



Comparative Analysis of Fumonisin Contamination and Infant Dietary Exposure in Kongwa District, Tanzania: A Sub-Study Within a Two-Arm Cluster-Randomized Trial

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Abstract

In central Tanzania, maize is frequently consumed in many households as a staple food. Reliance on maize-based complementary foods for infants and young children puts them at risk of Fumonisin (FUM) exposure. This observational study explores whether the provision of low-aflatoxin porridge flour as part of an intervention within a larger cluster randomized control trial, Mycotoxin Mitigation Trial (MMT), had any effect on the intake and exposure to FUM in complementary foods consumed by children aged 12 to 13 months. Sampling was done for randomly selected 12-13-month-old children from 20 of the 52 trial clusters within the two arms of MMT. The households within the intervention arm of the MMT were provided with low aflatoxin blended flour, 4 parts maize, and 1 part ground nuts to feed the index child, every month for 12 months. The Standard of Care (SoC) households were educated on preparing the 4:1 blended flour from locally produced maize and groundnuts. Blended and maize flours consumed by the index child in the past 24 hours were collected for FUM analysis. The samples were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Data analysis was done through STATA version 16 software at a 5% significance level. The levels of FUM in blended flour samples were not significantly different between the intervention and the SoC arm $p > 0.05$. Similarly, differences existed in FUM intake and exposure through blended flour between arms ($p > 0.05$). There were no significant differences in FUM intake and exposure between arms from maize ($p > 0.05$). This study suggests that the provision of low-aflatoxin flour to households in the intervention arm did not impact differences in intake and exposure to FUM across the two arms.



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Introduction

Fumonisin is a type of mycotoxin produced by *Fusarium verticillioides* and *Fusarium proliferatum* fungi species.^{1,2} They are ubiquitous and contaminate several foods that pose detrimental health hazards to humans.³ Fumonisin consists of different forms, such as fumonisin B1 (FUM1), fumonisin B2 (FUM2), and fumonisin B3 (FUM3), with FUM1 being the most toxic.⁴ Maize is used as a staple food for the majority of the population in Tanzania who live in rural areas. Maize and maize-based products are susceptible to fumonisin contamination.^{5,6} Due to this fact, fumonisin has been mentioned as one of the challenges affecting foods in Tanzania. A study conducted in each of the four main maize-producing regions of Ruvuma, Kilimanjaro, Iringa, and Tabora estimated the total fumonisin (FUM₁ + FUM₂) and found contamination of up to 11,048 µg/kg (median: 363 µg/kg) from the samples collected from 120 households.⁷

Fumonisin is found globally, with particularly high concentrations reported in regions such as Southern Africa and China.^{8,9} Fumonisin concentrations in "home-grown" corn from Linxiang, China, and areas of the Transkei, southern Africa, are continuously high, and concentrations exceeding 100,000 µg/kg have been occasionally reported.¹⁰ The extremely high fumonisin produced in Benin, West Africa, by two species, *Fusarium verticillioides* (68%) and *Fusarium proliferatum* (31%), was most commonly isolated from maize with total fumonisin levels ranging from 8240 µg/kg to 16,690 µg/kg.¹¹

Factors promoting the occurrence and growth of fumonisin species include higher temperatures and humid climates.¹² Fumonisin climatic conditions regions experience extreme rainfall patterns, temperature, as well as longer durations of drought.⁵ Fumonisin toxins are predominant in temperate and Mediterranean climatic regions.¹³ Fumonisin becomes the predominant contaminant of maize and maize-based products.¹⁴ Fumonisins' growth and accumulation are associated with drought and insect stress, and growing hybrids outside their areas of adaptation.^{15,16}

Infants and young children who consume maize-based foods are at a high risk of exposure to fumonisin.¹⁷ Fumonisin exposure during the first

trimester of pregnancy has been associated with an increased risk of neural tube defects in infants, particularly through the consumption of maize-based foods containing high levels of the toxin.¹⁸⁻²¹ Children are at greater risk of toxic effects as compared to adults because of their lower body mass and do not have a mature detoxification system.²² The first 1000 days of life, the period from the fetal stage to 2 years old, is characterized by accelerated growth and developmental plasticity.²³ This early-life period is very sensitive to any event that alters the programming of the main body functions and represents a window for intervention to improve child health.²⁴ In this regard, pregnant women, fetuses, and infants are particularly vulnerable to exposure to food contaminants.²⁴ Fumonisin from contaminated food can cross the placental barrier and affect fetal health.²⁵

Throughout the world, different countries set regulations to protect clients from fumonisin contamination. The Maximum Tolerable Limit (MTL) in developed countries, including Europe, is 1.0 µg kg⁻¹ body weight per day.²⁶ In developing countries, including the East African community, Tanzania, being one among them, has 2000 µg/kg.^{27,28} There are no established Maximum Tolerable Limits (MTLs) of fumonisin concentrations specifically for children in low- and middle-income countries.

To assess the causal evidence between aflatoxin exposure and linear growth faltering, a two-arm cluster randomized control trial (Mycotoxins Mitigation Trial; MMT) was conducted in Kongwa District, in Dodoma region, Tanzania. The intervention arm households were provided with low aflatoxin maize and groundnuts (4:1) blended and groundnut flour for the index child every month for 12 months. The comparison or standard of care (SoC) arm participants were educated on preparing the 4:1 blended flour from locally produced maize and groundnuts. During the preparation of low aflatoxin blended flour, rigorous sorting was done to reduce AF in maize and groundnuts. Sorting was also done to ensure that the blended flour provided to households in the intervention arm did not exceed the legal limit for fumonisin in Tanzania. Maize was not provided by the MMT project in all arms but study participants utilized maize acquired at household's level.

The primary objectives of the sub-study were (i) to assess fumonisin contamination of foods fed to infants and the consequent dietary exposure of the infants based on body weight, and (ii) to compare the infant food contamination and infant dietary exposures among selected households from the two arms of the trial. This observational study aimed at exploring whether the provision of low aflatoxin

flour led to differences in intake and exposure to fumonisin among children from households across the two arms of MMT.

Materials and Methods

This sub-study took place in the Kongwa District of Dodoma region among infants recruited in the Mycotoxin Mitigation Trial (Figure 1).

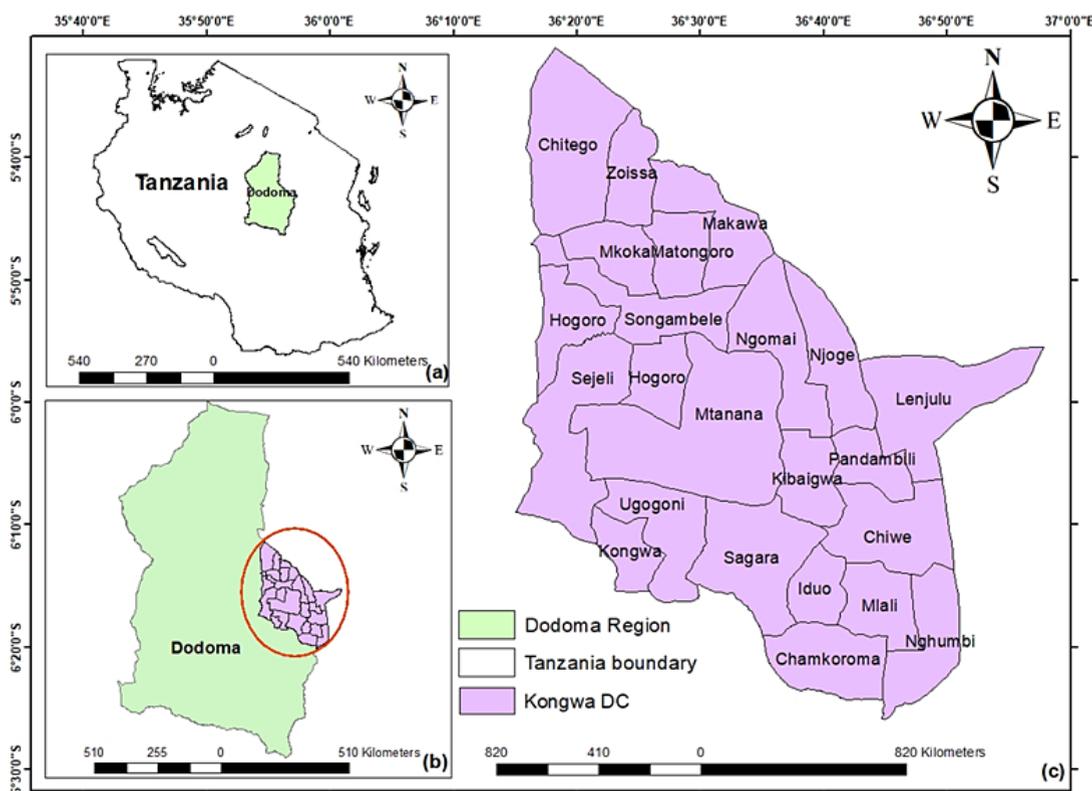


Fig. 1: A map of (a) Tanzania showing Dodoma region, (b) Dodoma region showing Kongwa DC, (c) Kongwa district showing wards

**Background and Study Population
Mycotoxin Mitigation Trial**

Children were recruited into the MMT from health facilities. When the infant turned 6 months of age, the intervention was introduced to the mother. Pre-blended porridge flour made of low-aflatoxin maize and groundnut in the ratio of 4:1, and low-aflatoxin groundnut flour were provided to participants in the intervention arm along with the infant feeding education. The low-aflatoxin maize and groundnut flours were processed through a collaboration with a local food producer in Arusha, Tanzania, known

as Halisi Products Limited. The threshold for low aflatoxin was 5 µg/kg²⁹ and 2000 µg/kg for fumonisin. Participants in the SoC arm received the same infant feeding education along with the promotion of infant porridge made from household-acquired maize and groundnut flours in the same 4:1 ratio as the intervention arm.

Sub-Study Participants' Selection

This sub-study was carried out in 20 of the 52 MMT clusters (10 matched pairs per arm). The 20 clusters were selected based on the accessibility

of the health facilities. A random number generator was used when infants turned 12 months of age, and researchers randomly selected and recruited study participants. One hundred forty mothers in the intervention arm and 142 from the SoC from these

clusters were selected randomly to participate in this sub-sample study. This sample size represented approximately 10% of the full trial sample planned for household visits and food sample collection (Figure 2).

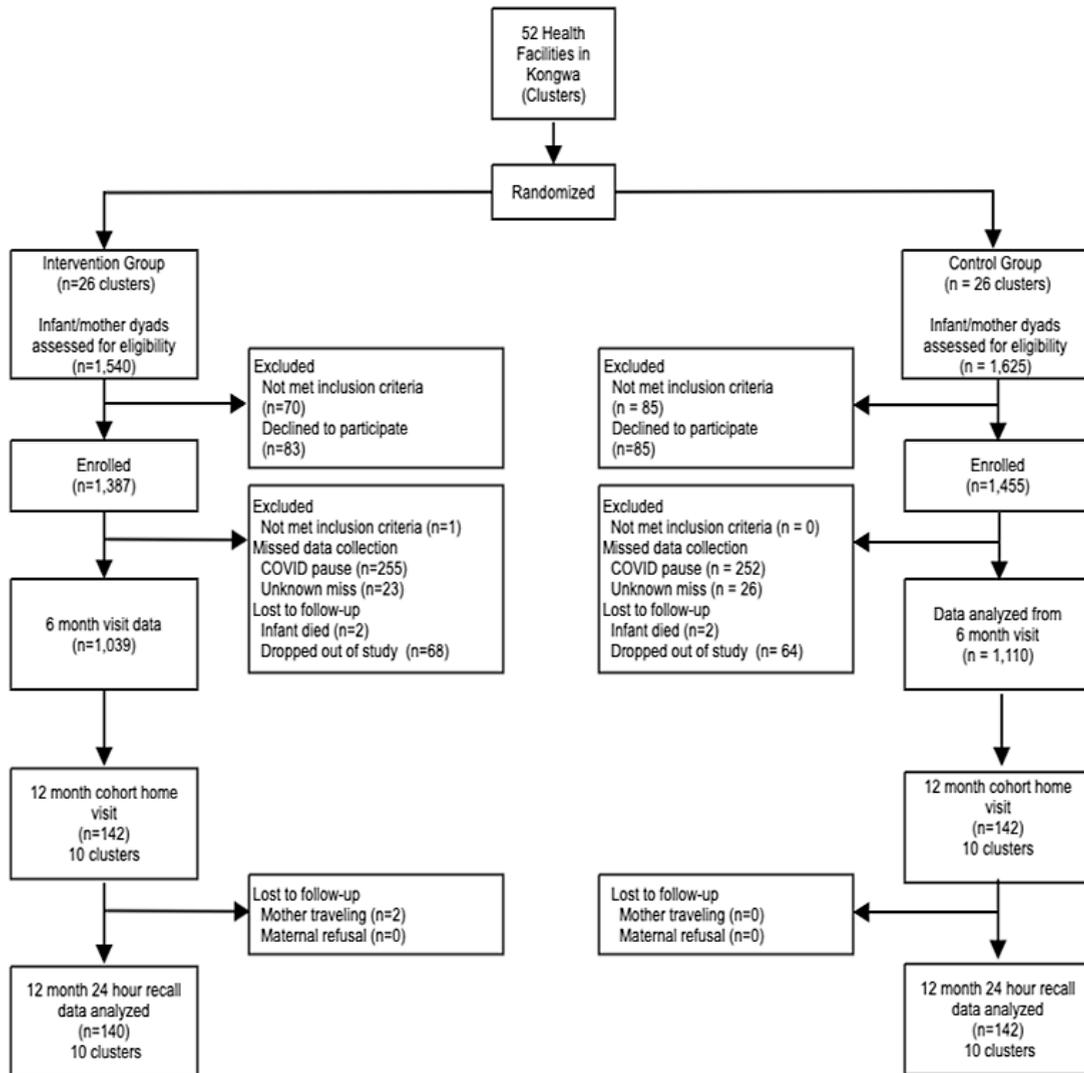


Fig.2: Flow diagram of participants

Infants were qualified for the study if (i) they participated in the 6-month visit within the MMT, (ii) they were between 11 and 13 months of age, and (iii) mothers were willing to provide informed consent for data collection, in addition to the informed consent for the larger MMT trial. Mother-infant dyads were excluded from this study if they

did not provide informed consent for additional data collection or dropped out of the MMT by the time they were visited. The age of 11 to 13 months was purposely targeted since the children had already started consuming porridge and other family foods, especially the stiff porridge, locally called ugali, made of maize and maize products.

Assessment of Food Intake and Collection of Food Samples

Pantry-level sample collection of fumonisin prone complementary food ingredients (maize and blended flours) from households in both arms was conducted during 24-hour dietary recalls. Maize (250 grams) and blended flour (100 grams) were sampled and weighed using a Pull-Out Display-5lb-(black)- food scale (OXO- model 1157100).

During food sampling, each batch of cereals or flour weighing less than 20 kg was mixed by flipping the storage bag or container up and down four times. Four random sub-samples (approximately 100-250 grams) were drawn from different sections of the stock bag/tin and carefully sealed in a food-grade, clean zip-lock bag. The air within the zip-lock bags was expelled during packing, and the bags were securely sealed and stored at -20 °C at Kongwa District Hospital. Subsequently, the samples were transported to the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory in Arusha and kept at -20 °C until analysis.

Compensation ranging from US\$0.50-0.65 was provided to households for each collected sample, depending on the type of food. Additionally, information regarding the source of food (market-sourced or home-grown) for the cereals and groundnuts was recorded.

Fumonisin Detection by ELISA

Fumonisin concentrations were quantified using commercially available low-matrix enzyme-linked immunosorbent assay (ELISA) kits (Helica Biosystems Inc., USA), following the manufacturer's protocol. Fumonisin was analyzed using total fumonisin ELISA (Catalogue number: 941AFL01M-96). Kit choices were determined by the manufacturer's recommendations for specific food types. The assay standards provided a 1-20 µg/kg range, and fumonisin samples exceeding the upper limit were diluted and retested. In each assay, a blank sample and two Quality Control Reference Materials (QCRM).

Estimation of the Daily Flour Intake

The estimates of dietary exposure of Infant Young Children (IYC) to fumonisin in the present study were based on flours consumed in stiff/thick porridge (ugali) or thin porridge only because they are not only

susceptible to contamination with fumonisin, but also the most commonly and frequently consumed foods in the study area. The proportion of flour consumed through a thin or stiff porridge was computed³⁰ estimated that the flour content of thin and stiff porridge consumed in Tanzania is 17 and 36% (w/w), respectively. The weight in grams of the thin or stiff porridge prepared from either cereal-only or cereal groundnut-based flour, as estimated from each of the two 24-hour recalls, was used to estimate the average flour intake per day per child. Calculating the amount of consumed flour and adjusting the volumes and weights of the thin porridge enabled us to estimate its specific gravity to be 1.1 g/mL. Therefore, the volumes (mL) of the thin porridge were multiplied by the specific gravity (g/mL) to obtain their consumed weight in grams.

Estimation of Fumonisin Dietary Exposure

The estimation of dietary exposure to fumonisin was estimated by the deterministic approach (Equation (1)) (JECFA, 2010). The estimated exposure of a child to fumonisin (ng/kg bw/day) was calculated through different CF ingredients consumed by the child. Exposure through an ingredient was estimated by multiplying the daily intake of contaminated ingredients and the contamination of fumonisin level in the consumed food ingredient, and dividing the result by the child's body weight.

$$\text{Dietary exposure (ng/kg bw/day)} = \text{Consumption (g/day)} \times \text{concentration (ng/g)} / \text{Body weight of the subject (kg)}$$

Statistical Analysis

Data analysis was conducted using STATA 16. A Chi-square test was used to compare differences in the distribution of fumonisin contamination for each type of food by study arm, using categories of fumonisin < 1 µg/kg (a value less than half of the legal limit), 1-2 µg/kg as intermediate, and >2 µg/kg (above the legal limit). An unpaired t-test was also conducted to compare mean fumonisin across arms; however, since fumonisin was not normally distributed, the data were additionally log transformed.

Ethical Clearance

Ethical approval for conducting this research was obtained from the Northern Tanzania Health Research Ethics Committee (KNCHREC) with registration number KNCHREC00041/02/2021.

Consent to participate in the study was obtained from mothers at the initial stages of study implementation.

Results

Demographic Information of Participants

Dietary recall and food sampling were conducted among 282 children aged 11 to 13 months (n=140 in the intervention arm and n=142 in the SoC arm). The demographic characteristics of the mothers were similar between arms. The average age of the recruited infants was 11.7 months, and 46.4% and

43.7% of the infants were female in the intervention and SoC arms, respectively. Most of the mothers were married and above the age of 18 years, with a mean of 27.8 and 27.9 in the intervention and SoC arms, respectively. 99.3% in the intervention arm and 97.2% were continuing breastfeeding while starting complementary feeding.³⁰

Types of complementary food flours and fumonisin results Food samples collected covered maize and blended flour as follows;

Table 1: Participants and household characteristics

Variable	Description	Intervention (n = 140)	Standard of Care (n=142)
Infant age (months)	Mean (SD, range)	11.7 (0.5, 11-13)	11.7 (0.4, 11-13)
Infant gender n (%)	Males	75 (53.6)	80 (56.3)
	Females	65 (46.4)	62 (43.7)
Breastfeeding n (%)	Currently breastfed	139 (99.3)	138 (97.2)
	No longer breastfed	1 (0.7)	4 (2.8)
Infant appetite n (%)	Normal	95 (67.9)	91 (64.1)
	Less than normal	43 (30.7)	49 (34.5)
	More than normal	2 (1.4)	2 (1.4)
Mother's age (years)	Mean (SD, range)	27.8 (7.2, 16-47)	27.9 (7.8, 16-47)
	Married	111 (79.3)	112 (78.9)
Marital status n (%)	Separated/Divorced	13 (9.3)	9 (6.3)
	Single/Never married	16 (11.4)	20 (14.1)
	Widowed	0 (0)	1 (0.7)
People in the house	Mean (SD, range)	5.3 (1.9, 1-11)	5.7 (1.9,1-11)
	Pit latrine	119 (85.0)	98 (69.0)
Type of toilet n (%)	Ventilated improved pit	20 (14.3)	37 (26.0)
	None, Bush	1 (0.7)	5 (3.6)
	Others	0 (0)	2 (1.4)
Water source n (%)	Protected source	107 (76.4)	108 (76.1)
		32 (23.6)	34 (23.9)

Table 2: Food sample collection between the intervention and SoC

Arm	Type of food	Sample collected	Samples analyzed
Intervention		Blended flour	181
SoC		Maize flour	205
Total			386

Table 3: Level of total fumonisin (µg/kg) in 4:1 blended flour sample between

Arm	Intervention	SoC
Mean (SD)	0.82 (0.74)	1.01 (1.25)
Median (range)	0.68 (0.0-4.81)	0.58 (0-5.2)
<1 µg/kg (%)	86 (80%)	52 (71%)
<2 µg/kg (%)	101 (94%)	61 (84%)
≥2 µg/kg (%) (range)	7 (6%)	12 (16%)
n	108	73

Table 4: Level of total fumonisin (µg/g) in maize between arms

Arm	Intervention	SoC
Mean (SD)	0.92 (1.55)	1.37 (1.56)
Median (range)	0.56 (0.01- 12.63)	0.78 (0.01- 6.9)
<1 µg/kg (%)	64 (78%)	47 (57%)
<2 µg/kg (%)	73 (89%)	64 (77%)
≥2 µg/kg (%) (range)	9 (11%)	19 (23%)
n	82	83

Table 5: Fumonisin intake and exposure levels between the intervention and SoC arms

Food type	Arm	fumonisin Intake (ng*g)	Intake mean	p-value	fumonisin Range (ng*g/kg)	fumonisin mean exposure	p-value
Blended flour	Intervention	0.0 - 0.27	0.030	0.6	0 - 4.811	0	0.6
	SoC	0.0 - 0.27	0.050				
Maize	Intervention	0.0 - 0.24	0.02	0.1	0 - 2.85	0.003	0.1
	SoC	0.0 - 0.36	0.05				

Discussion

Of great value, the MMT project ensures the safety of maize and blended flour from fumonisin during the process of reducing aflatoxin in maize and blended flour. Fumonisin contamination ranged from 0-4.8 µg/kg in the intervention and 0- 5.2 µg/kg in the SoC arm for blended flour; likewise, fumonisin levels in maize ranged from 0 -12.6 µg/kg in the intervention µg/kg and 0-3.9 µg/kg in the SoC arm. Fumonisin concentrations for blended flour samples were low in the intervention compared to SoC; this might be due to the pre-preparation of maize, including sorting and screening, which helped in lowering the concentration for blended flour. For maize

samples, fumonisin concentration was higher in the intervention arm than in the SoC because no process was done on both the intervention and SoC. Maize samples were not provided by the MMT project in all study arms, but all study participants utilized maize acquired from households. Generally, in these two arms, fumonisin levels in maize and blended flour were quite low compared to other studies. For example, results reported by Kimanya, who conducted a study in Tabora, Ruvuma, Kilimanjaro, and Iringa regions, show the fumonisin ranges (71-2763 µg/kg, 62-3560 µg/kg, 65-11048 µg/kg, and 61-3353 µg/kg), respectively. Likewise, results from our study were low to total fumonisin of 10,140

µg/kg determined in maize from the former Transkei region in South Africa.^{9,32} On the other hand, blended flour produced by the project and distributed to the intervention arm had low fumonisin compared to maize samples, which were directly acquired at family levels. These results demonstrated that Tanzanian home-grown maize and maize products were contaminated with fumonisin.

Results from this study also show that the occurrence and level of fumonisin in maize and blended flour did not differ significantly between the two arms. Similarly, there are no differences in exposure across households from the two arms.

There was no association between the weight of babies and fumonisin intake and exposure in this study because the weight of babies was almost the same between the SoC and intervention. The mean Weight of babies who consumed blended flour was (8.04, 9.18) kg, and (8.48, 8.75) kg in the maize intervention and SoC, respectively.

Furthermore, this study demonstrated that there is no evidence that the frequency of consumption of fumonisin level for blended samples was significantly different between the two arms ($p > 0.05$).

Fumonisin consumption from maize samples from the two arms is significantly different ($p = 0.05$).

Variation of fumonisin levels between the intervention and SoC arms was due to sorting done to the maize samples used by MMT to make blended flour for the intervention arms. High fumonisin levels were noted in maize samples because there was no treatment done to reduce fumonisin in maize, which was acquired at the household level. Thus, sorting as practiced on blended flour was effective enough to reduce fumonisin contamination in maize and subsequently minimize fumonisin exposures in these communities; likewise, these levels were low compared to fumonisin concentration reported in other African countries.

Maize grown in Africa has been reported to contain fumonisin concentrations as high as 10000 µg/kg.³² Fumonisin exposure assessments conducted in communities dependent on maize in South Africa and Tanzania indicated that consuming maize with fumonisin levels exceeding 155 µg/kg can lead

to exposure surpassing the provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg body weight (BW).⁷

Fumonisin exposure through diet poses a serious risk to children's health, as it can adversely affect growth and development. These mycotoxins are prevalent in staple foods and constitute a substantial portion of complementary foods for young children.³³ In some regions of sub-Saharan Africa, the prevalence of child growth impairment rises as children get older. This trend has been associated with the introduction of complementary foods, which increases fumonisin exposure due to the higher intake of maize and maize-based products.³⁴

Young children in rural areas of sub-Saharan Africa experience elevated levels of dietary fumonisin intake.³³ Two epidemiological studies have been carried out to examine the link between fumonisin exposure and child growth. In a study by Chen *et al.* (2018), it was observed that infants in Tanzania with fumonisin intakes exceeding the JECFA's PMTDI of 2 µg/kg bw/day, as estimated through caregivers' dietary recall questionnaires, were notably shorter and lighter than those with lower fumonisin intake. The study, based on validated urinary biomarkers of fumonisin exposure, found a negative correlation between fumonisin exposure and child growth in children from four villages in Tanzania.

Shirima *et al.* (2015) found a negative association between fumonisin exposure and child growth in children from four villages in Tanzania, with fumonisin levels assessed using validated urinary biomarkers.³⁰

This paper reports for the first time the level of fumonisin contamination in blended flour and maize samples after the MMT intervention implemented in central Tanzania. The study used an appropriate sample of 181 blended samples and 165 maize samples collected in the Kongwa District. The study aimed at identifying the extent of fumonisin levels in samples collected in the Dodoma region of central Tanzania, where the contamination is likely to be high and urgent intervention is needed. Additionally, the great value of this study was to examine whether the aflatoxin intervention did not impart fumonisin, and the study results confirmed that.

Based on the findings of this study, further research on fumonisin contamination in maize and maize-based products is essential, given that maize is a key component of complementary foods. Additional data will provide valuable insights for agricultural stakeholders working with rural communities, enabling them to offer informed guidance to farmers and households on strategies to minimize fumonisin contamination. Moreover, educating farmers on good agricultural and post-harvest management practices can play a crucial role in reducing mycotoxin exposure in rural populations.

Conclusion

Results from this sub-study confirmed that there were no significant differences in risks to fumonisin dietary exposure between selected households from the intervention and SoC arms. The results further confirmed that the process of low-aflatoxin blended flours contributed to lowering the levels of fumonisin in the food consumed by children in the household. This indicates that the production process, which involved procuring maize from different regions, did not raise fumonisin levels. Samples collected from this study had low fumonisin levels compared to other studies done in Tanzania. The preparation of low-aflatoxin complementary feeding flours also reduced fumonisin $\mu\text{g}/\text{kg}$ levels in blended and maize flour consumed by study participants in the intervention households.

We recommend the development of guidelines and education materials based on lessons from the processing of low aflatoxin flours that could contribute to the reduction of fumonisin. Winnowing, rigorous maize sorting, and slight decortication can offer low-cost, cost-effective fumonisin reduction strategies, especially in regions prone to high fumonisin.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The corresponding author will be happy to provide the data supporting the findings of this study upon request.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Materials from Other Sources

Not Applicable.

Author Contributions

- **Rosemary Alphonse Kayanda:** conceptualization, and methodology, and performed data collection, analysis, interpretation, and writing of initial and consequent drafts of the manuscript.
- **Francis Muigai Ngure:** designing of the study and provided a critical review of the manuscript, also engaged in oversight on lab analysis, data analysis and interpreting the results.

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