



Research Article



Ameliorative Effects of Vitamins A and E on Hematological and Biochemical Parameters in Aflatoxin B1 Intoxicated Broiler Chickens

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ABSTRACT

Introduction: Aflatoxin B1 (AFB1) stands out as one of the most damaging toxins in poultry, causing oxidative stress and immunosuppression in broiler chickens. The present study aimed to investigate the protective effects of vitamins A and E on hematological and biochemical parameters in broiler chickens intoxicated with AFB1.

Materials and methods: A total of 96 day-old unsexed broiler chickens, weighing approximately 40 grams, were randomly divided into six groups. Each group consisted of 16 chickens, with two replicates and eight chickens per replicate. The first treatment compromised commercial feed with 35 µg/kg of AFB1 and 10 mg of vitamin A (T1), the second treatment included commercial feed with 35 µg/kg of AFB1 and 15 mg of vitamin A (T2), third treatment included commercial feed with 35 µg/kg of AFB1 and 5 mg of vitamin E (T3), fourth treatment had commercial feed with 35 µg/kg of AFB1 and 10 mg of vitamin E (T4), the negative control had commercial feed with 35 µg/kg of AFB1 without any vitamins (T5), and the positive control group were given commercial feed only (T6). The entire study was conducted over 42 days, and hematological and serum biochemical parameters were assessed on day 42.

Results: Differences in hematological and biochemical parameters were not statistically significant across groups. However, T3 had the highest values of packed cell volume (31%), hemoglobin (10.05 g/dl), red blood cell ($3.30 \times 10^6/\mu\text{l}$), and white blood cell ($15.95 \times 10^3/\mu\text{l}$). Additionally, in T4, the serum biochemical parameters indicated the lowest values of aspartate aminotransferase (178.50 U/L), alkaline phosphatase (183.00 U/L), and blood urea nitrogen (1.25 mg/dL), numerically compared to the other groups.

Conclusion: The present study indicated that vitamins can be used as a strategic dietary ingredient in reducing the effects of aflatoxins. Supplementing with antioxidants such as vitamin E reduces oxidative stress, stabilizes liver and kidney functions, and supports poultry health under AFB1 exposure.

1. Introduction

The poultry industry produces substantial animal protein through its broiler chicken production activities¹. Broiler chickens achieve their health status and productivity through the quality of their dietary feed². To meet their specific needs, different classes of poultry have to be fed different types of diets; however, because each specific genotype has its own requirements, most commercial feed formulations use minimum requirements recommended by

the breeding companies that supply the chickens³. The major difficulty in poultry nutrition and health management arises from mycotoxins, particularly aflatoxin B1 (AFB1), that contaminate feed². The toxic compound AFB1 develops through two *Aspergillus* species (*A. flavus* and *A. parasiticus*) that mainly infect grains and feed ingredients when stored improperly under warm and humid conditions³. Aflatoxin B1 toxicity can affect broiler chickens when they

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consume contaminated feed, such as drops, because it leads to decreased feed conversion efficiency, weight gain, impaired immunity, and liver damage^{4,5}. The AFB1 mainly targets the liver, where it is metabolized into a highly reactive epoxide that damages DNA, disrupts necessary protein synthesis, and impairs essential liver enzyme functions⁶. Aflatoxin poisoning alters blood parameters, including packed cell volume (PCV), hemoglobin (Hb), and white blood cell (WBC) counts. Liver enzymes, total protein, albumin, and bilirubin are also affected⁷. The AFB1 can lead to different damages, including hepatic injuries, oxidative stress, bone marrow suppression, and the disruption of protein and lipid metabolism⁷. Studies on dietary antioxidant vitamins as countermeasures to aflatoxin toxicity present an emerging solution to reduce aflatoxin toxicity. The fat-soluble vitamin retinol (Vitamin A) serves as a crucial compound that regulates cell differentiation processes and epithelial maintenance with immune response and antioxidant defense⁸. The damage caused by aflatoxicosis targets both epithelial tissues and immune organs⁹, which makes vitamin A essential for maintaining their structural and functional integrity.

Vitamin E is a fat-soluble vitamin that contributes to the improvement of the physiological and immunological development of poultry. Vitamin E is the primary antioxidant component in poultry feed¹⁰, which helps to reduce lipid peroxidation and neutralize free radicals in both skeletal muscle and plasma¹¹. The antioxidant properties of Vitamins can serve two functions in the body by activating enzyme activities and neutralizing free radicals to protect organs such as the liver and spleen¹¹. Vitamin supplementation can provide blood stabilization effects while improving laboratory results for poultry subjected to environmental and dietary stress¹².

The present study aimed to determine the ameliorative effects of vitamin A and E supplementation on hematological and biochemical parameters in broiler chickens that consumed AFB1-contaminated feed.

2. Materials and Methods

2.1. Ethical approval

Approval for the use of animals as prescribed by the international and institutional guidelines was obtained from the University of Ibadan Animal Care and Use Research Committee, with the number of UI ACUREC NO: 017-0124/29, Ibadan, Oyo State, Nigeria.

2.2. Study location

The experiment was conducted at the Training and Research Unit of the School of Agriculture, Federal Polytechnic, Ilaro, located in the Yewa South Local Government Area of Ogun State, Nigeria.

2.3. Animals

A total of 96 day-old unsexed Ross broiler chickens, weighing 40 g on average, were obtained from Agrited commercial hatchery, Ibadan, Nigeria. Upon arrival, the chickens were examined for physical soundness and

randomly allocated into a brooding pen in which the initial temperature was 33-35°C with a relative humidity of 65%. The temperature was then reduced progressively by 2-3°C weekly until it reached 26°C by the end of the brooding period, while relative humidity was maintained between 60-70% to improve thermal comfort and reduce stress of the chickens during early growth and development as described by Cobb¹³. The brooding pen was compartmentalized into six sections with 16 chickens in each at the end of the brooding period. All management practices, including feeding, lighting, and sanitation, were conducted according to the established practice for tropical poultry production, as described by Cobb¹³. During the study, chickens were provided *ad libitum* access to clean drinking water and a commercial broiler starter diet (New Hope Feeds, Nigeria) containing 20% crude protein, 6% crude fiber, 0.9% calcium, 0.38% phosphorus, and 3050 kcal/kg metabolizable energy, in accordance with NRC¹⁴. At day 14, the weights of the chickens were obtained, and the chickens were divided into six treatment groups and kept for an additional 28 days in April 2025. The entire feeding trial was carried out in 28 days, and the whole study lasted for 42 days.

Routine vaccination was performed to protect the chickens against common tropical poultry diseases. On day 7, the chickens were vaccinated against Newcastle disease (ND) using the LaSota vaccine in drinking water (National Veterinary Research Institute, Vom, Nigeria). On day 14, the chickens were vaccinated against Infectious bursal disease (IBD) using the intermediate-strain IBD vaccine (Biovet, Bulgaria) by adding it to the drinking water. Chickens received a booster dose against ND by LaSota strain vaccine on day 21 through drinking water, and were given a second IBD vaccine by drinking water on day 28 to boost immunity. Prior to vaccination, chickens were withheld from water for one hour to enhance vaccine uptake¹⁵. Biosecurity protocols were strictly followed during the experiment as described by Mohammed¹⁶. Chickens were observed daily for any changes in behavior or health.

2.4. Experimental design

The study employed a completely randomized design consisting of six dietary treatments, each replicated twice, with eight broiler chickens per replicate. The treatments were formulated to evaluate the protective effects of vitamins A and E in broiler chickens exposed to AFB1. The different levels of vitamins A and E employed in the present study were due to the different nutritional requirements of chickens, based on the study of Yaripour et al.¹⁷, with some modifications, who used varying but not exact doses of vitamins A and E. Lower and higher inclusion levels of vitamins were used to assess the optimal protective concentration against oxidative and physiological alterations induced by AFB1 exposure. Experimental diets were formulated to meet the nutrient requirements of broiler chickens as outlined by the NRC¹⁴. Chickens in the first group received the basal diet containing 35 µg/kg of AFB1 and supplemented with 10 mg of vitamin A per kg of feed (T1). The second group received a 35 µg/kg AFB1 inclusion level supplemented with 15 mg of vitamin A per kg of feed (T2). The third group included the basal diet containing 35 µg/kg of AFB1 with 5 mg of vitamin E per kg of feed (T3), whereas

chickens in the fourth group received 35 µg/kg of AFB1 supplemented with 10 mg of vitamin E per kg of feed (T4). The fifth group was the negative control group, which included the basal diet containing 35 µg/kg of AFB1 without any vitamin supplementation (T5), while the sixth group was the positive control, fed the basal diet with no AFB1 or vitamin supplementation (T6). Both vitamins were added in powdered form, thoroughly mixed into the basal diet to ensure homogeneity before feeding. Chickens were checked daily for accurate feed intake by checking the feed remaining in the feeders at the end of the day.

2.4.1. Aflatoxin administration

Aflatoxins at 35 µg/kg, sourced from the Microbiology department at the Institute of Agricultural Research and Training in Ibadan, Nigeria, were added to the feed for broiler chickens in T1, T2, T3, T4, and T5. Fifty milliliters of AFB1 broth containing 700 µg/mL was thoroughly mixed into 1 kg of basal feed to achieve a final concentration of 35 µg AFB1 per kg feed. The toxin suspension was thoroughly blended into the feed using a mechanical mixer to evenly distribute AFB1 throughout the feedstuff. The treated feeds were air-dried at 25°C for 24 hours, placed in airtight polyethylene bags, and stored at 4°C before use. The same batch of feed was used for all treatment groups except T6 throughout the study to ensure a consistent AFB1 dose. The selected inclusion level (35 µg/kg) was determined through sub-clinical exposure doses documented in broiler chickens for mild hepatic and biochemical changes without mortality¹⁸.

2.5. Data collection

2.5.1. Blood collection

On day 42 (end of the experiment), two chickens from each replicate were randomly selected after a 12-hour fasting period. Two mL of blood was collected from the wing vein of each chicken with a sterile syringe via the brachial vein into well-labeled EDTA bottles hematological parameters, including PCV (%) using microhematocrit capillary tubes, Hb (g/dl) by the Coulter STKS method, red

blood cells (RBC, $\times 10^6/\mu\text{L}$), and WBC ($\times 10^3/\mu\text{L}$) using a hemocytometer were measured, according to methods described by Campbell¹⁹. An additional 2 mL of blood was collected from the wing vein of each chicken into a sterile sample bottle without anticoagulants for evaluation of serum biochemical parameters, including total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and blood urea nitrogen (BUN) using commercial diagnostic kits (Randox®, United Kingdom), following the method of Chand et al.²⁰.

2.6. Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) in SPSS (version 16). The Duncan test was used to find the statistically significant differences at a p-value less than 5% ($p < 0.05$). The standard deviation was measured for the analyzed data.

3. Results

Hematological and serum biochemical parameters were not statistically significant among treatment groups ($p > 0.05$), although differences in numerical values were noted. Hematological parameters in T3, which received vitamin E at 5 mg/kg of feed, indicated increased PCV ($31.00 \pm 1.22\%$), Hb ($10.05 \pm 0.18 \text{ g/dL}$), RBC ($3.30 \pm 0.15 \times 10^6/\mu\text{L}$), WBC ($15.95 \pm 0.41 \times 10^3/\mu\text{L}$), and platelet count (PLT, $127.50 \pm 2.34 \times 10^3/\mu\text{L}$) compared to other treatment groups ($p > 0.05$; Table 1). While differences among serum biochemical parameters were also not statistically significant ($p > 0.05$), for the treatment with a higher vitamin E concentration, T4 (10 mg of vitamin E), there was a trend toward improved liver and kidney function indicated by lower mean AST ($178.50 \pm 3.24 \text{ U/L}$), ALT ($20.50 \pm 1.22 \text{ U/L}$), ALP ($183.00 \pm 4.16 \text{ U/L}$), and BUN ($1.25 \pm 0.15 \text{ mg/dL}$) compared to the negative control (T5), which recorded the highest results for AST ($182.50 \pm 3.58 \text{ U/L}$), ALT ($23.50 \pm 1.60 \text{ U/L}$), ALP ($202.00 \pm 5.10 \text{ U/L}$), and BUN ($2.05 \pm 0.20 \text{ mg/dL}$; Table 2). None of the treatments significantly altered the hematological or serum biochemistry parameters ($p > 0.05$).

Table 1. Hematological parameters in aflatoxin-intoxicated broiler chicken fed diets supplemented with vitamin A and E at day 42

Groups Parameters	T1	T2	T3	T4	T5	T6
PCV (%)	24.00 ± 5.66	24.50 ± 6.36	31.00 ± 2.83	30.00 ± 0.00	29.50 ± 3.54	21.50 ± 2.12
Hb (g/dl)	7.50 ± 1.70	7.75 ± 1.90	10.05 ± 1.70	9.55 ± 1.70	9.50 ± 1.70	6.80 ± 1.70
RBC ($\times 10^6/\mu\text{L}$)	2.41 ± 1.00	2.45 ± 1.08	3.30 ± 0.12	2.83 ± 0.05	2.70 ± 0.71	1.76 ± 0.11
WBC ($\times 10^3/\mu\text{L}$)	13.73 ± 3.92	12.55 ± 4.95	15.95 ± 2.12	14.63 ± 9.35	14.18 ± 1.77	14.78 ± 2.23
Platelets ($\times 10^3/\mu\text{L}$)	108.00 ± 28.30	123.50 ± 13.40	127.50 ± 12.02	123.00 ± 28.28	124.00 ± 56.57	104.50 ± 63.64
Lymphocytes (%)	47.50 ± 2.12	50.00 ± 2.83	59.50 ± 9.19	61.00 ± 1.41	57.00 ± 9.80	48.00 ± 1.41
Heterophils (%)	45.50 ± 2.12	42.50 ± 2.12	32.00 ± 7.07	33.0 ± 2.83	36.00 ± 8.49	45.00 ± 1.41
Monocytes (%)	4.00 ± 1.41	2.50 ± 0.71	2.50 ± 0.71	3.00 ± 1.41	4.00 ± 0.00	2.50 ± 0.71
Eosinophils (%)	2.50 ± 0.71	4.50 ± 0.71	4.50 ± 0.71	3.00 ± 0.00	2.50 ± 0.71	4.00 ± 1.41
Basophils (%)	0.50 ± 0.71	0.50 ± 0.71	0.5 ± 0.71	None	0.50 ± 0.71	0.50 ± 0.71

PCV: packed cell volume, Hb: Hemoglobin concentration, RBC: Red blood cell count, WBC: White blood cell count. T1: Commercial feed + 35 µg/kg of AFB1 + 10 mg of vitamin A, T2: Commercial feed + 35 µg/kg of AFB1 + 15 mg of vitamin A/kg feed, T3: Commercial feed + 35 µg/kg of AFB1 + 5 mg of vitamin E, T4: Commercial feed + 35 µg/kg of AFB1 + 10 mg of vitamin E, T5 (Negative control): Commercial feed + 35 µg/kg of AFB1 without any vitamins, T6 (Positive control): Commercial feed without any vitamins or AFB1. The values are expressed as mean ± standard deviation. There is no significant difference in all the parameters among the treatment groups ($p > 0.05$).

Table 2. Biochemical parameters in aflatoxin-intoxicated broiler chicken fed diets supplemented with vitamin A and E at day 42

Parameters	T1	T2	T3	T4	T5	T6
Total Protein (g/dl)	3.65 ± 0.70	3.40 ± 0.57	3.50 ± 0.00	3.45 ± 0.07	3.75 ± 0.21	3.45 ± 0.07
Albumin (g/dl)	0.60 ± 0.14	0.70 ± 0.28	0.55 ± 0.07	0.55 ± 0.07	0.75 ± 0.21	0.50 ± 0.00
Globulin (g/dl)	3.05 ± 0.07	2.65 ± 0.21	2.95 ± 0.00	2.90 ± 0.00	3.00 ± 0.00	2.95 ± 0.07
A/G Ratio	0.15 ± 0.07	0.25 ± 0.07	0.20 ± 0.00	0.15 ± 0.07	0.25 ± 0.07	0.15 ± 0.07
AST (U/L)	179.50 ± 4.95	179.00 ± 4.24	176.50 ± 2.12	178.50 ± 0.71	182.50 ± 3.53	177.00 ± 0.00
ALT (U/L)	22.50 ± 0.71	21.00 ± 2.83	21.50 ± 0.71	20.50 ± 0.71	23.50 ± 2.12	19.50 ± 0.71
ALP (U/L)	194.00 ± 8.49	193.00 ± 11.31	193.50 ± 9.19	183.00 ± 4.24	202.00 ± 9.90	190.00 ± 2.83
BUN (mg/dL)	1.50 ± 0.00	1.45 ± 0.64	1.55 ± 0.35	1.25 ± 0.07	2.05 ± 0.07	1.60 ± 0.00
Creatinine (mg/dL)	0.50 ± 0.00	0.40 ± 0.00	0.60 ± 0.14	0.45 ± 0.07	0.60 ± 0.14	0.45 ± 0.07

A/G: Albumin/Globulin ratio, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, BUN: Blood urea nitrogen. T1: Commercial feed + 35 µg/kg of AFB1 + 10 mg of vitamin A, T2: Commercial feed + 35 µg/kg of AFB1 + 15 mg of vitamin A, T3: Commercial feed + 35 µg/kg of AFB1 + 5 mg of vitamin E, T4: Commercial feed + 35 µg/kg of AFB1 + 10 mg of vitamin E, T5 (Negative control): Commercial feed + 35 µg/kg of AFB1 without any vitamins, T6 (Positive control): Commercial feed without any vitamins or AFB1. The values are expressed as mean ± standard deviation. There is no significant difference in all the parameters among the treatment groups ($p > 0.05$).

4. Discussion

Based on the present findings, there were no considerable differences in hematological and biochemical parameters among dietary treatments. The absence of significant differences in hematological parameters across treatment groups might be attributed to the experimental conditions, including the dose of AFB1, the duration of exposure, and the concentration of vitamin supplements. These findings are consistent with the results of Hou et al.²¹, who demonstrated a clear dose-dependent relationship in the severity of aflatoxicosis, where higher AFB1 levels led to more severe oxidative damage, immunosuppression, and growth retardation in chickens.

Notably, the group supplemented with 5 mg of vitamin E (T3) had the highest values of PCV, Hb, RBC, and WBC counts in all the challenged groups, which suggested a protective hematopoietic function. The present findings align with the findings of Shlig²², who reported that Vitamin E at 30-50 IU/kg diet, notably improved PCV and Hb concentrations in AFB1-challenged broiler chickens. The present study demonstrated that vitamin E is a potent antioxidant that can mitigate the hematological alterations caused by AFB1, such as anemia and leukopenia, by inhibiting oxidative stress and stimulating haematopoiesis. In addition, Saleemi et al.²³ reported partial improvements in hematobiochemical indices in juvenile white leghorn when supplemented with Vitamin E at 100 mg/kg and *Moringa oleifera*.

The mild anemia observed in the positive control group during the present study was a result of the incidence of coccidiosis in this group during the experiment. Coccidiosis is known to cause intestinal hemorrhage, epithelial damage, and impaired nutrient absorption, which can lead to anemia due to blood loss and reduced hematopoiesis²⁴. The associated inflammation and oxidative stress can also contribute to decreased erythrocyte count and Hb levels. These findings are in agreement with the findings of Adamu et al.²⁴, who reported that broiler chickens infected with *Eimeria* species indicated a decreased RBC count, Hb, and haematocrit.

The control negative group indicated the highest mean values for AST, ALT, ALP, BUN, and creatinine, which indicated hepatic and renal injury. By contrast, the group receiving 10 mg of vitamin E had the lowest levels of these enzymes in comparison to the chicken receiving AFB1, showing that 10 mg of vitamin E had a hepatoprotective and nephroprotective effect. These findings align with the results of Saleemi et al.²³, who reported that juveniles receiving 400 ppb AFB1 and 100 ppm vitamin E exhibited partial improvements in serum biochemical parameters, thereby indicating a reversal of hepatic and renal toxicity, which was induced by AFB1.

In contrast, the chickens receiving vitamin A at 10-15 mg/kg had less biochemical protection, indicated by moderate levels of AST and ALT during the present study. Although vitamin A has been demonstrated to have antioxidant properties and play a role in promoting immune and epithelial health, the effects against AFB1-induced oxidative damage do not appear to be as pronounced as those of vitamin E. This observation suggested that while vitamin A possesses antioxidant properties and contributes to immune and epithelial health, its efficacy in mitigating the extensive oxidative stress caused by AFB1 may not be as pronounced as that of vitamin E, as stated by Rossi et al.²⁵. The moderate regulation of AST and ALT by vitamin A supplementation in the present study suggested that while vitamin A may offer some defense, it might be insufficient to entirely counteract the negative impacts of AFB1 on liver integrity and function. Thus, the minor biochemical changes in the current results indicated a low-grade, chronic exposure rather than an acute toxicosis.

5. Conclusion

The dosage and duration of exposure of chickens to aflatoxin B1 can determine the severity of damage to internal organs. While both vitamin A and vitamin E are beneficial in alleviating the impacts of aflatoxicosis in broiler chickens, 10 mg of vitamin E was more effective, particularly in preserving liver and kidney function due to

its superior antioxidant potential, ability to scavenge free radicals, and role in stabilizing cellular membranes, which were crucial in counteracting the oxidative damage induced by AFB1 exposure. Although vitamin E supplementation at 10 mg/kg of feed showed positive numerical trends in hematological parameters such as PCV, Hb, and RBC, and in decreasing biochemical markers of hepatic and renal stress, including AST, ALT, ALP, and BUN, the differences were not statistically significant. Further studies are suggested to investigate the effects of higher doses and longer durations of AFB1 exposure to determine the efficacy of vitamins A and E under severe toxicosis conditions, and also explore the synergistic potential of a combined supplementation of vitamins A and E to assess whether these vitamins provide superior protective effects than when used individually.

Declarations

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Authors' contributions

Phebe Oluwatoyin Okusanya and Theophilus Aghogho Jarikre developed the concept of the manuscript. Abdulrauf Adekunle Usman and Georgina Ijeoma Ukonu did the literature search. Phebe Oluwatoyin Okusanya and Ozomata Daniel Raji performed the experiment. The original draft was written by Phebe Oluwatoyin Okusanya and Abdulrauf Adekunle Usman, and reviewed by Theophilus Aghogho Jarikre and Georgina Ijeoma Ukonu. All authors have read and confirmed the final edition of the manuscript.

Availability of data and materials

The data obtained from the present study are included within the manuscript and are available from the corresponding author upon reasonable request.

Competing interests

All authors declared no conflict of interest.

Ethical considerations

The authors declared that this original study has not been published elsewhere. The manuscript underwent plagiarism screening using standard software. The authors confirmed that AI assistance was not used in the writing and execution of the present study.

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