Food and Chemical Toxicology 129 (2019) 458–465

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

The occurrence and co-occurrence of aflatoxin and fumonisin along the maize value chain in southwest Nigeria

Lenis Saweda O. Liverpool-Tasie[a,1], Nikita Saha Turna[b,1], Oluwatoyin Ademola[c], Adewale Obadina[c], Felicia Wu[b,*]

[a] Department of Agricultural, Food, and Resource Economics, Michigan State University, East Lansing, MI, 48823, USA
[b] Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI, 48823, USA
[c] Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, 2240, Nigeria

ARTICLE INFO

Keywords:
Aflatoxin
Fumonisin
Co-occurrence
Value chains
Maize
Nigeria

ABSTRACT

Aflatoxin and fumonisin are two major foodborne mycotoxins: toxic chemicals produced by fungi that contaminate food commodities including maize, a staple food in sub-Saharan Africa. Aflatoxin causes liver cancer, and is associated with acute liver toxicity and immunotoxicity; while fumonisin is associated with neural tube defects in infants and esophageal cancer. Both mycotoxins have been associated with child growth impairment. Previous studies suggest that co-occurrence of these mycotoxins may have potentially synergistic toxicological effects. Despite health risks associated with co-occurrence of these mycotoxins, no study has examined their co-occurrence along key food supply chains in Africa. This study is the first report that examines the occurrence and co-occurrence of aflatoxins and fumonisins along the maize value chain in Nigeria. All samples were analyzed using LC-MS/MS. About 52% and 21% of the samples had aflatoxin levels above the Nigerian and US standards for human food, respectively. Though no regulatory limits exist for fumonisin in Nigeria, 13% of the samples contained fumonisin levels higher than the US regulatory limit. Aflatoxin levels can become dangerously high in maize stored four months or longer. Adequately addressing mycotoxin risk requires consideration of the entire maize value chain and associated value chains for food production.

1. Introduction

Aflatoxins and fumonisins are two major groups of mycotoxins produced by *Aspergillus* and *Fusarium* fungi respectively. These mycotoxins frequently contaminate maize, mainly in countries with high temperature and humidity (Paterson and Lima, 2017). They have been implicated in multiple adverse human and animal health effects (Eze et al., 2018; Alshannaq and Yu, 2017; Wu et al., 2014; Shephard, 2008). In recent years, international organizations such as the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization and World Health Organization recognize the importance of the co-occurrence of aflatoxins and fumonisins in maize, because of potentially interacting toxicological effects (JECFA, 2017, 2018). But the nature of this co-occurrence in actual food for human consumption, and associated health effects, are still largely unstudied.

"Naturally occurring mixes of aflatoxins" are classified as a Group 1 human liver carcinogen by the International Agency for Research on Cancer (IARC, 2002). Aflatoxin contributes to causing hepatocellular carcinoma (HCC); additionally, the risk of aflatoxin-related HCC is multiplicatively higher for individuals who also have chronic hepatitis B virus (HBV) infection (JECFA, 1998; Wu et al., 2013). High doses of aflatoxin can result in acute aflatoxicosis, characterized by liver failure, edema, and even death. Aflatoxins are also associated with growth impairment in children (Wild et al., 2015; Khlangwiset et al., 2011). A recent study has found that aflatoxin exposure is significantly higher in stunted children compared to non-stunted children in Nigeria (McMillan et al., 2018). Aflatoxin exposure may also be associated with pregnancy loss and premature birth (Smith et al., 2017) and immunotoxicity (Bondy and Pestka, 2000).

Fumonisins were discovered initially through its association with equine leukoencephalomalacia outbreak and further investigations also found its association with causing porcine pulmonary edema (Marasas, 2001). Fumonisin is now classified as a Group 2B possible human carcinogen (IARC, 2002). It has been associated to a limited extent with

* Corresponding author. Michigan State University, Department of Food Science & Human Nutrition, East Lansing, MI, 48824, USA.
E-mail addresses: lliverp@anr.msu.edu (L.S.O. Liverpool-Tasie), sahaturn@msu.edu (N.S. Turna), ademolatoyin123@gmail.com (O. Ademola), fwu@msu.edu (F. Wu).
[1] Co-First Authors.

https://doi.org/10.1016/j.fct.2019.05.008
Received 31 January 2019; Received in revised form 3 May 2019; Accepted 5 May 2019
Available online 11 May 2019
0278-6915/

esophageal and liver cancers (Sun et al., 2007, 2011). Dietary fumonisin exposure in pregnant mothers has been linked to neural tube defects in infants (Missmer et al., 2005; Marasas et al., 2004). In the last decade, studies have associated fumonisin exposure with growth impairment in children (Kimanya et al., 2010; Shirima et al., 2015; Chen et al., 2018a; Chen et al., 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 (JECFA, 2018). A study in broilers indicated that co-exposure to aflatoxin and fumonisin had additive effects on body weight, liver structure and immunological response (Tessari et al., 2006). In a recent study, oral doses of pure aflatoxin and fumonisin in mice resulted in increased relative spleen weight and increased activity of enzymes that lead to oxidative stress, in a potentiating manner (Abbes et al., 2016). In a rat feeding study, exposure to pure aflatoxin or fumonisin 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 (Qian et al., 2016). Taken together, these studies suggest the possibility of increased hepatocarcinogenicity from co-exposure to aflatoxins and fumonisins (JECFA, 2018; JECFA, 2017; JECFA, 1998). The exact mechanism on how aflatoxins and fumonisins interaction leads to toxicity is not very clear yet. However, a previous rat study suggest that co-exposure may result in a decreased excretion of AFB1 through the urine and increased levels of serum AFB_1 –albumin adduct that forms the reactive AFB_1 -8,9-epoxide intermediates which ultimately leads to hepatocarcinogenicity (JECFA, 2017; Mitchell et al., 2014). Recent studies also show that chronic exposure to high levels of fumonisins may result in inhibition of ceramide synthase (Riley et al., 2015). This, in addition to increased sphingosine kinase activity, could enhance the development and progression of several human tumors (Espaillat et al., 2015); and possibly promote the tumorigenic potential of AFB_1 initiated DNA damage (JECFA, 2017).

Previous toxicological studies show solid evidence about the adverse human health effects from the consumption of aflatoxins. According to a dose response approach, it is estimated that 25,200–155,000 cases of liver cancer globally may be associated to aflatoxin exposure every year (Liu and Wu 2010). Even though the evidence for adverse health effects from fumonisin consumption in humans is currently not very conclusive, there are concerns that it may contribute to various serious adverse health outcomes including cancer and birth defects (WHO, 2018). Developing countries such as Nigeria are more at risk due to the climatic and crop storage conditions favoring the fungal growth and mycotoxin production. In addition, maize is often mixed with other commodities in the production of food and feed. These all create many opportunities for aflatoxin and fumonisin contamination during the production, handling, and storage of maize products.

Furthermore, the prevalence of chronic hepatitis B viral infection in Nigeria is also very high: about 12.2% (Olayinka et al., 2016). Since dietary exposure to aflatoxins among Nigerians is very likely, 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 μg/kg (SON, 2008). However, fumonisin levels are not known to be regulated in food and feed in Nigeria.

Maize is an essential crop for food security in Nigeria as well as an industrial crop (USDA, 2014). Maize in Africa is frequently contaminated with both aflatoxins and fumonisins (Kimanya et al., 2008). Nigeria, Africa's most populous nations is a major maize producer on the continent, second to South Africa (FAOSTAT, 2017). Over 75% of Nigeria's maize is consumed by humans, as maize is a staple of the Nigerian diet (USDA, 2014). 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 300,000 to 1.8 million tons: a 600% increase (Liverpool-Tasie et al., 2017).

Despite the health risks associated with co-occurrence of aflatoxins and fumonisins in diets, few 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. Most studies on mycotoxins explore their prevalence (and/or strategies to reduce them) at particular nodes (e.g. on farms or in food). Very few consider how the structure of commodity supply chains and their interconnectedness to other commodity value chains during conversion to food and feed could affect mycotoxin prevalence. This is important because the maize value chain in Nigeria (as in many parts of Africa) is often a long and fragmented supply chain with many players involved (Liverpool-Tasie et al., 2017).

Since several previous studies demonstrated how both of these mycotoxins, alone and in concomitance are real concerns in toxicology, the aim of this study was to determine the extent of occurrence and co-occurrence of aflatoxins and fumonisins in the supply chain of Nigerian maize and maize-based products for both human consumption and animal feed.

2. Materials and methods

2.1. 2Study area

In this study, the occurrence and co-occurrence of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and fumonisins (FB_1, FB_2 and FB_3) 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 and maize traders (after different lengths of storage), feed millers (maize and final feed) and retailers of maize based products.

The study area is Oyo State in Southwest Nigeria (Fig. 1). Oyo State covers over 28,000 square kilometers with geographic coordinates 8°00′N 4°00′E. We selected his area (See Fig. 1) 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 (USDA, 2018; Miller et al., 2006). Thus, this zone of the country is a major driver of increased maize demand (for animal feed) in the country. Second, the study area has a higher probability of human exposure to dietary mycotoxins. The majority of the maize in Nigeria is produced in the north, and then is moved 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. Study samples were collected from farmers, traders, feed millers and retailers with appropriate institutional review board protocol.

2.2. Sampling of maize and maize products

Within the state, supply chain segments were selected based on their role within the maize-poultry value chain. Thus the specific local government areas for each node reflect the major source of the maize based product in the state. More details are provided for each node in the subsections below.

Farmer's sample. Farmers from two local government areas (LGAs: the third level of government administration in Nigeria, similar to counties in the USA) of Oyo State, Atisbo and Saki West, were selected for the samples of maize (Table 1).

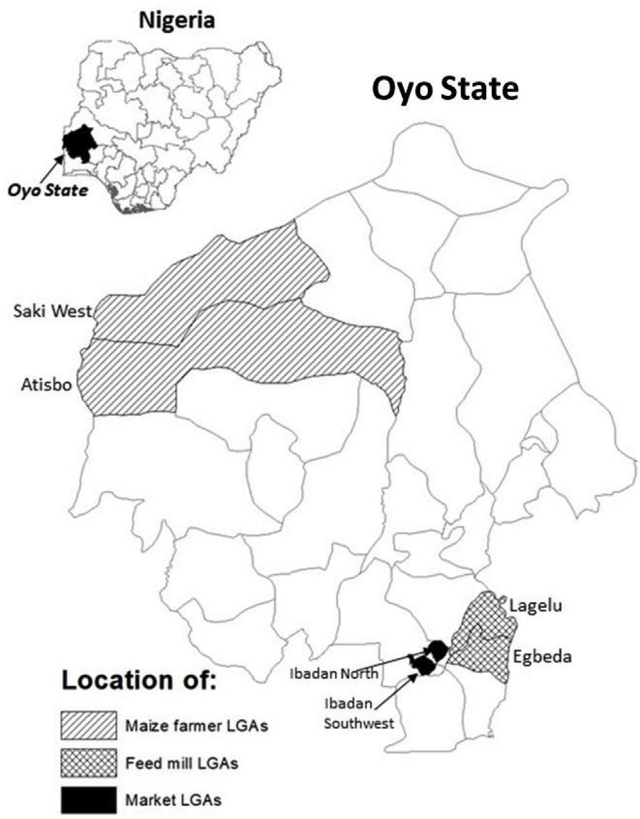

Fig. 1. Map of study locations.

Table 1
De-identified farmer maize samples and duration of maize storage in major maize producing local government areas.

Local government	Serial number of farmers	Number of maize samples	Samples collected
Saki West	1	2	Stored
	2	2	Stored
	3	4	Stored
	4	2	Stored
	5	2	Stored
	6	4	Field/stored
	7	4	Field/stored
	8	2	Stored
	9	4	Field/stored
	10	2	Stored
	11	2	Stored
	12	2	Stored
Atisbo	1	2	Stored
	2	4	Field/stored
	3	4	Field/stored
	4	2	Stored
	5	4	Field/stored
	6	4	Field/stored
	7	2	Stored
	8	2	Stored
	9	2	Stored
	10	2	Stored
	11	2	Stored
	12	4	Field/stored
	13	1	Stored
	14	1	Stored
	15	1	Stored
	16	1	Stored
	17	1	Stored
Total		**71**	

These two LGAs are the major maize producing LGAs in the state according to the Ministry of Agriculture. In each LGA, 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. The samples were collected in two batches; first in January, 2018 then in March, 2018. At least two samples (from different points in time) were collected from each farmer giving 71 maize samples with 0–4 months of storage (see Table 1). The maize grain from the 20 cobs was shelled, hand-mixed and 500 g 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). 500 g of each maize grain were grounded separately with a milling machine and subsamples of 50 g were further taken from the lots and placed in a well-sealed and labeled polythene bag for mycotoxin analysis. Samples were stored at 4 °C 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 (adjacent to but outside the actual market). Fifteen maize wholesalers were randomly selected from the three markets; five in each market. Samples consisting of 500 g maize grain were purchased from the sellers. The maize grains were ground separately with a milling machine and subsamples of 50 g were further taken from the lots and placed in a well-sealed and labeled polythene bag for mycotoxin analysis. Samples were stored at 4°c 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 500 g of finished feed and maize grain from the batch of maize used for producing the feed was collected from the feed-mills. Majority of these feed mills (90%) purchased their maize from the main maize producing regions of the state or the wholesale markets. 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 50 g 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 4 °C prior to analyses.

Maize based processed products. 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.

2.3. Mycotoxin analysis of maize samples

The maize samples were analyzed at Romer' lab (USA) using liquid chromatography tandem mass spectrometry (LC-MS/MS) for AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB_2 and FB_3. 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 g were weighed and extracted with 20 ml of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). For spiking experiments, 20 µl for AFB_1, AFB_2, AFG_1, AFG_2 and 50 µl for FB_1, FB_2 and FB_3 of the combined working solutions were consecutively added to 0.25 g of each 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. The filtered sample extract was diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). The samples (except FB_1, FB_2 and FB_3) were extracted, pushed through a Romer 228 MycoSep clean-up column, dried down, and reconstituted in internal standard out of which 40 µl were injected into the LC-MS/MS instrument. The fumonisin samples did not undergo any clean-up step. 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, 150 mm × 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. The analysis for all the mycotoxins were done in positive ion mode. For mobile phase A, DI H2O/formic acid with 1.2612 g ammonium formate was used as a solvent. Acetonitrile was used as the solvent for mobile phase B. Mycotoxin analyte identifications were confirmed by the acquisition of two MS/MS transition yielding 4 identification points. These are AFB_1 parent ion: 313.1 m/z; product ions: 241.1 m/z and 285.0 m/z, AFB_2 parent ion: 315.2 m/z; product ions: 287.0 m/z and 259.0 m/z, AFG_2 parent ion: 329.1 m/z; product ions: 243.1 m/z and 115.1 m/z, AFG_2 parent ion: 331.1 m/z, product ions: 313.0 m/z and 115.1 m/z, FB_1 parent ion: 722.4 m/z; product ions: 334.4 m/z and 352.4 m/z, FB_2 parent ion: 706.4 m/z; product ions: 336.4 m/z and 318.4 m/z, FB_3 parent ion: 706.3 m/z; product ions: 336.4 m/z and 318.5 m/z.

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 $p < 0.05$ was considered to be statistically significant for all the statistical tests.

3. Results

3.1. Farmers' samples

Table 2 shows how aflatoxin and fumonisin levels change over time in farmers' stored maize grain, from harvest through to four months and more of storage.

As these results show, while levels of each of the aflatoxins generally increased with increasing amounts of time in storage, levels of each of the fumonisins generally decreased over time. Furthermore, while fumonisin stayed at levels generally considered safe during the duration of storage time measured, the same cannot be said for total aflatoxins. Aflatoxin levels at four months or longer in storage were exceedingly high: about 250 µg/kg, over all acceptable limits set for human food by nations worldwide (FAO, 2004).

Table 3 shows the total 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 ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) in the samples tends to increase with time of storage. The geometric mean of total aflatoxin level at harvest was 4.2 µg/kg, but after 4 months of storage, the level went up to 42.7 µg/kg: much higher than the Nigerian maximum total aflatoxin regulatory limit in maize of 4 µg/kg. At harvest, 37.5% of the samples had aflatoxin levels more than 4 µg/kg and after 4 months of storage 87.5% of the samples had aflatoxin levels exceeding 4 µg/kg. The geometric mean levels of total aflatoxin in the samples at different storage times were statistically significantly different ($p < 0.05$) (Table 6); higher aflatoxin levels with higher storage time. Notably, at the higher end of ranges in maize stored for four months or longer, aflatoxin levels were found to be so high as to be dangerous in causing acute toxicity in humans or animals.

However, the total fumonisin levels ($FB1 + FB_2 + FB_3$) do not follow any specific pattern with length of storage time. The highest geometric mean level of total fumonisin was observed in samples collected at harvest (1682 µg/kg); 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 µg/kg (USFDA, 2000). The geometric means of total fumonisin level across the groups were not significantly different ($p > 0.05$) (Table 7).

3.2. Maize from local maize traders

Table 4 panel A shows the total aflatoxin ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) and fumonisin ($FB1 + FB_2 + FB_3$) 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 µg/kg but after 2 weeks of storage, the level went up to 5.6 µg/kg. 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 6). The geometric mean level of total fumonisin in samples collected at 1 week was 665 µg/kg and 677 µg/kg at 2 weeks which were both lower than the European Union (EU) regulatory limit of 1000 µg/kg and according to Mann-Whitney *U* test, the geometric means of total fumonisin level cross the groups were not significantly different ($p > 0.05$) (Table 7).

Table 2
Geometric mean levels of each of the aflatoxins and fumonisins in farmers' maize samples, from harvest to four months and more in storage.

Months in storage	Number of samples	AFB_1 (µg/kg)	AFB_2 (µg/kg)	AFG_1 (µg/kg)	AFG_2 (µg/kg)	FB_1 (µg/kg)	FB_2 (µg/kg)	FB_3 (µg/kg)
Harvest (0)	8	1.40	0.60	1.12	0.80	765	562	191
1	10	2.28	0.73	0.80	0.80	462	175.0	76.3
2	19	4.27	0.79	1.10	0.93	390	190	78.9
3	24	12.2	1.25	1.04	0.83	689	223.0	93.7
4	8	27.9	3.27	2.67	1.35	745	299	96.5

Table 3
Total aflatoxin and fumonisin levels (geometric mean and range) in maize stored for various lengths of time in farmers' households, Nigeria.

Months in storage	Mean total aflatoxin (μg/kg)	Range (μg/kg)	% > 4 μg/kg aflatoxin	Mean total fumonisin (μg/kg)	Range (μg/kg)	% > 2000 μg/kg fumonisin
Harvest (0)	4.2	2.7–26.5	37.5	1680	650–5800	37.5
1	5.3	2.7–42.5	50.0	671	200–3000	20.0
2	8.8	2.7–414	63.2	747	150–2300	21.0
3	17.5	2.7–180	91.7	1050	150–5800	20.8
4	42.7	2.7–1460	87.5	1230	650–2500	25.0

Table 4
Aflatoxin and fumonisin levels (geometric mean and range) in maize flour samples collected from maize traders and poultry feed millers.

Maize flour storage time	No. of samples	Mean total aflatoxin (μg/kg)	Range (μg/kg)	Mean total fumonisin (μg/kg)	Range (μg/kg)
Maize traders (Panel A)					
1 week	9	3.0	2.7–7.9	665	350–900
2 weeks	5	5.6	2.7–54.9	677	150–2100
Feed millers (Panel B)					
Maize in storage	10	3.1	2.7–6.8	1410	850–4400
Final feed	10	59.7	20.3–297	819	150–4600

3.3. Maize samples from feed millers

Table 4 panel B shows the total aflatoxin (AFB_1 + AFB_2 + AFG_1 + AFG_2) and fumonisin (FB1 + FB_2 + FB_3) 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 μg/kg) is much greater than that in the stored maize samples (3.1 μg/kg) and is statistically significantly different at $p < 0.05$ (Table 6). The geometric mean of total fumonisin level in the stored maize was 1040 μg/kg and 1330 μg/kg in the final feed, but the difference is not statistically significantly different ($p > 0.05$; Table 7).

3.4. Branded and non-branded maize-based food products

Table 5 shows the total aflatoxin (AFB_1 + AFB_2 + AFG_1 + AFG_2) and fumonisin (FB1 + FB_2 + FB_3) 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 μg/kg) is lower than that in the non-branded maize snack – corn roll (6.8 μg/kg). 4 out of the 34 (11.8%) 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 ($P < 0.05$).

The geometric mean total fumonisin level is also higher in the non-branded snacks (335 μg/kg) compared to branded snacks (0–94 μg/kg). Though the mean levels in both groups were much lower than the US regulatory limits for fumonisins, the difference is statistically significant.

As shown in Fig. 2, 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 μg/kg 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 μg/kg, as shown in Fig. 3.

4. Discussion

This work demonstrates the significant occurrence and co-occurrence of two important mycotoxins – aflatoxins and fumonisins – in Nigerian maize and maize products. This finding is important because maize is a staple food in Nigeria and many other sub-Saharan African nations, and these two toxins individually pose significant human health risks that may be increased by their co-occurrence in diets. Moreover, the co-occurrence is at multiple stages along the value chain of Nigerian maize: from harvest to postharvest storage to processing and final food and feed products in the marketplace.

The aflatoxin levels in samples collected from maize farmers indicate an increase in the aflatoxin levels with increasing time of storage. On average, total aflatoxins in farmer's samples stored for over 2 months–4 months exceeded the Nigerian regulatory limits for aflatoxins: 4 μg/kg, which is also considered unacceptable by European regulatory standards (EUC, 2006). There is no significant difference in 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 than the US regulatory limits for fumonisin (2000 μg/kg). The mean of total aflatoxin level in the samples collected from maize traders that are stored for two weeks is greater than the mean of samples stored for one week. This finding supports previous studies that show aflatoxin levels increase with the time of storage in hot and humid countries as the combination of heat and dampness favors the growth of *Aspergillus* fungi, which produce aflatoxins (Villers, 2014). The total fumonisin levels in the samples collected both at one and two weeks of storage did not change as much.

The samples collected from feed millers demonstrate that even though mean levels of total aflatoxins in stored maize is low, the levels in the final feed is significantly higher. The drastic increase in aflatoxins might be because other ingredients such as groundnut cake, which may also have aflatoxin contamination, are added to the 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 ppm in human food. The results from maize farmers and traders further confirm the potential for aflatoxin contamination during storage. This implies that efforts to reduce exposure

Table 5
Aflatoxin and fumonisin levels in branded vs non-branded snacks.

	Sample type	No. of samples	Mean total aflatoxin (μg/kg)	Range (μg/kg)	% > 4 μg/kg aflatoxin	Mean total fumonisin (μg/kg)	Range
Non-branded	Corn roll	10	6.8	4.0–10.9	80.0	311	150–1050
Branded	Cereal mix	20	3.1	2.7–5.3	20.0	195	150–400
	Custard	14	2.7	2.7–2.7	0	150	150–150

Table 6
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	0.00330	-	-
Trader's flour (1 week–2 weeks storage)	Mann-Whitney U	0.424	16	−0.8
Feed millers (stored maize to final feed)	Mann-Whitney U	0.000180*	0	−3.74
Branded and non-branded maize snacks	Mann-Whitney U	< 0.0000100*	8	−4.52

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

Table 7
Statistical analyses for fumonisin levels across the groups.

Group	Statistical test used	P-value	U-value	Z-score
Farmer's flour (harvest to 4 months storage)	Kruskal-Wallis	0.125	-	-
Trader's flour (1 week–2 weeks storage)	Mann-Whitney U	0.944	21.5	−0.0670
Feed millers (stored maize to final feed)	Mann-Whitney U	0.197	32.5	1.29
Branded and non-branded maize snacks	Mann-Whitney U	0.0128*	80.5	−2.49

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

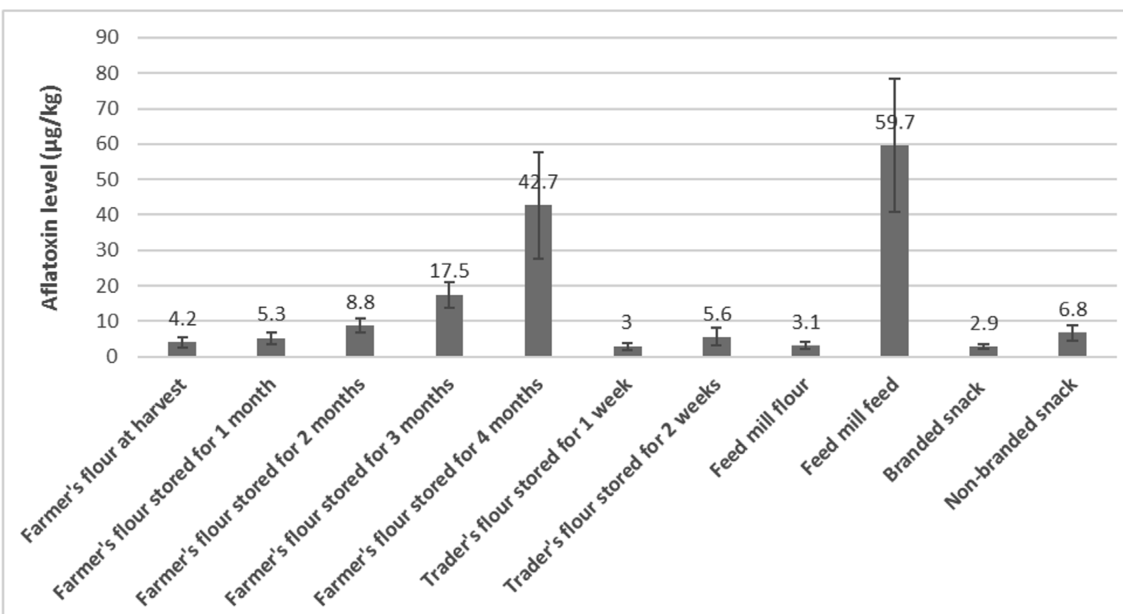

Fig. 2. Geometric means of total aflatoxin levels in Nigerian maize and maize products.

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.

The feed mill results also reveal the interrelated nature of food supply chains. Issues of food and feed contamination require attention to be paid to related supply chains. Even though feed millers make efforts to secure a safe input (the mean aflatoxins levels in their maize was lower than the recommended levels), this does not guarantee a safe final feed product. Focusing exclusively on the maize supply chain does not necessarily guarantee improved safety of maize based products when combined with other ingredients, such as groundnuts in the case of feed.

In 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. Eighty percent 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, if compared to USFDA regulatory limits.

This study confirms that aflatoxins and fumonisins are prevalent contaminants of maize for human consumption and animal feed in Nigeria. A significant fraction (52%, 76 out of 147 samples collected) of maize and maize products was contaminated with aflatoxin levels above the Nigerian maximum tolerable limit. In terms of fumonisins, 13% (19 out of 147 samples) of the total samples collected contained levels higher than the US regulatory limit of 2000 µg/kg. Regular routine checks by the Directorate of Food Safety and Nutrition (the directorate of the National Agency for food and drug administration agency mandated for such oversight) is still needed for the proper enforcement of existing standards. There is also a need for more oversight on fumonisins. This includes setting and enforcing standards on appropriate fumonisin levels.

Feasible and cost-effective methods to reduce aflatoxin risk in preharvest, postharvest, dietary, and clinical settings have been developed (Khlangwiset and Wu, 2010). 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). The International Institute of Tropical Agriculture (IITA) in Nigeria, along with other institutions worldwide such as the US Department of Agriculture, have

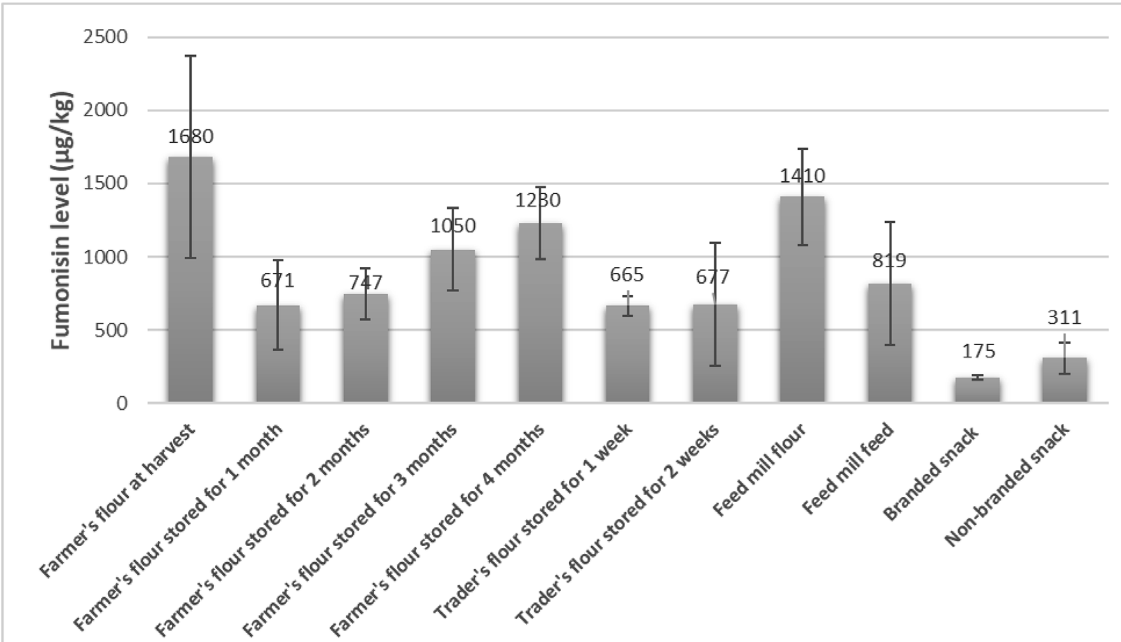

Fig. 3. Geometric means of total fumonisin levels in Nigerian maize and maize products.

worked on – among other strategies – developing aflatoxin-resistant maize hybrids with demonstrated efficacy in field conditions; although to date, none of these strains have been marketed (Brown et al., 2013). The absence of a price premium to compensate for investing in such technologies (e.g. AflaSafe, a biocontrol developed by IITA) limits their adoption in Nigeria (Ayedun et al., 2017). However, using such technologies alone is not enough to guarantee a safe maize product. In the absence of proper storage and handling practices or without taking into account the mycotoxin levels of other commodities mixed with maize in the production of final feed or food products, aflatoxin and fumonisin are likely to remain food safety challenges in maize based products. Thus, 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.

Funding information

This work was supported by the United States Agency for International Development (USAID) Feed the Future Initiative, and the United States Department of Agriculture (USDA) Hatch Fund Grant MICL02527. The contents are the responsibility of the study authors and do not necessarily reflect the views of USAID, USDA, or the United States Government. The authors would also like to acknowledge financial support of Michigan State University AgBioResearch.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.008

References

Abbes, S., Ben Salah-Abbès, J., Jebali, R., Younes, R.B., 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, 46–54.

Alshannaq, A., Yu, J.H., 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. Int. J. Environ. Res. Public Health 14, 632.

Ayedun, B., Okpachu, G., Manyong, V., Atehnkeng, J., Akinola, A., Abu, G.A., Bandyopadhyay, R., Abdoulaye, T., 2017. An assessment of willingness to pay by maize and groundnut farmers for aflatoxin biocontrol product in northern Nigeria. J. Food Prot. 80, 1451–1460.

Bondy, G.S., Pestka, J.J., 2000. Immunomodulation by fungal toxins. J. Toxicol. Environ. Health B Crit. Rev. 3, 109–143.

Brown, R.L., Menkir, A., Chen, Z.Y., Bhatnagar, D., Yu, J., Yao, H., Cleveland, T.E., 2013. Breeding aflatoxin-resistant maize lines using recent advances in technology – a review. Food Addit. Contam. 30, 1382–1391.

Chen, C., Mitchell, N.J., Gratz, J., Houpt, E.R., Gong, Y., Egner, P.A., Groopman, J.D., Riley, R.T., Showker, J.L., Svensen, E., Mduma, E.R., 2018a. Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania. Environ. Int. 115, 29–37.

Chen, C., Riley, R.T., Wu, F., 2018. Dietary fumonisin and growth impairment in children and animals: a review. Compr. Rev. Food Sci. Food Saf. 17, 1448–1464.

Dorner, J.W., 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.

Espaillat, M.P., Shamseddine, A.A., Adada, M.M., Hannun, Y.A., Obeid, L.M., 2015. Ceramide and sphingosine-1-phosphate in cancer, two faces of the sphinx. Transl. Cancer Res. 4, 484–499.

EUC, 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.

Eze, U.A., Routledge, M.N., Okonofua, F.E., Huntriss, J., Gong, Y.Y., 2018. Mycotoxin exposure and adverse reproductive health outcomes in Africa: a review. World Mycotoxin J. 11, 321–339.

FAO (Food and Agriculture Organization of the United Nations), 2004. Worldwide Regulations for Mycotoxins in Food and Feed in 2003. FAO, Rome, Italy.

FAOSTAT, 2017. Food and Agricultural Commodities Production. FAO Statistics Retrieved from. http://fao.org/faostat/en/#data/QC.

IARC, 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In: International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans, vol 82. pp. 1–556.

JECFA, 2018. Co-exposure of fumonisins with aflatoxins. Food Safety Digest February 2018, World Health Organization, WHO/NHM/FOS/RAM/18.3. Retrieved from: http://www.who.int/foodsafety/Food_Safety_Digest_Fumonisins_aflatoxins_EN.pdf.

JECFA, 2017. Eighty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Evaluation of Certain Contaminants in Food, Rome, Italy. https://apps.who.int/iris/bitstream/handle/10665/254893/9789241210027-eng.pdf;jsessionid=22FD197AE7E749B139F5905FC63D6854?sequence=1.
JECFA, 1998. Forty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety Evaluation of Certain Food Additives and Contaminants in Food. Aflatoxins, Geneva, Switzerland.
Khlangwiset, P., Shephard, G.S., Wu, F., 2011. Aflatoxins and growth impairment: a review. Crit. Rev. Toxicol. 41, 740–755.
Khlangwiset, P., Wu, F., 2010. Costs and efficacy of public health interventions to reduce aflatoxin-induced human disease. Food Addit. Contam. 27, 998–1014.
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 Addit. Contam. 25, 1353–1364.
Kimanya, M.E., De Meulenaer, B., Roberfroid, D., Lachat, C., Kolsteren, P., 2010. Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. Mol. Nutr. Food Res. 54, 1659–1667.
Liverpool-Tasie, L.S.O., Omonona, B., Sanou, A., Ogunleye, W., Padilla, S., Reardon, T., 2017. Growth and transformation of chicken and eggs value chains in Nigeria. Nigerian J. Agric. Econ. 7, 1–15.
Liu, Y., Wu, F., 2010. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ. Health Perspect. 118, 818–824.
Marasas, W.F., 2001. Discovery and occurrence of the fumonisins: a historical perspective. Environ. Health Perspect. 109 (Suppl. 2), 239.
Marasas, W.F., Riley, R.T., Hendricks, K.A., Stevens, V.L., Sadler, T.W., Gelineau-van Waes, J., 2004. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. J. Nutr. 134, 711–716.
McMillan, A., Renaud, J.B., Burgess, K.M., Orimadegun, A.E., Akinyinka, O.O., Allen, S.J., Miller, J.D., Reid, G., Sumarah, M.W., 2018. Aflatoxin exposure in Nigerian children with severe acute malnutrition. Food Chem. Toxicol. 111, 356–362.
Miller, J., Atanda, T., Asala, G., Chen, W.H., 2006. Integrated irrigation-aquaculture opportunities in Nigeria: the special programme for food security and rice-fish farming in Nigeria. In: Halwart, M., van Dam, A.A. (Eds.), Integrated Irrigation and Aquaculture in West Africa: Concepts, Practices and Potential. FAO, Rome, pp. 117–124 181pp.
Missmer, S.A., Suarez, L., Felkner, M., Wang, E., Merrill Jr., A.H., Rothman, K.J., Hendricks, K.A., 2005. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environ. Health Perspect. 114, 237–241.
Mitchell, N.J., Xue, K.S., Lin, S., Marroquin-Cardona, A., Brown, K.A., Elmore, S.E., Tang, L., Romoser, A., Gelderblom, W.C., Wang, J.S., Phillips, T.D., 2014. Calcium montmorillonite clay reduces AFB1 and FB1 biomarkers in rats exposed to single and co-exposures of aflatoxin and fumonisin. J. Appl. Toxicol. 34, 795–804.
Olayinka, A.T., Oyemakinde, A., Balogun, M.S., Ajudua, A., Nguku, P., Aderinola, M., Egwuenu-Oladejo, A., Ajisegiri, S.W., Sha'aibu, S., Musa, B.O., Gidado, S., 2016. Seroprevalence of hepatitis B infection in Nigeria: a national survey. Am. J. Trop. Med. Hyg. 95, 902–907.
Paterson, R.R.M., Lima, N., 2017. Thermophilic fungi to dominate aflatoxigenic/mycotoxigenic fungi on food under global warming. Int. J. Environ. Res. Public Health 14, 199.
Qian, G., Tang, L., Lin, S., Xue, K.S., Mitchell, N.J., Su, J., Gelderblom, W.C., Riley, R.T., Phillips, T.D., Wang, J.S., 2016. Sequential dietary exposure to aflatoxin B1 and fumonisin B1 in F344 rats increases liver preneoplastic changes indicative of a synergistic interaction. Food Chem. Toxicol. 95, 188–195.
Riley, R.T., Torres, O., Matute, J., Gregory, S.G., Ashley-Koch, A.E., Showker, J.L., Mitchell, T., Voss, K.A., Maddox, J.R., Gelineau-van Waes, J.B., 2015. Evidence for fumonisin inhibition of ceramide synthase in humans consuming maize-based foods and living in high exposure communities in Guatemala. Mol. Nutr. Food Res. 59, 2209–2224.
Shephard, G.S., 2008. Impact of mycotoxins on human health in developing countries. Food Addit. Contam. 25, 146–151.
Shirima, C.P., Kimanya, M.E., Routledge, M.N., Srey, C., Kinabo, J.L., Humpf, H.U., Wild, C.P., Tu, Y.K., Gong, Y.Y., 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.
Smith, L.E., Prendergast, A.J., Turner, P.C., Humphrey, J.H., Stoltzfus, R.J., 2017. Aflatoxin exposure during pregnancy, maternal anemia, and adverse birth outcomes. Am. J. Trop. Med. Hyg. 96, 770–776.
SON, 2008. National Industrial Standard. https://www.tobaccocontrollaws.org/files/live/Nigeria/Nigeria%20-%20NIS%204632008%20-%20national.pdf.
Sulyok, M., Krska, R., Schuhmacher, R., 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. Anal. Bioanal. Chem. 389, 1505–1523.
Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., Tang, L., Gao, W., Wang, J.S., 2007. Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. Food Addit. Contam. 24, 181–185.
Sun, G., Wang, S., Hu, X., Su, J., Zhang, Y., Xie, Y., Zhang, H., Tang, L., Wang, J.S., 2011. Co-contamination of aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. Food Addit. Contam. 28, 461–470.
Tessari, E., Oliveira, C., Cardoso, A., Ledoux, D., Rottinghaus, G., 2006. Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Br. Poult. Sci. 47, 357–364.
USDA, 2014. Nigeria grain and feed annual report. Global Agriculture Information Network. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Lagos_Nigeria_3-13-2014.pdf.
USDA, 2018. Nigeria grain and feed annual report. Global Agriculture Information Network. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Lagos_Nigeria_4-12-2018.pdf.
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.
Villers, P., 2014. Aflatoxins and safe storage. Front. Microbiol. 5, 158.
WHO, 2018. Fumonisins. Food Safety Digest. https://www.who.int/foodsafety/FSDigest_Fumonisins_EN.pdf.
Wild, C.P., Miller, J.D., Groopman, J.D., 2015. Mycotoxin Control in Low-And Middle-Income Countries. International Agency for Research on Cancer, Lyon, France Working Group Report 9.
Wu, F., Groopman, J.D., Pestka, J.J., 2014. Public health impacts of foodborne mycotoxins. Ann. Rev. Food Sci. Technol. 5, 351–372.
Wu, F., Stacy, S.L., Kensler, T.W., 2013. Global risk assessment of aflatoxins in maize and peanuts: are regulatory standards adequately protective? Toxicol. Sci. 135, 251–259.