Nigeria Agricultural Policy Project

THE OCCURRENCE AND CO-OCCURRENCE OF AFLATOXIN AND FUMONISIN ALONG THE MAIZE VALUE CHAIN IN SOUTHWEST NIGERIA
By

Lenis Liverpool-Tasie*, Nikita Saha Turna*, Oluwatoyin Ademola, Adewale Obadina, Felicia Wu
*Co-First Authors

Food Security Policy *Research Papers*

This *Research Paper* series is designed to timely disseminate research and policy analytical outputs generated by the USAID funded Feed the Future Innovation Lab for Food Security Policy (FSP) and its Associate Awards. The FSP project is managed by the Food Security Group (FSG) of the Department of Agricultural, Food, and Resource Economics (AFRE) at Michigan State University (MSU), and implemented in partnership with the International Food Policy Research Institute (IFPRI) and the University of Pretoria (UP). Together, the MSU-IFPRI-UP consortium works with governments, researchers and private sector stakeholders in Feed the Future focus countries in Africa and Asia to increase agricultural productivity, improve dietary diversity and build greater resilience to challenges like climate change that affect livelihoods.

The papers are aimed at researchers, policy makers, donor agencies, educators, and international development practitioners. Selected papers will be translated into French, Portuguese, or other languages.

Copies of all FSP Research Papers and Policy Briefs are freely downloadable in pdf format from the following Web site: www.foodsecuritylab.msu.edu

Copies of all FSP papers and briefs are also submitted to the USAID Development Experience Clearing House (DEC) at: http://dec.usaid.gov/

AUTHORS

Saweda Liverpool-Tasie is an Associate Professor in the Department of Agricultural, Food, and Resource Economics (AFRE) at Michigan State University (MSU).

Nikita Saha Turna is a PhD student in the Department of Food Science and Human Nutrition, Michigan State University, East Lansing Michigan 48824, USA

Oluwatoyin Ademola is an MSc student in the Department of Food Science and Technology, Federal University of Agriculture, Abeokuta 2240, Nigeria.

Adewale Obadina is Associate professor in the Department of Food Science and Technology, Federal University of Agriculture, Abeokuta 2240, Nigeria.

Felicia Wu is professor in the Department of Food Science and Human Nutrition and the Department of Agricultural, Food, and Resource Economics (AFRE), Michigan State University, East Lansing Michigan 48824, USA

Contents

ABSTRACT

Aflatoxins and fumonisins are two major mycotoxins: toxic chemicals produced by fungi that contaminate a variety of food commodities including maize, a key staple food in sub-Saharan Africa. Aflatoxin causes liver cancer (hepatocellular carcinoma, HCC) and has been associated with acute liver toxicity and immunotoxicity, while fumonisin has been associated with neural tube defects in infants and esophageal cancer. Both mycotoxins have been associated with child growth impairment. Previous studies have demonstrated that co-occurrence of these mycotoxins may have potential synergistic toxicological effects in humans. Therefore, this study examines the occurrence and co-occurrence of fumonisin and aflatoxin along the maize value chain in southwest Nigeria. Despite regulatory limits in Nigeria for aflatoxins in maize products, 51.70% of the samples were found had aflatoxin levels above those limits. Though no regulatory limits currently exist for fumonisins, 12.93% of the samples contained total fumonisin levels higher than the United States regulatory limit. We found that aflatoxin and fumonisin contamination in maize products extends beyond production to storage and final food products. Thus, adequately addressing the mycotoxin challenge requires consideration of the entire maize value chain. This study encourages further research to generate data on the exposure of Nigerians to fumonisin and aflatoxin and potential adverse health effects.

Key words: Aflatoxin, fumonisin, co-occurrence, value chains, maize, Nigeria

1. INTRODUCTION

Aflatoxins and fumonisins are two major groups of mycotoxins produced by the *Aspergillus* and *Fusarium* genera of fungi respectively. These naturally occurring toxins frequently contaminate maize, mainly in countries with high temperature and humidity (Paterson and Lima 2017). Several studies have demonstrated these mycotoxins as potential risk to human and animal health (Wu et al. 2014).

Aflatoxin B1 (AFB1) is classified as a group 1 human carcinogen (IARC, 2002). It is known to be the second leading cause of hepatocellular carcinoma (HCC) worldwide and the risk of HCC is multiplicatively higher (30 times) for individuals who have chronic hepatitis B virus (HBV) infection (JECFA 1998, Wu et al. 2013). High doses of aflatoxin can also result in acute aflatoxicosis, severe liver damage, edema and even death. Aflatoxins are associated with inducing adverse immune system and growth effects in animals (Bondy and Pestka, 2000) and growth impairment in children (Khlangwiset et al. 2011). Recent studies have also found that aflatoxin exposure may be associated with risks of prematurity and pregnancy loss (Smith et al. 2017).

Fumonisin B1 (FB1) is classified as a group 2B carcinogen (IARC, 2002). It's contamination in maize has been associated with the incidence of esophageal and liver cancers (Sun et al., 2007, 2011). Dietary exposure of fumonisin in pregnant mothers has also been associated with the incidence of neural tube defects in infants (Missmer et al., 2006). Recent studies have associated fumonisin exposure with growth impairment in children (Shirima et al. 2015, Chen et al. 2018a, 2018b).

Several animal and *in vitro* studies of aflatoxin-fumonisin co-exposure indicate additive or synergistic effects on the development of precancerous lesions or liver cancer in laboratory animals and in vitro studies (WHO, 2018). A study in broilers (chicks) indicated that co-exposure to AFB1 and FB1 had primarily additive effects on body weight, liver structure and immunological response (Tessari et al., 2006). In a recent mouse study, oral doses of pure AFB1 and pure FB1 in mice resulted in increased relative spleen weight and increased activity of enzymes that lead to oxidative stress, in a potentially additive or potentiating manner (Abbes et al., 2016). In a rat feeding study, exposure to pure AFB1 and pure FB1 alone or sequentially showed effects on body weight to be less than additive, but effects on some liver enzymes were synergistic; supporting the theory that fumonisins may act as a promoter for aflatoxin-initiated liver cancer (Quian et al., 2016). These studies support the hypothesis of increased hepatocarcinogenicity under the condition of co-exposure to aflatoxins and fumonisins (JECFA, 2017). In another study, chickens fed diets containing both aflatoxins and fumonisins experienced changes in plasma triglycerides, very low-density lipoprotein levels and low percentage of total liver lipids (Siloto et al., 2013). In a rat liver hepatoma cell line, the FB1-AFB1 combination showed greater increase in CYP1A transcription and upregulation of the aryl hydrocarbon receptor in a dose-dependent manner (Mary et al., 2015). Although several animal models have indicated higher incidence of liver cancer following consecutive exposure to both aflatoxins and fumonisins and other known liver carcinogens, currently no data is available on similar effects in humans (WHO, 2018). However, dietary exposure to these two mycotoxins can still be considered a significant health concern based on the results of toxicological studies above.

Despite these potentially dangerous effects of the co-occurrence of aflatoxins and fumonisins on humans and animals, only a limited number of studies have explored the co-occurrence of these

mycotoxins in foods consumed as key staples, and no such studies exist along supply chains in sub-Saharan Africa. This study attempts to begin to fill this gap by exploring the occurrence and co-occurrence of aflatoxins and fumonisins in the supply chain of maize, a key staple in sub-Saharan Africa. Aflatoxins and fumonisins have a widespread occurrence in maize globally, and maize in Africa is frequently contaminated with both aflatoxins and fumonisins (Kimanya et al., 2008). Nigeria leads Africa's maize production with around ten million tons produced in 2014 (FAOSTAT, 2017). The majority of Nigeria's maize (over 75%) is used for direct human consumption, as maize is a staple of the Nigerian diet (USDA, 2012). With urbanization, higher incomes and increased animal protein consumption, Nigeria's demand for maize for feed has also been increasing rapidly. Between 2003 and 2015, the volume of maize used for feed in Nigeria increased from 300 thousand to 1.8 million tons; a 600% increase (Liverpool-Tasie et al. 2017). Thus, maize is an essential crop for food security in Nigeria as well as an industrial crop (USDA, 2014).

Furthermore, the prevalence for hepatitis B virus in Nigeria is also very high (~ 12.2%) (Olayinka et al., 2016); Concomitant hepatitis B virus (HBV) infection and aflatoxin exposure greatly increase the risk of HCC (Wu et al., 2013). Therefore, since dietary exposure to aflatoxins and fumonisins among Nigerians is very likely, resulting health outcomes from its consumption is an important concern for the country. The Standards Organization of Nigeria (SON) has set standards for maximum total aflatoxin concentrations in maize for 4 ppb (SON, 2008). However, fumonisin levels are not known to be regulated in food and feed in Nigeria.

In this study, the occurrence and co-occurrence of aflatoxins (AFB1, AFB2, AFG1 and AFG2) and fumonisins (FB1, FB2 and FB3) along the maize value chain in southwest Nigeria is reported. Rather than just focusing on maize samples from one node of the value chain (e.g. maize from farmers or maize based products in retail outlets), we explore this phenomenon in samples collected from actors all along the maize supply chain. This includes farmers, maize traders (after different lengths of storage), feed millers (maize and final feed) and retailers of maize based products. This is important because the maize value chain in Nigeria (as in many parts of SSA) is often a long and fragmented supply chain with many actors (Liverpool-Tasie et al. 2018). This creates many opportunities for aflatoxin and fumonisin contamination during maize production as well as during handling and storage. Looking at maize based products along the maize value chain provides key insight as to where along such value chains (and in what form of maize based products) the challenge of mycotoxin contamination occurs generally and where the co-occurrence of aflatoxins and fumonisins presents particular health concerns.

2. MATERIAL AND METHODS

Study area

The study area is the Greater Ibadan Area of Oyo State in Southwest Nigeria. This area was selected for several reasons. First, in addition to maize consumption by humans, southwest Nigeria (and Oyo State particularly) is a major zone for poultry production and aquaculture. Thus, this zone of the country is a major driver of increased maize demand (for animal feed) in the country. Second, the study area was selected because of its higher probability of exposure to mycotoxin challenges. For example, majority of the maize in Nigeria is produced in the north and then travels all over the country; often over a thousand kilometers to the south. Having to transport maize over such long distances

creates potential additional opportunities for exposure to various molds. In addition to being a major consumption zone, the study area reflects the maize producing area of southwest Nigeria. Due to the very humid conditions in the southwest, the maize produced there is likely to face more challenges associated with exposure to moisture compared to the drier north. Though the study area is not nationally representative, it is largely representative of maize consumption and production areas in southwest Nigeria.

Sampling of maize and maize products

Farmer's sample
Farmers from two local government areas (LGAs) of Oyo State, Atisbo and Saki West were selected for the samples of maize. These two LGAs are the major maize producing LGAs in the state according to the Ministry of Agriculture. In each LGAs, four dominant maize producing villages were selected and maize cobs were collected from 30 randomly selected farmers from the four main maize producing villages. For each farmer, 20 maize cobs were randomly selected from the farmer's field and store. Where available, unharvested maize cobs were randomly selected on farmer's field. Samples of maize cobs stored for minimum of one and maximum of four months were collected from each of the farmer's stores, where available. At least two samples (from different points in time) were collected from each farmer giving 71 maize samples with 0-4 months of storage. The 20 maize cobs collected in each period (from each farmer) were treated as separate samples. The maize grain was shelled from its cobs, hand-mixed and 500g of grain were taken from each lot as a separate sample. A total of 71 maize samples were collected from the farmers (field and stored samples). 500g of each maize grain were grounded separately with a milling machine and subsamples of 50g were further taken from the lots and placed in a well-sealed and labeled polythene bag for mycotoxin analysis. Samples were stored at 4oc prior to analyses.

Market samples
Three major maize wholesale markets in the Greater Ibadan area of Oyo State, Nigeria were selected for collection of maize samples from traders. One wholesale market is located in an urban area (Bodija market), one in a rural-near-city area (Ojaoba market) and the other in an off-market area . Fifteen maize wholesalers were randomly selected from the three markets; five in each market. Samples consisting of 500g maize grain were purchased from the sellers. The maize grains were ground separately with a milling machine and subsamples of 50g were further taken from the lots and placed in a well-sealed and labeled polythene bag for mycotoxin analysis. Samples were stored at 4oc prior to analyses.

Feed-mill samples
Ten feed-mills from two LGAs (Lagelu and Egbeda) of the greater Ibadan area of Oyo state (identified by stakeholders in the poultry subsector as the areas with high concentrations of feed mills) were selected for the collection of poultry feed and maize samples. Five feed mills were randomly selected from a list of feed mills in each LGA and a sample of 500g of finished feed and maize grain from the batch of maize used for producing the feed was collected from the feed-mills. The maize and feed samples from each feed miller were treated as separate samples linked to the same feed mill. A total of 10 maize grain and 10 poultry feed samples was collected from the feed-mills. The maize grains were grounded separately with a milling machine and subsamples of 50g were taken from each lot and placed in a well labeled polythene bag for mycotoxin analysis. The poultry feed was also labeled separately in polythene bag. Samples were stored at 4oC prior to analyses.

Maize based processed products: These are samples of final consumer products made from maize. These processed maize based products were purchased from the two main wholesales markets (Bodija and Ojaoba) in the study area. The identified products were broadly categorized into branded and unbranded maize based products. The branded products include cereals such as corn flakes, golden morn and custard while the unbranded products were largely maize based snacks sold informally called Kokoro and Aadun. A total of 44 processed maize products (34 branded and 10 unbranded) were purchased. They were well labeled and stored appropriately for mycotoxin analysis.

Mycotoxin analysis of maize samples
The maize samples were analyzed at Romer' lab (USA) using liquid chromatography tandem mass spectrometry (LC-MS/MS). The extraction of mycotoxins from the maize samples was carried out according to the method described by (Sulyok et al., 2007). For each sample, 5 grams were weighed and extracted with 20 ml of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). For spiking experiments, appropriate amounts of the combined working solutions were consecutively added to 0.25 g samples. The spiked sample was stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and the sample. Samples were extracted for 90 min on a GFL 3017 rotary shaker followed by filtration and sample clean up. The filtered sample extract was diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). 40 µl of the diluted extracts were injected into the LC-MS/MS instrument. Apparent recoveries of the analytes were crosschecked by spiking a sample (multi-analyte standard on a fixed concentration level with no mycotoxin contamination). The corresponding peak areas of the spiked samples were then used to determine the apparent recoveries by comparison to a standard prepared and diluted in neat solvent. The concentrations of samples contaminated with aflatoxins and fumonisins were corrected by a factor equivalent to the reciprocal of apparent recovery (1/R; where R is the apparent recovery value) for each analyte.

LC-MS/MS parameters
The samples were screened for aflatoxin and fumonisin contamination using a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V electrospray ionization (ESI) source and a 1290 Series UHPLC System (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed on a Gemini R _ C18-column, 150mm × 4.6 mmi d., 5 µm particle size, equipped with a C18 security guard cartridge, 4 mm × 3 mmi. d. (all from Phenomenex, Torrance, CA, USA) at room temperature. Mycotoxin analyte identifications were confirmed by the acquisition of two MS/MS transition yielding 4 identification points.

3. DATA ANALYSIS

For samples whose aflatoxin and fumonisin levels were less than the limit of detection (LOD), the values were replaced with half of the limit of detection (LOD). All statistical analysis was done using MS Excel and the JMP 14 for Windows software. Kruskal–Wallis tests were performed to test the statistical significance for total aflatoxin and fumonisin levels among samples collected from farmers at different storage times. A Mann–Whitney test was used to compare the difference between two groups. A $p \leq 0.05$ was considered to be statistically significant for all the statistical tests.

4. RESULTS

Farmer's stored maize

Table 1 shows the aflatoxin and fumonisin levels in maize samples collected from farmers, from harvest to 4 months of storage with 1-month intervals. The total aflatoxin level in the samples tends to increase with time of storage. The geometric mean of total aflatoxin level at harvest was 4.2 ppb but after 4 months of storage, the level went up to 42.7 ppb which is much higher than the Nigerian maximum total aflatoxin regulatory limit in maize of 4 ppb. At harvest, 37.5% of the samples had aflatoxin levels more than 4 ppb and after 4 months of storage 87.5% of the samples had aflatoxin levels > 4 ppb (Figure 1). The geometric mean levels of total aflatoxin in the samples at different storage times were statistically significantly different ($p < 0.05$) (Table 5); higher aflatoxin levels with higher storage time. However, the total fumonisin levels do not follow any specific pattern with length of storage time (Figure 2). The highest geometric mean level of total fumonisin was observed in samples collected at harvest (1682.3 ppb); 37.5% of the samples collected at harvest had total fumonisin levels higher than the United States Food and Drug Administration (USFDA) regulatory limit of 2000 ppb (USFDA, 2000) (Figure 2). The geometric means of total fumonisin level across the groups were not significantly different at $p < 0.05$ (Table 6).

Table 1: Aflatoxin and fumonisin levels in maize stored for various lengths of time in farmers' households, Nigeria.

|	Number of samples	Mean total aflatoxin level (ppb)	Standard deviation	%>4 ppb aflatoxin	Mean total fumonisin level (ppb)	Standard deviation	%>2000 ppb fumonisin
At harvest	8	4.2	8.2	37.5	1682.3	1953.2	37.5
Stored for 1 month	10	5.3	12.9	50.0	671.2	962.6	20.0
Stored for 2 months	19	8.8	93.2	63.2	737.5	747.7	21.0
Stored for 3 months	24	17.5	42.2	91.7	1035.4	1383.0	20.8
Stored for 4 months	8	42.7	498.7	87.5	1220.8	703.0	25.0

Note: All means are geometric
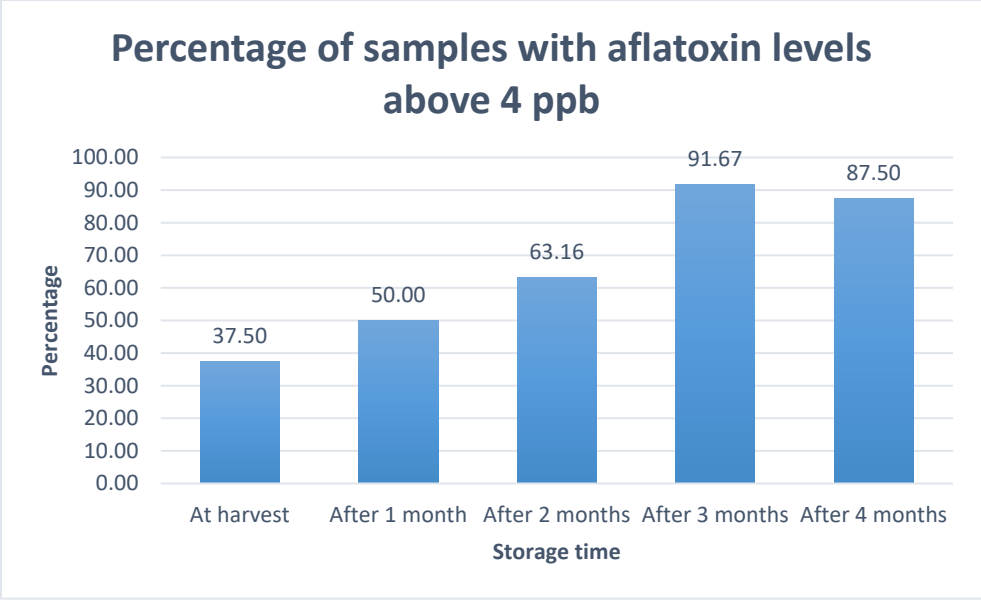
Figure 1: Bar graph showing the percentage of farmer's samples containing total aflatoxin levels above the allowable limit in Nigeria
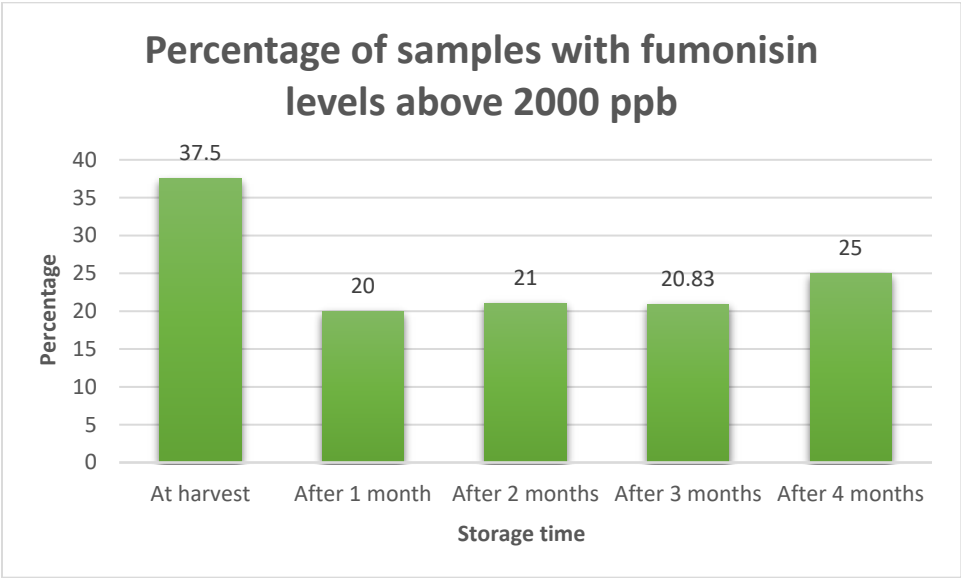

Figure 2: Bar graph showing the proportion of farmer's samples containing total fumonisin levels above maximum acceptable level set by the USFDA

Maize from local maize traders
Table 2 panel A shows the total aflatoxin and fumonisin levels in maize samples collected from maize traders after 1 week and 2 weeks of storage. The geometric mean of total aflatoxin level in maize stored for 1 week was only 3.0 ppb but after 2 weeks of storage, the level went up to 5.6 ppb, and two out of the five samples collected at 2 weeks of storage had aflatoxins contamination higher than Nigerian aflatoxin regulatory limit of 4 ppb. However, the geometric mean levels of total aflatoxin in the maize trader's samples at different storage times were not statistically significantly different ($p > 0.05$) (Table 5).

The geometric mean level of total fumonisin in samples collected at 1 week was 665.1 ppb and 676.6 ppb at 2 weeks which were both lower than the EU regulatory limit of 1000 ppb and according to Mann-Whitney U test, the geometric means of total fumonisin level cross the groups were not significantly different at $p < 0.05$ (Table 6).

Table 2: Aflatoxin and fumonisin levels in maize flour samples collected from maize traders and poultry feed millers

Maize flour storage time	No. of samples	Geomean of total aflatoxin (ppb)	Standard deviation	%>4ppb aflatoxin	Geomean of total fumonisin (ppb)	Standard deviation	%>2000 ppb fumonisin
Maize traders (Panel A)							
1 week	9	3.0	1.7	11.1	665.1	197.6	0
2 weeks	5	5.6	23.1	40	676.6	931.0	20
Feed millers (Panel B)							
Maize in storage	10	3.1	1.4	0	1413.6	1037.4	10
Final feed	10	59.7	87.1	100	819.1	1331.5	10

Maize samples from feed millers
Table 2 panel B shows the total aflatoxin and fumonisin levels in maize flour samples collected from feed millers from their storage and feed samples produced out of their stored maize. The geometric mean total aflatoxin level in the final feed (59.7 ppb) is much greater than that in the stored maize samples (3.1 ppb) and is statistically significantly different at $p < 0.05$ (Table 5). All of the total feed samples collected from the feed millers had aflatoxin levels higher than 20 ppb which is the maximum allowable limit in feed set by the USFDA (USFDA, 2000).

The geometric mean of total fumonisin level in the stored maize was 1037.4 ppb and 1331.5 ppb in the final feed but is not statistically significantly different at $P < 0.05$ (Table 6). 10% of the stored maize samples and also the final feed samples contained fumonisins levels higher than the USFDA regulatory limits of 2000 ppb in feed.

Branded and non-branded maize-based food products

Table 4 shows the total aflatoxin and fumonisin levels in branded and non-branded snacks and cereals made from maize. The geometric mean total aflatoxin level in branded snacks- cereal mix and custard combined (2.9 ppb) is lower than that in the non-branded maize snack – corn roll (6.8 ppb). 4 out of the 34 (11.76%) branded snacks and 8 out of the 10 (80%) non-branded snacks contained total aflatoxin levels higher than the Nigerian regulatory limits. The geometric mean of total aflatoxin levels between the branded and non-branded groups were significantly different at P<0.05 (Table 5).

The geometric mean total fumonisin level is also higher in the non-branded snacks (335.03 ppb) compared to branded snacks (0-94 ppb). Though the mean levels in both groups were much lower than the US regulatory limits for fumonisins, the difference is statistically significant.

Table 4: Aflatoxin and fumonisin levels in branded vs non-branded snacks.

	Sample	No. of samples	Geomean of total aflatoxin (ppb)	Standard deviation	%>4 ppb aflatoxin	Geomean of total fumonisin (ppb)	Standard deviation	%>2000 ppb fumonisin
Non-branded	Corn roll	10	6.8	2.1	80.0	310.6	335.03	0
Branded	Cereal mix	20	3.1	0.96	20.0	194.5	94.0	0
	Custard	14	2.7	0	0	150.0	0	0

Table: 5 Statistical analyses for aflatoxin levels across the groups

Group	Statistical test used	P-value	U-value	Z-score
Farmer's flour (harvest to 4 months storage)	Kruskal-Wallis	0.0033	-	-
Trader's flour (1 week to 2 weeks storage)	Mann-Whitney U	0.4237	16	-0.8
Feed millers (stored maize to final feed)	Mann-Whitney U	0.00018*	0	-3.74185

| | Mann-Whitney U | <0.00001* | 8 | -4.5229 |
| Branded and non-branded maize snacks | | | | |

*values significant with respect to a P-value of 0.05

Table 6: Statistical analysis results for <u>fumonisin</u> levels across the groups

Group	Statistical test used	P-value	U-value	Z-score
Farmer's flour (harvest to 4 months storage)	Kruskal-Wallis	0.1254	-	-
Trader's flour (1 week to 2 weeks storage)	Mann-Whitney U	0.9442	21.5	-0.06667
Feed millers (stored maize to final feed)	Mann-Whitney U	0.1971	32.5	1.28508
Branded and non-branded maize snacks	Mann-Whitney U	0.0128*	80.5	-2.4925

*values significant with respect to a P-value of 0.05

As shown in Figure 3, the geometric means of total aflatoxin levels in farmer's flour samples stored for 2-4 months, samples from maize traders stored for over 2 weeks, final feed samples from feed millers and the non-branded maize snacks were higher than 4 ppb which exceeded the Nigerian set maximum limit for total aflatoxin level in maize. The geometric means of total aflatoxin levels in other groups were comparatively lower and can be considered safe or acceptable. However, the geometric means of total fumonisin levels in all the group of samples collected were much less than the USFDA regulatory limit of 2000 ppb, as shown in Figure 4.
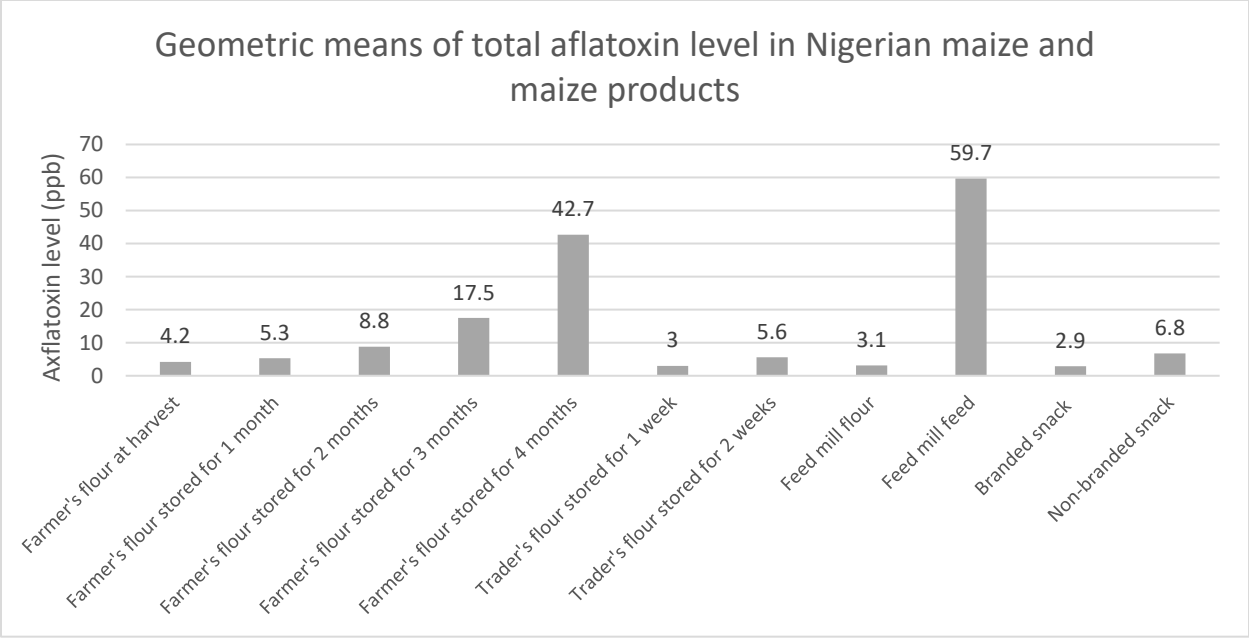
Figure 3: Bar graph showing the geometric means of total aflatoxin levels in Nigerian maize and maize products
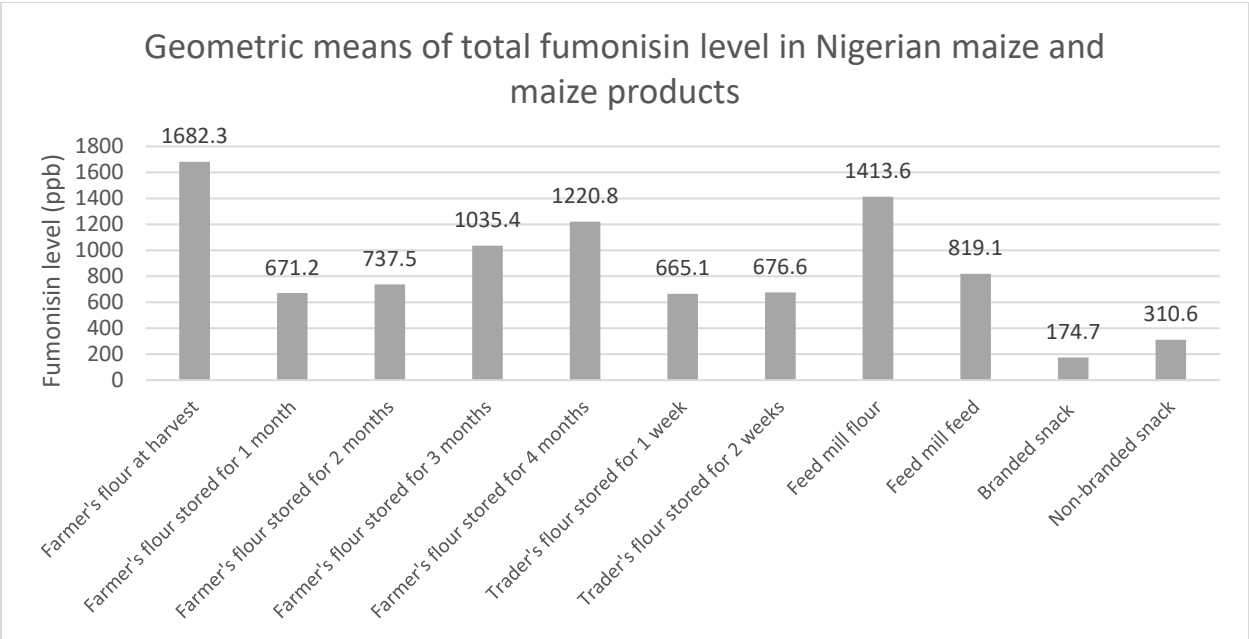

Figure 4: Bar graph showing the geometric means of total fumonisin levels in Nigerian maize and maize products

5. DISCUSSION

The aflatoxin levels in samples collected from maize farmers indicate an increase in the aflatoxin levels with increasing time of storage. The geometric mean of total aflatoxins in farmer's samples stored for over 2 months to 4 months exceeded the Nigerian regulatory limits for aflatoxins - 4 ppb, which is also considered risky according to European regulatory standards (EU, 2006). However, there is no significant difference in the geometric mean levels of total fumonisins with the length of storage time but almost 20.5% of the samples collected from the farmers and traders contained fumonisin levels higher that the US regulatory limits for fumonisin (2000 ppb).The geometric mean of total aflatoxin level in the samples collected from maize traders, that are stored for 2 weeks is greater than the geometric mean of the ones that are stored for 1 week which again supports previous studies that show aflatoxin levels increase with the time of storage in hot and humid countries (Villers, 2014). The total fumonisin levels in the samples collected both at 1 week and 2 weeks of storage did not change as much.

The samples collected from feed millers demonstrate that even though the geometric mean levels of total aflatoxins in stored maize is very low, the levels in the final feed is significantly higher and all of the final feed samples contained aflatoxin levels exceeding 20 ppb which is the allowable maximum level for total aflatoxins set by the USFDA. The drastic increase in aflatoxins might be due to the fact that other ingredients such as, ground nut cake (made from peanuts) which tend to be contaminated with high levels of aflatoxins, are added to the actual feed. The total fumonisin levels were found to be lower in feed than in stored maize, and the geometric mean levels of total fumonisin in both the stored maize and final feed were much lower than the strictest US regulatory fumonisin level of 2 mg/kg.

The results from maize farmers and traders confirm the potential for contamination of maize postproduction during storage. This implies that efforts to reduce exposure to aflatoxins among maize consumers cannot only focus on one set of actors in the value chain. To focus only on maize production in the field is not likely to guarantee a safe product for the final maize consumer. Feasible and cost-effective methods to reduce aflatoxin risk in postharvest conditions have been developed (Khlangwiset and Wu 2010, Wu and Khlangwiset 2010).

The feed mill results reveal the interrelated nature of food supply chains. Issues of food and feed contamination require attention to be paid to related supply chains. Focusing exclusively on the maize supply chain does not necessarily guarantee improved safety of maize based products when combined with other ingredients as in the case of feed. This calls for a more holistic approach to food safety that recognizes the often-interrelated nature of food and feed supply chains.

In terms of the Nigerian branded and non-branded maize snacks, the geometric means of both total aflatoxin and total fumonisin levels tend to be much higher in the non-branded snacks than in branded snacks. 80% of the non-branded snacks contained risky levels of total aflatoxins according to Nigerian and EU regulations. However, both the branded and non-branded snacks contained safe or allowable levels of total fumonisins according to USFDA regulatory limits.

6. CONCLUSIONS

This study confirms that aflatoxins and fumonisins are prevalent contaminants of maize for human consumption and animal feed in Nigeria. Despite the existence of regulatory limits in Nigeria for aflatoxins in maize, a significant fraction (51.70% of the total samples collected) of maize and maize products were contaminated with risky levels of aflatoxins. In terms of fumonisins, 12.93% of the total samples collected contained levels higher than the US regulatory limit of 2000 ppb. The findings of this study show that people in Nigeria are at risk of exposure to both of these mycotoxins which is a real concern in toxicology because based on previous studies, the co-occurrence of these toxins may have potential synergistic impacts on humans. Also, Nigeria has high prevalence of HBV and synergistic interactions of aflatoxin exposure and HBV infection increases the risk of HCC. Therefore, our study should encourage further research that will generate data on the exposure of the fumonisin and aflatoxins among Nigerians and verify if stricter regulatory limits for these mycotoxins in food and feed should be introduced alongside better enforcement mechanisms.

Research and policy interventions that support the development and dissemination of improved maize varieties that are resistant to fungal infection and mycotoxin control on maize fields are important (Dorner and Horn 2007, Khlangwiset and Wu 2010). These efforts may need to be accompanied by measures to prevent the exposure of grain to the fungi along the entire value chain, from harvest to food products in stores and homes. Due to the prevalence of multiple ingredients in most food and feed, minimizing human and animal exposure to dangerous mycotoxins requires consideration of multiple related supply chains such as maize and groundnut products in the case of animal feed. Efforts to understand and address challenges associated with mycotoxins in maize based products need to be more holistic and to consider the potential for exposure of the grain to these harmful fungi along the entire supply chain and across related supply chains.

7. REFERENCES

Abbès S, Salah-Abbès JB, Jebali R, Younes RB, Oueslati R.2016. Interaction of aflatoxin B1 and fumonisin B1 in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. J Immunotoxicol.13(1):46–54.

Bondy, G. S., &Pestka, J. J. 2000. Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health Part B: Critical Reviews, 3(2), 109-143.

Chen C, Mitchell NJ, Gratz J, Houpt ER, Gong Y, Egner PA, Groopman JD, Riley RT, Showker JL, Svensen E, Mduma ER, Patil CL, Wu F. (2018a). Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania. Environment International 115:29-37.

Chen C, Riley RT, Wu F (2018b). Dietary Fumonisin and Growth Impairment in Children and Animals: A Review. Comprehensive Reviews in Food Science and Food Safety, DOI:10.1111/1541-4337.12392.

Dorner, J. W. and Horn, B. W. 2007. Separate and combined applications of nontoxigenic Aspergillus flavus and A. parasiticus for biocontrol of aflatoxin in peanuts. Mycopathologia 163, 215–223.

European Union Commission 2006. Commission Regulation (EC) No. 1881/2006 of 19th December 2006. Setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union L364:15–24

FAO Statistics Division (FAOSTAT). Food and Agricultural Commodities Production. 2017. Available online: http://fao.org/faostat/en/#data/QC

Food and Agriculture Organization (FAO) 2004. Worldwide Regulations for Mycotoxins in Food and Feed in 2003. Available at:
http://www.fao.org/docrep/007/y5499e/y5499e0i.htm#bm18.1.3 Accessed August 17, 2018

International Agency for Research on Cancer (IARC). 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC MonogrEvalCarcinog Risks Hum, 82: 1–556.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 1998. Forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Safety Evaluation of Certain Food Additives and Contaminants in Food: Aflatoxins. WHO Food Additive Series, 40. Geneva, Switzerland.

Khlangwiset P, Wu F (2010). Costs and efficacy of public health interventions to reduce aflatoxin–induced human disease. Food Additives & Contaminants 27:998-1014.

Khlangwiset P, Shephard GS, Wu F (2011). Aflatoxins and Growth Impairment: A Review. Critical Reviews in Toxicology 41:740-755.

Kimanya, M. E., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., Van Camp, J., &Kolsteren, P. 2008. Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. Food additives and contaminants, 25(11), 1353-1364.

Liverpool-Tasie, L.S.O., Omonona, B., Sanou, A., Ogunleye, W., Padilla, S. and Reardon, T. 2017. Growth and transformation of chicken and eggs value chains in Nigeria. Nigerian Journal of Agricultural Economics 7(1):1-15

Liverpool-Tasie, T. Reardon, A. Sanou, W.Ogunleye, I, Ogunbayo and B.Omonona. 2018. The transformation of value chains in Africa: Evidence from the first large survey of maize traders in Nigeria. Feed the Future Innovation Lab for Food Security Policy Research Paper. April 2018

Mary VS, Valdehita A, Navas JM, Rubinstein HR, Fernandez-Cruz ML. 2015. Effects of aflatoxin B1, fumonisin B1 and their mixture on the aryl hydrocarbon receptor and cytochrome P450 1A induction. Food Chem Toxicol.75:104–11.

Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill Jr, A. H., Rothman, K. J., & Hendricks, K. A. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environmental health perspectives, 114(2), 237.

Olayinka, A. T., Oyemakinde, A., Balogun, M. S., Ajudua, A., Nguku, P., Aderinola, M., ...&Gidado, S. 2016. Seroprevalence of hepatitis B infection in Nigeria: A national survey. The American journal of tropical medicine and hygiene, 95(4), 902-907.

Paterson RRM, Lima N. 2017. Thermophilic fungi to dominate aflatoxigenic/mycotoxigenic fungi on food under globalwarming. Int J Environ Res Public Health. 14(2):199.

Qian G, Lili L, Shuhan L, Xue KS, Mitchell NJ, Su J et al. 2016. Sequential dietary exposure to aflatoxin B1 and fumonisin B1 in F344 rats increases preneoplastic changes indicative of a synergistic interaction. Food ChemToxicol. 95:188–95.

Standards Organization of Nigeria (SON), National Industrial Standard. 2008.
https://www.tobaccocontrollaws.org/files/live/Nigeria/Nigeria%20-%20NIS%204632008%20-%20national.pdf

Shirima CP, Kimanya ME, Routledge MN, Srey C, Kinabo JL, Humpf HU, et al. 2015. A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environ Health Perspect 123:173-178.

Siloto EV, Oliveira EFA, Sartori JR, Fascina VB, Martins BAB, Ledoux DR et al. 2013. Lipid metabolism of commercial layers fed diets containing aflatoxin, fumonisin, and a binder. Poult Sci.92:2077–83.

Smith LE, Prendergast AJ, Turner PC, et al. 2017. Aflatoxin Exposure during Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. Am J Trop Med Hyg 96:770-776.

Sulyok, M., Krska, R., Schuhmacher, R.A. 2007. Liquid Chromatography/tandem mass spectrometric multi-mycotoxin method for quantification of 87 analytes and its application to semi-quantitative screening of mouldy food samples. Anal BioanalChem 389:1505–1523.

Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., ...& Wang, J. S. 2007. Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. Food Additives and Contaminants, 24(2), 181-185.

Sun, G., Wang, S., Hu, X., Su, J., Zhang, Y., Xie, Y., ...& Wang, J. S. 2011. Co-contamination of aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. Food additives and contaminants, 28(4), 461-470.

Tessari, ENC, Oliveira, CAF, Cardoso, ALSP, Ledoux, DR and Rottinghaus, GE. 2006. Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Brit Poultry Sci, 47: 357–364.

United States Food and Drug Administration (USFDA). 2000. Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm#afla

United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS). 2014. Nigeria grain and feed annual report. Global Agriculture Information Network (GAIN). https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Lagos_Nigeria_3-13-2014.pdf

Villers, P., 2014. Aflatoxins and safe storage. Frontiers in microbiology, 5, p.158.

World Health Organization (WHO). 2018. Co-exposure of fumonisins with aflatoxins. http://www.who.int/foodsafety/Food_Safety_Digest_Fumonisins_aflatoxins_EN.pdf

Wu, F., Groopman, J. D., &Pestka, J. J. 2014. Public health impacts of foodborne mycotoxins. Annual review of food science and technology, 5, 351-372.

Wu F, Khlangwiset P (2010). Evaluating the technical feasibility of aflatoxin risk reduction strategies in Africa. Food Additives & Contaminants 27:658-676.

Wu, F., Stacy, S. L., & Kensler, T. W. 2013. Global risk assessment of aflatoxins in maize and peanuts: Are regulatory standards adequately protective? Toxicological sciences, 135(1), 251-259.