



Article History

Received: 28th Feb, 2020
Revised: 25th May, 2020
Accepted: 27th May, 2020
Published: 30th June, 2020

Incidence of Aflatoxin B1 in Commercial Poultry Feed and Tissues of Broiler Chickens in Ibadan, Nigeria

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ABSTRACT

Aflatoxin B1 (AFB1) belongs to a group of hepatocarcinogenic and hepatotoxic mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* found in food or feed products. In this study we analyzed 180 feed of six different brands of commercial poultry feeds from three feed distribution outlets in Ibadan, southwest Nigeria for the occurrence and concentrations of AFB1. In addition, to the transfer of AFB1 to broiler meat from chicken fed for four weeks with one brand of feed associated with the highest AFB1 contamination. The study was carried out between April and June, 2019. The presence/concentration of AFB1 was determined using High Performance Liquid Chromatography (HPLC) following solid phase extraction of sample and preparation. Aflatoxin B1 was detected in all (100 %) the brands of feed and 48 % of chicken samples tested. The concentration of AFB1 in feed ranged between 10.5 ± 4.0 and 47.78 ± 6.4 , while the mean AFB1 residues obtained in chicken muscle, gizzards and liver after four weeks were 0.07 ± 0.02 , 0.18 ± 0.05 and 0.13 ± 0.02 $\mu\text{g}/\text{kg}$, respectively. The presence of AFB1 in all the brands of poultry feeds tested in this study and its possible transfer into chicken meat poses food/feed safety and public health concern. There is therefore, the need to screen feed and apply Hazard Analysis Critical Control Point to feed manufacturing, storage and broiler chicken production to prevent aflatoxicosis.

Keywords: Incidence; Commercial poultry feed; Broiler chicken; Aflatoxicosis; Mycotoxin

INTRODUCTION

Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus* and *A. parasiticus* (Sarma *et al.*, 2017). These fungi are relatively ubiquitous (Adeniran *et al.*, 2013). Climatic factors such as high temperature and humidity aid the growth and spread of these fungi (Moss, 1992; Rodrigues and Nosanchuk, 2020). They are commonly found in cereals such as maize, sorghum, guinea corn and plant protein such as soya bean meal, groundnut cake and cotton seed cake (Halfon-Meir and Barki, 1990; Herrman, 2002). These are the major constituents of commercial poultry feed in Nigeria (Akande *et al.*, 2006; Hanif *et al.*, 2006). Feed storage in tropical humid high temperature also enhances the growth of Aflatoxin producing *Aspergillus* resulting in contamination (Marijani *et al.*, 2019).

Aflatoxin B1 (AFB1) is the most prevalent toxin in cereals used in feeds and presents the greatest toxigenic threat to chicken and humans (Prithi *et al.*, 1992; Azzam and Gabal, 1997; Kpodo *et al.*, 2000; Osho *et al.*, 2007; Leslie *et al.*, 2008). Animal feed contamination with Aflatoxin B1 (AFB1)

constitute a significant public health risk along the food chain (IARC, 1993; Azzam and Gabal, 1998; Verma, 2004; Del *et al.*, 2005; Njobeh *et al.*, 2009; Essono *et al.*, 2009; Herzallah, 2013). AFB1 is known to be carcinogenic and teratogenic in addition to being the most harmful form of aflatoxin due to its direct link to the cause of human liver cancer (Leslie *et al.*, 2008; USAID 2012; Sarma *et al.*, 2017).

Poultry production in Nigeria has its hub in Ibadan, Nigeria where poultry birds and inputs, including different commercial brands of feed are produced and distributed across the country (Oluwole *et al.*, 2012). Feed ingredients are obtained from different sources that are of variable qualities which could also determine the contamination and safety of poultry feeds (Maciorowski *et al.*, 2007). Aflatoxin B1 is a poultry feed contaminant that impairs all important poultry production parameters including feed intake, feed conversion efficiency, weight gain, pigmentation, male/female reproductive performance, processing yield and egg production (Klich, 2002; Rathod *et al.*, 2013). The toxin

has been shown to be transferred from poultry feed to muscles, kidney, liver tissues and eggs of chicken via consumption (Chang and Hamilton, 1982; Herzallah, 2013). It is therefore important to ensure safety of feed to poultry and ultimately the consumers of poultry products. This study determined the occurrence and contamination levels of AFB1 in major brands of commercial poultry feeds commonly sold in Ibadan and the concentration of AFB1 in the tissues of broilers chickens fed with a brand of the contaminated feed.

MATERIALS AND METHOD

Ethical Statement

Ethical approval was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan with assigned number UI-ACUREC/18/0105.

Sample Area

Ibadan, southwest Nigeria was chosen for this study due to high volume of poultry production and consumption in this region of the country (Oluwole *et al.*, 2012). Several commercial poultry farms, feeds manufacturers and distributors are located in this region. Also, it has a favorable climate for the growth of *Aspergillus* spp. and subsequent production of AFB1 with an average annual temperature of 26°C and humidity of 85 %.

Sample Collection

A Complete Randomized Design (CRD) was employed for data collection. A total of 180 feed samples from six commonly available commercial brands of broiler feed were randomly collected from three retail outlets in Ibadan. One pooled sample comprising ten samples of each brand of feed were collected from each of the six brands of feed (A – F) in each visit. Making six pooled samples comprising of sixty feed samples per visit and a total of eighteen pooled samples culminating in one hundred and eighty samples from the six brands of feed from the three visits. The samples were obtained in April, June and July 2019 with a minimum of four weeks in between each visit for sample collection. Each pool of samples was analysed in triplicate to obtain the mean concentrations in each brand/visit. Samples were collected in Nasco^(R) sample whirl bags and tightly sealed. Also, a total of 75 fresh broiler meat samples (25 each for breast muscle, gizzard and liver) were obtained from 25 market sized broilers randomly obtained from commercial farms that fed chicken with one of the brands of sampled commercial feeds for a minimum of four weeks. All samples were transported to Food and Meat Hygiene Laboratory, University of Ibadan for sample preparation. Aflatoxin B1 analysis was subsequently carried out using High Performance Liquid Chromatography (HPLC) at the Nigerian Institute of Laboratory Science Technology (NILST) in Ibadan.

Sample Preparation and Clean-up Procedures

The feed and meat samples were grinded into fine powder and paste with Warin blender and stored at -4°C and -18°C respectively until subsequent extraction and analysis (Urraca *et al.*, 2004). Extraction process involved homogenization of 20 g of each feed sample with 2 g NaCl and 100 ml of methanol: water solution (80:20) in a blender for 3 minutes. After which one cover was removed and the extract filtered

with Whatman No. 1 filter paper (Ige *et al.*, 2012; Wacoo, 2014). Approximately 20 ml of the filtrate was transferred into a conical flask to which 60 ml of 10% phosphate buffer solution (pH 7.4) was added and mixed thoroughly. The diluted extract was filtered into another conical flask using the glass microfilter after which 20 ml of the solution was passed through Solid Phase Extraction (SPE) in Immuno Affinity Column (IAC). Aflatoxin B1 analyte was eluted into 5 mL volumetric flask in addition with 1 mL of methanol ready for HPLC analysis (AOAC, 2000; Abd El Monem *et al.*, 2015).

HPLC Determination

Detection and quantification of AFB1 was performed by Agilent HPLC equipped with binary HPLC pump. Thermo LC-Si model column of 250 x 4.6 mm kept in column oven at 40°C and fluorescence detector (Model FL 2475) set at wavelength 365 and 425 nm for excitation and emission respectively were used. The mobile phase for isocratic comprised of toluene, ethyl acetate, formic acid and methanol (90:5:2.5:2.5, v/v/v/v) at flow rate of 2.0 mL/ min. The results were confirmed by Agilent HPLC equipped with fluorescent detector and run under similar conditions.

Standard Curve Preparation and Recovery of AFB1 Spiked Aflatoxin B1 standard (0.26 ng/μl) (Supelco # 46304-U) was prepared according to AOAC Official methods (AOAC, 2000). The solution was diluted in ratio 1:10 (100 μl of 2.6 ng/μl stock with 900 μl methanol) to make a 0.26 ng/μl aflatoxin B1 standard. Afterwards, the 0.26 ng/μl aflatoxin B1 standard was further diluted at ratio 1:10 (100 μl of 0.26 ng/μl stock with 900 μl methanol) to make a 0.026 ng/μl aflatoxin B1 standard. The calibration curve of peak is as against the corresponding concentrations of aflatoxin B1 standards was created using Microsoft® Excel® 2013 version 15.0.5249.1000. The peak areas of the analytes from the samples unknown were used to calculate the concentrations in (ppb) using linear regression ($Y = mX + b$) where: Y = peak area/height; x = analyte concentration in ppb/μ/kg; m = slope of curve; and b = intercept of y.

Recovery of Aflatoxin B1

The solutions were stored in Teflon capped 2 mL amber vials away from light and at -18°C. Blank feed samples (corn) were spiked with serially diluted standard AFB1. Spiked samples were allowed to dry for 30 minutes in the hood before assaying following same extraction above. Recovery of the aflatoxin B1 was done using a blank feed sample spiked with standard solution of AFB1 and the resultant contamination level recorded. A linear AFB1 calibration curve was plotted and the correlation coefficient determined.

Statistical Analyses

The AFB1 residues concentrations in the selected brands of poultry feed and different meat types were subjected to ANOVA with Duncan's Multiple Range Test using the general linear model (GLM) procedure in PC-SAS version 9.0. Values were considered significant if $p < 0.05$.

RESULTS

Feed Sample Aflatoxin B1 Contamination

Out of the tested feed samples brand D feed samples had the highest AFB1 contamination of 47.78 ± 6.4 while the lowest

mean concentration 10.56 ± 4.0 $\mu\text{g}/\text{kg}$ was obtained from brand F. Table 1 showed mean AFB1 concentrations in the six brands of poultry feed tested. A linear AFB1 calibration curve was obtained with correlation coefficient of 0.999 and the coefficient of variation (CV) of 1.32% with minimum detection limit of 0.05 $\mu\text{g}/\text{kg}$. The concentration of AFB1 in the meat samples ranged from 0.07 - 0.18 $\mu\text{g}/\text{kg}$ with gizzards having the highest concentration of 0.18 ± 0.05 $\mu\text{g}/\text{kg}$ (Table 2). The recoveries were generally higher than 80 %, ranging from 85 to 93 %.

Analysis of Feed and Tissue Samples

Table 3 showed the results of analysis of variance of AFB1 concentrations in the six brands of feeds. There was

significant difference ($p < 0.05$) in the concentration of Aflatoxin B1 (AFB1) between commercial feed A and that of commercial feeds D and feed F. There was significant difference ($p < 0.05$) between the level of AFB1 in commercial feeds B and A, D, E and F, except brand C where the p value > 0.05 . Feed brand C showed significant difference ($p < 0.05$) in the level of AFB1 compared to brands D and F while there was no significant difference ($p > 0.05$) when compared with feed brands B and E. The level of AFB1 in brand D feed was significantly different ($p < 0.05$) compared to brands E and F. There was also significant difference ($p < 0.05$) in the level of AFB1 between feed brands E and F (Table 3).

Table 1: Aflatoxin B1 Contamination Level ($\mu\text{g}/\text{kg}$) in Commercial Poultry Feed.

S/N	Commercial Brands of Broiler Finisher (N = 180)	Mean AFB1 Levels ($\mu\text{g}/\text{kg}$)
1	Feed A (n=30)	28.67 ± 8.9
2	Feed B (n=30)	35.00 ± 3.6
3	Feed C (n=30)	30.00 ± 3.7
4	Feed D (n=30)	47.78 ± 6.4
5	Feed E (n=30)	27.11 ± 5.8
6	Feed F (n=30)	10.56 ± 4.0

Table 2: Aflatoxin B1 Content of Chicken Meat from Broilers Fed with Brand D Commercial Feeds for 4 Weeks

S/N	Chicken Tissue N=75	Mean AFB1 Levels ($\mu\text{g}/\text{kg}$)
1	Chicken breast (n=25)	0.07 ± 0.02
2	Gizzard (n=25)	0.18 ± 0.05
3	Liver (n=25)	0.13 ± 0.02

DISCUSSION

The importance of food safety cannot be over emphasised. The danger posed by AFB1 in animals and humans is immense because of its carcinogenic and teratogenic nature. The occurrence of AFB1 in the sampled commercial poultry feeds was confirmed with positive samples discovered in all the sampled brands. The mean levels of AFB1 in five of the sampled brands were higher than the United States Food and Drug Administration (FDA, 2019) recommended level of 20 $\mu\text{g}/\text{kg}$. The contributory factor to the level of incidence of AFB1 in samples analysed in this study maybe the time of collection of samples was between the months of April and July and it coincided with rainy season in Nigeria. It is an established fact that conditions of high humidity contribute to increased production of Aflatoxins by *Aspergillus* spp. In a similar study carried out by Rashid *et al.* (2012), the maximum level of AFB1 detected in finished commercial feed was 166 $\mu\text{g}/\text{kg}$ while the minimum was 10 $\mu\text{g}/\text{kg}$. The significant difference in the levels of AFB1 in the six brands of feed highlighted the differences in production practices maintained by the different producers.

The findings of this study confirmed the presence of AFB1 in commercial poultry feed which may be attributed to prevalence of poor harvesting techniques, insect infestation of crops, poor storage practices and environmental conditions hence the need for regular monitoring of mycotoxin levels in animal feeds by relevant regulatory agencies (Jones *et al.*, 1994). The transfer of AFB1 from feed unto the muscle tissues of broilers was confirmed by the detection of trace quantities of the toxin in the meat samples of broilers fed with a brand of sampled commercial feeds for four weeks. In another study carried out by Herzallah (2013), where laying birds were fed with AFB1 contaminated feed in increasing dosages for 7 weeks, their eggs and meat were found to be contaminated in the same increasing order of AFB1 dosage in feed served. It was also observed that the levels of AFB1 in the chicken meat parts; gizzard and liver were higher than chicken breast meat which was in tandem with the work of Saltana and Hanif (2009) and Oyero and Oyefolu (2010), where transfer of AFB1 from contaminated concentrates to beef was assessed. According to the study, organs like liver and kidney were more contaminated than beef.

The same pattern was observed by Herzallah (2013). In this research work however, the level of AFB1 found in the meat samples; chicken breast, gizzard and liver were below the International Safety Limit which is 5 µg/kg (FAO, 2003).

Feeding birds with diets high in AFB1 for long periods of time could lead to higher level of AFB1 residues in the meat and other products such as eggs (FAO, 2003).

Table 3: Analysis of Variance (ANOVA) of AFB1 Concentrations between the Six Brands of Poultry Feed

Brands	Groups	P-value	F-value	Mean Diff.	95% C.I.	
A	A	B	0.23	39.91	6.33	11.76 – 0.89
		C	0.63		1.33	6.76 – 4.10
		D	<0.001		19.11	-24.54 – 13.67
		E	0.57		1.56	-3.88 – 6.99
		F	<0.001		18.11	-12.67 – 23.54
B	B	A	0.02	39.91	6.33	0.89 – 11.76
		C	0.07		5.00	0.44 – 10.43
		D	<0.001		12.77	18.21 – 7.34
		E	0.005		7.88	2.45 – 13.32
		F	<0.001		24.44	19.00 – 29.88
C	C	A	0.62	39.91	1.33	-4.10 – 6.76
		B	0.07		5.00	0.43 – 10.43
		D	<0.001		17.77	23.21 – 12.34
		E	0.29		2.88	2.54 – 8.32
		F	<0.001		19.44	14.00 – 24.88
D	D	A	<0.001	39.91	19.11	13.67 – 24.54
		B	<0.001		12.77	7.34 – 18.21
		C	<0.001		17.77	12.34 – 23.21
		E	<0.001		20.66	15.23 – 26.10
		F	<0.001		37.22	31.78 – 42.65
E	E	A	0.56	39.91	1.56	-6.99 – 3.88
		B	0.005		7.89	2.45 – 13.32
		C	0.29		2.89	2.54 – 8.32
		D	<0.001		20.67	15.23 – 26.10
		F	<0.001		16.56	11.11 – 21.99
F	F	A	<0.001	39.91	18.11	12.67 – 23.54
		B	<0.001		24.44	19.00 – 29.88
		C	<0.001		19.44	14.00 – 24.88
		D	<0.001		37.22	31.78 – 42.65
		E	<0.001		16.55	11.11 – 21.99

Conclusions

The presence of AFB1 in poultry feeds and its possible transfer into chicken meat is of food safety and public health importance. It is also a concern to both the poultry and food industry. Aflatoxin B1 could be potential health risk to both chicken and the consumers of chicken and its products. Export of chicken feed with AFB1 concentration of 20 µg/Kg is not acceptable in most part of the world especially Europe and United States of America. There is need to screen chicken feed possibly apply hazard analysis critical control point (HACCP) to feed manufacturing, storage and broiler chicken production in order to prevent the presence of mycotoxins and ensure food safety. It is recommended that monitoring and surveillance of mycotoxin levels in animal feeds and animal products meant for human consumption

should be carried out for longer periods and routinely to enhance poultry production.

Authors' contributions

OIO and AJO designed and carried out the research. OIO AJO and OOO were involved in the analysis, writing and proof reading of the manuscript.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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