



Aflatoxin in Commercial Poultry Feeds and Clinico-Pathological Manifestation of Aflatoxicosis in Poultry in Southwest, Nigeria

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SUMMARY

Aflatoxin remains the most studied mycotoxin with aflatoxin B1 making up 66 to 82% of total aflatoxin found in feed. In poultry, it can cause high production losses and vaccine failure resulting from its hepatocarcinogenic, mutagenic, teratogenic and immunosuppressive effects. This study aimed to determine the level of aflatoxin in commercial feed and feed ingredients (FFIs) use in poultry production in Southwest Nigeria. The study also tends to describe the clinico-pathological presentations of birds fed with aflatoxin-contaminated feed. A total of 142 commercial FFI (commercial feed, maize, cassava, wheat offal, soya cake etc) were collected randomly from poultry farms and feed mills in Southwest Nigeria. Total aflatoxin levels in FFI were measured using competitive ELISA. The study showed that 34 (23.9%), 24 (16.9%) and 67 (47.2%) of FFI had 21-60 µg/g, 61-100 µg/g and ≥ 101 µg/g of total aflatoxin levels respectively. These levels are above the maximum permissible limit (20 µg/g) as recommended by United State Food and Drug Administration (USFDA). Most of the clinico-pathological findings such as decrease in egg production, friable liver, gastrointestinal mucosal erosion and fistulations were consistent with published reports on aflatoxicosis. History revealed that feed millers engaged in malpractices such as mixing of feed ingredients from different sources etc. The socioeconomic implications of aflatoxin in FFI are enormous, thus measures to reduce fungi contamination at every stage of production should be a collective effort. Our study showed that out of 10 FFIs, approximately 9 had >20 µg/g total aflatoxin. Therefore, studies aimed at prevention of fungal growth and mycotoxin production in FFI as well as mycotoxin absorption through the gastrointestinal wall should be strongly facilitated. Malpractices among feed millers should be abolished by concerned government agencies.

Key words: Mycotoxin, Aflatoxin, Aflatoxicosis, Feed, Ingredients, ELISA.

INTRODUCTION

Toxins produce by fungal organism are generally called Mycotoxins and the most important mycotoxin producers are: *Aspergillus species*, *Fusarium species*, *Penicillium species* and *Alternaria species* (Varga *et al.*, 2009). These moulds grow in the feed and feed ingredients (FFIs) during growth, harvest and storage, causing spoilage and release of toxic secondary metabolites. These toxins act as poisons or carcinogens which are harmful to humans and animals (Frisvad *et al.*, 2007). There are several classes of mycotoxins depending on their chemical nature and toxicity. Some of the identified mycotoxins include aflatoxin, ergot alkaloids, amanitins, ochratoxin, cyclopiazonic acid, citrinin, Tricothecenes and slaframine (Frisvad *et al.*, 2007).

Mycotoxicology evolved as a result of 1960 Turkey X disease outbreak in United Kingdom (Coker, 1979). The United Nation's Food and Agriculture Organization (FAO) gave an estimate of 25% world's grain supply contaminated with mycotoxins with aflatoxin ranking highest (FAO, 2002). Aflatoxin, a potent carcinogen and probably most studied mycotoxin is produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Beg *et al.*, 2006). It is classified into types B1, G1, G2 and B2 (in decreasing order of potency and toxicity) (Reo, 1978; Galvano *et al.*, 2001). Aflatoxin B1 has been reported to contribute to 66 to 82% of total aflatoxins in feed (Becha and Devi, 2013).

Generally, aflatoxin is connected with several diseases including aflatoxicosis in livestock and humans, and it causes a worldwide problem with high economic losses (Beg *et al.*, 2006). It has been reported to have hepatocarcinogenic, mutagenic, teratogenic and immunosuppressive effects (CAST, 2003). The immunosuppressive damage by aflatoxin results into increasing infection

susceptibility and vaccine failure due to its ability to depress Tor B lymphocyte activity; and suppression and impairment of macrophage/neutrophil effector functions (Hatori *et al.*, 1991). This culminates into reduce antibody levels following infection or vaccination. Some clinico-pathological features seen include decrease egg production, increasing morbidity and mortality, friable liver, multifocal hepatic necrosis, haemorrhagic hepatitis, enteritis etc (Sklan *et al.*, 2001; Bentvihok *et al.* 2002; Giacomini *et al.*, 2006; Becha and Devi, 2013; Kehinde *et al.*, 2014).

The chronic morbidity and mortality effects of aflatoxin with accompanied economic losses underline its significance in poultry industry. The reported residues of aflatoxin and its metabolites in poultry products such as meat, egg and offals like gizzard, liver and blood (Oliveira *et al.*, 2002; Anjum *et al.*, 2012) also emphasized its public health significance as these products become indirect sources of aflatoxin to human consumers.

Today, several countries are aware of these deleterious effects of aflatoxin and other mycotoxins on humans and animals, thus maximum permissible limit of mycotoxin in food and feedstuffs are set in order to safeguard human and animal health. United State Food and Drug Administration (US-FDA) recommended maximum permissible aflatoxin level of 20 µg/g in corn, peanut products, other animal feeds and feed ingredients for immature or dairy animals or when the intended use is not known (US-FDA, 2000).

This study aimed to determine the levels of total aflatoxin in commercial FFIs use in poultry in Southwest Nigeria. Farmer's observations and post-mortem findings of some of the collected carcasses in relation to the feed were also reported.

MATERIALS AND METHODS

Sample collection

A random sample of one hundred and forty two FFI, such as commercial feed, maize/corn grit, soya cake/oil, cassava etc, were collected from poultry farms and feed mills in Southwest Nigeria. About 100g of FFIs were collected into a sterile container for aflatoxin level measurement. Also, recent history and carcasses of birds that died naturally after being fed with commercial feed above were taken. Post-mortem examinations were done and findings were juxtaposed with aflatoxin levels in FFIs collected.

Sample analysis

Samples were analysed by competitive enzyme linked immunosorbent assay (cELISA) using AgraQuant[®] Total Aflatoxin Assay 4/40 kit (Romer Lab Singapore Pte. Ltd). Aflatoxin extraction was carried out based on manufacturer's instruction. Briefly, 20g of ground sample was measured into a clean glass conical flask containing 100ml of 70% (v/v) methanol. This was shaken vigorously for 3 minutes and allowed to settle. The top layer of extract was then filtered through Whatman #1 filter and the filtrate was tested with ELISA kit. ELISA reader set at 450nm filter was used to obtain the optical density (OD) of the final reaction. A dose-response curve was constructed using the five

standards provided with the kit. Since the amount of aflatoxin in each standard is known, the sample aflatoxin amount was measured by interpolation from this standard curve.

Statistical analysis

SPSS program version 15.0 (2006) was used for statistical analysis (SPSS Institute Inc. Chicago, Illinois). Descriptive statistical analysis was reported as mean \pm standard deviation.

RESULTS

A total of one hundred and forty two (142) FFIs were obtained including commercial feed 112 (78.9%), maize/corn grit 16 (11.3%), soya cake/oil 6 (4.2%), cassava 3 (2.1%), wheat/ wheat offal 3 (2.1%), malta grain 1 (0.7%) and groundnut cake 1 (0.7%) (Table I). Out of 142 samples analysed, only 17 (12.0%) contained $\leq 20 \mu\text{g/g}$ aflatoxin while 21-60 $\mu\text{g/g}$, 61-100 $\mu\text{g/g}$ and $\geq 101 \mu\text{g/g}$ of aflatoxin were found in 34 (23.9%), 24 (16.9%) and 67 (47.2%) FFI respectively (Table I). The range of aflatoxin level is 0 to 452 $\mu\text{g/g}$ with a mean \pm SD of 112.5 \pm 101.1 $\mu\text{g/g}$. Table II summarised the distribution of total aflatoxin level in "already in use feed" and "about to be used FFIs". Out of the feed already in use, 92.2% (47/51) had total aflatoxin level $>20 \mu\text{g/g}$ while 85.7% (78/91) of "about to be used feed" had total aflatoxin level $>20 \mu\text{g/g}$.

Table I: Distribution of total aflatoxin in feed and feed ingredients

SN	Feed and feed ingredients	Total aflatoxin			
		$\leq 20 \mu\text{g/g}$	21-60 $\mu\text{g/g}$	61-100 $\mu\text{g/g}$	$\geq 101 \mu\text{g/g}$
1	Commercial feed (n= 112)	16	30	19	47
2	Maize/ Corn grit (n= 16)	1	3	2	10
3	Soya cake/ Oil (n= 6)	0	1	1	4
4	Cassava (n= 3)	0	0	1	2
5	Wheat/ Wheat offal (n= 3)	0	0	1	2
6	Malta grain (n= 1)	0	0	0	1
7	Groundnut cake (n= 1)	0	0	0	1
Total (n= 142)		17 (12.0%)	34 (23.9%)	24 (16.9%)	67 (47.2)

Table II: Distribution of total aflatoxin in “already in use” and “about to be used” feed

Total aflatoxin level ($\mu\text{g/g}$)	Feed already in use	Feed and feed ingredients about to be fed to birds
≤ 20	4 (7.8%)	13 (14.3%)
21-60	16 (31.4%)	18 (19.8%)
61-100	10 (19.6%)	14 (15.4%)
≥ 101	21 (41.2%)	46 (50.5%)
Total	51	91

Varying history of drop in egg production, morbidity and mortality were reported (Table III). Generally, among carcasses obtained from birds fed with feed containing $>20 \mu\text{g/g}$ total aflatoxin, 18 (35.3%) had necrotic streaks on liver, friable liver, increasing mortality and or egg production drop, 12 (23.5%) had erosion, ulceration or fistulation of the gastrointestinal tract and 17 (33.3%) had all the signs and lesions previously mentioned (Table III). However, some of the carcasses obtained from birds fed with $\leq 20 \mu\text{g/g}$ total aflatoxin FFIs presented few clinico-pathologic abnormalities earlier stated (Table III).

Oral history by farmers revealed that mixed feed and feed ingredients from different sources are sometimes sold by feed millers to the farmers especially when the needed quantity is inadequate from a single source. The sales of packaged (ready to use) FFIs of unknown aflatoxin level were also reported.

DISCUSSION

The analyses of the surveyed samples showed that 34 (23.9%), 24 (16.9%) and 67 (47.2%) of FFI had 21-60 $\mu\text{g/g}$, 61-100 $\mu\text{g/g}$ and $\geq 101 \mu\text{g/g}$ aflatoxin respectively. These levels are higher than the maximum and permissible limit of 20 $\mu\text{g/g}$ recommended by United State Food and Drug Administration (US-FDA, 2000), thus making them unsuitable for their use in poultry. There are past reports indicating various levels of aflatoxin contamination of FFIs used in poultry industry in Nigeria and other countries (Pandey *et al.*, 2001; Wang

et al., 2003; Manafi *et al.*, 2007; Manafi, 2009; Manafi, 2010; Kehinde *et al.*, 2014). Our study showed the out of 10 FFIs, approximately 9 of them had $> 20 \mu\text{g/g}$ total aflatoxin contamination. In fact, 85.7% (78/91) of FFI obtained from feed mills (not yet bought to feed poultry) had $> 20 \mu\text{g/g}$ aflatoxin level (Table II). These suggest that virtually all FFIs had aflatoxin level that can be injurious to the body system.

Birds fed with $>20 \mu\text{g/g}$ aflatoxin contaminated feed presented clinical signs of decrease egg production, increasing mortality and inactiveness (Table III). These are in consonance with published reports on clinical manifestations of aflatoxicosis in poultry which include increase susceptibility to infection, poor growth, decrease egg production, increasing morbidity and mortality rates etc (Sklan *et al.*, 2001; Bentvihok *et al.* 2002; Giacomini *et al.*, 2006; Resanovic *et al.*, 2009; Becha and Devi, 2013). The postmortem lesions found, such as necrotic streaks on liver, friable liver, blood clots on the liver, gastrointestinal mucosal erosion, ulceration and fistulation (Table III and Figure A) were in agreement with previous reports indicating the poisonous effects of aflatoxin (Jewers, 1990; Anjum *et al.*, 1997; Saif *et al.*, 2003; Dhanasekaran *et al.*, 2009; Sumit *et al.*, 2010).

The socioeconomic implications of aflatoxin in FFIs are enormous, thus measures to reduce fungi contamination of crops and cereals during pre-harvest, harvest and storage periods must be considered. The

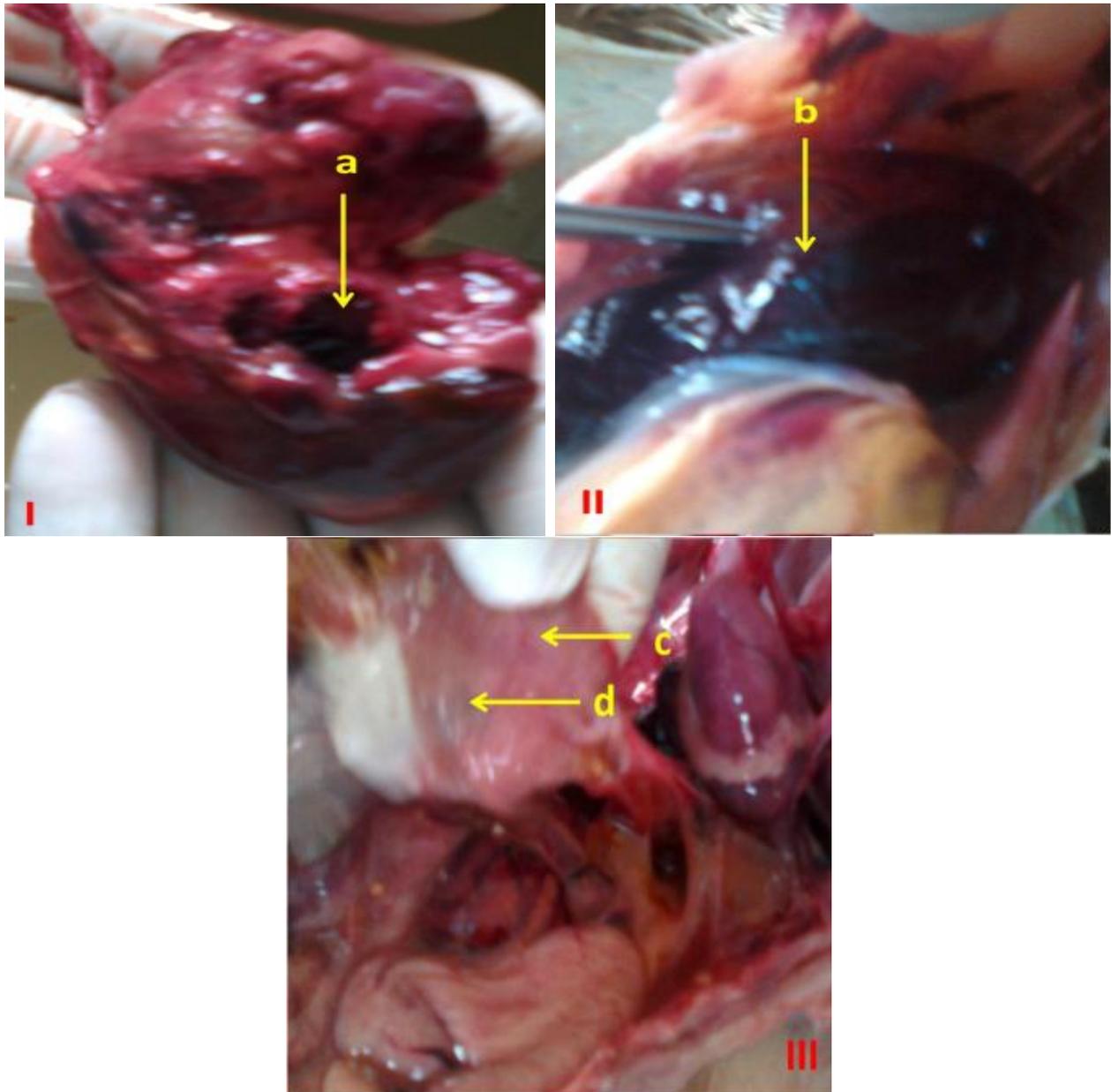


Plate I-III: Gross lesions of poultry carcasses fed with Aflatoxin containing feed

- a- Haemorrhagic plug in the friable liver
- b- Blood clot on the liver in-situ
- c- Ulceration of the proventricular mucosa
- d- Erosion of the proventricular mucosa

availability of an affordable, fast and sensitive analytical techniques and continuous advocacy to farmers to quantify the amount of aflatoxin and other mycotoxins in FFIs before use will be of great value. Malpractices among feed

millers should be deterred by the relevant government agency.

Although, FFIs free of aflatoxin are hard to come by, the reduction and or avoidance of feed ingredients with reported high aflatoxin levels (e.g. groundnut cake and maize)

should be encouraged (Becha and Devi, 2013). The application of various studied biologic and chemical aflatoxin detoxifying agents such as zeolites, bentonite, sodic montmorillonite, hydrated sodium calcium aluminosilicate, esterified glucomannan, monensin, aluminosilicate, phytogenic substances, plant charcoal from specific species, plant herbal extracts, yeast, different yeast cell extracts etc can be adopted in feed mills as recommended by Manafi et al (2009) and Oguz (2012).

Conclusion

This study showed that there were high levels of aflatoxin contamination in FFIs sampled from Southwest Nigeria, the major hub of poultry industry in country. Thus, continuous research into developing practicable, non toxic, non anti-nutritive and cost effective means of aflatoxin detoxification in FFIs and or means of impeding their absorption through the gastrointestinal mucosal should be strongly facilitated.

Competing interest: The authors affirm that this study and its interpretations were not under any financial or otherwise competing interest.

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