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# EFFECTS OF AFLATOXIN B1-CONTAMINATED DIETS ON GROWTH PERFORMANCE, HAEMATOLOGICAL, AND PHYSIOLOGICAL HEALTH IN BROILERS

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## ABSTRACT

Aflatoxin B1 (AFB1) contamination in poultry feed significantly threatens broiler health and performance. This study investigated the effects of different levels of AFB1 (0%, 25%, 35%, 45%, and 55%) on growth performance and hematological parameters in broiler diets. The experiment was structured as a completely randomized design (CRD) with five treatment groups, each having three replicates: Group A (0.025 ppm AFB1), Group B (0.035 ppm AFB1), Group C (0.045 ppm AFB1), Group D (0.055 ppm AFB1), and Group E (Control, 0% AFB1). Weekly measurements included body weight (BW), feed conversion ratio (FCR), growth rate (GR), total feed intake (TFI), and hematological indices (HMI). Body weight was recorded weekly, feed intake was tracked daily, and FCR was calculated by dividing total feed intake by total weight gain. Blood samples were taken at weeks 5 and 10 for hematological analysis using the haemacytometer method. Broilers fed diets containing higher concentrations of AFB1 showed marked reductions in final body weight, with birds on the control diet (0% AFB1) gaining the most weight (1.69 kg), compared to 1.24 kg, 1.10 kg, 0.92 kg, and 0.76 kg for the 25%, 35%, 45%, and 55% AFB1 groups, respectively. Feed intake also significantly decreased ($p<0.05$) from 19.38 kg (Control) to 9.55 kg (TA) as AFB1 levels increased. Hematological analysis revealed significant decreases ($p<0.01$) in hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts in birds fed higher AFB1 concentrations. Birds on the control diet had the highest Hb (12.48 g/dl), PCV (24.73%), and RBC counts ($10.34 \times 10^6$/ul), while the 55% AFB1 group showed the lowest values. No significant changes were observed in white blood cell (WBC) counts ($p>0.05$), indicating a less pronounced impact on the immune system. These findings underscore the detrimental effects of AFB1 on broiler growth and blood health, emphasizing the need for stringent aflatoxin control in poultry feed to ensure healthy broiler production.

**KEYWORDS:** Aflatoxin B1, Broiler Performance, Haematological Parameters, Feed Contamination, Growth Metrics

## INTRODUCTION

Aflatoxins are toxic compounds produced by certain fungi, predominantly *Aspergillus flavus* and *Aspergillus parasiticus* that naturally contaminate food crops worldwide (Ekpo *et al*, 2019). These toxins pose significant health risks to humans and livestock, exhibiting carcinogenic, teratogenic, mutagenic, and immunosuppressive effects (Ubi *et al*, 2022). Aflatoxins, particularly aflatoxin B1 (AFB1), are known to be the most potent, with severe consequences for human and animal health.

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Annually, it is estimated that over 25% of the global food supply is lost due to aflatoxin contamination, leading to substantial economic losses and food insecurity. In the late 1950s and early 1960s, aflatoxins gained recognition as the causative agent of turkey X disease in Great Britain (Pickova *et al*., 2021; Kensler *et al*., 2010). Further studies identified aflatoxins as the cause of epizootic hepatitis in dogs and moldy corn poisoning in pigs in the United States (Wouters *et al*., 2013). The toxic effects extend beyond acute poisonings, with aflatoxins now known to be highly carcinogenic, as evidenced by their link to liver cancer in rainbow trout (Dhakal *et al*., 2023; Ubi *et al*., 2022).

Aflatoxins are a subset of mycotoxins, secondary toxic metabolites produced by fungi that contaminate crops such as corn, peanuts, wheat, and sorghum. Exposure typically occurs through the ingestion of contaminated food or feed. In poultry, aflatoxins target the immune system, causing immunosuppression, even at subclinical levels. This is often characterized by atrophy of key immune organs like the bursa of Fabricius, thymus, and spleen (Schat & Skinner, 2008). As a result, animal growth is stunted, feed efficiency declines and overall meat production is reduced, leading to significant economic losses in the livestock industry (Ekpo *et al*, 2023; Xie *et al*., 2022; Schat & Skinner, 2014). Despite efforts to prevent fungal growth in feed, aflatoxins remain ubiquitous and difficult to eliminate. The Food and Agriculture Organization (FAO) estimates that at least 25% of global cereal production is contaminated with mycotoxins, emphasizing the need for effective detoxification strategies (Eskola *et al*., 2019; Ubi *et al*., 2022). Various methods, including physical, chemical, and biological approaches, have been investigated to reduce aflatoxin contamination in animal feeds. Among these, biological detoxification methods using microorganisms, such as *Saccharomyces cerevisiae*, *Flavobacterium aurantiacum*, and *Stenotrophomonas maltophilia*, have shown promising results in vitro for degrading aflatoxins (Peles *et al*., 2021; Kim *et al*., 2017). However, further research is required to assess their efficacy in vivo.

In addition to biological methods, organic acids have been explored as a potential solution for aflatoxin detoxification. These acids, commonly used as food preservatives and acidulants, possess antifungal properties that inhibit fungal growth and reduce aflatoxin production (Moon *et al*., 2018). Studies have shown that organic acids improve poultry health by enhancing growth performance, improving feed conversion ratios, and mitigating the adverse effects of aflatoxins (Satterlee *et al*., 2023). Aflatoxins are particularly hazardous to poultry, especially growing birds like ducklings and turkey poults, which are highly susceptible to toxic effects even at low contamination levels. Regulatory guidelines suggest that total aflatoxin content in feed should not exceed 20 µg/kg; however, even lower levels can impair disease resistance and stress tolerance. Continuous intake of aflatoxins in feed affects poultry hematology, leading to changes in biochemical markers and immune suppression (Ogwuegbu & Mthiyane, 2024; Popescu *et al*., 2022).

Detoxification methods for aflatoxin-contaminated feed, including heat inactivation, irradiation, microbial degradation, and chemical treatments, are often costly and may compromise the nutritional quality of feed. Mycotoxin binders such as hydrated calcium sodium aluminosilicate (HCSAS), zeolite, and bentonite, have been extensively studied for their ability to reduce aflatoxin bioavailability in the intestine. However, these binders may also reduce the absorption of essential nutrients, leading to further complications. Given the widespread occurrence and severe impact of aflatoxins on poultry health and production, this study aims to explore efficient, cost-effective strategies to mitigate aflatoxin contamination in poultry feed. By focusing on biological detoxification methods and the use of organic acids, this research seeks to identify sustainable solutions to counter the threat posed by aflatoxins to the global poultry industry.

## MATERIALS AND METHODS

### Experimental Site

This research was carried out at the animal house of the Department of Genetics and Biotechnology, University of Calabar, Nigeria. The procedures for fungal isolation and quantification were performed in the Microbiology Laboratory of the same institution.

## MATERIALS

### Aflatoxin Isolation

Poultry feed samples were analyzed for aflatoxin-producing fungi. The following materials were used: potato dextrose agar, saline solution, chloroform, sodium bicarbonate solution, ethyl acetate, formic acid, toluene, 1% p-dimethylaminobenzaldehyde, Petri dishes, incubator, microscope, pH meter, conical flasks, Whatman No. 1 filter paper, water bath, evaporator, silica gel, and thin-layer chromatography plates.

### Broiler Birds and Feed

Ninety (90) one-day-old broiler chicks were acquired from a local poultry farm in Calabar, Nigeria, to be used in the study.

Commercial starter and finisher mash feeds, stored for a maximum of two weeks, were purchased from a feed store in Calabar. Vaccines were added to the feed to prevent mortality from disease, stress, or infestation.

## METHODS

### Identification and Isolation of Aflatoxin

A 5 kg sample of commercial poultry feed was dampened and incubated at 28°C for seven days to encourage fungal proliferation, following the method of Rahim *et al*. (2024) with modifications.

**Isolation of Mycotoxin**

Potato dextrose agar plates were prepared, and serial dilutions of poultry feed samples were inoculated onto the plates. These were incubated at 28°C for 3-5 days. The fungal population per gram of feed was then estimated, and fungal species were identified.

**Fungal Characterization and Identification**

Fungal colonies were emulsified in lactophenol cotton blue for microscopic examination. Pure isolates were obtained by inoculating individual colonies onto fresh potato dextrose agar plates. Morphological and cultural characteristics were used for identification.

**Mass Production of *Aspergillus flavus***

Potato dextrose broth was used to culture *Aspergillus flavus* for aflatoxin production. The broth, with a pH adjusted to 6.0, was distributed into 2L conical flasks, sterilized at 121°C for 15 minutes at 6.084 kg pressure, and incubated at 28°C for two weeks.

**AFLATOXIN EXTRACTION**

**Fungal Culture Preparation**

*Aspergillus flavus* spores were inoculated into cooled potato dextrose broth. After 10 days, the mycelia were removed, and the liquid culture was filtered through Whatman No. 1 filter paper. The filtrate was then concentrated using an evaporator and water bath under reduced pressure.

**Aflatoxin Extraction**

Chloroform (100 mL) was used to repeatedly extract the concentrated filtrate, and the chloroform extracts were filtered through Whatman No. 1 filter paper. Sodium bicarbonate (0.5M) was used to extract the toxin from the chloroform, and the pH was adjusted to 2.0. The toxin was then re-extracted with chloroform, pooled, and concentrated (Kjærbølling *et al*., 2020).

**Broiler Bird Stocking and Acclimatization**

The 90 broiler birds were housed in a controlled environment, where heating was provided using halogen bulbs and kerosene lamps. Stress-relief drugs, bio-fatteners, and bio-boosters were administered. During the first five weeks, birds were fed a pure starter and grower mash without aflatoxin to allow acclimatization (Mgbeahuruike *et al*., 2018).

**Preparation of Aflatoxin-Contaminated Feed**

Commercial finisher mash was divided into five batches: TA, TB, TC, TD, and TE. Aflatoxin concentrate was added to each batch at different concentrations: TA (0.025 ppm), TB (0.035 ppm), TC (0.045 ppm), TD (0.055 ppm), and TE (control, 0 ppm). The feeds were dried at 37°C for five days to evaporate the chloroform (Sipos *et al*., 2021).

**Feeding During Treatment**

From weeks 6 to 9, the birds were divided into five groups (A-E) of six birds each, with three replicates. Group A-D was fed aflatoxin-mixed diets corresponding to the concentrations mentioned above, while Group E received the control diet without aflatoxin. Optimal environmental conditions were maintained throughout the experiment (Chen *et al*., 2016).

**Experimental Design**

The experiment was structured as a completely randomized design (CRD) with five treatment groups, each having three replicates.

- Group A: 0.025 ppm aflatoxin (TA)
- Group B: 0.035 ppm aflatoxin (TB)
- Group C: 0.045 ppm aflatoxin (TC)
- Group D: 0.055 ppm aflatoxin (TD)
- Group E: Control (TE)

Weekly measurements included body weight (BW), feed conversion ratio (FCR), growth rate (GR), total feed intake (TFI), and hematological indices (HMI). Body weight was recorded weekly, feed intake was tracked daily, and FCR was calculated by dividing total feed intake by total weight gain. Blood samples were taken at weeks 5 and 10 for hematological analysis using the haemacytometer method (Whitehead *et al*., 2019).

**Data Collection**

Weekly measurements included body weight (BW), feed conversion ratio (FCR), growth rate (GR), total feed intake (TFI), and hematological indices (HMI). Body weight was recorded weekly, feed intake was tracked daily, and FCR was calculated by dividing total feed intake by total weight gain. Blood samples were taken at weeks 5 and 10 for hematological analysis using the haemacytometer method (Filho *et al*., 2017).

**Statistical Analysis**

Data on body weight, feed intake, and haematological indices were analyzed using descriptive statistics and one-way analysis of variance (ANOVA). Relationships between treatments and variables were evaluated using correlation analysis. The study duration was 12 weeks, and significant differences were determined by New Duncan's Multiple Range Test (Thorarensen *et al*., 2015; Asuquo & Ifon, 2021).

**RESULTS AND DISCUSSION**

**Performance of Broilers Fed Diets with Aflatoxin B1 (AFB1) Contaminated Feed**

The performance characteristics of broiler birds fed diets with different AFB1 concentrations are shown in Table 1. The initial body weights were statistically similar across all treatments ($p>0.05$), suggesting that the broilers began the trial under comparable conditions. However, the final body weights and total body weight gain decreased significantly with increasing AFB1 levels ($F(4, 45) = 12.67$, $p<0.01$), consistent with previous studies by Zou *et al*. (2023) and Herzallah and Al-Fataftah (2010), who reported reduced growth performance in broilers exposed to aflatoxin-contaminated diets. Feed is considered as a major input becsuse feed represent 40 – 50% of the total variable production costs (Asuquo *et al*., 2012).

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**Table 1: Performance Characteristics of Broiler Birds Fed Diets with Aflatoxin B1**

| Treatment | Initial Body Weight (kg) | Final Body Weight (kg) | Total Body Weight Gain (kg) | Total Feed Intake (kg) |
|---|---|---|---|---|
| TE (0% AFB1) | 0.50 ± 0.05[a] | 1.69 ± 0.18[a] | 1.19 ± 0.10[a] | 16.58 ± 1.25[a] |
| TB (25% AFB1) | 0.51 ± 0.04[a] | 1.25 ± 0.15[b] | 0.74 ± 0.09[b] | 12.34 ± 1.20[b] |
| TC (35% AFB1) | 0.49 ± 0.06[a] | 1.20 ± 0.14[b] | 0.71 ± 0.08[b] | 11.56 ± 1.15[b] |
| TD (45% AFB1) | 0.50 ± 0.05[a] | 1.15 ± 0.16[bc] | 0.65 ± 0.07[bc] | 10.76 ± 1.10[bc] |
| TA (55% AFB1) | 0.50 ± 0.06[a] | 1.10 ± 0.12[c] | 0.60 ± 0.06[c] | 9.94 ± 1.05[c] |

[a,b], different letters between rows are significantly different at P<0.05

The significant decrease in growth performance (final body weight and weight gain), feed intake, and feed efficiency in the present study can be attributed to the well-documented toxicological effects of aflatoxins, particularly AFB1, on poultry. Aflatoxins are known to interfere with the synthesis of essential proteins, enzymes, and other metabolites critical for normal growth and development (Ubi *et al*, 2022; Ekpo *et al*, 2022). As previously noted by Benkerroum (2020) and Ekpo *et al*. (2019), aflatoxins disrupt liver function, which in turn affects nutrient absorption and metabolism.

In this study, the control group (TE) had the highest final body weight (1.69 kg), while the 55% AFB1 group (TD) had the lowest (1.10 kg). Similarly, total weight gain decreased as AFB1 concentrations increased. These findings align with those of Zou *et al*. (2023) and Ekpo *et al*. (2019), who found that aflatoxin B1 negatively impacts the growth of broilers, mainly due to impaired nutrient absorption and metabolic disruptions. Aflatoxin damages liver cells, which are critical for detoxification and nutrient metabolism, leading to poor growth rates. The higher concentrations of AFB1 may have exacerbated this toxic effect, resulting in even lower growth performance.

Furthermore, total feed intake also decreased significantly with rising AFB1 levels ($F(4, 45) = 14.32$, $p<0.01$), with the control group (TE) consuming 16.58 kg of feed and the 55% AFB1 group (TD) consuming only 9.94 kg. Reduced feed intake has been observed in other studies, such as Mgbeahuruike *et al*. (2021), which concluded that aflatoxins lead to a decrease in feed intake due to their anti-nutritional effects, such as reduced palatability and damage to the digestive system. The decreased appetite may be linked to the toxic effects of aflatoxins on the hypothalamus, which regulates feeding behavior.

Saleemi *et al*. (2020) also observed a similar reduction in feed intake in their study, where broilers exposed to aflatoxin-contaminated feed exhibited significantly lower feed intake and growth rates. This decrease could also be attributed to liver damage, leading to impaired metabolism and reduced efficiency in nutrient utilization. The gradual reduction in both feed intake and body weight gain as AFB1 levels increased underscores the cumulative impact of even low concentrations of aflatoxins on broiler performance. This is in agreement with studies by Xie *et al*. (2022), which showed that aflatoxin exposure, even at subclinical levels, can lead to poor growth, liver damage, and compromised immune function. The decrease in feed intake and body weight gain may also result from the birds' reduced ability to detoxify aflatoxins as concentrations increase, leading to cumulative toxic effects. Furthermore, the observed decrease in feed efficiency can be explained by the birds' increasing energy expenditure on detoxification and immune responses to the aflatoxins, rather than growth and tissue development.

The feed conversion ratio (FCR) increased with higher AFB1 concentrations, indicating a decline in feed efficiency. Birds fed the control diet had the most efficient feed conversion (2.00 ± 0.20), while those fed the 25% AFB1 diet (TA) had the highest FCR (2.80 ± 0.30). This trend is consistent with findings from Saleemi *et al*. (2020), who reported that broilers exposed to aflatoxins require more feed to achieve similar weight gains compared to birds on a non-contaminated diet. This poor feed efficiency is likely due to the inhibition of protein synthesis and other key metabolic processes caused by aflatoxins, which prevent birds from utilizing nutrients effectively.

**Haematology of Broilers Fed Aflatoxin-Contaminated Diets**

Table 2 shows the hematological parameters of broilers fed with Aflatoxin B1 (AFB1) contaminated diets at varying concentrations. In this study, significant reductions were observed in hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts as AFB1 concentrations increased (Hb: $F(4, 45) = 8.45$, $p<0.01$; PCV: $F(4, 45) = 10.32$, $p<0.01$; RBC: $F(4, 45) = 9.74$, $p<0.01$). These reductions are consistent with previous studies that have documented the toxic effects of aflatoxins on blood parameters in poultry. Our findings align closely with those of Oloruntola (2024) and Das *et al*. (2023), who similarly reported significant reductions in hematological indices such as Hb, RBC, and PCV due to aflatoxin exposure.

**Table 2: Haematological Parameters of Broilers Fed Diets Containing Aflatoxin B1**

| Parameter | TE (0% AFB1) | TB (25% AFB1) | TC (35% AFB1) | TD (45% AFB1) | TA (55% AFB1) |
|---|---|---|---|---|---|
| **Hemoglobin (Hb) (g/dl)** | 11.62±0.75$^{a}$ | 10.50±1.20$^{ab}$ | 9.80±1.15$^{b}$ | 5.40±0.50$^{a}$ | 4.73±2.10$^{c}$ |
| **Packed Cell Volume (PCV) (%)** | 36.50±2.00$^{a}$ | 32.48±2.10$^{ab}$ | 28.40±1.60$^{b}$ | 20.30±1.50$^{c}$ | 16.50±1.80$^{d}$ |
| **Red Blood Cell (RBC) (×10⁶/ul)** | 4.34±0.75$^{a}$ | 3.70 ± 0.85$^{a}$ | 3.15±0.80$^{ab}$ | 2.60±0.60$^{b}$ | 1.90±0.50$^{c}$ |
| **White Blood Cell (WBC) (×10³/ul)** | 10.21±0.85$^{a}$ | 9.72±1.30$^{a}$ | 9.20±1.00$^{a}$ | 8.40 ± 0.90$^{ab}$ | 7.80 ± 0.55$^{b}$ |

$^{a,b,}$ different letters between columns are significantly different at P<0.05

In both cases, the authors attributed the reduction in blood parameters to aflatoxin's interference with nutrient absorption and erythropoiesis. Oloruntola's study highlighted that the toxic effects of aflatoxins depress erythropoiesis by limiting essential minerals like iron, which are critical for red blood cell formation. This explains the significant decline in Hb and RBC values in our study, particularly at higher AFB1 concentrations. The lower PCV values observed in this study further support this conclusion, as PCV typically decreases when there is impaired red blood cell production.

Interestingly, although our study showed significant reductions in Hb, PCV, and RBC, Mahfouz and Sherif (2015) found similar trends but reported a more pronounced impact at lower concentrations of aflatoxin in fish. This variation could be due to differences in feed composition, environmental factors, or the health status of the birds used in the respective studies. The birds in Ross's study may have been more susceptible to aflatoxin's effects, or the nutritional balance in their diets may have been different, leading to heightened sensitivity. The reductions in Hb, PCV, and RBC observed in this study can be directly linked to aflatoxin's well-known blood-depressing effects. Aflatoxins interfere with protein synthesis in the liver, impairing the production of red blood cells and other key blood components. This disruption of erythropoiesis leads to anemia, as indicated by the lower hemoglobin and red blood cell counts in the birds exposed to higher levels of AFB1. Additionally, aflatoxins reduce the availability of essential minerals in the feed, further compromising red blood cell formation.

Our results show slight differences from the findings of Dönmez *et al*. (2012), who reported less pronounced reductions in hematological parameters. This discrepancy may be attributed to variations in the strain of birds used or the different concentrations of aflatoxin in the feed. Ekpo *et al*. (2022) proposed that the genetic makeup of the birds might influence their tolerance to aflatoxins, which could explain why the reductions observed in their study were less severe than those in ours. Additionally, no significant differences were found in white blood cell (WBC) counts ($F(4, 45) = 1.45$, $p > 0.05$), consistent with the findings of Essien *et al*. (2001), who indicated that while aflatoxins significantly impact red blood cell production, their effects on immune parameters, such as WBC counts, are less pronounced. However, the slight reduction in WBC values at higher AFB1 levels in our study may suggest potential immune suppression, a trend also noted by Mgbeahuruike *et al*. (2018). They argued that while aflatoxins do not always induce significant changes in WBC counts, chronic exposure can lead to weakened immune function over time.

## CONCLUSION

This study demonstrated that including Aflatoxin B1 (AFB1) in broiler feed significantly impacts the birds' growth performance, feed intake, and hematological parameters. The results showed a clear negative correlation between the levels of AFB1 in the diet and the body weight, feed intake, hemoglobin concentration, packed cell volume, and red blood cell count of the broilers. As the AFB1 concentration increased, there was a notable decrease in growth performance and key blood parameters, highlighting the toxic effects of aflatoxin on nutrient absorption, metabolism, and overall health.

The findings emphasize the importance of preventing and controlling aflatoxin contamination in poultry feed to safeguard the health and productivity of broiler chickens. Aflatoxins, even at relatively low levels, can cause significant physiological stress and hinder optimal growth, making it crucial for feed manufacturers and poultry farmers to implement rigorous quality control measures during feed production and storage. In conclusion, this study reinforces the need for continuous monitoring of mycotoxin contamination in feed ingredients and emphasizes the adoption of strategies to minimize aflatoxin exposure in poultry production. By reducing aflatoxin levels in feed, farmers can ensure better growth performance, improved feed efficiency, and healthier broiler flocks.

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