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Detection and Quantification of *Aflatoxins* from Commercial Poultry Feed in the Federal Capital Territory, Nigeria

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Abstract

This study aimed to extract and quantify Aflatoxins from commercial poultry feed collected from the local markets within the Federal Capital Territory (FCT), Nigeria. Seven commercial poultry feeds were sampled using a convenient sampling method from six Area Councils of the FCT, Nigeria, and analyzed using High Performance Liquid Chromatography attached to a Fluorescence Detector (HPLC-FLD). Out of the seven samples analyzed, two types of Aflatoxins were quantified, with Aflatoxin B1 having a concentration of 1.00 parts per billion (ppb) in feed D and 10.81 ppb in feed E, while Aflatoxin B2 was found at a concentration of 2.11 ppb in feed A and 9.12 ppb in feed E. There is a need for further studies involving other types of mycotoxins that can be detected in commercial poultry feed, which will provide insight into the current state of mycotoxicosis as baseline data for future research and possible control measures to eradicate Aflatoxicosis in FCT poultry farms.

Keywords: Aflatoxin, HPLC-FLD, Poultry feed, Nigeria.

INTRODUCTION

Aspergillus niger is commonly found in cereals, grapes, coffee, and processed foods and drinks, such as red wine, and has been reported to produce mycotoxins, including aflatoxins, which are potent human and animal carcinogens (Awuchi *et al.*, 2022). They are widely distributed worldwide and are important food contaminants that may be present at various stages of processing, including the pre-harvesting stage, dehydration stage, storage stage, and transfer stage (Adah *et al.*, 2024). Mycotoxins are secondary metabolites produced by a wide variety of filamentous fungi that harm humans, animals, and crops, causing disease and financial losses. Mycotoxins are believed to contaminate approximately 25% of the world's food-grade grains, particularly those used as ingredients in commercial chicken feed (Adah *et al.*, 2024). *Aspergillus niger* is reported to be responsible for 75% of the deaths that occur in animals and humans globally due to Aspergillosis (Navale *et al.*, 2021; Xu, 2022).

Most fungi are aerobic and are found almost everywhere in extremely small quantities due to the minute size of their spores (Xiong *et al.*, 2022). They consume organic matter wherever humidity and temperature are sufficient. Under optimal conditions, fungi proliferate into colonies, and mycotoxin levels become high (Fashola *et al.*, 2023). The reason for the production of mycotoxins by fungi is not only necessary for growth or development, but also to weaken the receiving host (Awuchi *et al.*, 2021). As such, fungi may use them as a strategy to improve the environment for further fungal proliferation and increased mycotoxicosis in animals (Navale *et al.*, 2021), leading to 85% of production and economic losses globally (Ezekiel *et al.*, 2020).

Food security and safety are acknowledged to be seriously threatened by fungal contamination (Fones *et al.*, 2020). This is due to the fact that filamentous fungi have the ability to create mycotoxins, which are extremely hazardous secondary metabolites (Ezekiel *et al.*, 2020). The most common agricultural product contaminants in Sub-Saharan Africa are aflatoxins, which account for a substantial portion of the 1.3 billion metric tons of food lost each year worldwide (Xu, 2022). Due to fungal contamination during cultivation, aflatoxins can be detected throughout the food chain, and infestation can also persist during food processing and storage (Fouche *et al.*, 2020), which may lead to aflatoxicosis and a decrease in agricultural production yield (Xiong *et al.*, 2022).

Aflatoxins may have an adverse effect on a country's economy by killing farm animals or making management more difficult, thereby leading to significant agricultural losses (Awuchi *et al.*, 2021). They may also make a commodity unmarketable in the domestic or international market if it does not meet national standards for the highest acceptable concentrations of specific mycotoxins (Ezekiel *et al.*, 2020). Mycotoxigenic molds, such as Aspergillus niger, can invade crops or plants during either pre-harvest or post-harvest periods, producing aflatoxins in human food or animal feeds due to their pathogenic or symbiotic interaction with the plant, leading to aflatoxin development (Abdel-Azeem *et al.*, 2019). These toxins are hazardous to both humans and animals.

Mycotoxins, which possess teratogenic, carcinogenic, mutagenic, and estrogenic properties, can be toxic to animals and are produced by many Aspergillus species in food (Awuchi *et al.*, 2021). The majority of Aspergillus species are saprophytes, or soil fungi, but some can also degrade stored goods, infect plants, or spread diseases to people and other animals (Nji *et al.*, 2023). Corn, peanuts, cottonseed, rice, tree nuts, cereal grains, and fruits are among the main agricultural commodities affected by fungal development and mycotoxins, either before or after harvest, leading to contamination of animal products, including meat, milk, and eggs (Marrez and Ayesh, 2022). The mycotoxins produced by *Aspergillus flavus, A. parasiticus, A. fumigatus, A. ochraceus, A. niger,* and other aspergilli cause major concerns globally as they produce potentially harmful metabolites like patulin, sterigmatocystin, aflatoxin, gliotoxin, fumonisin, citrinin, cyclopiazonic acid, and ochratoxins, respectively (Navale *et al.*, 2021), hence the need for proper public health awareness and enlightenment, so as to aid in control and total eradication of aflatoxicosis globally.

MATERIALS AND METHODS

Study Area

The Federal Capital Territory is located north of the Niger and Benue Rivers lying between latitude 8.25 and 9.20 north of the equator and longitude 6.45 and 7.39 east of the Greenwich Meridian (Ameh *et al.*, 2022). The Federal Capital Territory has a land area of approximately 7,315 km^2, situated within the Savannah region with moderate climatic conditions. The territory is currently comprised of six Area Councils, namely: Abaji, Abuja Municipal, Gwagwalada, Kuje, Bwari, and Kwali Local Council (Mailafia *et al.*, 2023).

Study Design and Sample Size

This is a cross-sectional study conducted to determine the occurrence and quantification of aflatoxins from commercial poultry feed sold at the local markets in the six (6) Area Councils of the Federal Capital Territory, Nigeria. Seven different types of commercial poultry feed samples were collected weekly using a convenience sampling method (Golzar *et al.*, 2022).

A total of 42 Commercial poultry feed Samples were collected from the 6 Area Councils in the FCT from July to August, 2023. This sample size was determined using the formula recommended by Thrusfield (2018), using a calculated prevalence of 50% reported in a previous study by Ezekiel *et al.* (2014).

Sampling and Processing

Sampling was conducted using the Convenient Sampling Method (Golzar *et al.*, 2022), a non-probability sampling technique where subjects are selected due to their convenient accessibility and proximity to the researcher. It would be ideal to test the entire population, but in most cases, the population is simply too large, making it impossible to sample every commercial poultry feed. This sampling technique is fast, inexpensive, easy and the subjects are readily available. (Golzar *et al.*, 2022). The sampling method will be used to collect 42 commercial poultry feeds from the local markets of each Area Council in the FCT, Nigeria, for this research.

Fifty grammes (50g) of seven (7) commercial poultry feed (Hybrid feed, Vital feed, Ultima feed, Chikun feed, Local feed, Top feed and Nutria feed) were collected aseptically weekly for 6 weeks (July, 2023 to August, 2023) from the six area councils using sterile polythene bagsby hand – grab sampling method (Jones *et al.*, 2018). All feed samples collected were sealed, labelled appropriately, and transported to the Veterinary Microbiology Laboratory of the Faculty of Veterinary Medicine for storage.

Twenty grammes (20g) of 7 pooled different commercial brands of poultry feed from the six area councils designated as Feed A, Feed B, Feed C, Feed D, Feed E, Feed F and Feed G were weighed and submitted to the Chemistry

Department of Sheda Science and Technology Complex (SHESTCO) in Kwali, for mycotoxins (Aflatoxins, Ochratoxin and Fumonisin) extraction and quantification analysis using High Performance Liquid Chromatography – Fluorescence Detection (HPLC – FLD).

Data Analysis

The data obtained from this study were presented using simple descriptive statistical tables, and the occurrence rate was calculated for the different aflatoxins detected (Ubah *et al.*, 2024).

RESULTS

Aflatoxin B1 was detected from six (6) feed samples out of the 7 feed samples investigated and their various concentrations after quantification were Feed B (vital feed) 1.10ppb, Feed C (Ultima feed) 1.01ppb, Feed D (Chikun feed) 1.00bbp, Feed E (Local feed) 10.81bbp, Feed F (Top feed) 1.91bbp and Feed G (Nutria) 1.97bbp. Feed D (Chikun feed) had the lowest concentration of 1.00 bbp, while the highest concentration of 10.81 bbp was observed in Feed E (Local feed), as shown in Table 1.

Aflatoxin B2 was detected in this study from three (3) feed samples investigated with various concentrations after quantification was 2.11bbp for Feed A (hybrid poultry feed), 2.83bbp for Feed D (Chikun feed), and 9.12 bbp for Feed E (Local feed). Feed A (hybrid poultry feed) had the lowest concentration of 2.11 bbp, while the highest concentration of 9.12 bbp was observed in Feed E (Local feed), as shown in Table 1 below.

Table 1: Aflatoxins quantified from the commercial poultry feed

Poultry feed (g)	Aflatoxin B1 (Toxin) (ppb)	Aflatoxin B2 (Toxin) (ppb)
Feed A	Nil	2.11
Feed B	1.10	Nil
Feed C	1.01	Nil
Feed D	1.00	2.83
Feed E	10.81	9.12
Feed F	1.91	Nil
Feed G	1.97	Nil

DISCUSSION

The importance of detecting mycotoxins from animal feed or food cannot be under estimated as mycotoxicosis led to high morbidity and mortality in livestock especially in poultry (Gonenc *et al.*, 2020) and humans respectively due to its devastating effects (hepatotoxic, teratogenic, nephrotoxic, and carcinogenic effects) on organs (Hagos and Dechassa, 2020) leading to reduced sale/production yield arising from compromised weight and productivity (Daou *et al.*,2021), reduced feed intake, nervousness, abortions, kidney damage, liver damage, and increased carcass condemnation (Ali, 2020).

The detection of Aflatoxin B1 from poultry feed using HPLC-FLD is in line with the findings of Omeiza *et al.* (2018) and Beyene *et al.* (2019), *who detected Aflatoxin B1 from feed using the* same technique. It has been verified that the HPLC-FLD test can detect and identify the concentration levels of aflatoxins in feed (Beyene *et al.*, 2019), which will help determine the standard permissible concentration of Aflatoxin B1 acceptable for animal and human consumption in feed and food, respectively, and hence serve as a guide in food safety measures.

The highest concentration of 10.81ppb obtain from Feed E (Local feed) is within the limit of the lowest concentration allowed by the European Union limit (4–15 ppb) (Yakubu and Vyas, 2020) and the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) standard (15 ppb) (FAO/WHO, 2020), and hence all the seven types of feed sampled for Aflatoxin B1 detection/quantification for this study is said to be safe for animal and human consumption, since all their Aflatoxin B1 concentration is lower and within the global standard permissible limits by the Codex Alimentarius Commission for food safety, and this implies that the poultry feed sampled are probably safe for both animal and human consumption at the time of sampling. This finding of high level of Aflatoxin B1 and Aflatoxin B2 in Local feed (Feed E) popularly referred to as Dhusa compared to other commercial feeds sampled might be attributed to the fact that most commercial poultry feed industries practice the addition of mycotoxin binding feed additives like Bentonite, Kaolinite and Zeolite, which helps with the binding, adsorption and inactivation of mycotoxins and water from poultry feed thereby preventing the proliferation of microorganisms and their toxins (Prasai *et al.*, 2017), from the poultry feed while this practice is absent in the formulation of the locally homemade Dhusa produced by pheasant and small scale farmers in Nigeria.

Aflatoxin levels found in Feed A and Feed D samples were lower than the 4.00µg/kg (4bbp) European Union (EU) acceptable limit. Still,

Aflatoxin concentration observed in Feed E sample is higher than the EU acceptable limit. This increased level of aflatoxin detected in Feed E was attributed to the effect of storage temperature of the feed post-harvest predisposing it to high rate of contamination by *Aspergillus* species (Omeiza *et al.*, 2018: Adah *et al.*, 2024) hence leading to increased aflatoxin production (Valencia – Quintana *et al.*, 2020) especially in areas with high humidity (Awuchi *et al.*, 2022)and high temperature which increase the possibility of feed becoming contaminated with aflatoxin (Kumar *et al.*, 2021), since it was discovered that the spread of aflatoxin and aflatoxigenic *Aspergillus* was mostly temperature-dependent. This finding is also consistent with previous research carried out by Schmidt *et al.* (2010), which similarly found that temperatures above 20 °C were a critical component in the development of aflatoxin, as this temperature provides a conducive environment for the proliferation of Aspergillus organisms, leading to high production of the mycotoxin. The detection of Aflatoxin B2 from poultry feed using HPLC-FLD is in agreement with the report of Fapohu *et al.* (2018), who reported a low concentration of Aflatoxin from maize grains in Abuja, which was lower than the regulatory limits for aflatoxins.

CONCLUSION

The study quantified two types of Aflatoxin in various concentrations: Aflatoxin B1 and Aflatoxin B2. All seven types of commercial poultry feed sampled for Aflatoxin B1 and Aflatoxin B2 detection/quantification in this study were found to be safe for animal consumption.There is a need for further studies involving other types of mycotoxins that can be detected in commercial poultry feed in the FCT and other States in Nigeria, as this will provide insight into the current state of mycotoxicosis in the Federal Capital Territory, Nigeria.

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