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Occurrence of mycotoxigenic fungi in poultry feeds at live-bird markets, Zaria, Nigeria

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Abstract

Contamination of poultry feeds with mycotoxin-producing fungi such as *Aspergillus* spp is a major threat to animal and human food. This study was conducted to determine the occurrence of aflatoxigenic strain of fungi in feeds, fed to birds in live-bird markets. Feed samples were collected from feeding troughs and feeder in cages of birds and were inoculated on Sabouraud dextrose agar and Czypeck dox agar. Of 300 feed samples, 283 yielded various fungal growth belonging to seven genera, four of them known to be mycotoxigenic. *Aspergillus, Rhizopus*, *Mucor*, *Dermatophyte*, *Yeast, Fusarium* and *Penicillium*, whose isolation frequencies were 78%, 6%, 5.67%, 2%, 2%, 0.33% and 0.33% respectively. The aflatoxin producing *Aspergillus* spp isolated were *A. flavus*, *A. parasiticus* and *A. nomius* 126 (42%), 27 (9%) and 3(1%) respectively. In conclusion *A. flavus* was the most frequently isolated, and it is a known aflatoxin producer. It is recommended that mycotoxin binders should be added to poultry feed to mitigate the effect of aflatoxin contamination of feed in live-bird market.

Keywords: Aflatoxin, *Aspergillus* species, Feed, Live bird markets, Mycotoxin

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Introduction

Mycotoxins are poisonous chemical compounds and secondary metabolites produced by fungi (Tola and Kebede, 2016). These secondary metabolites which are produced by filamentous genera of fungi have deleterious effects on human and animal consumers following consumption of contaminated animal feeds and the economy of the country (WHO, 2006; Mostafa *et al*., 2012). Globally, they cause diseases and human deaths annually such as liver damage, esophageal cancer, reduced digestive enzyme activity, acute toxicosis, immune suppression, and stunted growth in children (Liu and Wu, 2010; Salim *et al.,* 2011). Sufficient quantities of mycotoxins in food and feedstuff can adversely affect human and animal health. However, these toxic effects vary depending on intake dose, toxin type, duration of exposure, metabolism, mode of action, and defense mechanism (Hussein and Brasel, 2001). The significant mycotoxins of public health concern are aflatoxins, ochratoxin, trichothecenes, patulin, penicillium, fumonisins, fusarium, zearalenone, deoxynivalenol and ergot alkaloids (Iqbal *et al.,* 2014).

Mycotoxins affect feed quality by reducing the nutritive value and producing unpleasant smell. In addition, they contaminate feed, thereby serving as vehicle for animal and human infection (Maciorowski *et al.,* 2007). Feed contaminated with mycotoxins negatively affect poultry performance and their health (Monson *et al.,* 2014). The primary mycotoxins of concern in poultry feedstuffs are aflatoxins, which have four major forms: aflatoxin B1 (AFB_1), aflatoxin B2 (AFB_2), aflatoxin G1 (AFG_1), and aflatoxin G2 (AFG_2) (Monbaliu *et al.,* 2010; Lereau *et al*., 2012). Aflatoxin AFB_1 is the most potent and is derived from sterigmatocystin a naturally occurring carcinogen (Xu *et al.,* 2000). Aflatoxin M_1 is a metabolite and derivative of AFB_1 that is formed and excreted in the milk of humans and animals following ingestion of feedstuffs contaminated with AFB_1 (Xu *et al.,* 2000). Several studies revealed that *A. flavus* and *Aspergillus parasiticus*

are of major concern in poultry production and the most common producers of aflatoxin (Magnoli *et al.,* 2011; Ghadeer & Al-Delamiy, 2012). Of these two *Aspergillus* species, *A. flavus* is found frequently in contaminated feed (Varga *et al.,* 2011).

Preventing mycotoxicoses relies mostly on feed management practices at live bird markets; this reduces the level of exposure of birds to aflatoxin. However, feed may leave the manufacturers free of mycotoxin contamination and get exposed to contamination at the level of live bird market. Facilities in live bird markets are limited with poor hygienic conditions especially stores where feeds are kept, points of sale and slaughter (FAO, 2008). Most birds in live bird markets receive feed from containers which are poorly kept giving rise to contamination of feed. It is therefore important to understand the level of exposure to mycotoxin contaminated feed. Information obtained in this regard could form the basis for extending mycotoxin avoidance to cover the entire poultry value chain. This study was conducted to assess occurrence of mycotoxigenic fungi in poultry feeds in Zaria, Nigeria.

Materials and Methods

Study area

The study area was Zaria, Kaduna state, Nigeria. The area has six major live bird markets (Sabon Gari, Samaru, Tudun wada, Kwangila, Zaria city and Dan Magaji). The population of Zaria is estimated at 547,000 of the 2006 Nigerian census. It is situated on latitude 11°7", 11°12"N and longitude 7°41"E (Mamman *et al*., 2000). Relative humidity in Zaria is between 63.2- 68.8 %, average rainfall of 155.9-182.1mm, temperature range of 25-30.2°C, and with a low evaporation rate (154.2-163.91mm). In addition, the vegetation is within the guinea savannah (Mamman *et al*., 2000).

Study design

The study was a cross sectional approach. Six major live bird markets within Zaria metropolis were used for sample collection between August 2015 and January, 2016. Majority of the feed at these markets were sourced from feed stores across Kaduna state. The sample size was calculated, based on an estimated prevalence rate of 78% (Habib *et al.,* 2015). The sample size was 263 but was increased to 300 to increase precision and minimize sampling error. Therefore, 50 feed samples were collected at each of the live-bird markets.

Samples and Sampling

Three hundred poultry feed samples were collected from six live bird markets from August, 2015 to January, 2016 and cultured for fungi. The feed samples were collected randomly from feeding troughs and feeders per stand in live bird market with a sterilised spoon and polythene bag. Preparation of feed samples was as described by Makun *et al.* (2010) and Udom *et al.* (2012). One gram of feed was added into 9 mL of sterile distilled water as one fold dilution in a sterile polythene bag and homogenized with stomacher (Stomacher® Bag, Seward, USA). A loopful of each suspension was inoculated into a labelled sterile Sabouraud Dextrose Agar (CM41-0xoid, U.K.) medium impregnated with Chloramphenicol and incubated at room temperature for 3-5days. Plates were examined grossly for characteristic growth of *Aspergillus* species such as obverse and reverse colour according to the method described by James & Natalie (2001), Mycology-Critique (2004), Giorni *et al.* (2007) and Bandh *et al.* (2012). Czypeck Dox Agar (CM0097-Oxoid, U.K.) was used as secondary differential media for specific identification and growth of *Aspergillus* section *flavi*. All the media were prepared following the manufacturer's instruction and sterilised by autoclaving at 121 °C for 15 minutes. The growths were stained using lactophenol on clean glass slide. The slides were observed under x 10 and x 40 magnifications of a light microscope.

Data analysis

Data generated were analyzed using descriptive statistics (Snedecor & Cochran, 1989).

$$\text{Fr (\%)} = \frac{\text{number of samples with a species or genus} \times 100}{\text{Total number of samples}}$$

where Fr is isolation frequency

Results

A total of 300 feed samples were analysed for the presence of fungal contamination in live bird markets. A total of 283(94.33%) revealed the presence of fungal organisms where *Aspergillus* sp having the highest isolation frequency rate of 234(78%), while other fungi account for 49(16.33%). Similarly, 6 (2%) were *dermatophyte*, 17 (5.67%) *mucor,* 18 (6%) *rhizopus,* 1 (0.33%) *penicillium*, 6 (2%) *yeast* and 1 (0.33%) *fusarium* (Table 1). Similarly, of the 234 *Aspergillus* spp. *A. flavus* was the most frequently isolated 136(42%), 48(16%) *A. fumigatus,* 27(9%) *A. parasiticus*, 9(3%) *A. nidulans,* 15(5%) *A. niger,* 5(1.67) *A. terreus*, 3(1%) *A. nomius* and 1(0.33%) was *A. caelatus* (Table 2).

In this study, the macroscopic view of *Aspergillus flavus* on Sabouraud dextrose agar was yellow green colony at room temperature and biseriate vesicle microscopically as shown on plate I. *Aspergillus parasiticus* on microscope had a

Table 1: Isolation frequency (Fr) of different genera of fungi from poultry feeds used in live bird markets in Zaria

Isolation frequency	*Aspergillus*	*Dermatophyte*	*Mucor*	*Rhizopus*	*Penicillium*	*Yeast*	*Fusarium*
No. of Isolates	234	6	17	18	1	6	1
Fr (%)	78	2	5.67	6	0.33	2	0.33

Table 2: Prevalence of *Aspergillus* spp isolated from poultry feed in live bird market Zaria

Aspergillus isolated	No. of Isolates	Isolation Frequency (%)	Prevalence (%) (n=234)
A. caelatus	1	0.3	0.4
A. flavus	126	42.0	53.9
A. fumigatus	48	16.0	20.5
A. nidulans	9	3.0	3.9
A. niger	15	5.0	6.4
A. nomius	3	1.0	1.3
A. parasiticus	27	9.0	11.54
A. terreus	5	1.7	2.1
Total	234	78.0	100.00

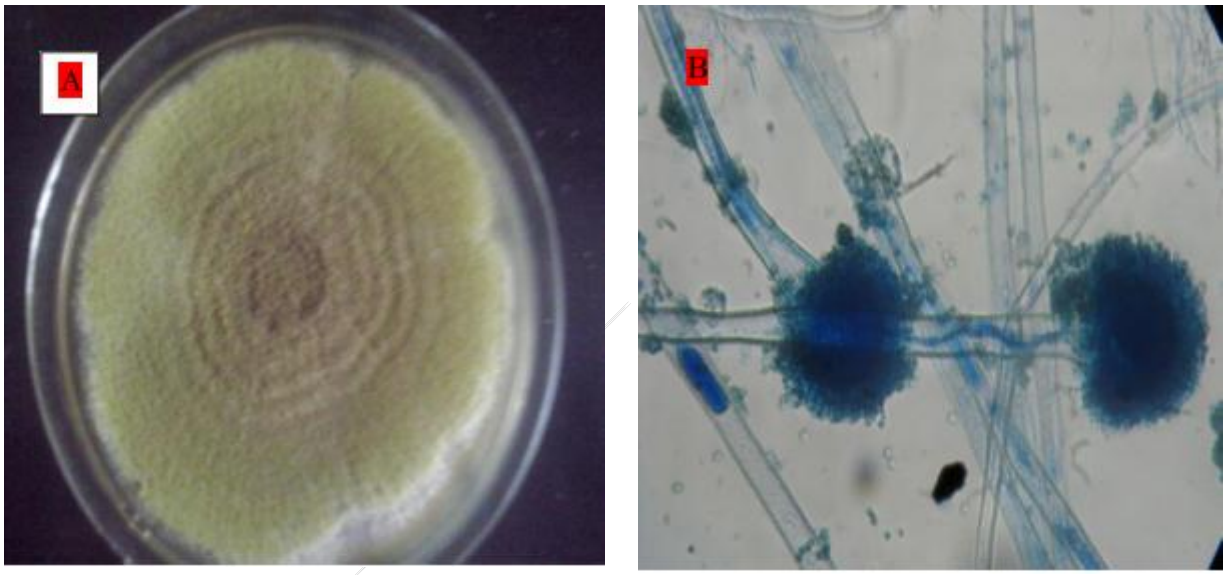

Plate I: (Macro (A) and Microscopic (B) *A. flavus*): A: Yellow to green colony at 27°C after 7 days on Sabouraud dextrose agar. B: Biseriate head with globose vesicle

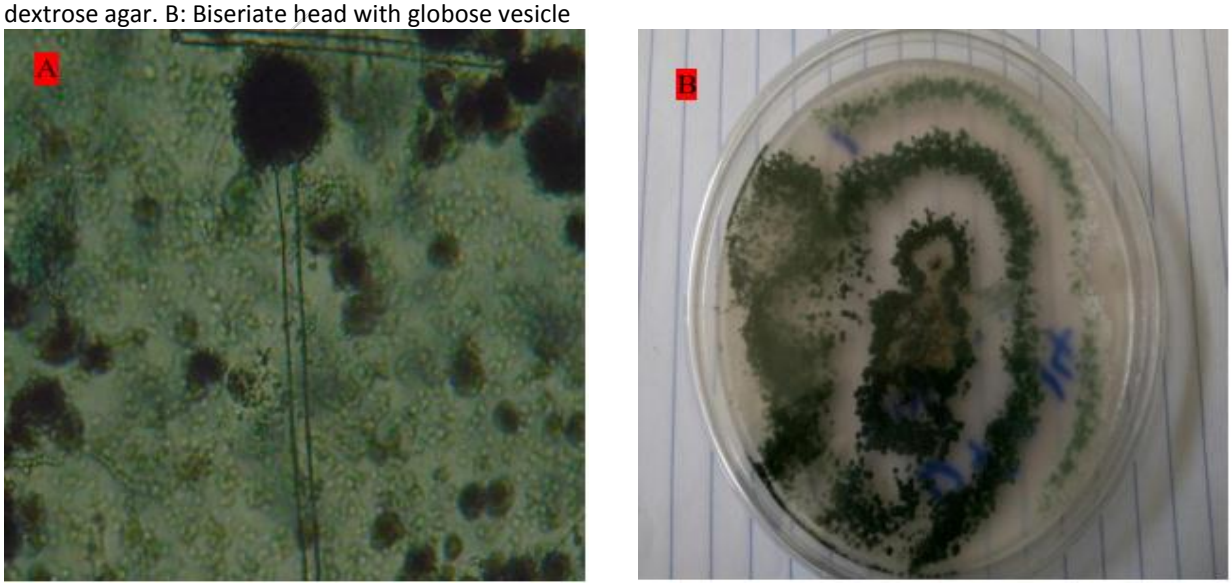

Plate II: (Micro(A) and Macroscopic(B) *Aspergillus parasiticus*); A: Biseriate head with subglose and globose vesicle (Mag×40) B: Dark green colony at 27 °C after 7 days on Czypeck dox agar

biseriate head with subglobose vesicle and the colony on Czypeck dox agar with dark green color macroscopically (Plate II). Plate III shows *Aspergillus nomius*, a golden yellow colony appearance on Czypeck dox agar macroscopically and biseriate head with globose vesicle under the microscope. Mixed fungal contamination, yeast and *Fusarium* stained with lactophenol cotton blue are shown on plate IV(A), IV(B) and IV(C), respectively.

Discussion

The results of this study showed that there was a high level of fungal contamination (94.67%) in feeds fed to birds in live bird markets which agrees with other findings in Nigeria (Obi and Ozugbu, 2007; Osho *et al*., 2007; Uwaezuoke and Ogbulie, 2008; Habib *et al.*, 2015; Aliyu *et al.*, 2016). This result is in agreement with the researches conducted by Dalcero *et al.* (1997), Oliveira *et al.* (2006), Rosa *et al.* (2006), Krnjaja *et al.* (2007) and Saleemi *et al.* (2010), where they reported high levels of fungal contamination in feed.

In this study, isolation frequency of different genera of contaminating fungi ranked in decreasing order; *asperillus*, *rhizopus*, *mucor, yeast*, *dermatophyte, fusarium* and *penicillium* which was in concordance with Saleemi *et al.* (2010), Sivakumar *et al.* (2014) and Bhuyan *et al.* (2015). These fungal isolations might have been as a result of the season in which the research was conducted which agrees with Murugesan *et al.* (2015), that fungal growth is dependent on factors such as seasons, location of grain cultivation, drought and time of harvest. Some of the feed might be poorly processed and handled. Most of poultry sellers add water to their feed, to increase the intake volume of feed, which encourages mould growth and subsequent aflatoxin production.

The high contamination level of *Aspergillus* spp., could be as a result of poor hygienic status of live-

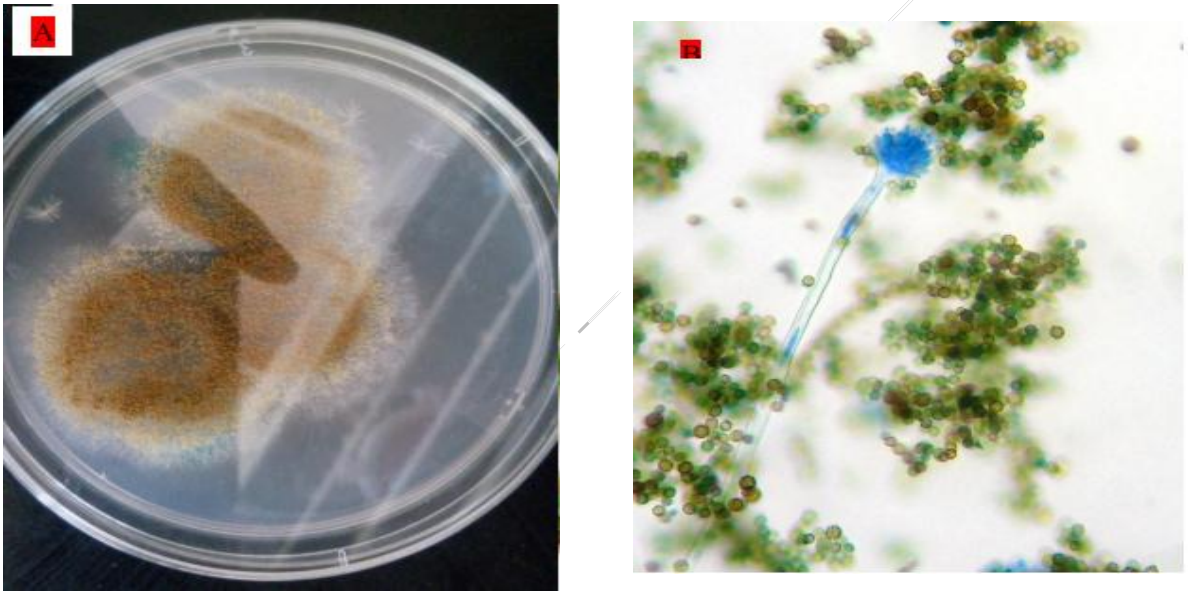

Plate III: (Macro(A) and Microscopic(B) *Aspergillus nomius*); A: Golden yellow colony at 27 °C after 5 days on Czypeck dox agar. B: biseriate head with globose vesicle (Mag×40)

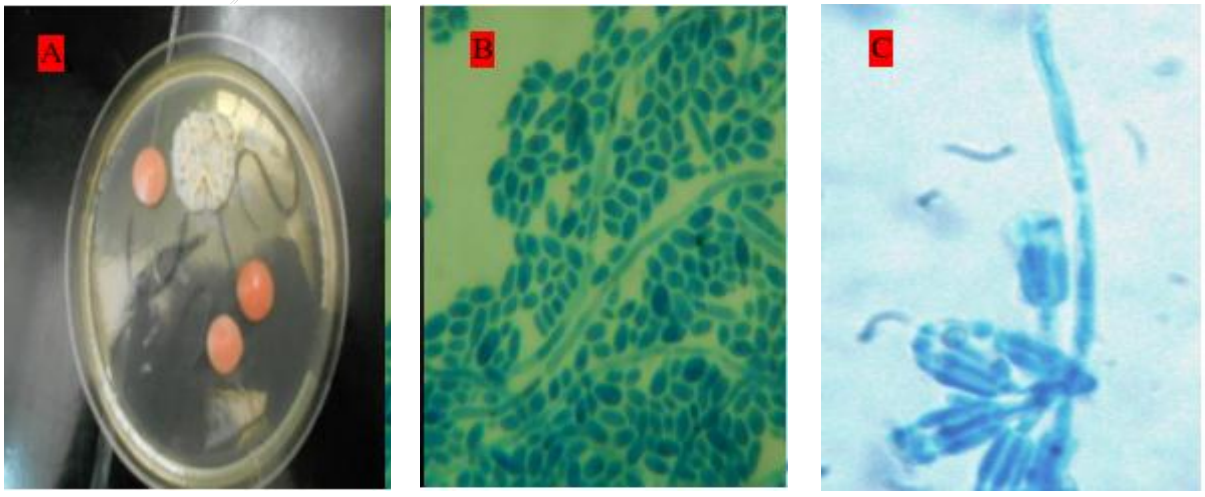

Plate IV: (A)Isolates showing mixed fungal contamination (B)microscopic view of yeast (C) microscopic view of *Fusarium* (Mag×40)

live-bird markets, the containers use in feeding the birds, poor storage facility and the study area been in northern guinea savanna with moderate to high rainfall which might have been responsible for the high frequency rate of *Aspergillus* spp in live bird markets.

The predominant *Aspergillus* species observed in this study was *A. flavus* 126(42%). This agreed with the findings of Fapohunda *et al.* (2012), Davari *et al.* (2015), Fakruddin *et al.* (2015) and Ghaemmaghami *et al.* (2016). This might have been that *A. flavus* can adapt to different geographical locations especially the sub-tropical and tropical regions of a country. Generally, high water activity and high humidity are conducive for *Aspergillus* growth (Fernandez-Cruz *et al.,* 2010). Conditions for the production of aflatoxins by *A.* section *flavi* are 33 °C and 0.99a_w (Milani, 2013). Thus, typical hot and humid atmosphere and substandard storage conditions are required to synthesize aflatoxins in agricultural products (Atanda *et al*., 2013). Some of the metabolites produced by these members of *Aspergillus* section *flavi* are known to possess encoding genes for aflatoxin production.

The predominance of *A. flavus* isolated from poultry feed in the six live bird markets in the study area is in agreement with previous reports of Atehnkeng *et al.* (2008), Saleemi *et al.* (2010), Azarakhsh *et al.* (2011), Ezekiel *et al.* (2014), Fakruddin *et al.* (2015) and Aliyu *et al.* (2016). That *A. flavus* is one of the most common fungi in poultry feed samples showed that it can easily adapt itself to various geographical regions, high temperature tolerance and high (64-74%) humidity levels. They possess a higher adaptability to grow on substrates in a wide range of environment and the production of spores that remain viable even under extremely hard conditions (Saleemullah *et al.,* 2006).

In the present study, the contaminating mycotoxin was *Aspergillus* spp., *Yeast* spp., *Penicillium* spp. and *Fusarium* spp which are known mycotoxigenic species contaminating poultry feeds. *Aspergillus flavus*, *A. parasiticus* and *A. nomius* are known aflatoxigenic species when present can be passed to the bye-products of poultry such as meat or egg, therefore having a negative effect on human health.

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