British Journal of Applied Science & Technology
4(12): 1759-1771, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Hematological Parameters of Broilers Fed with Feed Contaminated with Aflatoxin

Y. A. Jeff-Agboola[1*]

[1]Department of Biological Sciences, Ondo State University of Science and Technology, PMB 353 Okitipupa, Nigeria.

Author's contribution

This work was carried out by author YAJA. Author YAJA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches, managed the analyses of the study and literature searches. The author read and approved the final manuscript.

Original Research Article

Received 4th May 2013
Accepted 16th October 2013
Published 25th February2014

ABSTRACT

Aims: The study was design to show the effect of aflatoxicosis in hematological parameters of broiler fed with different doses of aflatoxin contaminated diets
Place and Duration of Study: Sample: A day old broilers were purchased from a poultry farm in Oyo State Nigeria and the heamatological analysis were conducted in Federal University of Technology Akure, Nigeria in the Department of Microbiology and Animal production and Health in Department between January–August 2012.
Methodology: A day old broilers were fed with normal commercial starter feed for 6 weeks. Thereafter were divided into 11 groups and fed with the different feed samples. (A= Basal diet free from mycotoxin contamination, B= diet containing mycotoxin standard AFB1(0.5µg/Afkg), C=diet containing standard AFB1(1µg/Afkg), D=diet containing standard AFB2(0.5µg/Afkg), E=diet containing standard AFB2(1µg/Afkg), F=diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H=diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing toxicgenic *Aspergillus flavus* (1µg/AFkg),K= diet containing toxicgenic *Aspergillus* (1µg/AFkg) *parasiticus)* for 4 weeks. The initial body weight of the birds were recorded at 7 days interval up to the end of the experimentation.
Results: The result obtained from this research showed that, the average weight gain (g) increased in birds fed with normal diet from day 0 up to day 21 (39. 31±0.13^{a}, 60.72±0.99^{a}, 89.82±0.24^{c}, 121.22±0.62^{a}). There were reduction in weight of birds fed with

**Corresponding author: E-mail: yjeffagboola@yahoo.com;*

feed containing the aflatoxigenic moulds and standard with increase in concentration of the toxin. The highest reduction was found in sample containing toxigenic *A. parasiticus* (25.33 ± 0.19^{k}, 38.45 ± 0.09^{i}, 49.12 ± 0.02^{n} and 62.34 ± 0.32^{n}) *and A. flavus* (28.15 ± 0.65^{i}, 31.32 ± 0.02^{i}, 57.43 ± 0.05^{m} and 78.45 ± 0.21^{l}) respectively after feeding up to 21 days. Significant decreases were noted in the following hematologic parameters: Red blood cell (RBC), Hemoglobin (Hb), Packed cell volume (PCV), and White blood count (WBC) heterophils and monocytes counts. There was increase in lymphocytes and eosinophils count compared with control.

Conclusion: The effects of the exposure of six-week-old broiler to doses of Aflatoxin correspond to a marked decrease in the weight of broilers, decrease in the Red blood cell (RBC), Hemoglobin (Hb), Packed cell volume (PCV), and White blood count (WBC).

Keywords: Aflatoxicoses; hematology; toxigenic fungi; feed.

1. INTRODUCTION

Aflatoxins (AF) are mycotoxins produced by the toxigenic fungi mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Toxicity of these fungi was first reported in samples of groundnut meal used for livestock and poultry feed [1]. Aflatoxins ingested by the birds accumulate in different tissue/organs and eggs and entered into human food chain which posses a major risk to human health, as these aflatoxins are not inactivated by dry heating at 160ºC for one hour or by steam heating [2] and are unlikely to be affected by cooking. In poultry the severity of clinical disease and lesions of aflatoxicosis may vary with varying levels of dietary aflatoxins [3,4]. In tropical and subtropical areas, aflatoxin is the number one mycotoxin problem. [5].

Aflatoxicosis in poultry is primarily a disease of the liver and it showed the typical lesions on it, which ultimately cause production problems and mortality. The main clinical signs in affected birds are decreased feed intake, decreased body weight, poor skin, decreased egg production and decreased immunity [6]. The disease may be fatal and resulted in heavy mortality. The main lesions of aflatoxicosis in birds are also appeared on liver and kidneys which are, Jaundice, generalized oedema and hemorrhages, tan or yellow discoloration of the liver, periportal necrosis with bile duct proliferation and fibrosis and depletion of lymphoid organs. [7] and there is a little awareness of harmful effects of aflatoxins and it is a common practice of feed mill owner, that damaged and mouldy food grains rejected as unfit for human consumption are mixed in poultry feed. Similarly other cereal grains, oilseeds cake and their products, particularly corn and its bye product contamination with AF are mixed in poultry ration [8,9]. Continuous reports of heavy contamination of poultry feed ingredient and finished feed with AF contamination, a little work has been done on experimental production of aflatoxicosis in Nigeria. Removing AF from contaminated food and foodstuffs remains a major problem and there is a great demand for effective decontamination technology. Decontamination procedures have focused on degrading, destroying, inactivating or removing AF byphysical, chemical or biological methods [10,11,12,13].

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL PLAN

The experiment was conducted considering all the national legislation regarding animal protection and welfare. A day old broilers were purchased from a poultry farm in Oyo State and fed with normal commercial starter feed for 6 weeks [14]. Thereafter were divided into groups and fed with sample feeds for 4 weeks. The initial body weights of the birds were recorded at 7 days interval up to the end of the experimentation. In other to prevent stress, shock, deficiencies and infections vitamin-mineral premix and antibiotic were used as per recommendation of manufacturer. The birds were immunized against gumboro and ranikhet disease (BCRD) on the 5^{th} and 18^{th} day and also with Nobilis gumboro D-78 on days 11 and 21. The basal diet was tested for presence of mycotoxin and there was no detectable limit present.

The animals were disinfected with 70% ethanol then they were bled by cardiac puncture [15,16]. Samples of blood were collected for the determination of total WBC differential counts and other haematological parameters.

2.2 Aflatoxins Production and Quantification

2.2.1 Processing of contaminated feed

Broiler's commercial feed contaminated were inoculated with 0.5 and 1µg aflatoxin/kg feed (10ppb) of different types AFB1, AFB2, AG1, AG2 which were purchased from VICAM through NMASN and toxigenic mould of spores of *Aspergillus flavus and Aspergillus parasiticus (*1µgAF/kg) were also inoculated separately. Their toxins were quantified and level of their contents was determined by High Pressure Liquid Chromatography (HPLC) method 990.33 [17]. The toxins were subjected for dilution with autoclaved mycotoxin-free diet to adjust the required limits of treatment for this study. The dietary treatment was: zero (control), 5 and 10µg total aflatoxins/kg feed (ppb). The broiler's birds were exposed to the contaminated feed at 6wk-old of age and throughout the study this contaminated feed and clean water were provided.

2.2.2 Determination of aflatoxins in feed

Total aflatoxin was quantified in uncontaminated layer's feed, using immunoaffinity method which is applicable for mycotoxins that have fluorescence [18]. Series-4 Fluorometer (VICAM) was used in this procedure which is summarized as follows:

2.2.3 Sample extraction

50g sample + 5g NaCl + 100ml. methanol (80%), Blended at high speed (1 min), Filtered with fluted filter paper. Ten ml. extract was diluted with 40 ml distilled water and filtered with glass.

2.2.4 Column chromatography

Ten ml (= 1g sample microfibre filter paper equivalent) of filtered extract was passed through Afla Test-p affinity column with a rate of 1-2m drops/second. Column washed twice with 10

ml. distilled water. The toxin was eluted with 1 mlHPLC methanol, to which 1ml. of freshly prepared aflatoxin developer was added spontaneously. Reading of total aflatoxin was obtained after 60 second as part per billion (ppb).

2.3 Determination of White Blood Cell Counts and Relative Differential Counts

Blood samples were collected at weekly intervals for the enumeration white blood cell such as lymphocytes, monocytes and neutrophils. A volume of 20 L blood was added to a 180 L1 volume of blood previously deposited in a Petri dish. The mixture was mixed gently before a volume of 20 ml was charged in a haemocytometer counting chamber. Blood samples were taken by cardiac puncture from birds in each group, and heparin was used as anticoagulant. Samples were taken to the laboratory under refrigeration as recommended [20]. Identification and proportions of leukocytes were determined in Giemsa-stained blood smears using 100 x magnifications according to [19].

2.4 The Total WBC Count was Calculated by Using the Following Formula

Total Number of cells counted in 4 corners/4 X 10 X104 cells/ml Replicates of blood smears were likewise made on clean slides for the differential counts. These were allowed air-dried and stained. Each individual cell type has been recorded on a tally sheet until a total of 100 cells had been counted. The relative differential count was computed by the formula:

Average cell count X 100% X Total WBC count (at a given time interval)

2.5 Study of Clinical Signs, Behavioral Alterations

Clinical signs and behavioral alterations in birds of each group were subjectively recorded by observing twice daily for dullness, attraction to feed, attraction to water, condition of feces, appearance of feathers. The presence or absence of nervous signs was also observed [20].

2.6 Statistical Analysis

The results obtained were subjected to ANOVA Using SPSS 15 version as a completely randomized block design. Treatment means were compared by the Duncan Multiple Range Test Statistical significance was accepted at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Clinical Signs and Behavioral Alterations

The results of clinical signs and behavioral alterations showed that birds in group A (control), did not exhibit any abnormal signs and their behavior remained normal throughout the length of the experiment. All the birds rushed towards feed and water. The birds remained active and became alert upon tapping of the cage wall. Birds of Groups B-K showed depression which was progressive with dietary concentration of aflatoxin being highest at day 21 of the experiment and then gradually decreased. Attraction towards feed decreased in a dose related manner and was lowest on day 14 of the experiment.

3.2 Effect on Body Weight

The mean body weights of broilers in different groups showed that the average weight gain (g) increased in birds fed with normal diet from day 0 up to day 21 (39. 31±0.13[a], 60.72±0.99[a], 89.82±0.24[c], 121.22±0.62[a]). There were reduction in weight of birds fed with feed containing the aflatoxinmoulds and standard with increase in concentration of the toxin. The highest reduction was found in sample containing toxigenic *A. parasiticus* (25.33±0.19[k], 38.45±0.09i, 49.12±0.02[n] and 62.34±0.32[n]) and *flavus* (28.15±0.65[i], 31.32±0.02[i], 57.43±0.05[m] and 78.45±0.21[l]) respectively after feeding up to 21 days. This report was in accordance with the findings of [21] who observed reduction in weight of chicks fed on the aflatoxn -amended diets compared to controls. There were significant ($P < 0.05$) decreases in weight gains (60-80%) in chicks at the 2 higher feed concentrations of mycotoxins and reduced weight gains (38-44%) in chicks at the lowest level.

3.3 Effect on Blood Parameters

Heamatological parameters are presented in Tables 1- 9. The mean values of each of the blood parameters vary significantly ($p< 0.05$). Significant decreases were noted in the following hematologic parameters: Red blood cell (RBC), Hemoglobin (Hb), Packed cell volume (PCV), and White blood count (WBC) heterophils, and monocytes counts. There was increase in lymphocytes and eosinophils count compared with control.

The effects of the exposure of broilers to doses of Aflatoxin correspond to a marked increase in the percentage of circulating lymphocytes. This prevents the birds mount adequate inflammatory responses against the different pathogens from the environment. Haemoglobin status was observed to be 15.27±3.55[a], 11.71±0.08[b], 13.75±0.09[ca] and 12.43±0.13[a] for broiler birds fed on the control (A) diets respectively. When compared to the control, the Hb values obtained for the experimental diets were significantly low even at higher concentration in each case, the values fall in the physiological range of poultry [22].

The lowest count was observed in birds fed with diet containing standard AG1 (1µg/Afkg) fed up to 21 days (9.21±0.04[1], 9.57±0.08[h] and 9.16±0.28[f]).

The same trend was observed for PCV, RBC and WBC status, with birds fed on the control diet having significantly higher values than those fed on the experimental diets. However, broiler birds fed on the meal diet containing standard aflatoxin had values that are relatively similar to those of the broilers fed on the diet containing aflatoxin mould isolated from laboratory Haematological parameters are used in the assessment of the qualitative and quantitative composition as well as the biochemistry of the blood cells, are warning signals on, and is indicative of an impaired function. The result observed in red blood cell counts and hematocrit and hemoglobin concentrations were different to those reported by [24], who did not observe differences between hens and broiler chickens fed 2,500g of AFB1/kg and the control group. In fact, it appears that RBC and hemoglobin levels were only adversely affected when chickens were exposed to much higher concentrations of AFB1 as in the experiment conducted by [23]. However, [23] found a decrease in RBC and hemoglobin levels of broilers fed 125 and 274 mg of AB1/kg from 1 to 14 d of age. Contradictory results were also observed for other avian species [23] reported an increase in RBC count in young turkey poults fed high AB1 levels. The reasons for those discrepancies remain unclear.

Clinical bleeding and abnormal coagulation have been noted in animals intoxicated by aflatoxin B1 both experimentally and naturally [24] reported that aflatoxicosis caused a decrease in haemoglobin, haematocrit values and thrombocyte counts in broilers.

However, it is important to note that purified AB1, AB2, AG1, AG2 and toxicgenic moulds were used in the present study, but those previous works were conducted using *A. flavus and A. parasiticus* culture material. Therefore, the culture material may be the source of unknown *Aflatoxin* metabolites that may be responsible for confounding effects in each study. A significantly higher percentage of lymphocytes was observed in the differential leukocyte count of birds fed with diet containing standard AFB1 (1µg/Afkg) (62.37 ± 0.06^{a}, 62.40 ± 0.32^{a}, 62.62 ± 0.13^{a} and 62.83 ± 0.04^{a}) when compared with controls (41.81 ± 0.01^{j}, 41.52 ± 0.02^{k}, 41.15 ± 0.07^{k} and 40.75 ± 0.21^{L}). Therefore, the significant increase seen in the percentage of lymphocytes suggest that, due to the great aflatoxin availability, a direct toxic effect might have occurred on the populations of circulating lymphocytes (mostly T helper lymphocytes), probably because it is related to the blood surface determinants, or through the activation of caspases and consequent induction of cellular death by apoptosis . Although the mean lymphocyte percentage was higher in the treated groups than in the non-inoculated animals, the number of quantified lymphocytes in the vehicle-treated animals was higher than the mean number recorded for non-treated animals. There was observation of anemia condition (reported elseware in-press) observed in broiler chickens during aflatoxicosis is characterized by a decrease in while red blood cell Therefore, the anemia seen during aflatoxicosis is categorized as a type of hypochromic-microcytic anemia. Hypochromic- microcytic anemias are divided into three major groups: iron deficiency anemia, thalassemia, and sideroblastic anemia, these three groups of hypochromic-microcytic anemias can be distinguished by their differential effect on serum iron. Serum iron levels are normal to slightly elevated during thalassemia, which is a hereditary defect in peptide synthesis of hemoglobin, elevated during sideroblastic anemia, which is a hereditary defect in synthesis, and depressed during iron deficiency anemia, which is a nutritional disease The possibility that the growth-inhibitory effect of aflatoxin is a consequence of lowering the serum iron seems unlikely because iron deficiency anemia has been reported to occur independently of growth in chickens.

The variation in the percentage of basophils (Table 9). Support the report of [24]. Since normal birds may have percentage values varying between 0 and 6% for basophils alterations seen for this cell type in the different groups were considered non-statistically different. Conversely, the circulating eosinophil population varied significantly in the birds exposed to aflatoxins this might indicate important clinical-hematological alterations resulting from the presence of parasites. The high numeric difference found between the non-inoculated animals and those inoculated with the toxin, marked increase in the number of circulating eosinophils, suggests that the percentage of circulating granulocytes might indicate important alterations due to bird handling. Therefore, the differences found between the toxin-administered and the vehicle-administered groups indicated that the variation resulted from the inoculation stress.

Table 1. Red blood cell (X 10^{-6}/mm^3) of broilers fed with normal and toxigenic feeds

Samples	Day (0)	Day 7	Day 14	Day 21
A	3.20±0.00	3.10±0.0^{a}	3.00±0.00a^{c}	3.10±0.0^{a}
B	3.20±0.00	2.55±0.07^{g}	1.60±0.00^{g}	1.60±0.00^{e}
C	3.20±0.00	2.30±0.00^{h}	1.40±0.00^{h}	1.40±0.00 ^{fa}
D	3.20±0.00	2.60±0.14^{f}	2.60±0.00^{d}	2.50±0.00 ^{dc}
E	3.20±0.00	2.60±0.00^{f}	2.45±0.07^{e}	2.50±0.10 ^{dc}
F	3.20±0.00	3.00±0.14^{f}	3.05±0.07^{a}	2.75±0.07 ^{b}
G	3.20±0.00	2.85±0.07^{c}	2.70±0.00^{dc}	2.70±0.00 ^{cb}
H	3.20±0.00	2.75±0.07^{e}	2.70±0.00^{dc}	2.55±0.07 ^{dc}
I	3.20±0.00	2.85±0.07^{c}	2.75±0.07^{c}	2.70±0.00 ^{cb}
J	3.10 ±0.00	2.45±0.01^{g}	1.52± 0.00^{i}	1.96±0.06^{g}
K	3.02±0.00	2.63±0.03^{i}	2.61±0.06^{d}	2.49±0.21^{dc}

Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 2. Heamoglobin values of birds fed with normal and toxigenic feed Hb(g/dc)

Samples	Day (0)	Day 7	Day 14	Day 21
A	15.27±3.55^{a}	11.71±0.08^{b}	13.75±0.09^{ca}	12.43±0.13^{a}
B	12.06±0.07^{b}	10.17±0.08^{h}	10.09±0.01^{ag}	10.03±0.03^{de}
C	12.10±0.02^{b}	10.38±0.36^{h}	9.87±0.02^{g}	9.71±0.01^{ef}
D	12.66±.35^{b}	11.10±0.02^{de}	11.01±0.00^{e}	10.56±0.66^{cd}
E	12.21±0.03^{b}	10.65±0.05^{fg}	10.55±0.04^{f}	10.08±0.02^{de}
F	12.74±0.01^{b}	10.85±0.24^{ef}	13.02±0.13^{e}	10.81±0.06^{c}
G	12.73±0.03^{b}	9.21±0.04^{1}	9.57±0.08^{h}	9.16±0.28^{f}
H	12.69±0.01^{b}	11.66±0.04^{bc}	11.32±0.29^{d}	10.51±0.13^{cd}
I	12.74±0.03^{b}	10.32±0.13^{gh}	10.49±0.06^{f}	10.50±0.42^{cd}
J	12.35±0.01^{b}	11.31±0.45^{cd}	10.06±0.28^{ag}	10.01±0.53^{de}
K	12.21±0.03^{b}	10.65±0.13^{ef}	10.54±0.01^{e}	10.05±0.02^{de}

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.

Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2 (1µg/Afkg),J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 3. Packed cell volume of birds fed with the feed samples (%)

Samples	Day (0)	Day 7	Day 14	Day 21
A	42.700±0.00^{a}	43.45±0.01^{a}	42.70±0.28^{a}	42.20±0.14^{a}
B	42.55±0.07^{ab}	40.20±0.14^{a}	40.35±0.07^{d}	40.10±0.0^{c}
C	39.55±0.01^{g}	39.35±0.21^{e}	39.35±0.07^{e}	39.40±0.00^{d}
D	37.30±0.38^{i}	36.65±0.21^{e}	36.15±0.07^{g}	35.40±0.42^{f}
E	38.60±0.14^{h}	38.50±0.14^{f}	38.15±0.07^{f}	38.25±0.21^{e}
F	40.50±0.14^{g}	40.25±0.21^{d}	40.30±0.00^{d}	40.20±0.00^{c}
G	42.30±0.00^{bc}	42.10±0.00^{b}	42.10±0.00^{b}	42.10±0.00^{c}
H	42.30±0.01^{bc}	42.10±0.00^{b}	42.10±0.0^{b}	42.10±0.00^{c}
I	41.70±0.00^{d}	42.10±0.00^{b}	42.10±0.00^{b}	42.10±0.00^{a}
J	41.28±0.00^{e}	41.40±0.14^{c}	41.30±0.14^{c}	41.50±0.00^{b}
K	41.26±0.00^{e}	41.15±0.71^{c}	41.20±0.14^{c}	41.15±0.00^{b}

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg),J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 4. White blood cell of the birds fed with feed samples BC (X10^{3}/mm^{3})

Samples	Day (0)	Day 7	Day 14	Day 21
A	34.60±0.00^{a}	34.55±0.07^{a}	34.65±0.07^{a}	34.75±0.07^{b}
B	33.45±0.07^{d}	33.30±0.14^{e}	33.05±0.07^{e}	32.60±0.00^{b}
C	31.65±0.07^{g}	31255±0.21^{i}	31.15±0.07^{i}	31.10±0.00^{b}
D	32.45±0.07^{f}	32.10±0.00^{h}	32.00±0.00^{g}	32.0±0.0o^{b}
E	32.60±0.00^{f}	32.45±0.07^{g}	32.40±0.00^{f}	32.10±0.00^{b}
F	33.75±0.21^{c}	33.65±0.07^{d}	33.45±0.07^{d}	33.15±0.07^{b}
G	32.60±0.00^{f}	32.10±0.00^{h}	31.75±0.21^{h}	31.20±0.14^{b}
H	33.80±0.14^{c}	33.70±0.00^{d}	33.45±0.07^{d}	33.20±0.00^{b}
I	33.10±0.00^{e}	32.80±0.14^{f}	32.40±0.14^{f}	32.20±0.14^{b}
J	34.15±0.07^{b}	34.10±0.00^{c}	33.85±0.07^{c}	33.35±0.07^{b}
K	34.50±0.00^{a}	34.40±0.00^{ab}	34.15±0.07^{b}	38.60±6.36^{a}

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing spores of toxicgenic Aspergillusflavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus(1µg/AFkg) parasiticus.*

Table 5. Lymphocytes (%) count of poultry bird with feed samples

Samples	Day (0)	Day 7	Day 14	Day 21
A	41.81±0.01^{J}	41.52±0.02^{k}	41.15±0.07^{k}	40.75±0.21^{L}
B	52.21±0.14^{e}	52.43±0.02^{e}	52.63±0.03^{d}	52.94±0.01^{d}
C	62.37±0.06^{a}	62.40±0.32^{a}	62.62±0.13^{a}	62.83±0.04^{a}
D	51.51±0.00^{f}	51.97±0.03^{f}	52.43±0.03^{e}	52.97±0.01^{d}
E	53.24±0.03^{d}	53.06±0.02^{d}	52.73±0.42^{d}	52.05±0.06^{e}
F	48.20±0.16^{g}	47.35±0.23^{h}	46.38±0.02^{g}	45.50±0.09^{g}
G	44.76±0.04^{h}	48.69±0.05^{g}	49.21±0.14^{f}	50.69±0.09^{g}
H	55.78±0.27^{c}	56.32±0.27^{c}	57.61±0.02^{c}	58.11±0.03^{c}
I	60.31±0.01^{b}	60.77±0.01^{b}	60.99±0.01^{b}	61.09±0.04^{b}
J	41.27±0.07^{k}	41.15±0.05^{L}	41.54±0.01^{j}	41.05±0.06^{k}
K	42.11±0.04^{I}	42.83±0.04^{I}	43.16±0.00^{h}	43.73±0.03^{h}

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 6. Heterophils (%) of poultry birds fed with feed samples

Samples	Day (0)	Day 7	Day 14	Day 21
A	47.16±0.07^{a}	47.54±0.03^{a}	47.57± 0.03	47.71±0.00
B	38.80±0.14^{i}	36.87±0.06^{l}	35.64±0.05	34.25±0.18
C	32.67±0.08^{m}	32.73±0.00^{n}	32.76±0.01	32.95±0.05
D	43.70±0.11^{e}	42.38±0.06^{g}	41.50±0.16	40.62±0.09
E	38.64±0.01^{i}	37.52±0.02^{k}	36.10±0.01	35.32±0.01
F	45 40±0.03^{c}	44.25±0.10^{d}	42.39±0.03	40.21±0.04
G	40.61±0.04^{g}	39.22±0.15^{I}	38.02±0.01	37.57±0.04
H	37.62±0.08^{j}	35.87±0.06^{m}	35.06±0.05	34.39±0.08
I	33.62±0.04^{l}	32.43±0.02^{o}	32.26±0.01	32.04 ±0.04
J	32.65±0.05^{m}	32.44±0.01^{o}	30.20±0.01	30.03±0.01
K	46.65±0.02^{b}	47.10±0.01^{b}	45.64±0.01	43.62±0.01

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg),J=diet containing spores of toxicgenic Aspergillusflavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 7. Monocytes (%) of poultry birds fed with feed samples

Samples	Day (0)	Day 7	Day 14	Day 21
A	6.75^{n} ± 0.04	6.71^{o} ± 0.01	6.61^{a} ±0.03	6.49± 0.01^{k}
B	3.13^{b} ± 0.03	3.91^{b} ± 0.01	3.08^{a} ± 0.02	3.08± 0.00^{b}
C	3.02^{a} ± 0.01	3.91^{a} ± 0.01	2.90^{a} ±0.02	2.60± 0.07^{a}
D	5.62^{j} ± 0.02	5.46^{k} ± 0.02	5.24^{a} ± 0.04	5.14± 0.04^{g}
E	5.43^{i} ± 0.04	5.24^{j} ± 0.04	5. 12^{a} ± 0.01	5.07± 0.01^{f}
F	6.23^{m} ±0.04	6.26^{n} ± 0.04	6.18^{a} ± 0.01	5.06 ±0.04^{f}
G	5.82^{k} ± 0.01	5.71^{m} ± 0.02	5.46^{a} ±0.06	5.32± 0.02^{ı}
H	5.22^{g} ± 0.01	5.18^{ı} ± 0.00	5.09^{a} ± 0.01	5.03±0.01^{f}
I	4.90^{d} ± 0.04	4.62^{d} ± 0.01	4.54^{a} ± 0.02	4.06± 0.04^{c}
J	5.23^{g} ± 0.02	5.13^{h} ± 0.02	5.05^{a} ± 0.000	5.02± 0.01^{f}
K	5.32^{h} ± 0.01	5.23^{j} ± 0.02	5.19^{a} ± 0.01	5.03± 0.01^{f}

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 8. Eosinophil (%) of poultry feed fed with feed samples

Samples	Day (0)	Day 7	Day 14	Day 21
A	1.07±0.00	1.05±0.00	4.57±0.04	1.02±0.01
B	4.43 ±0.01	4.47±0.00	3.91±0.12	4.48±0.02
C	4.55±0.02	4.56±0.04	4.01±0.00	4.59 ±0.01
D	3.63±0.01	3.77±0.02	4.23±0.03	3.97±0.01
E	4.00 ±0.05	3.99±0.01	4.28±0.02	4.07±0.04
F	3.91±0.00	3.99±0.00	3.99±0.01	4.35±0.04
G	4.01±0.01	4.12±0.01	4.36±0.05	4.31±0.01
H	3.66±0.01	3.85±0.06	3.99±0.01	4.09±0.03
I	4.14±0.04	4.31±0.02	4.36±0.05	4.48±0.01
J	2.31±0.01	2.67±0.01	2.87±0.02	2.98±0.01
K	1.84±0.18	1.57±0.03	1.96±0.01	2.08±0.01

Values (mean ± SE) followed by different superscripts within a row differ (P≤0.05) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg),J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

Table 9. Basophils (%) of poultry birds fed with feed samples

Samples	Day (0)	Day 7	Day 14	Day 21
A	5.93^{h}± 0.00	5.95^{l}±0.01	5.98^{o}±0.01	5.99^{l}± 0.00
B	3.45^{b}±0.01	3.47^{b}±0.06	3.34^{b}±0.04	3.28^{b} ±0.01
C	3.25^{a}±0.01	3.23^{a}±0.03	3.21^{a}±0.01	3.14^{a} ±0.04
D	5.73^{g}±0.01	5.54^{j}±0.01	5.51^{m}±0.01	5.43^{k} ±0.03
E	5.66^{g}±0.04	5.51^{J}±0.01	5.44^{l}±0.01	5.42^{k}±0.00
F	5.43^{f}±0.01	5.24^{h} ±0.04	5.16^{i}±0.04	5.07^{g} ±0.01
G	5.05^{d}±0.05	5.07^{g}±0.01	5.05^{h}±0.01	5.02^{f}±0.01
H	4.22^{c}±0.15	4.29^{i}±0.01	4.24^{e}±0.03	4.06^{d}±0.04
I	4.12^{c}±0.05	4.08^{c} ±0.00	4.01^{d}±0.02	4.02^{d}±0.00
J	4.21^{c}±0.01	4.08^{j}±0.01	3.94^{c}±0.01	3.56^{c}±0.01
K	5.27^{e}±0.08	$5.01^{†}$ ±0.01	4.67^{g}±0.01	4.22^{e}±0.01

Values (mean ± SE) followed by different superscripts within a row differ ($P \leq 0.05$) significantly.
Key: *A = Basal diet free from mycotoxin contamination, B= diet containing standard AFB1(0.5µg/Afkg), C= diet containing standard AFB1(1µg/Afkg), D= diet containing standard AFB2(0.5µg/Afkg), E= diet containing standard AFB2(1µg/Afkg), F= diet containing standard AG1(0.5µg/Afkg), G= diet containing standard AG1(1µg/Afkg), H= diet containing standard AG2(0.5µg/Afkg), I= diet containing standard AG2(1µg/Afkg), J=diet containing spores of toxicgenic Aspergillus flavus (1µg/AFkg), K= diet containing toxicgenic Aspergillus (1µg/AFkg) parasiticus.*

4. CONCLUSION

The effects of the exposure of six-week-old broiler to doses of Aflatoxin correspond to a marked decrease in the weight of broilers, decrease in the Red blood cell (RBC), Hemoglobin (Hb), Packed cell volume (PCV), and White blood count (WBC) heterophils, and monocytes counts. However there was increase in percentage lymphocytes and eosinophils. This finding reveals that prevention of aflatoxin contamination of poultry feeds is the best way to control aflatoxicosis in poultry.

ACKNOWLEDGEMENTS

The Federal University of Technology Akure, Nigeria is acknowledged.

COMPETING INTERESTS

Author declares that there are no competing interests.

REFERENCES

1. Abarca ML, Accensi F, Bragulat MR, Cabanes FJ. Current importance of ochratoxin A-producing *Aspergillus* spp. Journal of Food Protection. 2001;64(6):903-6.
2. Abdel-Wahhab MA, Nada SA, Khalil FA. Physiological and toxicological Responses in rats fed aflatoxin-contaminated diet with or without sorbent materials. Animal Feed Sci. Tech. 2002;97:209-219.
3. FAO/WHO, Aflatoxin contamination in foods and feeds in the Philippines. Regional Conference onfood safety for Asia and Pacific. Seremban, Malaysia. 2004;24-27.

4. Stander MA, Bornscheneuer UT, Henke E, Steyr PS. Screening of commercial hydrolases for degradation of Ochratoxin A. Journal of Agriculture and Food Chemists. 2000;48:5736-5739.
5. Lawlor PG, Lynch PB. Mycotoxin management. African Farming and Food Processing. 2005;46:12-13 (January/February, issue).
6. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE. Nutritional and Health Implications of Mycotoxins in Animal Feeds: A Review Pakistan Journal of Nutrition. 2006;5:398-403. ISSN 1680-5194.
7. Oguz, HT Kececi YO, Birdane F, Onder V, Kurtoglu O. Effect of clinoptilolite On serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Res. Vet. Sci. 2000;69:89-93.
8. O'Keeffe M. Mycotoxins in Foods and Feeds. In: Farm and Food - The Teagasc Research and Digest. Ashtown; 2003. Available: http://ww.food assurance.teagac.
9. Bennett JW, Klich M Mycotoxins. Clinical microbiology. 2003;16(3):497-516
10. Gezen SS, Eren M, Deniz G. The effect of zeolite on broiler performance. Indian Vet. J. 2004;81:411-415.
11. Boranic M. What a physician should know about zeolites. Lijec. Vjesn., 2000;122:292-298.
12. Doubek J. Veterinary haematology. 1st ed., Novicoa.s., Brno. Czech Republic. 2003;464, (in Czech).
13. Jain NC. Essentials of Veterinary Hematology. Philadelphia (PA): Lea &Febiger;. 2003;417.
14. Hawkey CM, Dennett TB. Atlas of comparative veterinary hematology. London: Wolf Publishing. 2005;365.
15. Moura MAI, Machado CHII, Porfírio LCIII, Freire RB. Effects of ochratoxin a on broiler leukocytes Revista Brasileira de Ciência Avícola Avic. 2004;63: Campinas July/Sept. 2004.
16. Tariq J, Mary A, Dombrink K, John L, Richard-Glenn A, Bennett L, Marie C, William B, Buck B. Serohematologic alterations inbroiler chicks on feed amended with *Fusarium proliferatum* culture material or fumonisin B1 and moniliformin J Vet Diagn Invest 1995;7:520-526.
17. Attia YA, Abd El-Hamid AE, Ellakany HF, Bovera F, Al-Harthi MA., Sharehan A, Ghazal SA. Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. Ital. J. Anim. Sci. 2013;12:222-230.
18. Tung HTFW, Cook R, Wyatt D, Hamilton PB. The anemia caused by aflatoxin.Poult. Sci. 1975;54:1962-1969.
19. Strakova E, Suchy P, Herzig I, Šerman V, Mas N. The long-term administration of a clinoptilolite supplemented feed to layers and its effect on performance, haematological parameters and metabolic profile Czech J. Anim. Sci. 2008;5:212-218.
20. Nurcan D, Ercan K. The effects of aflatoxin and glucomanna on coagulation parameters in rabbits VETERINARSKI ARHIV. 2009;79 6:555-560.
21. Freire RB. Micotoxinas e Respostaimune. In: Perspectiva Latino-americana. Rio de Janeiro: UFRRJ Editora; 1996;261.
22. Anwar MI, Khan MZ, Muhammad G, Bachaya A, Babar AM. Effects of dietary formalin on the health and testicular pathology of male Japanese quails (coturnix japonica). Vet. Hum. Toxicol. 2001;43:330-333.

23. Basmacioglu H, Oguz H, Ergul M, Col R, Bridane YO. Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin. Czech. J. Anim. Sci. 2005;50:31-39.

24. Betina V. Mycotoxins-chemical, biological and environmental aspects. Amsterdan (NL): Elsevier. 1989;325-421

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=445&id=5&aid=3829