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Growth, Blood and Histopathological Changes in Broiler Fed Aflatoxin

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Abstract

Aflatoxins are lethal secondary products that have negative influences on growth, health, and organ functioning of broiler chickens, especially in diets based on maize. This study was focused on the protective effect of activated charcoal against the toxicity of aflatoxins on broilers. A total of one hundred one-day-old Arbor Acres chicks were randomly allocated to five diet treatments based on T1: basal control diet, and T2-T5: basal diet with supplementation of aflatoxin, 200 ppb, activated charcoal, 0, 0.5, 1.0, 1.5, and 2.0%. At day 42, blood samples would be taken to assess serum biochemical and hematological parameters, and 6 birds would be euthanised to evaluate the liver and kidney histopathology. Biochemistry of serum did not have any significant differences ($P > 0.05$) between ALT (2.93-6.48 IU /L), total protein (2.97-3.65 g/dL), albumin (0.87-1.27 g/dL), cholesterol (108.86-138.79 mg/dL), glucose (132.61-148.11 mg/dL), or calcium (8.94-9.47 mg/dL). AST levels showed tremendous disparities in comparison with treatments, and HDL, LDL, uric acid, alkaline phosphatase, creatinine, and phosphorus showed significant (P 0.05) alterations, which is suggestive of a dose-dependent physiological reaction to activated charcoal. This did not significantly affect haematological indices such as packed cell volume (28.83-32.83 %), hemoglobin (9.27-10.62%), and red blood cell count ($2.9–3.34 \times 10^6$/µL). The count of white blood cells ranged between 15.36 - 17.83 x 10^9/µL, platelet between 13.52 - 16.80 x 10^3, and lymphocyte percentages were highest in T5 (65.17%) and lowest in T4(59.33%). Dose-dependent hepatic and renal lesions were observed by histopathology with moderate degeneration in 1.0 percent charcoal (T3) and almost all tissue being preserved in 2.0 percent (T5), as in the control group. The results show that the protective effect on the negative effects of aflatoxin-contaminated diets is induced by the addition of activated charcoal to the food and reduces the negative effects on broiler chicken growth, physiological indices, and protein integrity. Maximum charcoal supplementation improves the resistance to aflatoxicosis and promotes the health of poultry in polluted feeding environments.

Keywords: *Aflatoxin, Broiler chicken, Activated charcoal, Growth performance, histopathology, Serum biochemistry.*

Introduction

The aflatoxins are one of the most dangerous mycotoxins that put food and feed systems at risk worldwide, and aflatoxin B1 (AFB1) was recognised as the most powerful and most common one (Gobikanila *et al*., 2025). The observed types of secondary metabolites are mainly the products of the fungal taxa of Aspergillus flavus and A. parasiticus in the case of exposure to the warm and humid environmental conditions that favour fungal growth (Klich, 2007; Guo *et al*., 2023). It is particularly prone to tropical and subtropical areas, since the climate patterns of these areas favor the development of fungi in crop growing, harvesting, storage, and processing stages. Therefore, aflatoxin pollution continues to limit maize-based poultry production systems, especially in developing economies.

The nutritional dietary intake with aflatoxins has been associated with impaired performance, reduced feed efficiency, immunosuppression, hepatotoxicity, and increased incidences of mortality in broiler chickens (Moloi *et al*., 2024). Chronic aflatoxicosis, which is not only prevalent in field conditions but also more common than acute toxicity, normally develops insidiously and compromises physiological resistance over time (Peles *et al*., 2019; Nazhand *et al*., 2020). Feed contamination impairs protein synthesis, causes oxidative stress, and upsets metabolic homeostasis, which eventually affects the declining productivity and profitability of poultry enterprises (Jobe *et al*.,2023; Moloi *et al*., 2024). These negative effects highlight the importance of using effective methods of mitigating diets.

Mycotoxins, such as aflatoxins, ochratoxins, fumonisins, deoxynivalenol, or zearalenone, are structurally heterogeneous, low-molecular-weight, fungal metabolites with severe toxicological effects (Solis-Cruz *et al*., 2018). These are ubiquitous, which makes it difficult to prevent them entirely and requires other mitigation measures (Salvo *et al*., 2018). Out of these toxins, aflatoxins are especially worrisome as they are hepatotoxic and carcinogenic in nature, having been proven to have a well-associated correlation with hepatocellular carcinoma in humans (Pyshyev *et al*., 2021).

Several strategies have been put forward to reduce the toxicity imposed by aflatoxins in poultry feeds, such as better agronomic systems, superior storage systems, and the addition of toxin binders. Adsorbents like bentonite, hydrated sodium -calcium aluminosilicates, and products of yeast work by adsorbing aflatoxin to absorb aflatoxin in the gastrointestinal tracts, thus lowering the systemic absorption (Kolawole *et al*., 2022; Mgbeahuruike et al., 2023). Among them, one has been drawn to activated charcoal because of its high adsorption capacity, porosity, and the ability to bind to the spectrum (Ahmed *et al*., 2022).

Activated charcoal works by binding aflatoxins in the digestive system, reducing bioavailability and toxic effects (Genli *et al*., 2022)

This study aims to advance resilience in the poultry production system by helping to develop resilient systems to dose-dependent protective potential of activated charcoal in addition to its application in sustainable feed safety actions.

Materials and Methods

Study Location

The current experiment was conducted in the Teaching and Research Farm of the University of Ibadan, Ibadan, Oyo State, Nigeria. The location is a tropical climate with a distinct rainy and dry season. The experiment was run in the period between 22 ^{0}C and 30 ^{0}C ambient temperature, the relative humidity was 40 per cent to 55 per cent or less, and it was carried out during the dry season (December 2024 to January 2025). These were deemed to be favorable to the production of broilers and continued to be monitored within the period of the experiment.

Management and Experimental Birds

One hundred 1-day chicks were purchased in a well-known commercial hatchery located in Ibadan. The birds were randomly allocated to five dietary treatment groups on arrival, with each consisting of 20 chicks and five birds per replicate in a randomised complete block design. The experiment lasted 42 days. The poultry pen was well-ventilated; the size of the pens was 2 m x 2m. Wood shavings was used as bedding material. Feed and water were given *ad libitum*. The normal (routine) management procedures, such as check-ups, inoculation, deworming, and strict biosecurity procedures, were established to provide maximum welfare and to limit the chances of exogenous contamination.

Production of Aflatoxin and Maize Preparation with Inoculums

Aspergillus flavus (isolate 3228), a toxigenic strain of this fungus, was obtained in the Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. The fungus culture was cultivated on V8 agar medium, which had 50 mL of V8 vegetable juice diluted by 950 ml of sterile distilled water and corresponding pH adjusted to 6.0. Plates were inoculated following autoclaving at 121 ^{0}C and incubated at 37 ^{0}C over a period of seven days. The spores were picked in 0.1% Tween 20 solution and diluted to 2 x 10 6spores/mL with a hemocytometer. Maize grains were washed with clean water for 10 minutes and autoclaved at 121 °C for 45 minutes, then cooled, and 50 ml of spore suspension was added to each 500 g. The inoculated maize was then incubated at 28-30 ^{0}C at intervals of shaking to allow growth of the fungi to be uniform everywhere.

Nomination for Experimental Diets and Structure of Treatments

The experimental diets were prepared by blending maize grains inoculated with Aspergillus flavus with aflatoxin-free maize (Aflasafe) to create a basal feed containing 200 parts per billion (ppb) of aflatoxin per kilogram of diet. Diets were created and used experimentally to meet the nutrient demands of broilers at the starter, grower, and finisher stages. The starter diet had 3104.76kg/kg -1 metabolizable energy (ME) and 23.00 percent crude protein (CP); the grower diet had 3100kcal/kg -1 ME and 20.01 percent CP; and the finisher diet had 3150kcal/kg -1 ME and 19.20 percent CP. Five dietary treatments were established as follows:

- Diet 1 (Control): Basal diet without activated charcoal
- Diet 2: Basal diet + 200 ppb aflatoxin + 0.5% activated charcoal
- Diet 3: Basal diet + 200 ppb aflatoxin + 1.0% activated charcoal
- Diet 4: Basal diet + 200 ppb aflatoxin + 1.5% activated charcoal
- Diet 5: Basal diet + 200 ppb aflatoxin + 2.0% activated charcoal.

Weight and feeds were made weekly. The weights of the body were measured on a digital scale with a precision of 1~g. The amount of the feed fed in was calculated by subtracting the amount of unused feed. The calculation of performance indices was done as follows:

i. Weight Gain (WG) = Final body weight -Initial body weight.
ii. Average Daily Gain (ADG) =weightgain/days.
iii. Average Daily Feed Intake (ADFI)= total weight of feed fed: number of days.
iv. Feed Conversion ratio (FCR): = total feed eaten/total increment of weight.

Blood Laboratory Analyses and Collection

At the end of week 6, Birds were chosen randomly from the 5 treatments to sample their blood. The selected birds were humanely euthanized using cervical dislocation. 5mL of blood was taken through the jugular vein with sterile syringes. Half of each of the samples was moved into EDTA tubes to get hematologic analysis,while the other half was moved into plain tubes to get serum separation. Hematological parameters examined were packed cell volume (PCV), level of hemoglobin (Hb), the number of red blood cells (RBCs), white blood cells (WBCs), and the number of differential leukocytes. Biochemical parameters that were analysed in serum were the following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, glucose, and creatinine.

Tissue Collection and Histopathological Examination

At the end of the experimental period, two birds were randomly selected from each replicate for tissue sampling. The liver and kidney were carefully excised and rinsed in physiological saline to remove residual blood. Tissue samples were then fixed in 10% neutral buffered formalin for preservation. Standard histological procedures involving dehydration, embedding, sectioning, and staining were carried out. The prepared sections were examined under a light microscope to evaluate possible pathological lesions associated with dietary aflatoxin exposure and the ameliorative effects of activated charcoal.

Statistical Analysis

All data were examined according to SPSS version 25.0. One-way analysis of variance (ANOVA) was conducted, to find out the impact of diet treatments. Separating differences between means was done with Duncan Multiple Range Test with significance level of $p< 0.05$. Correlations analyses were performed to determine associations between aflatoxin exposure, physiological indices and growth performance parameters.

Results

Performance of broiler chickens fed aflatoxin feed with varied inclusion levels of activated charcoal

The performance indices of broilers fed aflatoxin-contaminated diets supplemented with graded levels of activated charcoal are presented in Table 4.1. Birds on the control diet (T1) recorded significantly higher average daily feed intake (106.84 ± 3.39 g) compared with those on aflatoxin-containing treatments T2 (81.21 ± 5.58 g), T3 (65.48 ± 2.61 g), T4 (64.69 ± 4.55 g), and T5 (63.92 ± 4.55 g). Similarly, average daily weight gain was highest in T1 (48.17 ± 7.57 g) and markedly reduced across T2 (29.32 g), T3 (24.89 g), T4 (19.48 g), and T5 (20.53 g). Feed conversion ratio (FCR) was lowest and most efficient in T1 (2.25 ± 0.29), moderate in T2 and T3 (2.78 ± 0.10 and 2.78 ± 0.62, respectively), and poorest in T4 (3.44 ± 0.76), followed by T5 (3.14 ± 0.37). These findings indicate that aflatoxin exposure adversely affected feed intake, weight gain, and feed utilisation, with the greatest inefficiency observed in T4. However, statistical analysis revealed no significant differences among treatments ($P > 0.05$).

Table 1: Performance of broiler chickens fed aflatoxin feed with varied inclusion levels of activated charcoal

Parameter	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*
ADFI (g/bird)	106.84^{a} ± 3.39	81.21^{b} ±5.58	65.48^{c} ±6.25	64.69^{c} ± 2.61	63.92^{c} ±4.55	2.34
ADWG (g/bird)	48.17^{a} ±7.57	29.32^{b} ±3.08	24.89^{b} ±7.94	19.48^{b} ± 4.22	20.53^{b} ± 2.61	2.78
FCR	2.25^{a} ± 0.29	2.78^{ab} ± 0.10	2.78^{ab} ± 0.62	3.44^{b} ±0.76	3.14^{ab} ±0.37	0.24

^{abc} Treatment means with different superscripts on the same row significantly differ ($P<0.05$), T1- T5 (Treatment 1-5), ADFI- Average daily feed intake, ADWG- Average daily body weight gain, FCR- Feed conversion ratio, g/bird- gram per day.

Haematology of broiler chicken fed aflatoxin feed with varied inclusion levels of activated charcoal.

The effects of graded levels of activated charcoal on the haematological parameters of broilers fed aflatoxin-contaminated diets are presented in Table 4.2. Packed Cell Volume (28.83–32.83%), Hemoglobin (9.27–10.62 g/dL), and Red Blood Cell counts (2.91–3.34 $\times 10^{6}$/µL) did not differ significantly among treatments ($P > 0.05$). White Blood Cell counts ranged from 15.36 to 17.83/µL, while platelet values varied between 13.52 and 16.80/µL. Lymphocyte percentages were highest in T5 (65.17%) and lowest in T4 (59.33%), whereas heterophils ranged from 28.00% to 31.17%. Monocyte values were lowest in T5 (2.67%) and highest in T1 (3.17%), while eosinophils ranged from 3.67% to 4.17%. Overall, activated charcoal inclusion did not significantly influence haematological indices. Basophils (BA) were low across the various treatments from 0.00 ± 0.00% (T3 and T5) to 0.50 ± 0.55% (T1). Mean corpuscular volume (MCV) ranged from 97.19 fL (T3) to 100.40 fL (T2), while mean corpuscular hemoglobin (MCH) ranged from 31.34 pg (T3) to 32.49 pg (T2). The heterophil to lymphocyte (H/L) ratio ranged from 0.43 (T5) to 0.56 (T4). The statistical evaluation of the measured haematological indices used a significance threshold of $P<0.05$.

Serum biochemical indices of broiler chickens fed aflatoxin diets with varied inclusion levels of activated charcoal

The effects of graded activated charcoal inclusion on serum biochemical parameters are presented in Table 4.3. Alanine aminotransferase (ALT), total protein (2.97–3.65 g/dL), albumin (0.87–1.27 g/dL), cholesterol (108.86–138.79 mg/dL), glucose (132.61–148.11 mg/dL), and calcium (8.94–9.47 mg/dL) were not significantly affected ($P > 0.05$) by dietary treatments. Although aspartate aminotransferase (AST) showed overall significance ($p < 0.05$), post-hoc analysis revealed no significant pairwise differences among treatments. Conversely, significant differences ($P < 0.05$) were observed in high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, alkaline phosphatase (ALP), creatinine, and phosphorus. HDL was highest in T5 (77.52 mg/dL), while LDL peaked in T1 (61.77 mg/dL) and was lowest in T3 (38.92 mg/dL). Uric acid and creatinine increased progressively with higher charcoal inclusion, with peak values in T4. ALP was highest in T1 (58.94 IU/L) and lowest in T3 (40.82 IU/L), indicating differential biochemical responses to activated charcoal supplementation under aflatoxin challenge.

Histological analysis of the kidney of broiler chicken fed aflatoxin feed with varying inclusion levels of activated charcoal

Histological examination of kidney sections (Plate 4.1) revealed treatment-dependent alterations in broilers fed aflatoxin-contaminated diets supplemented with activated charcoal. Birds in the control group (T1) showed normal renal architecture with intact tubular and glomerular structures. Mild diffuse tubular degeneration was observed in T2 (0.5% charcoal), while T3 (1.0%) exhibited moderate periglomerular cellular infiltration, indicating localised inflammation. Birds in T4 (1.5%) showed mild to moderate cortical congestion. Notably, T5 (2.0%) presented no visible lesions, with renal morphology comparable to the control group, suggesting improved protective effects at higher charcoal inclusion.

Histopathological report of liver in broiler chickens fed aflatoxin diets with varying inclusion levels of activated charcoal

Histological sections of livers from broiler chickens that received some level of aflatoxin in their diets with different levels of activated charcoal are shown in Plate 4.5. The birds that were in the control diet, which were observed to be free of aflatoxin and charcoal in the (T1), had no visible lesions. Their hepatocytes were arranged in normal cords, and the sinusoids were intact. In contrast, sections from broilers on an aflatoxin diet with 0.5% activated charcoal (T2) displayed mild diffuse hydropic degeneration and necrosis of hepatocytes. This was characterised by cellular swelling and cytoplasmic breakdown. Chickens on a diet with 1.0% charcoal (T3) showed severe diffuse hepatic degeneration and necrosis, resulting in significant loss of hepatic structure.

Table 2: Haematology of broiler chicken fed aflatoxin feed with varied inclusion levels of activated charcoal.

Parameter	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*
PCV (%)	32.00 ± 4.86^{ab}	32.83 ± 4.92^{a}	32.17 ± 3.43^{ab}	28.83 ± 3.31^{b}	32.67 ± 3.72^{ab}	2.37
H.B (g/dl)	10.43 ± 1.47	10.62 ± 1.66	10.37 ± 1.13	9.27 ± 1.11	10.60 ± 1.14	0.76
RBC ($x10^6$/uL)	3.27 ± 0.33	3.27 ± 0.35	3.31 ± 0.26	2.91 ± 0.50	3.34 ± 0.25	0.2
WBC ($x10^9$/L)	17.83 ± 2.70	16.60 ± 1.72	16.98 ± 1.52	15.36 ± 1.38	17.38 ± 2.57	1.18
PLATELET($x10^3$/L)	16.10 ± 3.97	14.18 ± 2.24	13.52 ± 7.96	14.20 ± 2.47	16.80 ± 2.73	1.53
LYM (%)	62.50 ± 5.24	64.33 ± 5.20	62.33 ± 5.35	59.33 ± 7.20	65.17 ± 3.97	3.17
HET (%)	29.83 ± 6.59	28.67 ± 5.01	31.17 ± 5.85	33.33 ± 6.25	28.00 ± 3.41	3.2
MON (%)	3.17 ± 0.98	3.00 ± 0.89	2.83 ± 0.98	3.00 ± 0.63	2.67 ± 0.52	0.48
EO (%)	4.00 ± 2.00	3.67 ± 1.86	3.67 ± 1.63	4.00 ± 2.28	4.17 ± 0.98	1.04
BA (%)	0.50 ± 0.55	0.33 ± 0.52	0.00 ± 0.00	0.33 ± 0.52	0.00 ± 0.00	0.24
MCV(fL)	97.8	100.40	97.19	99.08	97.80	-
MCH(pg)	31.89	32.49	31.34	31.85	31.74	-
H/L(Ratio)	0.48	0.45	0.50	0.56	0.48	-

[abc] Treatment means with different superscripts on the same row significantly differs (P<0.05), T1- T5 (Treatment 1-5), SEM- standard error of means,PCV- packed cell volume, Hb- Hemoglobin, RBC- Red blood cell, WBC- white blood cell, LYM- lymphocytes, HET - heterophiles, MON- Monocytes, EO - Eosinophils, BA- Basophil

Table 3: Serum biochemical indices of broiler chickens fed aflatoxin diets with varied inclusion levels of activated charcoal

Parameter	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*
AST (IU/L)	86.33±27.95	82.18±11.20	89.54±20.84	101.92±22.68	107.30±20.97	12.37
ALT (IU/L)	4.43±2.21	2.93±0.81	5.15±2.01	4.43±2.47	6.48±3.46	1.36
TP (g/dL)	2.97±0.50	3.00±0.54	3.08±1.06	3.65±0.55	3.43±0.38	0.38
ALB (g/dL)	0.97±0.13	1.14±0.19	1.24±0.08	1.15±0.16	1.27±0.32	0.11
CHOL (mg/dL)	138.79±40.67	108.86±26.71	111.78±12.44	135.83±11.52	137.11±15.80	13.92
GLUC (mg/dL)	146.89±29.26	132.61±35.40	148.11±31.26	137.41±9.34	141.57±18.94	15.35
HDL (mg/dL)	68.08^{ab}±7.83	68.51^{ab}±7.93	62.21^{b}±9.14	72.07^{ab}±5.58	77.52^{a}±9.11	4.63
LDL (mg/dL)	61.77^{a}±7.37	46.76^{b}±4.09	38.92^{b}±6.18	45.67^{b}±7.93	48.89^{b}±6.08	3.73
U.A. (mg/dL)	3.80^{b}±0.69	3.55^{b}±0.62	4.29^{ab}±0.41	4.79^{a}±0.43	4.32^{ab}±0.45	0.31
ALP (IU/L)	58.94^{a}±2.47	45.01^{bc}±4.98	40.82^{c}±4.19	47.70^{bc}±6.14	50.40^{b}±4.32	2.64
CRT	0.14^{c}±0.03	0.43^{b}±0.11	0.63^{ab}±0.13	0.79^{a}±0.04	0.62^{ab}±0.24	0.08
PHOS (mg/dL)	13.98^{ab}±1.34	11.29^{b}±1.98	11.85^{b}±2.06	34.05^{a}±14.55	27.72^{a}±13.23	5.14
CAL (mg/dL)	26.52^{a}±3.59	27.27^{a}±2.82	26.50^{a}±3.24	28.81^{a}±3.46	28.31^{a}±3.44	1.92

abc Treatment means with different superscripts on the same row significantly differs (P<0.05), T1- T5 (Treatment 1-5), SEM- standard error of means, AST- Aspartate amino transferase, ALT - Alanine amino transferase, TP - Total protein, CHOL -Choline, HDL - High density lipoprotein, LDL - Low density lipoprotein, U/A- Uric acid, ALP- Alkaline phosphatese, CRT -Creatinine, PHOS - Phosphorus, CAL -Calcium

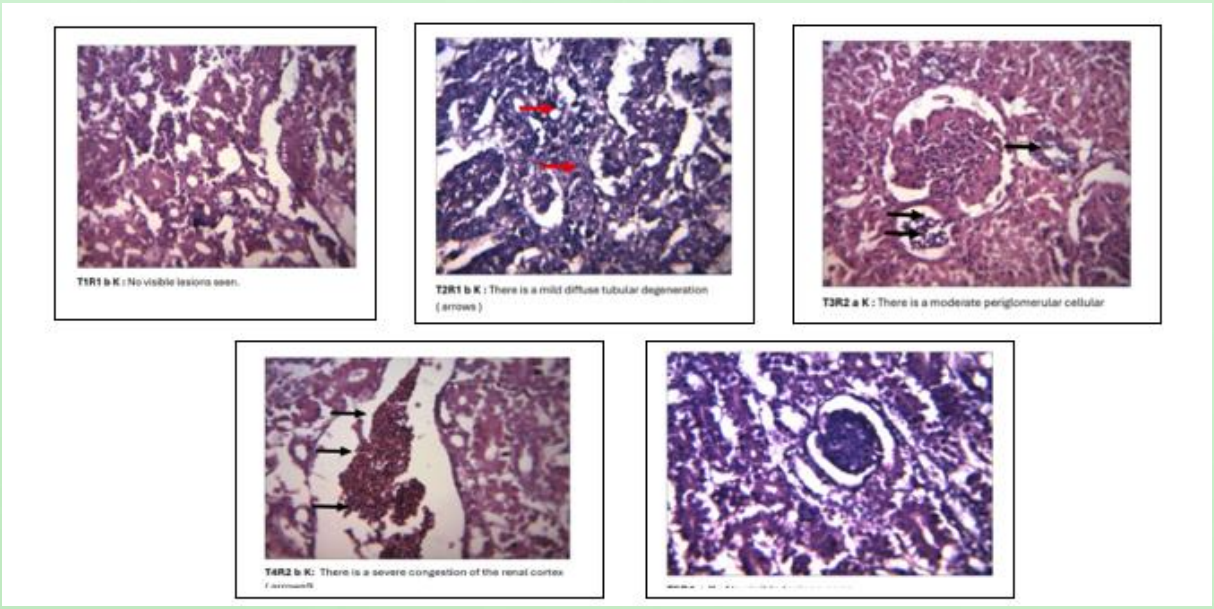

Plate 1 Histological section of the kidney of a broiler fed aflatoxin poultry feed

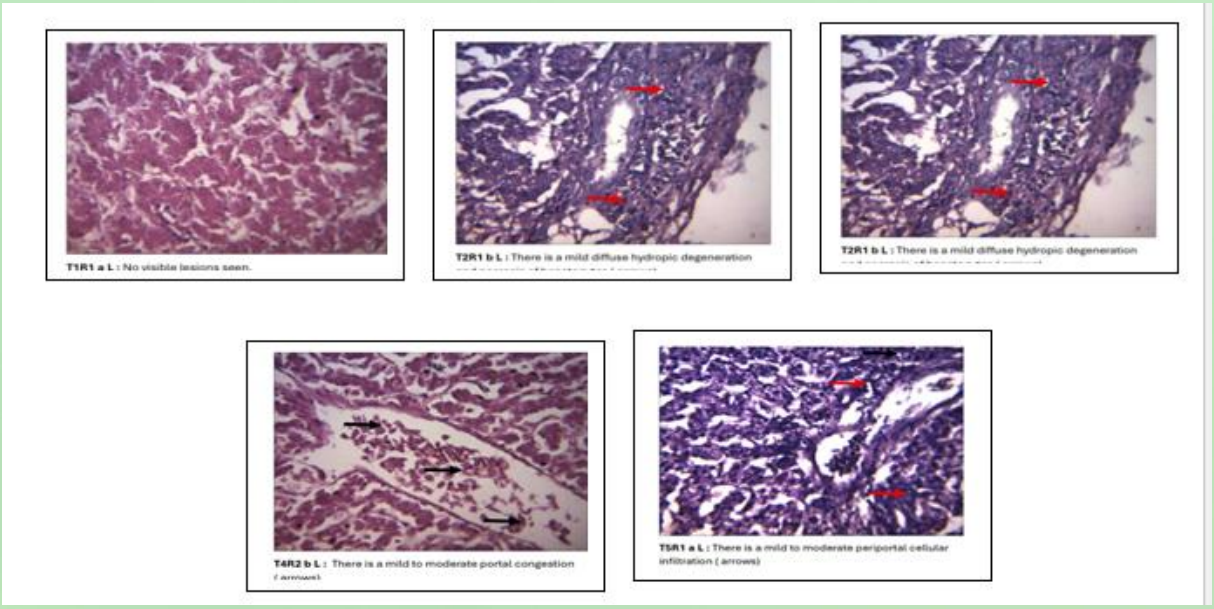

Plate 2: Histological section of the liver of a broiler fed aflatoxin poultry feed

Discussion

Growth Performance

The study supports the already known growth-depressive action of aflatoxin among broiler chickens. Birds kept on the control diet, free of toxins, had a better feed consumption, weight gain and feed efficiency which are signs of optimum nutrient efficiency in a nutritional context. On the other hand, aflatoxin exposure led to reduced feed intake and retarded gain ratio thus confirming earlier studies that aflatoxin damages intestinal integrity, interferes with nutrient absorption and mitigates metabolic efficiency (Yakout, 2024; Oloruntola *et al*., 2025). Furthermore, the high feed ratios of aflaxosin-challenged make additional witness to lower biological efficiency, which can be probably ascribed to decreased protein synthesis, as well as oxidative stress induced by the aflatoxin metabolites (Jobe *et al*., 2023). The dose-dependent non-linear protective response was discovered in activated charcoal supplementation. Proportional improvements in growth performance were not experienced with increased dosages but levels of inclusion were lower.

Haematological Indices

The haematological parameters act as the indicators of the systemic toxicity and physiological stress that are sensitive. It was indicated by the absence of major differences in packed cell volume, haemoglobin concentration,

and erythrocyte counts that activated charcoal counteracted the extreme haematopoietic repression despite the presence of aflatoxin. It is known that aflatoxin interrupts the process of erythropoiesis by inhibiting protein synthesis and promoting the occurrence of oxidative damage (Fujii *et al*., 2021; Yuan *et al*., 2024). This means that the preservation of near physiological indices of red-cells in charcoal supplemented groups implies partial safeguarding against anaemic states. The differences in the leukocyte profiles also gave a bit more hints on the issue of immune modulation. The low counts of white blood cells in some treatments, are consistent with immunosuppression instigated by aflatoxin (Ekpo *et al*., 2024). An increase in lymphocyte proportions at increase in charcoal inclusion may be an indicator of a restoration of immune competence due to a reduction in toxin bioavailability. These finding are in line with those that attest that dietary adsorbent reduces aflatoxin-induced immune compromise (Jose Mendes *et al*., 2024; Kipkoech *et al*., 2025). Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) are erythrocyte indices that describe the average size of red blood cells and the average amount of hemoglobin present in each cell, respectively. In the present study, MCV and MCH values were relatively similar across treatments, indicating that dietary inclusion of activated charcoal did not adversely affect erythrocyte morphology or hemoglobin concentration in the red blood cells. Stable MCV and MCH values suggest that the birds maintained normal erythrocyte characteristics despite exposure to aflatoxin, possibly due to the toxin-binding properties of activated charcoal. Similar findings were reported by Adeyemi *et al*. (2023) and Kipkoech *et al*. (2025), who observed that dietary toxin binders helped maintain normal erythrocyte indices in broiler chickens exposed to mycotoxins.

Biochemical Responses in the Serum

Additional signs of organ specificity were serum biochemical indices. The constancy of alanine aminotransferase, total protein, albumin, glucose, and calcium in the treatments implies that hepatocellular integrity and primary metabolic events were not very disturbed. Other studies have also obtained comparable results in which the adsorbent supplementation inhibits excessive hepatic leakage of enzymes during aflatoxin challenge (Bhatti *et al*., 2022; Jobe *et al*., 2023). Nevertheless, the considerable change in aspartate aminotransferase indicates minor hepatic stress. It is believed that AST tends to be more sensitive compared to ALT to identify hepatocellular changes due to aflatoxin (Sang *et al*., 2023). Changes in lipid fractions, especially the corresponding higher levels of the high-density lipoprotein with higher charcoal addition can reflect better lipid metabolism of reduced hepatic load. On the other hand, high uric acid and creatinine levels in high inclusion levels indicate that the kidney may be strained, or may have a defective nitrogen metabolism. Similar reports have been carried out in previous studies, which have indicated mild renal loading and high toxin binders (Selle *et al*., 2021; Forni *et al*., 2022).

Renal Histopathology

Histological observation of renal tissues showed that progressive changes were observed with the increase of exposure to aflatoxins and to amounts of charcoal. The incomplete detoxification of the charcoal supplementation was manifested by mild tubular degeneration and inflammatory infiltration at the lower levels, which allowed charcoal-mediated nephrotoxicity to occur. Lesions of this type are typical of oxidative injury and vascular deviation that is caused by aflatoxins (Dabuo *et al*., 2022). It is important to note that the architecture of the kidneys of birds with the largest charcoal addition had similarities with the control album, which suggested that the protection effectiveness was significant at this scale. This finding indicates better adsorption of aflatoxin in the gastrointestinal tract hence averting systemic circulation and kidney deposition. The broilers have been reported to have similar protective renal outcomes when subjected to effective amounts of adsorbents (Bhatti *et al*., 2022).

Hepatic Histopathology

Liver being the most prominent organ in terms of aflatoxin metabolism showed different degrees of degeneration in the treatments. The condition of hydropic degeneration, necrosis, and vascular congestion in those groups challenged with aflatoxin is associated with deteriorated protein synthesis and lipid peroxidation (Moloi *et al*., 2023; Wang *et al.*, 2024). Suppression of lesion severity with increased charcoal addition, however, only indicated a mild degeneration change, which is a sign of partial hepatoprotection. The presence of mild lesions being persistent is an indication that the hepatic detoxification mechanisms are active even when the systemic toxicity levels have lowered. A dose responsive protective trend can be observed when hepatic and renal results are viewed as one. Less than optimal inclusion levels of charcoal (0.52 -1000) moderately mitigated, and higher inclusion (2.0) were associated with a renal intact state and a significant decrease in hepatic pathology. On the whole, the results indicate that activated charcoal has an effect of protection against aflatoxin-induced toxicity on broiler chicken in a dose-dependent manner. Maximal inclusion however is not simply proportional to performance improvement, thus, the importance of optimisation. The results provide a useful piece of knowledge regarding the application of practical feed safety interventions, especially in tropical production systems where aflatoxin contamination is still prevalent (Ochieng *et al*., 2021).

Conclusion

This study evaluated the effect of activated charcoal on broiler chickens fed aflatoxin contaminated diets. Birds in the control group (T1) received a diet free of aflatoxin and activated charcoal, while birds in T2, T3, T4, and T5 received aflatoxin-contaminated diets supplemented with 0.5%, 1.0%, 1.5%, and 2.0% activated charcoal respectively. The results showed that aflatoxin caused negative effects on the performance and tissue structure of the birds. However, the inclusion of activated charcoal reduced these effects. Hematological parameters remained within normal physiological ranges across treatments, while serum biochemical indices showed some variations due to aflatoxin exposure. Histopathological examination revealed that birds fed lower levels of activated charcoal showed some kidney and liver lesions, whereas birds fed 2.0% activated charcoal (T5) showed improved tissue structure similar to the control group. Therefore, activated charcoal was effective in reducing the toxic effects of aflatoxin in broiler chickens, with 2.0% inclusion level showing the best protective effect.

Recommendation

Activated charcoal should be included in broiler diets contaminated with aflatoxin to reduce its toxic effects. An inclusion level of 2.0% activated charcoal is recommended based on the results of this study. However it is recommended that broilers ration should include activated charcoal at optimum levels (1.0 -2.0%). Poultry farmers should ensure proper storage of feed ingredients to prevent aflatoxin contamination.

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