

Effects of aflatoxins and fumonisins exposure in infants under six months of age in Tanzania

Happy Steven Magoha

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Effects of aflatoxins and fumonisins exposure in infants under six months of age in Tanzania

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Dedication

To my husband

Rodrick Meena

Our sons

Rodney Meena and Roland Meena

& My Mum

Martha Magoha

Acknowledgements

First of all I thank God for keeping me healthy throughout the period of my study and, for providing the opportunity to do a PhD. I know I did all this under his guidance.

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List of abbreviations

AF-alb	Aflatoxin-albumin adduct
AFB ₁	Aflatoxin B ₁
AFB ₂	Aflatoxin B ₂
AFG ₁	Aflatoxin G ₁
AFG ₂	Aflatoxin G ₂
AFM ₁	Aflatoxin M ₁
AOAC	Association of Official Analytical Chemists
BTC	Belgium Technical Cooperation
BMDL	Bench Mark Dose Lower Limit
bw	Body weight
CAC	Codex Alimentarius Commission
CI	Confidence interval
DDS	Dietary diversity score
DHS	Demographic Health Survey
DNA	Deoxynucleic acid
DON	Deoxynivalenol
EC	European Commission
EE	Environmental enteropathy
EFSA	European Food Safety Agency
ELEM	Equine Leucoencephalomalacia
EU	European Union
FAO	Food and Agriculture Organisation
FB ₁	Fumonisin B1
FB ₂	Fumonisin B2
FB ₃	Fumonisin B3
FFQ	Food frequency questionnaire
GEMS	Global Environmental Monitoring Systems
HAZ	Height-for-age z-score
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LAZ	Length-for-age z-score
LC-MS	Liquid chromatography – mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
ML	Maximum limit
MOE	Margin of exposure
NBS	National Bureau of Statistics, Tanzania
NIMR	National Institute for Medical Research, Tanzania
NSGRP	National strategy for growth and reduction of poverty

OPA	<i>Othor</i> -phthaldehyde
OTA	Ochratoxin A
PMTDI	Provisional maximum tolerable daily intake
SAX	Strong Anion Exchange
SD	Standard deviation
TBS	Tanzania Bureau of Standards
TDHS	Tanzania Demographic Health Survey
TFDA	Tanzania Food and Drugs Authority
TFNC	Tanzania Food and Nutrition Centre
TOL	Tanzania oxygen limited
UHT	Ultra High Temperature
URT	United Republic of Tanzania
UNICEF	United Nations Children's Funds
US	United States
US EPA	United States Environmental Protection Agency
V/V	Volume over volume
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score
WHO	World Health Organization

Table of Contents

Contents

Acknowledgements.....	v
Summary.....	xv
Samenvatting.....	xix
Introduction and objectives of the study	1
1. Background information.....	1
2. Conceptual framework and objectives of the study	4
I. To identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants.....	5
II. To assess dietary exposure to aflatoxins and fumonisins in infants under six months	6
of age	6
III. To assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk	6
IV. To assess the effects of concomitant exposure of aflatoxins and fumonisins in breastfeeding and/or complementary feeding practices on infants' growth	7
V. To recommend measures for protecting infants in Tanzania against aflatoxins and fumonisins exposure	7
CHAPTER 1	11
Growth status, feeding practices and potential risk of exposure to aflatoxins and fumonisins in infants in Tanzania.....	11
Summary.....	12
Growth status of under six months of age infants in Tanzania	13
1.1. Growth status	13
1.2. Factors causing malnutrition in the first six months of infants' life in Tanzania	16
1.3. Strategies used for reduction of Malnutrition in Tanzania	21
1.4. The occurrence of mycotoxin in food.....	23
1.5. Occurrence of mycotoxins in breast milk	27
1.6. Occurrence, exposure and effects of mycotoxin in Tanzania.....	29
1.7. Effect of mycotoxin exposure in Health	32
1.8. Mycotoxin exposure estimation	35
1.9. Control measures for mycotoxin contamination in Maize in Africa.....	36
1.10. Conclusion	39
CHAPTER 2	41

Methods for the research and subject characteristics.....	41
Summary.....	42
2.1. Study area, selection criteria and sample collection.....	42
2.2. Mycotoxins analyses.....	46
2.3. Characteristics of the study population.....	55
2.4. Food intake of the lactating mother and the infants.....	56
2.5. Food intake of the infants.....	57
2.6. Data management and analysis.....	59
Chapter 3.....	61
Risk of aflatoxins and fumonisins exposures from breast milk.....	61
Summary.....	62
3.1. Introduction.....	63
3.2. Exposure assessment.....	63
3.2.1. AFM ₁ exposure estimation and risk management.....	63
3.2.2. FB ₁ exposure estimation and risk management.....	64
3.2.3. Assessment of seasonality variation on AFM ₁ and FB ₁ contamination.....	64
3.2.4. Data management and analyses.....	65
3.3. Results.....	65
3.3.1. Dietary intake for lactating mothers.....	65
3.3.2. Occurrence of AFM ₁	65
3.3.3. Exposure of AFM ₁	66
3.3.4. Occurrence of FB ₁ in breast milk.....	66
3.3.5. Exposure of FB ₁	67
3.4. Discussion.....	67
3.5. Conclusion.....	71
Chapter 4.....	73
Risk of aflatoxins and fumonisins exposure from complementary foods.....	73
Summary.....	74
4.1. Introduction.....	75
4.2. Estimation of maize flour intake.....	76
4.3. Extraction and analysis of aflatoxins (AFB ₁ , AFB ₂ , AFG ₁ and AFG ₂) and fumonisins (FB ₁ and FB ₂) from maize flour.....	76
4.4. Exposure estimation for aflatoxins and fumonisins.....	76
4.5. Risk characterization for aflatoxins.....	77

4.6. Risk characterization for fumonisins	77
4.7. Data interpretation, management and analysis.....	77
4.8. Results	78
4.8.1. Subjects.....	78
4.8.2. Type of food introduced to infants.....	78
4.8.3. Risk of exposure to aflatoxins and fumonisins	79
4.8.4. Aflatoxins and fumonisins in maize flour	79
4.8.5. Risk of exposure above tolerable limits.....	80
4.9. Discussion	80
4.10. Conclusion	84
Chapter 5	85
Relationship between impaired growth and exposure of infants to aflatoxins and fumonisins through breast milk	85
Summary.....	86
5.1. Introduction.....	87
5.2. Assessment of the feeding practices with growth	87
5.3. Assessment of association between AFM ₁ and FB ₁ exposures and growth indicators.....	88
5.4. Results	88
5.5. Discussion	92
5.6. Conclusion	94
Chapter 6	95
Concomitant exposure of aflatoxins and fumonisins from food and breast-milk	95
Summary.....	96
6.1. Introduction.....	97
6.2. Estimation of breast milk intake.....	97
6.3. Results	98
6.4. Discussion	101
6.5. Conclusion	103
Chapter 7	105
General Discussion	105
7.1 Introduction.....	106
7.2. Measures for protecting infants in Tanzania against aflatoxins and fumonisins exposure	108
7.2.1. Awareness creation on the health risks of mycotoxin	109

7.2.2. Reduction of mycotoxin content in foods at both household/community level and national levels	109
7.2.3. Educate lactating mothers on dietary diversification and quality issues	111
7.2.4. Reduction of mycotoxin exposure as a strategy to prevent malnutrition	112
7.2.5. Involving all stakeholders in addressing mycotoxin challenges	112
7.3 How to achieve the recommendations on the reduction of mycotoxin exposure in infants	112
7.4 Limitation of the study	114
7.5 Suggestions for future research	115
References	117
Annexes	133
Curriculum Vitae	141

Summary

Tanzania is experiencing a high rate of malnutrition with inadequate feeding practices being one of its major causes, just like in other African countries. A particular example of these inadequate practices is the introduction of infants to maize-based complementary food before the age of six months. Despite the presence of mycotoxins, especially for aflatoxins and fumonisins, in Tanzanian maize, a majority of lactating mothers especially in rural Tanzania consume home grown- maize which is poorly processed thus increasing their risk of exposure to mycotoxins. The toxins are thereafter excreted into their breast milk thus exposing the breastfed infants to the same. This implies that Tanzanian infants below six months of age can be exposed to mycotoxins from the breast milk as well as from the complementary foods. The presence of these mycotoxins in food has been associated with poor child growth in some countries and is also implicated with inverse linear growth in children above six months of age in Tanzania. However, up to now Tanzania as a country has neither standards for aflatoxins and fumonisins in infants' food nor standards for fumonisins in food in general. Setting up of maximum limits requires sufficient data on the occurrence and exposure to these toxins (including the effects on infants) which is also missing, currently. The present study targeted the infants under six months of age with the aim of assessing their aflatoxin and fumonisin exposure and the associated effects. The specific objectives were to:

- Identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants under six months of age.
- assess dietary exposure to aflatoxins and fumonisins in infants under six months of age
- assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk
- assess the concomitant exposure of breastfeeding and/or complementary feeding practices on infants' growth
- recommend measures for protecting infants in Tanzania from aflatoxins and fumonisins exposure

The first chapter of this document presents the literature review regarding malnutrition in Tanzania and the strategies for addressing malnutrition in Tanzania. Infants' feeding practices including the duration for exclusive breastfeeding, age of introduction of complementary foods as well as composition of complementary food are presented. The occurrence of mycotoxins in food, worldwide, and in Tanzania is also been presented based on different studies previously conducted regarding the contamination, exposure and effects of the mycotoxins being discussed.

Chapters 2 covers the methodology of the study including the criteria used in selection of the study site and procedures for recruitment of mothers and their infants. Collection of data is also presented, such as anthropometric measurements for the infants and data on dietary assessment. Procedures for breast milk and maize flour collections are also been discussed in this chapter together with the procedures for laboratory analyses of mycotoxins, aflatoxin M₁ (AFM₁) and fumonisin B₁ (FB₁) from breast milk and total aflatoxins i.e. the total of aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂) and total fumonisin i.e. the total of fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) from maize flour.

Chapter 3 demonstrates the prevalence and exposure of aflatoxins and fumonisin in breast milk. High performance liquid chromatograph (HPLC) technique was used to determine AFM₁ and FB₁ content in breast milk. Exposure assessments have been carried out using the deterministic approach. All the breast-milk samples were contaminated by AFM₁ at levels ranging from 0.01 to 0.55 ng/ml. More than 90% of samples exceeded the EU limit of 0.025 ng/ml for infants' foods while over 76% exceeded the EU limit of 0.05 ng/ml for dairy milk and milk products. Only 1% of the samples exceeded the limit of 0.5 ng/ml set for dairy milk in the United States and several other countries in Asia. Above 40% of samples contained FB₁ at levels ranging from 5.58 – 471.05 ng/ml. Of the contaminated samples, 10% and 2% had FB₁ levels above the EU limit of 200 ng/ml for fumonisins in infants' food in lactation stages of month 1 and 5 respectively. Aflatoxin M₁ exposures in infants ranged from 1.13 – 66.79 ng/kg body weight (bw)/day. Whereas FB₁ exposures ranged from 0.60 – 64.93 µg/kg bw/day and exceeded the provisional maximum tolerable limit of 2 µg/kg bw/day in 29% and 35% of the infants during month 1 and 5 respectively.

Dietary mycotoxin exposures for infants receiving foods other than breast milk is reported in Chapter 4. A 24 hour dietary recall technique was used to estimate flour intake for infants who had been introduced to maize-based foods by the age of six months. Aflatoxins and fumonisins in the flours were analysed using HPLC technique. Exposures of aflatoxins or fumonisins was estimated using the deterministic approach. Results indicated that, by the age of 3 months, 98 out of 143 infants had started taking food; 67 of them, maize flours at levels ranging from 0.57 - 37.50 g/infant/day (average; 8 g/infant/day). Fifty eight percent of 67 maize flour samples contained detectable aflatoxins (range, 0.33 - 69.47 µg/kg; median, 6 µg/kg) and 31%, detectable fumonisins (range, 48 - 1224 µg/kg; median, 124 µg/kg). For infants who consumed contaminated flours, aflatoxins exposures ranged from 0.14 to 120 ng/kg bw/day (all above the health concern level of 0.017 ng/kg bw/day recommended by the European Food Safety Agency) and fumonisins exposures, from 0.005 to 0.88 µg/kg bw/day.

Chapter 5 presents the growth effect which resulted from AFM₁ and FB₁ exposure from the breast milk. Weight and length of infants recorded in each visit were computed to growth indicators namely weight for age z-score (WAZ), length for age z-score (LAZ) and weight for height z-score (WHZ) according to WHO (2006) using the Stata program. The growth status at month five was compared for exclusively and non-exclusively breast fed infants until month 3 of age. Also mixed effect linear regression models was used to assess growth effect using WAZ, LAZ and WHZ and the level of exposure. Infants who were exclusively breastfed until the age of three months were less likely to be stunted at the fifth month of age compared to those who had been introduced to other foods by the age of three months (odds ratio = 1.30; 95% confidence interval = 0.34 – 4.93). Growth impairments have been observed in both toxins. Inverse association (P<0.05) has been observed between AFM₁ exposure levels and WAZ or LAZ. Whereas for FB₁ the inverse association has been observed with WAZ and WHZ.

In chapter 6 the effect of total exposure from breast milk and food with growth indicators is reported. The breast milk intake was estimated based on the total energy requirements per day for infants with regard to age and sex as recorded by Butte, (2005). Deterministic exposure assessment was done for both toxins from food and breast milk. The exposures were zero skewed

log transformed and fitted into a mixed-effect linear regression models for each variable; WAZ, LAZ and WHZ. Fumonisin had significant negative association with WAZ and WHZ ($P < 0.05$).

In conclusion the present study reveals that mycotoxin exposure in infants starts before the age of six months and that the exposure levels are associated with impaired growth. This situation calls for intervention to reduce mycotoxin content in food at both household and national levels. The intervention should involve all stakeholders at every stage such as in food production, processing, trade and health sectors. Lactating mothers should also be educated on the importance of dietary diversification by using foods which are less susceptible to fungi attacks such as finger millet, sorghum and rice. Also to facilitate the elaboration of the effects of mycotoxin it is important to follow up infants involved in this study.

Samenvatting

Tanzania kampt met een hoge mate van ondervoeding waarbij, net zoals in andere Afrikaanse landen, inadequate voedingsgewoonten één van de belangrijkste oorzaken is. Een specifiek voorbeeld van deze inadequate voedingsgewoonten is het opstarten van aanvullende maïs-gebaseerde voeding bij zuigelingen vóór de leeftijd van zes maanden. Ondanks de aanwezigheid van mycotoxines, vooral omwille van de aflatoxinen en fumonisinen, in Tanzaniaanse maïs, consumeert de meerderheid van zogende moeders, in het bijzonder op het platteland in Tanzania, zelfgekweekte maïs die slecht verwerkt is waardoor hun risico op blootstelling aan mycotoxines verhoogt. Deze toxines worden daarna uitgescheiden in hun moedermelk, waardoor het zogende kind hier ook aan blootgesteld wordt. Dit betekent dat Tanzaniaanse zuigelingen van jonger dan zes maanden blootgesteld kunnen worden aan mycotoxines, zowel via de moedermelk als via de aanvullende voeding. De aanwezigheid van deze mycotoxines in voedsel wordt geassocieerd met gebrekkige kindergroei in bepaalde landen en is ook betrokken bij de invers lineaire groei van kinderen ouder dan zes maanden.

Maar tot nu toe heeft Tanzania geen normen op nationaal vlak opgelegd voor aflatoxinen en fumonisinen voor voedsel bestemd voor zuigelingen, noch normen voor fumonisinen in voeding in het algemeen. Het implementeren van maximum normen vereist voldoende gegevens over de aanwezigheid van en de blootstelling aan deze toxines (met inbegrip van de gevolgen voor zuigelingen) die op dit moment ook ontbreekt. Deze studie is gericht op baby's jonger dan zes maanden met de bedoeling om hun blootstelling aan aflatoxine en fumonisine en de daarmee geassocieerde effecten te bepalen. De specifieke doelstellingen waren:

- Het identificeren en kwantificeren van aflatoxines en fumonisines in moedermelk en het evalueren van de blootstelling van zuigelingen onder de zes maanden oud eraan.
- Het evalueren van de blootstelling van zuigelingen onder de zes maanden oud aan aflatoxines en fumonisines in voeding.
- Het bepalen van het verband tussen verminderde groei en blootstelling van zuigelingen jonger dan zes maanden aan aflatoxines en fumonisines in moedermelk.

- Het evalueren van hiermee gepaard gaande blootstelling aan de praktijken van borstvoeding en/of aanvullende voeding op de groei van zuigelingen.
- Maatregelen aanbevelen om zuigelingen in Tanzania te beschermen tegen blootstelling aan aflatoxines en fumonisines.

Het eerste hoofdstuk van dit document behandelt het literatuuronderzoek met betrekking tot ondervoeding in Tanzania, samen met strategieën voor het bestrijden van ondervoeding in Tanzania. De voedingsgewoontes voor zuigelingen met inbegrip van de periode waarin alleen borstvoeding gegeven wordt, de leeftijd voor het opstarten van aanvullende voeding alsook de samenstelling van de aanvullende voeding worden uiteengezet.

De aanwezigheid van mycotoxines in voedsel wereldwijd en in Tanzania komt ook bod, op basis van verscheidene studies die eerder uitgevoerd werden met betrekking tot de verontreiniging, blootstelling aan en gevolgen van mycotoxines.

Hoofdstuk 2 bespreekt de methodologie van de studie met inbegrip van de criteria die gebruikt werden voor de keuze van de studiesite en de procedures voor het rekruteren van de moeders en hun zuigelingen. Ook de datacollectie wordt behandeld, zoals antropometrische metingen van de zuigelingen en gegevens betreffende de evaluatie van de voeding. Procedures voor het verzamelen van stalen moedermelk en maïsmeel worden ook besproken in dit hoofdstuk samen met de procedures voor laboratorium analyses van mycotoxines, aflatoxine M₁ (AFM₁) en fumonisine B₁ (FB₁) van moedermelk en de totale aflatoxines i.e. het totaal van de aflatoxine B₁ (AFB₁), aflatoxine B₂ (AFB₂), aflatoxine G₁ (AFG₁) en aflatoxine G₂ (AFG₂) en de totale fumonisines i.e. het totaal van fumonisine B₁ (FB₁) en fumonisine B₂ (FB₂) in maïsmeel.

Hoofdstuk 3 toont de prevalentie en risico op aflatoxines en fumonisines in moedermelk aan. De techniek van hogedrukvlloeistofchromatografie (high performance liquid chromatograph, HPLC) werd gebruikt om de hoeveelheden aan AFM₁ en FB₁ in moedermelk te evalueren. De evaluatie van de mate van blootstelling werd uitgevoerd aan de hand van de deterministische benadering. Al de stalen moedermelk bleken verontreinigd met AFM₁ met waarden van 0.01 tot 0.55 ng/ml. Meer dan 90% van de stalen overschreden de EU-norm van 0.025 ng/ml voor voeding voor zuigelingen terwijl meer dan 76% de EU-norm van 0.05 ng/ml voor koemelk en zuivelproducten overschreden. Slechts 1% van de stalen overschreed de norm van 0.5 ng/ml voor koemelk

vooropgesteld in de Verenigde Staten en verscheidene landen in Azië. Meer dan 40% van de stalen bevatten FB₁ met waarden die varieerden van 5.58 tot 471.05 ng/ml. Van de verontreinigde stalen, hadden 10% en 2% FB₁ waarden boven de EU-norm van 200 ng/ml voor fumonisines in voeding voor zuigelingen in de zoogperiode van maand 1 en 5 respectievelijk. Blootstelling aan aflatoxine M₁ bij zuigelingen varieerde van 1.13 – 66.79 ng/kg bw/dag. Terwijl blootstelling aan FB₁ varieerde van 0.60 tot 64.93 µg/kg lichaamsgewicht (body weight, bw) per dag en de voorlopige maximum toelaatbare norm van 2 µg/kg bw/dag bij 29% en 35% van de zuigelingen in de loop van maand 1 en 5 respectievelijk overschreed.

Blootstelling aan mycotoxine in voeding voor zuigelingen die ander voedsel dan moedermelk ontvangen wordt besproken in Hoofdstuk 4. Een repetitieve voedingstechniek voor een periode van 24 uur werd gebruikt om de meelinname te schatten bij zuigelingen die vanaf de leeftijd van zes maanden maïs- gebaseerde voeding gekregen hadden.

Aflatoxines en fumonisines in het meel werden geanalyseerd door gebruik te maken van de HPLC-techniek. Blootstelling aan aflatoxines of fumonisines werd geschat door gebruik te maken van deterministische benadering. De resultaten tonen aan dat, op de leeftijd van 3 maanden, 98 van de 143 zuigelingen voedsel waren beginnen innemen; 67 onder hen maïsmeel met waarden die variëren van 0.57 tot 37.50 g/zuigeling/dag (gemiddeld: 8 g/zuigeling/dag). Achtenvijftig procent van de 67 stalen maïsmeel bevatten detecteerbare aflatoxines (waarde 0.33 - 69.47 µg/kg; mediaan, 6 µg/kg) en 31% detecteerbare fumonisines (waarde, 48 - 1224 µg/kg; mediaan, 124 µg/kg). Voor zuigelingen die verontreinigd meel hadden gegeten, varieerde de blootstelling aan aflatoxines van 0.14 tot 120 ng/kg bw/dag (allen boven de gezondheidsgrenswaarde van 0.017 ng/kg bw/dag aanbevolen door de Europese Autoriteit voor Voedselveiligheid) en blootstelling aan fumonisines van 0.005 tot 0.88 µg/kg bw/dag.

Hoofdstuk 5 bespreekt de gevolgen voor de groei die voortvloeien uit blootstelling aan AFM₁ en FB₁ in de moedermelk. Gewicht en lengte van de zuigelingen die bij elk bezoek genoteerd waren, werden omgerekend tot groei indicators namelijk gewicht per leeftijd z-score (WAZ), lengte per leeftijd z-score (LAZ) en gewicht voor lengte z-score (WHZ) in overeenstemming met de WHO (2006) door gebruikmaking van het Stata-programma. De groeistatus op maand vijf werd

vergeleken voor zuigelingen die uitsluitend en niet-uitsluitend met moedermelk gevoed waren geweest tot de leeftijd van 3 maanden. Het model van het gemengd lineair regressie-effect werd ook gebruikt om het groei effect te evalueren aan de hand van WAZ, LAZ en WHZ met de mate van blootstelling. Zuigelingen die tot de leeftijd van drie maanden uitsluitend met moedermelk gevoed werden, hadden minder kans om in hun vijfde maand een groeiachterstand te hebben in vergelijking met deze die vanaf de leeftijd van drie maanden aanvullend voedsel kregen (odds ratio = 1.30; 95%, betrouwbaarheidsinterval = 0.34 – 4.93). Groeibeperking werd bij beide toxines vastgesteld. Er werd inverse associatie ($P < 0.05$) gedetecteerd tussen de waarden van blootstelling aan AFM₁ en WAZ of LAZ. Voor FB₁ werd er inverse associatie vastgesteld bij WAZ en WHZ.

In hoofdstuk 6 wordt het effect van totale blootstelling in moedermelk en voedsel met groei indicators uiteengezet. De inname van moedermelk werd geschat aan de hand van de totale energiebehoefte per dag voor zuigelingen, rekening houdende met hun leeftijd en geslacht zoals genoteerd door Butte, (2005). Er werd een deterministische bepaling van blootstelling gedaan voor beide toxines in voedsel en moedermelk. De mate van blootstelling werd omgezet in een zero skewed log en aangepast aan een model van gemengd lineair regressie-effect voor elke variabele: WAZ, LAZ en WHZ. Fumonisine had een aanzienlijk negatieve associatie voor WAZ en WHZ ($P < 0.05$).

De conclusie van deze studie onthult dat de blootstelling aan mycotoxine bij zuigelingen begint voor de leeftijd zes maanden en dat de waarden van blootstelling geassocieerd worden met groeibeperking. Deze situatie vergt een interventie om de hoeveelheid mycotoxine in voedsel te verminderen, zowel op huishoudelijk als op nationaal niveau. De interventie zou alle stakeholders bij elke fase moeten betrekken zoals voedselproductie, voedselverwerking, handel- en gezondheidssector. Zogende moeders zouden ook voorgelicht moeten worden over het belang van een gevarieerde voeding door voedsel te gebruiken dat minder vatbaar is voor aanvallen van schimmels als gierst, sorghum en rijst. Om de uitwerking van de effecten van mycotoxine te vergemakkelijken is het belangrijk om de zuigelingen die betrokken waren bij deze studie op te volgen.

Introduction and objectives of the study

1. Background information

Growth faltering is a major public health problem affecting infants and young children. Recent estimates suggest that stunting, wasting, and intrauterine growth restrictions are responsible for 2.2 million deaths and 21% of disability-adjusted life-years lost among children younger than 5 years (Black et al., 2008; Black et al., 2010). In addition, stunted linear growth is associated with poor infant motor and mental development (Grantham-McGregor et al., 2007). Most growth faltering occurs from birth until 2 years of age but may have already started during the gestation period (Victora et al., 2010).

In Tanzania, among children of less than 5 years of age, malnutrition as measured by rates of stunting, underweight and wasting, stands at 42, 16 and 5%, respectively in 2010 (NBS and ICF Macro, 2011). Among the main causes of child growth retardation are poor complementary feeding and breastfeeding practices (Kulwa et al., 2006; Bhutta et al., 2008; Muhimbula and Issa-Zacharia, 2010).

Exclusive breastfeeding for six months is rarely practiced in Tanzania (Shirima et al., 2001, Mamiro et al., 2005, Kulwa et al., 2006, Muhimbula and Issa-Zacharia, 2010). Maize being the staple food in Tanzania, is used as the major ingredients in the formulation of a baby's first food (Mamiro et al., 2005; Nyaruhucha et al., 2006). Unfortunately, mycotoxin contamination in maize has been reported in different parts of Tanzania including Tabora, Iringa, Ruvuma and Kilimanjaro (Kimanya et al., 2008a). Mycotoxins are toxic secondary metabolites of fungi. Though there are thousands species of fungi only of the genera *Aspergillus*, *Penicillium* and *Fusarium* are known to produce mycotoxins. Aflatoxins are produced as secondary metabolites by fungi belonging to several *Aspergillus* species, mainly *A. flavus* and *A. parasiticus*. *Fusarium moniliforme* (presently known as *F. verticillioides*) and *F. proliferatum* are responsible for the production of fumonisins (Bhat et al., 2010).

The mycotoxin contaminations in Tanzania are associated with its temperate and humid climate which favour fungal growth responsible for mycotoxin production (Kimanya et al., 2008a; Manjula et al., 2009) as is the case in West Africa (Bankole and Adebajo, 2004). Processing techniques such as poor harvesting, improper storage conditions, poor transportation can also contribute to fungal growth as well as increasing the risk of mycotoxin production (Wagacha and Muthomi, 2008; Miller, 2008). Therefore mycotoxin can enter in the food chain at the field level, harvesting, transportation, storage and processing of foodstuffs (Bhat et al., 2010).

Mycotoxins are of different types, but the important ones are aflatoxins B₁ (AFB₁) and its metabolites aflatoxin M₁ (AFM₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), aflatoxin G₂ (AFG₂), and fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) which are the major contaminants found in maize and ground nuts and are considered to be of primary importance to children's health (Wagacha and Muthomi, 2008; Sherif et al., 2009). Aflatoxins and fumonisins have been generally reported to cause serious health effects. AFB₁ and AFM₁ are classified by the International Agency for Research on Cancer (IARC) as a human carcinogen group 1, and FB₁ is classified as a group 2B carcinogen (IARC, 2002). Apart from carcinogenicity of AFB₁, the toxin is also reported to be teratogenic, immunotoxic and genotoxic in various studies (Williams et al., 2004; Turner et al., 2007; Shephard, 2008; Prandini et al., 2009; El Morsi et al., 2010; Elzupir and Elhoussein, 2010). In Kenya several cases have been reported for the acute aflatoxin poisoning (aflatoxicosis) which eventually led to the death of a number of people. In 2004 the worst outbreak was reported; 317 cases were reported and out of these 125 died, while in year 2005, 75 cases were reported and 32 died (Shephard, 2008). AFM₁ is cytotoxic as it was demonstrated in human hepatocytes *in vitro*. AFM₁ can also damage Deoxyribonucleic acid (DNA), cause gene mutation, chromosomal abnormalities and cell transformation in mammalian cells *in vitro* (Prandini et al., 2009).

FB₁ is reported to cause liver and kidney cancers in rats and mice (Gelderblom et al., 2001). It has also been observed that there is an association between consumption of fumonisin contaminated maize and high incidences of oesophageal cancer in the former Transkei

region of South Africa (Rheeder et al., 1992), Iran (Shephard et al., 2000), and China (Li et al., 2001).

Aflatoxins and fumonisins have been linked with profound health effects in children. Aflatoxin exposure in children has been associated with the incidence of kwashiorkor (Adhikari et al., 1994). A study which was conducted in Cameroon showed the presence of AFB1 in 35.5% of kwashiorkor children and in 45.5% of marasmic children compared with the control group with only 11% AFB1 positive (Tchana et al., 2010). Gong et al. (2003) showed growth faltering in children exposed to aflatoxin in Benin and Togo indicating a strong association between aflatoxin-albumin adduct (AF-alb) levels and stunted growth and underweight children. This association was also observed in the study of the effect of aflatoxin exposure in utero and early growth faltering of Gambian infants (Turner et al., 2007).

Recent studies in Tanzania have shown that 10% of young children (above 6 months of age) had fumonisin intakes above the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight, through eating contaminated maize (Kimanya et al., 2009a). In this report, Kimanya et al. (2009a) demonstrated that this percentage would increase to 24% if the maize used to prepare complementary foods was not sorted prior to use. In addition, Kimanya et al. (2010) observed from a cohort study that growth of infants in the same community was inversely associated with fumonisins exposures. The infants with an exposure higher than the PMTDI were on average 1.3 cm shorter and 328 g lighter than those below the limit (Kimanya et al., 2010). While, this study showed the association between mycotoxin exposure and stunting it did not take into account other confounding factors such as the poverty status, micronutrient deficiencies, infectious diseases etc . The causal effect is yet to be studied.

2. Conceptual framework and objectives of the study

This study was thus undertaken in view of the current situation that infants in Tanzania are not exclusively breastfed as recommended by WHO 2001 (Shirima et al., 2001, Mamiro et al., 2005, Kulwa et al., 2006, Muhimbula and Issa-Zacharia, 2010). Several studies have shown that complementary foods introduced at first weaning are mainly composed of maize (Mamiro et al., 2005; Nyaruhucha et al., 2006; Kulwa et al., 2006; Kimanya et al., 2009a; Muhimbula and Issa-Zacharia, 2010). Recently, using biomarkers, Shirima et al. (2013) showed that Tanzanian infants aged between 12 – 22 months receiving maize a based diet were exposed to aflatoxins and fumonisins. Since it was observed that infants were introduced to maize-based complementary food much earlier than six months of age it was hypothesized that infants younger than this age in Tanzania are exposed to mycotoxins.

There is limited data on mycotoxin exposure in infants less than six months of age worldwide. Therefore, the aim of this study was to investigate the mycotoxin exposure in infants under six months of age in Tanzania and also assess their effects on growth. The evidence that aflatoxins can be excreted in breast milk (Polychronaki, 2007; Gürbay et al., 2010a) is another motivator for studying mycotoxins exposure in infants who rely on milk as food. Infants are considered more susceptible to mycotoxin effect than adults, due to their low body weight, high metabolic rate, low ability to detoxify toxins and incomplete development of some vital organs and tissues, especially central nervous system (Sherif et al., 2009). Thus the following specific objectives were set:

- I. Identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants under six months of age
- II. Assess dietary exposure to aflatoxins and fumonisins in infants under six months of age
- III. Assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk
- IV. Assess the concomitant exposure of breastfeeding and/or complementary feeding practices on infants' growth

- V. Recommend measures for protecting infants in Tanzania from aflatoxins and fumonisins exposure.

I. To identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants.

AFM₁ occurrence in cow's milk in Tanzania was reported in 2006 (Urio et al., 2006), where up to 92% of milk samples collected from retail shop in Dar es Salaam and analysed for AFM₁ were positive with AFM₁ at levels ranging from 0.005 to 0.855 µg/kg. No other study has focused on AFM₁ contaminant in milk in Tanzania. In other countries, several assessments have been conducted on AFM₁ in dairy milk and milk products; skimmed milk powder, for example in Turkey (Devec and Sezigin, 2005), dairy milk and ice cream in Nigeria (Atanda et al., 2007), pasteurized liquid milk, infant formula and milk-based cereal weaning food consumed in Tehran, Iran (Oveisi et al., 2007). Assessments made for AFM₁ in human breast milk include (Navas et al., 2005) in Brazil, (Polychronaki et al., 2006) in Egypt, (Gürbay et al., 2010a) in Turkey, (Mahdavi et al., 2010) and (Ghiasain and Maghsood, 2012) in Iran and (Adejumo et al., 2013) in Nigeria. All these studies shows the occurrences of AFM₁ in milk and milk products. They also indicate that in the regions where there is mycotoxin contamination in food/feed there is a higher possibility of having milk and milk products contaminated.

In view of the earlier observation regarding mycotoxin contamination of maize in Tanzania, and the fact that it is one of the staple foods, there is a high possibility that maternal diet of Tanzanian mothers is contaminated with AFB₁ and FB₁. This could be associated with AFM₁ in breast milk as reported in other countries, and fumonisin carry-over to the breast milk as described by Fink-Gremmels (2008) in ruminants.

The determination of the levels of AFM₁ and FB₁ in breast milk during the first 6 months of lactation is thus important. It is in this regard that the exposure assessment was made to establish if the contamination levels in breast milk would lead to exposure above the set PMTDI. The findings of the present study provide information to better understand of the

extent of the problem and allow appropriate interventions, hence, improving the nutritional wellbeing of our infants. Results obtained in this investigation are presented in **Chapter 3**.

II. To assess dietary exposure to aflatoxins and fumonisins in infants under six months of age

Exclusive breastfeeding is rarely practised (Shirima et al., 2001; Mamiro et al., 2005; Kulwa et al., 2006; Nyaruhucha et al., 2006) and infants below six months of age are introduced to complementary foods which are mainly composed of maize flour (Mamiro et al., 2005; Kulwa et al., 2006; Nyaruhucha et al., 2006; Muhimbula and Issa-Zacharia, 2010). Therefore, it was necessary to sample ready-to-cook maize flour which was specifically meant for infants below six months of age in order to analyse them for aflatoxins and fumonisins. To assess the dietary exposure a 24 hour dietary recall technique, Tanzania food composition table (Lukmanji et al., 2008) and Lucille food intake software of Ghent University (Lucille, 2012) were used to determine maize flour consumption per child from either a thin porridge (*uji*) or stiff porridge (*ugali*) consumed by them. The results of dietary exposure are reported in **Chapter 4**.

III. To assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk

This study was conducted to explore the relationship between infants' exposure to aflatoxin and fumonisin through breastfeeding and their effects on growth. The growth impairment assessment was executed in relation to the contamination level observed; AFM₁ and FB₁ contamination in breast milk as reported in Chapter 3. The growth effects on infants under six months of age due to their exposure to aflatoxins and fumonisins was thus worth examining. In the earlier study conducted in Benin, growth impairment was recorded in children aged 16-37 months (Gong et al., 2004). Similarly in another study which was conducted in Tanzania, mycotoxins proved to have growth effects on children of 6 months to one year (Kimanya et al., 2010). Results of growth impairment and exposure through breast milk are presented in **Chapter 5**.

IV. To assess the effects of concomitant exposure of aflatoxins and fumonisins in breastfeeding and/or complementary feeding practices on infants' growth

The effect of the overall contamination from AFM₁, total aflatoxin and fumonisins on infants' exposure through breast milk and/or the complementary food was also assessed. This was done in view of the variation of the level of the exposure with feeding practices to examine the overall effect of aflatoxins and fumonisins exposure in infants' growth. The results are presented in **Chapter 6**.

V. To recommend measures for protecting infants in Tanzania against aflatoxins and fumonisins exposure

Based on the present findings and the established data on the extent of mycotoxin contamination in breast milk and complementary feeding and the associated risks of infant exposure to mycotoxin contamination, recommendations were developed. Their focus is on minimising mycotoxin contamination in foods and hence reducing the exposure of both lactating mothers and infants under six months of age. Reduction of mycotoxins in foods is likely to improve the nutritional status of infants and minimise the latter problems caused by malnourishment at the young age. The recommendations are for the implementation by the Government of Tanzania via its two ministries. Ministry of Agriculture, Food security and Cooperatives and Ministry of Health and Social Welfare and also to Tanzanian residents, especially lactating mothers. All these are described in **Chapter 7**.

The layout of this study is presented in Figure 0.1. A visual diagram demonstrating the link between the objectives of the study and the methods used to achieve them is shown in figure 0.2.

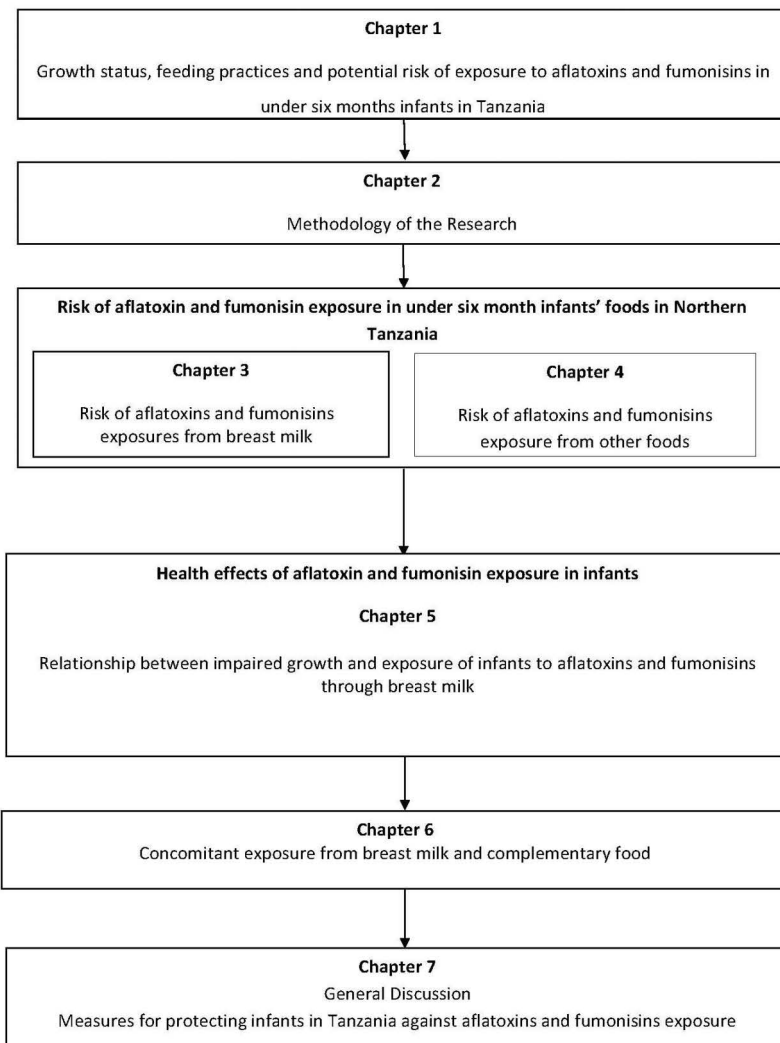


Figure 0.1. Outline of the PhD study

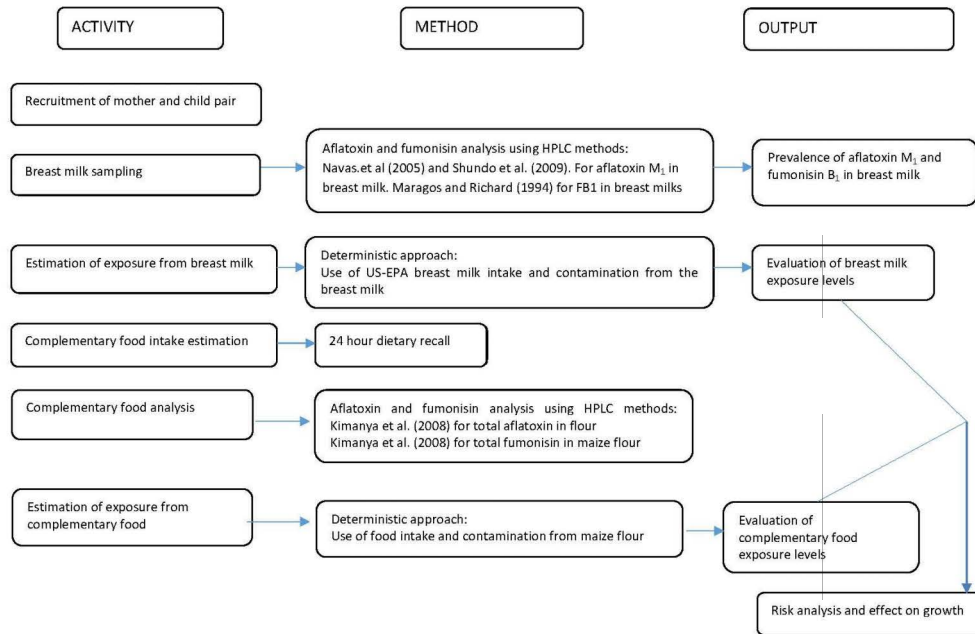


Figure 0.2: Diagram demonstrating the relationship between objectives and methodology of the study

CHAPTER 1

Growth status, feeding practices and potential risk of exposure to aflatoxins and fumonisins in infants in Tanzania

Summary

This chapter presents a literature review on various aspects related to infants feeding practices in Tanzania and similar experiences from other countries. It demonstrate the problem of malnutrition in Tanzania from 2004 to 2010, and also discusses problems associated with malnutrition. Infants' feeding practices in Tanzania are described together with the composition of complementary foods given to them. Strategies for the reduction of malnutrition in Tanzania are also highlighted in this chapter.

The occurrence of mycotoxin in food worldwide and in Tanzania is also presented. Different methods for mycotoxin exposure assessment have been discussed. Mycotoxin exposure data available in Tanzania and other East African countries have also been discussed. Furthermore, the health effects of mycotoxins have been described as examined in in earlier studies. In line with the occurrence and effects of mycotoxins, many countries have set regulations for aflatoxins and fumonisin as mentioned in this chapter.

The Chapter concludes that Tanzanian infants under six months of age are at a risk of being exposed to aflatoxins and fumonisin. Though exposure to mycotoxins can be part of the several causes of malnutrition in Tanzania, till now Tanzania, as a country, has neither standards for aflatoxins or fumonisins for infants' food nor standards for fumonisins in foods in general. This reflects a need to assess mycotoxin exposure in infants under six months of age in Tanzania.

Growth status of under six months of age infants in Tanzania

1.1. Growth status

1.1.1. Malnutrition in Tanzania

Tanzania like other sub-Saharan countries is faced with malnutrition. Malnutrition is defined as an imbalance between the body's supply of nutrients and the body demand for growth, maintenance and specific activities. Malnourishment relates to the quality as well as the quantity of food. Individuals can become malnourished from either excess calorie intake (overnutrition) or insufficient nutrient intake whereby the body starts to waste; losing fat and then muscle (undernutrition) (Manaray and Solomons, 2004; UNICEF, 2009). Undernutrition contributes to more than one third of deaths in children under the age of five (UNICEF, 2009). There are two types of under-nutrition: micronutrient malnutrition which involves deficiencies in different micronutrients such as iron, iodine and vitamin A, and growth failure malnutrition which is characterised by wasting, stunting and underweight (UNICEF, 2009). The latter of these types of under-nutrition is assessed in this study.

There are several measurements for undernutrition (growth failure), physical measurements such as height and weight are one of them. In children malnutrition is often measured by comparing their nutrition status with a reference for healthy children. References are used to compare a child's measurements with the median for children of the same sex and age for height-for-age (HAZ), weight-for-age (WAZ) or weight-for-height (WHZ) z-score. Z-score is the difference between child's weight or height and the median value at that age and sex in the reference population, divided by the standard deviation (SD) of the reference population. Whereby, a child is considered as stunted if his/her HAZ is less than -2 SD; wasted if his/her WHZ is less than -2 SD and underweight if WAZ is less than -2 SD (Caulfield et al., 2006). Children with a WHZ below -3 SD are considered severely wasted, HAZ below -3 SD severely stunted and with WAZ below -3 SD are considered severely underweight (NBS and ICF Macro, 2011).

There are two clinical forms of severe undernutrition which are known as marasmus and kwashiorkor. Marasmus is characterised by severe wasting of fat and muscles which break

down to make energy. Typical characteristics include skin and bones appearance; ribs are easily seen, loose skin of upper arms and thighs (image 1). Kwashiorkor is characterised by excessive accumulation of fluids in body tissues (oedema) in the lower legs and feet. As it progresses it becomes more generalised to the arms, hand and face and patches of skin abnormally light or dark (image 1) (Manary and Solomons, 2004).

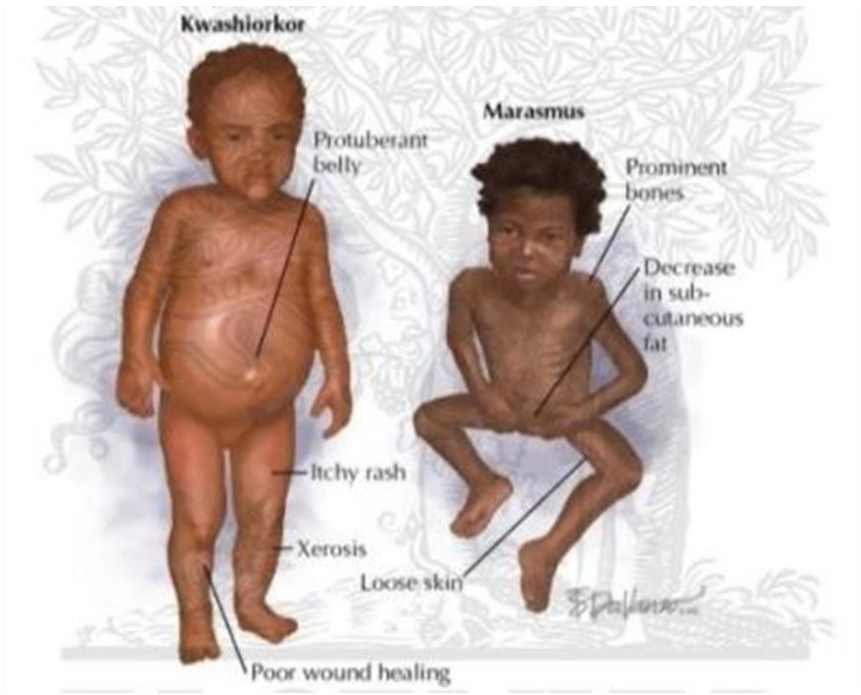


Image 1: Kwashiorkor and marasmic children

Source: <http://healthdrip.com/wp-content/uploads/2012/05/Kwashiorkor-and-Marasmus.jpg>

Tanzania is the 3rd worst affected country for undernutrition in Africa after Ethiopia and the Democratic Republic of Congo. Worldwide It is ranked 10th in its contribution to all chronically undernourished children (UNICEF, 2009). The malnutrition trend of children under five years children in Tanzania as extracted from the TDHS of 2004, 2009 and 2010 is summarised in Table 1.1. From table 1.1, it is observed that the malnutrition was still a

problem in Tanzania. At the beginning of this study, the malnutrition status was 42 % stunting, 5 % wasting and 16% underweight, as reported by the Tanzania Demographic and Health Survey (TDHS) in NBS and ICF Macro (2011).

The TDHS provides up-to-date information on the country's population and health status from the regular surveys it conducts. The 2010 TDHS was the fifth national Demographic and Health survey to be conducted in the country. Repeated surveys allow for an analysis of trends over time. The survey is based on a nationwide representative sample.

Table 1.1. Trend of malnutrition for under five children in Tanzania for 2004, 2009 and 2010

Year	Stunting (%)	Wasting (%)	Underweight (%)
2004 ^a	38	3	22
2009 ^b	44	4	22
2010 ^c	42	5	16

Source: ^aTDHS (NBS and ORC Macro, 2005), ^b(UNICEF 2009), ^cTDHS (NBS and ICF Macro, 2011)

Table 1.2 presents the 2010 malnutrition status in Tanzania. It can be observed from the table that growth faltering had started before 6 months of age. Stunting for under six months of age stands at 18.3%, wasting 6.4% and underweight 8.7%. The table also presents the malnutrition status of children above 6 months of age.

Table 1.2. Percentage of children under five years classified as malnourished according to three anthropometric indices of nutritional status, 2010

Background characteristics	Stunting			Wasting			Underweight		
	Percentage below -3 SD	Percentage below -2 SD ¹	Mean Z-score (SD)	Percentage below -3 SD	Percentage below -2 SD ¹	Mean Z-score (SD)	Percentage below -3 SD	Percentage below -2 SD ¹	Mean Z-score (SD)
Age in months									
<6	7.7	18.3	-0.7	2.2	6.4	0.3	2.3	8.7	-0.4
6-8	7.0	20.6	-0.9	3.7	10.9	-0.1	5.1	13.3	-0.7
9-11	8.1	28.1	-1.3	2.0	8.6	-0.1	4.0	16.8	-0.8
12-17	17.2	43.2	-1.6	2.1	7.0	-0.1	3.7	16.0	-0.8
18-23	24.8	55.0	-2.1	0.8	7.1	-0.0	5.7	20.5	-1.0
24-35	21.3	53.0	-2.0	0.9	3.6	0.1	3.2	16.0	-1.0
36-47	18.6	47.7	-1.9	0.6	2.1	0.0	3.5	16.0	-1.1
48-59	13.9	38.9	-1.8	0.7	2.9	-0.1	3.9	16.8	-1.2

¹ Includes children who are below -3 standard deviations (SD) from the WHO Child Growth standards population median

Source: TDHS 2010(NBS and ICF Macro 2011)

1.1.2. Problems associated with malnutrition

Malnutrition is linked to reduced immunity and thus increased mortality rate. The majority of malnourished children who survive are often locked in a cycle of recurring illness and growth faltering (Muhimbula and Issa-Zacharia, 2010). This situation is worse for children under two years of age, since malnutrition is negatively associated with cognitive development hence retards their ability to learn, thus impacting negatively during their entire lives, furthermore it is known that a child who is malnourished in the first stage of life is likely to remain stunted in his/her adulthood. (Alderman et al., 2009). A study conducted in the Kagera region of Tanzania indicated that malnourished children had lower schooling and were delayed in starting schooling (Alderman et al., 2009).

Malnutrition hurts the economy as farmers and other laborers, often women, are weakened by stunting, inadequate energy intake and anemia (Black et al., 2013). Because of this they are unable to exert much effort, leading to smaller harvests and reduced labor productivity. Malnutrition also contributes to lost opportunities for economic growth as adults with stunted brain development caused by inadequate nutrition during childhood are less innovative and less likely to respond to new market opportunities (UNICEF, 2009; Twaweza, 2010). It is also reported that maternal short stature and iron deficiency anaemia increase risk of death of the mother at delivery (Black et al., 2008; 2013).

1.2. Factors causing malnutrition in the first six months of infants' life in Tanzania

1.2.1. Duration of exclusive breastfeeding in Tanzania

Although Tanzania is among the countries that advocate exclusive breastfeeding, as recommended by WHO for up to 6 months of age (WHO, 2001). The breastfeeding pattern and practices vary across the population and differ significantly between individual mothers. According to the available data, it appears that exclusive breastfeeding during the first 6 months is poorly adopted. The study which was conducted in both urban and rural areas of Morogoro, Tanzania indicated that exclusive breastfeeding is rarely practised, although the duration of breastfeeding is longer in rural areas than urban areas (Shirima et al., 2001). It

was observed that exclusive breastfeeding practice was worse in the urban areas of Dar es Salaam (Kulwa et al., 2006) where exclusive breastfeeding was adhered to by only 9% of the subjects. Table 1.3 presents the feeding practices in Tanzania for under six months of age. The table indicates that a very minimal number of infants are exclusively breast fed for up to 6 months and above, and complementary foods are introduced before six months of age. The median duration of exclusive breastfeeding at the national level is 2.4 months while the median duration of predominant breastfeeding, which is defined as exclusive breastfeeding, or breastfeeding in combination with plain water, water-based liquids, or juices is 3.2 months (NBS and ICF Macro, 2011).

Table 1.3. Exclusive breastfeeding and early introduction of complementary foods in Tanzania by age groups for the years 1996, 1999, 2005 and 2010

Practice	Year	Age in months			
		0 - 1	2 - 3	4 - 5	6 - 7
Not breastfeeding (%)	1996	0.6	1.3	2.7	0.7
	1999	1.1	3.0	3.0	1.9
	2005	2.4	0.3	3.8	2.0
	2010	0.8	2.0	2.2	3.3 ^a
Exclusive breastfeeding (%)	1996	55.2	27.5	8.0	4.1
	1999	57.8	25.4	15.5	1.5
	2005	70.0	42.4	13.5	1.7
	2010	80.5	51.1	22.9	2.3 ¹
Complementary feeding (%)	1996	19.9	50.2	77.0	91.3
	1999	18.5	47.6	70.3	91.0
	2005	7.2	32.1	57.9	88.5
	2010	10.9	33.4	63.5	91.6 ¹

^a Data is for 6-8 months

Source: TDHS, 2005(NBS and ORC Macro 2005), 2010(NBS and ICF Macro, 2011)

This breastfeeding practice observed in Tanzania matches well with that made in Bangladesh, which indicated that lactating mothers residing in the rural areas and coming from the poorest households were more likely to implement exclusive breastfeeding than women from the richest household, that is, employed mothers and older mothers (Rasheed et al., 2009). The poor practice of exclusive breastfeeding has also been reported in other African countries, such as Uganda where 7% of the studied population practiced exclusive breastfeeding for up to three months of infant's age (Engebretsen et al., 2007). In Malawi exclusive breastfeeding was also maintained for up to 2.5 months (Vaahtera et al., 2001). The issue of not practising exclusive breastfeeding for 6 months is not an isolated problem

for developing countries alone, but also for developed countries (Arora et al., 2000; Lande et al., 2003).

1.2.2. Initiation and types of complementary foods

Complementary feeding as described by WHO is any nutrient containing foods or liquids other than breast milk given to young children from six months of age. Children are feeding on foods or liquids along with breast milk up to two years or beyond. However, appropriate complementary feeding should have the following four properties: timely introduction, i.e. foods are introduced when the need for energy and nutrients exceeds what can be provided through exclusive breastfeeding; adequate, i.e. the food must provide sufficient energy, protein and micronutrients to meet a growing child's nutritional needs; safe i.e. the food is hygienically stored and prepared; and last is properly fed, i.e. meaning that food should be consistent with the child's signals of appetite and satiety, meal frequency and feeding method (WHO, 2003).

Complementary foods should include adequate quantities of meat, poultry, fish or eggs as well as vitamin A rich fruits and vegetables. The consistency of complementary foods should change from semisolid to solid foods and the variety of foods offered should increase as the infant grows (WHO, 2003). By eight months, infants can eat 'finger foods' and by twelve months, most children can eat the same types of food as the rest of the family (WHO, 2003). It is suggested by a WHO report (2003) on complementary feeding that the energy requirements from complementary food with an average breast milk intake should be, 200 kcal/day for infants aged 6–8 months, 300 kcal/day for infants aged 9–11 months, and 550 kcal/day for children aged 12–23 months. The prevalence of undernutrition in Tanzania indicates that the energy requirement of infants is not met in some cases. However, infants in this study were under six months of age and were recommended exclusive breastfeeding, thus there was no energy requirement available from complementary foods

In Tanzania the type of foods introduced to infants at the recommended age of six months includes grains (92%), vegetables and vitamin A rich fruits (67%), legumes and nuts (39%), meat, fish, poultry and eggs (37%), roots and tubers (28%), milk and milk products (27%)

(NBS and ICF Macro, 2011). The data presented in Table 1.4 further demonstrates grains as the main components of infants' complementary food for different age groups in the following percentages 7%, 20.6%, 60.3% and 86.2% for infants of 0-1, 2-3, 4-5 and 6-8 months respectively. By the age of 6-8 months very few infants (0.9%) were still receiving infant formula and 4.5% fortified baby foods.

Table 1.4. Food and liquid consumed by breastfeeding infants in percentage recorded in the day or night preceding interview according to type of food and age, Tanzania 2010

Type of foods	Infants age in months				
	0-1	2-3	4-5	6-8	
Liquids	Infant formula	0.6	0.0	2.3	0.9
	Other milk	3.8	16.0	15.4	24.7
	Other liquid	2.1	10.0	24.6	34.5
Solid or semi-solid foods	Fortified baby foods	0.6	0.0	1.5	4.5
	Food made from grains	7.0	20.6	60.3	86.2
	Fruits and vegetables rich in Vitamin A	0.5	1.3	11.9	47.5
	Other fruits and vegetables	0.0	0.0	2.2	14.5
	Food made from roots and tubers	0.0	0.1	5.0	22.0
	Food made from legumes and nuts	0.5	0.2	5.5	28.3
	Meat, fish, poultry and eggs	0.0	0.6	5.7	23.3
	Milk, cheese, yogurt, other milk products	3.2	15.6	16.0	22.1
	Any solid or semi-solid food	11.0	34.1	64.9	94.7

Source: TDHS, 2010(NBS and ICF Macro 2011)

According to Shirima et al. (2001) the introduction of solid food to infants in Tanzania starts at the age of 1.8 months in rural and 2.6 months in urban areas. On the other hand, Nyaruhucha et al. (2006) found that 54% of mothers in Simanjiro district in Tanzania weaned their children as early as two months after birth and they were introduced to maize porridge mixed with cow's milk. Similarly, Mamiro et al. (2005) reported the frequent use of the same food in early weaned infants of 3 – 5 months of age in the Kilosa district of Tanzania. The mean age for the introduction of complementary food in urban areas reported was 3 months, and thin cereal based porridge was also the main food for infants even in urban areas like Dar es Salaam (Kulwa et al., 2006). The most recent information regarding exclusive breastfeeding and introduction of complementary foods in Tanzania (2010) as compiled by TDHS (NBS and ICF Macro, 2011) indicates that, 11% of children below 2 months

of age, 34% of children age 2-3 months, and 65% of children aged 4-5 months are given complementary foods (Table 1.4).

Generally, the majority of infants are introduced to complementary food before the age of six months and the main food to which infants are introduced is mainly composed of maize and is in the form of a thin porridge (uji) (Mamiro et al., 2005; Nyaruhucha et al., 2006). Although the main ingredients in the porridge is maize flour, other additional ingredients can be added and differ between households, such ingredients include sugar or salt, and other cereal grains like finger millet, rice and peanut composite flour porridge (Muhimbula and Issa-Zacharia, 2010).

1.2.3. Occurrence of infectious diseases and environmental pollutants

The frequency and duration of infectious diseases is also recorded as one of the causes of malnutrition. Diarrhoea reduce fluid balance and other nutrients such as zinc and copper from the body. When these nutrients are not replaced they may result to severe dehydration, malnutrition and growth faltering (Dewey and Mayers, 2011). Tanzania prevalence of diarrhoea reported for infants less than age 6 months is 11 percent in TDHS 2010 (NBS and ICF Macro, 2011), which is mainly caused by frequent use of contaminated water and unhygienic practices in food preparation and disposal of excreta, however prevalence varies with season (NBS and ICF Macro, 2011) .

There is also subclinical infection, which has no obvious signs or symptoms but with physiological evidence of abnormality associated with growth faltering known as environmental enteropathy (EE) (Dewey and Mayers, 2011). EE is likely to occur in developing countries and is linked with poor sanitation and hygiene conditions, it causes nutrient malabsorption by changing the structure and function of the small intestine (Dewey and Mayers, 2011). Exposure to mycotoxins has been linked to conditions similar to EE (Smith et al., 2012), this is because the intestine is a major target for the toxin effects. Aflatoxins exposure may inhibit protein synthesis leading to impaired metabolism and enterocyte damage which leads to systemic immune inactivation. Whereas fumonisin exposure decreases food intake and inhibits sphingolipid metabolism, which could lead to

degradation of the epithelia barrier and stimulate inflammatory immune response. All these effects may results in growth impairment. Due to the reported cases of mycotoxin prevalence in Tanzania, the poor nutritional status of infants may be attributed to mycotoxin exposure.

1.3. Strategies used for reduction of Malnutrition in Tanzania

Various studies of the reduction of malnutrition have been conducted in Tanzania. Leach and Kilama (2009), Alderman et al. (2009) which have come up with strategies for the prevention of malnutrition. The main focus of those studies were on health protection, promotion and sustaining improved nutrition in children under two years of age. The country through its national institute namely Tanzania Food and Nutrition Centre (TFNC) has been promoting the conceptual framework for nutrition (Leach and Kilama, 2009). The priority interventions proposed in the framework with regard to infant and young children feeding include promoting exclusive breastfeeding for six months, and extended breastfeeding with complementary foods for children up to two years (URT, 2011). In order to achieve malnutrition reduction, the following interventions have been proposed by the Ministry of Health and Social Welfare for implementation (URT, 2011):

- i. *Accessing quality nutrition services*: Nutrition interventions must be delivered at a high scale of impact especially where prevalence of malnutrition is high.
- ii. *Advocacy and behaviour change communication*: Intense advocacy at the household and community levels will raise the visibility and profile of malnutrition, hence, improving knowledge on caring practices for children and women of child-bearing age.
- iii. *Legislation for a supportive environment*: include measures to prevent unethical marketing of breast-milk substitutes, safeguard breastfeeding rights of employed women, ensure adequate labelling and quality of infant and child food products and the fortification of food.

iv. *Mainstreaming nutrition into national and sectoral policies, plans and programs:* Nutritional indicators have been included in the National Strategy for Growth and Reduction of Poverty (NSGRP). But further efforts are needed so that nutrition is firmly part of policies and strategies in the health, agriculture, education, community development and industry sectors.

v. *Institutional and technical capacity for nutrition:* For health service providers both at the central and local government levels, pre-service and in-service training courses need to keep pace with latest policies, strategies, guidelines and scientific thinking.

vi. *Resource mobilization:* Reduce the budget gap by mobilizing adequate and sustainable financial resources and ensure efficient spending to deliver nutrition interventions and collaborations with other sectors and programs.

vii. *Research, monitoring and evaluation:* Research ensures evidence-based nutritional interventions, programs and policies.

viii. *Coordination and partnerships:* A coordinated multi-stakeholder and multi-sectorial response maximizes the use of available technical and financial resources for greater synergy of efforts. Public-private partnerships can increase the opportunities for delivering and scaling up nutrition services.

An effective implementation of the strategy however requires the participation and involvement of stakeholders at all levels from the community to the national level, including the public sector (sectoral ministries and institutions, regional secretariats and local government authorities), higher learning and training institutions, professional bodies, private sector, development partners, civil society, media and the community. All concerned parties should thus share the responsibilities for the successful implementation of the Strategy and acknowledge and embrace their responsibilities (URT, 2011).

1.4. The occurrence of mycotoxin in food

Mycotoxin contamination in food is a worldwide problem (Falade, 2011); however it is mostly prevalent in countries with high humidity and a temperate climate which favours the growth of fungi. Fungi are responsible for the spoilage of food as well as production of secondary metabolites referred to as mycotoxins (Magan and Olsen, 2004). Although different fungi are favoured by different environmental conditions, the humidity above 0.7, with temperature range of 10 - 40°C, and pH range of 4 to 8 is conducive for their growth (Bhat et al., 2010). However, it is important also to note that it is not always the case that wherever there is fungal growth, there is production of mycotoxins. The production of mycotoxins depends on the interactions of several factors such as the temperature of the environment, moisture content, pH, nutritional availability, maturity of the fungal growth, other insects' activities, co-occurrence of other fungi, competition from other microbes and other related factors (Bhat et al., 2010).

Although there are thousands of fungi species, it is mainly the genera *Aspergillus*, *Penicillium* and *Fusarium* which are known to produce mycotoxins (Bryden 2007; Bhat et al., 2010). While there are number of mycotoxins identified, the most proven to be toxic are AFB₁, AFB₂, AFG₁ and AFG₂ which are the metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*; AFM₁ is the metabolite of AFB₁. As for fumonisins more than ten types have already been isolated and characterised, of these FB₁, FB₂ and FB₃ are the major types produced by *Fusarium moniliforme* (*F. verticillioides*) and *Fusarium proliferatum*, ochratoxin A (OTA) is produced by *Penicillium verrucosum* and *Aspergillus ochraceus*. It has also been reported that isolates of *Aspergillus niger* as well as *A. carbonarius* are capable of producing OTA (Bhat et al., 2010), zearalenone and deoxynivalenol (DON) are associated primarily with *Fusarium graminearum* and *Fusarium culmorum*, Patulin on the other hand is produced by fungi belonging to *Aspergillus* spp., *Penicillium expansum*, and *Paecilomyces* and *Byssoschlamys* spp. (*Byssoschlamys nivea*, *B. fulva*), T-2 and HT-2 are also produced by *Fusarium* species.

Fungi can enter into the food before and/or after harvest if there are favourable environmental conditions. Fungi contamination is also facilitated by poor agricultural practices, improper transportation, poor processing as well as poor storage facilities (Wagacha and Muthomi, 2008). Apart from the climatic conditions, developing countries are at a higher risk simply because the majority of the population consume home grown food which is not subject to food safety and quality standards, as are foods bought from markets (Shephard, 2008; Zinedine and Mañes, 2009). Once the food has been infested with fungi, mycotoxin can be found within 24 hrs. These mycotoxins cannot be physically observed. Laboratory tests are required to determine and quantify their presence.

Different types of foods are infested by fungi and hence contaminated with mycotoxins. Approximately 25% of the world food crops are infested each year with fungi (Bryden, 2007; Falade, 2011). Aflatoxins are found in a wide range of food but mainly in maize and maize products, peanut and also in dried fruits. Fumonisin is found mainly in maize and maize products while OTA is found mainly in cereal, coffee, cocoa dried fruits and may also occur in beverages such as wine, beer and grape juices. T-2 toxin mainly occurs in grains such as barley, corn and cereal-based products. Patulin is affecting mostly fruits such as apples and pears (Bhat et al., 2010). Worldwide occurrence of fumonisin and aflatoxins are presented in Tables 1.5 and 1.6 respectively. Table 1.5 shows the prevalence of fumonisin in maize food/feed while Table 1.6 shows aflatoxin contamination levels in various food products such as maize, peanuts and spices.

Table 1.5. Occurrence of fumonisin worldwide according to type of food

Product	Region/Country	Detected/Total samples	Fumonisin B1 (mg/kg)
North America			
Maize	Canada, USA	324/729	0.08 – 37.9
Maize flour, grits	Canada, USA	73/87	0.05 – 6.32
Miscellaneous maize foods	USA	66/162	0.004 – 1.21
Maize feed	USA	586/684	0.1 – 330
Latin America			
Maize	Argentina, Brazil, Uruguay	126/138	0.17 – 27.05
Maize flour, alkaline-treated kernels, polenta	Peru, Uruguay, Venezuela	5/7	0.07 – 0.66
Miscellaneous maize foods	Uruguay, Texas-Mexico border	63/77	0.15 – 0.31
Maize feed	Brazil, Uruguay	33/34	0.2 – 38.5
Europe			
Maize	Austria, Croatia, Germany, Hungary, Italy, Poland, Portugal, Romania, Spain, United Kingdom	248/714	0.007 – 250
Maize flour, maize grits, polenta, semolina	Austria, Bulgaria, Czech Republic, France, Germany, Italy, Netherlands, Spain, Switzerland, United Kingdom	181/258	0.008 – 16
Miscellaneous maize foods	Czech Republic, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland, United Kingdom	167/437	0.008 – 6.10
Imported maize, grits and flour	Germany, Netherlands, Switzerland	143/165	0.001 – 3.35
Maize feed	France, Italy, Spain, Switzerland, United Kingdom	271/344	0.02 – 70
Africa			
Maize	Benin, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, Zimbabwe	199/260	0.02 – 117.5
Maize flour, grits	Botswana, Egypt, Kenya, South Africa, Zambia, Zimbabwe	73/90	0.05 – 3.63
Miscellaneous maize foods	Botswana, South Africa	8/17	0.03 – 0.35
Maize feed	South Africa	16/16	0.47 – 8.85
Asia			
Maize	China, Indonesia, Iran, Nepal, Philippines, Thailand, Viet Nam	380/633	0.01 – 155
Maize flour, grits, gluten	China, India, Japan, Thailand, Viet Nam	44/53	0.06 – 2.60
Miscellaneous maize foods	Japan, Taiwan	52/199	0.07 – 2.39
Maize feed	Korea (Republic of), Thailand	10/34	0.05 – 1.59
Oceania			
Maize	Australia	67/70	0.3 – 40.6
Maize flour	New Zealand	0/12	-

Source: Fumonisin B1. IARC MONOGRAPHS (IARC, 2002)

Table 1.6 Occurrence of aflatoxins worldwide according to food commodity

Country	Food commodity	Levels (range)	Reference
Argentina	Maize	0–3.19 µg/kg	Broggi <i>et al.</i> , 2007
	Maize	< 2 µg/kg	Solovey <i>et al.</i> , 1999
Bangladesh	Maize	33 µg/kg	Dawlatana <i>et al.</i> , 2002
	Groundnut	65 µg/kg	
Bahrain	Spices	27.7–69.2	Musaiger <i>et al.</i> , 2008
Botswana	Peanut	12–329 mg/kg	Barro <i>et al.</i> , 2002
	Peanut butter	0.3–23 µg/kg	Siame <i>et al.</i> , 1998
Brazil	Corn	0.2–129 µg/kg	Vargas <i>et al.</i> , 2001
	Peanuts and products	43–1099 µg/kg	Freitas and Brigido, 1998
	Sorghum	7–33 µg/kg	Da Silva <i>et al.</i> , 2000
China	Corn	9–2496 µg/kg	Li <i>et al.</i> , 2001
	Rice	0.99–3.87 µg/kg	Liu <i>et al.</i> , 2006
	Hazel nut	25–175 µg/kg	Williams <i>et al.</i> , 2004
Egypt	Soybean	5–35 µg/kg	Kadt <i>et al.</i> , 1993
	Wall nut	15–25 µg/kg	
	Corn	2.7–7.5 µg/kg	Amra, 2007
	Sorghum	0.1–11.2 µg/kg	Ibrahim <i>et al.</i> , 1998
Ethiopia	Cereals	trace–26 µg/kg	Ayalew <i>et al.</i> , 2006
Gambia	Ground nut source	162 µg/kg	Williams <i>et al.</i> , 2004
Ghana	Kernels	5.7–22,168 µg/kg	Awuah and Kpodo, 1996
India	Pistachio nuts	15–259 µg/kg	Candlish <i>et al.</i> , 2001
	Maize	96–8164 µg/kg	Bhat <i>et al.</i> , 1997
	Rice	0.1–308 µg/kg	Reddy <i>et al.</i> , 2009
	Corn	0.02–7.4 µg/g	Ramakrishna <i>et al.</i> , 1990
	Maize	0–26.8 µg/kg	Janardhana <i>et al.</i> , 1999
	Groundnut	>30 µg/kg	Kishore <i>et al.</i> , 2002
Irish	Spices	18.5–27.5 µg/kg	Riordon and Wilkinson, 2008
Italy	Spices	0.57–26.9 µg/kg	Romagnoli <i>et al.</i> , 2007
Japan	Peanut butter	2.59 µg/kg	Kumagai <i>et al.</i> , 2008
Kenya	Maize	Up to 46,000 µg/kg	CDC, 2004
	Maize flour	1210 µg/kg	Kenji <i>et al.</i> , 2000
	Wheat	2–7 µg/kg	Muthomi <i>et al.</i> , 2008
Korea	Corn	20 ng/g	Park <i>et al.</i> , 2002
	Spices	0.08–4.66 µg/kg	Cho <i>et al.</i> , 2008
	Rice	1.8–7.3 ng/g	Park <i>et al.</i> , 2005
Kuwait	Cereals	2.7–4.1 µg/kg	Dashti, 2005
	Spices	0.48–10.1 µg/kg	
Malaysia	Wheat flour	25.6–289 µg/kg	Abdullah <i>et al.</i> , 1998
	Peanut	1–378 µg/kg	Ali <i>et al.</i> , 2000
Mexico	Corn	1.6–465.3 µg/kg	Torres <i>et al.</i> , 1992
Morocco	Spices	0.03–2.88 µg/kg	Zinedine <i>et al.</i> , 2006
Nepal	Peanut	>30 µg/kg	Koirala <i>et al.</i> , 2005
Nigeria	Yam chips	4–186 µg/kg	Bankole and Adebajo, 2003,
	Pre harvest maize	3–138 µg/kg	Bankole and Mabekoje, 2004,
	Shelled melon	5–20 µg/kg	Bankole <i>et al.</i> , 2004,
	Corn and corn based snacks	25–770 µg/kg	Adebajo and Idowu, 1994
	Cereals	17–48.6 µg/kg	Odoemalam and Osu, 2009
Pakistan	Chilli	0.1–96.2 µg/kg	Paterson, 2007
Philippines	Rice bran and rice	0.27–11 µg/kg	Sales and Yoshizawa, 2005

	hull		
	Corn	130 µg/kg	Ali <i>et al.</i> , 2000
Qatar	Cereals and cereal products	0.14–81.6 µg/kg	Abdulkadar <i>et al.</i> , 2004
Senegal	Peanut	40 µg/kg	Ndiaye <i>et al.</i> , 1999
Sudan	Peanut butter and peanut	87.4–197.3 µg/kg	Omer <i>et al.</i> , 1998
Thailand	Corn	73 µg/kg	Lipigorngoson <i>et al.</i> , 2003
	Peanut oil	102 µg/kg	
Turkey	Red pepper	–97.5 µg/kg	Erdogan, 2004
	Spices	0.025–2.0 µg/kg	Aydin <i>et al.</i> , 2007
Vietnam	Rice	3.31–29.8 ng/g	Nguyen <i>et al.</i> , 2007
	Maize	0–126.5 µg/kg	Trung <i>et al.</i> , 2008
United States	Maize	21–699 µg/kg	Abbas <i>et al.</i> , 2006

Source: An overview of mycotoxin contamination in foods and its implications for human health (Reddy *et al.*, 2010)

1.5. Occurrence of mycotoxins in breast milk

1.5.1. Dietary intake of AFB₁

Intake of aflatoxin contaminated food is a starting point of the presence of AFM₁ in human milk since AFM₁ is a secondary metabolite of AFB₁. The oral intake is reported to be the main route of the aflatoxin exposure (Polychronaki, 2007). However the concentration of the AFB₁ and the food matrix are the factors that influence an individual's exposure. These can affect the bio-accessibility and bioavailability of AFB₁ in the body. Bio-accessibility is the amount of contaminant that is released from the food and can be absorbed by the body while bioavailability is the fraction of this amount which is most likely to reach systemic circulation in the body. Furthermore the interaction of toxin with other dietary components can also affect its absorption which means that individual lifestyle does interfere with the risk assessment of the contaminant. AFB₁ is concentrated in the liver after absorption because of the high permeability of the hepatocyte membrane for AFB₁ plus its active metabolism and subsequent covalent binding with hepatic macromolecules. The distribution of AFB₁ to other areas of the body is by blood circulation and is affected by the binding of the compound to plasma proteins, however only the free AFB₁ is available to pass through the capillary membranes (Polychronaki, 2007). This process is also specific to individuals depending on the nature of the enzymes present in the gut, local microorganisms as well as the mode of transport across the intestinal epithelium.

1.5.2. Metabolism of AFB₁

Human cytochrome P450 (CYP 450) enzymes are involved in the AFB₁ metabolism in the liver (Wild and Gong, 2010). Among the CYP 450 enzymes, CYP1A2 and CYP 3A4 are mainly responsible for the metabolism of absorbed aflatoxins to form AFB₁ epoxides (AFB₁-8, 9-exo-epoxide and AFB₁-endo-epoxide). Both the exo- and endo-epoxides can undergo rapid non-enzymatic hydrolysis to AFB₁-8,9-dihydrodiol that can react with lysine (an amino group) in serum albumin resulting in aflatoxin–albumin adducts. These are used as biomarkers (Wild and Gong, 2010). AFB₁ exo-epoxide is also the only known genotoxic product of AFB₁ (Wang and Groopman 1999). When added to DNA or guanosine containing double-stranded oligodeoxynucleotides, it readily adducts to the N7 position of guanine by covalently bonding to C8 of AFB₁ epoxide. This reaction yields the predominant AFB₁–DNA adduct identified as trans-8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB₁ (AFB₁-N7-guanine). The majority of the AFB₁-N7-Gua adducts are removed from DNA rapidly as they are relatively unstable within the DNA structure and are excreted exclusively into urine (Wang and Groopman 1999).

CYP 3A4 and 1A2 also oxidize AFB₁ to form AFM₁ and aflatoxin Q₁, and the demethylated metabolite of aflatoxin P. CYP 1A2 readily oxidizes AFB₁ to form AFM₁ while CYP 3A4 oxidizes AFB₁ to form aflatoxin Q₁ (Raney et al., 1992). AFB₁ metabolites can be excreted into the urine and feces. AFM₁ is the major aflatoxin metabolite in humans and has been detected in cord blood, maternal blood and breast milk. AFM₁ in breast milk can be the main source of aflatoxin to newborn infants.

1.5.3. Factors affecting the AFM₁ concentration in breast milk

Factors affecting chemical transportation to breast milk can be grouped into two categories namely (i) Chemical characteristics and (ii) Physiological and maternal characteristics. Chemical characteristics involve the aspects of the compounds that affect their ability to be transported such as lipid solubility, degree of ionization, molecular weight, ability to bind to maternal blood and/or milk component. While physiological and maternal characteristics include the physiological state of the mother such as maternal age, number of pregnancies,

the level of maternal exposure, milk composition and volume and breast milk pattern. For example when the milk has a high fat content, the transfer of highly lipophilic chemicals would be likely to occur during lactation, likewise if the protein content is high then chemicals with high affinity for proteins may be more prone to be transported to the milk (Clewell and Gearhart, 2002). It is reported that at the start of feeding sessions milk contains less fat than milk obtained at the end, these changes in milk composition directly affect the amount of chemicals transferred through milk (Clewell and Gearhart, 2002).

1.5.4. Occurrence of FB₁ in breast milk

In view of the available literature, fumonisin in human breast milk is not well investigated. However, reports from dairy milk studies have shown that cattle fed on contaminated feeds are likely to have fumonisin in their milk. Fumonisin remained unchanged after undergoing rumen metabolism and its biological potency also did not change with an estimated carry-over rate of 0-0.05% (Fink-Gremmels, 2008). But there is no information on the metabolism of fumonisin with regard to its excretion in breast milk in humans.

1.6. Occurrence, exposure and effects of mycotoxin in Tanzania

Several studies on mycotoxin exposure which have been conducted in Tanzania (Kimanya et al., 2008b; Kimanya et al., 2009a,b; Shirima et al., 2013) have revealed mycotoxin contamination and exposure for older children and adults. In the study conducted in four regions namely Tabora, Kilimanjaro, Iringa and Ruvuma, Kimanya et al. (2008a) reported a higher magnitude of aflatoxin and fumonisin contamination in maize. The habit of eating defective maize is likely to contribute to higher fumonisin and aflatoxin exposure to the respective consumers (Kimanya et al., 2008b). The data showed that 53% of the surveyed population was consuming defective maize. Furthermore it was observed that the concentration of fumonisin in 15% of the positive samples exceeded 1000 µg/kg which is the maximum limit for human consumption as indicated in other countries (Van Egmond and Jonker, 2004). Twelve percent of aflatoxin positive samples exceeded the Tanzania limit for total aflatoxin in maize of 10 µg/kg for human consumption. Manjula et al. (2009) investigated aflatoxin and fumonisin contamination in cassava and maize in three other

regions of Tanzania namely, Zanzibar, Unguja and Mtwara and found that both aflatoxin and fumonisin are a problem, but was more prevalent in maize compared to cassava. Maize samples from different storage conditions in Rungwe district were also reported to have mycotoxin contaminations at above permissible levels (Mboya et al., 2011).

Shirima et al. (2013) also reported dietary exposure to aflatoxin and fumonisin in three villages of Tanzania namely, Kigwa, Nyabula and Kikelelwa for children of age above one year using biomarker. AF-alb was detectable in 84% children while urinary fumonisin was detectable in 96% of children. AF-alb was higher to fully weaned children. The mean concentration levels for AF-alb were 43.2, 19.9 and 3.6 pg/ml albumin in children of Kigwa, Nyabula and Kikelelwa respectively. For urinary fumonisin the mean concentration levels were 327.2, 211.7, and 82.8 pg/ml in Kigwa, Nyabula and Kikelelwa respectively. About 82% of all children were exposed to both mycotoxins.

Multiple exposure of aflatoxin, fumonisin and DON has been recently reported to infants of 18–24 months old through maize based complementary foods (Kimanya et al., 2014). Maize flour consumption ranged from 16 to 254 g/child/day. Thirty two percent of children who consumed flour with detectable aflatoxin levels which ranged from 0.11 – 386 µg/kg resulted in the exposures ranging from 1 to 786 ng/kg bw per day, 44% of those who consumed flour with detectable DON levels of 57 - 825 µg/kg resulted in the exposure ranging from 0.38 – 8.87 µg/kg bw per day and 83% who consumed flour with fumonisin detectable level of 63 – 2,284 µg/kg resulted in exposure ranging from 0.19 – 26.37 µg/kg bw per day. Sixty six percent of the DON exposed children and 56% of the fumonisins exposed children exceeded the respective PMTDI of 1 µg/kg bw per day and 2 µg/kg bw per day respectively. Co-exposures was reported for aflatoxins, DON and fumonisin in 10% of the children and co-exposures of aflatoxins with fumonisins alone in 29% and for fumonisins with DON alone in 41% of the children

Mycotoxin contamination has also been reported in neighbouring countries of Tanzania. In a review study by Kaaya and Warren (2005) in Uganda, they indicated that contamination has been reported mainly in groundnuts and maize, with AFB₁ being the most prevalent one.

More aflatoxin contamination was encountered in foods sampled from the market level than at farm level. In some samples, aflatoxin was above the maximum limit (ML) of 20 ppb. In Makueni district, Kenya the mean aflatoxin level in maize was 53 ng/g with the highest contamination level of 5400 ng/g (Shephard, 2008). Kenya was faced with an aflatoxicosis outbreak in years 2005 and 2006, and even in 2007 there was still aflatoxin contamination in maize above 20 ng/g in 16% of samples investigated. This indicates high aflatoxin contamination (Daniel et al., 2011). AFM₁ in cow's milk samples was also reported in Kenya in 2006/2007 (Kang'ethe and Lang'a, 2009).

Apart from studies conducted in East African countries, several other studies on AFM₁ contamination in milk, dairy products and breast milk have been conducted elsewhere. Lin et al. (2004) examined AFM₁ contamination in pasteurised milk, powdered milk and yoghurt in Taiwan; Kamkar (2005) assessed the AFM₁ in raw milk in Iran; Devec and Sezigin (2005) conducted a study on AFM₁ levels in skimmed milk powder in Turkey; Oveisi et al. (2007) examined pasteurised milk, infant formula and milk based cereals for weaning in Iran; Atanda et al. (2007) examined milk and ice cream in Nigeria; Shundo et al. (2009) examined powder milk, pasteurized milk and ultra-high temperature (UHT) treated milk in Brazil; Elzupir and Elhussein, (2010) examined dairy cattle milk in Sudan; Rahimi et al. (2010) examined raw milk in Iran; Fallah (2010), AFM₁ in UHT and pasteurized milk in Iran. While Hassan et al. (2006); Polychronaki (2007); Mahdavi et al. (2010); Gürbay et al. (2010a); Tchana et al. (2010); El-Tras et al. (2011); Ghiasain and Maghsood (2012) examined AFM₁ contamination in breast milk. All studies have reported various levels of AFM₁ contamination among the studied samples. While Lin et al. (2004) and Ghiasain and Maghsood (2012) reported concentrations below the EU limit of 0.05 ppb (European-Commission, 2006), other studies reported concentrations below and above the 0.05 ppb in the studied samples.

Polychronaki et al. (2007) reported AFM₁ contamination in 56% of the samples collected from Egyptian mothers. Another small experiment which was done in Egypt (Dashti et al., 2009) also indicated the contamination of AFM₁ in the range of 8.83 – 15.2 ng/kg. Gürbay et al. (2010b) indicated the contamination of AFM₁ in breast milk in Turkey to be in the range of 60.90–299.99 ng/l, with all the collected samples showing positive results for AFM₁. A

recent study in Egypt (El-Tras et al., 2011) indicated a higher risk of AFM₁ exposure through breast milk than infant formula powder milk (Relative risk; 1.6, 95% CI; 1.28–2.03, p = 0.0001). The average daily exposure of newborns to AFM₁ was 52.684 ng through consumption of breast milk and 8.170 ng through infant formula powder milk. However, Ghiasain and Maghsood (2012), showed low contamination of AFM₁ in breast milk in Iran; with 6% positive samples at a mean concentration of 9.45 ng/L.

1.7. Effect of mycotoxin exposure in Health

Mycotoxins are associated with various health effects depending on the type of mycotoxin and exposure level to human. Two types of health effects are identified, which are acute and chronic effects. Acute diseases are observed following high levels of mycotoxin ingestion while chronic effects occur due to high frequency intake of low levels of mycotoxin.

1.7.1. Acute and chronic effects of aflatoxin

The disease occurring as a result of consuming high levels of aflatoxins is referred to as aflatoxicosis. Aflatoxicosis is a result of direct liver damage and can lead to death (Williams et al., 2004). This has been reported in different countries including Malaysia (Lye et al., 1995) and Kenya (Shephard, 2008).

Chronic aflatoxicosis has been extensively associated with liver cancer and also with nutritional and immunological effects (IARC, 2002). There is also a strong synergy between aflatoxin exposure and hepatitis B (HBV) virus and hepatitis C virus (HCV) for liver cancer. In China for example a study which was conducted by Wang et al. (2001), indicated that in Zhuqing village where there was HBV prevalence and aflatoxin exposure, the hepatocellular carcinoma was predominant (64%) compared to other cancers. In animals, chronic aflatoxicosis were also found to cause growth retardation and impairment of the immune system (Raisuddin et al., 1993). In humans, aflatoxins were shown to retard both *in utero* and postnatal growth (Gong et al., 2002; Turner et al., 2007) and higher levels of AF-alb in maternal blood were significantly associated with lower weight and height gain. A longitudinal study in Benin demonstrated a strong negative correlation between blood AF-

alb and growth in infants between 16 and 37 months of age. The results demonstrated a difference in height of 1.7 cm over the eight month period between the highest and lowest AF-alb quartile (Gong et al., 2004). Strong correlation was also observed between occurrence of aflatoxins and incidence of kwashiorkor in infants (Hatem et al., 2005). Findings from other studies (Adhikari et al., 1994; Tchana et al., 2010) on growth impairment as a result of exposure to aflatoxin have been discussed in the introductory part section 1.

1.7.2. Effect of AFM₁ on human health

AFM₁ poses similar effects as AFB₁ as its mechanisms of action are the same as that of AFB₁. AFM₁ can cause DNA damage, gene mutation, cell transformation although it was reported to be less toxic compared to AFB₁ (Galvano et al., 1996; Prandini et al., 2009). The carcinogenic potency of AFM₁ in sensitive species was found to be about one order of magnitude less than that of AFB₁ (Creppy, 2002). JECFA (2001) and Zinedine et al. (2007); reported the carcinogenicity of AFM₁ of 2 – 10% to be the same as that of AFB₁. Previously, AFM₁ was classified as human carcinogenic group 2B, but after several studies and evaluation it was relocated to group 1 (IARC, 2002).

1.7.3. Acute and chronic health effects of fumonisin

There are various studies conducted using animals in assessing the effect of fumonisin, however such studies cannot be performed in humans as it is unethical. It has been revealed that fumonisins are poorly absorbed from the gastrointestinal tract and are therefore rapidly cleared from the blood. Little fumonisin accumulated in the tissue and low amount were found in liver and kidney of laboratory animals (Voss et al., 2007). The main reported target organs of fumonisin are liver and kidney, however the toxin is reported to affect liver in all species but not for kidney (Voss et al., 2007). Acute toxicosis of fumonisin was reported by Edrington et al. (1995) in lambs treated with fumonisin culture material. Lambs were observed with diarrhoea on the third day after consuming fumonisin and lambs which received a higher dose died on day 3. Similar symptoms were observed in India in 1995, where people had abdominal pain and diarrhoea after consuming damaged moldy sorghum

and maize which were confirmed to contain high levels of FB₁ compared with samples from unaffected households (Bhat et al., 1997).

Chronic toxicosis of fumonisin has been reported to cause cancer and neural tube effects. Fumonisin inhibits ceramide synthase causing accumulation of bioactive intermediates of sphingolipid metabolism (sphinganine and sphingoid bases) and depletion of complex sphingolipids. The depletion of sphingolipids interferes with the function of folate binding protein, hence causing neural tube defects (Marasas et al., 2004).

Horses have also been reported to be affected by fumonisin, the target organs being heart, central nervous system as well as liver. They were found to have equine leukoencephalomalacia (ELEM). Different areas have been reported to face the problem of ELEM including South America, China, Egypt, Germany and South Africa. ELEM syndrome is characterized by the presence of liquefactive necrotic lesions in the white matter of the cerebrum, the first symptoms are lethargy, head pressing and decreased feed intake, followed by convulsions and death after several days (Morgavi and Riley, 2007). There are other effects recorded in animals such as immune suppression in poultry, this was observed by Li et al. (2001). The impact of fumonisin in growth retardation was reported in pigs, whereby with each mg/kg increase of FB₁ in the diet resulted in growth rate depression of 0.4% (Dersjant-Li et al., 2003).

For human toxicity, fumonisin has been associated with high incidence of oesophageal cancer in South Africa as reported earlier (Rheeder et al., 1992) and FB₁ as a cancer promoter was also reported in China (Chu and Li, 1994; Ueno et al., 1997). Health effects of aflatoxins and fumonisins are summarised in Table 1.7.

Table 1.7. Health effects of aflatoxins and fumonisins exposure

Mycotoxin	System affected	Effects
Aflatoxins	Gene expression	Teratogenic effect (birth defect of the offsprings) Carcinogenic effect (higher incidence of cancer)
	Pathological changes	Weight variations of internal organs (liver and kidney)
	Urinary system	Kidney inflammation
	Immune system	Immunosuppression(increase susceptibility to diseases)
	Digestive system	Impaired rumen function(decrease cellulose digestion, decrease protein breakdown into smaller polypeptides or amino acids)
Fumonisin	Pathological changes	Kidney and liver weight increase, pancreatic and liver damage
	Immune system	Immunosuppression (increase susceptibility to diseases)
	Digestive system	Gastro-intestinal effect (diarrhoea)
	Hepatotoxic effect	Liver damage
	Nervous system	Neurotoxic effect

As summarised in www.mycotoxins.info

1.8. Mycotoxin exposure estimation

Exposure is defined as contact over time and space between a person and one or more biological, chemical or physical agents (WHO, 2000). Exposure assessment involves collection of data on the route, magnitude, duration, frequency and distribution of exposures to hazardous agents for individuals and populations. Human exposure data have been used for the evaluation and protection of environmental health in four interrelated disciplines: 1) epidemiology, 2) risk assessment, 3) risk management, and 4) status and trends analysis. In assessing exposure through food, chemical compounds in food are measured directly from the diet (ready-to-eat foods) and this takes into account the procedures in the preparation of that particular food that can alter the contamination level. However there are many indirect methods that can also be used for estimating exposure to food chemicals, such as use of legislative levels, industry use level, predicted, proposed or analysed levels or any combination of these. Thereafter matching the contamination level in the food with the food consumption data.

Food consumption data can be obtained using five different approaches: 1) food record/diary survey, 2) meal-based diet history, 3) food habit questionnaire, 4) food frequency questionnaire (FFQ), and 5) 24 hour dietary recall. For assessing exposure, there

are two main types of models: deterministic and probabilistic. Deterministic models are based on a logical expression of the physical environment and the human behaviour, it uses points of estimates to represent input parameters (e.g. consumption, concentration, processing effect, use frequencies etc.). Use of average values to represent these parameters could result in underestimates of the exposures, while use of upper bound estimates would result in overestimates of these exposures. A deterministic model can be used to predict exposure of a new population and settings. A probabilistic exposure model is normally based on a deterministic model, but it incorporates the measured or estimated distributions of input variables, therefore, it produces more realistic population exposure distributions than a deterministic model (WHO, 2000).

1.9. Control measures for mycotoxin contamination in Maize in Africa

1.9.1. Pre and post-harvest practices for reducing mycotoxin contamination

The contamination of mycotoxins in food is favoured by food processing techniques. As stated before, fungi responsible for mycotoxin contamination can enter the food in the field, during harvesting, transportation and storage. Insect damaged maize are most vulnerable and can easily get infested with fungi. Fungi mycelium can spread among the kernels and penetrate through the pericarp. Insects that feed on maize in the field and stored maize predispose kernels to fungal infection through physical damage, while storage insect pests open the kernels to fungal invasion. Therefore the presence of insect pests in farm produce can be an indicator of the risk of contamination (Wagacha and Muthomi, 2008).

The time of harvest can also be the source of mycotoxins if agricultural products are prematurely harvested or are harvested late. Harvesting early is associated with high moisture which favours the growth of fungi during storage. However, the delayed harvest of maize for 4 weeks after physiological maturity has also been reported to increase mycotoxin contamination in Uganda (Kaaya et al., 2005). The author demonstrate that delays in harvest did not result in dry maize safe storage but it significantly increased the insect infestation, and mould infection hence resulting in increased aflatoxin contamination (Kaaya et al.,

2005). Therefore timely harvest is important as far as mycotoxin contamination is a concerned.

Improperly dried maize also facilitates fungal growth during storage. Maize must be dried to attain less than 14% moisture content for the best storage. This can be ensured if storage structures are designed in such a way that they prevent further fungal infestation. The maize storage survey which was conducted in Rungwe, Tanzania documented that common storage methods were placing on the roof and in sacks, which were also associated with the environmental conditions of rainfall throughout the year and temperature between 10 - 25°C. It was also observed that these storage techniques favoured fungal growth on maize. Of the samples collected, 88% were contaminated with either aflatoxin, fumonisin, ochratoxin or a combination of toxins (Mboya et al., 2011). All in all, agronomic factors are also important in controlling microbial growth and hence mycotoxin contamination, which includes, the type of maize variety, e.g. varieties resistant to insect infestation, timely sowing, proper balance of fertilizers and effective control of pests (Magan and Aldred, 2007).

Other simple control measures can be implemented at a household level to minimize mycotoxins contamination in maize. These include sorting out infested maize, washing and dehulling before milling. In one study which was conducted in Benin (Fandohan et al., 2005), 34% of aflatoxin in the maize was removed with the discarded hulls and germ and the mean fumonisin content were decreased from 2,890 µg/kg in the raw maize to 1,450 µg/kg in the cleaning of maize. Similar results were reported in Kenya where the effect of processing *muthokoi/kande* (a traditional dehulled maize dish) showed that dehulling decreased aflatoxins content by 46.6% (5.5-70%) in maize containing 10.7-270 µg/kg of aflatoxin concentrations (Mutungi et al., 2008). Furthermore, it was reported that reduction of fumonisin after hand sorting and washing of maize prior to milling had reduced fumonisin exposure by 62% to 2.55 (1.94–3.35) µg/kg body weight per day (van der Westhuizen and Wild, 2010).

1.9.2. Regulations of aflatoxins and fumonisin in different countries

Many countries have developed the minimum tolerable limits for mycotoxin contaminants in foods, so as to protect the health of consumers. The formation of regulatory limits depends on several factors such as: availability of toxicological data, availability of data on the occurrence and distribution of mycotoxin in various food products, the availability of analytical methods, need for sufficient food supply as well as the legislation in other countries in which trade contracts exist (van Egmond, 2007). The limit set up of mycotoxin contamination in food started with few countries but now many countries have developed theirs. In 1981 only 33 countries had set up the contamination limits and the number increased to 100 in 2003; it is aflatoxin which was the first to have its limits set in 1960s (van Egmond, 2007). Table 1.7 shows the number of countries that had legally established limits for aflatoxins in foodstuffs by 2003. In food, the regulatory limit for AFB₁ is 2 µg/kg and for total aflatoxins 4 µg/kg mainly for EU countries (van Egmond and Jonker, 2004). Another commonly used limit for AFB₁ is 5 µg/kg which is followed by 21 countries, spread over Asia/Oceania, Latin America, Europe and Africa. However, it is not indicated how many of those countries are in Africa. Tanzania also applies 5 µg/kg for maize flour (TBS, 2010). The mostly used limit for total aflatoxin is 4µg/kg , which is also commonly applied in EU countries, followed by 20 µg/kg applied in 17 countries, half of them in Latin America and several in Africa (van Egmond and Jonker, 2005). In Tanzania the limit for total aflatoxin for maize flour is 10 µg/kg (TBS, 2010). Regulatory limits for AFM₁ also varies between countries, however 0.05 µg/kg for dairy and dairy products is commonly used; it is applied in 34 countries mostly EU countries, followed by 0.5 µg/kg which is applied in 22 countries including USA, Asian and mostly frequently in Latin America (van Egmond and Jonker, 2005). The AFM₁ limit for infants' food in EU countries is 0.0255 µg/kg (European-Commission, 2006).

Table 1.7 Number of countries with general limits of aflatoxins in foodstuffs, by 2003

Aflatoxin B ₁		Total aflatoxins		Aflatoxin M ₁	
Limit in µg/kg	Number of countries	Limit in µg/kg	Number of countries	Limit in µg/kg	Number of countries
20	3	35	2	15	1
15	2	30	3	5	1
10	5	20	17	0.5	22
5	21	15	8	0.2	1
2	29	10	8	0.05	34
1	1	5	3		
		4	29		
		3	1		
		1	3		
		0	2		

Source: Worldwide regulation of aflatoxin (*van Egmond and Marco A. Jonker, 2005*) in aflatoxin and food safety (*Abbas, 2005*).

Fumonisin is not regulated in many countries as reported by Van Egmond and Jonker (2004). Up to 2002, only six countries were documented to regulate fumonisin in maize with a limit set in a range between 1000 – 3000 µg/kg, four of them applied the limit of 1000 µg/kg. The official limit for EU countries by 2006 for the sum of FB₁ and FB₂ are; 2000 µg/kg for unprocessed maize, 1000 µg/kg for maize flour, 400 µg/kg for maize based food for direct human consumption and 200 µg/kg for processed maize based foods and foods for infants and young children (European-Commission, 2006). However, there is no regulatory limit of fumonisin in Tanzania.

1.10. Conclusion

Generally, there are no regulatory limits for aflatoxins (AFB₁, AFM₁ or aflatoxin total) or fumonisins for infants food specific to Tanzania. This situation calls for more studies in order to establish a data bank on the occurrence and distribution of these toxins in other foods such as cereals, peanuts, cow milk (Reddy et al., 2010) which are commonly consumed in several parts of Tanzania Data should also be collected on the occurrence of aflatoxicosis, the effect of aflatoxins and fumonisins on growth and their association with cancer cases.

In conclusion, with regard to the contents of this chapter, it can be clearly seen that, in Tanzania maize is a staple food which is also prone to aflatoxins and fumonisins contamination. It is possible that lactating mothers consuming contaminated maize can excrete aflatoxins and fumonisin in breast milk thus exposing their children to these toxins. The information also demonstrates that infants in Tanzania are introduced to maize-based food before six months of age. Our concern is therefore that the introduction of complementary foods may also expose these infants to mycotoxins. The exposure level could rise up to unacceptable levels, hence, adversely affect their growth and health. It is because of these concerns that the research objectives of this study were set. These objectives are:

- To identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants.
- To assess dietary exposure to aflatoxins and fumonisins in infants under six months of age
- To assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk
- To assess the effects of concomitant exposure of aflatoxins and fumonisins in breastfeeding and/or Complementary feeding practices on infants' growth
- To recommend measures for protecting infants in Tanzania against aflatoxins and fumonisins exposure

We needed to respond to such questions like, do lactating mothers excrete aflatoxin and fumonisin in breast milk? and at what level?, to what extent will the infants under six months be exposed?, is the level of exposure of health concern? Do breast-fed infants introduced to maize based foods also get exposed to mycotoxin? What are the levels of exposure? Does the exposure to mycotoxin affect their growth status?

CHAPTER 2

Methods for the research and subject characteristics

Summary

This chapter covers the background information of the study area including the agro ecological zones and the main economic activities in the district. The study follows ethical guidelines on working with human subjects, therefore ethical approval was obtained prior to the start of the research and this is also reported in this chapter. The whole report of this study is based on the same subjects and samples therefore to avoid repetition of similar information in the following chapters, the majority of methodology is covered in this chapter. This includes: procedures for recruitment of mothers and their infants, recording of anthropometric measurements for the infants, dietary assessment, collection of breast milk and maize flour samples and, procedures for laboratory analyses of mycotoxins (AFM₁ and FB₁) from breast milk and (AFB₁, AFB₂, AFG₁, AFG₂, FB₁ and FB₂) from maize flour. The descriptive characteristics, socio-economic status and food intake of the subjects are also reported.

2.1. Study area, selection criteria and sample collection

2.1.1. Study area

The study was conducted in Rombo district in Kilimanjaro region in Tanzania (figure 2.1). Kilimanjaro Region is located in north-eastern Tanzania and consists of six districts: Rombo, Mwanga, Same, Moshi vijijini (Rural), Hai and Moshi mjini (Urban). Rombo is bordered to the north and east by the Republic of Kenya, to the north west by the Hai and Longido districts and to the south west by the Moshi Rural district. Rombo district has an area of 1,440km² with a population of 267,092, out of it 136,217 (51%) are males according to Rombo district council data (2014).

Rombo receives a mean annual rainfall of about 500mm/m² per annum. The rainfall pattern is bimodal, unreliable, erratic and poorly distributed. These characteristics mean that Rombo experiences food shortages every year. The district has three agro-ecological zones namely, highland zone, middle zone and lowland zone. Highland zone ranges between 1600 and 2000 meters above mean sea level. The main crops grown are coffee, maize and bananas. Other

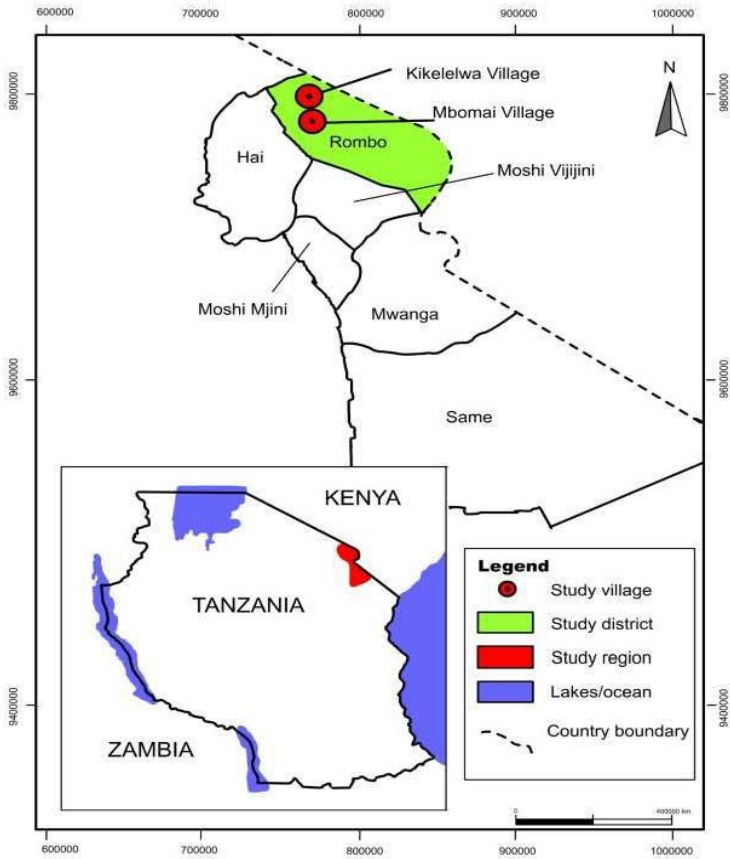


Fig 2.1. A map of Tanzania indicating the study area in Kilimanjaro region, Rombo district.

Figure 2.1. A map of Tanzania indicating the study area in Kilimanjaro region, Rombo district.

2.1.2. Ethical consideration

This study was conducted according to the guidelines of the Helsinki Declaration following the approval and clearance from the Ethics Committee of the National Institute for Medical Research in Tanzania (certificate number NIMR/HQ/R.8a/Vol.IX/1142). Mothers were made aware of the objectives of the study and clarification was given on all procedures for data collection. Confidentiality of the information obtained and all other rights of the subjects were ensured. Written informed consent was obtained from each mother who agreed to participate in the study.

2.1.3. Recruitment of subjects

All mothers who delivered from November 2011 to February 2012 were progressively recruited based on set inclusion criteria and clinical records available at the Kikelelwa dispensary (for Kikelelwa village) and Tarakea health center (for Mbomai Village) including mothers who delivered at home but registered for post natal clinic services. The inclusion criteria were: (i) not planning to leave the study zone during the next 6 months; (ii) mother being able to breast feed her child. The targeted sample size was 144 mother/infants pairs. The sample size was calculated based on PASS 2008 (NCSS, Kaysville, UT, USA) for a longitudinal study with a power of 90% and a 2-sided significance level of 5%, assuming a standard deviation (SD) of 1.13 for the exposed and 1.05 for non-exposed and 20% loss to follow-up.

Three follow up visits were made at age-defined intervals: 1st, 3rd and 5th months after birth. This meant the field work lasted for 8 months, from November 2011 to June 2012. The collection of samples therefore cut across both the dry season (January, February, June) and rainy season (March, April, May, November and December). Methods of data and sample collections from follow-up visits are elaborated on in sections 2.1.4, 2.1.5, 2.1.6, and 2.1.7.

2.1.4. Anthropometric measurements

Infant anthropometry was recorded in each visit using daily calibrated instruments. Infant's weight and height were recorded at each visit by trained health workers. Weight was measured to the nearest 0.1kg using SALTER scale model 2356M (Salter, Tonbridge, UK), length was measured to the nearest 0.1cm using SECA infantometer model: 416 1821009 (Chino, CA, US). "Weight for height" Z score (WHZ) and "Length for age" Z score (LAZ) were computed in Stata 12.0 (College Station, TX, USA) with reference to the WHO 2006 growth standards (WHO, 2006).

2.1.5. Dietary assessment

A 24 hour food intake recall questionnaire was administered by the principal investigator at each visit to assess the different foods consumed by each mother the day before breast milk sampling. A 24 hour dietary recall for the infants were also recorded in which information about the food eaten, sources of maize used in complementary food and whether the maize was dehulled, or not (dehulling is known to reduce mycotoxins contamination in food), prior to milling for flour were recorded. Estimates of liquid food intake were made using a graduated bottle, while solid food consumption was determined using number of spoons. The frequency of maize consumption in a week for each child that had started maize based food was also recorded in each visits. In a separate questionnaire, information about breastfeeding practices, socio-economic status of the mother including age, education, marital status, maternal age, employment status, income per month and number of household members including children was documented.

2.1.6. Breast milk sample collection

At each visit, 75 ml of breast milk were collected from each mother during the morning period by self- expression into a sterile plastic container. Samples were kept at 4°C and frozen within one day at -20°C until analysis as described by Polychronaki et al. (2006) and El-Tras et al. (2011). Prior to analysis samples were thawed and maintained in a water bath at 37°C.

2.1.7. Collection of maize flour samples

Maize flour samples (250g) used for making porridge for the infants was collected in each visit from those families that had started feeding complementary food to their infants. The flour was collected in paper bags, then kept in polythene bag sealed and stored at -20°C until analysis (Kaaya et al., 2006).

2.2. Mycotoxins analyses

2.2.1. Chemicals and reagents

A variety of chemicals and reagents were used for the analysis. For HPLC analysis all chemicals were of HPLC grade and were supplied by different companies as follows: acetonitrile, methanol and acetic acid, hydrochloric acid (Merck KGaA-Darmstadt, Germany), ortho-phthaldehyde (Sigma-Aldrich, St. Louis, MO, USA), sodium tetraborate (BDH, Poole, UK), acetone (Fisher scientific, Leicestershire, UK), 50µl β-mercaptoethanol (Fluka, Steinheim, Germany) and sodium dihydrogen orthophosphate, ammonium acetate and phosphoric acid (BDH - Poole, UK), nitrogen gas was supplied by Tanzania oxygen limited (TOL). Mycotoxins analyses were carried out using standard procedures and some with modifications, the procedures followed are described in section 2.2.2, 2.2.3, 2.2.4 and 2.2.5.

2.2.2. Determination of AFM₁ in breast milk

2.2.2.1. Extraction of AFM₁ from the breast milk samples

The extraction and HPLC analyses were done as reported earlier (Navas et al., 2005; Shundo et al., 2009) with some modifications. Milk samples were centrifuged at 2000 g for 15 min and the upper (fat) layer was removed prior to filtration using filter paper (Whatman paper #4; Whatman, Maidstone, UK). Ten ml of milk was applied on an immunoaffinity column (AFLAPREP M; from R-biopharm Rhône Ltd, Glasgow, UK) through a syringe. After the milk had passed through the column, the column was washed with 20 ml of deionized water. The elution of AFM₁ was done using 4 ml of pure acetonitrile. The elute was collected in a conical

glass tube and the solvent was evaporated using a gentle stream of nitrogen from a Nitrogen Evaporator (Reacti-Vap model 18780; Pierce, Rockford, IL, USA). The dried elute was then reconstituted by 500 μ L of the mobile phase and injected into the HPLC.

2.2.2.2. HPLC analysis of AFM₁ from breast milk

The stock standard of AFM₁ at a concentration of 100 ng/ml was prepared in acetonitrile. From the stock standard, an intermediate standard solution of 10 ng/ml was also prepared in acetonitrile. The working standard solutions were prepared from the intermediate standard solution with appropriate dilutions. The calibration curve was prepared in the range of 0.005 ng/ml to 0.275 ng/ml.

A HPLC (Shimadzu, Tokyo, Japan) equipped with an HPLC pump LC 20AD and a fluorescence detector model RF-10AXL set at wavelength 365 nm for excitation and 450 nm for emission, was used. The HPLC column used is LiChrosorb C18 column (250x4 mm, 10 mm, Merck Germany). The mobile phase used was composed of 2% acetic acid in water/acetonitrile/methanol (40/35/25; v/v/v).

2.2.2.3. Validation of the method

A plot of the AFM₁ calibration curve showed a linear line, and the calibration curve was used for the determination of AFM₁ in samples. Spiking of AFM₁ in the samples was done at the lowest, middle and higher levels of the calibration curve; 0.005, 0.125 and 0.275 ng/ml. The average recovery of the spiked samples was 87.4% (RSD 3%). The limit of detection (LOD) was 0.001 ng/ml and limit of quantification (LOQ) 0.003 ng/ml on matrix basis. The average RSD for repeatability and reproducibility of the method were 1.6 and 5.2% respectively. For all the analyses, a 0.125 ng/ml working standard was inserted in the sample stream for quality assurance purposes.

2.2.3. Determination of fumonisin in breast milk

2.2.3.1. Extraction of fumonisin from breast milk

The extraction procedure was modified from the method reported earlier (Maragos and Richard, 1994) as elaborated herein. Breast milk (from stocks stored at -20°C) was warmed in the water bath maintained at 30°C to attain a room temperature (25°C). Fifteen ml of methanol:acetone (1:1) was added to 5 ml breast milk and shaken for 10 min using a multi-purpose rotator – Thermo Fisher Scientific Model 2346 – ICE (Langensfeld, Germany). The mixture was then cooled to -40 °C for 10 min and then centrifuged using Hettich ROTOFIX 32 A (Lawrence, MA, USA) at 4000 rpm for 15 min. The supernatant was transferred into a 50 ml Teflon centrifuge tube and held at -40°C. The 15 ml of extraction solvent methanol:acetone (1:1) was added to the pellet and mixed and then centrifuged as above. The supernatant solution from the first and second extract was pooled together and cooled at -40°C. The combined extract was again centrifuged at 4000 rpm for 10 min and stored in polypropylene centrifuge tubes at -20°C ready for the cleanup procedure.

On the day of analysis the extract was evaporated by rotary evaporator of BUCHI (Flawil, Switzerland) (fixed with vacuum controller V-855, rotorvapor[®] R-215, vacuum pump V-700 and heating bath B-491) to 1-2 ml, and 20 ml of methanol:water in a 3:1 ratio was added. Clean up was done using Strong Anion Exchange (SAX) columns (Varian Bond elut LRC 500mg 10ml, Varian Belgium NV/SA, Belgium). A column was first conditioned by 5 ml of methanol and then 5 ml of methanol:water (3:1). Twenty ml of sample filtrate was poured into the column, followed by 2.5 ml of methanol:water (3:1) which was used to rinse the sample flask. The column was then washed by 5 ml of pure methanol. Fumonisin was eluted by 10 ml of 5% acetic acid glacial in methanol. The elute was evaporated to dryness at 60°C under gentle stream of nitrogen using a Nitrogen Evaporator (Reacti-Vap model 18780; Pierce, Rockford, IL, USA).

2.2.3.2. HPLC analysis of fumonisin from breast milk

A supplied mixed analytical standard of fumonisin (FB1 and FB2) (Romer labs, Cheshire, UK) which contained 50 µg/ml of each in 50:50 acetonitrile/water was used. A working standard

solution of 5000 ng/ml was prepared and used for preparation of solutions for the calibration curve. The calibration curve was prepared in the range of 24 ng/ml to 120 ng/ml.

Twenty μl of the mixture of 200 μl of acetonitrile:water (1:1) and 200 μl of derivatising reagent which were added into the dried fumonisin and vortexed for 1 minute, was injected into an HPLC system (Shimadzu, Tokyo, Japan). The derivatising reagent was prepared by dissolving 40 mg of orthophthaldehyde in 1 ml of methanol, 5 ml of 0.1 M sodium tetraborate and 50 μl β -mercaptoethanol. The HPLC equipment fitted with a pump LC 20AD and fluorescence detector model RF-10AXL was set at wavelength of 335 nm excitation and 440 nm emission. The oven temperature was operated at a range of 19°C to 30°C, maximum. HPLC Column was a stainless steel, Waters Spherisorb® 5 μm ODS 1, 4.6 x 200 mm (Milford, MA, USA) and the mobile phase was composed of 750 ml of methanol and 250 ml of 0.1 M NaH_2PO_4 with its pH adjusted to 3.35 by using phosphoric acid solution. Mobile phase was filtered under vacuum with a 0.45 micron filter paper before use, each time it was prepared. The flow rate of the mobile phase was set at 1 ml/min.

2.2.3.3. Validation of the method

A plot of the FB_1 calibration curve showed a linear line, which when statistically tested with a residual plot, demonstrated random scatter of the residual points, supporting the linearity. For determination of the recovery levels, some of the breast milk was first analysed, the blank samples (5 ml each) were spiked with FB_1 at levels of 24, 72, and 120 ng/ml and mixed for 10 min. The spiking was carried out in triplicate for each level. The average recovery of FB_1 was 93.2% with a standard deviation of 4.8%. Mean coefficient of variations of within-day and between-day of the method were 5.1% and 9.2% respectively at the three concentration levels tested. Limit of detection (LOD) was calculated based on the recoveries from spiked samples. The LOD and LOQ for FB_1 was 5.5 ng/ml and 19.5 ng/ml respectively.

There was also some indication of the presence of FB_2 in breast milk samples; however the validation data for FB_2 in the method used were not credible. The average recovery obtained was 63%, with the repeatability of above 20% RSD and 19.10 ng/ml LOD. The criteria for

acceptance was above 80% for recovery and RSD below 20%. As such the results of FB₂ are not reported in this study.

2.2.3.4. Confirmation of fumonisin in breast milk

To confirm the presence of fumonisins in breast milk, a few breast milk samples were extracted and dried under nitrogen and transferred (under the agreement for material transfer with National Institute for Medical Research in Tanzania) to the Department of Food Safety and Food Quality, Ghent University, Belgium. The samples were analysed using High resolution liquid chromatographic separation technique (RSLC) on an Ultimate 3000 RSLC system (Dionex, Amsterdam, The Netherlands), consisting of a vacuum degasser, binary pump, cooled auto sampler and, column oven (30°C). A Zorbax Eclipse XDB C₁₈ column RRHD (1.8 µm, 2.1 x 100 mm) (Agilent Technologies, Waldbronn, Germany) was used. Mobile phase A consisted of water/methanol/acetic acid (94/5/1, v/v/v) and mobile phase B of methanol/water/acetic acid (97/2/1, v/v/v), both containing 5 mM of ammonium acetate. A binary gradient was applied with flow rate of 0.2 ml/min. The RSLC system was interfaced split less to a time-of-flight mass spectrometer (microTOF II, Bruker Daltonics, Bremen, Germany) equipped with an orthogonal electrospray ionization (ESI) source operating in both positive and negative mode using a mass to charge ratio range of 50–1000 for acquisition. The peak for FB₁ (C₃₄H₅₉NO₁₅) with mass/charge ratio of 722.4 was detected at 11.3 minutes.

2.2.4. Determination of aflatoxins in maize flour

2.2.4.1. Extraction of aflatoxins from maize flour

Two samples of maize flours collected from a family, at three and five months of age, were thoroughly mixed at the laboratory to make a sample for analysis. The method used to determine aflatoxin contamination is described by Stroka et al. (2000). Minor modifications to the process were made according to Kimanya et al. (2008a). To extract aflatoxins in a flour sample 12.5 g was weighed in a conical flask. Extraction solvent, 50 ml of methanol and water at a ratio of 6:4, was added to the flour and shaken for one hour using a horizontal laboratory shaker (SF1-R00100545). The mixture was then filtered through filter paper. Ten

ml of the extract was diluted with 30 ml of phosphate buffer saline. The diluted extract was adjusted to pH 7.4 by adding 0.1 M sodium hydroxide (NaOH).

The pH adjusted extract was then applied to an immunoaffinity column (Aflastar) for clean-up purposes. Once the extract had gone through, the column was washed with 20 ml of deionized water which was followed by a vacuum to remove any remaining water. One point five (1.5) ml of methanol was subsequently added, and allowed to remain within the column for approximately one minute prior to the elution of aflatoxins. The eluate was collected into vials, ready for injection into the HPLC unit.

2.2.4.2. HPLC analysis of aflatoxin from maize flour

Ten μ l of the eluate was injected into an 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 μ m, 4.6x 150 mm was used, with a mobile phase composed of methanol/acetonitrile/water (15/20/65, v/v/v). One hundred and nineteen mg of potassium bromide and 100 μ l of 65% nitric acid was added for derivatisation. The flow rate of the mobile phase was set to 0.8 ml/min.

2.2.4.3. Validation of the method

A standard of aflatoxins with concentrations of 1000 ng/ml for AFB₁ and AFG₁ and 300 ng/ml for AFB₂ and AFG₂ was used in the preparation of working standards. Working standard solutions of 50 ng/ml for AFB₁ and AFG₁ and 15 ng/ml for AFB₂ and AFG₂ were prepared in methanol and used as solutions for a calibration curve. Five levels of concentrations were prepared for the calibration curve. The concentrations, on dry weight basis, ranged from 0.75 to 6.75 μ g/kg for AFB₁ and G₁, and from 0.225 to 2.025 μ g/kg for AFB₂ and AFG₂. The LOD were 0.53 μ g/kg for AFB₁, 0.15 μ g/kg for AFB₂, 0.24 μ g/kg for AFG₁ and 0.01 μ g/kg for AFG₂. AFB₁ and AFG₁ were spiked into blank maize flour samples, each at 0.75, 3.75 and 6.85 μ g/kg. On average AFB₁ was recovered by 89% (three samples; RSD within a day 4.1% and between a day of 15.7%) and AFG₁ by 102% (three samples; RSD of 4.1% within a day and 19.8% between a day). The blank maize flour samples were also spiked with AFB₂ and AFG₂, each at 0.23, 1.13 and 2.03 μ g/kg. On average the toxins were recovered by 87%. The RSD

for three maize flour samples spiked with the AFB₂ was 3.7% and 14.3% (within a day and between a day respectively) and AFG₂ was 4.6% within a day and 19.9% between a day.

2.2.5. Determination of fumonisins in maize flour

2.2.5.1. Extraction of aflatoxins from maize flour

Extraction 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). Fifteen grams of flour was used for the extraction of fumonisins. The flour was mixed with 40 ml of methanol:water (3:1) in a 100 ml capped conical flask, and then shaken overnight using a horizontal laboratory shaker (Stuart scientific SF1 R00100545). The mixture was thereafter filtered using filter paper (Whatman paper # 1) into a 100 ml beaker. Ten ml of methanol and water (3:1) mixture was used to ensure that all contents were rinsed out from the conical flask. The pH of the filtrate was adjusted to fall between 5.8 and 6.5. This was done using 0.1M potassium hydroxide

SAX columns were used for the cleanup of toxins. A SAX column was first conditioned with 5 ml of methanol and then by 5 ml of the methanol:water (3:1). This was followed by pouring 10 ml of the sample filtrate into the column. The column was then washed with 8 ml of methanol:water (3:1) followed by 3 ml of pure methanol. Fumonisins were eluted by 10 ml of 1% acetic acid glacial in methanol. The eluate was evaporated to dryness at a temperature 60°C under a gentle stream of nitrogen using a Nitrogen Evaporator (Reacti-Vap model 18780; Pierce, Rockford, IL, USA).

2.2.5.2. HPLC analysis of fumonisin from maize flour

Two hundred µl of acetonitrile:water (1:1) and 200 µl of a derivatising reagent were added to the dried fumonisins and vortexed for one minute. Derivatising reagent was prepared by dissolving 40 mg of ortho-phthaldehyde into 1 ml of methanol, 5 ml of 0.1 M sodium tetraborate and 50 µl of β-mercaptoethanol. Twenty µl of this mixture was injected into 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. A HPLC column Stainless

steel, Waters Spherisorb[®] 5 µm ODS-1, 4.6 x 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 by using phosphoric acid, and the mobile phase was filtered under vacuum with a 0.45 micron filter paper. The flow rate of the mobile phase was set at 1ml/min.

2.2.5.3. Validation of the method

A supplied mixed analytical standard of fumonisin (FB₁ and FB₂) (Romer labs, Cheshire, UK) which contained 50 µg/ml of each in 50:50 acetonitrile:water was used. A working standard solution of 5000 ng/ml was prepared and used for preparation of solutions for the calibration curve. The recovery for fumonisin in maize was determined by spiking the blank samples of maize flour with mixed standard of FB₁ and FB₂ at concentration levels ranging from 50 - 250 µg/kg. The average recovery value for FB₁ was 89% for five samples, with relative standard deviation (RSD) of 15.5% and for FB₂ 81% for five samples with RSD of 15.4% (between-day). The limit of detection and quantification for FB₁ was 53 µg/kg and 102 µg/kg and for FB₂ 47 µg/kg and 94 µg/kg respectively.

Table 2.1 presents a summary of the analytical methods including the equipment used, verification of the linearity of the calibration curve, accuracy (as percent recovery), precision (as repeatability and /or reproducibility), LOD and LOQ of the methods

Table 2.1 Summary of the analytical method used

METHOD	
Specific equipment	High Performance Liquid Chromatography with fluorescence detector
Linearity	Visual straight line Regression analysis ($R^2 \geq 0.99$) Lack-of-fit test at $\alpha = 0.05$ significance level
Specificity	Spiked sample; contaminant peak detected Blank sample; no contaminant peak detected
Statistical techniques	Mean, % Relative Standard Deviation (%RSD) and/or Standard deviation (SD)
Matrices	Breast milk
Quantification parameter	Aflatoxin M ₁
% Recovery ($\geq 80\%$)	87.4
LOD^a	0.001 ng/ml
LOQ^b	0.003 ng/ml
Repeatability (%)	1.6
Reproducibility (%)	5.2
	Breast milk Fumonisin B ₁
	93.2
	5.5 ng/ml
	19.5 ng/ml
	5.1
	9.2
	Aflatoxin B ₁
	89
	0.53 µg/kg
	1.62 µg/kg
	4.1
	15.7
	Aflatoxin B ₂
	87
	0.15 µg/kg
	0.46 µg/kg
	3.7
	14.3
	Aflatoxin G ₁
	102
	0.24 µg/kg
	0.73 µg/kg
	4.1
	19.8
	Aflatoxin G ₂
	87
	0.01 µg/kg
	0.02 µg/kg
	4.6
	19.9
	Fumonisin B ₁
	89
	53 µg/kg
	102 µg/kg
	2.6
	15.5
	Fumonisin B ₂
	81
	47 µg/kg
	94 µg/kg
	1.5
	15.4

^a:LOD (limit of detection) were calculated using: 3:3x (residual standard deviation (standard error) of the calibration curve /mean of slope of the calibration curve)

^b:LOQ (limit of quantification) were calculated using 10 x (residual standard deviation (standard error) of the calibration curve /mean of slope of the calibration curve)

2.3. Characteristics of the study population

The descriptive information of subjects presented in Table 2.2 shows the age of the parents, the age range of the mothers at first bearing, number of children in a family, number of any child death encountered in a family and, total members in a household. Age of the mothers involved in the study ranged from 17 – 45 years old with their husbands' age in the range of 19 – 60 years. The mean age of mothers to start bearing children is 20 years. In a family the number of children ranged from 1 – 9, with the mean number of children to be 3. Generally the birth weight of their infants ranges from 2 - 4.5 kg. The observed households had family members ranging from 2 – 15. During recruitment infants were 73 girls and 72 boys

The majority (81.4%) of the mothers involved in the study were married or living together with their husbands. Primary level of education is the highest level attained by as many as 82.1% of mothers, and 75.9% of the fathers. In terms of economic activities, participants depend mainly on agriculture to support their life; however a high percentage of fathers have permanent employment compared to mothers. On the other hand, fathers have higher monthly earnings but with only a few individuals earning above 101,000 Tshs (47€) per month, (5.5% of mothers and 15.2% fathers) (Table 2.3). This figure is important because from verbal communication it is the amount that can sustain the individual needs in rural settings of Rombo at least for a month.

Each economic status variable (Table 2.3) was grouped into two categories and then coded as 0 and 1 for data analysis purposes. Ages of the parents were grouped for young parents and older parents where any age above 27 yrs was regarded as older. For the education level the groups were those with only primary education and those with secondary education and above. Earning was categorised as earners of less than 50,000 Tshs and earners of 50, 000 Tshs and above. Marital status was grouped for single and families with two parents. These newly created variables were included in the multilevel mixed effect linear regression as confounding factors for mycotoxin exposure in the growth assessment.

Table 2.2. Characteristics of infants and their parents available for the study*

	N	Mean ± SD	Range
Age of mother (yr)	143	28 ± 7	17 – 45
Age of father (yr)	130	33 ± 8	19 – 60
Age at the birth of first child (yr)	130	20 ± 3	15 – 33
Number of children	143	3 ± 2	1 – 9
Number of died children	128	0 ± 1	0 – 3
Family size	143	6 ± 2	2 – 15
Children birth weight (kg)	135	3 ± 0.48	2 – 4.5

*results are from those who responded to that particular question

Table 2.3. Social economic factors of the parents*

	Mother		Father	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Marital status: n 145				
Missing	1	1		
Single	22	15		
Married/living together	118	81		
Widow	2	1		
Divorced	2	1		
Education level				
Missing	1	1	6	4
No education	4	3	1	1
Primary level	119	82	110	76
Secondary level	19	13	27	19
College	2	1	1	1
Working status				
Missing	6	4	9	6
Employed	6	4	39	27
Small business	41	28	26	18
Farmer	89	61	67	46
Other	3	2	4	3
Earning per month				
Missing	37	26	48	33
20,000 – 50,000 Tshs	83	57	47	32
51,000 – 100,000 Tshs	17	12	28	19
Above 101,000 Tshs	8	6	22	15

*results are from those who responded to that particular question

2.4. Food intake of the lactating mother and the infants

Foods from the 24 hour recall were grouped in the expanded questionnaire format of FANTA food group classification system (Hoddinott and Yohannes, 2002). These food groups enabled the computation of dietary diversity scores (DDS) which provided a clearer picture of dietary diversity and mycotoxin contamination during lactation stages, and is a good indicator of food groups commonly consumed by the study population. Different food

groups were eaten by mothers 24hrs before the collection of breast milk samples as presented in Table 2.4. The mean diet diversity score was 5, 6 and 6 during first, third and fifth month of lactation stage out of 12 DDS score, respectively, indicating that half of the number of food groups mentioned in Table 2.4 were consumed daily.

Table 2.4. Food group frequency distribution for lactating mothers diet 24hr before expressing breast milk (%) as recorded during lactation month 1, 3 and 5

Diet diversity – 24h recall	Lactation stage		
	Month 1 n = 143	Month 3 n = 121	Month 5 n = 118
interviewees who ate cereals (mainly maize)	81	94	94
interviewees who ate green banana cooked	90	73	58
interviewees who ate roots and tuber	15	27	19
interviewees who ate vegetables	27	56	76
interviewees who ate fruits	4	5	4
interviewees who ate meat,poultry	56	43	32
interviewees who ate eggs	8	9	6
interviewees who ate fish and sea food	18	20	24
interviewees who ate pulse/legumes/nuts	30	43	45
interviewees who ate milk and milk products	63	69	62
interviewees who ate oils/fat	95	93	92
interviewees who ate sugar	64	78	82
miscellaneous (local beer/softdrink)	2	3	3.
Household diet diversity mean score (DDS)	5.5	6.1	6.0

2.5. Food intake of the infants

The food intake results (Table 2.5) reveals that exclusive breastfeeding for up to six months is rarely done. Exclusive breastfeeding was practiced for only 19% of infants at the 3 months and only 3% at 5 months are exclusively breastfed. The majority of infants are introduced to solid food as early as three months of age, although breastfeeding continued even after the introduction of solid foods. The most common infants' food recorded during the survey is cow milk and maize porridge (Table 2.5). Porridge was mainly prepared from plain maize flour and in some few cases from mixed grain flour in which also maize was the main ingredient. Additionally, infants were given water, fruits, cow milk, potatoes porridge, banana porridge, rice, roasted banana with ghee, and stiff porridge with either milk, fish or green vegetables as their accompaniment. Table 2.6 shows the average daily energy and nutrients intake for infants from complementary food as recorded in the 3rd and 5th months

of age. Nutrients assessed were vitamin, iron, zinc, sodium and fibre and the total energy from the complementary food.

Table 2.5. The average daily infant food intake likely to contain mycotoxin as recorded in the 3rd and 5th months of age

	Month 3 n = 98	Month 5 n = 115
Maize (g)	1.6 ± 5.0	12.0 ± 21.3
Millet (g)	0.5 ± 2.9	6.8 ± 19.3
Groundnuts (g)	0.04 ± 0.5	0.1 ± 0.9
Cow milk (g)	8.4 ± 23.7	43.5 ± 74.1

Table 2.6. The average daily energy and nutrient intake for infants from complementary food as recorded in the 3rd and 5th months of age

	Month 3 n = 80	Month 5 n = 115
Kcalories	61.1 ± 73.1	174.5 ± 151.8
Protein (g)	0.7 ± 1.5	2.8 ± 2.7
Fat (g)	3.0 ± 5.3	6.1 ± 6.4
% Energy from fat	0.4 ± 0.3	0.3 ± 0.2
Sodium Na (mg)	19.0 ± 89.6	51.7 ± 75.2
Vitamin A (mg)	17.8 ± 44.7	161.0 ± 593.2
Iron Fe (mg)	0.1 ± 0.2	0.8 ± 1.1
Zinc Zn (mg)	0.1 ± 0.2	0.5 ± 0.8
Fibre (g)	0.5 ± 2.7	6.0 ± 21.0

Table 2.7 presents infants growth characteristics. Percent wasting was 4 at first month and 3 at the fifth month. Stunting was 11% at month 1 and peaked up to 17% at month 5.

Table 2.7. Infants' growth characteristics (mean ± standard deviation)

	Weight (kg)	HAZ ^a	%stunting	WAZ ^b	%under- weight	WHZ ^c	%wasting
Month1 (n=143)	4.33±0.7	-0.3±1.3	11	-0.04±1.3	4	1.0±8.4	4
Month3 (n=121)	6.10±0.8	-0.6±1.4	13	-0.06±1.1	9	0.6±1.3	1
Month5 (n=118)	7.09±1.0	-0.9±1.2	17	-0.19±1.2	10	0.6±1.3	3

^aHAZ: height for age z-score; stunting = HAZ < -2

^bWAZ: weight for age z-score; underweight = WAZ < -2

^cWHZ: weight for height z-score; wasting = WHZ < -2

2.6. Data management and analysis

Data from questionnaires were entered into Epidata 3.1 (Odense, Denmark). Mycotoxin levels obtained from HPLC analysis were recorded in Microsoft Excel 2013; all values below LOD were treated as negative (uncontaminated) samples. Data were analysed in Stata 12.0. Skewed data were presented as median and inter-quartile ranges, in analysis they were natural log transformed. Mixed-effect linear regression models were used to determine factors related to the children growth: quadratic models were fitted separately for each dependant growth variable (i.e. WAZ, LAZ and WHZ) and the mycotoxin exposure and social economic variables as explained in the following chapters.

Chapter 3

Risk of aflatoxins and fumonisins exposures from breast milk

This chapter has been redrafted from two publications:

H. Magoha, M. Kimanya, B. De Meulenaer, D. Roberfroid, C. Lachat and P. Kolsteren. (2014). Association between M1 exposure through breast milk and growth impairment in infants from Northern Tanzania, *World Mycotoxin Journal* 7: 277 - 284.

H. Magoha, B. De Meulenaer, M. Kimanya, D. Hipolite, C. Lachat and P. Kolsteren. (2014). Fumonisin B1 contamination in breast milk and its exposure in infants under six months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology* 74: 112 - 116.

Summary

Infants breastfeeding from mothers consuming aflatoxin and or fumonisins contaminated foods may be exposed to aflatoxin M₁ (AFM₁), a metabolite of aflatoxin B₁ (AFB₁) and or fumonisin B₁ (FB₁). Therefore, this study assessed AFM₁ and FB₁ contamination in breast milk and associated exposures of infants under six months of age. Breast milk samples were collected from lactating mothers during 1st, 3rd and 5th month of lactation stages. Both AFM₁ and FB₁ contamination in the samples were determined using HPLC. AFM₁ and FB₁ exposure by an infant was estimated using a deterministic approach. All the breast-milk samples were contaminated by AFM₁ at levels ranging from 0.01 to 0.55 ng/ml. More than 90% of samples exceeded the EU limit of 0.025 ng/ml for infants' foods while over 76% exceeded the EU limit of 0.05 ng/ml for dairy milk and milk products. Only 1% of the samples exceeded the limit of 0.5 ng/ml set for dairy milk in the United States and several countries in Asia. Aflatoxin M₁ exposures ranged from 1.13 – 66.79 ng/kg bw/day. Above 40% contained FB₁ at levels ranging from 5.58 – 471.05 ng/ml. Of the contaminated samples, 10% and 2% had FB₁ levels above the EU limit of 200 ng/ml for fumonisins in infants' food in lactation stages of month 1 and 5 respectively. FB₁ exposure in infants ranged from 0.60 – 64.93 µg/kg bw/day and exceeded the provisional maximum tolerable daily intake of 2µg/kg bw/day in 29% and 35% of the infants during month 1 and 5 respectively. Appropriate strategies should be applied to prevent aflatoxins and fumonisins exposure in lactating mothers in order to protect infants from these exposures.

3.1. Introduction

Occurrence of mycotoxin in breast milk has been reported in various studies as it has been explained in Chapter 1. Tanzania uses maize as a staple food as well as a main ingredient in the preparation of complementary food for children. Tanzania dietary energy consumption as pointed out by Komba and Moltedo (2012) shows that 70% of dietary energy consumed is from cereals, maize in particular. Maize flour, maize grain, rice and cassava flour contribute to half of the national dietary energy consumption (Komba and Moltedo, 2012). The existing records in Tanzania also show a high consumption rate of maize and the presence of mycotoxins in maize for human consumption (Kimanya et al., 2008a; Manjula et al., 2009; Mboya et al., 2011). Furthermore, (Kimanya et al., 2008a; Kimanya et al., 2014) reported co-occurrence of multiple mycotoxins in maize, aflatoxin, DON and fumonisin inclusive. Fumonisin contamination in maize in Tanzania was reported up to a level of 11,048 µg/kg. Recently, Shirima et al. (2013) also reported AFB₁ and fumonisin exposure in different parts of Tanzania including the area of this study, Rombo district in Northern Tanzania. There is a possibility for these mycotoxins to be carried over to the breast milk. Taking into consideration the adverse health effect of aflatoxin and fumonisin, their presence in breast milk could affect infants under six months of age who are solely depending on breast milk for their nutritional requirement. This chapter reports on the occurrence and exposure of AFM₁ and FB₁ to infants aged below 6 months through breast milk.

3.2. Exposure assessment

3.2.1. AFM₁ exposure estimation and risk management

To estimate the exposure of a child, the AFM₁ contamination obtained from his or her mothers' breast milk was multiplied by the breast milk intake as documented by the United States Environmental Protection Agency (US-EPA, 2011) for infants of his or her age divided by his or her body weight. The average breast milk intake used by age was 510 ml/day; 690 ml/day and 770 ml/day for month 1, 3 and 5, respectively.

There is no existing international limit for AFM₁ in breast milk. Neither is there a maximum limit set for the toxin in baby foods for use in Tanzania. Therefore, the AFM₁ levels in this study were compared to the maximum regulatory limits for AFM₁ set for infants' food or dairy milk and milk products for other countries (FAO, 2004). These are 0.025 ng/ml for infants food in EU (European-Commission, 2006), 0.05 ng/ml for dairy milk and milk products in EU (Lin et al., 2004; European-Commission, 2006; Alonso et al., 2010) or 0.5 ng/ml for dairy milk and milk products in the USA (Lin et al., 2004), Korea (Lee et al., 2009) and Brazil (Lin et al., 2004; Shundo et al., 2009).

3.2.2. FB₁ exposure estimation and risk management

Estimation of FB₁ exposure of a child involved the multiplication of FB₁ contamination obtained from his or her mothers' breast milk with the breast milk intake as documented by the United States Environmental Protection Agency (Table 3.1) for infants of his or her age divided by his or her body weight as explained in AFM₁ exposure. The level of exposures were compared with WHO Provisional maximum tolerable daily intake (PMTDI) of 2µg/kg body weight per day (WHO, 2002).

There is no existing international limit for FB₁ in breast milk. Neither is there a maximum limit set for the toxin in baby foods for use in Tanzania. Generally, there is no official set maximum limit for fumonisin in Tanzania. In that case, the FB₁ levels in this study were compared to the EU maximum regulatory limits (200 µg/kg) for total fumonisin as set for infants' food (European-Commission, 2006).

3.2.3. Assessment of seasonality variation on AFM₁ and FB₁ contamination

Seasonality variation assessment of AFM₁ and FB₁ for the rainy and dry season was done. As previously stated in chapter 2 section 2.1.3, collection of samples cut across the two seasons. The data for AFM₁ and FB₁ were natural log transformed as they were not normally distributed prior to their analysis.

3.2.4. Data management and analyses

Data were entered and analyzed in Stata 12.0. Results were presented as median and inter-quartile range. For seasonality assessment, the mean AFM₁ and mean FB₁ contaminations for the rainy and dry seasons were compared using Student's t-test.

3.3. Results

3.3.1. Dietary intake for lactating mothers

Different food groups were consumed by the mothers 24hr before the collection of breast milk samples. Consumption of maize (81%, 94% and 94%) and vegetables (27%, 56% and 76%) increased over time while the intake of green banana (90%, 73% and 58%) and meat (56%, 43% and 32%) declined, reflecting the customary dietary practice of the post-partum period.

3.3.2. Occurrence of AFM₁

All the breast milk samples contained AFM₁ concentration at levels ranging from 0.01 – 0.55 ng/ml (Table 3.1). At all lactation stages, AFM₁ levels in more than 90% of samples were above the EU limit of 0.025 ng/ml set for infants' food. More than 70% samples exceeded the EU limit of 0.05 ng/ml set for dairy products. Only 1% of samples from month one exceeded the limit of 0.5 ng/ml set for dairy milk in USA and other countries. The percentages of samples exceeding 0.05 ng/ml increased with the length of time the mother had been lactating (Table 3.1), but this increase was not statistically significant. Likewise, as shown in Table 3.1, there was a very small increase in median of AFM₁ levels over the lactation stages; from 0.07 ng/ml at month 1 to 0.08 ng/ml at month 3 or 5.

The results also showed marginal insignificant (P=0.05) seasonality differences in AFM₁ contamination between samples collected during the rainy and dry seasons. Geometric mean of AFM₁ (95% confidence interval) during the rainy season was 0.07 (0.06 – 0.08) ng/ml and dry season, 0.08 (0.07 – 0.09) ng/ml.

Table 3.1. Aflatoxin M₁ (AFM₁) in breast milk: occurrence, exposure and percentage of the milk exceeding regulatory limits at different lactation stages

	Lactation stage		
	Month1	Month3	Month5
Number of samples	143	121	118
Range of AFM ₁ contamination (ng/ml)	0.01 - 0.55	0.01 - 0.47	0.01 - 0.34
Median (ng/ml)	0.07	0.08	0.08
Interquartile range (ng/ml) (25%, 75%)	(0.05, 0.11)	(0.05, 0.13)	(0.06, 0.12)
% milk exceeding regulatory limit:			
0.025 ng/ml ^a	96	91	96
0.05 ng/ml ^b	76	82	88
0.5 ng/ml ^c	1	0	0
Milk intake per day (ml/day) ^d	510	690	770
Mean AFM ₁ Exposure (ng/kg bw per day)	11.08 ± 10.13	11.94 ± 9.69	10.91 ± 6.82
Range of AFM ₁ exposure (ng/kg bw per day)	1.13 - 66.79	0.81 - 58.96	1.08 - 34.90

^a: EU limit for AFM₁ contamination in infants food (European-Commission, 2006)

^b: EU limit for AFM₁ contamination in dairy milk and milk products (European-Commission, 2006)

^c: AFM₁ limit for dairy milk; USA (Lin et al., 2004), Korea (Lee et al., 2009) and Brazil (Shundo et al., 2009).

^d: Intake levels documented by United States Environmental Protection Agency (US-EPA, 2011)

3.3.3. Exposure of AFM₁

The AFM₁ exposures ranged from 0.8 – 66.8 ng/kg body weight per day. The highest exposure rates were observed in the 1st month of lactation while minimum exposure rates were in the 5th month. The mean exposure for all the three lactation stages was around 11 ng/kg body weight per day (Table 3.1).

3.3.4. Occurrence of FB₁ in breast milk

The occurrence of FB₁ in breast milk of lactating mothers in Rombo is presented in Table 3.2. Over 40% were contaminated with FB₁ in the two lactation stages, lactation stage at month 1 and month 5. The variation in contamination levels ranged from 5.6 to 471 ng/ml. About 10% and 2% of positive samples in month 1 and 5 respectively exceeded 200 ng/ml the EU limit for total fumonisin in infants foods. The results also showed insignificant (P=0.79) seasonality differences in FB₁ contamination between samples collected during the rainy and dry seasons. The mean of FB₁ (95% confidence interval) during the rainy season was 26.26 ng/ml and dry season, 24.03 ng/ml.

Table 3.2. Fumonisin B1 (FB₁) in breast milk: occurrence and exposure at different lactation stages

	Lactation stage	
	Month 1	Month 5
Number of samples	131	91
Number of positive samples (n, %)	(58, 44)	(52, 57)
Range of FB ₁ contamination (ng/ml)	6.57 – 471.05	5.58 – 257.43
Median contamination for positive samples (ng/ml)	26.23	31.05
Interquartile range of positive sample (ng/ml) (25%, 75%)	(11.60, 64.35)	(12.72, 59.94)
Positive sample exceeding regulatory limit: 200 ng/ml ^a (n, %)	(6, 10)	(1, 2)
Milk intake per day (ml/day) ^b	510	770
Infants exposed to FB ₁ (%)	44	57
Median of FB ₁ Exposure (µg/kg bw per day)	3.08	3.21
Range of FB ₁ exposure (µg/kg bw per day)	0.78 – 64.93	0.60 – 28.32
Infants exposed below PMTDI of 2 µg/kg bw per day ^c (n, %)	(20, 15)	(19, 21)
Mean ± Standard deviation	1.14 ± 0.32	1.20 ± 0.40
Infants exposed above PMTDI of 2 µg/kg bw per day ^c (n, %)	(38, 29)	(32, 35)
Mean ± Standard deviation	10.91 ± 14.89	7.24 ± 5.71

^a: Total fumonisin level exceeded EU limit for infants food 200 ng/ml (European-Commission, 2006)

^b: Intake levels documented by US Environmental Protection Agency

^c: PMTDI (Provisional maximum tolerable daily intake) for fumonisin set by WHO (WHO, 2002)

3.3.5. Exposure of FB₁

The range of FB₁ exposure was 0.6 – 64.9 µg/kg bw per day (Table 3.2). Similar to AFM₁, the highest exposure was observed in the 1st month of lactation. The median exposure for both lactation stages were around 3 µg/kg bw per day.

3.4. Discussion

The present findings have demonstrated contamination of breast milk with both AFM₁ and FB₁ in lactating mothers of northern Tanzania. The higher the contamination in breast milk the higher the expected exposure to infants. This is the first study of AFM₁ and FB₁ exposure from breast milk in Tanzania and the first study that has followed up the exposure from lactating mothers during the first 6 months of child life.

In the present study, all milk samples were contaminated with AFM₁ with over 90% exceeding 0.025 ng/ml, the EU limit for infants' food. Almost half of the samples were

positive for FB₁, with 471 ng/ml being the highest level recorded. The occurrence of FB₁ in breast milk is in line with the findings by (Fink-Gremmels, 2008) who indicated that upon intake of FB₁ in animals, the toxin remains unchanged in the rumen after metabolism and its biological potency also remain the same, with estimated carry over rates from 0 to 0.05%. These findings also suggests that reports of fumonisins contamination in cow's milk (Gazzotti et al., 2009) are applicable to human milk.

The high prevalence of AFM₁ contamination observed in this study was similar to the findings of Gürbay et al. (2010a). However, the recorded contaminations, 0.01 – 0.55 ng/ml were higher than those of 0.06 – 0.299 ng/ml reported previously (Gürbay et al., 2010a). Higher levels of AFM₁ (0.02 -1.816 ng/ml) in human milk have also been reported in other countries such as Ghana, (Lamplugh et al., 1988). The median (0.08 ng/ml) AFM₁ level in our samples was higher than that of 0.0135 ng/ml reported by Polychronaki et al. (2006). There is no study of FB₁ in breast milk known worldwide to compare findings with.

The higher contamination observed in the present study could be due to high aflatoxin and fumonisin contamination in maize with an assumption that the exposure of mycotoxins in lactating mothers is from the diet. Even though the study did not analyze AFB₁ and FB₁ contamination in the food consumed by mothers, reports of aflatoxins and fumonisins contamination in maize from Kikelelwa and Mbomai as reported in Kimanya et al. (2008a, b) indicate high likelihood of high contamination of both toxins. Adejumo et al. (2013) reported on the correlation between dietary mycotoxin exposure and the contamination in breast milk. The area with high prevalence of AFB₁ had higher AFM₁ in breast milk. Occurrences of AFM₁ and FB₁ in breast milk calls for immediate action to prevent AFB₁ and fumonisins contamination of foods consumed by lactating mothers, although the rate of conversion and transfer of AFB₁ to AFM₁ in breast milk at an individual level is still not clearly defined (Turner, 2013).

The findings of this study showed a marginal insignificant seasonal variation in AFM₁ and insignificant seasonal variation for FB₁ in breast milk collected during rain and dry seasons. Though, the contaminations were insignificantly different, the contamination in the dry

season was slightly higher than that in rain season for AFM₁ and vice versa for FB₁. This slight difference in AFM₁ contamination between the dry season and rain season could be attributed to the higher temperature and humidity which favour the fungal growth and influence toxin production in stored foods, like maize.

The present study has demonstrated the exposure of infants to AFM₁ and FB₁ via breast milk during their very early months of life. The highest recorded exposure for both mycotoxins was observed during the 1st month of lactation, 66.79 ng/kg bw per day for AFM₁ and 64.93 µg/kg bw per day for FB₁. The exposure estimation was done using average milk consumption for age groups (1, 3 and 5 months) as stated in the US EPA, 2011 assuming that milk intake does not vary from one child to another in an age group. Furthermore, the fat content of the breast milk was also not estimated though it is expected to vary depending on the type of food consumed by the lactating mother. The recorded median of exposure for FB₁ of 3 µg/kg bw per day is higher than reported earlier in the assessment of fumonisin dietary exposure to infants of 6 – 8 months old, in which the median of exposure was 0.47 and 0.15 µg/kg bw per day for the study conducted in 2005 and 2006 respectively (Kimanya et al., 2009a). The difference in exposures between the two scenarios can be explained by the differences in complementary food and milk consumptions. The average maize flour intake for the infants studied by Kimanya et al. (2009a) was about 43 g/day, a level which is by far less than the milk intake of 510 ml/day (equivalent to 510g/day) for month 1 and 770 ml/day (equivalent to 770g/day) for month 5 used for the exposure estimation in this study. Considering the findings of the present study with regard to infants' exposure to mycotoxins through breastfeeding it is therefore, of paramount importance to ensure the mycotoxin safety of breast milk.

AFM₁ contamination levels can also be used as biomarker indicators for AFB₁ intake by the mothers. However, the extent of AFB₁ exposure to mothers and the route of AFM₁ transportation into the breast milk in each individual is difficult to determine (Turner, 2013). Although studies have established that the rate of excretion of AFM₁ in human and dairy cattle's is between 0.3 – 6.1% of AFB₁ consumed (Oveisi et al., 2007), the exact rate may differ between and within individual. This can apply to FB₁ as well. It is stated that the rate of

excretion of chemicals into the breast milk in humans is affected by chemical factors such as pH value, molecular weight, degree of ionization and capacity of binding to plasma protein and/or milk component (Somogyi and Beck, 1993; Polychronaki, 2007) or physiological factors such as, maternal age, adipose tissue levels, number of pregnancies, milk composition and volume and breast-feeding pattern (Clewell and Gearhart, 2002), all of which vary among individuals. Therefore studies are needed to examine rate of these toxin excretion in humans' breast milk while taking these factors into account.

A small increase in the number of samples for AFM₁ exceeding the EU limit of 0.05ng/ml was observed over lactation stages. This implies an increase in intake of AFB₁ contaminated diet by mothers (Galvano et al., 2008). In this study, the percentage of mothers who consumed maize increased from 81 to 94% reflecting the specific dietary practice of the post-partum period. For a newly delivered mother in Rombo, a special diet is normally introduced which include mainly cooked green bananas, meat, grain porridge and high fat food with little dairy milk maintained for the first three months. After this period the diet is modified by reducing the aforementioned food groups while increasing cereals, and in particular maize which are prone to aflatoxins. However no defined trend was observed in FB₁ contamination between lactation stages.

We need to take note that, mycotoxin level in breast milk is an indication of the mothers' exposure to this mycotoxin (Prandin et al., 2009). Current evidence shows that only a small amount of mycotoxins ingested by a mother is transferred to her breast milk (Oveisi et al., 2007; Fink-Gremmels, 2008). These results, therefore, show that mothers in the study area are highly exposed to mycotoxins. In this case prevention of lactating mothers against mycotoxins will minimise the problem of transferral to infants.

Even though it is unreasonable to set a limit of AFM₁ and FB₁ in breast milk, preventive measures against mothers' exposure to mycotoxins should be the approach in reducing contamination. As for the dietary sources of aflatoxins, the results indicate that cereals especially maize, dairy milk and groundnuts are the major food sources, for a dietary source of fumonisins mainly maize is involved. The maize used by the majority is home grown and

locally processed whereas the dairy cow milk is hand-milked and is drunk without first ascertaining its quality.

3.5. Conclusion

In conclusion newly born infants in Rombo are exposed to AFM₁ and FB₁ via breast milk and the exposure can exceed the recommended PMTDI (EU) of 2 µg/kg bw for fumonisins. While breast milk continues to be the best source of nutrients to infants, there are difficulties in regulating AFM₁ and FB₁ in it. It is therefore important to protect infants from AFM₁ and FB₁ by preventing maternal exposure to aflatoxins and fumonisins. Education on safety measures, formulation of supportive policies and regulatory activities are thus crucial.

As previously stated in chapter 2, infants were also introduced to complementary foods as early as three months of age. There is a possibility of these infants being exposed to mycotoxins via complementary foods. Therefore chapter 4 presents the study on the mycotoxins dietary exposure in infants under six months of age.

Chapter 4

Risk of aflatoxins and fumonisins exposure from complementary foods

This chapter has been redrafted from the article:

Happy Magoha, Martin Kimanya, Bruno De Meulenaer, Dominique Roberfroid, Carl Lachat, and Patrick Kolsteren. (2014). Risk of dietary exposure to aflatoxins and fumonisins in under six months of age infants in Rombo, Northern Tanzania. *Maternal and child nutrition*. DOI:10.1111/mcn.12155

Summary

Under 6 months of age infants 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 under 6 months infants in Northern Tanzania. A total of 143 infants were progressively recruited and three follow up visits were made at the 1st, 3rd and 5th months of age. A 24 hour dietary recall technique was used to estimate flour intake for infants who had been introduced to maize foods. Aflatoxins and fumonisins in the flours were analysed using HPLC technique. Exposures of aflatoxins or fumonisins were estimated using the deterministic approach. By the age of 3 months, 98 infants had started taking food; 67 of them had maize flour intake at levels ranging from 0.57 - 37.50 g/infant/day (average; 8 g/infant/day). Fifty eight percent of 67 maize flour samples contained detectable aflatoxins (range, 0.33 - 69.47 µg/kg; median, 6 µg/kg) and 31%, detectable fumonisins (range, 48 - 1224 µg/kg; median, 124 µg/kg). For infants who consumed contaminated flours, aflatoxins exposures ranged from 0.14 to 120 ng/kg bw/day (all above the health concern level of 0.017 ng/kg bw/day recommended by the European Food Safety Agency) and fumonisins exposures, from 0.005 to 0.88 µg/kg bw/day. Reducing aflatoxins and fumonisins contamination of maize and dietary diversification can prevent infants and the public, in general, from exposure to the toxins.

4.1. Introduction

Under 6 months of age infants 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 major mycotoxin contaminants of maize based foods. Aflatoxins and fumonisins are the major mycotoxin contaminants found in maize from Tanzania (Kimanya et al., 2008a; Manjula et al., 2009; Kimanya et al., 2014). Aflatoxin exposure in children has been associated with the presence of kwashiorkor and marasmus (Adhikari et al., 1994; Tchana et al., 2010), although no causal link has been established to date. 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 defects (Marasas et al., 2004) and cardiovascular problems (Fincham et al., 1992) in populations consuming relatively large amounts of food made with contaminated maize.

It has been shown in previous studies that exclusive breastfeeding is rarely practiced in Tanzania (Shirima et al., 2001; Mamiro et al., 2005; Nyaruhucha et al., 2006), where, as stated before, the maize used for complementary foods is often contaminated with both aflatoxins and fumonisins (Kimanya et al., 2008a; Manjula et al., 2009; Kimanya et al., 2014). This state of affairs suggests that infants under 6 months of age are at a risk of exposure to aflatoxins and fumonisins. This study was therefore conducted to estimate the extent of maize consumption amongst infants younger than 6 months in Northern Tanzania, to 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.

The study area and subjects involved in the study are as documented in Chapter 2. Three follow up visits were made at the 1st, 3rd and 5th month of the infant's 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 using 24 hour dietary recall. Anthropometric measurements were recorded at each visit.

4.2. Estimation of maize flour intake

With the estimated 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, 2012). This was also done in accordance to the quantity of food consumed and the food preparation techniques described in the Tanzania food composition tables (Lukmanji et al., 2008).

4.3. Extraction and analysis of aflatoxins (AFB1, AFB2, AFG1 and AFG2) and fumonisins (FB1 and FB2) from maize flour

Extraction and determination of aflatoxins and fumonisins involved the use of standard procedures as described in chapter 2.

4.4. Exposure estimation for aflatoxins and fumonisins

Dietary levels 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 24h dietary recalls; at third and fifth 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 done 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).

4.5. Risk characterization 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 month. The risk associated with the consumption of foods other than breast milk is presented in the respective results section (4.8.3). 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 falls below the limit of 10,000 recommended by the European Food Safety Authority (EFSA) (EFSA, 2007 and Barlow et al., 2006) for prioritization of risk management actions. According to EFSA, the margin of exposure (MOE) of aflatoxins for an individual is calculated by dividing the Bench Mark Dose Limit (BMDL); (170 ng/kg bw/day) by the infant's estimated aflatoxin exposure. The BMDL of 170 ng/kg bw/day represents the lower limit of the bench mark dose estimate at 95% confidence. This was established by modelling and represents an estimate of the dose required to produce a small response (10%) above the control for rodents A MOE of 10,000 which is equivalent to exposure of 0.017 ng/kg bw/day is considered a cut-off point whereby an MOE below 10,000 or exposure above 0.017 ng/kg bw/day indicates public health concern (EFSA, 2007).

4.6. Risk characterization 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 month. The risk was also characterized in terms of exposures above the Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg body weight (bw) per day, as per WHO recommendation (WHO, 2012).

4.7. 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 spread-sheet.

Food intake data were entered in food intake software. All data were imported and processed into Stata version 12.0. The information generated is presented as range, mean and median.

As there are no maximum limits set in Tanzania for aflatoxins in baby foods, AFB₁, and total aflatoxin (sum of AFB₁, AFB₂, AFG₁, AFG₂) concentrations were compared to Tanzania ML (5 µg/kg for AFB₁; 10 µg/kg for total aflatoxins) set for maize flour (TBS, 2010) and the EU limits for cereal foods, specifically 2 µg/kg for AFB₁ and 4 µg/kg for total aflatoxin content (European-Commission, 2006). Total fumonisins (sum of FB₁ and FB₂) results were compared to the EU regulation limit (200 µg/kg for total fumonisins) for infant's maize-based food (European-Commission, 2006). There are no maximum limits set in Tanzania for fumonisins in maize based baby foods or general purpose maize flour

4.8. Results

4.8.1. Subjects

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

4.8.2. Type of food introduced to infants

At three 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 the third month 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 included banana porridge, cow milk, home-made butter, sardines, beef or fruits. However, none of the infant was exclusively supplemented by the age of 5 months.

4.8.3. Risk of exposure to aflatoxins and fumonisins

Based on the percentage of infants receiving complementary foods, the risk of exposure to aflatoxins or fumonisins increases with age. Infants introduced to complementary foods this risk rises from 15% to 81% and 97% at ages 1, 3 and 5 months, respectively. Amongst them, the risk of exposure to mycotoxins of those introduced to maize-based foods increased over time from 5%, 68% to 87%.

4.8.4. Aflatoxins and fumonisins in maize flour

Maize flour samples were collected from 67 infants who had started maize complementary feeding by third month of age and analysed for aflatoxin and fumonisin contamination. As shown in Table 4.1, of the 67 samples, 58% were positive for aflatoxins and the contamination ranged from 0.33 to 69.47 µg/kg. Contamination in 23% of the positive samples exceeded the Tanzania Maximum limit of 10 µg/kg set for total aflatoxins in maize flour for human consumption (TBS, 2010) and 56 % the 4 µg/kg limit set for total aflatoxin content in the EU. AFB₁ contaminations in the positive samples ranged from 0.85 to 55.73 µg/kg. Contamination in 35% of the positive samples exceeded the ML of 5 µg/kg set for AFB₁ for maize flour for human consumption in Tanzania and 68%, 2.0 µg/kg EU limit for processed cereals.

Thirty one per cent of samples were positive for fumonisins (total fumonisin), with a contamination range of 48.4 – 1224.6 µg/kg. FB₁ and FB₂ contamination in those samples ranged between 52.7 – 974.1 µg/kg and 48.4 – 250 µg/kg respectively. Contamination levels in 48% of the positive samples were above the EU limit of 200 µg/kg set for infant's maize-based food (European-Commission, 2006). Of the 67 maize flour samples, 23% were found to be contaminated by both aflatoxins and fumonisins.

Table 4.1. Distribution of aflatoxins and fumonisins in maize flour

	n	Positive samples n (%)	Range of positive sample (µg/kg)	Median (µg/kg)	Percentage of positive samples exceed regulatory limit n (%)
AFB ₁	67	34 (50.75)	0.85 - 55.73	3.50	12 (35.29) ^a 23 (67.64) ^b
Total aflatoxin (AFB₁+AFB₂+AFG₁+ AFG₂)	67	39 (58.21)	0.33 - 69.47	5.58	9 (23.08) ^c 22 (56.41) ^d
FB ₁	67	20 (29.85)	52.70 - 974.10	140.30	
FB ₂	67	11 (16.41)	48.40 – 250.50	74.3	
Total fumonisin (FB₁+FB₂)	67	21 (31.34)	48.40 - 1224.60	123.80	10 (47.62) ^e

^a: AFB₁ level exceeded Tanzania limit of 5 µg/kg for maize flour (TBS, 2010)

^b: AFB₁ level exceeded EU limit of 2 µg/kg for cereal based food (European-Commission, 2006)

^c: Total aflatoxin level exceeded Tanzania limit of 10 µg/kg for maize flour (TBS, 2010)

^d: Total aflatoxin level exceeded EU limit of 4 µg/kg for processed cereal (European-Commission, 2006)

^e: Total fumonisin level exceeded EU limit for infants food 200 µg/kg (European-Commission, 2006)

4.8.5. 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 g/infant/day to 37.50 g/infant/day, with an average of 8.0 and median of 5.0 g/infant/day. The total exposures to aflatoxins ranged from 0.14 to 120 ng/ kg bw/ day. The MOE to aflatoxins were below 10,000 (1.41 - 1176) or above 0.017 ng/kg bw/day. Exposures to fumonisins ranged from 0.005 to 0.88 µg/kg bw/day, levels which are below the PMTDI of 2 µg/kg bw/day recommended by JECFA. Median exposure to aflatoxins and fumonisins were 3.88 ng/kg bw/day and 0.14 µg/kg bw/day, respectively.

4.9. Discussion

The present findings show that the failure to comply with WHO's (2001) recommendation of exclusive breastfeeding for the first 6 months of life puts infants at risk of exposure to dangerous levels of aflatoxins and fumonisins. Exclusive breastfeeding would certainly prevent infants under the age of six months from dietary-induced health hazards, including those related to mycotoxins, especially when mothers themselves are not exposed to mycotoxins. As previously explained by Sherif et al. (2009), infants between zero to six

months of age are prone to the adverse effects of toxins as their body's systems have not fully developed to allow for detoxification. Considering the engagement of lactating mothers in socio-economic activities, exclusive breastfeeding can be effectively practiced if the mothers are also taught to self-express breast milk and store it safely. However, due to the different poverty backgrounds of the mothers the authors acknowledge that not all of them could comply to the hygiene requirements of breast milk self-expression. Yet, its use can still bring about a positive change to a considerable number of lactating mothers.

The extent of the risks of dietary 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 aflatoxins exposed infants exceeded the daily intake of 0.017 ng/kg bw which is given by EFSA (2007).

The main source of aflatoxins and fumonisins exposures in these infants is early introduction of baby foods. As found in this study, at the age of three months, 80% of infants had already been introduced to foods other than breast milk. It is important to note that the average maize flour intake of 8 g/infant estimated in this study is comparable to the average consumption for adults in 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/person.

The findings of this study are also relevant to other communities in Africa where maize intake among children is high, particularly because aflatoxins and fumonisins contamination in Africa is common. Mostert and 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) and (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 FB₁ in urine compared to 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 found for the infants that were studied. The higher risk is expected in communities where de-hulling of maize before milling is not practiced. An example of such areas is the Tabora region in Tanzania where Kimanya et al. (2008a), reported that 27% of respondents did not de-hull maize prior to milling for food. Again this emphasizes 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 µg/kg set by EU for cereal based food. Given that AFB₁ is the most potent form of aflatoxins (Do and Choi, 2007) and that it reacts with nuclear acids to generate the polynucleotide-base adducts responsible for carcinogenicity and that it affects liver and blood proteins, 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 µg/kg for total aflatoxins and 123.80 µg/kg for total fumonisins) in maize flours tested by this study are lower than those reported by Kimanya et al. (2008a); 24.00 µg/kg for aflatoxins and 363.00 µg/kg 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. (2008a) used maize directly sampled from stores. Reports show that treatments applied in preparation of maize such as sorting and milling can reduce mycotoxin contamination levels in maize. Mutungi et al. in a study (2008) of the effect of processing *muthokoi/kande* (a traditional dehulled maize dish) in Kenya showed that dehulling decreased aflatoxins content by 46.6% (5.5-70%) in maize containing 10.7-270 µg/kg of aflatoxin concentrations. Similar results were reported by Fandohan et al. (2005) in a study of 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 fumonisins content decreased from 2,890 µg/kg to 1350 µg/kg. Recently, Limbikani (2014) reported 95% reduction of mycotoxin through sorting.

The best approach to minimize 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 fertilizers, well-timed planting, timely harvests, the use of appropriate drying and processing techniques such as sorting, cleaning and milling (Hell and Mutegi, 2011; Limbikani, 2014). The FAO/WHO Codex Alimentarius Commission (CAC/RCP 51-2003) guidelines for reducing of mycotoxin contamination in foods can also be applied.

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 and Bullerman, 1996). However, with the levels of poverty and other constraints in Tanzania, one cannot rely on these measures to protect infants against mycotoxins exposure. This implies that we should continue to rely on exclusive breastfeeding as the most reliable approach to prevent infants from dietary mycotoxins exposures. Nonetheless, like in other African countries and as shown by this study, exclusive breastfeeding during the first 6 months of life is rarely practiced. Therefore, results of this study should be used by nutrition extension services to emphasize the need for observance of exclusive breast-feeding.

Much as we advocate for exclusive breastfeeding, it should be noted that, as previously stated, infants may also be exposed to mycotoxins, AFM₁ and FB₁ 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 such as rice, sorghum, millet, green bananas and potatoes. 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 for safeguarding the general population from mycotoxin's exposures. However, this strategy is only possible in communities where food is traded. Although aflatoxin limits are set in Tanzania, enforcement still remains 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. Presence of a mycotoxin limit may however influence contamination reduction specifically for those who may voluntarily implement the standards. This explains why society should advocate for formulation of maximum limits of mycotoxins such as fumonisins which are not officially regulated in Tanzania.

4.10. Conclusion

The results reveal that feeding maize to infants in the first 6 months results in exposure to aflatoxin and fumonisin at potentially harmful levels. Since maize is often used despite the WHO's recommendation of exclusive breast-feeding, exposure to mycotoxins starts earlier than 6 months. The study also demonstrates the need for more studies to establish the effect of the mycotoxins dietary exposure in growth of these infants. The following chapters will present the effects of feeding practices and mycotoxins exposure to growth.

Chapter 5

Relationship between impaired growth and exposure of infants to aflatoxins and fumonisins through breast milk

Redrafted from:

H. Magoha, M. Kimanya, B. De Meulenaer, D. Roberfroid, C. Lachat and P. Kolsteren. (2014). Association between M1 exposure through breast milk and growth impairment in infants from Northern Tanzania, *World Mycotoxin Journal* 7: 277 - 284.

H. Magoha, B. De Meulenaer, M. Kimanya, D. Hipolite, C. Lachat and P. Kolsteren. (2014). Fumonisin B1 contamination in breast milk and its exposure in infants under six months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology* 74: 112 – 116

Summary

In this part of the study, the data of aflatoxin M1 (AFM₁) and fumonisin B1 (FB₁) exposure presented in chapter 3 are used to estimate the association between AFM₁ and FB₁ exposure levels and growth indicators, for infants under six months of age. The deterministic approach was used for exposure assessment. The growth indicators namely weight for age z-score (WAZ), length for age z-score (LAZ) and weight for height z-score (WHZ) were computed in Stata programme as explained in chapter 2 according to WHO (WHO, 2006). The growth status at month five were compared for exclusively and non-exclusively breast fed infants until 3 months of age. Mixed effect linear regression models were also used to assess growth effect using WAZ, LAZ and WHZ as growth indicators. Infants who were exclusively breastfed until the age of three months were less likely to be stunted at the fifth month of age compared to those who had been introduced to other foods by the age of three months (odds ratio = 1.30; 95% confidence interval = 0.34 – 4.93). Growth impairments were observed in both toxins. Inverse association (P<0.05) was observed between AFM₁ exposure levels and WAZ or LAZ. While for FB₁ the inverse association was observed with WAZ and WHZ. This emphasize on the reduction of these mycotoxins in lactating mothers diet to minimise its carry over in breast milk and infants exposure and hence improve the nutrition status of the infants.

5.1. Introduction

Presence of mycotoxin in foods has been associated with various health effects including growth impairment (Gong et al., 2004; Shephard, 2008; Kimanya et al., 2010). Various mycotoxin contaminants in breast milk have been reported such as AFM₁ (Polychronaki, 2007; Mahdavi et al., 2010), AFB₁, AFG₁, AFG₂, OTA (Jonsyn et al., 1995; Gürbay et al., 2010b) supporting results of this study as reported in Chapter 3. Occurrence of these mycotoxins in breast milk pose a great risk to infants especially those who are exclusively breastfed and who need to obtain much of their nutritional requirements from breast milk. WHO (2001) recommends exclusive breastfeeding in infants under six months of age which has proved to have advantages when comparing with the early introduction of other foods.

As previously stated in chapter 1, growth faltering is a major public health problem affecting infants and young children in developing countries, Tanzania inclusive. According to Tanzania demographic health survey of 2010 the rate of malnutrition among children of less than 5 years of age stands at 42% for stunting, 16% underweight and 5% wasting (NBS and ICF Macro, 2011). In view of these data on growth faltering, the consequence of mycotoxin exposure through breastfeeding cannot be undermined. The presence of mycotoxins (AFM₁ and FB₁) in breast milk has been evidenced as reported in chapter 3. The association between exposure level of AFM₁ and FB₁ in breast milk and growth of these infants is now assessed.

5.2. Assessment of the feeding practices with growth

Effect of feeding practices was conducted by comparing the growth status at months five, for infants who were exclusively breastfed until 3 months of age with those introduced to complementary foods by the age of three months.

5.3. Assessment of association between AFM₁ and FB₁ exposures and growth indicators

The assessment of the growth effects was done in two different scenarios; one involved exposure from month 1, 3 and 5 and the second one excluded the exposure at month 5. Results of AFM₁ and FB₁ reported in chapter three are used for the determination of growth effects. To account for the repeated measurements associated with each child for AFM₁ and FB₁, mixed-effect linear regression models with random intercept were used to determine factors related to the children growth. Quadratic models were fitted, separately, for each dependent variable, i.e. WAZ, LAZ and WHZ, and AFM₁ or FB₁ exposure, child age and age square, birth weight, age of mother, age of the father, education level of the mother and father, dietary diversity score and income of both mother and father (Rabe-Hesketh and Skrondal, 2012). The random effects were estimated for each child level using a unique identification of a child. The correlation matrix was unstructured. Significance level of 5% was considered for all analyses.

5.4. Results

5.4.1. Feeding practices and the growth status of infants at the fifth month of age

Table 5.1 shows the growth status at the fifth month of age in relation to their feeding practices. The results indicate that infants who were introduced to foods other than breast milk by the age of three months were more likely to be stunted at the fifth month of age (16% stunted) compared to those who were exclusively breast fed until the age of three months (13% stunted) (odds ratio = 1.30; 95% confidence interval = 0.34 – 4.93).

Table 5.1. Infants' growth status (mean ± standard deviation) at the fifth month of age according to feeding practices at third month of age.

	n	LAZ ^a	% stunting	WAZ ^b	% underweight	WHZ ^c	% wasting
Exclusive breast feeding until 3months of age	23	-0.62±1.09	13	-0.25±1.39	13	0.27±1.30	9
Infants introduced to complementary foods	98	-0.88±1.15	16	-0.15±1.13	9	0.65±1.23	2

^a LAZ: length for age Z-score, n =207

^b WAZ: Weight for age Z-score, n =208

^c WHZ: Weight for height Z-score, n =207

5.4.2. AFM₁ and FB₁ exposures association with growth indicators

The results in Table 5.2 shows that AFM₁ exposures had significant ($P < 0.05$) inverse association with LAZ or WAZ, the association became stronger when the exposure of month 5 was excluded (Table 5.3). Whereas FB₁ exposures had a significant ($P < 0.05$) negative association with WAZ and WHZ in both scenarios (Table 5.4 and 5.5).

Table 5.2. The association between levels of exposure to aflatoxin M₁ from breast milk at month 1, 3 and 5 and growth indicators

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Exposure (ng/kg bw/day)	-0.011 (-0.021, -0.000)*	-0.018 (-0.033, -0.004)*	0.010 (-0.006, 0.027)
Age of infant(months)	-0.035 (-0.254, 0.184)	-0.346 (-0.716, 0.025)	0.535 (0.100, 0.970)*
(Age of infant) ² (months) ²	-0.005 (-0.040, 0.030)	0.040 (-0.022, 0.100)	-0.086 (-0.157, -0.015)
Infant's birth weight (kg)	1.020 (0.598, 1.441)*	0.812 (0.374, 1.250)*	0.685 (0.185, 1.184)*
Age of the mother (yrs)	0.016 (-0.042, 0.732)	0.052 (-0.008, 0.111)	-0.044 (-0.112, 0.024)
Age of the father (yrs)	-0.002 (-0.045, 0.040)	-0.022 (-0.067, 0.022)	0.015 (-0.035, 0.066)
Sex	0.579 (0.151, 1.007)*	0.470 (0.026, 0.914)*	0.169, (-0.338, 0.675)
Education of the Mother	-0.236 (-0.745, 0.274)	-0.036 (-0.569, 0.498)	-0.177 (-0.782, 0.427)
Education of the father	-0.157 (-0.743, 0.428)	-0.025 (-0.640, 0.590)	-0.072 (-0.767, 0.622)
Earning of the mother	-0.060 (-0.446, 0.326)	-0.043 (-0.446, 0.360)	-0.036 (-0.493, 0.420)
Earning of the father	-0.020 (-0.349, 0.309)	0.141 (-0.199, 0.482)	-0.273 (-0.663, 0.116)
Dietary diversity score	0.035 (-0.037, 0.108)	-0.020 (-0.123, 0.082)	0.029 (-0.090, 0.149)

^a WAZ: Weight for age Z-score, n =208

^b LAZ: length for age Z-score, n =207

^c WHZ: Weight for height Z-score, n =207

CI: Confidence interval 95%

*Denotes a significant difference ($P < 0.05$)

NB: These models were quadratic as growth trajectories are typically nonlinear (Rabe-Hesketh and Skrondal 2012)

Table 5.3. The association between levels of exposure to aflatoxin M₁ from breast milk at month 1 and 3 and growth indicators

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Exposure (ng/kg bw/day)	-0.020 (-0.034, -0.005)*	-0.024 (-0.042, -0.006)*	0.003 (-0.016, 0.022)
Age of infant (months)	-4.830 (-9.939, 0.278)	-2.980 (-9.079, 3.118)	-4.054 (-10.483, 2.374)
(Age of infant) ² (months) ²	1.18 (-0.090, 2.460)	0.689 (-0.833, 2.211)	1.057 (-0.547, 2.661)
Infant's birth weight (kg)	1.013 (0.543, 1.482)*	0.618 (0.096, 1.140)*	0.887 (0.336, 1.438)*
Age of the mother (yr)	0.011 (-0.052, 0.075)	0.063 (-0.008, 0.134)	-0.056 (-0.132, 0.020)
Age of the father (yr)	-0.000 (-0.047, 0.047)	-0.013 (-0.066, 0.040)	0.014 (-0.042, 0.071)
Sex	0.687 (0.211, 1.162)*	0.628 (0.098, 1.159)*	0.186 (-0.375, 0.747)
Education of the Mother	-0.346 (-0.912, 0.221)	-0.274 (-0.902, 0.354)	-0.143 (-0.805, 0.519)
Education of the father	-0.171 (-0.820, 0.477)	-0.108 (-0.825, 0.609)	0.043 (-0.713, 0.798)
Earning of the mother	-0.096 (-0.522, 0.330)	-0.100 (-0.572, 0.373)	-0.060 (-0.560, 0.440)
Earning of the father	0.022 (-0.344, 0.388)	0.332 (-0.076, 0.740)	-0.378 (-0.809, 0.534)
Dietary diversity score	0.095 (-0.020, 0.210)	0.059 (-0.085, 0.203)	0.074 (-0.080, 0.229)

^a WAZ: Weight for age Z-score, n = 143

^b LAZ: Length for age Z-score, n = 142

^c WHZ: Weight for height Z-score, n = 142

CI: Confidence interval 95%

*Denotes a significant difference (P < 0.05)

NB: These models were quadratic as growth trajectories are typically nonlinear (Rabe-Hesketh and Skrondal 2012)

Table 5.4. The association between levels of exposure to fumonisin B₁ from breast milk at month 1, 3 and 5 and growth indicators

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Exposure (µg/kg bw/day)	-0.030 (-0.047, -0.012)*	-0.016 (-0.037, 0.006)	-0.038 (-0.061, -0.016)*
Age of infant (months)	-0.583 (-3.385, 2.218)	1.565 (-2.001, 5.131)	-3.228 (-6.923, 0.468)
(Age of infant) ² (months) ²	0.084 (-0.382, 0.549)	-0.277 (-0.871, 0.316)	0.534 (-0.081, 1.148)
Infant's birth weight (kg)	1.035 (0.525, 1.544)*	0.758 (0.251, 1.264)*	0.666 (0.130, 1.201)*
Age of the mother (yr)	0.019 (-0.049, 0.087)	0.042 (-0.026, 0.109)	-0.023 (-0.094, 0.048)
Age of the father (yr)	-0.012 (-0.061, 0.037)	-0.007 (-0.056, 0.042)	-0.012 (-0.063, 0.040)
Sex	0.754 (0.254, 1.253)*	0.376 (-0.117, 0.869)	0.572 (0.050, 1.094)*
Education of the Mother	-0.272 (-0.888, 0.345)	-0.195 (-0.805, 0.415)	-0.148 (-0.794, 0.498)
Education of the father	0.057 (-0.619, 0.733)	0.114 (-0.553, 0.781)	-0.032 (-0.739, 0.674)
Earning of the mother	-0.069 (-0.525, 0.385)	0.010 (-0.440, 0.461)	-0.081 (-0.558, 0.396)
Earning of the father	-0.116 (-0.490, 0.258)	0.143 (-0.225, 0.510)	-0.321 (-0.711, 0.068)
Dietary diversity score	0.010 (-0.097, 0.115)	-0.063 (-0.193, 0.068)	0.040 (-0.096, 0.177)

^a WAZ: Weight for age Z-score, n = 123

^b LAZ: Height for age Z-score, n = 123

^c WHZ: Weight for height Z-score, n = 123

CI: Confidence interval 95%

*Denotes a significant difference (P < 0.05)

NB: These models were quadratic as growth trajectories are typically nonlinear (Rabe-Hesketh and Skrondal 2012)

Table 5.5. The association between levels of exposure to fumonisin B₁ from breast milk at month 1 and 3 and growth indicators

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Exposure (µg/kg bw/day)	-0.039 (-0.067, -0.011)*	-0.020 (-0.051, 0.010)	-0.044 (-0.075, -0.013)*
Age of infant (months)	-1.698 (-4.011, 0.615)	0.520 (-2.005, 3.045)	-3.016 (-5.547, -0.486)
Infant's birth weight (kg)	0.919 (0.359, 1.479)*	0.632 (0.020, 1.243)*	0.676 (0.063, 1.289)*
Age of the mother (yr)	0.002 (-0.073, 0.785)	0.042 (-0.041, 0.124)	-0.054 (-0.136, 0.029)
Age of the father (yr)	0.005 (-0.048, 0.057)	-0.001 (-0.058, 0.056)	0.008 (-0.049, 0.066)
Sex	0.749 (0.209, 1.290)*	0.481 (-0.109, 1.071)	0.381 (-0.210, 0.972)
Education of the Mother	-0.397 (-1.078, 0.283)	-0.356 (-1.099, 0.387)	-0.157 (-0.902, 0.587)
Education of the father	0.024 (-0.698, 0.746)	0.000 (-0.789, 0.789)	0.065 (-0.726, 0.855)
Earning of the mother	-0.082 (-0.572, 0.409)	-0.135 (-0.670, 0.401)	0.039 (-0.497, 0.576)
Earning of the father	-0.080 (-0.485, 0.326)	0.245 (-0.198, 0.687)	-0.411 (-0.855, 0.032)
Dietary diversity score	0.028 (-0.178, 0.235)	0.001 (-0.218, 0.233)	0.032 (-0.194, 0.258)

^a WAZ: Weight for age Z-score, n = 75

^b LAZ: Height for age Z-score, n = 75

^c WHZ: Weight for height Z-score, n = 75

CI: Confidence interval 95%

*Denotes a significant difference (P < 0.05)

NB: These models were quadratic as growth trajectories are typically nonlinear (Rabe-Hesketh and Skrondal 2012)

5.5. Discussion

This part of the study demonstrates for the first time the effects of mycotoxin contamination in breast milk on the infant's growth. In this study it was observed that there is an inverse association between AFM₁ exposures and child growth, as indicated by either LAZ or WAZ, and also between FB₁ exposure and WAZ or WHZ. The significant inverse association recorded in this study between AFM₁ levels of exposures and LAZ or WAZ is in accordance with the reports of other studies (Marin et al., 2002; Gong et al., 2004; Turner et al., 2007; Turner, 2013) which also observed an association between aflatoxin exposure through food

and poor child growth. It is also in accordance with the growth effect of fumonisin exposure reported by Kimanya et al. (2010).

The inverse relationship between the exposure to mycotoxin and growth is much stronger when the exposure at month 5 is excluded. This observation can be generally explained by the fact that the mycotoxin contamination in breast milk was higher during the first lactation stages but was decreasing with time. Different reasons are highlighted (Clewell and Gearhart, 2002; Polychronaki, 2007) for the variation of these mycotoxins in breast milk with lactation stage, the level of maternal exposure to the respective mycotoxins, interaction of mycotoxin with other dietary components, milk composition, volume and breast milk pattern.

The present study also demonstrates that apart from the negative effects of mycotoxin on the growth of breastfeeding infants, infants who were exclusively breastfed until third month of age were less likely to be stunted (13%) than those who had been introduced to complementary foods (16%). This observation further emphasises the consideration of the safety of both complementary foods and breast milk for the betterment of our infants' health.

Available reports (Muhimbula and Issa-Zacharia, 2010) show that early introduction of complementary foods which are poor in nutritional quality and which are contaminated with microbes is the main cause of persistent malnutrition problem in Tanzania. However with the present findings, mycotoxin exposure is another cause of malnutrition. In view of the importance of breast milk as the only source of balanced nutrients to newly born infants, the prevalence of mycotoxin in breast milk needs to be taken seriously. The health and economic importance of breast-feeding is incomparable to other alternative methods like formula milk and complementary feeding which require money and time for preparation but are also associated with high health risks which might be caused by cross contamination during preparations.

The social economic factors assessed, including the literacy levels and earning of the child's both parents and the dietary diversity score, did not show any association with either AFM₁ or FB₁ occurrence in breast milk as reported in previous studies (Polychronaki et al., 2006; Ghiasain and Maghsood, 2012). This lack of association could possibly be due to the insignificant differences in social-economic status among the study population.

5.6. Conclusion

These findings underscore the need to create awareness on the effects of mycotoxins on infants' growth, amongst community members and in particular the expectant mothers. The awareness creation and trainings should also focus on the methods of mycotoxins prevention and exposure reduction in lactating mothers. Sources of mycotoxins contamination in food should be clearly defined so that the preventions techniques are well developed and effectively adhered to. Recommendations for prevention of mycotoxin exposures are presented in detail in chapter 7.

Since the infants enrolled in this study were also exposed to mycotoxins through complementary feeding, it is also important to assess the growth impairment attributes by both breast milk and dietary exposure. This total exposure assessment is reported in the following chapter.

Chapter 6

Concomitant exposure of aflatoxins and fumonisins from food and breast-milk

Summary

The objective of this study was to determine the relationship between child growth and the total exposures of aflatoxins and fumonisins from both breast milk and food. Breast milk intake was computed based on the total energy required by infants per day with regard to age and sex and the amount of energy the infant received from food per day. The exposure from food and breast milk was determined using a deterministic approach. The exposures were then zero skewed log-transformed and fitted into a mixed-effect linear regression model for each variable; weight for age z-score (WAZ), length for age z-score (LAZ) and weight for height z-score (WHZ).

The study found an inverse association between the total fumonisin exposures from food and breast milk with WAZ and WHZ ($P < 0.05$). Although the total of aflatoxins exposures from food and breast milk was not significantly associated with growth indicators, the negative coefficient was observed with LAZ. These findings suggest the need for a long-term cumulative fumonisin and aflatoxin exposure assessment for their effects in infants and young children.

6.1. Introduction

This chapter presents the assessment of the overall relationship of mycotoxin exposure and growth. The relationship of total exposure of aflatoxin and fumonisin from both breast milk and food was determined. According to available documentation (IARC, 2002) the long term effect of mycotoxin exposure includes liver, kidney and hematopoietic toxicity, immune toxicity, reproduction toxicity, foetal toxicity and teratogenicity, and mainly carcinogenicity. However, the concomitant toxicity requires a more advanced study as indicated by (Speijers and Speijers, 2004). Different results have been reported regarding immunobiological effects caused by concomitant mycotoxin toxicity (Theumer et al., 2003).

6.2. Estimation of breast milk intake

To account for concomitant exposure of aflatoxin and fumonisin from both food and breast milk, breast milk intake was estimated based on the universal total energy requirements per day (TER) for infants with regard to age and sex as recorded by Butte (2005). TER used for boys were 113, 95 and 81 kcal/kg bw/day and for girls were 107, 94 and 83 kcal/kg bw/day for month 1, 3 and 5, respectively. First the energy requirement from breast milk for an infant per day was calculated using the following formula:

$$EBMi \text{ (kcal/kg bw/d)} = TERi \text{ (kcal/kg bw/d)} - ECFi \text{ (kcal/kg bw/d)}$$

Where: $EBMi$ = Energy required from breast milk for infant i

$TERi$ = Total energy required by infant i per day

$ECFi$ = Energy for infant i obtained from food as recorded during the 24hr dietary recall

Therefore from the above equation breast milk intake was calculated as follows:

$$BM \text{ intake } i \text{ (g)} = EBMi \text{ (kcal/d)} / 0.67 \text{ kcal/g}$$

Where: $BM \text{ intake } i$ = Breast milk intake for infant i

$bw \ i$ = body weight of infant i

0.67 = is the kcal obtained from 1g of milk (WHO, 1998)

The exposure of AFM₁ and FB₁ from breast milk was calculated by multiplying the estimated breast milk intake (BM intake *i*) with the aflatoxin and/or fumonisin contamination of his or her mothers' breast milk (as reported in chapter 3), divided by his or her body weight. Exposure from food was calculated using the contamination levels of maize flour and a 24 hour dietary recall for months 3 and 5.

Contaminant exposure from milk and from food were summed up at month 1, month 3, month 5 for aflatoxin. For fumosins, this was done only at month 1 and month 5 as exposure from breast milk was not performed at month 3. For both contaminants, all the exposure was from breast milk at month 1. We used mixed-effect linear regression models with the individual as random intercept to account for repeated individual measurements. Models were fitted, separately, for each dependent variable, i.e. WAZ, LAZ and WHZ at month 1, month 3 and month 5. The main independent variable was exposure by aflatoxin or fumonisin and adjustment was done for child age, birth weight, and sex. Exposure variables were zero skewed log transformed (Stata command: `lnskew0 newvar= var`) as they were not normally distributed. Level of significance used was 5% for all analyses.

6.3. Results

The results of mycotoxin exposure from both milk and food are summarised in Table 6.1. The exposure results were skewed to the right and high median of exposure was observed from breast milk in both toxins; aflatoxins and fumonisins. Median of exposures to AFM₁ from breast milk in all visits were above 8 ng/kg bw/day whereas median of exposure to aflatoxin from food were below that value. Similarly the median of exposure to fumonisin from breast milk were all above 2 µg/kg bw/day, exceeding the PMTDI recommended by JECFA while the median of fumonisin exposure from food were below 1 µg/kg bw/day in both visits.

Table 6.1 Mycotoxins exposure of infants from breast milk and from food at month 1, 3, and 5 of lactation stages

Type of exposure	Positive sample (n)	Range of exposure	Mean \pm SD	Median
Aflatoxin exposure (ng/kg bw/day)				
From breast milk:				
Aflatoxin M1 at month 1	141	1.13 – 66.79	11.08 \pm 10.13	8.11
Aflatoxin M1 at month 3	118	0.81 – 58.96	11.94 \pm 9.70	9.17
Aflatoxin M1 at month 5	116	1.08 – 34.91	10.91 \pm 6.82	8.62
From food				
Aflatoxins at month 3	38	0.14 – 120.24	14.80 \pm 28.02	3.88
Aflatoxins at month 5	42	0.35 – 229.87	22.42 \pm 47.75	5.43
Fumonisin exposure (μg/kg bw/day)				
From breast milk				
Fumonisin B1 at month 1	58	0.78 – 64.93	7.54 \pm 12.88	3.08
Fumonisin B1 at month 5	51	0.61 – 28.32	4.99 \pm 5.38	3.21
From food				
Fumonisin at month 3	21	0.01 – 0.87	0.17 \pm 0.18	0.14
Fumonisin at month 5	22	0.01 – 1.65	0.24 \pm 0.34	0.16

The association of the growth indicators; WAZ, LAZ and WHZ with total aflatoxins exposure is presented in Tables 6.2. No significant association was observed between total aflatoxin exposure with WAZ, LAZ or WHZ. However, a negative coefficient was observed between the total aflatoxin exposure and LAZ.

Table 6.2 The association between the total exposure from breast milk and food and growth indicators at the fifth month of age

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Aflatoxins (ng/kg bw/day)	0.055 (-0.044, 0.153)	-0.070 (-0.197, 0.058)	0.324 (-0.400, 1.048)
Age of infant (months)	-0.035 (-0.075, 0.005)	-0.143 (-0.196, -0.091)*	-0.112 (-0.459, 0.234)
Infant's birth weight (kg)	1.117 (0.738, 1.495)*	0.993 (0.596, 1.391)*	-0.773(-2.092, 0.546)
Age of the mother (yrs)	-0.011 (-0.038, 0.016)	-0.008 (-0.037, 0.019)	0.057 (-0.035, 0.150)
Sex	0.361 (0.021, 0.701)*	0.316 (-0.041, 0.673)	0.709 (-0.463, 1.880)

^a WAZ: Weight for age Z-score, n = 349

^b LAZ: Height for age Z-score, n = 347

^c WHZ: Weight for height Z-score, n = 347

CI: Confidence interval 95%

*Denotes a significant difference (P < 0.05)

The relation between the total exposures of fumonisin with growth are presented in Tables 6.3. The total fumonisin exposure was found to have significant negative association with WAZ and WHZ.

Table 6.3 The association between the total fumonisins exposure from breast milk and food and growth indicators at the fifth month of age

	WAZ ^a Coefficient (CI)	LAZ ^b Coefficient (CI)	WHZ ^c Coefficient (CI)
Fumonisin (µg/kg bw/day)	-0.177 (-0.343, -0.011)*	-0.112 (-0.298, 0.074)	-0.263 (-0.534, 0.007)*
Age of infant (months)	-0.066 (-0.117, -0.016)*	-0.161 (-0.222, -0.101)*	0.073 (-0.000, 0.146)*
Infant's birth weight (kg)	1.108 (0.666, 1.550)*	0.987 (0.558, 1.416)*	-3.012 (-6.488, 0.464)*
Age of the mother (yrs)	-0.019 (-0.049, 0.012)	-0.013 (-0.043, 0.017)	0.179 (-0.063, 0.420)
Sex	0.445 (0.061, 0.830)*	0.288 (-0.084, 0.661)	2.032 (-1.012, 5.075)*

^a WAZ: Weight for age Z-score, n = 206

^b LAZ: Height for age Z-score, n = 205

^c WHZ: Weight for height Z-score, n = 205

CI: Confidence interval 95%

*Denotes a significant difference (P < 0.05)

6.4. Discussion

The main finding of this study indicates that total fumonisin exposure had negative association with WAZ or WHZ. This means fumonisin exposure may contribute to underweight and wasting of infants in areas where there is consumption of fumonisin-contaminated food. According to the Tanzania Demographic Health Survey (TDHS; 2010), the underweight and wasting rate for under five infants is 16% and 5% respectively (NBS and ICF Macro, 2011). Globally, Black et al. (2013) reported the underweight and wasting rate for under five children in 2011 stood at 16% and 8% respectively, Africa being the second from Asia in wasting prevalence.

The current results reveal that infants who are introduced to maize based foods are also exposed to mycotoxins from breast milk. The findings reveal that, infants of below six months of age get higher mycotoxin exposures from breast milk in comparison with foods, as reflected by the median of exposure in Table 6.1., thus the presence of AFM₁ and FB₁ in breast milk should be urgently dealt with. WHO recommends infants to be exclusively breast fed for the first six months of life because, in addition to other benefits, breast milk provides all the nutrients required for growth (WHO, 2001).

The association between fumonisins exposure and impaired growth in children above six months of age has been reported in Tanzania (Kimanya et al., 2010) and for aflatoxins, in other countries (Gong et al., 2004). The result of the effect of aflatoxins and fumonisins exposure from breast milk for infants below six months has also been reported in Chapter 5. The total effect of aflatoxin and fumonisin exposure from both breast milk and complementary foods which was assessed in this chapter shows that fumonisins have a significant negative association with WAZ or WHZ. Lack of a significant effect between aflatoxins exposure and growth could be due to individual variation of AFM₁ in breast milk samples. The variation in individual samples could be due to a number of reasons as stated earlier, such as the level of maternal exposure, milk composition, milk volume and intra-individual differences in the conversion of AFB₁ to AFM₁ (Clewell and Gearhart, 2002; Polychronaki, 2007). Furthermore, lack of correlation between the exposures from the two

sources, possibly diluted the combined toxins' effect. The exposure assessment for complementary food was done for those infants who ate maize with detectable levels only, and the interval between exposure and the growth assessment can also affect the association. Theumer et al. (2003) observed that the body weight gain for an exposed rat with AFB₁ was higher during the first 30th day and declined as the days went on.

Due to variation in breast milk contamination, it could have been more appropriate to do repeated collection and analysis of the breast milk in a month. This would have given a more appropriate picture on the cumulative effect of mycotoxins exposure. However, inadequate resources in terms of time, money, human resource and willingness of the lactating mothers to offer breast milk more frequently could be a challenge.

It has been shown in various studies that the effect produced by individual mycotoxins may be different from the effect produced by a mixture of mycotoxins. A study of nutritional effect of FB₁ in rats (Theumer et al., 2002) showed that intake of FB₁ reduced both body weight and body weight gain at the 90th day while the intake of AFB₁ (Theumer et al., 2003) showed an increase in both body weight at the 30th, 60th and 90th day and a body weight gain during the first 30 days. While the mixture of both AFB₁ and FB₁ had a similar behaviour of FB₁ i.e. lowering body weight but supporting body weight gain at the 90th day when the effect of AFB₁ were predominant with respect to FB₁, Weibking et al. (1994) demonstrated that exposure to AFB₁ and FB₁ whether individually or in combination affected the poultry health and performance. Speijers and Speijers (2004) elaborated the difficulties encountered in the issue of combined mycotoxin toxicity that mycotoxins with similar mode of action would be expected to have at least additive effects, but some interaction could have subtractive effects. It is the toxicokinetic behaviour, metabolism and the toxicodynamic aspects that influence on the final outcome of the exposed animal/person. Therefore, further studies with long duration (at least 2 years) are needed to estimate the cumulative effects from a bigger sample size and more repeated measurements on both contamination levels and anthropometrics, are needed.

It is also important to note that there are other effects of the combined exposure to AFB₁ and FB₁ which have been evaluated in different animals such as increased weights of both liver and spleen (Weibking et al., 1994) which cannot be tested in humans. Effects of this kind are more dangerous despite the fact that they cannot be easily noticed. All in all, the occurrence of mycotoxins in infants food should be prevented since the chronic exposure of these mycotoxins can lead to life threatening disorders as different cancer types have been reported to be associated with mycotoxin exposure (IARC, 2002). Miller et al. (2002) elaborated that the earlier life exposure to carcinogens may result in greater lifetime risk of cancer. Therefore despite the insignificant growth effect observed with total aflatoxin exposure in this study, the effect of exposure of these mycotoxins in infants cannot be ignored.

6.5. Conclusion

The general overview of the results from this chapter indicate that Tanzanians are more prone to fumonisin from maize in comparison to aflatoxins. This can be justified by the fact that Tanzanian maize is highly contaminated with fumonisin as previously reported (Kimanya et al., 2008a). It is high time now for the Tanzanian government to set the regulatory limit for fumonisin in foods.

Chapter 7

General Discussion

7.1 Introduction

This PhD study aimed at assessing the exposure and effects of aflatoxins and fumonisins in infants under the age of six months. The study examined mycotoxin content in infants' foods, in particular in breast milk and maize flour used for preparation of infants' porridge. Infants' growth status was also assessed in the follow up visits at the age of 1, 3 and 5 months. The specific objectives of this study were to:

- I. Identify and quantify aflatoxins and fumonisin in breast milk and assess their exposure in infants under six months of age.
- II. assess dietary exposure to aflatoxins and fumonisins in infants under six months of age
- III. assess the relationship between impaired growth and exposure of infants to aflatoxin and fumonisin through breast milk
- IV. assess the concomitant exposure of breastfeeding and/or complementary feeding practices on infants' growth
- V. recommend measures for protecting infants in Tanzania from aflatoxins and fumonisins exposure

Based on the main findings of the research the following conclusions are made:

Breast milk in Rombo is contaminated with both AFM₁ and FB₁ to an extent that exceeds the regulatory limits set for countries by the EU. With this as a benchmark, in Tanzania breastfed infants are exposed to mycotoxins well before the age of six months. It was also evidenced that exposure to these mycotoxins from breast milk is associated with impaired growth. This is an alarming call as far as nutrition is concerned.

It was further observed that maize flour used for preparation of infants' food is contaminated with both aflatoxin and fumonisins. This indicates that the early introduction of complementary foods especially maize-based foods exposes infants to mycotoxins. The exposure of dietary aflatoxins was of public health concern since all the MOE computed were below 10,000 recommended by EFSA, 2007.

Socio-economic status did not show a significant difference in infants' mycotoxin exposure. This could be due to an insignificant difference in socio-economic status among the study's population also some mothers could not tell how much their family earned per month. However, the impact of socio-economic status can probably be clearly seen when two communities with very different standards of living are involved, such as rural versus urban. In this study all the communities involved were from rural areas.

In the assessment of total exposure from breast milk and food, total aflatoxin did not show any significant growth effect contrary to fumonisin. However as explained in chapter one, aflatoxin had growth effects in children above 6 months of age. Lack of a significant effect on the growth observed in this study could be a result of the small sample size and the short duration for cumulative effect observation. It has to be noted that, aflatoxin exposure is also associated with other health effects such as hepatotoxicity (effect on liver), mutagenicity (effect on genetic materials), prevention of protein synthesis and immune suppressions as documented previously in section 1.7 though have not been assessed in the present study. Immune suppression will enhance susceptibility of infants to the infectious agents. Therefore, presence of aflatoxins and other mycotoxins in infants' food should be avoided.

The significant growth effect observed in infants due to their exposure to total fumonisin is an indication that infants are more susceptible to fumonisins than aflatoxins in Tanzania. This observation is supported by previous findings (Kimanya et al., 2008) that reported higher prevalence of fumonisin in Tanzanian maize as compared to aflatoxin. Shephard et al. (2007) also reported high exposure to fumonisin for the population studied in South Africa, with mean fumonisin exposures all above PMTDI of 2 µg/kg bw/day.

The malnutrition status in Tanzania indicates that something has to be done to improve the nutritional status of children under five years. The results of this study support the view that, prevention of mycotoxin exposure in infants can improve their nutritional well-being in one way or another and also reduce the number of cases of infectious diseases given the fact that, infants below six months of age are exposed to mycotoxins in Rombo.

Exposure of mycotoxins in infants below six months of age can also be observed in other regions of Tanzania and other African countries where maize is used as a staple food. Presence of mycotoxins in infants' food may result into chronic exposure which will lead these infants into other health defects related to these toxins such effects on liver and kidney at their later ages as documented by previous researchers (Miller et al., 2002). Therefore, all efforts should be made to prevent the presence of mycotoxins in infants' food as one of many measures to improve the health and development of children and ultimately to the building of a healthy society.

7.2. Measures for protecting infants in Tanzania against aflatoxins and fumonisins exposure

The present study revealed the exposure of infants under the age of six months, to fumonisins and aflatoxins through breast milk and food. In order to protect infants under 6 months from mycotoxins exposure, several approaches can be adopted. These include protection of mothers from dietary mycotoxin exposure, since diet is the main source of these mycotoxins in humans. In Tanzania and other sub-Sahara Africa countries, maize is the main ingredient for complementary foods used by infants and young children and is in most cases contaminated with aflatoxins and fumonisins. Infants under six months of age are being introduced to maize based foods regardless of a WHO recommendation that children of that age should be exclusively breastfed. It is worth noting that studies have shown that even if infants are exclusively breastfed they can still be exposed through aflatoxins and fumonisins through breast milk. The results of the present study also show that even though mycotoxins in breast-milk forms just a fraction of the exposure of the mothers, it largely accounts for the infant's exposure to mycotoxins, possibly due to the larger intake of breast milk compared to the amount of maize consumed by these infants. This observation underscores the importance of preventing contamination in lactating mothers' diet as well as complementary foods. In achieving this, the following recommendations are made:

7.2.1. Awareness creation on the health risks of mycotoxin

In view of mycotoxin prevalence in breast milk in Tanzania, as revealed for the first time in the present study, it is important to create awareness amongst all stakeholders in the country about the possibility of exposing infants to mycotoxins at very early ages and about the associated health risks. This could help them to take on board the advocated measures in reducing infants' exposure and consequently lowering health risks.

7.2.2. Reduction of mycotoxin content in foods at both household/community level and national levels

The corrective measures for the reduction of mycotoxin contamination in food have to be taken at all levels; that is from household level to the national level. This can be achieved by observing both pre and post-harvest measures. These include adherence to good agricultural practices as described in Chapter 1 under the methods for the reduction of mycotoxin. The measures encompass the use of insect resistant varieties, improved irrigation, use of fertilizers and pesticides, timely harvesting, and good storage facilities and practices to minimise fungal infestations, hence minimizing mycotoxin production. Furthermore maize milling should also involve the procedures which have been proven to reduce mycotoxin content such as sorting of bad maize, washing and dehulling prior to milling. The combined use of pre and post-harvest measures is likely to have a positive impact in the reduction of mycotoxin and the associated health risks, especially in rural areas where people usually consume maize milled in the small local mills and has not undergone any quality assurance measure.

The government should also set regulatory limits for mycotoxin contents in infants' foods such as the maximum limits for AFB₁, total aflatoxin, FB₁ and total fumonisin while also ensuring their enforcements. Though it is impractical to set limits for breast milk, setting the limits for mycotoxins in foods like maize which is mainly used as staple food could further reduce the levels of transfer of the same into breast milk, hence, reduce the rate of infants' exposure. The existing limits in Tanzania are 5 µg/kg for AFB₁ and 10 µg/kg for total aflatoxins in maize generally. But there is none specifically set for infants' foods. Furthermore, even the enforcement of the two limits is still poor.

In the ideal situation, using the maximum level of 5 µg/kg set for AFB₁ in Tanzania and the recommended maize consumption of 771 g/person/day (TFNC, 1997), the exposure of a lactating mother with an average body weight of 60 kg (Kimanya et al., 2008b) can be calculated as follows:

$$\text{Exposure (ng/kg bw /day)} = [5 \mu\text{g/kg} \times 0.771 \text{ kg} / 60 \text{ kg}] / 1000 = 0.06 \text{ ng/kg bw/day}$$

Therefore, for the whole day a total of 3.6 ng/day of AFB₁ is absorbed by the mother. Considering that 510ml/day of breast milk is required for the infant per day at the first lactation stage (US-EPA, 2011) and that the maximum conversion rate for AFB₁ to AFM₁ in milk is 6.2% (Oveisi et al., 2007), the level of AFM₁ transferred into breast-milk at the first lactation stage can be calculated as follows:

$$6.2\% \times 3.6 \text{ ng} = 0.2232 \text{ ng of AFM}_1 \text{ carried over to breast milk}$$

Hence the concentration of the AFM₁ in milk per day is (0.2232 ng divide by 510 ml) 0.0004 ng/ml. This concentration is below the 0.025 ng/ml set for AFM₁ in infants' food (European-Commission, 2006).

Suppose an infant of 3 kg body weight takes 510 ml in a day as recommended, he/she will be exposed by 0.068 ng/kg bw/day. This exposure is below the rate observed in this study. It therefore means that the exposure will be reduced to a certain extent. However, it is still above the 0.017 ng/kg bw/day limit which has been considered as an exposure of public health concern (EFSA, 2007). The estimation by using 5 µg/kg as a regulatory limit for AFB₁ and the exposure to infants for the three lactation stages: 1, 3 and 5 is presented in Table 7.1. Nevertheless, it is important to note that during set up of the AFB₁ limit in Tanzania, the concern was more about the available exposure data, capacity to determine that level in food, as well as the prevailing food insecurity issues of that time. It is therefore high time this limit is re-examined as it is not valid with regard to AFM₁ in breast milk.

Table 7.1. Estimation of the exposure of infants from aflatoxin M₁ using 5 µg/kg as a regulatory limit for aflatoxin B₁ in lactating mother diet.

Estimate of the exposure for lactating mother:			
Aflatoxin B ₁ maximum limit for Tanzania (µg/kg)	5		
Exposure of a 60 kg lactating mother consuming 771g per day (ng/kg bw/day) ^a	0.06		
The exposure for lactating mother (ng/day)	3.6		
AFM ₁ carried over to breast milk ^b	0.2232		
Estimate of the exposure for infant:			
	Lactation month 1	Lactation month 3	Lactation month 5
Milk produced per day (equal to milk intake per day) (ml) ^c	510	690	770
Concentration of aflatoxin M ₁ in breast milk per day (ng/ml) ^d	0.0004	0.0003	0.0003
Average infants body weight (kg) ^e	3	6	8
Exposure of infants from breast milk ^f (ng/kg bw/day)	0.068*	0.035*	0.029*

^a: Exposure (ng/kg bw/day) = [5 µg/kg x 0.771 kg/ 60 kg]/1000

^b: Conversion rate of AFB₁ to AFM₁ is 6.2% (Oveisi et al., 2007)

^c: Milk intake level (US-EPA, 2011)

^d: Aflatoxin carried to milk (ng)/milk produced per day

^e: Average infants body weight (http://www.who.int/childgrowth/standards/Technical_report.pdf)

^f: Exposure (ng/kg bw/day) = [concentration of aflatoxin M₁ (ng/ml) x milk intake (ml)]/ average body weight

*: Exposure above 0.017 ng/kg bw/day is of public health concern.

Table 7.1 shows that infants will be exposed above 0.017 ng/kg bw/day, a level which is of public health concern if 5 µg/kg is used as a limit for aflatoxin B₁ in food.

7.2.3. Educate lactating mothers on dietary diversification and quality issues

The use of other cereal grains in combination with maize so as to attain the nutritional intake, required per day but with minimal mycotoxins exposure, is recommended as suggested by previous researchers (Kimanya et al., 2012). Generally, foods highly contaminated with mycotoxins should be avoided and replaced with foods which are rarely contaminated with mycotoxins such as millet, sorghum and rice (Munimbazi and Bullerman, 1996), if available. This will not only ensure a balanced diet and a nutritional well-being for both mothers and infants but also minimize mycotoxin exposure to a large extent. However this recommendation may be more applicable in those areas where these cereals are grown. Not all cereals can be available in all places but I believe in one are there can be more than one cereal that can be grown, as indicated in the agricultural activities of Rombo district. Since mycotoxins are carcinogenic, the use of diets rich in vitamins, antioxidants and other

substances known to prevent carcinogenesis is important to prevent the effect of its exposure.

7.2.4. Reduction of mycotoxin exposure as a strategy to prevent malnutrition

It is also important for responsible institutions and the government of Tanzania to take into account the issue of mycotoxin exposure in their national nutrition interventions and programs addressing malnutrition issues. The existing evidence from this study reveals that mycotoxin exposure is associated with growth impairment. Therefore, when focusing on the care practices for infants, young children and women of child bearing age the methods for the reduction of mycotoxins in food at the household and community level should also be introduced.

7.2.5. Involving all stakeholders in addressing mycotoxin challenges

Mycotoxins issues are cross-cutting. They cut across various sectors, from household level, communities, to public sectors such as Ministry of Agriculture, Food Security and Cooperatives, Ministry of Health and Social Welfare, Ministry of Industries and Trade, private sector, learning institutions and the media. If all the stakeholders take full responsibility within their different capacities, the reduction of mycotoxin contaminants in foods would be achieved and thus reduce the extent of infants' exposure in Tanzania. This will in turn improve infants' nutrition as well as it will reduce poverty caused by malnutrition in the country.

7.3 How to achieve the recommendations on the reduction of mycotoxin exposure in infants

Awareness creation can be done in different ways such as use of media like radios, televisions, local newspaper etc. Nowadays in Tanzania many regions have private radio stations. I therefore do believe that stakeholders involved in delivering nutritional education to the communities can work closely with the local radio stations in creating awareness and thus reach a wider community. Leaflets can also be prepared and made available in

churches, mosques and districts hospitals where they can be easily shared with people paying visits for any reason.

Furthermore, nutritionists who are now available in district hospitals can participate in the awareness creation of mycotoxins to clinics attendees. For example mothers who are attending ante natal and post natal clinics. Also the issue of dietary diversification can be discussed with mothers attending the clinics. It should be noted that the diversification of diet depends on what is available in that particular area.

The government can also use the agriculture and livestock officers who are available in every district to educate farmers on the methods highlighted for the reduction of mycotoxins. These officers are well informed of the government's operations regarding the issues of fertilisers and pesticides subsidies. The proper use of fertilisers and pesticides can also be instructed with this officer.

The issue of mycotoxin contamination is now being much investigated and different results have been generated. It is a high time for stakeholders involved in the food chain and health to be educated about mycotoxins exposure and its effect.

The obstacles to practising exclusive breastfeeding for 6 months are as follows; some of the mothers engage in employment and could not therefore find time to practice exclusive breastfeeding since they are to resume work after just three months of a child's age. Furthermore, the nature of general activities in which mothers in rural areas are involved like farming could be an obstacle to exclusive breastfeeding e.g. farms situated far away from residential areas force mothers to spend the whole day in the farming. These mothers need to be trained about expression of breast milk, though poverty can also be another challenge to expression of breast-milk. This can be in terms of lack of cold storage facilities for expressed breast milk.

7.4 Limitation of the study

The present study did not analyse the mycotoxins content of lactating mothers' foods which contribute to the quality of the breast milk. However, the available data on the mycotoxins contamination in the area of the study was sufficient to support the discussion on the results of breast milk contamination.

Another limitation of the study is that the mycotoxin exposure in breast milk was also computed by using the documented milk intake levels appropriate for the infant's age. In reality some of the infants might have taken more and some less than the documented amounts. However it was our believe that the documented amount is optimum intake necessary for the proper growth of the infants, and in that case, the mycotoxin exposure computed using those levels gives the mostly representative results as far as breast milk contamination is concerned.

The use of HPLC made it difficult to analyse all mycotoxins of public health concern as would have been possible if LC-MS was available. However the aflatoxins and fumonisins analysed are commonly reported to be present in maize in Tanzania

It was also difficult to get other relevant data from the available literature for comparing with the results for fumonisins prevalence in breast milk found in the current study. The available literature regarding breast milk (Maragos and Richard, 1994) was for developing analytical methods. While Fink-Gremmels (2008) reported the possibility of fumonisin carry over into breast milk in ruminants, Gazzotti et al. (2009) analysed only 10 samples of bovine milk, hence, no relevant data on fumonisin prevalence in human breast milk is available.

Difficulty in estimating the proportion of ingredients used in the preparation of infants' food during the recording of the 24 hour dietary recall was one of the limitation. Therefore in estimating the maize flour used in making infants food, the Tanzania food composition table (Lukmanji et al., 2008) was used.

Another limitation was in estimating the household earnings. Some of the mothers could not tell how much their monthly earnings were. This may have affected the “income” as one of the confounding factors for mycotoxin exposure

The sample size was small to make conclusive recommendations on the concomitant exposure from breast milk and food. However, this was an exploratory study demonstrating the effect of mycotoxins in infants under six months of age. It therefore provides room for further studies.

7.5 Suggestions for future research

In view of the findings of the present study the following studies are needed in order to supplement or extend it and thus understand how big the problems are and the best way of implementing the suggested recommendations:

To follow up the growth development of the infants under this study in comparison with feeding practices up to two years, in order to find out how the extent exposure early in life affects health later in life.

To determine other mycotoxins in breast milk. In depth assessment of mycotoxin contaminants in breast milk should be done. With the results of this study there is a possibility of other mycotoxins to be excreted in breast milk as reported in studies of other countries. This should include analysis of AFB₁, AFG₁, AFG₂ and OTA.

In depth study using biomarkers such as urine and blood should be done. Studies on other outcomes like gut microbiota and immunological parameters in determining the effect of mycotoxin exposure in infants is also important. This is because intestinal cells are the most prone to mycotoxins in comparison with other tissues. The effect of mycotoxins on the gastro intestinal tract could probably demonstrate the growth effects occurring in infants.

To expand geographical coverage of the study. In depth analysis of mycotoxins in breast milk should also be conducted in other areas of Tanzania with different agro-ecological zones. This will enable identification of the interventions programs for the reduction of mycotoxins exposure in lactating mothers.

Understanding the extent of exposure to dietary mycotoxins affects contamination level in breast milk. The correlation of mycotoxin contaminants in food and breast milk as a biomarker should also be studied in depth.

With regard to the mycotoxins in foods and exposure to infants, more research is needed to test the following hypotheses:

- I. Reduction of mycotoxins in maize will reduce the mycotoxin content of breast milk
- II. Reduction of mycotoxin content in infant food will improve the nutritional status of infants
- III. There is a correlation between dietary exposure to aflatoxins and fumonisins in infants under six months of age and their biomarkers
- IV. Exposure of aflatoxin and fumonisin in infancy influence nutritional status later in life

Though this study suggests the ways for reducing mycotoxin exposure in infants (section 7.2 and 7.3), further studies are still needed to evaluate the effectiveness of the methods described.

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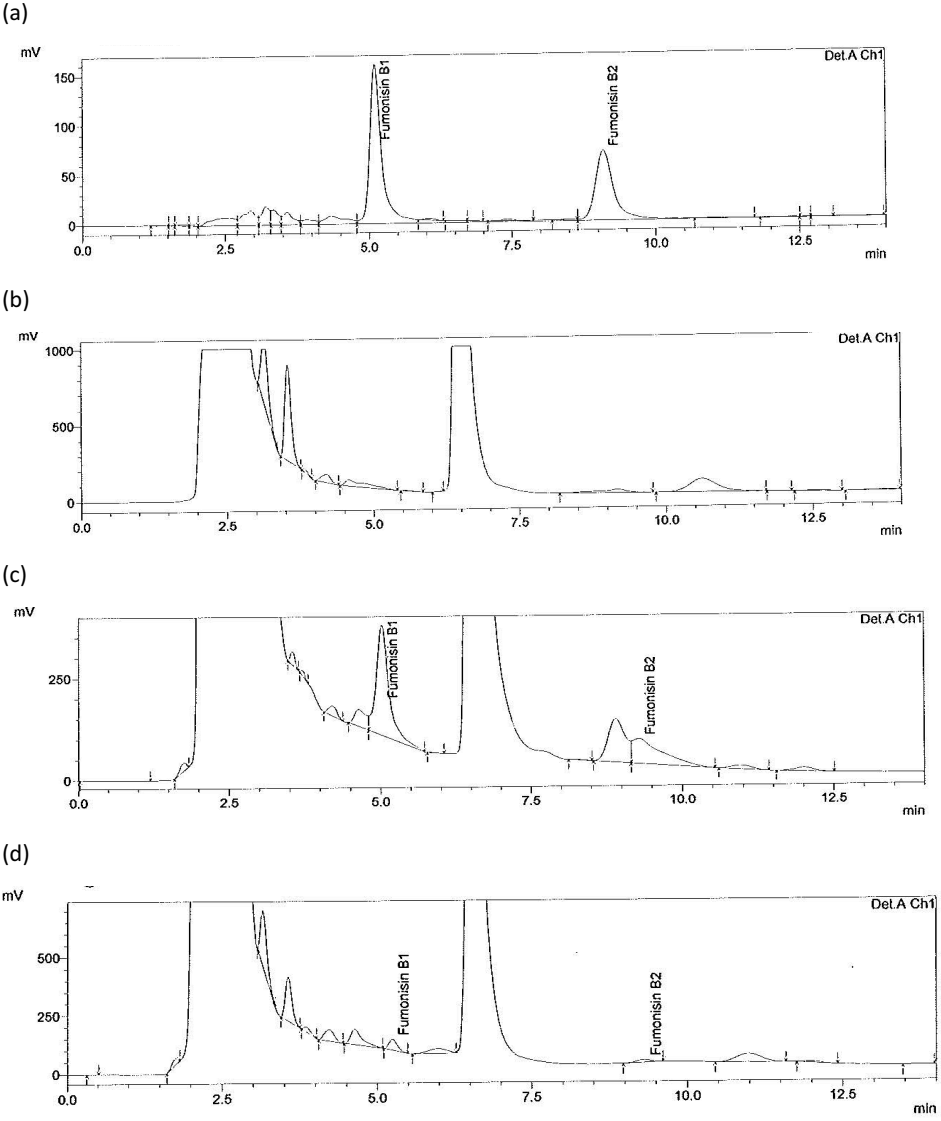
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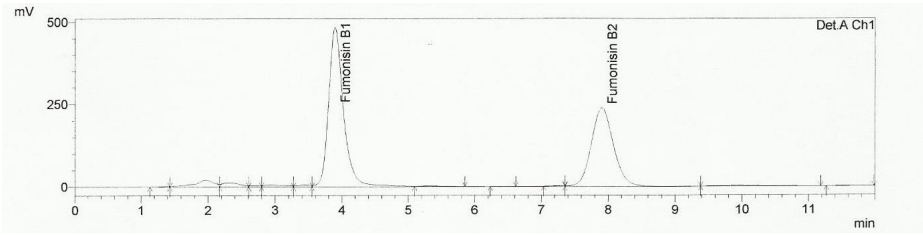
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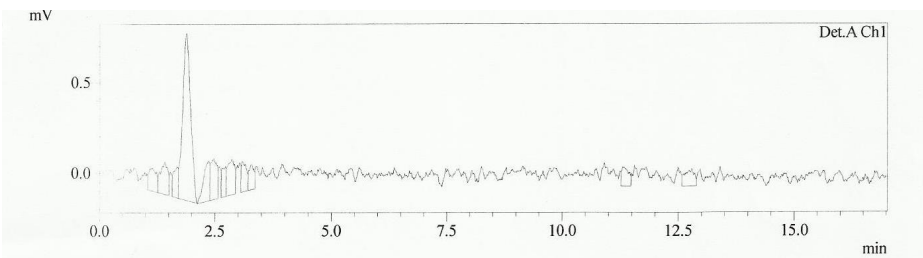
Annexes



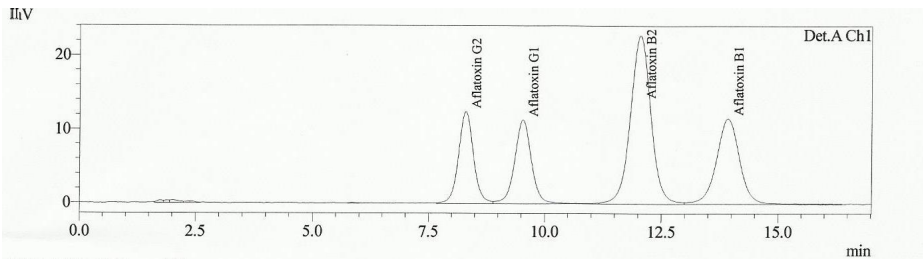
Annex I: HPLC chromatograms. (a) Fumonisin B1 standards of 72ng/ml in methanol:water (1:1). (b) Blank sample of human breast milk. (c) Sample of human breast milk spiked with fumonisin B1 at 72ng/ml. (d) Sample of human breast milk containing fumonisin B1.



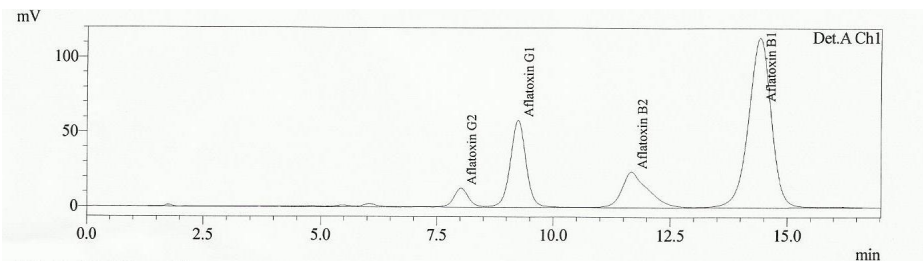
Annex II: HPLC chromatogram for fumonisin standard (FB₁ and FB₂) of 250µg/kg



Annex III: HPLC chromatogram of aflatoxins free maize flour



Annex IV: HPLC chromatogram for aflatoxin standard



Annex V: HPLC chromatogram of maize flour contaminated with aflatoxins.

Annex VI: Questionnaire for evaluation of dietary exposure to mycotoxin in infants under six months of age – Rombo, Tanzania

ID No.

EVALUATION OF DIETARY EXPOSURE TO MYCOTOXIN IN INFANTS UNDER 6MONTHS OF AGE

Date:..... Name of interviewer:.....

Name of the street/suburb:Street/suburb leader.....

A. MOTHER PARTICULARS

- 1. Full name.....Age.....years
- 2. Marital status (a) single (b) married/living together (c) widow (d) divorced/separated
- 3. Education level (a) No education (b) primary (standard.....) (c) Secondary (form.....) (d) Others (specify).....
- 4. Number of births.....
- 5. Maternal age.....
- 6. Family size.....
- 7. Working status (a) employed (b) unemployed (c) small business (d) farmer (e) others (specify).....
- 8. Earning per month (a) 20,000 – 50,000Tshs. (b) 51,000 – 100,000 Tshs.(c) 101,000 – 200,000 (d) more than 200,000Tshs

B. FATHER PARTICULARS

- 1. Full name.....Age.....
- 2. Education level (a) No education (b) primary (std.....) (c) Secondary (form.....) (d) others (specify).....
- 3. Working status (a) employed (b) unemployed (c) small business (d)farmer (e) others (specify).....
- 4. Earning per month(a) 20,000 – 50,000Tshs. (b) 51,000 – 100,000 Tshs.(c) 101,000 – 200,000 (d) more than 200,000Tshs ???

C. CHILD PARTICULARS

- 1. Full name.....Sex (M/F).....date of birth.....
- 2. Birth weight.....
- 3. Anthropometric measurements:

Age (months)	Weight (kg)	Length (cm)

D. FOOD FREQUENCY QUESTIONNAIRE

- 1. When did you initiate breastfeeding (a) within 1hr of birth (b) within 24hr of birth
- 2. Is your child still breast feeding? Yes/No
- 3. If no at what age did the child stop breast feeding? month
- 4. At what age did you introduce weaning food to your child? month
- 5. Breast feeding pattern:
.....
- 6. What type of food do you give your child in a week? Mention all
.....
.....
- 7. In the last one week, how many days did you give your child maize based meals.....

E. MAIZE PROCESSING

- 1. What is the source of your maize? (a) Home grown (b) From the market
- 2. How do you process maize used for preparation of child’s food?
(a) Sorting (b) washing (c) soaking (d) dehulled (e) undehulled (f) Milling (g) other (specify)

Annex VII: 24 hour dietary recall for the child and the mother

ID No.....

24 HOUR DIETARY RECALL FOR THE CHILD – MONTH(1,3,5)

DATE:.....INTERVIEWER:.....

CHILD NAME:..... MOTHER:.....

Time	All types of food	All ingredients used in preparation	Quantity of each food that the child ate in gm/ml/pieces
Morning			
Mid-morning			
Lunch			
Mid-evening			
Dinner			

ID No.....

24 HOUR DIETARY RECALL FOR THE MOTHER

DATE:.....INTERVIEWER:.....

MOTHER'S NAME:.....CHILD NAME:.....

Time	All types of food	All ingredients used in preparation	Quantity of each food that the child ate in gm/ml/pieces
Morning			
Mid-morning			
Lunch			
Mid-evening			
Dinner			

Curriculum Vitae

Personal information

Full Name: Happy Steven Magoha

Birth date: 7th March 1975

Place of birth: Dar es Salaam, Tanzania

Marital status: Married

Address: Happy Magoha

P.O.Box 32627

Dar es Salaam

Mobile phone: +255 754 299116 ; +255 715 299116

E mail: hmagoha@yahoo.com, happy.magoha@out.ac.tz

Academic qualifications

2010-2014: PhD in Applied Biological Sciences – Faculty of Bioscience Engineering, Ghent University

PhD. “Effects of aflatoxins and fumonisins exposure in infants under six months of age in Tanzania”. (18th December 2014)

2002-2004: Masters in Applied Science (Food Science and Technology). Auckland University of Technology (AUT). New Zealand.

Thesis: “Destruction of Polycyclic Aromatic Hydrocarbon (PAHs) and long chain aliphatic hydrocarbons in soil using ball milling”.

1996-2000: BSc. (Food Science and Technology). Sokoine University of Agriculture, Tanzania.

Dissertation: Effect of grading on dehulling quality and chemical composition of different sorghum varieties.

Peer-reviewed publications

H. Magoha, M. Kimanya, B. De Meulenaer, D. Roberfroid, C. Lachat and P. Kolsteren. (2014). Association between aflatoxin M1 exposure through breast milk and growth impairment in infants from Northern Tanzania. *World Mycotoxin Journal* 7:277 - 284.

H. Magoha, B. De Meulenaer, M. Kimanya, D. Hipolite, C. Lachat and P. Kolsteren. (2014). Fumonisin B1 contamination in breast milk and its exposure in infants under six months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology* 74: 112 - 116.

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Other publications

Kimanya, Martin E., Candida P. Shirima, Happy Magoha, Danstan H. Shewiyo, Bruno De Meulenaer, Patrick Kolsteren, and Yun Yun Gong.(2014). Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize based complementary foods in Rombo, Northern Tanzania. *Food Control* 41:76-81.

H. Magoha, A. Makokha, L.Mghweno, A. Nakajugo, and J. Wekesa. (2008). Lead pollution in the environment and contamination in food around Mwanza, Tanzania. *Journal of Applied Biosciences* 9: 396 – 402. www.biosciences.elewa.org

A. Makhoka, L. Mghweno, H. Magoha, A. Nakajugo and J. Wekesa. (2008). Environmental lead pollution and contamination in food around Lake Victoria, Kisumu, Kenya. *African Journal of Environmental Science and Technology* 2:349–353. www.academicjournals.org/AJest

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Poster presentation

Happy S. Magoha, Patrick Kolsteren and Martin Kimanya. Concomitant exposure of aflatoxins and fumonisins to infants under six months of age in Rombo, Tanzania. Schlumberger Foundation Faculty for the Future Fellows Forum in Cambridge UK, from May 19th to May 22nd, 2013.

Happy S. Magoha, Martin Kimanya, Bruno De Meulenaer, Dominique Roberfroid, Carl Lachat and Patrick Kolsteren. Risk of aflatoxin M1 Exposure from breast milk in infants under six months of age in Rombo, Tanzania. Nutrition and nurture in infancy and childhood conference in Cumbria UK from June 10th to 12th, 2013.

Work experience

Since 2009: Lecturer at the Open University of Tanzania.

2005 – 2009: Assistant Lecturer at the Open University of Tanzania.

2004 – 2005: Senior Researcher at Tanzania Industrial Research and Development Organization (TIRDO). Involved in the Food Traceability Project, arranging several workshops in creating awareness and training the stake holders in coffee (Kilimanjaro), fish (Mwanza), tea (Njombe) and cashew nuts (Mtwara) industries on the traceability requirements in food products EU law 2002. I was a member of the laboratory accreditation committee at TIRDO – Tanzania

Other activities

2006 – 2009: Lake Victoria research on the “Environmental lead pollution and contamination in food around Lake Victoria basin” funded by the Inter University Council for East Africa (IUCEA)

2012 – 2013: Provided technical guidance and advice into the aflatoxin country assessment in Tanzania. With Abt Associate Inc

2013 to date: International Foundation for science project titled; Monitoring of the reduction of aflatoxin M1 and fumonisin contamination in breast milk after the intervention of maize production and storage.