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Study of Supplementation of Various Levels of Biochar on Health and Production Performance of Growing Local Turkey (*Meleagris gallopova)* Poults

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ABSTRACT

The study determined the hematobiochemical, lipid peroxidation and antioxidant enzyme parameters of local day-old male turkey poults (n=24), during two feeding periods, grower phase (6-15 weeks) and finisher phase (16-24 weeks). A commercial (Top® brand) chick mash served as the basal diet, while biochar supplementation levels were 5, 15 and 25g/kg at the start of the grower phase, until slaughter. Poults fed 5 and 15g/kg biochar had higher (P<0.05) final body weight and average daily weight gain when compared with poults fed other treatment diets. White blood cell count was higher (P<0.05) in poults fed 25g/kg biochar diet compared with those of the control group. Poults fed 5 and 15g/kg biochar diets had reduced serum (P<0.05) aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, creatinine, and bilirubin concentrations when compared with those of the control birds, while the lowest concentrations of uric acid were recorded in poults fed 5g/kg biochar diet. Increased (P<0.05) activities of serum catalase and glutathione peroxidase were observed in 15 and 25g/kg biochar groups, coupled with decreased (P<0.05) malondialdehyde values which were comparable (P>0.05) with those of the control group. The results of the present study revealed that 15g/kg biochar can be incorporated in the diet of local turkey poults for improved growth performance and serum-biochemistry and reduced lipid peroxidation. However, improvement in hematology and antioxidant enzyme activity in local turkey poults require 25g/kg biochar supplementation.

Key words: Biochar, Turkey poults, lipid peroxidation, Serology, Antioxidant capacity, Hematology.

INTRODUCTION

Poultry producers continue to seek for marketable products, while maintaining costs in the production cycle. This has led to the advent of feed additives, which are cheap dietary protocols with the potential to improve feed efficiency and overall performance of poultry (Dhama et al. 2015). Feed additives are known to enhance blood indices and endogenous antioxidants and inhibit lipid peroxidation in poultry birds. Notwithstanding, the growth promoting benefits of some of these feed additives usually come at a metabolic cost, thus, affecting the health and welfare of the animals (Al-Dobaib and Mousa 2009; Oloruntola et al. 2019). It is therefore necessary and justifiable to investigate the effect of natural, locally sourced, cheap, and readily available feed additives such as biochar with no attendant harmful effect on birds.

Biochar is the thick carbon material that results from partial incineration of biomass that occur through pyrolysis in the presence of no-or-low oxygen at a temperature range of 300-1000^{o}C (International Biochar Initiative 2015; Weber and Quicker 2018). The physicochemical attribute of biochar is affected by the feedstock type, prevailing conditions, and the temperatures under which the pyrolysis occurred (Weber and Quicker 2018). Biochar enhances growth, health and feed utilization efficiency in livestock and poultry due to its redox activity and absorption capacity (Sun et al. 2017; Schmidt et al. 2019). Flores et al. (2021) reported that biochar supplementation improved growth in turkey birds. However, there is still a dearth of literature on the positive effects of biochar supplementation in turkey birds. Against these backdrops, the present study was conducted to determine the hematological, serological and antioxidant profiles of growing turkeys.

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MATERIALS AND METHODS

Study Location and Duration and Ethical Statement

The study was carried out at the Turkey Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. The study spanned a period of 24 weeks. The experimental procedures employed in the study adhered strictly to the provisions of the Ethical Committee on the Use of Animals and Humans for Biomedical Research University of Nigeria, Nsukka.

Experimental Diets

The poults were fed with a commercial feed (Top® brand) for the first 0-5 weeks of age (brooding phase), and throughout the duration of the feeding trial (24 weeks). The basal diet was maize-soybean-based and had no inclusion of biochar. Biochar was incorporated into the commercial basal diet at 5, 15 and 25g/kg at the grower (6-15 weeks) and finisher (16-24 weeks) phases respectively. Table 1 shows the percentage compositions of the experimental grower and finisher diets.

Preparation of Biochar

Poultry organic waste materials were collected and sun-dried for 2 weeks. After that, the materials were emptied into an improvised kiln to generate the biochar. The char was discharged from the kiln after uniform pyrolysis was achieved and spread on a bare floor to cool. The biochar was ground, sieved to fine powder and included in the diets of the birds at various inclusion levels according to their treatment tags.

Chemical Analysis of Biochar

The biochar was analyzed to determine its proximate composition based on the methods of AOAC (2006) and the results are presented in Table 2.

Management of Experimental Birds

A total of 24, day-old male turkey poults with initial mean weights of 60g were used for the study. The poults were vent-sexed, brooded for five weeks in deep litter system and fed commercial (Top® brand) chick mash. During the brooding phase, the walls of the brooding pens were high enough to conserve heat, ensure proper brooding environment and permit free flow of air within the brooding house. Sequel to brooding, the allocation of poults to four treatment groups followed the completely randomized experimental design. The grower diet was introduced to the birds at their 6th week of age, whereas they were introduced to the finisher diet at the 16th week until the slaughter age of 24th weeks. The birds were fed *ad libitum* and allowed access to clean drinking water by providing ample number of drinking troughs. Vaccination of the poults was done routinely.

Parameters Studied

Growth Performance

The average daily feed intake (ADFI) was determined by the difference between the amount of feed previously offered to the birds and the amount left unconsumed in the feeding trough the next day. The weight readings obtained on a weekly basis (from 6-24 weeks) were used to determine the average daily weight gain (ADWG). The feed conversion ratio (FCR) was determined as a measure of the amount of feed that was consumed which was utilized in reaching a unit of weight gain.

Hematology and Serum-biochemical Parameters

At the 16th (end of grower phase) and 24th week (end of finisher phase) of the study, blood samples were collected from 16 poults and used for hematological and serological studies. Blood samples (2.5mL) were collected from the wing veins of the birds into ethylene-diamine tetra acetic acid (EDTA) bottles. The blood was collected using sterile syringes and needles and used for the determination of hematological indices. Another set of blood was collected in plain tubes without EDTA used to determine serum metabolite concentrations. The red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb) and packed cell volume (PCV) were determined based on the methods of Ochei and Kolhatkar (2008). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957), while the activities of alkaline phosphatase (ALP) were assayed by the method of Babson et al. (1966) as outlined in the Randox kit. Total protein was determined based on the methods described by Tietz (1994), whereas serum creatinine and urea concentrations were assayed based on the method of Tietz (1994). The calorimetric method was employed to assess the total and direct concentrations of serum bilirubin (Garber 1981). The activity of catalase (CAT) was assayed by the method of Sinha (1972), while superoxide dismutase (SOD) activity was determined based on the method of Misra and Fridovich (1972). The activity of glutathione peroxidase (GSH-Px) was determined based on the methods described by Paglia and Valentine (Paglia and Valentine 1967).

Lipid Peroxidation

Lipid peroxidation was estimated in the serum by measuring the level of the lipid malondialdehyde (MDA) via a spectrophotometer as described by Wallin et al. (1993). Briefly: 10µL of serum, and 10µL distilled water (DW) was added into three well-labelled test tubes. After that, 0.5mL of 25% TCA (trichloroacetic acid) and 0.5mL of 1% TBA (thiobarbituric acid) in 3% NaOH were added. The mixture was boiled for 40 minutes in a water bath and cooled in cold water. Then, 0.1mL of 20% sodium dodecyl sulphate was added to the cooled solution and mixed properly. The absorbance was then recorded at wavelengths of 532nm and 600nm against a blank.

Statistical Analysis

Data generated from the study were subjected to analysis of variance (ANOVA) as described for completely randomized design (Steel and Torrie 1980). The significant differences between treatment means were separated using Duncan's New Multiple Range Test as cited by Obi (2002).

RESULTS

Growth Performance

The results on growth performance indices of growing local turkey poults fed biochar supplemented diets are shown in Table 3. The final body weight and ADWG were

Table 1: Percentage composition of commercial grower and finisher diets

Ingredients	Grower (%)	Finisher (%)
Maize	48.00	50.00
Soybean meal	37.00	24.10
Fish meal	4.50	1.50
Bone meal	4.00	17.55
Oyster shell	3.20	3.65
Vitamin premix*	2.50	2.50
Salt	0.25	0.25
DL-Methionine	0.25	0.25
Lysine	0.20	0.10
Calculated components %	0.10	0.10
Crude protein	22.40	19.50
Crude fibre	4.26	4.65
Ether extract	4.52	4.31
Energy (Kcal/Kg ME)	3100.00	3150.00

*Each 2.5kg of vitamin premix contains: 10,000,000 IU Vitamin A; 2,200,000 IU Vitamin D3; 10,000mg; Vitamin E; 2000mg Vitamin K3; 1500mg Vitamin B1; 5000mg Vitamin B2; 1500mg Vitamin B6; 10mg; Vitamin B12; 15,000mg Niacin; 20mg biotin; 125,000mg Antioxidant; 500mg Folic acid; 5000mg Calpan

Table 2: Proximate compositions of biochar

Parameters (%)	Quantity
Moisture	10.00
Crude fibre	36.00
Crude protein	-
Ash	16.00
Nitrogen-free extract	38.00

Table 3: Performance of local toms fed varying dietary inclusions of biochar (9-24wks)

Parameters	T1	T2	T3	T4	SEM
Initial body weight (g)	1020	1000	1010	1003	0.11
Final body weight (g)	6470b	7270a	7010a	6390b	1.79
ADWG (g/bird)	51.90b	59.71a	57.14a	51.04b	2.06
ADFI (g/bird)	313.47	322.43	329.12	337.37	3.38
FCR (g/g)	6.03ab	5.39b	5.75ab	6.60a	1.96

Values (Mean±SEM) bearing different alphabets in a row differ significantly (P<0.05). T1=Control (0% biochar), T2=5g/kg biochar, T3=15g/kg biochar, T4=25g/kg biochar, BW=Body weight; ADWG=Average daily weight gain, ADFI=Average daily feed intake, FCR=Feed conversion ratio, SEM=Standard error of mean.

Table 4: Hematological indices of local toms fed varied dietary inclusions of biochar

Parameters	T1	T2	T3	T4	SEM
Grower phase					
WBC ($x10^6/mm^3$)	1.07b	1.07b	1.13ab	1.27a	3.45
RBC ($x10^{12}/L$)	8.40a	7.35b	8.55a	9.10a	0.25
Hb (g/dL)	9.88a	9.03b	9.55ab	9.83a	0.14
PCV (%)	40.50a	36.50b	38.50ab	40.00ab	0.69
Finisher phase					
WBC ($x10^6/mm^3$)	1.07b	1.09ab	1.15ab	1.30a	4.02
RBC ($x10^{12}/L$)	9.57a	7.66c	8.81b	9.42ab	0.29
Hb (g/dL)	10.01ab	9.75c	9.92b	10.04a	0.04
PCV (%)	41.50	38.50	39.50	40.50	0.53

Values (Mean±SEM) bearing different alphabets in a row differ significantly (P<0.05). T1=Control (0% biochar), T2=5g/kg biochar, T3=15g/kg biochar, T4=25g/kg biochar, WBC=White blood cell, RBC=Red blood cell, Hb=Hemoglobin concentration, PCV=Packed cell volume, SEM=Standard error of mean.

decreased at the 0% (control) and at the highest inclusion level of biochar (T4) i.e., 25g/kg dietary group. Poults fed 5g/kg (T2) and 15g/kg (T3) biochar had the highest (P<0.05) final body weight and ADWG. Although, the FCR was improved (P<0.05) in the T2 birds compared with the T4 group, the value was the same (P>0.05) with those of poults on T1 and T3. The ADFI values were not influenced by biochar treatments.

Hematology

The hematological profile of poults at the grower and finisher phases are shown in Table 4. At the grower phase, WBC count was higher (P<0.05) in T4 birds when compared with those of the T1 and T2 groups. The highest (P<0.05) RBC counts were recorded in T1, T3 and T4 poults, whereas poults in T2 group had the lowest (P<0.05) value for RBC. Higher Hb values were recorded in T1 and T4 birds, whereas birds fed the T2 diet had lower Hb values. Whereas the highest PCV values was recorded in T1 birds and the lowest in birds in the T2 group, their PCV values were similar (P>0.05) with those of birds fed the T3 and T4 diets. At the finisher phase, higher (P<0.05) WBC value was recorded in T4, whereas, T1 birds had lower WBC. Birds in the T1 and T4 groups had higher (P<0.05) RBC and Hb values than T2 and T3 birds. PCV values were the same (P>0.05) across treatment groups.

Serum Biochemical Results

Liver Function Tests

Fig. 1 shows the liver function parameters of local toms fed biochar supplemented diets. At the grower phase, poults fed various inclusion levels of biochar had similar (P>0.05) albumin concentration with poults in the control group. Effect of treatments on albumin concentrations during the finisher phase were not significant while total protein levels were also similar across treatments. Direct and total bilirubin values were lower (P<0.05) in the T2 and T3 birds during both phases. The concentrations AST, ALT and ALP were lower (P<0.05) in T2 and T3 birds, whereas at the finisher phase, T2 birds consistently had lower (P<0.05) AST, ALT and ALP concentrations compared with birds on other treatment diets.

Kidney Function Tests

The kidney function parameters of local turkey toms fed biochar supplemented diets are shown in Fig. 2. The birds fed T2 consistently had lower (P<0.05) concentrations of creatinine and urea concentrations during their grower phase. Throughout the finisher phase, T1 and T2 had lower creatinine, even as T2 and T3 had lower (P<0.05) urea concentrations than T4. Uric acid concentration of poults in the control and 25g/kg biochar groups were highest (P<0.05) while those fed 5g/kg biochar diet had the lowest (P<0.05) value for uric acid concentration.

Antioxidant Profile

Fig. 3 shows the lipid peroxidation and antioxidant enzyme activity parameters of local turkey toms fed biochar supplemented diets at both grower and finisher phases. During both phases of the study, CAT activity was consistently higher (P<0.05) in T4 birds when compared with T1 and T2 groups. The concentration of MDA was lower (P<0.05) in birds fed T1 and T2 diets at the grower phase, when compared with T4 birds with higher (P<0.05) MDA value. The MDA concentrations of poults in T1, T2 and T3 were significantly reduced (P<0.05) during the

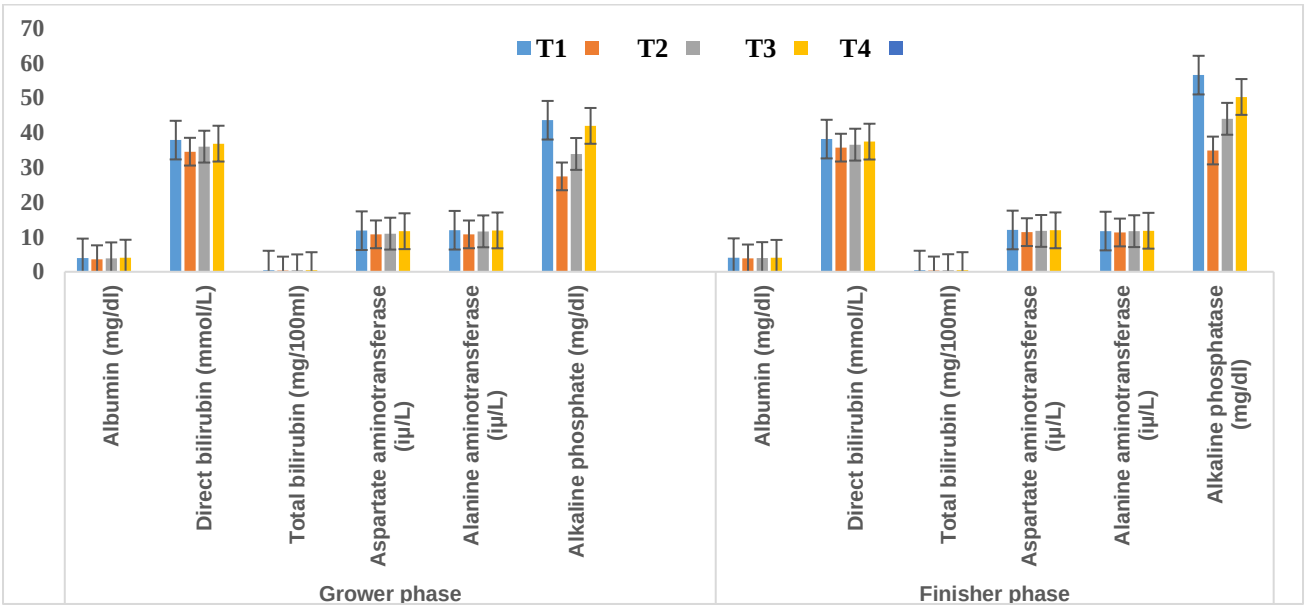

Fig. 1: Serum liver functions of local toms fed varying dietary levels of biochar.

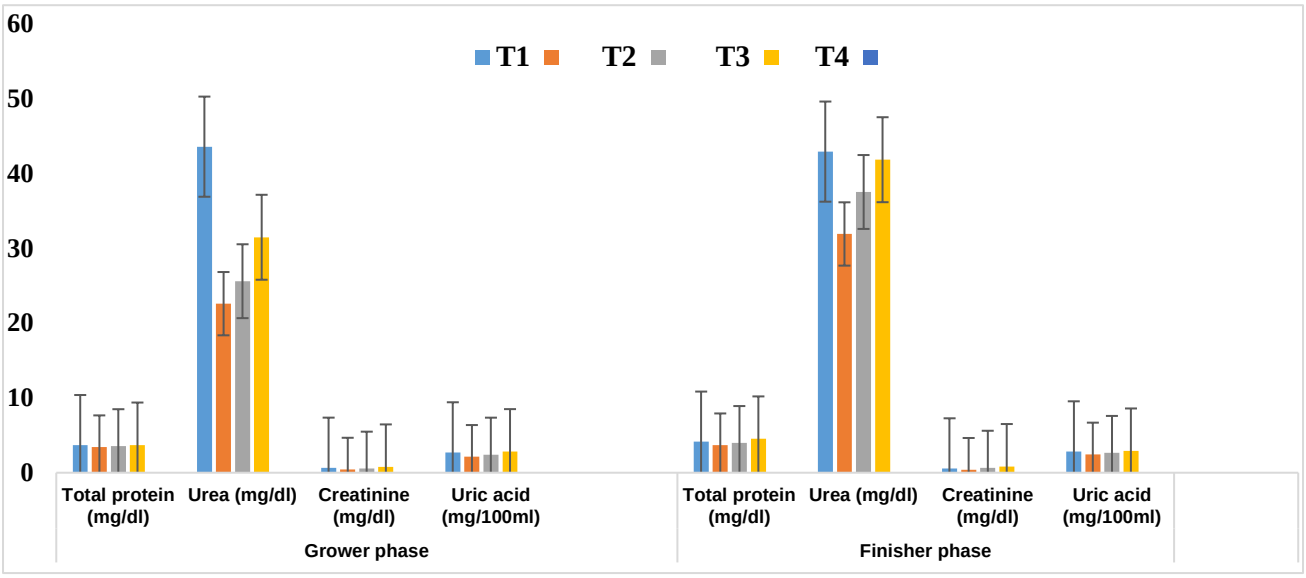

Fig. 2: Effect of biochar inclusion on serum kidney functions of local toms.

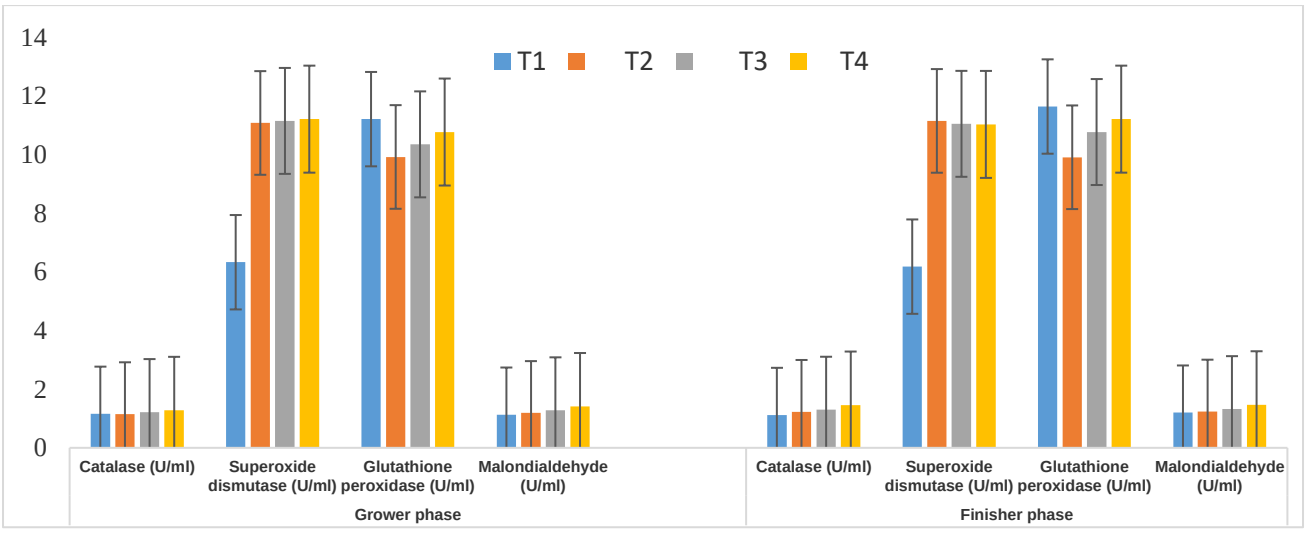

Fig. 3: Serum antioxidant profile of local toms fed varied inclusions of biochar.

finisher phase while higher (P<0.05) MDA values were recorded in T4 birds. Higher (P<0.05) GSH-Px values was recorded in T1 birds while T2 birds had lower (P<0.05) concentrations of GSH-Px during both phases. Similar (P>0.05) GSH-Px values existed among the T3, T4, T1 and T2 groups. Dietary biochar had no significant (P>0.05) effect on serum SOD activity at both grower and finisher phases of the study.

DISCUSSION

The results in Table 3 showed that 5 and 15g/kg biochar supplementation improved FBW and ADWG of local turkey poults. Flores et al. (2021) also made similar observation that male turkeys fed with 20% biochar litter-treated diets had higher body weight gain when compared with the control birds. There are similar reports on improved growth upon biochar feeding in broiler birds (Evans et al. 2015; Prasai et al. 2017; Rashidi et al. 2020; Kalus et al. 2020). In the present study, it is evident that turkey poults can tolerate up to 15 g/kg inclusion of biochar. Nevertheless, there are previous recommendations that biochar should not be incorporated beyond 2% (Rashidi et al. 2020) and 6% (Evans et al. 2015) in broiler diets, due to the suppressed growth performance of the birds. Biochar improves growth because it binds to toxins and reduce their bioavailability. Biochar also slows down the rate of digesta passage via the gastrointestinal tract, leading to an enhanced villi function and a decreased harmful gut bacteria population (Kalus et al. 2020; Rashidi et al. 2020).

Our results on increased RBC, PCV and Hb at 15 and 25g/kg biochar inclusion (Table 4) agree with the findings of Dim et al. (2018) who reported that biochar supplementation increased the Hb, RBC and PCV of broiler birds. There are very limited literatures on the dietary effect of biochar on hematological indices of turkey poults. However, a previous work showed that biochar inclusion did not have any influence on RBC, Hb and PCV values of turkeys (Majeswka et al. 2009). Similarly, Odunsi et al. (2007) and Kana et al. (2010) reported that dietary biochar supplementation did not influence the hematological parameters of broiler birds. Interestingly, the 25 g/kg biochar-treated birds had higher white blood cell count, suggesting increased antibody production, and by extension, disease resistance. Biocharcoal supplementation of broiler diets had no effect on their WBC values (Kana et al. 2010; Dim et al. 2018).

Turkey poults fed 5 and 15g/kg biochar diets had reduced AST, ALT and ALP concentrations (Fig. 1). Elevated AST and ALT concentrations had been attributed to the toxicity of some feed formulation ingredients. This toxicity causes cellular damage owing to necrotic or altered permeability of the cell membrane, coupled with muscle damage (Rajput et al. 2017). The reduction in serum AST, ALT and ALP levels may be linked to the toxin-binding ability of biochar. Similar works exist on the positive effects of biochar in reducing AST, ALT and ALP concentrations in broiler birds (Kana et al. 2010; Jiya et al. 2013; Rashidi et al. 2020).

Serum urea, in addition to total protein, albumin and glucose are important biomarkers for evaluating hepatic injury and function. From the present findings, serum urea levels were reduced in poults fed the 5 and 15g/kg biochar diets (Fig. 2), suggesting that there was no incidence of hepatic dysfunction in the poults. The increase in serum urea levels had previously been linked to impaired synthesis of protein due to decreased amino acid use (Shannon et al. 2017). Higher uric acid levels were noted among the 15 and 25g/kg biochar groups and the control birds, coupled with decrease in creatinine levels. Higher serum uric acid values could be due to increased radical scavenging ability of the test material that increases the antioxidant defence capacity of animals (Simoyi et al. 2002). On the other hand, increase in serum creatinine levels is an indication of muscular wastage (Adeleye et al. 2018), as well as nephrotoxicity (Benjamin 1978). Nevertheless, Fafiolu (2007) attributed high uric acid levels to low efficiency of protein utilization that leads to protein wastage.

In the current study, there was an increase in serum CAT and GSH-Px activities and a decrease in the MDA noted in the biochar-treated groups (Fig. 3). This improvement in serum antioxidant parameters is an indication that turkey birds fed biochar supplemented diets had minimal exposure to lipid peroxidation due to an enhanced antioxidant defence capacity. There is a dearth of literature on the dietary effects of biochar on serum MDA and antioxidant enzyme activity in turkey poults. However, the results of an earlier study revealed that dietary poultry-litter biochar decreased the MDA concentrations in the breast meat of broiler birds but had no effect on the serum MDA levels (Rashidi et al. 2020).

Conclusion

Results from the present study revealed that biochar can be supplemented at 15g/kg in the diets of local turkey poults for improved growth performance and serum-biochemistry and reduced lipid peroxidation. On the other hand, improvement in hematology and antioxidant enzyme activity in local turkey poults require dietary supplementation of 25g/kg biochar.

Authors Contributions

ECD: Designed and conducted the study, collected and analyzed the data, wrote the first draft, EAA: Wrote the first draft, revised manuscript; MFN: Assisted in data collection and laboratory analysis, CEO: Revised manuscript; ABF: Revised manuscript; FBL: Revised manuscript; AOE: Designed and supervised study. All authors read and approved the final manuscript.

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