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Exploring the Physiological and Biochemical Investigation of Nile Tilapia by Application of Biochars

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Abstract

Fresh water aquaculture is considered as the most prominent type of aquaculture in the world. Nile tilapia aquaculture is favored because it can adopt to wide range of environments and can tolerate various abiotic stresses. However, the success, profitability of aquaculture and nutritional composition of fishes depends upon nutritional values of meal provided to them. For this purpose, aquafeeds industries are trying to prepare complete meal for favorable nutrition. An experiment was performed to examine the effectiveness of five biochar experimental diets on growth, body composition, hematological and mineral content of Nile tilapia fingerlings. These fingerlings were acclimatized for ten days under laboratory conditions and were fed with commercial diet. After acclimatization 30 fingerlings were distributed randomly in each tank and there were three replicates of each experimental diet. Fingerlings were fed with their respective biochar experimental diet twice a day for 60 days. Data were recorded for growth, body composition, hematological and mineral content attributes. Analysis of data revealed that D3 (CCBC) experimental diet performed exceptionally well for all growth, hematological and mineral attributes of Nile tilapia. D3 caused 44% increase in final weight and provided highest value of SGR (0.96) as compared to controlled. D3 (CCBC) fed fingerlings also showed significant increase in RBC's, WBC's, platelates, hemoglobin and PCV. Supplementation of 2% CCBC also provided the maximal absorption efficiency of minerals (Ca, Na, K, Fe and Zn). So form these findings it can be assumed that supplementation of 2% CCBC has the potential to be used as envirnonment friendly, cost effective and sustainable supplement in Nile tilapia diets.

Keywords: Nile tilapia, Biochar, Sustainable aquaculture, Cost-effective fishmeal.

1. INTRODUCTION

Aquaculture provides 50% of food fish utilization globally that's why it is growing at faster rate [1]. Aquaculture industry grows each year at the rate of 5.8% which shows enormous growth of this industry [2]. As the population of developing countries increasing rapidly which lead to higher demands of healthy diet and results in expansion of this industry [3]. Fish is known as the most prominent source of best quality minerals, vitamins and its is also important source of easily digestible proteins [4]. Fish is one of the most important products of aquatic industry as it provides essential nutrients for human food [5]. Natural habitats of fish, such as lakes, rivers are manipulating by anthropogenic activities and these habitats are not producing enough fish to fulfill human needs. Fish provides poly unsaturated fatty acids which protects and strenthen human cardic. As compared to other food industries, aquaculture is growing at exponential rate and trying to perform their role against global hunger and malnutrition [6]. Indeed, fish is recommended in diets due to its requirement of protein, minerals, unsaturated fats, and vitamins.

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The American Heart Association mentioned that consuming fish two times a week is adequate in making sure that one's required dose of omega-3 fatty acids is met [7]. The major input and one of the key factors that are rate limiting in aquaculture is feed. Due to the aforementioned positive characteristics, fishmeal (FM) and fish oil (FO) are viewed as the most valuable feed ingredients [8]. Animal protein which is considered to be of high quality is primarily contributed from aquaculture and thus underlines an extremal importance to global food security. Out of the world's aquaculture food productions, 70% are derived from the freshwater aquaculture system. It has been turned into an important business in most countries primarily due to the high consumption of fish and other sea foods in the world. Based on the above nutritional advantages of FM, it is incorporated in fish feed including balance of polypeptide chain and vitamin content, digested substances, growth promoting characteristics and good smell [9]. However, the cost of FM has shifted up in over the last decade owing to the growing demand of the services.

As for the changes of population density within the last 20 years, it has significantly grown, and the rates of industrialization and urbanization has grown as well. Because of such factors, environmental pollution and short supply of clean water has also risen in proportion as well. Some of the examples of spectrum of environmental pollution are toxic industrial chemicals, excess fertilizer, heavy metals food additives, insecticides, personal care items such as shampoos, lotions, and creams, and veterinary supplies like hormones etc. Since these materials are organic wastes, they release carbon dioxide, one of the green house gases when they decompose naturally [10]. BC is basically carbon a type of element that results from the pyrolisis of biomass. Therefore, the attempt to commodify waste through the production of biochar (BC) has the potential to remove this greenhouse gas out of environment [11]. Researchers are making BC, nano-biochar, other useful products from several wastes because feedstock is available easily to them [12].

BC is a carbon-based substance that is utilized coming from the process of heating organic matter in which oxygen is missing or is restricted in mere amount [13]. They are light in weight, usually black in color and contain more carbon as compared to other steels [14]. Some of the characteristics that make agriculture valuable include the following: enhancing the soil structure that reduce bulk density; enhances porosity, water reten- tion and plant growth [15]. Also, its main use is to control or treat ammonia concentrations in animal production facilities.

In aquaculture, O. niloticus is widely cultured and preferred over other species. This specie is adopted to various environmental stresses [16]. Its growth rate is also fast and is tolerant to various diseases. O. niloticus has the ability to adopt and utilize newly manufactured diets, can survive on less feeds and can also reproduce in captivity [17]. Nile tilapia can easily adopt to various abiotic stresses such as low oxygen levels, pH, temperature and salinity as compared to most freshwater species, which cant adopt to these abiotic stresses [18]. The year 2020 has been seen having a significant contribution with approximately 5% of the global aquaculture output. Thus, despite international success, high feed costs, particularly soybean meal, constrain tilapia and particularly its economics [19]. To the best of the present author's knowledge, there has been no study investigating the impacts of BC on growth performance, body composition, haematology and mineralization of O. niloticus. In the current investigation, on O. niloticus, the effectiveness of inclusion of 2% of the different BC levels on the growth size of fish, nutrient utilization, the carcass characteristics, hematology and mineral profile was evaluated.

2. MATERIALS AND METHODS

2.1. Experimental fish and design

This study was conducted in 2023 to examine the effeciveness of various biochars as an alternative to fishmeal in the diet of Nile tilapia. Nile tilapia fingerlings were purchased from fish seed hatchery. Initial weight of these fingerlings were 5.17 gram. These fingerlings were transported in polythene bags to labs, adequate aeration was provided in these bags. These fingerlings were acclimatized for ten days under laboratory conditions and were fed with commercial diet. After acclimatization 30 fingerlings were transported to each tank. NaCl solution was also applied to avoid ectoparasites [20].

2.2. Production of biochar

For the production of biochar, various biomass materials were obtained. Biomass materials used for production of biochar were:

1. Cotton sticks biochar (CSBC)
2. Wheat straw biochar (WSBC)
3. Corncob biochar (CCBC)
4. House waste biochar (HWBC)
5. Grass waste biochar (GWBC).

These materials were crushed and dried. Then top lit draft gasifier was used for pyrolyzation of these materials. These materials were passed through a 2 mm sieve to form fine particles [21]. These fine particles were stored in airtight container for being used in experimental diets later.

2.3. Feed Ingredients and processing

Five experimental diets were prepared by mixing 2% biochar from various sources such as D1 (CSBC); D2 (WSBC); D3 (CCBC); D4 (HWBC) and D5 (GWBC), while D0 (Controlled) diet didn't get any biochar. All ingredients were thoroughly mixed and fish oil was added gradually. Then water was added 10-15% of weight to prepare a homogenous dough [22]. With the help of pelleting machine, fine pellets were made from dough. After oven drying, these pellets were stored at − 20 °C. Table-1 shows the composition of all experimental diets.

Table 1: Percentage Composition of Test Diets

Ingredients	D0	D1	D2	D3	D4	D5
BC	0	2	2	2	2	2
Sunflower Meal	52	52	52	52	52	52
Fish Meal	16	16	16	16	16	16
Wheat Flour	12	10	10	10	10	10
Rice Polish	8. 5	8. 5	8. 5	8. 5	8. 5	8. 5
Fish Oil	7. 5	7. 5	7. 5	7. 5	7. 5	7. 5
Chromic Oxide	1	1	1	1	1	1
Vitamin Premix	1	1	1	1	1	1
Mineral Premix	1	1	1	1	1	1
Ascorbic Acid	1	1	1	1	1	1

2.4. Growth study

At the start of trials initial weight of fingerlings were noted and final weight was measured with the help of weight balance at the end of trials. While other growth attributes like weight gain, feed conversion ratio (FCR), specific growth rate (SGR) and survival rate were calculated by following formulas:

Weight gain = Final weight – Initial weight

SGR %d-1 = (log Final weight – log Initial weight) × 100 / Experimental period

FCR = Quantity of feed consumed / Weight gain

Survival = Final count of fish × 100 / Initial count of fish

2.5. Proximate analysis

Diet and fish body were homogenized with the help of motor and pestle. Crude proteins content was assessed by Kjehldahl apparatus. Petroleum ether extraction process was used to determine crude fats. While mineral ccontents of fingerlings were determined by using atomic absorption spectrophotometer [23].

2.6. Hematological study

After 60 days of trials, fishes were anaesthetized and blood samples were collected from caudal peduncle with the help of syringe rinsed in EDTA anti-coagulant. Then blood was stored in vials having 0.5 mg anti-coagulant. To count total number of white blood cells (WBC's) and red blood cells (RBC's), Blood was diluted in Turk's fluid and Hay-men's fluid respectively [24, 25]. While hemoglobin was estimated by cyanmethemoglobin method [26]. While PCV (packed cell volume) or hematocrit was measured by the centrifugation of hematocrit capillary tube.

2.7. Statistical analysis

This study was conducted in 60 days according to CRD by using three replicates of each diet. Data was noted for growth, body composition, hematological and mineralization attributes of Nile tilapia. Recorded data was analyzed by ANOVA with the help of Statistix 8.1 software. Then Tukey's HSD test was applied to analyze the multiple comparisons of means of all diets.

3. RESULTS

3.1. Growth parameters

Table-2 shows the growth attributes of tilapia fed with various biochar experimental diets. D3 experimental diet caused highest increase (19.37) in final weight followed by D1. Final weight of fingerlings fed with D3 were 44% higher as compared to fingerlings fed with controlled diet. Highest value (0.96) of specific growth rate (SGR) was also observed under D3 followed by D1 and D2. Feed conversion ratio (FCR) highest value was observed unde D4 diet. D3 showed lowest (1.53) FCR which means that D3 diet was efficiently utilized. Similarly survival rate was also highest in fingerlings fed with D3 experimental diet. D4 and D5 experimental diets didn't performed much better for growth attributes of tilapia fingerlings. Mean values of growth attributes under these diets were also significantly less as compared to controlled diet. From the results of growth attributes, it can be assumed that D3 (corncob biochar, CCBC) can be used as an effective replacement of fishmeal in the diet of tilapia fingerlings.

Table-2: Mean Values ± SE of Growth Attributes of Nile tilapia Fed with Various Biochar Experimental Diets

Diets	Initial Weight	Final Weight	Weight Gain	FCR	SGR	Survival
D0 (Control)	5.17	13.44	8.27	2.91	0.69	90.42
D1 (CSBC)	5.17	17.57	12.4	1.87	0.89	94.3
D2 (WSBC)	5.17	16.95	11.78	2.04	0.86	92.6
D3 (CCBC)	5.17	19.37	14.2	1.53	0.96	96.47
D4 (HWBC)	5.17	9.22	4.05	4.91	0.42	87.5
D5 (GWBC)	5.17	13.51	8.34	2.89	0.7	90.7

3.2. Body composition

Figure-1 shows the body composition of tilapia fingerlings fed with various biochar experimental diets. Figure-1 shows that body composition (crude protein and crude fats) of fingerlings were also significantly affected by all biochar experimenta diets. Highest values (16.04) of crude protein were observed in fingerlings fed with D3 experimental diet followed by D1 (15.91). While lowest value (11.77) of crude protein were observed in fingerlings fed with D4 experimental diet. While in case of crude fats totally opposite results were found. Figure-1 shows that highest value (9.24) of crude fats were observed in fingerlings fed with D4 experimental diet and lowest value (6.12) of crude fats were observed in fingerlings fed with D3 experimental diet.

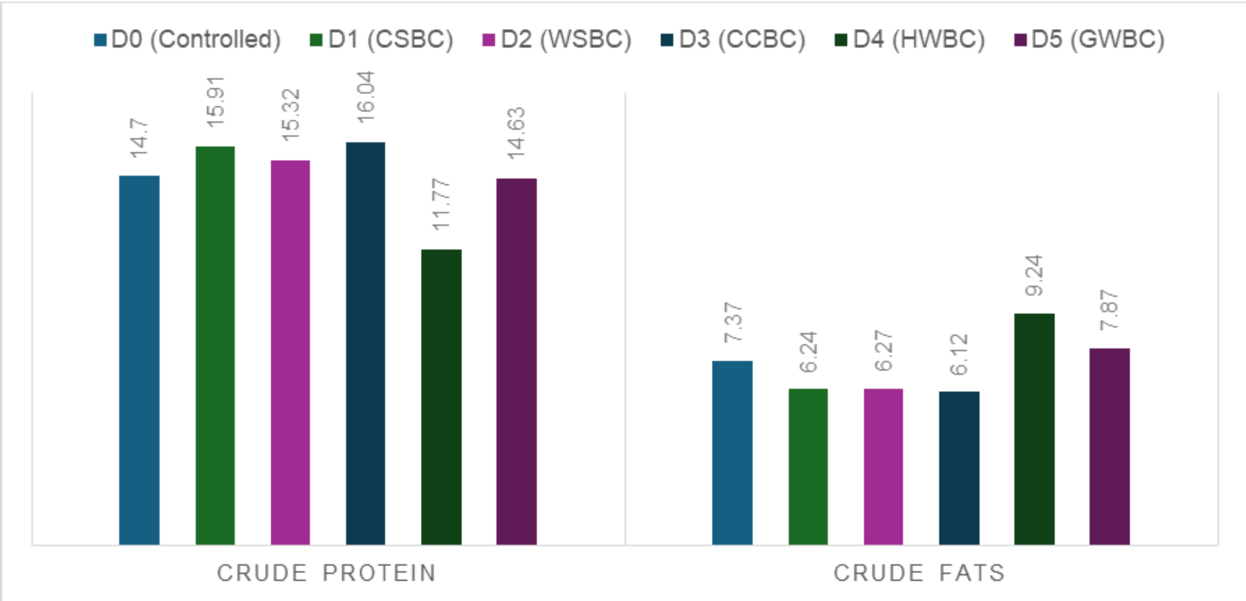

Figure-1: Graphical Representation of Mean Values of Crude Protein and Crude Fats of Nile tilapia Fed with Various Biochar Experimental Diets

3.3. Hematology

Hematological attributes of tilapia fingerlings fed with biochar experimental diets are represented in Figure-2. Figure-2 shows that D3 also provided highest values of hematological attributes just like growth attributes. Highest values (3.47) of red blood cells (RBC's) were observed in fishes fed with diet D3 followed by D1 and D4. These values were 41%, 29% and 27% were higher as compared to RBC's values of fingerlings fed with controlled diet respectively. Similar trends were also observed for white blood cells (WBC's). Highest value of WBC's (7.51) was observed in fingerlings fed with D3 followed by D1 (7.49). Platelats and hemoglobin highest values 75.37 and 8.16 respectively, were observed under D3 experimental diet. Similar trends were also observed for packed cell volume (PCV). Highest values (26.81) of PCV were also observed in fingerlings fed with D3 followed by D1 (25.91). These values were 36% and 32% higher than PCV values of fingerlings fed with controlled diet respectively. D4 and D5 experimental diets didn't performed much better for hematological attributes of tilapia fingerlings. Mean values of hematological attributes under these diets were also significantly less as compared to controlled diet except RBC's which were significantly higher in fingerlings fed with D4 diet. From the results of hematological attributes, it can be assumed that D3 (corncob biochar, CCBC) can be used as an effective replacement of fishmeal in the diet of tilapia fingerlings.

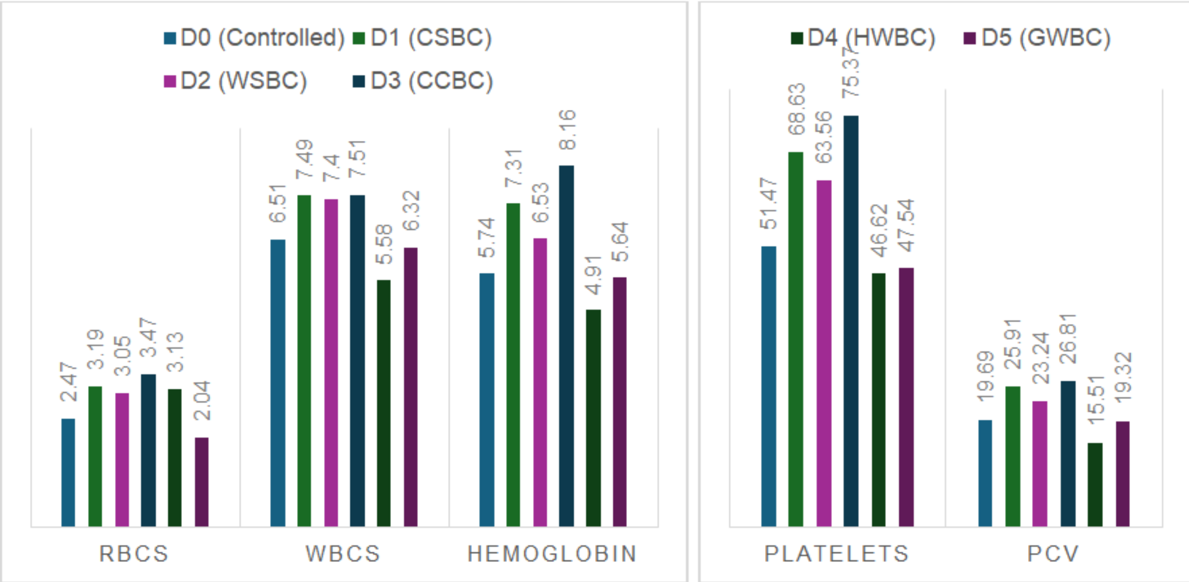

Figure-2: Graphical Representation of Mean Values of Hematological Attributes of Nile tilapia Fed with Various Biochar Experimental Diets

3.4. Mineralization

Minerals are very important for normal functioning of fish bodies. These minerals are found in the form of inorganic substances naturally. Figure-3 shows that minerals composition of fingerlings were also significantly affected by all biochar experimenta diets. In fingerlings fed with D3, the highest values of calcium, sodium, potassium, zinc and iron were observed. While lowest values of these minerals were observed in tilapia fingerlings fed with D4. Highest value of calcium (56.93) were observed in fingerlings fed with D3 followed by D1 (51.52). These values were 57% and 41% higher than calcium values of fingerlings fed with controlled diet. While calcium values of fingerlings fed with D4 were 35% less as compared to controlled. Similarly, sodium, potassium, zinc and iron values of fingerlings fed with D3 were 48%, 51%, 58% and 40% higher as compared to sodium, potassium, zinc and iron values of fingerlings fed with controlled diet respectively.

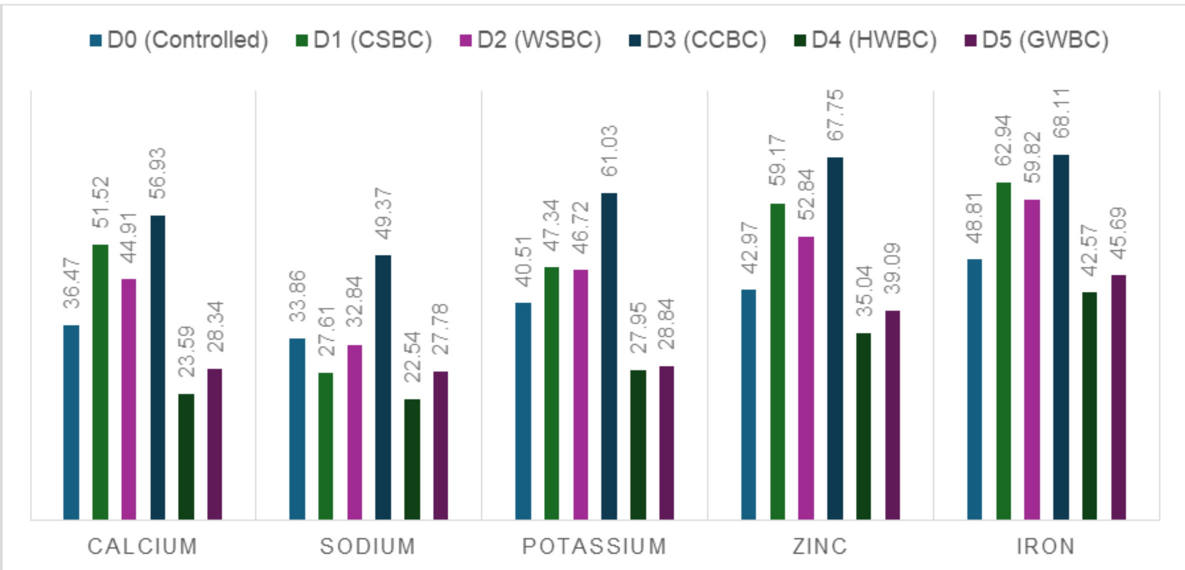

Figure-3: Graphical Representation of Mean Values of Minerals Percentage of Nile tilapia Fed with Various Biochar Experimental Diets

4. DISCUSSIONS

Aquaculture primary need is the identification and formulation of ideal diet. Fish meal which is obtained from animal protein sources is considered as the best diet but it not budget friendly. Its prices are increasing with time. In recent years addition of plant based diets in tilapia diet significantly increased tilapia performance and drawn significant attraction of researchers. In current study effects of various biochar sources on growth, body composition, hematological and mineral composition of Nile tilapia was evaluated. In previous researches on livestock including poultry, goats and cattles, benificial effects of biochar addition were found [27, 28].

Substituting 2% corn cob biochar in tilapia diet provided most significant results for all attributes. In previous trials of addition of 7g/Kg of activated charcoal in diet caused significant increase in Nile tilapia growth after 8 weeks [29]. This addition of activated charcoal in diet remove gases and other pollutants from the gastro intestinal tract, which optimizes the absorption and utilization of nutrients. This efficient utilization of nutrients cause improved feed efficiency and growth of fishes. Our findings were also in accordance with [30], who found similar results in Pangasius hypophthamus. He found that 2% substitution of bamboo charcoal caused significant increase in growth attributes of P. hypophthamus. He also found that 2% substitution of bamboo charcoal caused significant increase in weight gain, feed conversion ratio (FCR) and survival rate of trout and catfish. Najmudeen *et al.*, [31] conducted an experiment to substitute 0.5% and 1% Eichhornia crassipes biochar in O. mossambicus diet. He found that both levels of biochar substitution caused significant increase in weight and length, but maximum values of all growth attributes were observed at 1% Eichhornia crassipes biochar substitution. Furthermore, Michael *et al.*, [32] substituted 3% wood charcoal in the diet of red tilapia juveniles. He found that this 3% substitution of wood charcoal in the diet caused significant increase in all growth attributes of red tilapia juveniles. In current our experiment, it was revealed that all 2% replacements of biochar except D3 (HWBC) significantly improved the performance of Nile tilapia for all attributes. It also has been found that biochar supplementation in the diet of animals improves their health and production and also enhance the efficiency of nutrients utilization [33].

Thu *et al.*, [34] observed that body composition indices (crude protein: 17.5%, crude fats: 4.1%) were significantly enhanced by adding 4% bamboo charcoal to the diet of Japanese flounder and it also improved protein digestibility. In our findings similar results were found for body composition indices. D3 (CCBC) caused significant increase in crude protein and reduced the content of crude fats. Thu *et al.*, [34] also noted that the use of bamboo charcoal in P. olivaceus diets led to significant carcass improvements due to reduced ammonia excretion, which enhanced the protein content and overall quality of the fish. Similarly, Yoo *et al.*, [35] reported that a combination of wood vinegar and charcoal had a positive impact on the carcass quality of olive flounder (Paralichthys olivaceus).

This study's hematological observations revealed that all types of BC supplementation positively affected fingerlings, except for HWBC (D4). The most favorable hematological parameters were observed with CCBC (D3). Research on the impact of dietary BC on fish hematological indices is limited; however, some studies have been conducted in cattle and poultry farming. Mabe *et al.*, [36] reported that feeding bamboo BC to Cyprinus carpio did not affect growth indices but improved serum quality, indicating better overall fish health. Dim *et al.*, [37] observed significant improvements in RBC, HCT, Hb, and WBC when Meleagris gallopavo (turkeys) were given BC at 5 g kg^{-1}, 15 g kg^{-1}, and 25 g kg^{-1}, with the best results at 15 g kg^{-1}. Elghalid [38] found that hematological traits such as Hb, RBCs, and hemocytosis percentage (HCT%) improved in chicks fed diets containing varying levels of biochar (0%, 1%, 2%, 4%, 6%, or 8%). BC supplementation in red tilapia likely had a detoxifying effect, enhancing hematological parameters and reducing oxidative stress by decreasing the absorption of toxins and other harmful substances into the fish gut [32].

Mineral content analysis of fish bodies revealed that D3 (CCBC) caused maximum increase in minerals content. Fingerlings fed with D3 (CCBC) diet showed the highest percentage values calcium, sodium, potassium, zinc and iron. Biochar possesses high cation exchange capacity, which makes minerals easily available for absorption. Because of this high cation exchange capacity biochar increases the levels of minerals in fingerlings bodies [39].

5. CONCLUSIONS

In this experiment D3 (CCBC) experimental diet performed exceptionally well for all growth, hematological and mineral attributes of Nile tilapia. Other biochar diets also provided significant results except D4 (HWBC). So form this experiment it can be concluded that 2% of CCBC has the potential to be used as envirnonment friendly, cost effective and sustainable supplement in Nile tilapia diets.

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