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Fungal Profiles and Total Aflatoxin Concentrations in Rice and Animal Feeds Sold in Katsina Metropolis, Northwestern Nigeria: An Exploratory Market Survey

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Editorial

In warm tropical climates, aflatoxin exposure is a food and feed safety concern where cereals and compounded feeds may be exposed to moisture, heat and prolonged storage. The present exploratory survey assessed the total aflatoxin and culturable fungi in five commodity samples collected from three markets in Katsina Metropolis, Nigeria. The samples were local rice, imported rice, local rice, chicken feed and fish feed. Fungi were isolated by direct plating on chloramphenicol-supplemented potato dextrose agar and provisionally identified based on colony and microscopic morphology. The methanolic extracts were assayed by silica-gel thin-layer chromatography (TLC) and the total aflatoxin was determined by competitive enzyme-linked immunosorbent assay (ELISA) in triplicate. Representative morphotypes identified included *Aspergillus fumigatus* (local rice and chicken feed stored), *A. parasiticus* (local rice), *A. niger* (imported rice) and *A. flavus* (fish feed). Fluorescent zones were produced by TLC with R_f values of 0.13–0.36. Without the use of authenticated co-spotted standards and confirmatory chromatography these observations were treated as presumptive and not subtype specific. ELISA measurements reported concentrations ranging from 0.633 ± 0.152 to 1.423 ± 0.049 µg/kg, with the highest mean concentration in stored local rice and the lowest in imported rice. As only one market sample was analyzed for each commodity–location combination, the measurements are descriptive of the samples analyzed and do not provide estimates of market prevalence. However, the findings support the need for larger, adequately replicated surveillance using validated matrix-specific extraction, quality controls and confirmatory liquid chromatography–tandem mass spectrometry, with concurrent fungal contamination and measurable total aflatoxin.

INTRODUCTION

Aflatoxins are mainly produced by members of *Aspergillus* section Flavi and are difuranocoumarin metabolites. The most potent and studied of these is aflatoxin B₁ (AFB₁). However, the same commodity may contain AFB₂, AFG₁ and AFG₂. Aflatoxin M₁ is mainly a hydroxylated metabolite found in milk following consumption of AFB₁ and is not normally considered a primary fungal product in cereal or feed extracts [1–3]. Sole identification

by taxonomic studies are not enough as colony color cannot alone distinguish all species or determine toxigenic capacity. This is because aflatoxin production varies among species and strains [4–7]. Contamination may start before harvest and continue during drying, transport or storage. Fungal growth and toxin accumulation are affected by temperature, water activity, insect injury, drought stress and length of storage [5,8–11]. These interacting pressures are especially relevant to tropical and subtropical supply chains where smallholder production, open-

market handling and limited moisture control may repeatedly expose susceptible commodities to risk [2,3,12,13]. Although rice is not the commodity most frequently associated with acute aflatoxicosis, contamination has been reported in polished rice, rice fractions and rice by-products in several countries [11,14–19]. Animal feeds are often mixtures of cereal grains, oilseed cakes and other ingredients and may also lead to transfer of AFB 1 into animal products after metabolism [19–22] and present a related problem.

Aflatoxin is a public-health concern and is not only due to visible mold. Chronic exposure is causally associated with hepatocellular carcinoma and the risk is markedly modified by hepatitis B virus infection. Acute high dose exposure can also result in severe liver injury. Epidemiological studies have also looked at immune dysfunction and impaired growth in childhood [23–31]. Occupational inhalation or ingestion of contaminated dusts may be another route of exposure for feed mill and grain market workers [32]. Aflatoxin control is therefore a health and economic priority along with livestock productivity losses and rejected commodities [3,26,33].

No one analytical method answers every surveillance question. TLC is cheap and can provide a quick visual screen but reliable identification requires suitable standards and controlled development and, when regulatory decisions are being considered, a more selective confirmatory technique. Competitive ELISA can process many samples quickly, but validation of matrix effects, extraction efficiency, calibration range and cross-reactivity are needed for each commodity [34–42]. Chromatographic techniques combined with fluorescence or mass-spectrometric detection are still important for confirmation and multi-analyte measurement [34,35].

Aflatoxin or aflatoxigenic fungi have been reported in dairy feed, melon seed products, yam flour and other staples in published work from Nigeria [20,43–45]. However, data from market systems in northwestern part are still fragmented and few small studies have evaluated culturable fungi and aflatoxin together in rice and animal feeds. Therefore, this exploratory study was conducted to (i) recover and tentatively identify dominant fungal morphotypes from selected rice and feed samples sold in Katsina Metropolis, (ii) record presumptive fluorescent zones by TLC and (iii) quantify total aflatoxin by competitive ELISA. This was a preliminary market screen, not a prevalence survey.

MATERIALS AND METHODS

Study design and setting

An exploratory cross-sectional sampling exercise was conducted in Katsina Metropolis, Katsina State, northwestern Nigeria. Samples were purchased from Chake Market, Central Market and Tsohuwar Kasuwa. In the design, there were five combinations of commodity and location: stored local rice from Chake Market, local rice from Tsohuwar Kasuwa and imported rice, chicken feed and fish feed from Central Market. Each combination was represented by one market sample of 50 g. Thus, the experimental unit was the market sample ($n = 5$) and technical replication was achieved by triplicate laboratory measurements.

Collection and preparation of samples

Each sample was put into a sterile polypropylene bag, labeled and transported to the National Agency for Food and Drug Administration and Control laboratory and the Science Laboratory Technology laboratory, Al-Qalam University

Katsina. Each sample was milled for extraction of ~80% of the sample and the remaining portion saved for direct plating. Prepared material was stored at 4 °C until analysis.

Isolation and morphological identification of fungi

Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions with added chloramphenicol (500 mg L⁻¹). The medium was autoclaved at 121 °C for 15 min and distributed into Petri dishes after cooling. Directly selected portions of each commodity were placed on the agar and incubated at 27 °C for 5–7 days. Individual colonies were transferred to new medium until they appeared visibly pure. Macroscopic features included colony color, texture, margin and pigmentation. A small amount of growth was placed on a clean slide and mounted in lactophenol and examined at total magnifications of $\times 100$ and $\times 400$. *Aspergillus* species were identified on the basis of conidial head, conidiophore and conidia morphology. All identifications are reported as provisional [4–7,46] as molecular sequencing and validated species-level keys were not employed.

Aflatoxin analysis: Extraction

For the bulk extract, 20 g of each milled sample was mixed with 100 mL methanol-water (70:30, v/v) in a sealed flask and shaken vigorously for 3 min and then allowed to settle and filtered. The filtrate was concentrated in a rotary evaporator and kept for TLC analysis. For ELISA, 2 g of sample was extracted with 10 mL methanol on a horizontal shaker for 10 min and filtered through a Whatman filter paper. Filtrate (100 μ L) was mixed with 600 μ L distilled water and analyzed. The same extraction procedures were applied to the samples.

Thin-layer chromatography screening

Glass plates were coated with a slurry of 30 g silica gel in 75 mL distilled water, air-dried for 30 min and activated at 100°C for 30 m. Spotted extracts with capillary tubes. Plates were developed in chloroform-methanol (97:3, v/v) and removed before the solvent reached the upper edge and examined under ultraviolet light. The retention factor was calculated as $R_f = \text{distance migrated by the fluorescent zone} / \text{distance migrated by the solvent front}$. Fluorescent zones (blue or yellow) and R_f values were noted. Individual aflatoxin congeners were not assigned, as co-spotted standards were not authenticated and confirmatory chromatography was not used in this study [35,36,39,40].

ELISA competition

Microplate wells were filled with 50 μ L diluted extracts and 50 μ L kit standards and then 50 μ L enzyme conjugate and 50 μ L antibody solution were added. The plates were incubated for 30 min at room temperature, emptied and washed four times with the supplied wash buffer. Substrate solution (100 μ L) was added and allowed to react for 15 min and 100 μ L stop solution was added. The absorbance was read in a microplate reader and the results expressed as total aflatoxin in $\mu\text{g} / \text{kg}$ (numerically equivalent to ppb). Method performance has to be evaluated regarding the unreported kit identity, calibration range, detection and quantification limits, recovery and cross reactivity [37,38,42].

Analysis of data

ELISA measurements were done in technical triplicate and reported as mean $\pm$ standard deviation. Technical replicates do not provide independent estimates of between-sample variability. Thus, no inferential comparisons were made as there was only one independent market sample for each commodity–location

combination. Fungal observations and TLC observations were summarized in a descriptive manner.

RESULTS

Fungal morphotypes

Five representative fungal morphotypes were recovered, one from each market sample (Table 1). Two morphotypes were provisionally assigned to *A. fumigatus*; the remaining isolates were assigned to *A. parasiticus*, *A. niger* and *A. flavus*. These assignments indicate the presence of *Aspergillus* spp. but do not demonstrate that any isolate produced aflatoxin.

Table 1. Colony and microscopic features of representative isolates. Species names are provisional because no molecular confirmation was performed.

Code	Commodity	Market	Macroscopic appearance	Microscopic basis	Provisional identification
A	Stored local rice	Chake	Black to green-grey, hairy, flat colony	Predominantly columnar conidial heads	<i>A. fumigatus</i>
B	Local rice	Tsohuwar Kasuwa	Blue-green, hairy, flat colony	Globose conidia on a rough-walled conidiophore	<i>A. parasiticus</i>
C	Imported rice	Central	Black colony spreading across the medium	Branched conidiophore terminating in a vesicle with conidia	<i>A. niger</i>
D	Chicken feed	Central	Green colony with a flat margin	Columnar conidial heads and smooth-walled conidia	<i>A. fumigatus</i>
E	Fish feed	Central	Green mycelial growth with dark, flat-edged colonies	Radiate conidial head	<i>A. flavus</i>

TLC observations

Fluorescent zones migrated 0.6–1.6 cm while solvent fronts travelled 4.0–4.5 cm, producing R_f values of 0.13–0.36 (Table 2). Blue fluorescence was observed in the local and imported rice extracts; yellow fluorescence was recorded in stored local rice and both feed extracts. These signals constitute presumptive screening observations only.

Table 2. Thin-layer chromatographic observations. R_f values were recalculated from the recorded migration distances. Congener identity cannot be established without authenticated standards and confirmatory analysis.

Sample	Solvent front (cm)	Zone migration (cm)	R _f	Observed fluorescence	Interpretation
Stored local rice	4.5	1.0	0.22	Yellow	Presumptive fluorescent zone
Local rice	4.5	0.9	0.20	Blue	Presumptive fluorescent zone
Imported rice	4.5	0.6	0.13	Blue	Presumptive fluorescent zone
Chicken feed	4.5	1.6	0.36	Yellow	Presumptive fluorescent zone
Fish feed	4.0	1.1	0.28	Yellow	Presumptive fluorescent zone

Total aflatoxin by ELISA

Total aflatoxin was measurable in all five extracts (Table 3). Mean concentrations ranged from 0.633 µg/kg in imported rice to 1.423 µg/kg in stored local rice. The values describe technical repeatability within each extract; they do not estimate variability among vendors, lots or markets.

Table 3. Reported total aflatoxin concentrations by competitive ELISA. SD, standard deviation of three technical measurements.

Sample code	Commodity	Market	Total aflatoxin (µg/kg), mean ± SD
A	Stored local rice	Chake	1.423 ± 0.049
B	Local rice	Tsohuwar Kasuwa	1.200 ± 0.010
C	Imported rice	Central	0.633 ± 0.152
D	Chicken feed	Central	1.120 ± 0.020
E	Fish feed	Central	1.323 ± 0.049

DISCUSSION

Aspergillus morphotypes were recovered from each commodity tested and low levels of total aflatoxin were detected in all five extracts. The co-occurrence of fungi and toxin in a commodity is biologically plausible but the two observations should not be treated as a direct isolate-toxin link. Important aflatoxin-producing lineages are found in *A. flavus* and *A. parasiticus*, but toxigenic capacity varies within species; *A. niger* and *A. fumigatus* are more commonly associated with other mycotoxins or opportunistic disease than with routine aflatoxin production [1,4–7,10,46]. Culture-based toxin testing or molecular assays targeting the aflatoxin gene cluster would be necessary to prove the specific isolate made the toxin measured [5,47].

Morphological identification was a useful first screen but resolution was limited. Colony morphology overlaps in several members of section Flavi and phenotypic characters change with medium, temperature and incubation time. Thus, modern taxonomy integrates morphology with extrolite profiles and sequence data [6,7]. These species assignments should be interpreted at this level of certainty. Future work should include sequencing of an informative locus such as calmodulin or β-tubulin and testing for toxigenicity under controlled culture conditions.

The numerical ELISA values were low with means below 1.5 µg/kg. But studies similar to this one have demonstrated that aflatoxin distribution within a lot is heterogeneous and a small analytical portion may not be representative of the bulk commodity [2,3,34]. Distinct differences in grain fractions, origins and storage conditions have been reported in rice surveys [11,14,15,17,18]. Likewise, in feeds, risk is affected by composition of ingredients and history of storage [19,20,22]. These reasons make it impossible to generalize the present values to Katsina markets or certify compliance. A defensible compliance assessment would require a documented sampling plan, homogenization of a larger aggregate sample, a fully specified kit, matrix matched recovery experiments and confirmation of positive results by HPLC or LC–MS/MS.

The TLC record is an example of a second interpretive problem. Recalculated R_f values were internally consistent with recorded distances. Color and R_f alone do not establish congener identity. The historical TLC and HPTLC methods can be well-separated aflatoxins with authenticated standards, good extraction and controlled detection [39,40]. In this situation, no definite assignment of AFB₁, AFB₂, AFG₁, AFG₂ or AFM₁ was possible due to the absence of standards and wavelength data. Considering the observations as possible fluorescence avoids a false sense of analytical certainty and is consistent with modern principles for method validation [34–36]. The occurrence of *Aspergillus* in marketed foods and feeds is in agreement with Nigerian studies on melon seed, yam flour and dairy feed [20,43–45].

Other studies in Africa elsewhere also report that drying and storage structures, feed formulation and seasonal humidity can affect contamination [13,22]. Drought prior to harvest may increase susceptibility of crops, and warm storage with inadequate moisture control promotes fungal growth [8,9,11]. Therefore, practical risk reduction must encompass the whole value chain: timely harvest, rapid drying, protection against rain and insects, clean transport, moisture-resistant storage and regular monitoring are measures that reinforce each other [2,10,12,48,49]. Biological control with competitive atoxigenic *A. flavus* strains can also reduce preharvest contamination when locally adapted products and stewardship systems are in place [10].

Health effects are dependent on concentration and repeated dietary intake. AFB₁ causes genotoxic metabolites and DNA adducts and has synergistic effects with chronic hepatitis B infection in hepatocarcinogenesis [26,28–31]. In West African children, aflatoxin biomarkers have been associated with changes in immune measures and poorer growth, but nutrition, infection and socioeconomic conditions complicate causal interpretation [23–27]. Feed contamination is also important for animal performance and for carry-over of AFM₁ into milk [3,19,20,22]. Prevention and periodic surveillance are preferable to relying solely on visual quality, even when measured concentrations are low, as toxin can persist after visible fungal growth has ceased [1–3].

This work offers a useful pilot dataset but should be read within the framework of its design. The main limitation was the lack of independent replication; each commodity-location combination was represented by a single sample. Also, the study did not include moisture content and water activity, quantitative fungal counts, molecular identification, aflatoxigenicity testing, full ELISA validation data, authenticated TLC standards and confirmatory instrumental analysis. Only technical precision is represented by the triplicate ELISA readings and so inferential statistics and letter-based claims of significant difference were removed. For a follow-up survey, multiple vendors and lots should be selected per commodity across wet and dry seasons, target sample size should be pre-defined, laboratory order should be randomized, and blanks, spikes and certified reference materials should be included. Recovery corrected concentrations should be reported with limits of detection and quantification. Surveillance could be translated into safer purchasing and storage practices by means of market incentives and communication to consumers [33].

CONCLUSION

Each of the five rice or animal-feed samples analyzed from Katsina Metropolis contained culturable *Aspergillus* morphotypes and measurable total aflatoxin. ELISA values ranged from 0.633 to 1.423 µg/kg. This study should be considered an exploratory screen, not an estimate of prevalence or an assessment of regulatory compliance. Its major contribution is to acknowledge the rice and feed supply chains in the study area are worthy of a larger, independently replicated survey. Future analyzes should combine representative lot sampling with molecular identification of the fungi, matrix-specific validated immunoassays, and confirmatory LC-MS/MS. Rapid drying, storage under controlled humidity, hygienic handling and risk-based monitoring still remain appropriate preventive measures.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article.

ETHICS APPROVAL

Not applicable. The study analysed food and feed commodities and involved no human participants or experimental animals.

DATA AVAILABILITY STATEMENT

All numerical data analysed in this study are presented in the article.

AUTHORS' CONTRIBUTIONS

The conceptualization of the study was led by the corresponding author. Experimental works were carried out by first author and data analysis was conducted by the corresponding author, with the remaining authors contributing to review and editing.

AI USAGE DECLARATION

During the preparation of this work, the authors used OpenAI ChatGPT solely for language editing, grammatical correction, formatting support and refinement of readability. The authors reviewed and edited the resulting text and take full responsibility for the accuracy, integrity, originality and final content of the publication.

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