58 ± 0.94^{d}	52.65 ± 0.99^{e}	52.47 ± 0.43^{e}	46.68 ± 0.93^{e}	53.69 ± 0.95^{e}
II (CSBC)	60.72 ± 0.83^{ab}	67.42 ± 0.72^{b}	59.83 ± 0.92^{ab}	68.70 ± 0.70^{b}	64.67 ± 0.80^{b}	64.02 ± 0.70^{b}	66.66 ± 0.93^{b}
III (WSBC)	56.16 ± 0.80^{b}	63.39 ± 0.30^{c}	56.47 ± 0.50^{bc}	63.44 ± 0.81^{c}	60.40 ± 0.85^{c}	59.83 ± 0.73^{c}	63.53 ± 0.42^{c}
IV (CCBC)	65.77 ± 0.84^{a}	71.95 ± 0.48^{a}	63.23 ± 0.96^{a}	73.30 ± 0.63^{a}	69.80 ± 0.57^{a}	71.25 ± 0.21^{a}	70.99 ± 0.98^{a}
V (HWBC)	24.22 ± 3.67^{e}	45.66 ± 0.93^{g}	36.59 ± 5.01^{e}	44.07 ± 3.67^{f}	40.49 ± 1.76^{g}	36.81 ± 2.99^{g}	45.18 ± 1.37^{g}
VI (GWBC)	34.65 ± 4.94^{d}	48.62 ± 0.91^{f}	42.66 ± 0.48^{d}	47.53 ± 0.74^{f}	47.63 ± 0.71^{f}	40.98 ± 0.94^{f}	49.07 ± 0.99^{f}
VII (GwBC)	46.15 ± 0.96^{c}	58.91 ± 0.87^{d}	52.84 ± 0.87^{c}	58.80 ± 0.59^{d}	56.09 ± 0.97^{d}	54.03 ± 0.75^{d}	58.54 ± 0.74^{d}

Abbreviations: BC = Biochar, CON = Control; TD- I, CS = cotton stick; TD-II, WS = wheat straw; TD-III, CC = corn cob; TD-IV, HW = house waste; TD-V, GW = grass waste; TD-VI, and Gw = green waste; TD-VII

Pangasius hypophthalmus. Research conducted by Najmudeen et al. [46] noticed that supplementation of BC in fish feed significantly ($p < 0.05$) improved the growth of tilapia (*Oreochromis mossambicus)*, leading to increased length and WG. Our study in line with Thu et al. [41] who found that 0.5% bamboo biochar (BBC) was the ideal level for promoting growth in juvenile flounder. The WG and WG% of fish rise as the intrinsic microbial activity increases [47]. The increased surface area provided by biochar allows it to hold certain micro-minerals that are essential for improved growth performance, detoxification of pollutants and other harmful chemicals in the feed. Another reason might be the provision of conducive micro-habitats to harbor beneficial microorganisms in animal intestines and stomachs and aiding in the elimination of harmful microbes [22].

BC produced from corncobs typically has a higher nutrient content than BC derived from green waste, grass waste and house waste; attributed to the inherently higher nutrient density of corncobs and their superior ability to retain nutrients during the BC production process. In contrast, all other BCs tends to have lower nutrient levels due to the relatively lower nutrient content of the source materials [43].

In our research, it was noticed that biochar supplementation enhanced the nutrient digestibility of fish. BC supplementation as a feed additive in livestock farming, has been shown to increase animal health, increase nutrient utilization, and enhance overall production [48]. Khalid et al. [31] reported that 2 mg/kg PWBC addition enhanced the digestibility of CP, CF and GE in *C. catla*. Schubert et al. [49] results are in accordance with present study, they used two different types of BC and determined that 2% BC has a positive outcomes on the digestibility and improved the CP, GE, and CF of pigs. As reported by Thu et al. [41], the protein digestibility levels in Japanese flounder were shown significantly enhanced to 89% when fed with 4% BC. The increase in digestibility was due to the elevated levels of digestive enzymes which enhanced the digestion and absorption of essential nutrients [48].

In terms of body composition, CCBC gave the best results on the basis of CP, ash, CF, and moisture content. The incorporation of BC can influence the gut microbiota, leading to increased short-chain fatty acid production and energy availability forthe host animal. This can result in improved overall health of animal [50]. Another study suggested that the body composition of *Paralichthys ofivaceus*, the Olive Flounder, was improved through the use of a charcoal and wood vinegar combination [51]. Moreover, according to Thu et al. [41], bamboo charcoal (BCC) significantly enhanced the carcass of *P. olivaceus* by reducing ammonia excretion, leading to enhanced protein content and overall quality of the fish body.

In the current study, *C. carpio* exhibited improved hematological parameters with the notable exception of HWBC, which negatively impacted the hematological profile. In a study by Klahan et al. [52], the introduction of BC improved the blood profile of *Oreochromis niloticus*. Our study similar with Khalid et al. [44] that the hematological parameters of *Cirrhinus mrigala* were improved when PWBC *Moringa* *oleifer*a seed meal-based diet was applied. The research of Kara et al. [2] also concluded that hematological parameters were improved more with the supplementation of sugar bagasse biochar (SBBC) in Gift tilapia (*O. niloticus*). The BC supplementation minimizes the absorption of toxic substances and harmful compounds in the intestinal tract of fish, this detoxifying action positively affect hematological parameters and reduce the oxidative stress [48].

Based on the current study, the analyzed mineral composition of feces, showed that minimum minerals were discharged through feces in the TD-IV diet. The optimum digestibility values of Na (63.23%), K (73.30%), Ca (65.77%), P (71.25%), Fe (71.95%), Cu (70.99%), and Zn (69.80%) were obtained in *C. carpio* fed the CCBC diet. Biochar's potential to increase mineral content comes from its exceptional cation-exchange capacity, which improves the availability of related minerals. The presence of minerals like Ca, O, and Mg in BC enhances their bioavailability for fish, which may result in better absorption and utilization of these essential nutrients [53]. However, further study is required to elucidate the underlying mechanisms and to determine the consequences of different dietary biochar sources on fish health and productivity.

Conclusion

The findings of this investigation demonstrated that incorporating 2% corn cob biochar (CCBC) into a diet containing SFM improved the body composition, nutrient digestibility, growth performance, hematology, and minerals status of *C. carpio*. All sources of BC delivered generally positive results, with the exception of HWBC. Furthermore, improved growth performance, body composition, nutrient absorption, hematology, and minerals status in *C. carpio* fingerlings have proven BC as a cost-effective supplement for fish farming. Conclusively, the incorporation of BC derived from agro-waste as a dietary additive in aquaculture feed formulations offers a sustainable and eco-friendly approach to promote optimal fish growth, health, and productivity.

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Author Contributions Muhammad Amjad: Writing-original draft, Methodology. Syed Makhdoom Hussain: Writing-original draft, Data curation, Conceptualization, Supervision. Shafaqat Ali and Muhammad Rizwan: Writing-review & editing, Data curation, Investigation. Ajaz Ahmad: Data curation, Writing-review & editing, Conceptualization. Jean Wan Hong Yong: Writing-review & editing, Conceptualization, Funding Acquisition.

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Data Availability Data will be available on demand.

Declarations

Ethical Approval This study has been performed in a responsible and ethical manner. All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad (Ref No. GCUF/ERC/438).

Competing Interests The authors have no competing interests.

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