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RESEARCH ARTICLE

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Aflatoxin Contamination in Some Selected Grains, Feeds and Feed ingredients in Katsina and Zaria metropolis

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ABSTRACT

The prevalence and high susceptibility of aflatoxin contamination in agricultural commodities is alarming, posing great health concern to humans and animals. A total of twenty nine different types of Grains, Feeds and Feed ingredients were randomly obtained from two locations, Katsina (Sudan Savannah) and Zaria (Northern Guinea Savannah) metropolises. Raw samples were individually ground to pass through a 20-mesh screen and Total aflatoxins were extracted from the samples using 70% (v/v) methanol. Enzyme-linked immunosorbent Assay (ELISA) technique was used in analyzing aflatoxin concentration of the samples. Results shows that 79.3% of the entire sample were all positive with an aflatoxin level in the range of 0.1- >20 µg/kg. Amongst the grains, the highest concentration (>20 µg/kg) was found in white maize, while the least (0.1 µg/kg) was recorded in unmilled rice. For the feeds and feed ingredients, the highest concentration (>20 µg/kg) of aflatoxin was found in poultry feeds and maize chaff while the least concentration (0.85 µg/kg) was recorded in sorghum flour. Out of the positive samples 26.1% of them exceeded the SON and NAFDAC Aflatoxin permissible level. Though the aflatoxin concentration levels in samples were not disquieting, consistent consumption might result in long term accumulation of the toxin, causing an overt disease and an array of metabolic disturbances resulting in poor human and animal health.

Keywords: Aflatoxin, grains, feeds and feeds Ingredients, enzyme-linked immunosorbent Assay (ELISA)

INTRODUCTION

Fungi are ubiquitous organisms contaminating agricultural products which may occur in the field (during crop production), during harvest, drying, storage, transport, processing or in the market due to poor sanitary conditions. The contamination of agricultural products by fungi did not only results in reduction in quantity and quality of crop yield with significant economic losses, but also the production of mycotoxins. They are poisonous secondary metabolites which are harmful to animals and humans, resulting to mycotoxicosis upon exposure [1][2]. The most important mycotoxins in grains, feeds and feed ingredients are aflatoxins, ochratoxins and patulin produced mainly by *Aspergillus* and *Penicillium* species, as well trichothecenes, zearalenone and fumonisins toxins produced by *Fusarium sp.*[2].

Aflatoxins, produced by *Aspergillus* species including *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius*[3], are the best characterized and the most prevalent mycotoxins. Aflatoxins are generally considered a

potential hazard to public health due to their toxicity, mutagenicity, tetratogenicity and carcinogenicity [4][5]. Aflatoxins types B1, B2, G1, and G2 are listed as Group I carcinogens and Aflatoxin M1, is as toxic as aflatoxin B1 [6], which is listed as a Group 2B carcinogen by the International Agency for Research on Cancer [7]. Aflatoxins have also been known to cause sub-acute and chronic effects in humans. These effects include primary liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis, thus the presence of aflatoxin in grains and feed stock has far reached deleterious effects on animal and human health [8].

In Nigeria, 7,761 out of estimated 10,130 liver cancer cases in 2010 are attributed to aflatoxins [9], hence, the occurrence of liver cancer could be substantially reduced by lessening aflatoxin contamination. Lanyasunya *et al.*, [10] reported an outbreak of aflatoxin poisoning in Kenya (January – July 2004) resulting in 125 deaths and hospitalization of over 300 others. The assessment of the nation's economy conducted in 2012 by Abt Associates in collaboration with Mycotoxicology Society of Nigeria (MYCOTOXSON) and NAFDAC concluded that the largest impact of aflatoxins in Nigeria is on health, especially on humans, finding little awareness about aflatoxins among farmers, rural traders, and consumers. Researchers at the International Institute of Tropical Agriculture, the African Agricultural Technology Foundation, Kenya, and the United States Department for Agriculture have demonstrated the ability of natural Nigerian fungi to produce the concentrations of aflatoxins in maize and other grains, as a result, increases global trade losses estimated at $1.2bn [9].

Presently, more than 50 countries have established regulations for controlling aflatoxins in foods and feeds [11]; the U.S. Food and Drug Administration (FDA) has limits of 20 µg/kg for total aflatoxins. The Standards Organization of Nigeria (SON) has set a 4 µg/kg [12][13], 20 µg/kg [14] and 4 µg/kg [13] standards for maximum total aflatoxin concentrations for maize, raw groundnuts and groundnut cake (*kulikuli*) respectively. Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) enforces a standard of 4 µg/kg for ready-to-eat foods and 10 µg/kg for raw food items, but only for packaged goods and export-bound products. Despite aflatoxin regulatory standards, unpackaged food for domestic consumption are not regulated. This means that aflatoxin-contaminated grain can easily enter the Nigerian consumption stream due to low awareness about aflatoxins and their health impact among consumers and sellers. The vast majority of foods consumed by the Nigerian population are not regulated for aflatoxins. Field research conducted by Abt Associates in the Niger, Kogi, and Ondo states found no evidence of testing for aflatoxins in the domestic maize and groundnut sold in Nigerian markets.

A lot of methods have been described for aflatoxin determination in grains, feeds and feed ingredients that for human and animal usage. Techniques such as HPLC, GC and LC/MC have been used for aflatoxin detection [15-17] are very accurate but are expensive and low throughput research-oriented. In recent years, potentially advantaged Enzyme-linked Immunosorbent assay [18] has also been described and validated for aflatoxin detection and is said to be more preferred because of its simplicity, specificity, sensitivity, low cost and the use of safe reagents. Hence, this research was aimed at determining the aflatoxin contamination levels in grains, feeds and feed ingredients obtained from Katsina and Zaria metropolises using competitive ELISA technique.

MATERIALS AND METHOD

Sample Collection

A total of twenty nine samples comprising of 14 grains, 15 feeds and feed ingredients were randomly sampled (50g each) in clean labeled polyethene bags from local industries, markets, hawkers and farmer in Katsina (Sudan Savannah) and Zaria (Northern Guinea Savannah) metropolises. The collected samples were sealed and kept at room temperature until use.

Sample Extraction and Preparation

Sample extraction was performed according to manufacturer's instruction (Aqra Quant Total Aflatoxin Assay) test kit. Raw samples were individually ground using laboratory grinding submilling mill so that 75% would pass through a 20-mesh screen. One hundred ml of methanol/water (70/30) was added to 20g of each ground sample and was shaken for 30 minutes. Sample was allowed to settle and the supernatant was filtered through a whatman no. 1 filter paper. The filtrate was collected for further analysis.

Oil samples were extracted by dispensing 2ml of the oil sample into a test tube and 10ml of 70% (v/v) methanol was added to it (an extraction ratio of 1:5 of sample to extraction solution). The contents were mixed thoroughly and

then allowed to settle for 3 minutes, for which it separated into two layers. The methanol (supernatant) layer was taken for further analysis.

Aflatoxin Determination

The determination of aflatoxin was performed as described by the manufacturer's instructions using AqraQuant Total Aflatoxin Assay 1/20 test kit. Two hundred µL of enzyme conjugate was dispensed into each green-bordered Dilution well and 100µL of each standard (0, 1, 2, 4, 10 and 20 µg/kg)/sample (in duplicate) were added into the appropriate Dilution well containing the 200µL of conjugate. Each well was carefully mixed by pipetting it up and down three times and 100µL of the contents from each Dilution well was immediately transferred into a corresponding Antibody Coated Microwell. It was then incubated at room temperature for 15 minutes. The contents of the microwell strips were discarded, followed by a washing step of each microwell with distilled water. This was repeated for a total of five washes. Microwell strips were tapped using absorbent paper towels to expel as much residual water as possible after the fifth wash. The microwells were dried with a dry cloth or towel by tapping. One hundred µL of the substrate was added into each microwell and incubated at room temperature for 5 minutes and a blue color developed. One hundred µL of stop solution was added into each microwell strip and the color changed from blue to yellow. The optical density of each strip was read with a microwell reader using a 450 nm filter. A standard curve was generated using standard total aflatoxin concentrations in the range of 0 to 20µg/kg.

RESULTS

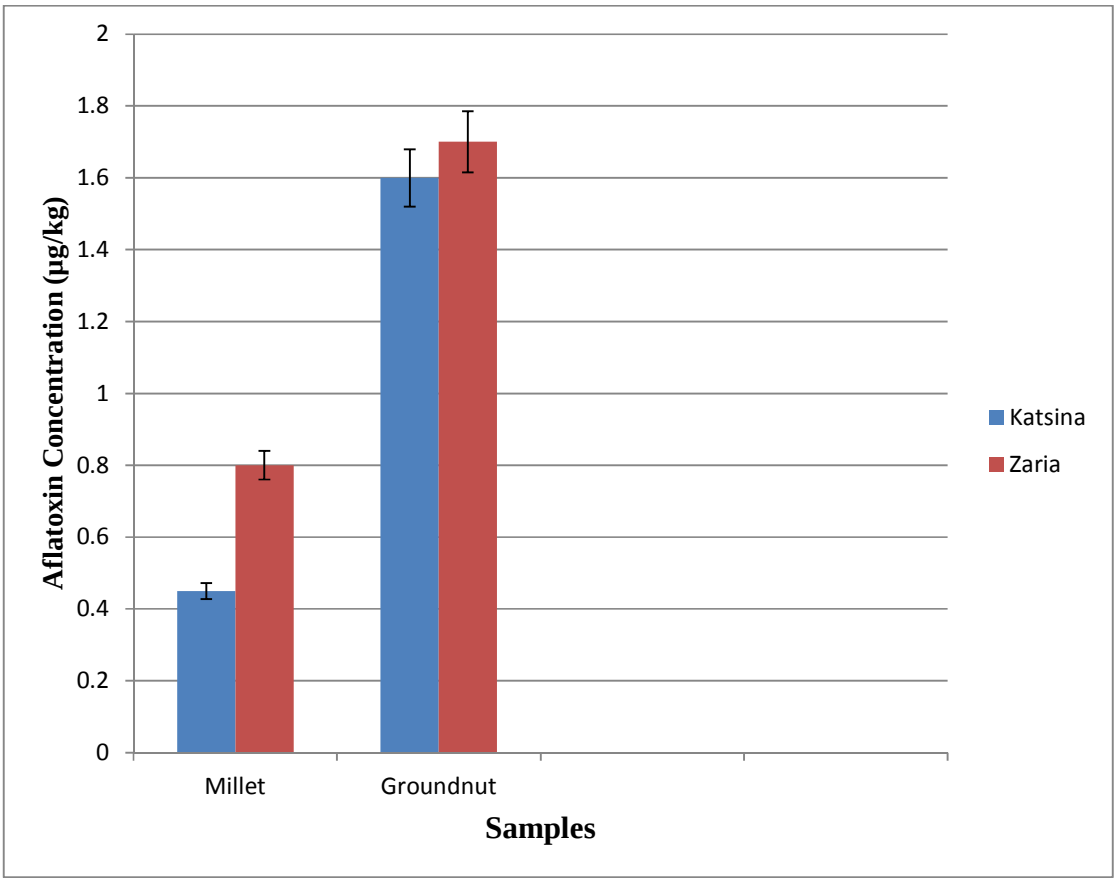

Figure 1.Aflatoxin concentration in Millet and Groundnut from the two study areas.

Aflatoxin contamination in grains

Table 1 shows the aflatoxin concentration level in the analysed grains. Among the 14 grain samples analysed, 12 were contaminated (85.7% incidence). The levels of aflatoxin in each sample ranged from; maize 0.45 - >20µg/kg

and sorghum 0.75-2.35µg/kg while wheat and beans had an aflatoxin concentration of 0.5 and 0.9µg/kg, respectively. Aflatoxin was not detected in two rice samples (per-boiled and milled) and a lowest concentration of 0.1µg/kg was recorded in unmilled rice. The millet and groundnut samples obtained from Zaria recorded higher aflatoxin concentrations (0.8µg/kg and 1.7µg/kg) than those obtained from Katsina (0.45µg/kg and 1.6µg/kg) as shown in Figure 1.

Table 1: Total aflatoxin concentration in grains (sampled from Katsina metropolis).

Sample	Total Aflatoxin Concentration (µg/kg)
Rice (Milled)	N.D
Rice (Unmilled)	0.1
Rice (Perboiled)	N.D
Millet (Dauro)	0.6
Wheat	0.5
White Maize	>20
Yellow Maize	0.45
White Sorghum	0.75
Red Sorghum	2.35
Beans	0.9

N.D : Not Detected

Aflatoxin contamination in feeds and feed ingredients

In this study, 73.3% of the feeds and feed ingredients were contaminated with aflatoxin as shown in Table 2. Both poultry feeds and maize chaff analysed had a >20 µg/kg aflatoxin concentration. The groundnut products (cake, kuli-kuli and oil) analyzed had an aflatoxin level ranging from 1.25-14.7, with the groundnut oil having the highest concentration and kuli-kuli having the lowest contamination level, whereas, the soya bean products (cake and oil) recorded an aflatoxin level of 2.7 and 1.9 µg/kg respectively. No aflatoxin contamination was recorded in all the wheat and maize flours analysed but the sorghum flour had a concentration of 0.85µg/kg. Garri, a cassava product, had an aflatoxin level of 1 and 1.4 µg/kg.

TABLE 2: Concentration levels of aflatoxins found in the feeds and feeds ingredients.

Samples	**Samples Tested**	**Samples Positive**	**Aflatoxin Concentration(µg/kg)**
Flour a. Maize	1	0	0.0
b. Sorghum	1	1	0.8
c. Wheat	3	0	0.0
TOTAL	**5**	**1**	**0 – 0.8**
Oil a. G/nut	1	1	14.7
b. Soyabean	1	1	1.9
TOTAL	**2**	**2**	**1.9 – 14.7**
Cake a. G/nut	1	1	14.2
b. Soyabean	1	1	2.7
TOTAL	**2**	**2**	**2.7 – 14.2**
Maize chaff	1	1	>20
Kuli-kuli	1	1	1.25
Garri	2	2	1.0 – 1.4
Poultry feed	2	2	>20

DISCUSSION

The worldwide occurrence of aflatoxins in agricultural products is well documented, with the major contamination occurring in countries with high temperature and humidity. While it is generally recognized globally that there is no safe level of aflatoxin exposure, the regulatory bodies, including The Standards Organization of Nigeria (SON) sets standards on many food commodities, taking into account global standards as well as national production and target export markets. Total aflatoxins were detected irrespective of the sample source.

Maize is one of the most widely distributed food plants in the world [19]. Two maize samples analyzed in this study revealed that the white maize sample had the highest contamination level (even among the grain samples) of >20µg/kg which exceeds the limits sets by NAFDAC and SON. The high susceptibility of maize to mycotoxin contamination than most grains as observed in this study has also been reported by [20] and [21] in Nigeria and at international level. Also, in agreement to our study, [22] reported that the mean values of aflatoxin contamination in

55 samples of maize ranged between (30.9µg/kg-507.9µg/kg) from 11 districts across three agro-ecological zones of Nigeria, which were far beyond all known acceptable limits. But the other maize (yellow) sample had an aflatoxin level of 0.45µg/kg. The difference in level of contamination between the two samples might be due to the agricultural practice and storage but not location. Also, for reason not known, [23] reported a higher mean aflatoxin levels in white maize (0.55µg/kg) than in yellow maize (0.46µg/kg). The Maize chaff, used as animal feed, had an aflatoxin level of >20.0µg/kg. It is produced by the removal of the outer surface of the maize before further processing to flour, as such, might be a reason why the contamination is high in maize chaff and a reason for the absence of aflatoxin in the maize flour sample.

Millet is a major staple food in northern Nigeria. In this study, the mean level of aflatoxin found in millet is 0.62µg/kg. The Zaria type Millet had the highest contamination level of 0.8µg/kg. The difference in contamination level might be due to the difference in environmental factors (temperature and relative humidity) that favors the growth of aflatoxigenic fungi and also agricultural practices between the two study areas. Sorghum is another staple food and an important starchy food for human and animal consumption Nigeria. The mean level of the aflatoxin found in the two sorghum and a flour samples was 1.32µg/kg. The aflatoxin concentration of the samples are within the permissible limit, thus are safe for human consumption. This might be due to the high phenol and tannin contents present in Sorghum [24] which are known to inhibit fungi infestation.

Wheat is the major source of flour for baked foods and other products. Aflatoxin concentration level in the wheat sample analyzed was 0.5µg/kg while the wheat flour samples (from 3 different flour mills in Nigeria) tested negative to aflatoxin. This might be due to the refined processes undergone by the wheat. Parker and Melnick [25] were the first to confirm that refining eliminates aflatoxin even from products obtained from moldy grains not suitable for human consumption. Aflatoxin contamination was not detected in par-boiled and milled rice samples which might be due to the refining process undergone by the samples. The unmilled rice and Beans samples had a low aflatoxin level of 0.1µg/kg and 0.9µg/kg respectively. Nigeria is the number one producer of cassava in the whole world. Garri, a roasted granular hygroscopic starchy food product, is consumed by several millions of people in the West African sub-region and in Nigeria in particular regardless of ethnicity and socio-economic class. However production and handling methods have not been standardized resulting in a product with varying quality and safety indices hence varying public health concern [26]. The two Garri samples analyzed in this study had an aflatoxin level of 1.0µg/kg and 1.4µg/kg, which is within the range reported elsewhere with a range of 0.3-4.4µg/kg in cassava products [23]. The aflatoxin concentration in garri might be due to its contamination by aflatoxigenic fungi by air in the market. This is in accordance with the work of Ogugbue*et al.,* [27] that reported the contaminated of post-processed garri during sale in portharcourt by fungi including *Aspergillus sp.* Also, the aflatoxin level of garri is known to increase with storage time [28][23].

The mean contamination level of groundnut and its products is 6.68µg/kg, with groundnut oil having the highest contamination level of 14.7µg/kg and fried groundnut cake (kuli-kuli) having the least level of 1.25µg/kg. Although the level of aflatoxin in groundnut and its products (cake, kuli-kuli and oil) was less below the range reported elsewhere [29] in Nigeria who reported an aflatoxin levels in Homemade and unrefined groundnut oil ranging between 20-2000µg/kg and [30] who reported an aflatoxin contamination in Nigerian groundnut cake at levels ranging between 20-455µg/kg. The difference between results obtained here and that of the previous investigations might be due the difference in geographical location, seasonal variations, weather conditions of the study areas, and also the method or technique used. The reason why the incidence of aflatoxin is more frequent in groundnut than in other agricultural commodities is not fully understood [31]. Also, the reason for low aflatoxin contamination in kuli-kuli might be due to the frying process undergone during its production. Soya bean cake products i.e. cake and oil were also analyzed. Soya bean cake serves as animal feeds and its oil is used as a substitute of groundnut oil due to its scarcity and expensiveness. The mean aflatoxin level of soya bean samples is 2.3µg/kg.

The poultry feeds are formulated from grains and other vital ingredients. Among the samples examined, the poultry feeds (from 2 different feeds in Nigeria) had the highest contamination level, with both samples recording a concentration of >20.0µg/kg each. In consonance with our study, [32] reported an aflatoxin contamination in over 7 out of 23 samples (30.4%) of South African animal feeds at the levels of >20µg/kg. These high levels of aflatoxin might be due to the grains and other ingredients contents that may harbour these toxins with high levels. Nigeria has no regulatory standards for maximum aflatoxin concentrations for animal feed [33]. But the level of >20.0µg/kg is within the EEC maximum permitted level of aflatoxin for poultry feed of 30-40µg/kg and exceeds the FDA maximum limit of 20.0µg/kg. The consistent consumption of this by poultry will often result in high economic loses

and lead to lack of interest by investors in poultry production. The need to improve and sustain poultry production in particular and animal husbandry in general, in underdeveloped countries where animal protein consumption per head is very low, cannot be over emphasized.

CONCLUSION

Among the types of mycotoxins, Aflatoxin is the most prevalent and the most potent environmental carcinogen. This study has shown that grains, feeds and feed ingredients are very prone to contamination by toxigenic *Aspergillus* resulting to the production of Aflatoxins. The results generally revealed the presence of aflatoxin contamination in 79.31% samples analysed with a trend in variability of the aflatoxin concentration level being poultry feeds>Feeds-and-feedingredients>grains. Out of the positive samples 26.1% of them exceeded the SON and NAFDAC Aflatoxin permissible level. Although aflatoxin levels may be low, the risk of aflatoxicosis resulting from the continuous ingestion of these foods may be high. Since the poultry feeds had the highest Aflatoxin concentrations; we suggest that the formulation of feeds should be compounded or incorporated with mycotoxin binders as additives, as well as, the establishment and enforcement of permissible or regulatory limits. Generally, creation of awareness and enlightenment to the public, especially in the rural areas and developing countries, about the health risks and hazards involved in consuming foods contaminated with aflatoxins. Moreover, Good Agricultural Practices (field, harvest, drying, transportation and storage) should be implemented widely among farmers, as well as, the selection and breeding of new seeds that are Aflatoxin resistant. This will enhance food and public health safety and in turn reduce the risk of Aflatoxicosis.

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