Original Article

Risk of dietary exposure to aflatoxins and fumonisins in infants less than 6 months of age in Rombo, Northern Tanzania

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Abstract

Infants less than 6 months of age receiving foods other than breast milk are at a high risk of exposure to mycotoxins. We surveyed food intake and estimated the risk of exposures to aflatoxin and fumonisin mycotoxins for infants less than 6 months of age in Northern Tanzania. A total of 143 infants were progressively recruited and three follow-up visits were made at 1, 3 and 5 months of age. A 24-h dietary recall technique was used to estimate flour intake of infants who had been introduced to maize foods. Aflatoxins and fumonisins in the flours were analysed using high-performance liquid chromatography technique. Exposure to aflatoxins or fumonisins was estimated using the deterministic approach. By the age of 3 months, 98 infants had started taking food; 67 of them, maize flours at levels ranging from 0.57 to 37.50 g per infant per day (average 8 g per infant per day). Fifty-eight per cent of 67 maize flour samples contained detectable aflatoxins (range 0.33–69.47 $\mu\text{g kg}^{-1}$; median 6 $\mu\text{g kg}^{-1}$) and 31% contained detectable fumonisins (range 48–1224 $\mu\text{g kg}^{-1}$; median 124 $\mu\text{g kg}^{-1}$). For infants who consumed contaminated flours, aflatoxin exposure ranged from 0.14 to 120 ng kg^{-1} body weight (BW) per day (all above the health concern level of 0.017 ng kg^{-1} BW per day as recommended by the European Food Safety Agency) and fumonisin exposure ranged from 0.005 to 0.88 $\mu\text{g kg}^{-1}$ BW per day. Insignificant association was observed between exposure to fumonisins or aflatoxins and stunting or underweight. Reducing aflatoxin and fumonisin contamination of maize and dietary diversification can prevent infants and the public, in general, from exposure to the toxins.

Keywords: aflatoxin, fumonisin, exposure, infants, maize, Tanzania.

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Introduction

Infants less than 6 months of age in Tanzania who are not exclusively breastfed are at a high risk of exposure to mycotoxins, particularly aflatoxins and fumonisins, which have been reported as the major mycotoxin contaminants of maize-based foods. Maize is used as a staple food in many parts of Tanzania, just like in other countries in the sub-Saharan Africa. It is also used as a major ingredient in the formulation of infant's foods. Aflatoxins and fumonisins are the major mycotoxin contaminants found in maize and

have been reported to co-occur in maize from Tanzania (Kimanya *et al.* 2008; Manjula *et al.* 2009; Kimanya *et al.* 2014).

There are four different forms of aflatoxins, namely, aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂), and all of them are known to have effects on health, with AFB₁ being the most potent form (IARC 2002). Aflatoxin exposure in children has been associated with the occurrence of kwashiorkor and marasmus (Adhikari *et al.* 1994). One study in Cameroon showed AFB₁ presence in 35.5% of kwashiorkor children, and 45.5% of marasmic

children, which were statistically significant ($P < 0.05$) in comparison with 11% of the control group (Tchana *et al.* 2010). Gong *et al.* (2003) showed growth faltering in children exposed to aflatoxins in Benin and Togo and also demonstrated a strong association between aflatoxin-albumin adduct levels and stunted growth in children. This association was also observed in a study of the effect of aflatoxin exposure in utero and early growth faltering of Gambian infants (Turner *et al.* 2007).

Fumonisin exist in three major forms, namely fumonisins B₁ (FB₁), B₂ (FB₂) and B₃ (FB₃), with FB₁ being the most potent form (Thiel *et al.* 1992; Samapundo *et al.* 2006). FB₁ has been associated with high incidence of human oesophageal cancer in Transkei, South Africa (Rheeder *et al.* 1992) and China (Zhang *et al.* 1997). FB₁ has also been reported to be a primary contributing factor to liver cancer in Haimen, China (Ueno *et al.* 1997). It has also been implicated in the high incidence of neural tube defect (Marasas *et al.* 2004) and cardiovascular problems (Fincham *et al.* 1992) in populations consuming relatively large amounts of food made with contaminated maize.

Infants are at a higher risk of exposure to these toxins than adults or older children due to lower body weights, higher metabolic rates and lower ability to detoxify toxins because of incomplete development of some organs and tissues (Sherif *et al.* 2009). It is therefore important to place a special emphasis on exclusive breastfeeding for infants less than 6 months of age as recommended by World Health Organization (WHO) in order to minimize the risk of mycotoxin exposure.

It has been shown in previous studies that exclusive breastfeeding is rarely practised in Tanzania

(Shirima *et al.* 2001; Mamiro *et al.* 2005; Nyaruhucha *et al.* 2006), as afore stated, in this country, the maize used for complementary foods is often contaminated with both aflatoxins and fumonisins (Kimanya *et al.* 2008; Manjula *et al.* 2009; Kimanya *et al.* 2014). This state of affairs suggests that infants less than 6 months of age are at risk of exposure to aflatoxins and fumonisins. However, there are no data on the extent of maize consumption in infants less than 6 months in Tanzania. Neither are there data on the extent of aflatoxins and fumonisins contamination in the maize-based foods used for less than 6-month-old infants. These data are necessary to estimate the risk of exposure to aflatoxins and fumonisins among infants less than 6 months of age, in Tanzania. This study was therefore conducted to estimate the extent of maize consumption among infants younger than 6 months in Northern Tanzania, determine the levels of aflatoxins and fumonisins in the infants' maize flour and then estimate the risk of dietary exposure of aflatoxins and fumonisins among the infants.

Materials and Methods

Study area and subjects

The study was conducted in two villages, Kikelelwa and Mbomai, of Rombo district in Northern part of Tanzania. This region was chosen based upon the reports of mycotoxin studies conducted earlier (Kimanya *et al.* 2008; Kimanya *et al.* 2014). All infants born from November 2011 to February 2012 and their mothers were progressively recruited in these villages. Information on the date of birth for the infants was obtained from their clinic cards and recorded.

Key messages

- At third month of age, 80% of infants in Northern Tanzania had started receiving complementary food and this proportion increased to 97% at fifth month of age.
- Based on maize intake, the risk of exposure to dietary aflatoxins or fumonisins, increased with age; from 5%, at first month, through 68% at third month to 87% at fifth month, of age.
- Infants who were exclusively breast fed were less likely to be stunted compared to their peers who were not (exposed to dietary aflatoxins or fumonisins).
- This findings justify the urgent need for the governments of Tanzania and other countries to formulate pre and post-harvest measures that can prevent mycotoxins contaminations and exposure in food.

Survey of infant's food intake and sampling of maize flour

Three follow-up visits were made at 1, 3 and 5 months of age to survey food intake and sample maize flour from families that had started giving maize-based foods to infants.

Food intake was estimated at each visit through a 24-h dietary recall. Mothers were asked to mention the types and estimate the quantity of food consumed by their infants in the 24 h prior to the interview. Estimates of liquid food intake were made using graduated bottle, whereas solid food consumption was determined using number of spoons. The name and proportion of each ingredient used in preparation of food were also recorded. In each visit, the frequency of maize consumption in a week for each child who had started maize-based food was also recorded. About 250 g of maize flour used for making infants' food was collected from families that had started feeding maize-based food to their infants. The flour was collected in paper bags, sealed and stored until analysis.

Anthropometry

Infants' body weight and length were recorded at each visit; weight was taken to the nearest 0.1 kg using SALTER scale model 2356M (Salter, Tonbridge, UK) and length to the nearest 0.1 cm using SECA infantometer model 416 1821009 (Chino, CA, USA). 'Weight for age z-score' (WAZ) and 'length-for-age z-score' (LAZ) were computed in Stata 12.0 (College Station, TX, USA) with reference to the WHO Growth Standards (WHO 2006).

Estimation of maize flour intake

With the estimates of the proportion of each ingredient, the amount of maize flour consumed by an infant from a thin (*Uji*) or stiff (*ugali*) porridge was assessed using the Lucille food intake software of Ghent University (Lucille: software to process food intake data, 2012) (Lucille, 2012). This was also carried out in accordance to the quantity of food consumed and the food preparation techniques

described in the Tanzania food composition tables (Lukmanji *et al.* 2008).

Ethical clearance

Ethical clearance certificate number NIMR/HQ/R.8a/Vol.IX/1142 was obtained from the National Institute for Medical Research in Tanzania. Mothers were made aware of the objectives of the study and clarification was given on all the procedures for data collections. A written informed consent was obtained from each mother who agreed to participate in the study. Confidentiality of the information obtained and all other rights of the subjects have been ensured.

Extraction and analysis of AFB₁, AFB₂, AFG₁ and AFG₂ from maize flour

For a child who had started receiving maize-based food at 3 months of age, the two samples of maize flours collected from a family, at 3 and 5 months of age, were thoroughly mixed at the laboratory to make a sample for analysis. The method used to determine contaminations of aflatoxin B₁, B₂, G₁ and G₂ is described by Stroka *et al.* (2000). Minor modifications to the process were made according to Kimanya *et al.* (2008). The limits of detection (LODs) were 0.53 µg kg⁻¹ for AFB₁, 0.15 µg kg⁻¹ for AFB₂, 0.24 µg kg⁻¹ for AFG₁ and 0.01 µg kg⁻¹ for AFG₂. The average recovery obtained for spiked samples was 86% for all aflatoxins with relative standard deviation of 15.70%.

The analysis was carried out using a high-performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan) equipped with pump LC 20AD and fluorescence detector model RF-10AXL set at a wavelength of 363 nm (excitation) and 440 nm (emission). HPLC column Spherisorb 80-3 ODS-1, 5 µm, 4.6 × 150 mm was used, with a mobile phase composed of methanol : acetonitrile : water (15:20:65). One hundred nineteen milligrams of potassium bromide and 100 µL of 65% nitric acid were added for derivatisation. The flow rate of the mobile phase was set at 0.8 mL min⁻¹.

Extraction and analysis of fumonisin B₁ and B₂

Fumonisin B₁ and B₂ were determined in maize flour samples constituted as explained in the "Estimation of maize

flour intake" section. Determination of FB₁ and FB₂ was carried out using the HPLC method described by Sydenham *et al.* (1992), with slight modifications made by Samapundo *et al.* (2006), as reported by Kimanya *et al.* (2008). The LOD for FB₁ was 53 µg kg⁻¹ and for FB₂ 47 µg kg⁻¹. The LODs for the method were based upon the mean value of the blank readings plus three standard deviations.

The analysis was carried out using the HPLC unit equipped with a pump LC 20AD and fluorescence detector model RF-10AXL, set at a wavelength of 335 nm for excitation and 440 nm for emission. HPLC column stainless steel, Waters Spherisorb® (Milford, MA, USA) 5 µm ODS-1, 4.6 × 200 mm was used with the mobile phase composed of 750 mL of methanol and 250 mL of 0.1 M NaH₂PO₄; pH was adjusted to 3.35 using phosphoric acid, and the mobile phase was filtered under vacuum with a 0.45-µm filter paper. The flow rate of the mobile phase was set at 1 mL min⁻¹.

Exposure estimation for aflatoxins and fumonisins

Dietary level of exposure to aflatoxins and fumonisins from maize flour was calculated for infants who consumed maize flours with detectable contaminations. Average maize flour intake by a child as estimated from two 24-h dietary recalls, at 3 and 5 months of age, was used in the exposure assessment. The maize intake for each infant was then adjusted to derive a more habitual intake. This was carried out by multiplying the estimated average maize flour intake with the number of days of maize flour consumption in the previous week divided by 7 (Kimanya *et al.* 2010).

For each child, exposure to aflatoxins or fumonisins was calculated by multiplying the detectable level of contamination in the flour (ng/g) by the adjusted amount of maize flour consumed (g of maize flour intake per day) and then dividing by the infant's body weight (kg).

Risk characterisation for aflatoxins

An infant was considered at risk of exposure to aflatoxins if he/she consumed foods (other than breast milk) before the WHO recommended age of 6

months. A child was considered at a risk of exceeding tolerable limit of aflatoxin exposure if, in addition to consuming aflatoxin-contaminated maize flour, the margin of exposure (MOE) falls below the limit of 10 000 recommended by the European Food Safety Authority (EFSA) (Barlow *et al.* 2006; EFSA 2007) for prioritisation of risk management actions. A MOE of 10 000, which is equivalent to exposure of 0.017 ng kg⁻¹ BW per day, is considered a cut-off point whereby an MOE below 10 000 or exposure above 0.017 ng kg⁻¹ BW per day indicates public health concern (EFSA 2007).

Risk characterisation for fumonisins

Like for the case of aflatoxins, an infant was considered at risk of exposure to fumonisins if he/she consumed foods (other than breast milk) before the WHO recommended age of 6 months. The risk was also characterised in terms of exposures above the provisional maximum tolerable daily intake (PMTDI) of 2 µg kg⁻¹ BW per day, as per WHO recommendation (WHO 2012).

Data interpretation, management and analysis

Data from questionnaires were entered into Epidata 3.1 (Odense, Denmark). Contaminants data acquired from laboratory analyses were entered into a Microsoft Excel spreadsheet. Food intake data were entered in food intake software. All data were imported and processed into STATA version 12.0, whereby a significance level of 5% was considered sufficient for interpretation of results from all analyses. The information generated is presented as range, mean and median or in a figure. The likelihood of association between aflatoxins or fumonisins or both aflatoxin and fumonisin exposure at 3 months of age, with growth status (in terms of proportions of stunted or underweight infants) at 5 months of age, was determined using Logistic regression analysis. The likelihood of association is presented as odds ratio with 95% confidence interval.

As there are no maximum limits (MLs) set in Tanzania for aflatoxins in baby foods, AFB₁, and total aflatoxin concentrations were compared to Tanzania's

ML ($5 \mu\text{g kg}^{-1}$ for AFB₁; $10 \mu\text{g kg}^{-1}$ for total aflatoxins) sets for maize flour (TBS 2010) and the European Union (EU) limits for cereal foods, specifically $2 \mu\text{g kg}^{-1}$ for AFB₁ and $4 \mu\text{g kg}^{-1}$ for total aflatoxin content (EC 2006). Total fumonisin (sum of FB₁ and FB₂) results were compared WITH the EU regulation limit ($200 \mu\text{g kg}^{-1}$ for total fumonisins) for infant's maize-based food (EC 2006). There are no MLs set in Tanzania for fumonisins in maize-based baby foods or general purpose maize flour.

Results

Subjects

A total of 143 infants, 49% male, participated in the study 1 month after birth. At 3 months of age, 121 infants were available for data collection as 22 had dropped out. The number of infants available for data collection at 5 months of age was 118 (three more infants had dropped out). The dropouts moved to another village or urban area.

Type of food introduced to infants

At 3 months of age, 80% (98 out of 121) of infants had started receiving complementary food and this proportion increased to 97% (115 out of 118) at the age of 5 months. The food given to 67 (68%) of infants, at 3 months of age, was prepared from plain maize or mixed cereal flours. The cereal flours contained maize

as the primary constituent. Other ingredients used were finger millet, wheat and/or rice. Food given to the rest (32%) of the infants includes banana porridge, cow milk, sip of home-made butter, sardines, beef and fruits. However, none of the infants were exclusively supplemented by the age of 5 months.

Risk of exposure to aflatoxins and fumonisins

Based upon the percentage of infants receiving all types of baby foods or maize-based foods, the risk of exposure to aflatoxin or fumonisin increased with age, as shown in Fig. 1. In terms of all types of food, the risk increased from 15%, 81% and 97% and in terms of maize-based food, from 5%, 68% and 87% at 1, 3 and 5 months of age, respectively.

Aflatoxins and fumonisins in maize flour

Maize flour samples were collected from 67 infants who had started maize complementary feeding by 3 months of age and analysed for aflatoxin and fumonisin contamination. As shown in Table 1, of the 67 samples, 58% were positive for aflatoxins and the contamination ranged from 0.33 to $69.47 \mu\text{g kg}^{-1}$. Contamination in 23% of the positive samples exceeded the Tanzania ML of $10 \mu\text{g kg}^{-1}$ set for total aflatoxins in maize flour for human consumption (TBS 2010) and 56%, $4 \mu\text{g kg}^{-1}$ limit set for total aflatoxin content in the EU. AFB₁ contaminations in the

Fig. 1. The risk of exposures to aflatoxin and/or fumonisin vs. infant's age.

Table 1. Distribution of aflatoxins and fumonisins in maize flour

	<i>n</i>	Positive samples, <i>n</i> (%)	Range of positive sample ($\mu\text{g kg}^{-1}$)	Median ($\mu\text{g kg}^{-1}$)	Percentage of positive samples exceed regulatory limit, <i>n</i> (%)
AFB ₁	67	34 (50.75)	0.85–55.73	3.50	12 (35.29)* 23 (67.64) [†]
Total aflatoxin (B ₁ + B ₂ + G ₁ + G ₂)	67	39 (58.21)	0.33–69.47	5.58	9 (23.08) [‡] 22 (56.41) [§]
FB ₁	67	20 (29.85)	52.70–974.10	140.30	
Total fumonisin (FB ₁ + FB ₂)	67	21 (31.34)	48.40–1224.60	123.80	10 (47.62) [¶]

*AFB₁ level exceeded Tanzania limit of $5 \mu\text{g kg}^{-1}$ for maize flour (TBS 2010). [†]AFB₁ level exceeded EU limit of $2 \mu\text{g kg}^{-1}$ for cereal-based food (EC 2006). [‡]Total aflatoxin level exceeded Tanzania limit of $10 \mu\text{g kg}^{-1}$ for maize flour (TBS 2010). [§]Total aflatoxin level exceeded EU limit of $4 \mu\text{g kg}^{-1}$ for processed cereal (EC 2006). [¶]Total fumonisin level exceeded EU limit for infants food $200 \mu\text{g kg}^{-1}$ (EC 2006).

Table 2. Infants' growth status at 5 months of age in relation to different feeding practices at 3 months of age

Feeding practice	<i>n</i>	Weight status			Length status		
		Weight gain, kg (mean $\pm$ SD)	WAZ [†] (mean $\pm$ SD)	Underweight (%)	Length gain, cm (mean $\pm$ SD)	LAZ [‡] (mean $\pm$ SD)	Stunting (%)
Exclusive breastfed	23	1.13 $\pm$ 0.48	−0.25 $\pm$ 1.39	13	4.32 $\pm$ 1.80	−0.62 $\pm$ 1.09	13
Maize-based food	67	0.96 $\pm$ 0.46	−0.13 $\pm$ 1.07	6	3.35 $\pm$ 2.28	−0.86 $\pm$ 1.11	18
Non-maize-based food	25*	0.93 $\pm$ 0.41	−0.23 $\pm$ 1.31	16	3.76 $\pm$ 2.93	−0.96 $\pm$ 1.27	12

[†]WAZ, weight for age *z*-score; underweight = WAZ < −2. [‡]LAZ, length for age *z*-score; stunting = LAZ < −2.

*Six infants had missing weights in one of the visits.

positive samples ranged from 0.85 to $55.73 \mu\text{g kg}^{-1}$. Contamination in 35% of the positive samples exceeded the ML of $5 \mu\text{g kg}^{-1}$ set for AFB₁ for maize flour for human consumption in Tanzania and 68%, $2.0 \mu\text{g kg}^{-1}$ EU limit for processed cereals.

Thirty-one per cent of samples were positive for fumonisins, with a contamination range of 48.4–1224.6 $\mu\text{g kg}^{-1}$. FB₁ contamination in those samples ranged between 52.7 and $974.1 \mu\text{g kg}^{-1}$. Contamination levels in 48% of the positive samples were above the EU limit of $200 \mu\text{g kg}^{-1}$ set for infant's maize-based food (EC 2006). Of the 67 maize flour samples, 23% were found to be contaminated by both aflatoxins and fumonisins.

Risk of exposure above tolerable limits

Exposure assessment involved infants who consumed maize flours with detectable contaminations, 39 infants for aflatoxin and 21 for fumonisin. The maize flour consumption in these infants ranged from 0.57 to 37.50 g per infant per day, with an average of 8.0

and median of 5.0 g per infant per day. The total exposure to aflatoxins ranged from 0.14 to 120 ng kg^{-1} body weight (BW) per day. The MOE to aflatoxins was below 10 000 (1.41–1176) or above 0.017 ng kg^{-1} BW per day. Exposure to fumonisins ranged from 0.005 to $0.88 \mu\text{g kg}^{-1}$ BW per day, levels that are below the PMTDI of $2 \mu\text{g kg}^{-1}$ BW per day as recommended by JECFA. Median exposure to aflatoxins and fumonisins were 3.88 ng kg^{-1} BW per day and $0.14 \mu\text{g kg}^{-1}$ BW per day, respectively.

Association between feeding practices and growth status

Table 2 shows the growth status at 5 months of age, in relation to infants' feeding practices at 3 months of age. Twenty-three infants were exclusively breastfed until 3 months of age and 67 were introduced to maize-based food in addition to non-maize-based foods by 3 months of age. Thirty-one infants were introduced to non-maize-based foods only. Infants who were exclusively breastfed had higher mean

Table 3. Infants' growth status at 5 months of age in relation to different forms of maize-based risk factors at 3 months of age

Maize-based risk factor	<i>n</i>	Length status			Weight status		
		Length gain, cm (mean $\pm$ SD)	LAZ* (mean $\pm$ SD)	Stunting (%)	Weight gain, kg (mean $\pm$ SD)	WAZ† (mean $\pm$ SD)	Underweight (%)
Maize-based food introduction	67	3.35 $\pm$ 2.28	-0.86 $\pm$ 1.11	18	0.96 $\pm$ 0.46	-0.13 $\pm$ 1.07	6
Aflatoxin exposure	39	3.42 $\pm$ 2.16	-0.07 $\pm$ 1.06	15	1.01 $\pm$ 0.48	0.01 $\pm$ 1.00	3
Fumonisin exposure	21	2.83 $\pm$ 1.82	-0.68 $\pm$ 0.69	5	0.99 $\pm$ 0.48	0.08 $\pm$ 0.74	0
Aflatoxin and fumonisin exposure	15	2.90 $\pm$ 1.97	-0.66 $\pm$ 0.76	7	0.99 $\pm$ 0.43	0.03 $\pm$ 0.83	0

*LAZ, length for age *z*-score; stunting = LAZ < -2. †WAZ, weight for age *z*-score; underweight = WAZ < -2.

Table 4. Odds ratio for the likelihood of association between growth status at 5 months of age and the exposure to maize-based risk factors at 3 months of age

Maize-based risk factor	<i>n</i>	Stunting*			Underweight†		
		Odds ratio	95% confidence interval	<i>P</i> -value	Odds ratio	95% confidence interval	<i>P</i> -value
Maize-based food intake	67	1.53	0.53–4.40	0.43	0.37	0.10–1.35	0.13
Aflatoxin exposure	39	0.97	0.33–2.82	0.96	0.17	0.02–1.41	0.10
Fumonisin exposure	21	0.23	0.03–1.81	0.16	NA	NA	NA
Aflatoxin and fumonisin exposure	15	0.35	0.04–2.83	0.32	NA	NA	NA

n, number of infants exposed to a particular risk factor; NA, not applicable, no underweight observed. *Length for age *z*-score < -2. †Weight for age *z*-score < -2.

weight and length gains compared with those who had been introduced to other foods (maize-based or non-maize-based). However, in the same group of exclusively breastfed infants, 13% were stunted and 13% were underweight. The highest rate of 18% for stunting was observed among infants who had been introduced to maize-based foods. Infants who were introduced to foods, other than maize, had the highest rate of underweight (16%).

Association between maize-based mycotoxin exposure and growth status

Table 3 shows the growth status of infants according to different forms of maize-based mycotoxin exposures. The rate of stunting or underweight among infants who were exposed to aflatoxins was higher than the respective rate among infants who were exposed to fumonisins. Table 3 shows further that none of the fumonisin exposed infants were underweight.

As shown in Table 4, using odds ratios, there was a weak and insignificant likelihood of association between stunting and exposure to aflatoxins or fumonisins. Table 4 shows further that the likelihood of an aflatoxin-exposed child to be underweight is 17% higher than that of a non-exposed child. However, this likelihood of association was marginally insignificant.

Discussion

The present study examined the risks of exposure to mycotoxins of infants less than 6 months of age in relation to their feeding practices. The present findings show that failure to comply with the WHO (2001) recommendation of exclusive breastfeeding for the first 6 months puts these infants at risk of exposure to aflatoxins and fumonisins at dangerous levels. Exclusive breastfeeding would certainly prevent infants less than 6 months of age from dietary health hazards including those related to mycotoxins.

As previously elaborated by Sherif *et al.* (2009) at the early stages of 0–6 months of age, infants are prone to different toxic effects as their body systems are not well developed to be able to detoxify the toxins.

The findings of this study found a relatively higher weight and length gain in exclusively breastfed infants than in infants who, in addition to breast milk, received other foods. Exclusive breastfeeding also appears to prevent the infants from stunting as the rate of stunting (13%) in these infants was lower than the rate of 18% in infants who received maize-based foods. However, it appears that introduction of maize-based foods, regardless of mycotoxin contamination status, prevents the infants from being underweight. Possibly, this suggests that exclusive breastfeeding practice in this community does not meet daily energy needs and this deficit was covered by maize-based foods. This is also explained by the observation that infants introduced to maize-based food had slightly higher mean weight gain than those introduced to other kinds of foods. Most of the infants who received foods other than maize based were fed on banana porridge, which is of lower caloric value than maize.

The extent of the risks of exposure to both aflatoxins and fumonisins of infants below 6 months of age is being reported for the first time in the present study. In view of the present findings, all the aflatoxin-exposed infants exceeded the daily intake of $0.017 \text{ ng kg}^{-1} \text{ BW}$, which is recommended by EFSA (2007). Although different weights and heights were recorded among infants, but infants who were exposed to aflatoxins were more likely to be stunted and underweight compared with their peers who were not exposed to the toxins. Although the likelihood of association was not significant, the findings are in line with previous observations by Gong *et al.* (2004) that aflatoxin exposure is associated with growth impairment.

Just like for the aflatoxin exposure, it is likely that fumonisin exposure was associated with impaired growth. It was observed that the likelihood of a child exposed to fumonisins to be stunted was 23% higher than that of a child who is not exposed to the toxins. This observation supports a previous observation by Kimanya *et al.* (2010) that fumonisin exposure in chil-

dren from this same community is associated with poor growth. Further analysis of the data from the present study shows that the likelihood of a child who is exposed to fumonisins to have an LAZ z -score less than zero is more than 300 times (odds ratio, 3.26, CI, 0.71–15.03, $P = 0.14$) (data not shown). This suggests that most, if not all, the fumonisin-exposed infants may in due course be stunted.

However, it is important to note that growth impairment in breastfed infants and other children can be attributed to many other factors. These include inadequate nutrient intake, frequent bacterial infections and other environmental factors. According to Magoha *et al.* (2014), the infants studied in this study were also exposed to aflatoxin M1 through breast milk. Thus, for a better understanding of the growth impairment effects of aflatoxins or fumonisins, a larger study is needed whereby all the confounding factors will be controlled for. While awaiting the planning and funding of such a larger study, efforts to minimise aflatoxin and fumonisin exposures through food and breast milk are worthwhile.

The main source of aflatoxin and fumonisin exposure in these infants is early introduction of baby foods. As found in this study, by 3 months of age, 80% of infants had already been introduced to foods other than breast milk. Importantly, average maize flour intake of 8 g per infant estimated in this study is comparable to the average consumption in adults of other communities. The average daily maize flour intake for adults as reported by WHO GEMS/Food Regional Diets (2003) for Europe is 8.8 g per person.

The findings of this study are also relevant to other communities in Africa where maize intake among children is high, particularly because aflatoxin and fumonisin contamination in Africa is common. Mostert & Steyn (2005) reported early introduction of solid food, maize meal porridge, being common among children living in Limpopo province, South Africa. Gong *et al.* (2003; 2004) also reported that the exposure to aflatoxin in children of West Africa increased following weaning onto mainly maize-based food, for the age group of 1–3 years. In a very recent study in Tanzania, Shirima *et al.* (2013) also reported that weaned children had higher mean aflatoxin markers in blood and FB_1 in urine compared

with those children receiving a mixture of breast milk and solid food.

The risk of aflatoxin and fumonisin exposure in other communities of Tanzania consuming similar amounts of maize flour per day may be higher than that found in the infants we studied. The higher risk is expected in communities where de-hulling of maize before milling is not practised. An example of such areas is the Tabora region in Tanzania, where Kimanya *et al.* (2008) reported that 27% of respondents did not de-hull maize prior to milling for food. Again, this emphasises the importance of advocating for reduction of mycotoxin contents in maize used for infant foods, and the dehulling of the maize before milling in places where one may be forced to introduce infants to maize-based foods.

More than 67% of the samples positive for AFB₁ had contamination levels above the $2 \mu\text{g kg}^{-1}$ set by EU for cereal-based food. Given that AFB₁ is the most potent form of aflatoxins (Do & Choi 2007) in that it reacts with nuclear acids to generate the polynucleotide-based adducts responsible for carcinogenicity and affects liver and blood protein, having high percentages of AFB₁-positive samples poses a greater health threat to the population concern.

As expected, the median levels of contamination ($5.58 \mu\text{g kg}^{-1}$ for total aflatoxins and $123.80 \mu\text{g kg}^{-1}$ for total fumonisins) in maize flours tested by this study are lower than those reported by Kimanya *et al.* (2008); $24.00 \mu\text{g kg}^{-1}$ for aflatoxins and $363.00 \mu\text{g kg}^{-1}$ for fumonisins for maize from the same community. The samples analysed in this study are maize flours that had been subjected to sorting and milling, whereas Kimanya *et al.* (2008) used maize directly sampled from stores. Reports show that treatments applied in the preparation of maize, such as sorting and milling, can reduce mycotoxin contamination levels in maize. Mutungi *et al.* (2008), in a study of the effect of processing *muthokoi/kande* (a traditional dehulled maize dish) in Kenya, showed that dehulling decreased aflatoxin content by 46.6% (5.5–70%) in maize containing $10.7\text{--}270 \mu\text{g kg}^{-1}$ of aflatoxin concentrations. Similar results were reported by Fandohan *et al.* (2005) in a study of the preparation of maize food products in Benin, where about 34% of aflatoxin in the maize was removed with the discarded

hulls and germ and the mean fumonisin content decreased from 2890 to $1350 \mu\text{g kg}^{-1}$.

The best approach to minimising mycotoxin contamination in maize and therefore its consumption is to control contaminations at each stage from farm to fork. This should involve educating farmers, producers, processors and consumers about appropriate handling and storage methods at all stages. There are various recommended methods; these include the use of aflatoxin-resistant maize varieties, practising crop rotation, the use of fertilisers, well-timed planting, timely harvests, the use of appropriate drying and processing techniques such as sorting, cleaning and milling (Hell & Mutegi 2011). The FAO/WHO Codex Alimentarius Commission (CAC/RCP 51-2003) guidelines for reducing of mycotoxin contamination in foods can also be applied (FAO/WHO 2003).

Moreover, the use of other types of grains, particularly those less susceptible to fungal infection, together with maize flour when preparing infants' food, can help reduce the contamination and exposure levels in maize (Munimbazi & Bullerman 1996). However, with the levels of poverty and other constraints in Tanzania, one cannot rely upon these measures to protect infants against mycotoxin exposure. This implies that we should continue to rely upon exclusive breastfeeding as the most reliable approach to prevent infants from mycotoxins exposures. Nonetheless, like in other African countries and as has been shown by this study, exclusive breastfeeding during the first 6 months of life is rarely practised in Tanzania (Shirima *et al.* 2001; Mamiro *et al.* 2005; Nyaruhucha *et al.* 2006). Therefore, results of this study should be used by nutrition extension services to emphasise on the need for observance of exclusive breastfeeding.

Much as we advocate for exclusive breastfeeding, it should be noted that, as previously stated, infants may also be exposed to mycotoxins, aflatoxin M₁ in particular, through breast milk. Thus, in addition to advocating for exclusive breastfeeding nutrition education, workers should also advise lactating mothers to exercise extra care and select, for their own consumption, foods that are mycotoxin free or less susceptible to mycotoxins contamination. Perhaps, Tanzania and other countries should develop guidance on the diets of lactating mothers that

reduces risks and levels of the mycotoxins found in maize.

Application of regulations is one of the means to prevent the general population from mycotoxin exposures, but this strategy is only possible in communities where food is traded. In Tanzania however, although aflatoxin limits are set, enforcement is still a challenge. Most rural people consume own grown maize and resources are not sufficient to ensure effective enforcement of the limits in areas where food is traded.

The presence of a mycotoxin limit may however influence contamination reduction specifically for those who may voluntarily implement the standards. This explains why the society should advocate for formulation of maximum limits of mycotoxins such as fumonisins, which are not officially regulated in Tanzania.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

HM participated in designing the research, carried out all the field work, analysed the samples and drafted this manuscript. MK participated in designing the study, supervised all the field work and laboratory experiments conducted in Tanzania, and critically reviewed the manuscript before submission. BM participated in designing the study, reviewed results of aflatoxin and fumonisin analyses, and critically

reviewed the manuscript before submission. DR and CL participated in designing the research and carried out statistical analysis of the data. PK conceptualised and led the designing of the study, safeguarded the scientific and ethical aspects of the study, and critically reviewed the manuscript before submission.

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