

Aflatoxin contamination of locally-processed cereal-based complementary foods in Tanzania

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SUMMARY

Feeding children with cereal-based foods has potential to expose them to aflatoxins (AFs). This study was conducted to determine the occurrence and levels of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) in 64 commercial locally produced cereal-based complementary foods obtained from producers and popular markets in the two cities of Dar es Salaam (DSM) and Arusha. The High Performing Liquid Chromatographic method was employed to determine level of the toxin in the samples. Aflatoxin was detected in 38 (59.4%) samples, consisted of 21 (70%) Arusha samples (with mean aflatoxin concentration of 11.0 µg/kg; range: 0.2–23.2 µg/kg) and 17 (50%) Dar es Salaam samples (mean aflatoxin concentration of 8.4 µg/kg; range: 2.2–17.5 µg/kg). Considering the standard limit, 19 samples (30%) had levels above the maximum tolerance limit for total Aflatoxin (10 µg/kg). Aflatoxin B₁ was detected in 35 samples (55%) with a mean concentration of 1.6 µg/kg (range: 0.1–10.9). Two samples (3.3%) had AFB₁ well beyond the maximum tolerable limit. However, considering the EU limit (0.1 µg/kg), the figure increased to 31 (48%) for all samples, with Arusha leading with 19 samples (63%). The results indicated that the contamination with AFB₁ in such a level could pose health problems to consumers. Further studies need to be conducted to assess the multi-occurrence of mycotoxins, exposure of infants to aflatoxins and associated risks of consumption of cereal-based complementary foods in Tanzania.

Keywords: Aflatoxin, *Aspergillus*, Complementary Foods, Contamination.

INTRODUCTION

Aflatoxin are the potent toxic, mutagenic, heterogenic and carcinogenic metabolites produced by species of *Aspergillus flavus*, *A. paraciticus* and *A. nomius* in food and feed ,especially maize, oil seeds and their products, both at pre- and post harvest conditions (Banu and Muthumary, 2010; Imperatos *et al.*, 2011; Zinedine and Manes, 2009). Aflatoxins consist of four naturally occurring compounds namely aflatoxin B₁, B₂, G₁ and G₂ which are biosynthesized by the toxigenic stains of *A.flavus*, *A. parasiticus* and *A. nominius*, with *A.flavus* exclusively producing the B aflatoxins and the other responsible for aflatoxins B and G production (Bittencourt *et al.*, 2005; CiSmileanu *et al.*, 2008; Sidhu *et al.*, 2009; Fallah, 2010; Mardan *et al.*, 2011). Among the four groups of these aflatoxins, AFB₁ has been extensively studied and has shown to be the most potent toxic of all aflatoxins and they were classified by the International Agency on Research in Cancer as Group 1 human carcinogens (Turner *et al.*, 2003; McKean *et al.*, 2006; El-Sayed and Khalil, 2009; Mardan *et al.*, 2011; Cano-Sancho *et al.*, 2012a).

The presence of these toxins in food or feed may pose significant health risks in human and animals, as well as huge economic losses to the producers (Turner*et al.*, 2003; Sanchez*et al.*, 2005; Griessler *et al.*, 2010). Hepatocellular carcinoma or liver cancer has been highly linked to aflatoxins exposures and is mostly prevalent in areas with conducive environment such as high humidity, high temperature and poor

storage conditions, especially in Africa and Some part of Asia, where crops are exposed to fungal contaminations, resulting into aflatoxins production (Kaaya and Kyamuhangire, 2006; Banu and Muthumary, 2010; Imperatos *et al.*, 2011). Contamination of cereals and grains especially maize with *A. flavus* and subsequent production of aflatoxins during storage is considered as one of the most serious safety problems throughout the world and given the facts that it is the main staples and major ingredient in weaning food formulations in developing countries (Bankole and Adebajo, 2003; Williams *et al.*, 2004; Van Egmond *et al.*, 2007; Ibanez-Vea *et al.*, 2011). According to Wagacha and Muthoni (2008), human can be exposed to aflatoxins by the periodic consumption of contaminated foods, contributing to an increase in nutritional deficiencies, immunosuppression and hepatocellular carcinoma.

Aflatoxins is reported to be associated with impaired childhood growth and development among children fed contaminated food (Gong *et al.*, 2004; Egal *et al.*, 2005; Kimanya *et al.*, 2009) which may reduce their productivity in adult life and reduces country's human resources. Despite the public health significance of mycotoxins and aflatoxins in particular to consumers, there are limited studies on aflatoxins occurrence and contamination in cereals and cereal-based products such as complementary foods in Tanzania, although the contamination levels reported in maize and groundnuts are significantly high (URT, 1989; KDAO, 2009; Kimanya *et al.*, 2008; 2009).

The objective of this study was

therefore to assess the occurrence and contamination of aflatoxins in locally processed cereal-based complementary foods. The findings of this study are very important to policy makers, control authorities and food producers to put more efforts and strategies in protecting the health of the population, particularly young children.

MATERIALS AND METHODS

Study area

The study was conducted in Arusha and Dar es Salaam, Tanzania between July 2011 and March 2012. Arusha region is located in the Northern-eastern part of Tanzania. It lies south of the equator between latitude 2° and 6° and longitudinally the region is situated between 35° and 38° east of Greenwich meridian. It is a cool dry air with temperature ranging from 13 to 30°C, with average annual temperature of 21°C. Dar es Salaam is located between latitudes 6°36' and 7° south of Equator and longitudes 33°33' and 39° east of Greenwich. It is bordered by the Indian Ocean to the East and by the Coast region on the other sides. It is generally hot and humid (67- 96%) throughout the year with an average temperature of 29°C.

Samples

A total of 64 samples of cereal-based complementary foods were purchased randomly from producers, retail shops and large supermarkets in Arusha and Dar es Salaam cities. Samples weighed between 500 g and 1000 g and were collected, transported and stored under cool conditions and away from direct

sunlight until analysis.

Analytical method

Mycosep® 228 Aflapat immunoaffinity cleanup columns (Romer Labs® Washington, MO, USA) were used to extract AFB₁, AFB₂, AFG₁ and AFG₂) from all analytical samples. Twenty-five grams of homogenized sample was mixed with 100 mL of extractant solution (84% Acetonitrile, 16% water) for 60 minutes at 500 rpm on a shaker, and filtered with Whatman filter paper (No. 4, Ø 150 mm). Six milliliters of filtrate was drained through Mycosep® 228 Aflapat columns and 4 mL of toxin extract were collected in vials. Then the content was dried in the thermo-evaporators under Nitrogen gas. The dried sample was dissolved into 400 µL of mobile phase (20% Methanol, 15% Acetonitrile, 65% water) and was vortexed for 2 minutes. HPLC system consisted of an Agilent 1100 HPLC system, equipped with a quaternary pump with vacuum degasser, a temperature controlled column oven and a fluorescence detector with time programming (Schimadzu Co. Tokyo, Japan). A post-column derivatization system was employed using a Cobra cell (R-Biopharm, Rhone Ltd., Darmstadt, Germany). Fluorescence detection was carried out at excitation and emission wavelengths of 365 nm and 435 nm for aflatoxins. Aflatoxins B₁, B₂, G₁ and G₂; columns A, B, C, D and E operated using a mobile phase of methanol, acetonitrile, water (20 + 15 + 65 vol/vol) + 119 mg KBr + 100 µl 65% AgNO₃ at a flow rate of 1 ml min⁻¹ operated at 40°C. The injection volume was 10 µL. All analytical chemicals were supplied by Sigma-Aldrich Company (Vienna,

Austria) and Aflatoxin standards were supplied by RomerLabs® Washington, MO, USA. Aflatoxin concentration was expressed in µg of aflatoxin per kg of each assayed food sample. Aflatoxin contamination was calculated by using the following formula:

$$\text{Aflatoxin conc (}\mu\text{g/kg)} = \left(\frac{C_{\text{samp}} \times \text{Solvent} \times \text{Elution} \times \text{Dilution}}{W \times \text{Aliquot}_{\text{ex}} \times \text{Aliquot}_{\text{iac}}} \right)$$

Where:

C_{samp} = Concentration of aflatoxin calculated from the graph; Solvent = Solvent taken for extraction; Elution = Final volume achieved after elution; Dilution = Volume achieved after dilution; $\text{Aliquot}_{\text{ex}}$ = Aliquot taken from extraction; $\text{Aliquot}_{\text{iac}}$ = Aliquot taken for clean-up column; W = weight of sample taken.

Validation of the method

Recovery, repeatability, limit of detection (LoD) and limit of quantification (LoQ) were carried out to validate the method used in this study. Selectivity was checked by injecting 10 µL of aflatoxin standard solution five times before extracted sample was injected. Comparison was made between the peak retention times and the fluorescence spectra of the substances, and the standard curves were produced by linear regression of concentration and peak areas. Recovery was established by determining AFB1, AFB2, AFG1 and AFG2 levels from spiked samples in triplicate. The mean retention time for aflatoxin B₁, B₂, G₁ and G₂ were 8.68, 9.95, 12.28 and 14.17 minutes, respectively. Recovery was determined by comparing the absolute responses of each aflatoxin with absolute responses of calibration

standards. Under these conditions, the mean recovery in spiked food samples was 82-95%, relative standard deviation (%RSD) 1.2- 4.7 %, LoD ranged from 0.001 to 0.009 µg/kg and LoQ was 0.01- 0.03µg/kg, as shown in Table 1.

Statistical analysis

Basic statistics were performed to determine mean, standard deviation, minimum, maximum, range and t-test was done to compare the means using Microsoft Excel software (2007 version) after triplicate injections, and the percentage, mean and standard deviation were calculated on spiked samples from three injections.

RESULTS

The occurrence and levels of aflatoxins in locally produced commercial cereal based complementary foods in Tanzania are presented in Tables 2-5. Aflatoxins B1 and G2 was detected in 55% of all samples analyzed whereas B₂ and G₁ contamination was 36 and 33%, respectively. Aflatoxin G2 had the highest range of occurrence (1.1-22.9 µg/kg) followed by Aflatoxin B1 (Table 2). Table 3 presents the results of AFB1 analysis in samples of complementary foods. Two samples (3.1%) exceeded the maximum legal limit for AFB1 set by Tanzania Food and Drugs Authority (TFDA). Considering the maximum limit for AFB1 set by the EU and US, 86% of all tested samples exceeded the limit.

Table 1. Recovery of different aflatoxins in spiked food samples

Aflatoxins	LoD/LoQ(μg/kg)	Spiking level (μg/kg)	Recovery (%)	RSDr (%)
AFB1	0.009/0.03	1.2 - 10.8	95±1.2	1.2
AFB2	0.003/0.01	0.3 - 2.7	82±5.7	6.9
AFG1	0.001/0.03	1.2 - 10.8	94±1.4	1.5
AFG2	0.006/0.02	0.3 - 2.7	89±4.2	4.7

Table 2. Aflatoxins B1, B2, G1 and G2 contamination in individual food samples

AF Type	Tested samples	Number positive samples	(%)	Mean±SD (μg/kg)	Range (μg/kg)
B1	64	35 (55)		1.6± 2.4	0.1-10.9
B2	64	23 (36)		1.0 ±1.6	0.1-7.0
G1	64	21 (33)		1.5±1.2	1.1-22.9
G2	64	35 (55)		7.6±5.7	0.1-4.1

Table 3. Occurrence of aflatoxin B1 in locally produced complementary foods from Arusha and DSM

Source	Samples tested	Positive samples, n (%)	Aflatoxin conc. (µg/Kg)	Range	Exceed Legal limit, n (%) ^a
			(Mean± SD)	Min - Max	TFDA EU
Arusha	30	19(63.0)	1.6 ± 2.2	0.2 - 8.6	1(3.3)
DSM	34	16(47.0)	1.7 ± 2.7	0.1 - 10.9	1(2.9)
Total	64	35(55.0)	1.6 ± 2.4	0.1 - 10.9	2(3.1)

^aLegal limit set at 5μg/Kg for TFDA and 0.1 μg/Kg for EU
Among the two locations sampled, Arusha had the highest aflatoxin B₁ contamination (63%) with levels exceeding the EU maximum tolerable limit than those from Dar es Salaam (35%). One sample (2%) from Dar es Salaam had more than 2-fold aflatoxin B₁ contamination level (10.9μg/kg) than

the TFDA set limit whereas it was more than 100-fold the EU limit for cereal based complimentary foods. Overall, nearly half of all samples exceeded the EU standards for maximum limit for AFB₁. However, the level of aflatoxin B₁ distribution indicated that 48% of samples had B₁ levels between 0.1 and 4.0 μg/kg and less than 4% were close to the maximum limit as per TFDA

(Table 4).

About 60 percent of all samples were contaminated with aflatoxins (B_1 , B_2 , G_1 and G_2) with levels ranging from 0.2 to 23.2 $\mu\text{g/kg}$. Thirty percent of all samples exceeded the 10 $\mu\text{g/kg}$ for AFT set by National Food Authority but with EU limit, it increased to 45%. Again, a higher number of samples (21/30) from Arusha region had AFT with the highest range (0.2-23.4 $\mu\text{g/kg}$) and more than 50% exceeding the EU limit for AFT (Table 5). Seven samples (20%) from DSM region had AFT contamination level between 10 and 20 $\mu\text{g/kg}$, whereas 3 samples (30%) from Arusha were contaminated with AFT with more than 2 and 5-fold levels permitted by TFDA and the EU, respectively, for AFT in cereal-based foods

Table 5. Occurrence of total Aflatoxins (AFT) in locally produced complementary foods from Arusha and Dar es Salaam

Source	tested	Positive		E x c e e d	
		Samples	Concentration	Legal limit,	
		n (%)	($\mu\text{g/kg}$)	Range	n(%) ^b
			(Mean $\pm$ SD)	Min - Max	TFDA EU
Arusha	30	21(70.0)	11 $\pm$ 6.9	0.2 - 23.2	12(40.0) 16(53.0)
DSM	34	17(50.0)	8.4 $\pm$ 4.7	2.2 - 17.5	7(21.0) 13(38.0)
Total	64	38(59.4)	9.8 $\pm$ 6.1	0.2 - 23.2	19(30.0) 29(45.0)

^bLegal limit set at 10 $\mu\text{g/kg}$ (TFDA) and 4 $\mu\text{g/Kg}$ EU)

DISCUSSION

Aflatoxin contaminated food presents one of the biggest global health problems and consumption of contaminated food is unavoidable in many African countries, because they don't test crops and/ or products for aflatoxins, coupled with poor harvesting and storage conditions (Kimanya *et al.*, 2008; Shephard, 2010; Wild and Gong, 2010). Presence of aflatoxin is contributed by use of maize and peanuts in complementary food formulation which are highly susceptible to aflatoxin-producing fungi which has been proved to be high

potent carcinogens that affect millions of people world-wide including children (Kaaya and Kyamuhangire, 2006; Bankole *et al.*, 2006; Katerere *et al.*, 2008; Scundamore *et al.*, 2009; Heitaniemi *et al.*, 2009; Van der Fels-Klerx and Stratakou, 2010).

The presence of significant amount of aflatoxins in complementary foods produced in Tanzania is an indicator of a hidden problem that may be affecting thousands of exposed children. This toxin affects liver as well as brain which can lead to significant reduction of children performance at school. Infants and children are considered

to be more susceptible to different toxins than adults, because of their lower body weight, higher metabolic rate, lower ability to detoxify, and because of incomplete development of some organs and tissues such as the central nervous system (WHO, 1986; Sherif *et al.*, 2009). In children, exposure to aflatoxins is a causative factor for stunted growth, underweight, neurological impairment, immunosuppression and mortality (Bankole *et al.*, 2006; Katerere *et al.*, 2008; Scundamore *et al.*, 2009; Van der Fels-Klerx and Stratakou, 2010; Kew, 2012).

Immunosuppression caused by exposure to aflatoxins can increase susceptibility of the victims to other diseases such as HIV/AIDS. It has also been reported that aflatoxins interact synergistically with hepatitis B virus (HBV) and thus increasing the risk of hepatocellular carcinoma (HCC), the fifth most common human cancer that affect about 750,000 people every year, 80% of which occurs in developing countries (Kew, 2012). Chronic exposure to aflatoxin-contaminated diets in HBV patients has been shown to increase 30 times the risks to HCC than non HBV individuals, and given the fact that HBV infections occur in infancy and early childhood, feeding children with aflatoxin contaminated foods could put them at high risk of HCC in their early adulthood (Wild and Gong, 2010; Ferlay *et al.*, 2010; Szymanska *et al.*, 2009). Moreover, exposure to AFB₁ is a serious problem in countries, where hygiene and food

safety are not strictly and vigorously enforced (Liu *et al.*, 2006; Sugita-Konishi *et al.*, 2006). This is especially important in developing countries like Tanzania where food safety and hygiene are not strongly enforced in small scale food processing where most of these foods are produced.

Unlike in the developed countries, exposure to aflatoxin-contaminated foods is significantly higher in developing countries (Jestoi *et al.*, 2004; Razzazi-fazeli *et al.*, 2004; Wild and Gong, 2010; FSA, 2011; Cano-Sancho *et al.*, 2012a, 2012b). This signifies the role of high temperature and relative humidity in this region and therefore constant monitoring is imperative, if we are to reduce the negative impacts of aflatoxin exposure among the infants and young children.

Moreover, results of this study are comparable to those obtained in other developing countries, which have indicated high a level of aflatoxins contamination in cereal-based products (Egal *et al.*, 2005; Shephard, 2008). However, higher aflatoxins contamination than those obtained in this study was reported in maize-based weaning foods fed to infants in Nigeria, with a mean level 19.2 µg/kg in 25% of all sampled foods (Oyelami *et al.*, 1996). In another study by Tam *et al.* (2006) lower levels of aflatoxins contamination was detected in 50% of infant cereal food in study conducted in Canada between 2002 and 2005. In that study, 1% (2/177) of all infant cereal foods tested exceeded the EU maximum

limit (0.1 µg/kg AFB₁) compared to this study (48% of samples were above EU limit).

Soubra *et al.* (2009) reported lower levels of contamination with total aflatoxins in cereal-based products consumed by children in Lebanon in 13% of 1160 samples examined for total aflatoxins (range 0.5- 4 µg/kg). In a study done in Turkey by Baydar *et al.* (2007) on infant cereal foods, they reported detection of aflatoxins in 87% of 60 samples analyzed with high AFB₁ (mean 0.89 µg/kg and range 0.10-6.04 µg/kg) and eight samples exceeded the maximum allowable limit for baby foods. In Spain, Hernandez-Martinez and Navarro-Blasco (2010) reported occurrence of aflatoxins in 66% of 91 samples of Spanish infant cereal products with lower levels of AFB₁ (mean: 0.09± 0.40 µg/kg) and AFT (mean: 0.12± 0.51 µg/kg) as compare this study. Alvito *et al.* (2010) reported lower level of aflatoxins (0.09 µg/kg) in 7% (1/27) of samples of cereal based baby foods in Portugal which was within the maximum limit for baby foods.

Abdulkadir *et al.* (2002) reported a similar result in corn and peanut-based food products in Qatar in which case 20% of samples were contaminated with aflatoxin in the range 0.1 to 20 µg/kg. Aidoo *et al.* (2011) reported a higher aflatoxin contamination level (19-70 µg/kg) in cereal-based commercial baby foods in North Africa than what was obtained in this study, though only 2.4 % of samples were detectable. Findings

of this study suggest that children in developing world particularly sub-Saharan Africa, South East Asia and the Pacific, are at higher risk due to constant consumption of aflatoxin-contaminated food than their fellows in developed world. Concerted efforts and strategies need to be devised in order to protect them including promotion of HBV immunization.

Within the prevailing warm humid climatic conditions and the available poor storage environment of agricultural products, the contamination of mycotoxins in cereals and nuts is inevitable. The data presented in this study indicate that the diets of Tanzanian infants could contain cocktail of other mycotoxins, apart from the significant level of aflatoxins observed. However more studies are needed to assess multiple occurrences of mycotoxins and estimation of exposure risks due to consumption of complementary foods in Tanzania.

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INSTRUCTIONS TO AUTHORS (EDITED 2013)

The Journal

The Tanzania Veterinary Journal (The Tropical Veterinarian) is a biannual Journal, which publishes original contribution to knowledge on Veterinary Science, Animal Science and Production, and allied sciences including new techniques and developments in Veterinary Medicine. The target readers of the Journal are the Research Workers, Veterinary Clinicians, Animal Scientists, Field Officers, as well as Policy Makers. Papers are accepted on the understanding that their content has not been published elsewhere and that they are subject to editorial revision. The Editors reserve the right to make minor alterations in the lay out or phraseology without reference to the author.

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Manuscript should be submitted electronically to the: The Chief Editor, Tanzania Veterinary Journal, Email: ekarimu@yahoo.co.uk OR nongahezron@yahoo.co.uk / hezron@suanet.ac.tz. Submitted manuscripts must be accompanied by a scanned copy of duly signed letter stating that the manuscript is not under review for publication elsewhere and that the data have not been published previously. The corresponding author certifies in the letter that all coauthors have read the manuscript and agree to its submission. Every coauthor should sign the submission letter to indicate his/her approval for submission.

Manuscripts involving recombinant DNA in prokaryotes, human materials and animals should be submitted only if the research protocols have been approved where required by the appropriate institutional committees. Circumstances related to animal experimentation must meet the International Guiding Principles for Biomedical Research Involving Animals.

Manuscripts under review are assigned to two experts in the respective field. Thus, two independent reviews are obtained and, using comments from these reviews, together with the third copy, which the Editor has read, the Editor makes a decision to accept, revise or reject the manuscript. An editorial decision on the manuscript is sent to the corresponding author by the

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Manuscripts should be in grammatically correct and consistent using either United Kingdom (UK) or United States (US) English. Manuscripts submission must be in MS word format. All pages of the manuscript should be numbered, double-spaced with font 12 of Times New Romans and line numbered. Table pages and figure captions should also be double-spaced. All acronyms (abbreviations) used must be spelled out in the first instance followed immediately by the acronym in parenthesis. Subsequent references to the relevant subject may be satisfied by use of the acronym alone.

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Assemble the manuscript in the following order: Title Page, Summary, Introduction, Materials

and Methods, Results, Discussion, Acknowledgements, References, Tables and Figure Legends. Subtitles may be used within sections. The total number of words in the whole manuscript should be between 2500 and 3000 excluding Tables, Figures, and References.

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The *Introduction* should provide sufficient background information to make the paper understandable to colleagues from a broad range of biological disciplines. It should provide adequate literature with relevant citations.

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