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

MJ Ibrahim¹*, J Kabir¹, CN Kwanashie², MT Salawudeen² & Z Joshua²

¹. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
². Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

*Correspondence: Tel.: +2348065826062: E-mail: muhammedibrahim407@gmail.com

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

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 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (Monbaliu et al., 2010; Lereau et al., 2012). Aflatoxin AFB1 is the most potent and is derived from sterigmatocystin a naturally occurring carcinogen (Xu et al., 2000). Aflatoxin M1 is a metabolite and derivative of AFB1 that is formed and excreted in the milk of humans and animals following ingestion of feedstuffs contaminated with AFB1 (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 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 mycotoxinogenic 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-Oxoid, 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 sterlised 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).

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

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, 3(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

<table>
<thead>
<tr>
<th>Isolation frequency</th>
<th>Aspergillus</th>
<th>Dermatophyte</th>
<th>Mucor</th>
<th>Rhizopus</th>
<th>Penicillium</th>
<th>Yeast</th>
<th>Fusarium</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Isolates</td>
<td>234</td>
<td>6</td>
<td>17</td>
<td>18</td>
<td>1</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Fr (%)</td>
<td>78</td>
<td>2</td>
<td>5.67</td>
<td>6</td>
<td>0.33</td>
<td>2</td>
<td>0.33</td>
</tr>
</tbody>
</table>

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

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

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 research conducted by Dalcero et al. (1997), Oliveira et al. (2006), Rosa et al. (2006), Knjaja 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-
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 0.99aw (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), Azarakshsh 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.

References


Fapohunda SO, Moore GG, Ganiyun OT & Beltz SB (2012). Toxigenic Aspergillus flavus and other fungi of public health concern in...


