RESEARCH ARTICLE

Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania

Martin E. Kimanya[1,2], Bruno De Meulenaer[2], Dominique Roberfroid[3], Carl Lachat[3] and Patrick Kolsteren[2,3]

[1] Tanzania Food and Drugs Authority, Dar es Salaam, Tanzania
[2] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[3] Nutrition and Child Health Unit, Department of Public Health, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium

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Infants consuming maize-based foods are at a high risk of exposure to fumonisins. This study explored the association between exposure of fumonisins from maize and growth retardation among infants in Tanzania. Mothers of 215 infants consented for their children to participate in this study. We estimated maize intake for each child by twice conducting a 24 h dietary recall and fumonisins level in the maize, using HPLC. Fumonisins exposure for each child was estimated by combining his/her maize intake and the fumonisins level in the maize. Of the infants, 191 consumed maize. The maize consumed by 131 infants contained fumonisins at levels varying from 21 to 3201 μg/kg. Fumonisins exposure in 26 infants exceeded the provisional maximum tolerable daily intake of 2 μg/kg body weight. At 12 months of age, infants exposed to fumonisins intakes above the provisional maximum tolerable daily intake of 2 μg/kg bodyweight were significantly shorter by 1.3 cm and 328 g lighter. It appears that the exposure to fumonisins is associated with growth retardation. This is the first study to report an association between fumonisins exposures and growth retardation.

Keywords:
Exposure / Fumonisins / Growth / Infants / Tanzania

1 Introduction

Growth faltering is a major public health problem affecting infants and young children in Tanzania. Among children of less than 5 years of age, growth failure, as measured by rates of stunting, underweight and wasting, stands at 38, 22 and 3%, respectively in 2004–2005 [1]. As in other African countries, infants in Tanzania experience growth retardation during the period of introduction of complementary foods.

In Tanzania, complementary foods are largely cereal based with maize being the main part [2, 3]. Maize from Africa contains fumonisins at concentrations which can be as high as 10 000 μg/kg [4–11]. Fumonisins exposure assessments performed for communities relying on maize in South Africa and Tanzania showed that consumption of maize containing fumonisins concentrations above 155 μg/kg can result in exposure above the provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight (bw) [9]. In a fumonisins exposure assessment for infants consuming maize-based foods in Tanzania,

Correspondence: Professor Patrick Kolsteren, Nutrition and Child Health Unit, Department of Public Health, Prince Leopold Institute of Tropical Medicine, 155 Nationalestraat, 2000 Antwerps, Belgium
E-mail: pkolsteren@itg.be
Fax: +32-3-247-6543

Abbreviations: bw, body weight; **FB_1,** fumonisin B_1; **FB_2,** fumonisin B_2; **FB_3,** fumonisin B_3; **MTL,** maximum tolerable limit; **LAZ,** length-for-age z-score; **PMTDI,** provisional maximum tolerable daily intake; **WAZ,** weight-for-age z-score

Kimanya *et al.* [11] showed previously that the infants are at a very high risk (up to 24%) of exceeding the PMTDI.

In Benin and Togo, researchers reported a striking association between exposures to another form of mycotoxins, aflatoxins and growth faltering whereby over a period of 8 months, children exposed to the highest level of aflatoxin had a 2 cm lower height gain than those exposed to the lowest levels [12]. Following these studies, Egal *et al.* [13] found that consumption of maize in the countries of Benin and Togo is an important cause of the high exposure of aflatoxins. Studies suggest that just like aflatoxins can do in humans, fumonisin B_1 (FB_1) can reduce growth rate in pigs [14–16] and rats [17–19]. These findings are a cause of worry that the poor growth among children in Tanzania is associated with high exposure to fumonisins and or aflatoxins in maize-based complementary foods.

Therefore, this study was performed to explore the influence of exposure to fumonisins on performance in growth among infants complemented with maize-based complementary foods in a community in Tanzania where maize is a staple food. Unlike the exposure assessment that was performed by Kimanya *et al.* [11] which used contamination in un-milled maize, this exposure assessment used contamination in ready-to-cook maize flour consumed by these infants.

2 Materials and methods

2.1 Study area

The study was conducted in four villages of Tarakea division, Northern Tanzania. In this area maize, coffee, banana, potatoes, kidney beans, finger millet and cassava are the primary sources of food and income. This division was chosen based on the outcome of a preliminary survey of fumonisins and aflatoxins contamination in maize from main maize producing parts of Tanzania [10]. The survey found that maize from a village (Kikelelwa) in Tarakea contained higher fumonisins contamination levels of up to 11 048 μg/kg compared with levels of up to 3560 μg/kg in maize from villages of Kigwa, Litapwasi and Nyabula surveyed in other parts of the country. The survey also showed that aflatoxin B_1 contamination in maize from Kikelelwa (just like in maize from Litapwasi and Nyambula) was lower than contamination in maize from Kigwa village. The occurrence of aflatoxin B_1 in samples of maize from Kikelelwa, Nyabula and Litapwasi was 3% and from Kigwa, 34%.

2.2 Recruitment of infants

All infants in Tarakea who attained the age of 6 months in July, August or September 2006, were eligible and recruited to participate in the study. Infants were identified using their registration number and dates of birth as recorded in registers of birth in seven reproductive child health clinics of the division. In Tanzania, all infants born in clinics are registered soon after birth and those born in homes are registered on the day the child is taken to the clinic for immunization. The study was approved by the Ethics Committees of the National Institute of Medical Research in Tanzania and Ghent University in Belgium.

2.3 Complementary food survey

Complementary food consumption data and maize flour samples were collected in September 2006. A repeated 24 h dietary recall was used to estimate consumption of complementary food for each infant. The procedure used to conduct the 24 h dietary recall is described in Kimanya *et al.* [11]. The mother of each child who consumed maize during the 24 h dietary recall, provided information about number of days she fed her child with maize in the previous week. During each visit, an amount of maize flour equivalent to that used in preparation of the complementary food in the previous day was packed in a khaki paper bag, sealed and then transported to the Tanzanian Food and Drugs Authority laboratory in Dar es Salaam for fumonisins determination.

2.4 Fumonisin determination in ready-to-cook maize flour

Two packages of maize flour, collected during the two 24 h dietary recalls, were opened and the contents were thoroughly mixed by using a laboratory mixer to constitute a composite sample. From the thoroughly mixed maize flour, 15 g of flour was taken and analyzed for FB_1, fumonisin B_2 (FB_2) and fumonisins B_3 (FB_3).

We determined FB_1, FB_2 and FB_3 in the maize flour by using a LC method based on Sydenham *et al.* [20] and slight modifications recommended by Samapundo *et al.* [21].

To evaluate suitability of the method, blank samples of maize flour were spiked with FB_1 at concentrations ranging from 50 to 150 μg/kg. The average recovery value for this toxin was 84% (four samples, RSD of 15.45%). Also the flour samples were spiked with FB_2 and FB_3 each at levels from 100 to 300 μg/kg. The average recovery was 86% (four samples, RSD of 9.16%) and 85% (four samples, RSD of 7.91%) for FB_2 and FB_3, respectively.

The LOD for FB_1 was 20 μg/kg and for FB_2 or FB3 was 18 μg/kg. The LOD for the method were based on the mean value of the blank readings plus three standard deviations. The results were corrected for recovery.

2.5 Anthropometric measurement

Information of each child's birth weight and date of birth were obtained from his/her mother and double checked

from birth records on his/her clinic card or a register of birth in the clinic. Initially (when the infants were 6 months old), and subsequently (when the infants attained 12 months of age) length and weight of each child were also measured. Recumbent length of the child was measured using an infant measuring board, which had a fixed head rest and a movable foot piece (Perspective Enterprises, Portage, MI). Length measurement was taken with the infant in supine position according to standard procedures and recorded to the nearest 0.1 cm. A weigh scale with a precision of 100 g (Seca, UK) was used to measure the weight with the child wearing light clothes only. Length-for-age *z*-scores (LAZ), weight-for-age *z*-scores (WAZ) and weight-for-length *z*-scores were calculated using the median value of the international reference population recommended by the World Health Organization (WHO) in 2005. According to the WHO criteria, a *z*-score < -2 for LAZ indicates stunting; for WAZ, being underweight and for weight-for-length *z*-scores, wasting or thinness.

2.6 Estimation of exposure

Fumonisins exposure assessment, for each of the child who consumed maize, was performed using the total fumonisins ($FB_1+FB_2+FB_3$) contamination data determined in this study and maize consumption data reported in Kimanya *et al.* [11]. In this study, we adjusted each of the child's average maize consumption reported by Kimanya *et al.* [11] by multiplying it with his/her weekly frequency (number of days in a week) of maize consumption divided by seven. This adjustment was done to obtain a better estimate of the habitual maize intake of the infants than what would be obtained from the repeat 24 h dietary recall alone.

In general, we determined fumonisins exposure for each child using the formula: $\mathbf{Y_i} = \mathbf{C_i}.\ \mathbf{D_i}.\ \mathbf{X_i}$, where $\mathbf{Y_i}$, the daily intake by an infant "*i*" of total fumonisins (μg/kg bw/day); $\mathbf{C_i}$, the total fumonisins ($FB_1+FB_2+FB_3$) in the maize flour (μg/kg) sample from the family of infant "*i*"; $\mathbf{D_i}$, the number of days the child received maize-based complementary food in the previous week divided by seven (number of days in a week); $\mathbf{X_i}$, the average daily consumption of maize (kg/kg bw/day) by the infant "*i*"; as estimated by Kimanya *et al.* [11] from the daily consumption of complementary food.

2.7 Categorization of infants to low and high exposure groups

At the end of the study, infants who consumed maize were categorized into two groups; the low and the high exposure groups. The low exposure group comprised infants who consumed maize that contained undetectable fumonisins and those exposed to fumonisins levels below the PMTDI value of 2 μg/kg bw. The high exposure group comprised infants exposed to fumonisins levels above the PMTDI. The PMTDI limit was derived by Joint FAO/WHO Expert Committee on Food Additives based on nephrotoxicity of these toxins in rodents and recommended for FB_1, FB_2 and FB_3 alone or in combination [22]. Joint FAO/WHO Expert Committee on Food Additives recommends this limit on the basis of no observable adverse effect level for renal lesions in male rats; which is about one-third of the lowest observable adverse effect level [22]. In the evaluation the no observable adverse effect level was 200 μg/kg bw/day but a safety factor of 100 was used to account for species (10) and interspecies (10) sensitivities thus deriving the PMTDI limit of 2 μg/kg bw. We chose to use this cut-off value to group the infants because international and national agencies responsible for formulation of food safety standards use this limit to derive the maximum tolerable limit (MTL) for fumonisins in maize; suggesting that being under PMTDI is safer than being above the limit.

2.8 Statistical analysis of data

The statistical package used was Stata version 10 (Stata 10.0; Stata, TX, USA). The error was set at 5% for all the tests done. Percentages of infants in the high and low exposure groups who consumed different types of maize-based dishes were compared using chi-squared test. First, chi-squared test was used to compare the distribution of gender and ethnic groups between the high and low exposure groups. The standard *t*-test was used in the bivariate analyses to compare mean of birth weight, energy intake, and LAZ and WAZ scores at 6 months of age between the high and the low exposure groups. Prior to comparison of the mean, the variables were tested for normality by using the Shapiro–Wilk test. In case of departure from normality the variables were log-transformed using the zero-skewness procedure. The association of fumonisin exposure on weight and length increase was analyzed using a multilevel mixed-effects linear regression model. The individual growth of each child was modeled with a random intercept and random slope (age was entered as covariate in the child level). Village was entered as an additional level and as a covariate. We analyzed the fumonisins exposure through a first model with the total fumonisins concentration as a continuous variable and a second model with fumonisins concentration expressed as dichotomous variable (higher and lower than 2 μg/kg bw/day). Other variables that could influence growth were entered in the model and retained as such. As additional covariates we entered energy and protein intake from complementary food, and gender, weight for height at 6 months. Birth weight was not retained in the model because the data cleaning revealed that the level of precision was too low. No substantial co-linearity was observed between the variables.

3 Results

3.1 Study subjects and recruitment

Mothers of 215 consented for their infants to participate in the growth assessment study. Of the 215 infants, 52% were males and 48% were females. The infants originated from three different tribes: Chagga (76%), Sambaa (14%) and Pare (10%). Of these infants, 99% were breastfed.

3.2 Amount of maize and type of maize dishes consumed

Eighty-nine percent (191 out of 215) of the infants consumed maize at varying levels from 1 to 106 g/child/day (mean; 32/day ± 24). The infants consumed maize in different type of dishes. Forty-four percent of the infants consumed a thin maize porridge, 20% consumed a combination of thin maize porridge and *ugali* (thick maize porridges), 15% consumed thin mixed cereal porridge, 5% consumed *ugali* and 5%, a combination of these dishes or these dishes and dehulled maize grits. The thin mixed cereal porridge contained, instead of pure maize flour, a mixture of flours from maize, rice, finger millet, groundnuts, legume beans, wheat, sorghum and bulrush millet at different combinations and proportions. In some families, sugar and cow's milk were added to thin porridges. Thick maize porridge and dehulled maize grits were prepared as family foods commonly served with some legume bean, vegetable or meat relishes.

3.3 Fumonisins in ready-to-cook maize flour

Of the 191 samples of maize flour, 131 (69%) contained total fumonisins ($FB_1+FB_2+FB_3$) at concentrations varying from 21 to 3201 μg/kg (median; 158 μg/kg). FB_1 was the most widespread and abundant of the three forms of fumonisins. FB_1 concentration in 67% of the samples ranged from 21 to 2375 μg/kg (median; 106 μg/kg), FB_2 in 52% of the samples ranged from 20 to 1076 μg/kg (median; 67 μg/kg) and FB_3 in 31% of the samples ranged from 18 to 604 μg/kg (median; 60 μg/kg). FB_1, FB_2 and FB_3 co-occurred in 27% of the samples. Eight percent of the maize flour contained total fumonisins concentrations in excess of the MTL of 1000 μg/kg set for maize flour for human consumption in the EU [23], Switzerland and Iran [24].

3.4 Fumonisin exposure

Of the 191 infants who consumed maize, 131 consumed maize that contained detectable fumonisin levels. Total fumonisin exposures in the 131 infants ranged from 0.003 to 28.838 μg/kg bw/day (median; 0.48 μg/kg bw/day and 90th percentile; 3.99 μg/kg bw/day). Of the 131 infants, 26 were exposed to levels above the PMTDI of 2 μg/kg bw. The 26 infants were classified into the high exposure group. The rest of the infants who consumed maize-based food were categorized into the low exposure group.

3.5 Baseline characteristics of the infants

Gender and ethnic groups' distributions between the high and low exposure groups were not significantly different (Table 1). Table 2 summarizes that, at the beginning of the study (6 months of age), LAZ and WAZ scores for the infants in the high exposure group were already significantly lower than the respective scores for those in the low exposure group.

3.6 The relationship between type of maize-based dishes and exposure to fumonisins

There was a significant association between consumption of a combination of thin maize porridge with *ugali* and high fumonisin exposures (Table 3). The association remained significant even after keeping only infants who consumed thin maize porridge in the dataset ($p = 0.013$). Table 3

Table 1. Similarity in proportions (percentages) of infants of different gender and ethnicity in the low and high exposure groups

Characteristic	Number of subjects (*n*)	Low exposure group (%)	High exposure group (%)	$p^{a)}$
Gender				
Males	95	85	15	
Females	93	87	13	0.694
Ethnicity				
Chagga	139	88	12	
Others	49	80	20	0.108

Low exposure: fumonisin intakes <2 μg/kg bw, high exposure: fumonisin intakes ≥2 μg/kg bw.
a) Proportions were compared using a *chi*-square test.

Table 2. Comparison of growth indicators at 6 months of age for infants in the low and high exposure groups

Characteristic	Low exposure group (mean ± SD)	High exposure group (mean ± SD)	$p^{a)}$
WAZ score	−0.32 ± 1.00	−1.77 ± 1.17	0.048
LAZ score	−0.97 ± 1.05	−1.60 ± 1.13	0.013
WLZ score	0.46 ± 1.01	0.44 ± 1.27	0.939

a) Means were compared using *t*-test, Low exposure: fumonisin intakes <2 μg/kg bw, high exposure: fumonisin intakes ≥2 μg/kg bw.

Table 3. Comparison of proportions (percentages) of infants who consumed different types of maize-based dishes in the high and low exposure groups

Type of dish	*n*	Low exposure group (%)	High exposure group (%)	*p*[a)]
Thin maize porridge versus other[b)] *dishes*				
Thin maize porridge	146	84	16	0.120
Other dishes[b)]	45	93	7	
Ugali versus other dishes				
Ugali	66	82	18	0.181
Other dishes	125	89	11	
Thin maize porridge with ugali versus other dishes				
Thin maize porridge with *ugali*	39	72	28	0.003
Other dishes	152	90	10	
Thin maize porridge with ugali versus thin maize porridge with other dishes				
Thin maize porridge with *ugali*	39	72	28	
Thin maize porridge with other dishes	107	89	11	0.013
Thin cereal[c)] *porridge versus other dishes*				
Thin cereal porridge	57	95	5	
Other dishes	134	83	17	0.028

a) Proportions were compared by *Chi*-square, Low exposure: fumonisin intakes $<2\,\mu g/kg$ bw, High exposure: fumonisin intakes $\geq 2\,\mu g/kg$ bw.
b) All other type of dishes, except the dish being compared with, considered together.
c) A mixture of maize with rice, finger millet, groundnuts, legume beans, wheat, sorghum or bulrush millet at different combinations and proportions; *ugali* is a stiff maize porridge.

further summarizes that the consumption of a thin porridge of mixed cereals was significantly associated with low fumonisin exposure ($p = 0.028$). Overall mean intake of maize ($49\,g/day \pm 27$) for the high exposure group was significantly higher than the respective amount of $28\,g/day \pm 23$ for the low exposure group. The mean energy intake for infants in the high exposure group ($448\,kcal/day \pm 212$) was significantly higher than for infants in the low exposure group ($353\,kcal/day \pm 230$).

3.7 The association between exposure of fumonisins and growth status

Using fumonisin exposure as a continuous variable demonstrated a dose-effect relation between exposure and growth in length, although not significant. Plotting the predicted values (using the mixed analysis) showed a definite decrease in length with increase in fumonsins exposure (Fig. 1). The predicted values are very much influenced by many zero values and the skewed distribution of the exposure values. Entering exposure as a dichotomous variable using $2\,\mu g/kg$ bw cut-off level, exposure was associated with a clear and very significant difference in growth between the high and the low exposure groups (Tables 4 and 5). Children who consumed complementary food with fumonisins concentrations $>2\,\mu g/kg$ bw were on average 1.3 cm shorter and 328 g lighter at 12 months. The effect on weight was mediated by length loss. The exposure–weight effect relationship disappeared when length was entered in the weight model. The exposure–village relationship

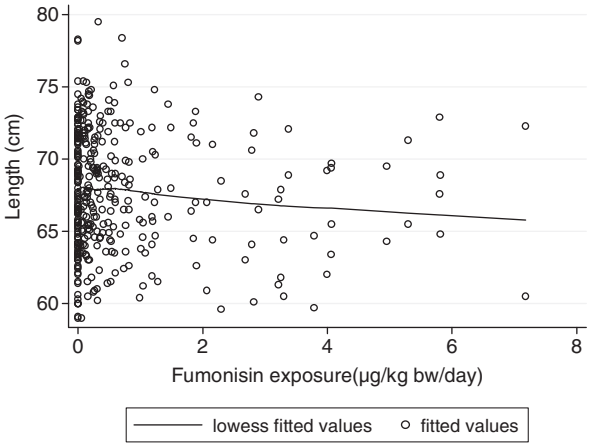

Figure 1. Relationship between fumonisin exposure from complementary food and attained length of infants at 12 months. Lowess: locally weighed regression curve.

is not equal in all villages as observed by the significant "village" effect in the model (Tables 4 and 5). In one village the effects are more pronounced. However, we did not collect village specific information and further multilevel analysis with covariates at village level is not possible.

4 Discussion

In the present study we demonstrated for the first time that there is a possibility that fumonisin exposure negatively

Table 4. Fumonisins exposure and its association with infant weight at 12 months

	β[a)]	SE[b)]	p[c)]	95% CI[d)]
Age (months)	0.245	0.006	<0.001	[0.233; 0.256]
Total energy intake from complementary foods (kcal)	−0.001	0.001	0.287	[−0.004; 0.001]
Gender (female is reference)	0.651	0.075	<0.001	[0.504; 0.797]
Fumonisins exposure[e)]	−0.328	0.109	0.002	[−0.541; −0.116]
Village	−0.132	0.048	0.006	[−0.226; –0.039]
Total protein intake from complementary foods (g)	−0.003	0.002	0.113	[−0.006; 0.001]
WHZ[f)] at 6 months of age	0.641	0.031	<0.001	[0.581; 0.701]

a) Regression coefficient.
b) Standard error.
c) Multilevel mixed-effects linear regression model with village as an additional level.
d) 95% CI.
e) Dichotomous; non-exposed = 0: fumonisin intakes <2 μg/kg bw, exposed = 1: fumonisin intakes ≥2 μg/kg bw.
f) Weight for height *z*-score.

Table 5. Fumonisins exposure and its association with infant length at 12 months, mixed analysis

	β[a)]	SE[b)]	p[c)]	95% CI[d)]
Age (months)	1.070	0.024	<0.001	[1.023; 1.118]
Total energy intake from complementary foods (kcal)	−0.00002	0.005	0.996	[−0.010; 0.010]
Gender (female is reference)	1.999	0.305	0.001	[1.400; 2.597]
Fumonisin exposure[e)]	−1.374	0.443	0.002	[−2.242; −0.506]
Village	−0.568	0.181	0.002	[−0.924; −0.213]
Total protein intake from complementary foods (g)	−0.014	0.007	0.036	[−0.028; 0.001]
WHZ at 6 months of age[f)]	0.368	0.171	0.031	[0.034; 0.702]

a) Regression coefficient.
b) Standard error.
c) Multilevel mixed-effects linear regression model with village as an additional level.
d) 95% CI.
e) Dichotomous; non-exposed = 0, exposed = 1.
f) Weight for height *z*-score.

affects linear growth of infants in rural Tanzania. Exposed children are both shorter and lighter, although the latter effect appears to be modulated by length. The data also suggest that this effect is noticeable at the age of 6 months. It is indeed well established that in rural societies, complementary feeds are introduced well before this age starting as early as 2–3 months.

Growth faltering in infants is caused by a wide range of factors [25–28], most of which relate to unsatisfactory food intake or severe and repeated infections, or a combination of the two [29, 30]. Energy intake in the high exposure group was significantly higher than in the low exposure group ($p = 0.015$) and ranged from 147 to 763 kcal/day (mean; 448 kcal/day ± 212) in the high exposure group and, from 28 to 1124 kcal/day (mean; 353 kcal/day ± 230) in the low exposure group. These mean energy intake values are well in accordance with the WHO recommended levels of energy intake from complementary foods in developing countries [31] assuming infants received average amounts of breast milk. Both energy and protein intake were used as covariates in the model making energy intake an unlike explanatory variable for the observations. The continuous effect of exposure on growth suggests that the effect on growth might already be present at levels lower than the cut-off used in this study. However the small numbers of exposed infants do not allow further analyses.

Being the first study to observe the effect of fumonisin exposure on linear growth, we have very little comparison possibilities for our findings. Research reports have predominantly shown that fumonisins have growth retardation effects in animals [14–19] and the mechanisms are still not well understood. According to Carratu *et al.* [17] the mechanisms responsible for FB_1 toxicity and carcinogenicity include lipid peroxidation (prevented by vitamin E) and inhibition of protein and DNA synthesis in both primary rat hepatocytes and C6 glioma cells. Furthermore, our observation is consistent with reports that mycotoxins could increase susceptibility of animals to infectious diseases and decrease vaccine efficacy [16, 31]. It was also reported that ingestion of FB_1 increases bacterial colonization of the

intestinal tract by a pathogenic strain of *Escherichia coli* [14]. In another study it was found that a low oral dose of fumonisin containing culture material may predispose piglets to the development of lung pneumonia induced by *Pasteurella multocida* in which animals treated with fumonisin extract and *P. multocida*, showed delay of growth, cough and lung inflammatory process [15]. Previously, Collin *et al.* [18, 19] described a dose-related decrease in overall feed consumption and bw gain in pregnant rats given FB_1 oral doses of up to 15 mg/kg bw/day on gestation days, 3–16. Although the doses used in these animal studies are higher than the highest exposure of 28.838 μg/kg bw/day estimated in the infants by this study, the association between exposure to fumonisins and growth retardation in the high exposure group is consistent with the findings from studies in animals.

The present study has some limitations. Even if we tried to get a good estimate on exposure by combining a repeat 24 h recall and an FFQ, one can argue that this gives a limited picture of a diet over 6 months. We know that the foods consumed are largely from own production and that large batches of flour are prepared to be used over a considerable period of time. Food diversity is low in this area and the diet is rather monotonous. All these elements account for the validity of our findings. We also did not collect information on the age when maize-based complementary food was introduced to these infants, nor do we have detailed information on early complementary feeding. However, available data on introduction of complementary foods in Tanzania show that mothers in Tanzania begin introducing complementary food as early as at 2–5 months of age [2, 32, 33].

We also found a relatively small number of exposed infants, which should caution us to declare with certainty the exposure–effect relationship. On the other hand, the possible effect underlines the importance to pay more attention to food contaminants as determinants of linear growth and even more so when one considers that vast population groups consume a predominantly maize-based diet. Larger studies will allow for collection of data on the age complementary foods are introduced to infants in Tanzania, thus providing a better assessment of the relationship between fumonisins exposure and child growth.

We also did not determine aflatoxins for the entire sample even if they have been associated with poor child growth in societies where maize is a staple food. On the basis of initial analyses (Kimanya *et al.* [10]) there was a low likelihood of finding aflatoxins in the maize consumed by the infants who participated in this study. However, we decided to verify this expectation by testing aflatoxins B_1, B_2, G_1 and G_2 in the samples of maize from 44 randomly selected families of the studied infants. There were no detectable aflatoxin levels in the samples.

Another limitation of our study is that exposure levels were determined by a food intake proxy. In the studies that reported association between exposure to aflatoxins and impaired child growth, aflatoxins were determined in blood, not in food [12]. Whereas the use of a biomarker is the most reliable method for determination of exposure to chemicals, biomarkers for fumonisins are not validated yet [34]. In this study, the fumonisins were relatively homogeneous in nature compared to aflatoxins in maize. Together with the possibly homogeneous food consumption pattern in rural Tanzania, this permitted a reasonable estimation of fumonisin exposure by combining the food intake estimation and fumonisin concentrations in maize. The families from which the subjects originate consume maize as staple food, mostly from home grown and stored stocks that last for up to 6 months or longer after harvest. It was possible, therefore, to get representative samples of the maize meal consumed by each infant during the study period.

The study used maize as the only source of fumonisins. This is because according to Miller [35, 36] fumonisins are found mainly in maize and sorghum as *F. verticillioides* and *F. proliferatum* (the fungi that produce fumonisins) rarely infect other crops. Other foods which were consumed together with maize in the study population include finger millet, rice, banana, bulrush millet and fresh cow's milk.

This being the first study to estimate effects of fumonisins exposure in complementary foods on growth of infants, the results are considered satisfactory enough to at least justify further research in this matter. These findings provide evidence for the need to set and enforce regulations that limit fumonisin levels in maize for human consumption in Tanzania. In Kimanya *et al.* [9] it was shown that in order to prevent fumonisins exposures exceeding the PMTDI in Tanzania, the MTL for fumonisins in maize should be set below 155 μg/kg. This observation supports our views that in communities relying on maize as main food, MTLs for fumonisins in maize flour must be set well below the current limit of 1000 μg/kg set in other countries [23, 24].

However, in addition to setting a maximum limit for fumonisins in maize, efforts should be made to ensure adoption of good agricultural practices that can reduce fumonisins contamination in agricultural produce. Regulations on maximum fumonisin levels cannot protect people in rural areas who consume what they harvest without official check of safety and quality. The efforts underway to identify affordable methods to reduce fumonisins contamination in maize merit support from the national, regional and international bodies. Peasants in the rural areas need to be sensitized on the existence of the fumonsins in their staple foods in order to prevent and reduce contamination in the food.

The association of fumonisins exposure on growth raises important new questions to be explored. Could it be that mycotoxins affect immunity in children as seen in animal studies and are responsible for the high infection stress of infants and children? Are fumonisins, thus, partly responsible for the high degrees of wasting and growth retardation that we observe? We have indeed observed infants in rural

Tanzania stunted even when having recommended intakes of complementary food [2]. Years ago, aflatoxins and their free radical generating properties were proposed as a causative agent in the pathogenesis of kwashiorkor [37]. This was however never confirmed, but at that time only aflatoxins were analysed. The observation that kwashiorkor is predominantly in communities that consume mainly maize, which is very prone to fumonisins contamination should renew our interest to explore further if there is a possible role for mycotoxins in the pathogenesis of kwashiorkor.

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