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Research Paper

INCIDENCE OF AFLATOXIN CONTAMINANT IN SOME POULTRY FEEDS SOLD IN SOUTH EASTERN NIGERIA

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Abstract

Some poultry feeds sold in parts of south – eastern Nigeria were screened for the presence of Aflatoxin. The aim was to ascertain their safety coefficient in the food web. Starters, growers, layers and Finishers mash collected from the study area, spanning Abia, Anambra, Ebonyi, Enugu and Imo states were used in the study. Standard detection methods, microscopy, thin layer chromatography (TLC) and Elisa-Spectrophotometer were used. The results showed that finishers mash obtained from Abia, Ebonyi and Enugu states contained mean quantities, 7.70 ± 0.64, 20.38 ± 0.64 and 9.5± 0.00 µg/Kg respectively of aflatoxin. In Anambra state, aflatoxin was detected only in Growers marsh at mean quantity of 16.08 ± 0.32µg/Kg while in Imo state, it was found in both starters and layers mash at mean quantities of 13.59 ± 0.65 and 16.32 ± 3.20 µg/kg respectively. The information produced in this study may be used in investigations involving bioaccumulation, food and feed production, trophic levels, mycotoxicosis and other health hazards issues. Livestock feeds should be properly screened by the appropriate Vetenary agency before administering to the animals.

Key words: Aflatoxin, Bioaccumulation, Mycotoxicosis, Health- hazard.

INTRODUCTION

Aflatoxins are a group of toxic secondary metabolites of fungal origin. They are low molecular weight secondary metabolites (mycotoxins) produced by certain molds of the genus *Aspergillus* mostly, *A. flavus and A. parasiticus* growing on a number of organic substrate (Bennett and Klick, 2003, Duru *et. al.,*2012). They can cause both acute and chronic toxicity in humans and many other animals. Their importance was first established in 1960 when 100,000 turkeys in Kenya and other poultry birds in UK died in a single event (Bankole and Mabekoje, 2004). The cause was eventually traced to a

toxic contaminant of groundnut meal used in the birds' feed. The contaminant was later found to be aflatoxin.

There are many types of aflatoxins but four are naturally found in food products (Fabian *et. al*., 2013). They are aflatoxin B_1, B_2, G_1, and G_2. The aflatoxin B_1 (AFB_1 or B_1) is the most toxic and commonly found in food (Atehnkeng *et. al*., 2008). The B and G in the nomenclature are based on their fluorescence under UV light (Adebayo and Ettah, 2010).

When lactating mammals ingest fungal contaminated foods or feeds, toxic metabolites such as aflatoxin M_1 (AFM_1) and AFM_2. can be found in their milk. Aflatoxin (AF) may be present in a wide range of food commodities such as cereals, oil seeds, nuts, dried fruits, black pepper, cheese, dairy products etc.

Prior to aflatoxin contamination, food must first be infested with aflatoxigenic fungi with the genetic potentials to synthesize and deposit toxins in the foods or feeds (substrate) before and after processing (Bondy and Peska, 2000).A strain of mold may have genetic potentials to produce aflatoxins but the levels of production depends on the environmental conditions such as the availability of nutrients, moisture content (humidity), moderate temperature, etc (Amadi and Adeniyi, 2009).

Nigerian animals are exposed to aflatoxins by feeding them with fungal infested feeds or domestic wastes. Humans are also exposed to the same by consuming the contaminated animal and or its products (Njobeh *et. al*., 2009). Aflatoxin exposure has high health and socio-economic implication. The AFB_1 are naturally powerful carcinogens, hence, has raised investigation by medical professionals.

Some of the diseases caused by AFB_1 include, acute and chronic toxicity, lungs cancer, immune-suppression, Kwashiokor and reproduction difficulties. (Oyelamin and Ogunleshi, 2007)

In view of the fact that these toxins get into human and livestock through ingestion of contaminated rations, this study therefore aim at screening for aflatoxin content of some poultry ration sold in south Eastern Nigeria, using standard methods with a view to releasing information to guide animal husbandry, public food safety and health

MATERIALS AND METHODS

Sample Collection

One-hundred grams (100g) of each of the 100 samples of the starters, growers, finishers and layers marsh were collected from artisan stores at Aba (Abia state), Awka (Anambara state), Abakaliki, Abakaliki (Ebonyi state) and Owerri (Imo state). The samples were collected in sterile polytene bags and taken to the laboratory at the Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambara state, Nigeria., where the study was carried out. The study area is located within the guinea savannah belt of south – eastern Nigeria and falls within latitude 4. 05^{o} N and longitude 7.30^{o}E.

Sample Preparation

Each of the feed samples was finely ground, homogenized and subjected to the TLC, following the methods described by Wacco et al. (2014). Twenty-five grams of each of the test feed powder was separately mixed with 50ml of the solvents, methanol and acetone mixture (1/ 2 v/v). The mixture complex was shaken thoroughly and allowed to stand for three hours at room temperature. It was then centrifuged at 5000rpm for ten minutes. The supernatants were decanted and used as the extracts for the thin layer chromatographic detection of aflatoxin.

Detection of Aflatoxin in the Test Samples using Thin layer Chromatography (TLC)

The thin layer has two phases: the Stationary phase with a plate and the mobile phase. The plate was applied with silica gel evenly and allowed to dry and stabilized. The mobile phase consisted of a mixture of acetonitrates and water in the ratio of (4:1). The solvent mixture was poured into the thin layer chamber to a height of 3cm above the base. A pencil marker was used to draw an inkless line at the height of 6cm from the plate base. The test crude extract (jelly form) was applied on the inkless line. The plate was oriented vertically in the solvent and the tank was closed. Sufficient time was allowed for spots to develop. When the solvent had risen to the tip of the plate. The plate was dried at 100^{o}C and developed in diethyl ether to a height of 12cm. This was allowed to dry in subdued light. It was taken to ultraviolent (UV) light box placed at about 30cm away and observed for the presence of the blue fluorescent spot. RF values of 0.50 to 0.55, which indicated the presence of AFB_1.

Determination of Aflatoxin content in the test samples using Elisa – spectrophotometry

The Elisa – spectrophotometer used was the direct competitive method kit, courtesy of Ghali*et. al*., (2010). Twenty grams of the pulverized test feed sample was mixed with 100ml of pure 70% methanol. The mixture was homogenized, filtered and the filtrate containing aflatoxin extracts were used in the next stage. Micro-wells were coated with antibody (Ab) and enzyme (Enz) conjugated (conj) aflatoxin (AF). The micro-wells were labeled as follows: **the Blank** (Ab + Enz conj.AF), **Standard Aflatoxin**(StdAF) (Ab + Enzconj AF + StdAF), **Test Sample** (Ab + Enzconj AF + Test Extract). The set up was allowed to stand for some time to compete separately with the conjugated aflatoxin for site binding. The reactions were stopped by washing the set up five times with light detergent. Enzyme extract provided by the kit was added to all for signal development. The absorbance of the blue colour that developed was read at 460nm wavelength.

The aflatoxin contents of the test samples were determined using the relation.

$$AFB_1 \mu g/Kg = \frac{1000}{W} \times \frac{au}{as} \times C$$

Where W: weight of the sample

C; concentration of the standard

Au; absorbance of the sample

As; standard AFBI solution.

Figure. 1 Map of Nigeria showing the study area

Legend: ---..... yellow colour indicating the study area.

RESULTS AND DISCUSSION

The screening protocol used in this study showed that aflatoxinB1 contaminant is present in some poultry feed sold within the study areas, Aba (Abia state), Abakaliki (Ebonyi state), Awka (Anambara state), Enugu (Enugu state) and Owerri (Imo state). (Table 1, Fig 1). Aflatoxin was reported to have been a low molecular weight secondary metabolite produced by certain fungi, mainly *Aspergillus flavus* and *A parasiticus* (Bennett and Klick (2003). The aflatoxin –contaminated poultry feed types were finishers mash, sold at Aba, Abakaliki and Enugu. Starters and layers mash sold at Owerri and growers mash sold at Awka. These feed types are prepared with active ingredients from whole grains and seeds like maize, rice, groundnuts, among other agro-commodities which may have been infested with the aflatoxigenic fungus, *A flavus,* detected in in the samples screened. This is supported by the findings of Duru and Anyadoh (2009) and Duru *et al* (2012) on the incidence of *A flavus* on seeds of *Arachis hypogea* (groundnut), *Phaseolus lunatus, sphenostylis stenocarpa, vigna unguiculata*, grains of *Zea mays* and kernels of Irvingia gabonensis sold in parts of Niger Delta of Nigeria. As well as Fabian *et al*. (2013) who reported that some fungi and bacteria population remain viable in the processed feed even at the time of marketing.

Table 1: occurrence of Aflatoxin in the Test Feed Samples (TLC assay)

Types of feed	**Location**				
	Aba	**Abakaliki**	**Awka**	**Enugu**	**Owerri**
Starters	-	-	-	-	+
Growers	-	-	+	-	-
Layers	-	-	-	-	+
Finishers	+	+	-	+	-

The presence of the blue fluorescence observed as the absorbance detection of aflatoxin in the study showed that the type of aflatoxin detected is AFB1. This also concurred with the report of Adebayo and Ettah (2010) Who found the blue fluorescence as a mark of absorbance for aflatoxin AFB1 and AFB2, yellow fluorescence for aflatoxin G1 and G2.

The quantity of aflatoxin detected per kilogram of the test feed is shown in Table 2. Starters and layers marsh from Owerri were approximately (0.014 mg / kg and 0.016mg /kg) respectively. Growers from Awka was (0.016 mg / kg), Finishers from Aba was (0.008mg / kg) while Abakaliki and Enugu were (0.020 and 0.010mg / kg) respectively. These quantities, though may exhibit some degree of toxicity, they are far below the WHO safety range of lethal dose (LD) of between (0.05 and 10mg / kg) (LD0.5 – 10) per body weight

Table 2: Aflatoxin Contents of Feed Samples in The Study Areas using Elisa – spectrophotometer.

Sample	**AFB_1 µg/kg**
	A
1 Enugu Finishers	9.5 ± 0.00
	B
2 Awka Growers	16.08 ± 0.32
	C
3 Abakaliki Finishers	20.38 ± 0.64
	A
4 Aba Finishers	7.70 ± 0.64
	B
5 Imo Starters	13.59 ± 0.65
	B
6 Imo Layers	16.32 ± 3.20

Values show means of duplicate analysis ± standard deviation. Figures having the different superscript down the column are significantly different (P < 0 .05)

CONCLUSION

Within limit of the scope of this study, it is evident that most of the agro-feed rations sold in Aba, Abakaliki, Awka, Enugu and Owerri were contaminated by a high potent aflatoxin (AFB1). This mycotoxin is a product of antibiosis arising from infestations of

aflatoxigenic molds, chiefly the *A flavus*. The isolation of *A flavus* from the test feed and the subsequent detection of aflatoxin confirm that the *A flavus* is responsible for the contamination. Since aflatoxin is a known carcinogen, effort should be made to screen grains and seeds components as well as disinfect and process feed / food in an aseptic environment.

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