AFLATOXIN CONTAMINATION OF POULTRY FEEDS IN NIGERIAN FEED MILLS AND THE EFFECT ON THE PERFORMANCE OF ABOR ACRE BROILERS

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ABSTRACT

The present study investigated the effect of aflatoxin contamination of poultry feeds from different commercial feed sources on the performance of broiler chicks. Seventy five 3 weeks old Abor acre broiler chicks were randomly divided into five groups (A – E), replicated thrice with each replicate having 5 birds. Each replicate constituted an experimental unit and the birds were separately housed in a 1 x 1 m^2 sized pen. The birds in each treatment group were fed feeds from 5 companies, A – E. Feed intake and body weights were measured in all the treatment groups. Blood samples from the birds were analyzed for different hematological parameters. Feed samples were analyzed for aflatoxin concentration using reverse phase HPLC. The total aflatoxin content (AFB1 + AFB2 + FG1 + AFG2) of the feeds ranged from 1.4 ± 0.4 - 224.2 ± 74.7 µg/kg. The total aflatoxin level in feed samples C and E were above the European community regulatory limit for poultry feeds (ECRL, 20 µg/kg). There was no statistically significant difference between the haematological values of the birds fed feeds from the different source (P > 0.005). Lymphocyte count was relatively low in all the treatment groups. However, the mixed blood populations were scantly detected in all the groups. Feed intake was positively correlated with body weight in the two treatment groups, A and E (P = 0.0001, R = 1). The high concentration of aflatoxin in some of the feeds caused reduced feed intake and poor body weight among the treated birds.

Keywords: Aflatoxin, Poultry feed contamination, Haematology, Body weight, Reverse phase HPLC

INTRODUCTION

Mycotoxin contamination is a major problem to agricultural products in Nigeria and over 40 % of agricultural products have been reported to be prone to mycotoxins (Pittet, 1998). Livestock feed quality may be affected by various mycotoxin contaminants. Contaminated cereals that are not healthy for human consumption usually find their ways into the animal feed manufacturing companies for use in feed production (Park *et al.,* 2005). Mycotoxin contamination of animal feeds is more common in countries with hot and humid environment (Paterson and Lima, 2011). Among the commonly known mycotoxins, ochratoxin A (OTA), deoxynivalenol, T-2, zearalenone (ZEN) and aflatoxins (AFs), are major contaminants of feed and feed ingredients. Aflatoxins are metabolites produced by some strains of *Aspergillus* species. The popularly known species include *Aspergillus flavus* and *Aspergillus parasiticus,* although other species such as *Aspergillus ochraceoroseus*, *Aspergillus nomius, Aspergillus bombycis* and *Aspergillus pseudotamari* have been reported (Goto *et al.,* 1996; Klich *et al.,* 2000; Peterson *et al.,* 2001). The investigated *Aspergillus* species, showed different strengths in aflatoxin producing abilities (Klich and Pitt, 1988). *Aspergillus flavus* was responsible for the death of over 100, 000 turkey poults in England in 1961. The dead turkeys were discovered to have been fed with groundnut meal contaminated with aflatoxins (Blount, 1961). Feed crops are more prone to aflatoxin contamination when they are still in the field because such crops are usually associated with drought stress before they are

harvested (Diener *et al.,* 1987; Klich, 1987). However, in storage, if the moisture content and the relative humidity of the environment is high, there is a likelihood of aflatoxin contamination (Wilson and Payne*,* 1994).

Grain contamination by aflatoxins could lower the value of such grains as animal feed and this could result to animal mortality when fed to animals (Smith and Moss, 1985). Aflatoxin contaminated feeds could have a huge economic impact on the farmer through increased veterinary care costs and reduced livestock production (Hussein and Brasel, 2001). Furthermore, aflatoxin-contaminated diet could cause biochemical, haematological and liver physiological changes and this could lead to economic losses to the farmer (Che *et al.,* 2011).

Haematological parameters give information on the health status of animals and man. However due to paucity of information, blood profile have not been commonly used in avian species for determining the health condition of birds (Mushi *et al.,* 1999; Kral and Suchy, 2000). Differences in the blood parameters of avian and human species have been reported (Smith *et al.,* 2000). Studies have also shown that some factors like environmental conditions (Vecerek *et al.,* 2002; Graczyk *et al.,* 2003), diet contents (Odunsi *et al.,* 1999; Kurtoglu *et al.,* 2005), fasting (Lamosova *et al.,* 2004), aging (Naziefy-Habibabadi, 1997; Furlan *et al.,* 1999; Seiser *et al.,* 2000), physiological conditions (Alodan and Mashaly, 1999), water and feed restriction (Galip, 1999; Al-Rawashdeh *et al.,* 2000; Iheukwumere and Herbert, 2003), anti-aflatoxin premixes (Oguz *et al.,* 2000), vitamin E supplementations (Tras *et al.,* 2000) and administration of drugs (Khan *et al.,* 1994; Zaman *et al.,* 1995) can affect the blood profiles of healthy birds. Birds given aflatoxin-contaminated feeds show reduced blood cell count, lower egg production, reduced feed intake, increased mortality, impaired resistance to infectious disease and reduced vaccination efficiency (Kamalavenkatesh *et al.,* 2005). Aflatoxins have been reported to have immunosuppressive effects and can act on cell-mediated and humoral immune components of the avian immune system (Aquino *et al.,* 2005). Studies have also shown that dietary aflatoxin induced genotoxic and mutagenic effects in male Swiss albino mice (Ezekiel *et al.,* 2011). Aflatoxin B1 (AFB1) was reported to affect the haematological parameters of broilers leading to depressed cellular immunity due to suppression of the phagocytic activity of macrophages and T-lymphocytes (Celik *et al.,* 2000). Other studies reported that AFB1 concentration in the liver caused considerable liver damage with resultant deficiency in humoral immunity (Fung and Clark*,* 2004).

In Nigeria, feed mill owners purchase large quantities of grains and other feed ingredients during the harvest seasons and these feed stuff are stored and used for feed production throughout the year without any regulatory measures for controlling aflatoxin contamination. Furthermore, the long post-harvest periods of agricultural products coupled with the tropical climate condition in Nigeria are known to favour aflatoxin production in feeds and feed ingredients. In addition, although the haematological parameters of different strains of birds have been documented by some studies (Uko and Ataja, 1996; Qaisar *et al.,* 1996; Onifade and Odunsi, 1998; Hauptmanova *et al.,* 2002), there is little or no information on the performance of Arbor-acre birds fed aflatoxin contaminated feeds from different feed mills in Nigeria. In the present, we intend to investigate the concentration of aflatoxin in poultry feeds produced in selected feed mills in Nigeria. The study will also investigate the effect of the aflatoxin contaminated feeds on the performance parameters of Arbor-acres broiler.

MATERIALS AND METHODS

Aflatoxins: Aflatoxins in feed were analyzed using Brera *et al.* (2007). Ten (10) grams of feed sample from each source was weighed and ground with mortar and pestle. The material was transferred quantitatively to extraction tubes with 80 % (v/v) acetonitrile. Extraction was performed by rotary shaking for 45 minutes, the extract was filtered through folded filter paper and a 1 ml aliquot was diluted with 40 ml de-ionized water and mixed thoroughly. The diluted extract was purified on immuno

affinity columns (Vicam Afla Test, Waters Corporation). The purified extract was analyzed by reversed phase HPLC (Shimadzu Corp.) with isocratic elution and fluorescence detection after post-column derivatization with bromine by the Kobra Cell (Rhone Diagnostics, Glasgow UK).

Broiler: Seventy-five Arbor acre broilers of 3 weeks old each were purchased from St Anthony's farm Akpugo Nike in Enugu State, Nigeria. The birds were acclimatized for two weeks in the Faculty of Veterinary Medicine Animal Farm. They were given routine vaccination in addition to antibacterial and anticoccidial treatments during the acclimatization period. Poultry feeds of 0 – 3 weeks old storage time were purchased from five commercial feed companies in Nigeria A, B, C, D and E. The broiler chicks were randomly divided into five groups (A – E), replicated thrice with each replicate having 5 birds. Each replicate constituted an experimental unit, the birds were wing marked and housed individually in separate pens of 1 x 1 m^2 size. At the 5^{th} week, the birds in the different groups were given feeds from the corresponding companies. Ambient temperature, lighting, ventilation and other environmental conditions were fully met according to the requirements laid down in the technical instructions for Arbor acre broiler breeding.

Body weight and Feed intake: Body weight measurements and feed intakes were also determined on individual bird at weekly intervals from the 7^{th} to 9^{th} week after the commencement of the treatments.

Haematology: At the 5^{th} week before the birds were placed on the feed treatments, all the birds were weighed to determine their body weights. Blood samples were collected from the jugular vein of birds in each group in EDTA anticoagulant treated syringes (Odunsi *et al.,* 1999; Iheukwumere and Herbert, 2003). The blood was transferred to 2 ml Eppendorf tubes containing anticoagulants, labeled and stored in the fridge; and were used as baseline. At the 6^{th}, 7^{th} and 8^{th} week respectively when the feeding treatment had fully commenced, subsequent blood samples were collected from the brachial vein of the birds from each group following the procedures described in Alcorn (2002) and Bermudez and Stewart-Brown (2003). The blood samples were transferred in anticoagulant treated Eppendorf tubes, labeled according to the date of collection, the identification number on each bird, the type of feed each bird was treated with and stored in the fridge for further analysis. Total numbers of RBC, WBC, PCV and Hb, together with the absolute counts of heterophils, lymphocytes, monocytes, eosinophils and basophils were determined following established procedures. The PCV and RBC were calculated using the microhaematocrit method (Cole, 1968). Total Hb was determined by the cyanmethaemoglobin method as described by Brown (1984). Total WBC count was determined as described by (Schalm *et al.,* 1975). Other haematological parameters were determined by routine methods (Campbell, 1988).

Statistics: For statistical analysis, body weight measurements, feed intake and hematological indices from individual bird in each group were analyzed using descriptive statistics and the result was represented as means and standard deviations. The aflatoxin data was analyzed and expressed as means and standard deviations. Other analysis were carried out on the hematological parameters, body weight and feed intake using One-way ANOVA. Relationships between treatments and other variables like the body weight, feed intake and haematological data were determined using Spearman correlations.

RESULTS

Aflatoxins: Each feed sample was analyzed for four aflatoxins, AFB1, AFB2, AFG1 and AFG2. Our analysis showed that, all the aflatoxins types were detected in all the feed samples but at different concentrations (Table 1). For example, the feed sample fed to group C birds had the highest concentration of AFB1 (170.0 µg/kg) and this level of aflatoxin is far much higher than the recommended limits in livestock feeds.

Table 1: **Aflatoxin contamination of poultry feeds in Nigeria**

Feed samples	AflaB1 (µg/kg)	AflaB2 (µg/kg)	AflaG1 (µg/kg)	AflaG2 (µg/kg)
A	1.6 ± 0.5	<0.2 ± 0.0	0.9 ± 0.3	<0.2 ± 0.0
B	5.4 ± 1.7	0.5 ± 0.2	1.2 ± 0.4	<0.2 ± 0.0
C	170.0 ± 56.0	25.0 ± 7.9	27.0 ± 8.6	2.2 ± 0.7
D	0.5 ± 0.2	<0.2 ± 0.0	0.5 ± 0.2	<0.2 ± 0.0
E	23.0 ± 7.2	2.6 ± 0.8	5.7 ± 1.8	0.6 ± 0.2

The same trend was maintained for the same feed sample for aflatoxin AFB2 and AFG1 but at relatively lower levels (Table 1). While some feed samples like A, B and D had very low contents of aflatoxins AFB1, AFB2 and AFG1, very minimal levels of aflatoxin AFG2 was found across all the feed samples analyzed in this study (Table 1). Total aflatoxin contents (AFB1+ AFB2+ AFG1 + AFG2) was highest in feed sample C (84.2%), followed by E (11.7%), B (2.6%) and A (1.4%) respectively (Figure 1). Feed samples A and B had very low levels of total aflatoxin content representing only 4 % of the total aflatoxins analyzed in this study (Figure 1).

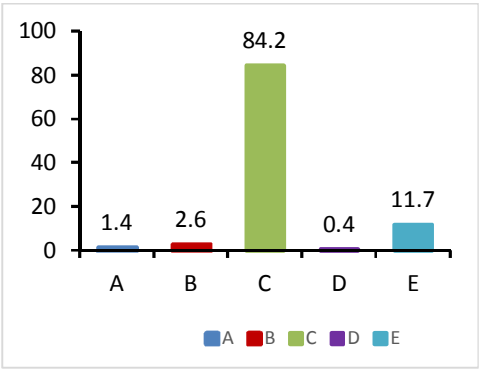

Figure 1: Percentage of total aflatoxin content of the analyzed feed samples

Body Weight: There was a corresponding increase in weight gain with increased feed intake, although in some of the groups, this was not statistically significant (p>0.05). However birds in groups A and E had more weight gains than the birds in groups B, C and D (Figure 2).

Feed Intake: All the feeds served to the birds in the different groups were finished by the birds a day after the feeds were served; as a result, there was no variation in the feed intake in all the groups.

In groups A and E, feed intake was positively correlated with body weights (p = 0.0001, R = 1).

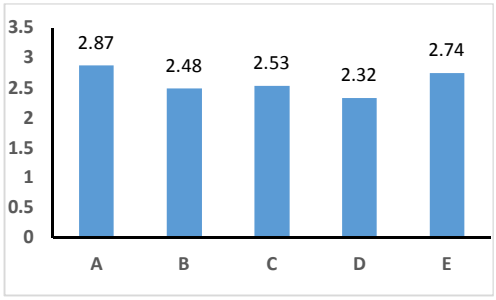

Figure 2: Body weights of Abor acre broilers fed feed from different sources

Haematology: There was no statistically significant difference between the haematological values of the birds in all the groups (p>0.05). However, the haematological parameters of the birds in all the treatment groups showed increased WBC counts, PCV values and haemoglobin levels. The lymphocyte counts were also high although there were some variations within the group. For example, birds in groups B and D had relatively high lymphocyte counts while those in A, C and E groups had relatively low lymphocyte counts. The average total RBC counts in all the groups were relatively low. The heterophils, monocytes, eosinophils and basophils were very low in all the groups and were counted as mixed populations of blood cells (Table 2).

DISCUSSION

There are documented evidences of the effect of aflatoxin contamination of livestock feeds on animal production (Saleemi *et al.,* 2010; Rawal *et al.,* 2010; Bryden, 2012; Oguz, 2012; Kana *et al.,* 2013). Aflatoxins can cause damage to animal cells at very low concentrations and can

Table 2: Haematological parameters of Abor acre broilers fed feeds contaminated with aflatoxins

Feed samples[a]	RBC Count (10^6/µl)	WBC Count (106/µl)	PCV Values (%)	Haemoglobin (g/dl)	Lymphocytes	Mixed
A	3.5 ± 0.8	47.9 ± 6.2	33.8 ± 5.7	11.3 ± 1.9	10.5 ± 8.3	0.0 ± 0.0
B	2.5 ± 0.3	64.1 ± 13.7	33.0 ± 4.2	11.0 ± 1.4	28.8 ± 26.0	2.0 ± 2.8
C	3.2 ± 0.4	44.1 ± 6.6	30.8 ± 11.3	10.3 ± 3.6	14.0 ± 3.3	1.8 ± 2.3
D	5.6 ± 3.8	56.4 ± 12.1	32.0 ± 3.9	10.7 ± 1.3	50.0 ± 12.83	6.3 ± 2.9
E	2.8 ± 0.7	36.8 ± 6.2	27.5 ± 7.1	9.2 ± 2.4	7.5 ± 5.20	2.8 ± 2.4

a= Feed samples fed to birds in different groups

depress growth performances in the affected livestock (Van Kessel and Hiang-Chek, 2004). The present study tried to investigate the effect of aflatoxin contaminated poultry feeds on the growth performance of Abor acre broilers. A very high concentration of AFB1 was recorded in the feed fed to the birds in group C when compared to the feeds fed to the other groups. Furthermore, a comparative analysis of the total aflatoxin content (AFB1 + AFB2 + AFG1 + AFG2) of each feed analyzed in this study showed that feed samples fed to group C birds had the highest total aflatoxin content (84.20 %) , followed by E(11.70 %), B(2.60 %) and A(1.40%) respectively. It is possible that the feed samples fed to the group C birds were stored for a longer time than the other feeds or the feed was processed with poor ingredients. Feed samples A and B had very low levels of total aflatoxin content representing only 4 % of the total aflatoxins analyzed in this study.

Heavy contamination of feed ingredients used in poultry feed production with aflatoxin producing fungi such as *Mucor* and *Rhyzopus* species have been reported in other studies (Okoli *et al.,* 2007; Ariyo *et al.,* 2013). Studies have also reported high contamination of common feed ingredients such as maize, rice, peanut meals and barley used in livestock feed production (Shah *et al.,* 2010; Niaz *et al.,* 2012; Firdous *et al.,* 2012; Majeed *et al.,* 2013; Sherazi *et al.,* 2014). These feed crops mature in the seasons characterized by hot temperatures and high humidity, as a result, the chances of infestation with aflatoxin producing fungi are quite high (Ratnavathi *et al.,* 2012).

In this study, AFB1 was found in concentrations far beyond the recommended limits in the feed samples fed to the group C and E birds (170 µg/kg) and (23 µg/kg) respectively. The FDA recommended average doses of 100 ppm AFB1 in poultry feeds in the United States (FDA, 2003). The European community recommended a maximum AFBI content of 20 µg/kg and 10 µg/kg for whole feed in poultry and chicks respectively (Jewers, 1987). AFB1 is the most toxic among the known aflatoxins and it induces hepatic cell necrosis, steatosis, hemorrhage and hepatocellular carcinomas in animals (Rawal *et al.,* 2010; Khan *et al.,* 2010). At levels of even less than one part per million (ppm), AFB1 is capable of damaging cells within an organism (Van Kessel and Hiang-Chek, 2004). The acute toxicity of AFBI in poultry varies from species to species. For ducklings and chickens, the LD_{50} single dose (mg/kg body weight) is 0.3 and 6.0 – 16.0 respectively. Poultry diets containing 250 – 500 µg/kg of aflatoxins have been shown to predispose birds to bacterial and viral infections (Edds *et al.,* 1973).

In the present study, although feed intake was high in all the groups, the body weights of the birds differed in each group of birds. For example, birds in groups B, C and D had reduced weight gains when compared with birds in groups A and E. The reduced body weight in these groups B, C and D could possibly be as a result of poor feed conversion rate due to high aflatoxin concentrations in the feeds. For groups A and E, it could be that the feeds were more palatable possibly because the ingredients used in the feed processing were rich in dietary composition.

Haematological values of avian species are used as performance index in determining the health condition of birds (Kral and Suchy, 2000). However, haematological values are influenced by poultry diseases and other health related conditions (Kokosharov, 2002). There

was no significant difference between the haematological parameters of the birds in each treatment group. It is possible that the effect of the aflatoxins on the blood cells could be time dependent and would manifest with longer time of exposure. However, the RBC counts were relatively low in all the treatment groups whereas the WBC and PCV counts were relatively high in all the groups. The total lymphocyte counts were almost the same in all the groups but the hemoglobin levels showed slight variations among the birds in all the treatments groups. In conclusion, the study has been able to establish the impact of aflatoxin contamination of poultry feeds on broiler performance. Most commercial feed mills in Nigeria do not have established regulatory mechanisms to check aflatoxin contamination of poultry feeds and this has been established from this study to affect the feed intake and growth performance of birds. This situation poses a huge public health implication as well as high veterinary care cost on the part of farmers.

ACKNOWLEDGEMENTS

The authors acknowledge the technical staff of the Mycotoxin Research Unit, National Veterinary Institute, Sweden.

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