

**Sokoine University of Agriculture**



**MSc Dissertation**

**Occurrence of Aflatoxins in Animal  
Feeds and Raw milk: The Case of  
Kondoa District in Dodoma  
Tanzania**

**Onesmo Andrew Robert**

**May, 2024**

**OCCURRENCE OF AFLATOXINS IN ANIMAL FEEDS AND RAW  
MILK: THE CASE OF KONDOA DISTRICT IN DODOMA TANZANIA**

**A Dissertation submitted in partial fulfillment of the  
requirements for degree of Master of Science in food quality and  
safety assurance of Sokoine University of Agriculture.  
Morogoro, Tanzania**

**Onesmo Andrew Robert**

**Supervisors**

**Dr. Alex Wenaty Ngunguru  
Dr. Frida Albinus Nyamete**

**School of Engineering and Technology  
Department Of Food Science and Agro-Processing  
Msc. Food Quality and Safety Assurance**

**May 2024**

## EXTENDED ABSTRACT

Aflatoxins in animal feeds and milk are common problem mostly reported among many regions in Tanzania, in Africa, and worldwide. Studies have revealed a high occurrence of aflatoxins in feeds and milk, which is largely due to a lack of knowledge among various stakeholders along the milk value chain, particularly smallholder dairy farmers (SHDF), animal feed processors, and feed suppliers. This has led to aflatoxins contamination of animal feeds during processing, storage, and feeding. The most common and highly carcinogenic aflatoxins is aflatoxin B1 (AFB1), which, when consumed, is metabolized into aflatoxin M1 (AFM1) and can be found in milk, meat, and eggs. Other types of aflatoxins include aflatoxin B2, G1, G2, and M2. The formation of aflatoxins is mostly caused by *Aspergillus flavus* or *Aspergillus parasticus*, which, under adverse environmental temperatures and humidity, can grow in livestock feeds such as maize bran, sunflower seedcakes, silage, hay, and, rarely, in the grass, leading to the production of toxins that referred to as aflatoxins.

This study was conducted to assess the level of awareness of aflatoxins in animal feeds and fresh milk among smallholder dairy farmers and to determine the contamination of AFB1 in animal feeds and the occurrence of AFM1 in milk. The study involved both qualitative and quantitative methodologies, including surveys and laboratory determinations of aflatoxins in animal feeds and milk. Semi-structured questionnaires were used, involving 120 SHDFs who had at least one dairy cow from six wards in Kondoa district namely Soera, Kikiro, Haubi, Kolo, Borisa, and Kondoa mjini. In the laboratory, 20 feed samples and 25 milk samples were collected from 25 SHDFs selected among 120 SHDFs and analysed for aflatoxin B1 and M1 respectively using High-Performance Liquid Chromatography (HPLC), with samples prepared following various steps before injection into the HPLC for aflatoxins detection and quantification. The results of this study provide a basis for the recommendation of more training programs and awareness campaigns on aflatoxins supplemented with close monitoring of the dairy value chain by extension officers across regions in Tanzania. The results of this study demonstrate that, majority of SHDFs have awareness of

aflatoxins. About 88.3% of the surveyed SHDFs have heard of aflatoxins through village meetings coordinated in collaboration with extension staff, where aflatoxins awareness was one of the agenda. Other sources of information included seminars, newspapers, radio, and television. Additionally, 77.5% were aware of the possible factors and control measures for aflatoxins in animal feeds and milk, and 68.2% understand that aflatoxins can harm both animal health and human health resulting in liver cancer. Furthermore, 63.3% knew the possible transmission of aflatoxins from animal feeds to milk. Additionally, more than 55% of the surveyed farmers were aware of at least one factor for aflatoxins contamination and control measures to prevent aflatoxins.

The results by High Performance Liquid Chromatograph (HPLC) analysis of animal feeds and milk revealed that 55% of all samples tested positive for AFB1 in animal feeds, where only 5% were above the permissible safe limit of 20  $\mu\text{g}/\text{kg}$  for dairy cattle feeding. The mean AFB1 was found to be 8.6 ( $\pm 5.9$ )  $\mu\text{g}/\text{kg}$ , ranging from the lowest limit of detection (LOD) to 21.1  $\mu\text{g}/\text{kg}$ . The analysis of milk samples revealed that 44% of the samples tested were positive for AFM1, with 12% of the sample above the level recommended by the Tanzania Bureau of Standards (TBS), the European Union (EU) and the World Health Organization (WHO). The mean AFM1 was found to be 0.07 ( $\pm 0.08$ )  $\mu\text{g}/\text{L}$ , ranging from the limit of detection to 0.23  $\mu\text{g}/\text{L}$ . However, there was no milk sample that exceeded the US Food and Drug Administration (USFDA) and Codex standards of 0.5  $\mu\text{g}/\text{L}$  for milk to be consumed by the public.

Findings from this study demonstrate that the majority of farmers had information about aflatoxins in animal feeds and raw milk, despite many awareness programs conducted for agricultural crop producers. Some farmers were aware of factors and the control measures for aflatoxins, also they were able to mention at least one sign of possible aflatoxins-contaminated feeds. This helped in their routine practices of feed preparation, storage, and feeding cattle. The findings of the survey reflect the real situation in the field, as the majority of feeds and milk samples were within the levels

recommended by TBS, EU, WHO, USFDA, and *Codex Alimentarius* standards for dairy cattle feeding and milk for public consumption.

In conclusion, it was found, smallholder dairy farmers were aware of aflatoxins animal feeds and milk. However, there are some fees and milk samples contain aflatoxin calling for more training programs to be established and implemented to spread awareness on aflatoxins causes and effects, and hence ensure that the community is aware of the problem and contribute to the effort to prevent aflatoxins. Monitoring of SHDFs practices along the dairy value chain is also recommended to encourage voluntary participation to prevent aflatoxins contamination in animal feeds and milk and throughout the food system in general

**Keywords:** *Aflatoxins, Awareness, Aflatoxin B1, Aflatoxin M1, Animal feeds, Raw/fresh milk, Occurrence, Smallholder dairy farmers, and High-Performance Liquid Chromatography.*

## IKISIRI KUU

Sumukuvu katika vyakula vya mifugo na maziwa ni tatizo sugu linalozikumba nchi nyingi duaniani ikiwemo Tanzania. Tafiti nyingi zilizofanyika kuhusu sumukuvu zimebaini viwango vya juu vya sumukuvu katika vyakula vya mifugo na maziwa kutokana na wadau wengi katika mnyororo wa thamani wa maziwa wakiwemo wafugaji wadogo wa ng'ombe wa maziwa, wachakataji wa vyakula na wasambazaji kukosa elimu juu ya sumukuvu, visababishi vyake na namna ya kukinga sumukuvu katika mifumo mbalimbali ya vyakula. Hii imesababisha uwepo wa sumukuvu katika hatua mbalimbali kuanzia kwenye uvunaji, uchakataji, utunzaji, na ulishaji wa vyakula hivyo. Mojawapo ya aina ya sumukuvu hatari zaidi kwa binadamu na mifugo ni Sumukuvu B1 ambayo hupatikana katika nafaka na hivyo katika vyakula vya mifugo. Sumukuvu hii inapokuwepo katika chakula anachokula ng'ombe au mnyama yeyote humengenywa na kuzalisha sumukuvu M1 ambayo hujitokea katika damu na kisha kwenda katika mazao ya mifugo ikiwa ni pamoja na maziwa, nyama na mayai. Aina nyingine a sumukuvu ni pamoja na sumukuvu B2, G1, G2 na M2. Hata hivyo shirika la kimataifa la utafiti wa magonjwa ya kansa imezitambua aina mbili za sumukuvu yaani sumukuvu B1 na sumukuvu M1 kama sumukuvu hatari zaidi na kuziweka katika daraja la kwanza la visababishi vya magonjwa ya kansa ulimwenguni. Uzalishaji wa sumukuvu hutokana na fangasi waitwao *Aspergillus flavus* or *Aspergillus parasticus*. Fungusi hawa wanapopata changamoto ya joto na unyevu kuliko kiwango stahiki huweza huzalisha sumu inayoitwa sumukuvu.

Utafiti huu umefanyika kupima uelewa wa jamii za wafugaji wadogo wa ng'ombe wa maziwa juu ya sumukuvu katika vyakula vya mifugo na maziwa pamoja na kubaini viwango vya sumukuvu B1 na sumukuvu M1 katika vyakula vya mifugo na maziwa. Utafiti huu ulitumia upimaji wa ubora na viwango ambazo zilijumuisha dodoso la uso kwa uso kwa washiriki waliochaguliwa pamoja na uchunguzi wa sampuli za vyakula vya mifugo na maziwa kutoka miongoni mwa washiriki wa dodoso. Maswali yaliyoandaliwa yalitumika katika kutambua kiwango cha uelewa kwa wafugaji wadogo 120 kutoka katika kata sita wilaya ya Kondoa ikiwemo kata ya Soera, Kikiro,

Haubi, Kolo, Borisa na Kondoa mjini wenye angalau ng'ombe mmoja anayekamuliwa. Katika uchunguzi wa kimaabara jumla ya sampuli 20 za vyakula na sampuli 25 maziwa zilizokusanywa kutoka miongoni mwa wafugaji 120 walioshikiri dodoso ambazo zilichambuliwa viwango vya sumukuvu kwa kutumia kifaa cha kisasa kiitwacho "*High Performance Liquid Chromatography (HPLC)*" kwa sampuli zilizoandaliwa ipasavyo kabla ya kuziweka katika kifaa hicho kwa ajili ya utambuzi wa uwepo wa sumukuvu na upimaji wa viwango vya sumukuvu. Matokeo ya utafiti huu ni muhimu katika kutoa kutoa msingi wa umuhimu wa kupanga, kuratibu na kutekeleza programu za utowaji elimu ya ufahamu wa sumukuvu katika mikoa mbalimbali ya Tanzania na nje ya nchi.

Matokeo ya utafiti huu yameonesha kwamba wafugaji wengi wa ng'ombe wa maziwa katika wilaya ya Kondoa wanauelewa juu ya sumukuvu kwenye vyakula vya mifugo na maziwa. Takribani asilimia 88 ya washiriki wote wamewahi kusikua kuhusu sumukuvu katika mikutano ya vijiji, semina zilizoandaliwa, magazetini, redioni na katika televisheni. Asilimia 79 ya washiriki wa dodoso wanafahamu kuwa sumukuvu inaweza kupatikana katika vyakula vya mifugo na maziwa. Pia, utafiti huu umeonesha kwamba asilimia 68 ya wafugaji walioshiriki wanaelewa kwamba sumukuvu inaweza kuathiri binadamu na mifugo na kusababisha kansa endapo binadamu au mifugo itakula chakula chenye sumukuvu. Aidha, asilimia 63 wanafahamu kwamba sumukuvu inaweza kusafiri kutoka katika vyakula vya mifugo vyenye vimelea vya sumukuvu kwenda hadi kwenye maziwa. Miongoni mwa washiriki wote, zaidi ya asilimia 55 wanafahamu visababishi vya sumukuvu na namna ya kukinga sumukuvu katika vyakula vya mifugo na maziwa. Pia asilimia 57 wanafahamu alama za kutilia mashaka juu ya uwepo wa vimelea vya sumukuvu kuwa chakula cha mifugo. Ambapo mabadiriko ya rangi ya kawaida ya vyakula kuwa rangi ya kijani, njano, nyeusi, na uvundo ni ishara zilizotajwa.

Aidha matokeo ya uchambuzi wa maabara kwa sampuli za vyakula vya mifugo na maziwa ziliokusanywa kutoka kwa wafugaji wilayani Kondoa yamebaini kwamba, asilimia 55 za sampuli za vyakula zina vimelea vya sumukuvu ambapo asilimia tano tu (5%) zilikuwa juu ya

kiwango stahimilivu kwa matumizi ya ng'ombe wa maziwa cha 20µg/kg. Wastani wa sumukuvu katika sampuli hizo ilikuwa 8.6 (±5.9) µg/kg ambayo ipo kati ya kiwango cha chini cha utambuzi wa sumukuvu hadi 21.1 µg/kg. Uchambuzi wa sampuli za maziwa umebaini asilimia 44 zinavimelea vya sumukuvu ambapo asilimia 12 zipo juu ya kiwango kinachoshauriwa na Shirika la Viwango la Tanzania, EU, and WHO kwa maziwa yanayokusudiwa kutumiwa na binadamu cha 0.05µg/L huku wastani wa sumukuvu katika maziwa ikiripotiwa kuwa 0.07 (±0.08) µg/L na kiwango cha juu cha sumukuvu kikiripotiwa kuwa 0.23 µg/L. Hakuna sampuli zilizokuwa na kiwango zaidi ya kinachoshauriwa na Shirika la Chakula na Dawa la Marekani (USFDA) na “*Codex Alimentarius*” cha 0.5 µg/L katika maziwa yakutumiwa na binadamu.

Matokeo ya utafiti huu yamebaini wafugaji waliowengi wanaufahamu kuhusu sumukuvu katika vyakula vya mifugo na maziwa japokuwa program mafunzo ya ufahamu zilijikita kwa wakulima wa mazao ya kilimo. Wafugaji wengi wanatambua sababu za sumukuvuna na namna ya kuzuia. Washiriki wa utafiti huu wameonesha kufahamu viashiria vya kutilia shaka chakula chenye sumukuvu. Hii inasaidia kuandaa, kutunza, na kulisha mifugo kwa kuinga sumukuvu. Matokeo haya yanaashiria hali halisi ya ufugaji na kiwango cha usalama wa mazoa wa mifugo. Japokuwa matokeo ya uchambuzi wa sampuli zilizo nyingi zilikuwa na viwango himilivu vinavyokubaliwa na TBS, EU, WHO, USFDA na *Codex Alimentarius* kwa vyakula vya kulisha ng'ombe wa maziwa na maziwa yanayolenga kutumiwa na binadamu, nivema kuongeza juhudi za kuzuia kabisa sumukuvu katika vyakula vya mifugo na maziwa kwani sumukuvu B1 na M1 ni hatari sana na husababisha kansa. Hivyo, ni muhimu kwa wataalamu wa mifugo kufanya ukaguzi wa mara kwa mara katika maeneo ya wafugaji wanaowasimamia ili kuhakikisha utendaji unaokidhi viwango wa kuinga sumukuvu katika vyakula, maziwa, na mazoa mengine ya mifugo.

Programu za mafunzo kwa wafugaji wadogo wa ng'ombe wa maziwa na wakulima wa mazao ya kilimo zina mchango mkubwa katika kuhakikisha usambazaji wa elimu ya ufahamu wa sumukuvu katika mazao, vyakula vya mifugo, maziwa, na mazoa ya mifugo kwa

ujumla. Mafunzo zaidi yanatakiwa kuanzishwa ili kusambaza elimu juu ya sumukuvu na namna ya kuzuia sumukuvu katika vyakula na hivyo kuhakikisha jamii kwa ujumla inaufahamu kuhusu sumukuvu pamoja na kusimamia shughuli za kila siku kuhakikisha kuzuia sumukuvu katika mifumo ya vyakula. Hii itasaidia kuongeza utayari wa jamii ya wakulima na wafugaji katika kuzuia sumukuvu katika vyakula vya mifugo na maziwa.

**Maneno Muhimu:** *Sumukuvu, Uelewa, Sumukuvu B1, Sumukuvu M1, Chakula cha mifugo, Maziwa, Kuonekana, Wafugaji wadogo wa maziwa, na "High-Performance Liquid Chromatography."*

## DECLARATION

I, **Onesmo Andrew Robert**, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration and that it has neither been submitted nor been concurrently submitted in any other institution.

\_\_\_\_\_  
Onesmo Andrew Robert

**(MSc. Food Quality and Safety Assurance Candidate)**

\_\_\_\_\_  
Date

The declaration above is confirmed by;

\_\_\_\_\_  
Dr. Alex Wenaty Ngungulu

**(Supervisor)**

\_\_\_\_\_  
Date

\_\_\_\_\_  
Dr. Frida Albinusi Nyamete

**(Supervisor)**

\_\_\_\_\_  
Date

**LIST OF PAPERS**

- PAPER 1: Assessment of Aflatoxins Awareness in Animal Feeds and Fresh Milk among Smallholders Dairy Farmers in Kondoa District, Dodoma Tanzania. *“The material contained in **paper one** in this chapter is online and has been accepted for publication by the Journal of Agriculture and Food research (JAFR) <https://doi.org/10.1016/j.jafr.2024.101051>.....10*
- PAPER 2: Aflatoxins Contamination in Animal Feeds and Fresh Milk in Kondoa District, Tanzania. *The material in **paper two** has been published by the European Journal of Nutrition and Food Safety (EJNF)” Volume 15, Issue 12, Page 10-21, 2023; Article no.EJNFS.109989 ISSN: 2347-5641.....18*

**COPYRIGHT**

No part of this dissertation may be reproduced, stored in any retrieval system or transmitted in any form or by any means without prior written permission of the author or the Sokoine University of Agriculture on the behalf.

## **ACKNOWLEDGEMENTS**

The authors acknowledge the potential support from various people, institutes and departments including my supervisors Dr. Alex Wenaty Ngungulu, Dr. Frida Albinusi Nyamete and the Head of Department of Food Science and Agro-processing, Dr. Sulemani Rashid for their close support. I also extend my acknowledgement to the Director General of Tanzania Livestock Research Institute (TALIRI) and all staffs, extension staffs from Department of Livestock and Fisheries Development at Kondoa DC & TC, and Tanzania Bureau of Standards for their contributions and support leading to the completion of this study.

## **DEDICATION**

This dissertation is dedicated to my lovely wife Leah Richard Mtunguja and all my children; Ndizeyima, Ndikumana and Jenitha, my father Mwl.Andrea Robert Ntagunga, brothers and sisters, my staff members at TALIRI Mpwapwa, Imani Choir members of St. Paul's Anglican church Mpwapwa and the rest of my relatives and friends for their extended prayers, support and encouragement during the entire period of my studies.

## **TABLE OF CONTENTS**

<b>EXTENDED ABSTRACT.....</b>	<b>ii</b>
<b>IKISIRI KUU.....</b>	<b>v</b>
<b>DECLARATION.....</b>	<b>ix</b>
<b>LIST OF PAPERS.....</b>	<b>x</b>
<b>COPYRIGHT.....</b>	<b>xi</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>xii</b>
<b>DEDICATION.....</b>	<b>xiii</b>
<b>TABLE OF CONTENTS.....</b>	<b>xiv</b>
<b>LIST OF FIGURE.....</b>	<b>xvi</b>
<b>LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS.....</b>	<b>xvii</b>
<b>CHAPTER ONE.....</b>	<b>1</b>
<b>1.0 GENERAL INTRODUCTION.....</b>	<b>1</b>
1.1 Background Information.....	1
1.2 Problem Statement and Justification.....	2
1.3 Research Questions.....	4
1.4 Objectives of the Study.....	4
1.4.1 General objective.....	4
1.4.2 Specific objectives.....	4
1.5 Literature Review.....	4
1.5.1 Mycotoxins.....	4
1.5.2 Aflatoxins.....	5
1.5.3 Occurrence of aflatoxins.....	6
1.5.4 Aflatoxins exposure and metabolism.....	7
1.5.4.1 Aflatoxins in hay feeds.....	7
1.5.4.2 Aflatoxins in grass forages.....	7
1.5.4.3 Aflatoxins in concentrate feeds.....	8
1.5.5 Effects of aflatoxins.....	8
<b>CHAPTER TWO.....</b>	<b>10</b>
<b>PAPER I.....</b>	<b>10</b>
<b>CHAPTER THREE.....</b>	<b>18</b>
<b>PAPER II.....</b>	<b>18</b>
<b>CHAPTER FOUR.....</b>	<b>30</b>

<b>4.0 GENERAL DISCUSSION.....</b>	<b>30</b>
4.1 Demographic Information.....	30
4.2 Awareness of Aflatoxins.....	30
4.3 Food Storage Practices.....	31
4.4 Feeding Practices.....	33
4.5 Occurrence of aflatoxins in Animal Feeds and Fresh Milk.....	34
5.0 Conclusion and Recommendations.....	35
References.....	36
<b>APPENDICES.....</b>	<b>41</b>

**LIST OF FIGURE**

**FIGURE IN CHAPTER FOUR**

Figure 1: Hay storage practice at one of the  
smallholder dairy farmer (SHDFs) at  
Kondoa town.....33

**LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS**

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AOAC	Association of Official Analytical Chemists
DC	District Council
DLFO	District Livestock and Fisheries Officer
EACH	East African Community
EAS	East African Standard
EC	European Commission
EU	European Union
FAO	Food and Agriculture Organization
FLD	Fluorescence Detector
GAP	Good Agricultural Practices
GDP	Gross Domestic Product
GMP	Good Manufacturing Practices
GPHP	Good Post-Harvesting Practices
HBV	Hepatitis B Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency Research for Cancer
ISO	International Organization for Standardization
LOD	Limit of Detection
LOQ	Limit of Quantification
QA	Quality Assurance
QC	Quality Control
SHDFs	Smallholder Dairy Farmers
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TC	Town Council
TALIRI	Tanzania Livestock Research Institute
TANIPAC	Tanzania Initiative for Prevention of Aflatoxin Contamination
TBS	Tanzania Bureau of Standards
TDB	Tanzania Dairy Board
MLFD	Ministry of Livestock and Fisheries Development
TFDA	Tanzania Food and Drug Authority

TFDA	Tanzania Food and Drugs Authority
URT	United Republic of Tanzania
USFDA	United States Food and Drugs Authority
WHO	World Health Organization

## CHAPTER ONE

### 1.0 GENERAL INTRODUCTION

#### 1.1 Background Information

The world is facing challenges regarding food safety due to contamination from various hazardous sources including aflatoxins. Aflatoxins are toxic substances produced as secondary metabolites by fungi, namely *Aspergillus flavus* or *Aspergillus parasiticus*, when exposed to adverse environmental conditions like temperature and humidity (Jiang *et al.*, 2021). These fungi can grow in soil, decaying vegetation, hay, and grains. Other *Aspergillus* species, such as *A. nominus*, *A. ochraceoroseus*, *A. bombycis*, *A. fumigatus*, and *A. pseudotamari*, have also been identified as potential causes of aflatoxins (Jallow *et al.*, 2021). Dietary aflatoxins have shown detrimental effects on animal health and production. The most common route of exposure is through the ingestion of contaminated food or feed. When contaminated feeds are consumed by livestock, they undergo metabolism, leading to the contamination of livestock products, including milk (Fink, 2008). Aflatoxin B1 is the most commonly occurring and most toxic type. When consumed in contaminated feeds, it is further metabolized within the body and excreted with livestock products as aflatoxin M1. Aflatoxins are considered a carcinogenic, mutagenic, and teratogenic agent. Therefore, its control and regulation in food systems are of great importance (Mughal *et al.*, 2017).

Milk consumption is recommended by many nutritional guidelines to meet daily requirements for proteins, fats, minerals and vitamin B12. However, the trend of individual milk consumption in Tanzania is increasing slowly (Wang *et al.*, 2022). Kurwijila (2010) reported that average per capita milk consumption in Tanzania was 45 litres per annum as compared to the current amount of 58 litres per annum as reported by The Ministry of Livestock and Fisheries Budget Report of 2024. This is still very low compared to the recommended amount of 267 liters per person per annum. Milk is likely to be contaminated with aflatoxins as a result of livestock consuming aflatoxin B1-contaminated feeds. Humans can be exposed to aflatoxin M1 when consuming contaminated milk and/or milk products. Several studies

have revealed that people, specifically children in urban and peri-urban areas, are the highest milk consumers, usually consuming more than twice the amount consumed by the rural population. Consequently, they are highly exposed to aflatoxin M1, which is excreted from milk after consumption (Makau, 2017).

Cattle feeding practices in Tanzania are defined by several production systems adopted by farmers, including zero grazing, mixed grazing, and free-ranging systems. The choice of production system influences feeding practices and, therefore, the likelihood of livestock products becoming contaminated with aflatoxin M1 from consuming aflatoxin B1-contaminated feeds. According to a report by the Tanzania Dairy Board (TDB) and Ministry of Livestock and Fisheries Development (MLFD) in 2015, 30% of the 2.5 billion litres of milk were supplied from improved dairy breeds, where supplementation is a common practice. This accounts for 70% of the milk sold, while most of the milk produced is consumed at the household level. The choice of production system determines aflatoxin B1 exposure in feeds and, consequently, aflatoxin M1 contamination in milk. Other determinants of aflatoxins contamination include feed handling and storage practices, geographical locations, the level of education, and aflatoxins awareness among livestock dairy farmers (Kitigwa *et al.*, 2023).

## **1.2 Problem Statement and Justification**

Aflatoxins level in grain food and feeds are strictly regulated in over 60 countries. The government of Tanzania through Tanzania Bureau of Standards (TBS) has established the minimum acceptable level of aflatoxin B1 in animal feeds as 5 µg/kg, while the maximum level for total aflatoxins count is 10 µg/kg (Tatfo Keutchatang *et al.*, 2022). However, for feeds intended for dairy cattle, the acceptable level of aflatoxin B1 is 20 µg/kg, and the level of aflatoxin M1 in milk is limited to 0.05 ng/mL. This is also the level recommended by Codex standard, and European Union standards. However, the recommended level of aflatoxin M1 in milk by USFDA is limited to 0.5 µg/L. Studies conducted in various parts of Tanzania, including Dar es Salaam, Morogoro, and Singida, have revealed higher levels of aflatoxin M1 in milk as reported by Mwakyosa & Mugula (2021);

Kajuna *et al.*(2013) and Mohammed *et al.*(2016) respectively. These levels range from the limit of detection to 2.007 ng/mL, compared to the recommended maximum daily intake of 1 ng/kg body weight/day for an adult (Mohammed *et al.*, 2016).

The Tanzania Initiative for Prevention of Aflatoxins Contamination (TANIPAC) project has been implementing various programs to prevent aflatoxin in some regions of the country. These programs include a baseline survey, awareness assessment, and education campaigns on aflatoxins prevention in the food systems especially in Dodoma, Katavi, Simiyu, Mbeya, Morogoro, Iringa, and Songwe. The aim of these programs is to increase awareness of aflatoxins and its prevention strategies in cereal crops and the food system in general. Kondoa, located in the Dodoma region, is one of the districts where TANIPAC and other related projects have been implemented. In Kondoa, smallholder farmers engage in mixed cultivation, primarily focusing on maize, sunflower and groundnuts, along with small herds of cattle, goats, pigs, or chickens.

It is worth noting that the first cases of aflatoxins outbreak in Tanzania were detected in Kondoa. These cases were a result of consuming maize contaminated with aflatoxins. In 2016, there were a total of 68 reported cases, with 20 deaths and more than 50% of the fatalities were children under the age of 15 (Kamala *et al.*, 2018). Various studies have been conducted to assess awareness of aflatoxins and its prevention strategies in cereal crops. However, limited studies has conducted on the prevalence of aflatoxins in animal feeds and milk. Dairy cattle are particularly susceptible to aflatoxin B1 exposure through maize grain, maize silage and maize by-products if farmers are unaware of aflatoxins contamination in animal feeds and milk. An initial assessment conducted in some parts of Tanzania revealed that 75% of farmers lack awareness of aflatoxins control practices and the carry-over effect in milk. As a result, consumers are highly exposed to aflatoxin M1, which can have carcinogenic, mutagenic and teratogenic effects (Jiang *et al.*, 2021).

Therefore, it was essential to conduct a study to determine the occurrence of aflatoxins in locally available and utilized animal feeds and its impact on milk safety in terms of aflatoxin M1 contamination. The findings of this study will provide crucial information on the extent of aflatoxins contamination in feeds and milk. This will enable basis for the development of recommendations aimed at promoting milk safety by reducing aflatoxin M1 levels in milk produced and consumed, primarily by reducing the use of feeds contaminated with aflatoxin B1 in the dairy value chain.

### **1.3 Research Questions**

- i. Are smallholder dairy farmers aware of aflatoxins and its potential contamination in animal feeds, as well as the factors that contribute to it and the effects it can have on livestock and human health?
- ii. Does their animal feed contain aflatoxin B1 levels that exceed the maximum limit, thus posing a potential risk for aflatoxin M1 contamination in fresh milk?

### **1.4 Objectives of the Study**

#### **1.4.1 General objective**

The general objective of this study was to determine the levels of aflatoxins contamination in animal feeds and fresh milk.

#### **1.4.2 Specific objectives**

The specific objectives of this study were to:

- i. To assess the awareness of aflatoxin in animal feeds and milk among smallholder's dairy farmers, as well as their harvesting, storing, and feeding practices.
- ii. To examine the levels of aflatoxins B1 and M1 in animal feeds and raw milk, respectively.

### **1.5 Literature Review**

#### **1.5.1 Mycotoxins**

Mycotoxins are secondary metabolites (chemicals) produced by certain mold species that can be found in foods before or after harvest. These mycotoxins can have toxic effects on both humans

and livestock (Rodríguez & Nunez, 2020). Molds can grow in various crops and foodstuffs, such as cereals (maize, sorghum, millet, and rice), nuts, spices, dried fruits, apples, and coffee beans, especially when these crops are exposed to warm or humid conditions. Maize and groundnuts are the most commonly affected crop species when it comes to aflatoxins contamination (Nagesh, 2018). Numerous studies have shown that mycotoxins can cause a range of serious health effects in both humans and livestock, from acute poisoning to long-term effects like immune deficiency, which can make individuals more susceptible to various diseases, including cancer. Mycotoxins are often referred to as "silent killers" because they are difficult to detect and some can be extremely toxic, posing risks to both humans and animals by compromising the immune system. Due to its significant impact on the economy and its potential hazards to human health, animal productivity, and trade, mycotoxins control has become a major concern (Balina *et al.*, 2018).

Some common mycotoxins include aflatoxins, ochratoxin A, patulin, fumonisin, zearalenone, and deoxynivalenol. The rate at which these substances enter the food system varies among countries due to differences in pre-harvest, during-harvest and post-harvest management practices. This exposure can occur through direct consumption of contaminated food or by consuming animal products from animals that have already been exposed to mycotoxins through contaminated feed (Mohamed *et al.*, 2021).

### **1.5.2 Aflatoxins**

Aflatoxins are the most common mycotoxins that pose health concerns for both livestock and humans when consumed in contaminated feeds and food, respectively. There are approximately 20 different types of aflatoxins identified worldwide, with the main group consisting of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Mahato *et al.*, 2019). Additionally, there are aflatoxin M1 and aflatoxin M2, which are hydrolyzed forms of Aflatoxin B1 and B2, respectively. These mycotoxins are commonly found in livestock products, particularly milk or milk products obtained from animals that have consumed significant amounts of aflatoxin B1-contaminated feed (Marimon

*et al.*, 2021). A study conducted by Prandini *et al.* (2009) revealed that AFM1 has approximately one order of magnitude lower potency compared to AFB1, which is considered the most toxic substance among the aflatoxins family. AFB1 accounts for approximately 75% of all aflatoxins contamination in food and feeds worldwide and is well-known for its role in hepatic carcinogenesis. Other adverse effects include immune weakness, reproductive issues, malnutrition, and growth impairment (Peles *et al.*, 2019). Overall, different types of aflatoxins have varying levels of toxicity, with aflatoxin B1 being highly toxic among all known types of aflatoxins (Navale *et al.*, 2021).

### **1.5.3 Occurrence of aflatoxins**

Outbreaks of aflatoxins poisoning have occurred in many countries across the world since the first detection of the disease in England in 1960, known as Turkey "X" disease. The most recent outbreaks were reported in dogs in the United States and humans in Kenya, India, Thailand, and Tanzania (Kamala *et al.*, 2018). From March to June 2011, over 75 dogs died after consuming pet food contaminated with aflatoxins in the U.S. (USFDA). The most severe outbreak in Kenya resulted in 125 deaths in 2004 (CDC). A study conducted by Ledo *et al.* (2020) revealed the occurrence of AFM1 in farm milk sampled from dairy farmers. This served as an indication of the level of performance of feed storage and monitoring practices, and the extent of risk in the chain. The results showed that the majority (63%) of the farm milk samples were below the lower detection limit (0.2 µg/L) of the dipstick method. Additionally, 14% were between the lower detection limit and the maximum limit of the USFDA legal standard (0.5 µg/L), while 22% of the farm milk samples exceeded the USFDA maximum limit. A previous study by Mohammed, Munissi, and Nyandoro (2016) in Tanzania, and Gizachew *et al.* (2016) in Ethiopia found that 16% and 26% respectively, of all milk samples, exceeded the USFDA maximum limit for AFM1. The majority of the milk samples were below the USFDA maximum limit of AFM1, but even at lower levels, AFM1 poses a risk to consumers as processing does not remove or reduce AFM1 in milk (Silva *et al.*, 2021).

#### **1.5.4 Aflatoxins exposure and metabolism**

Animals are exposed to several types of aflatoxins through the consumption of aflatoxins-contaminated feeds such as maize grain, maize silage, maize processing by-products, and hay (Rodriguez-Blanco *et al.*, 2020). Once consumed, the contaminants are absorbed with food in the small intestine and distributed to various parts of the body and organs, including the liver and kidneys (Bbosa *et al.*, 2013). Different metabolism takes place in the liver upon arrival, resulting in carry-over effects on animal physiological functions, reproductive functions, health well-being and ultimately, livestock products consumed by humans (Min *et al.*, 2021). Plant stress caused by drought, heat, or insect damage during fungus growth is a potential factor that increases aflatoxins levels (Magembe *et al.*, 2016).

##### **1.5.4.1 Aflatoxins in hay feeds**

A study conducted by Ceniti *et al.* (2021) revealed lower chances of aflatoxin B1 contamination in hay, although there were higher chances of contamination by other non-aflatoxigenic molds such as *Cladosporium cladosporioides*, *Alternaria alternata*, and *Rhizopus stolonifera*. These were fewer species closely related to aflatoxin (AF) contamination. Another study conducted in Italy further revealed that all hay samples were scarcely contaminated by AFB1, just below the limits established by the European Union (EU legislation) (0.02 mg/kg). None of the samples were positive for *Aspergillus*, and those tested for AFB1 showed results below the established limits. However, the higher the moisture content of the hay, the higher the chance of detecting aflatoxin B1, and vice versa. The recommended storage condition for hay is a moisture content of 15% (Ceniti *et al.*, 2021).

##### **1.5.4.2 Aflatoxins in grass forages**

Green forages are contaminated with some types of mycotoxins other than aflatoxins during the maturing stage and cutting. However, several studies have revealed limited levels of mycotoxins in forages compared to cereals (Nleya, Adetunji & Mwanza, 2018). Mycotoxins in forages are typically caused by filamentous fungi of the general *Fusarium*, while *Alternaria* grows normally in fresh forages. There have been no reports of the presence of *Aspergillus* fungi and

aflatoxins in fresh forages worldwide (Schenck *et al.*, 2019). However, there is a close association between aflatoxin B1 contaminants and aflatoxin M1 in milk when silage is used in the dairy cow diet (Puga *et al.*, 2022).

#### **1.5.4.3 Aflatoxins in concentrate feeds**

Concentrate feeds are the main means of dairy supplementation and consist of various feed ingredients, including energy sources, protein sources, vitamins, and minerals, compounded in a well-formulated ratio. The major proportion of concentrate feed is cereal by-products, specifically maize, which accounts for about 70%, while protein sources such as sunflower seedcakes constitute about 25% of the entire ration. The remaining portion contains mineral and vitamin sources (Dunshare *et al.*, 2019). Even though AFB1 has been detected in several types of cereals, cereal by-products remain dependable ingredients in concentrate feed formulation. Several studies have revealed that aflatoxin B1 is more stable when found in grains and resistant to degradation during normal processing such as milling and storage. This presents a high chance of causing toxicity and potential health hazards to both humans and livestock after consuming contaminated feedstuffs (Lalah *et al.*, 2019).

#### **1.5.5 Effects of aflatoxins**

The effects of aflatoxins in livestock depend on various factors, including age, genetics, physiology, and the environment. Young animals are more affected by aflatoxins than adults, as their livers are not mature enough for detoxification and other metabolic mechanisms (Min *et al.*, 2021). Aflatoxins reduce productivity and reproductive performance due to impaired nutritional and physiological functions and overall health (Gashaw, 2016). In addition to physiological impairment, aflatoxins cause loss of animal life, reduced livestock production, disposal of contaminated feeds and products, and increased veterinary care costs due to acute and chronic illness in livestock. Long-term exposure to aflatoxins can also lead to chronic health effects such as liver damage in both cows and humans (Senerwa, 2016). In humans, the degradation of aflatoxins and the interaction of the resulting toxic compounds with food components

can cause chronic diseases or severe cancers, increasing morbidity and mortality (Yin *et al.*, 2022).

Previous studies have addressed the occurrence of aflatoxins in agricultural crops, specifically maize, millets, sunflower and groundnuts, in relation to associated post-harvest practices in several regions of Tanzania, including Kondoia and Nchemba districts. However, there is limited information on the level of awareness of aflatoxins in animal feed and milk, as well as the prevalence of aflatoxins B1 and M1 in livestock feeds and fresh milk, respectively. This study was conducted to assess the level of awareness of aflatoxin among smallholder dairy farmers and determine the prevalence of aflatoxins B1 and M1 in animal feeds and milk, respectively. This was achieved through a survey conducted with 120 farmers using structured questionnaires and laboratory analysis of 45 samples of feeds (20) and fresh milk (25) using HPLC at the Tanzania Bureau of Statistics (TBS) in Dar es Salaam. However, the study was conducted in March during the rainy season and did not consider the seasonal variation of feed resources during the dry season, when post-harvest residues and dry grasses are the dominant source of livestock feeds.

**CHAPTER TWO**

## PAPER I



## Assessment of aflatoxins awareness in animal feeds and fresh milk among smallholder dairy farmers in Kondoa district, Dodoma Tanzania

A.R. Onesmo<sup>a,b,\*</sup>, A.N. Frida<sup>a</sup>, N.W. Alex<sup>a</sup>

<sup>a</sup> Sokoine University of Agriculture (SUA), School of Engineering and Technology, Department of Food Science and Agro-processing, P. O. Box 3006, Morogoro, Tanzania  
<sup>b</sup> Tanzania Livestock Research Institute (TALIRI), P.O. Box 202, Mtwapa, Dodoma, Tanzania

### ARTICLE INFO

**Keywords:**  
 Aflatoxins  
 Animal feeds  
 Fresh milk  
 Smallholder dairy farmers  
 Awareness

### ABSTRACT

The occurrence of aflatoxins in animal feeds and milk is a common problem that is affecting both livestock and human being leading to physiological disorders and cancer. Unfortunately, most livestock keepers are not aware of aflatoxins and their contamination in animal feeds, cause, effects, and control measures as most interventions on awareness creation have been involved with agricultural cereal crops. This study was conducted to assess awareness of aflatoxins among smallholder dairy farmers in Kondoa district to determine the effectiveness of an intervention to prevent aflatoxins. The study involved a survey using semi-structured questionnaires to 120 respondents purposively selected depending on having at least one dairy cattle in their herd. The results from this study indicated that the majority of respondents (88.3%) heard of aflatoxins through either village meetings, seminars, radio, television or from friends. About seventy eight (78.7%) of all respondents understand that aflatoxins can potentially contaminate animal feeds while 68.2% understand that aflatoxins can harm both animal health and human and results in liver cancer. Likewise, 63.3% of all respondents understand the possible transmission of aflatoxins to milk. On the other hand, about 55% of participants in this study understand factors and control measures for aflatoxins in animal feeds and milk. It is necessary to improve awareness and public training campaigns to prevent aflatoxins in the food system in many other regions in Tanzania.

### 1. Introduction

Aflatoxins are secondary metabolites of either *Aspergillus flavus* or *Aspergillus parasiticus* fungi which produce toxic substances when feed exposed to adverse environmental conditions such as temperature and humidity [1]. Other *Aspergillus* species including *A. nominus*, *A. ochraceoroseus*, *A. bombycis*, *A. fumigatus*, and *A. pseudotamari* are said to be little-identified causes of Aflatoxin [2]. These fungi are capable of growing in soil, decaying vegetation, hay and grains. Dietary aflatoxins have shown detrimental effects on animal health and production. Higher exposure causes fatal illness and sometimes death while chronic exposure has been shown to cause liver cancer, growth depression and immune suppression in both animal and human beings [3]. The most common exposure route occurs by ingestion of contaminated food or feeds. When contaminated feeds are consumed by livestock, they undergo metabolism leading to contamination of the livestock products including milk [4]. Aflatoxin B1 is the most commonly occurring and most toxic of all types when contaminated feeds are consumed, it is

further metabolized into the body and excreted with livestock products as Aflatoxin M1. Aflatoxins are considered carcinogenic, mutagenic and teratogenic agents hence high concern for control and regulation in food systems is of great importance [5].

The dairy sub-sector in Tanzania is exposed to aflatoxins through the production of contaminated milk resulting from consuming aflatoxin B1-contaminated feeds. Milk consumption is recommended by many nutritional guidelines to meet daily requirements for proteins, fat, minerals and vitamin B12. However, the trend of individual milk consumption in Tanzania is increasing slowly [6]. The average per capita milk consumption was 45 L per annum in 2010 which is very low compared to the recommended amount of 267 L per person per annum [7]. Children under five years of age are the most consumers of milk at the household level hence highly vulnerable to aflatoxins effect due to premature immune systems. The choice of cattle production system influences aflatoxins contamination in feeds and milk. Other factors are feed handling and storage practices, geographical locations, aflatoxins awareness, and the level of education [8].

\* Corresponding author. Sokoine University of Agriculture (SUA), School of Engineering and Technology, Department of Food Science and Agro-processing, P. O. Box 3006, Morogoro, Tanzania.

E-mail addresses: onesmoandrew288@gmail.com, onesmo.andrew@taliri.go.tz (A.R. Onesmo).

<https://doi.org/10.1016/j.jafr.2024.101051>

Received 2 September 2023; Received in revised form 18 January 2024; Accepted 14 February 2024

A.R. Onemo et al.

Maize bran and sunflower seed cakes are the most commonly used cereals processing by-products affected by aflatoxins. These are dependable raw materials in concentrate feed formulation. Likewise, maize postharvest residues contribute to the cattle basal diet during the dry season [9]. Therefore, cattle are likely to be exposed to aflatoxins through consumption of aflatoxins contaminated feeds including maize grain, maize silage, and maize by-products. This may be due to lack of sufficient knowledge on harvesting and post-harvest management of food and feeds against aflatoxins contamination. The early assessment conducted in several studies revealed that about 75% of the farmers lack awareness of aflatoxin control practices and the effects caused by aflatoxin-contaminated feeds. The study by Ref. [10] in Pakistan revealed that about 77.9% of farmers knew the negative impacts caused by moldy feeds but only 40% understood the possible transfer of the impacts to milk while 95% never heard of aflatoxins. Another study conducted [11] classified aflatoxins awareness according to the level of education whereby farmers with at least secondary education and formally employed were highly aware of the aflatoxins as compared to others with primary education and unemployed.

Kondoa is among districts in central zone Tanzania in Dodoma region whose farmers practice mixed agriculture producing maize, sunflower and groundnuts with herds of cattle, goats, pigs or chickens. The first aflatoxins outbreak cases in Tanzania were detected in Kondoa due to consumption of maize that was contaminated with aflatoxins. About sixty-eight (68) cases were reported in 2016 and twenty (20) people died of which above 50% were children below 15 years old [12]. Several studies have been conducted in cereal crops to assess awareness of aflatoxins among farmers. Tanzania Initiative for Prevention of Aflatoxins Contamination (TANIPAC) project, is among several interventions that have been conducted programs in the country ranging from baseline

surveys and assessment awareness, up to education campaigns on the prevention of aflatoxins contamination in food systems in Dodoma, Katavi, Simiyu, Mbeya, Morogoro, Iringa and Songwe regions [13]. These programs emphasize improving awareness of aflatoxins and its prevention strategies in cereal crops specifically maize, sunflower and groundnuts.

There are limited studies conducted to assess the awareness level of aflatoxins in animal feeds and livestock products specifically milk. This study was conducted to determine the awareness of smallholder dairy farmers of aflatoxins resulting from the consumption of contaminated feeds and milk to set up effective interventions and recommend necessary actions to better attain the objective of preventing aflatoxins exposure through feeds and food systems.

## 2. Materials and methods

### 2.1. Description of study areas

This study was conducted in Kondoa district in Dodoma region (Fig. 1). Currently, the district is divided into two administrative councils; Kondoa Town Council (916.13 Km<sup>2</sup>) and the Kondoa District Council (4640.87 km<sup>2</sup>) representing the semi-arid zone of Tanzania. This district is located at latitude -04<sup>o</sup> 54' 0" South and longitude 35<sup>o</sup> 47' 0" East about 169.9 km from Dodoma city boarded with Manyara region in the North, Nchemba district in the south, Bahi in the west and Chamwino in the East. Soils are of low fertility, Base Exchange Capacity, bulk density and water-retention capacity. Vegetation of the district comprises bushland with isolated bush grasslands. Kondoa district is wet savannah characterized by a long dry season between late April to December and a short wet season between early December and April.

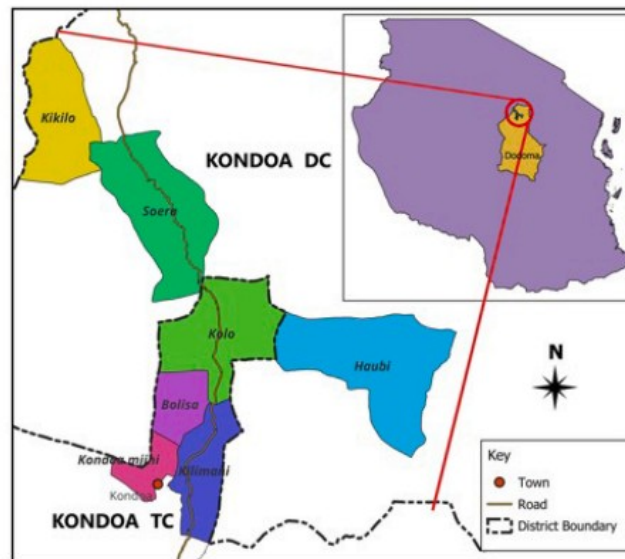


Fig. 1. Map of Tanzania showing the area where the study was conducted.

A.R. Onemo et al.

The average rainfall ranges from 400 mm in the plateau and up to 1000 mm in the highlands. The major crops grown include sorghum, millet and maize while the major cash crops are maize, groundnuts, sunflower, castor seeds and sesame.

## 2.2. Survey of smallholder dairy farmers' awareness of aflatoxins in animal feeds and fresh milk

Kondo district has approximately 18,257 smallholder dairy farmers. Out of these, 112 practice zero grazing management, 825 practice mixed grazing, and 17,320 practice pure grazing management where cattle are grazed on natural pastures or postharvest residues. To gather data, a cross-sectional survey was conducted in six purposively selected wards. These wards were chosen based on the high number of smallholder dairy cattle farmers. The selected wards were Soera, Kikiro, and Haubi from Kondo District Council, and Kondo mjini, Borisa, and Kolo wards from Kondo Town Council. From each ward, twenty (20) respondents were purposively selected to respond to the semi-structured questionnaires. These questionnaires aimed to capture farmer's awareness of aflatoxins in animal feeds and fresh milk. The questionnaires included sections on demographic information, feeding practices, handling and storage practices, and awareness of aflatoxins. Permission to conduct the survey was obtained to ensure the willingness of respondents to participate and answer the questionnaire. A total of 120 dairy farmers, each milking at least one cow, participated in this survey.

## 2.3. Data collection

The data were collected through direct interviews with respondents through a semi-structured questionnaire. Demographic information covered the age of the respondents, sex and marital status, level of education, occupation and the farmer's experience with dairy cattle keeping. Also, the questionnaire sought information on aflatoxins awareness among respondents. This part included questions seeking to know if they have ever heard of aflatoxins, the source of information, and the respondent's ability to recognize feed contaminated with aflatoxins. Through this part, the data for awareness on the possibility of aflatoxins causing cancer in both humans and livestock, harming animals and transfer to milk from contaminated feeds were collected and recorded. Respondents' awareness of factors influencing aflatoxins contamination, their control measures, possibility of feed detoxification, and if they have received any training covering aflatoxins were asked. Data were recorded instantly on the field each questionnaire was given a

specific identity during data entry and analysis.

## 2.4. Data analysis

The data for variables in the questionnaire were coded and analyzed using IBM Statistical Package for Social Sciences (SPSS) version 26 software (IBM Corp, Armonk, New York, NY, United States, 2011). Frequency distribution, percentage, p-value and chi-square were produced and assessed.

## 3. Results

### 3.1. Demographic information

#### 3.1.1. Number of respondents, age groups and marital status of smallholder dairy farmers involved in the study

A total of 120 smallholder dairy farmers 77.5% males and 22.5% females were involved in this study (Fig. 2). Participants were grouped into different age groups, 18–30 (5%), 31–40 (17.5%), 41–50(22.5%), 51–60 (31.7%), 61–70(13.3%) and the last group with age above 70 constituted 12 (10%) respondents. The study categorized the marital status of the dairy farmers as married 113 (94.2%), single 3 (2.5%), widow 3 (2.5%), and 1 divorced female (0.8%) (see Table 1).

#### 3.1.2. Respondents' main occupation, education level and experience in livestock keeping

The results in Fig. 3 (a) and (b) showed that the majority of respondents (84.2%) in this study had primary education while 5.8% had secondary education, respondents with college to university level were only 7.5% while 2.5% had no formal education. Of the main farmer's occupation, 93.3% conducted both farming and livestock and 6.7% of respondents were employed in different sectors and involved with livestock keeping as an alternative source of income. On the other hand, the duration they have been involved in livestock keeping was found to differ among themselves. The study showed that 70% of respondents had experience in livestock keeping for more than 10 years followed by 16.7% of respondents with less than 5 years while 13.3% were those with 5–10 years' experience in subsector.

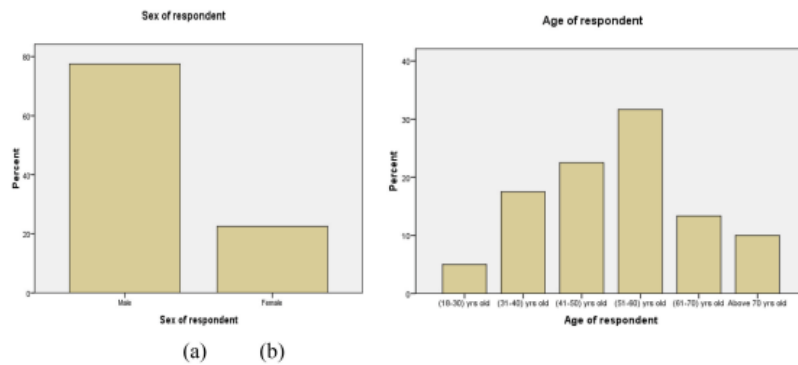


Fig. 2. Sex (a) and age groups of respondents (b) smallholder dairy farmers at Kondo.

**Table 1**  
Demographic information of the smallholder dairy farmers in Kondoa district council and Kondoa town council.

Variables	Categories	District Council Frequency (%)	Town Council Frequency (%)	Chi-squares	P values
Sex	Male	52 (55.9)	41 (44.1)	4.269	0.039*
	Female	9 (33.3)	18 (66.7)		
Age (yrs)	18-30	3(50.0)	3 (50.0)	4.963	0.420 <sup>ns</sup>
	31-40	11(52.4)	10 (47.6)		
	41-50	9 (33.3)	18 (66.7)		
	51-60	22 (57.9)	16 (42.1)		
	61-70	10 (62.5)	6 (37.5)		
	70 above	6 (50.0)	6 (50.0)		
Marital status	Single	0 (0.0)	3 (100.0)	3.189	0.203 <sup>ns</sup>
	Married	59 (52.2)	54 (47.8)		
	Divorced	0 (0.0)	0 (0.0)		
	Widow	2 (50.0)	2 (50.0)		
Education Level	No Formal	1 (33.3)	2 (66.7)	6.691	0.082 <sup>ns</sup>
	Primary	55 (54.5)	48 (45.5)		
	Secondary	4 (57.1)	3 (42.9)		
	College	1 (11.1)	8 (88.9)		
Occupation	Livestock keeping	0 (0.0)	0 (0.0)	5.039	0.025*
	Farming & Livestock	60 (53.6)	52 (46.4)		
Duration	Employed & livestock	1 (12.5)	7 (87.5)	0.357	0.836 <sup>ns</sup>
	Less than 5 years	9 (45.0)	11 (55.0)		
	5-10 years	8 (50.0)	8 (50.0)		
	10 years above	44 (52.4)	40 (47.6)		

Statistically significant at \* p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns = not significant.

**3.2. Smallholder dairy farmers' awareness of aflatoxins in animal feeds and milk**

**3.2.1. Farmers' awareness of aflatoxins and their potential to contaminate animal feeds**

The study showed that, respondents have heard of aflatoxins from various sources of information including village meetings which involved extension officers, newspapers, seminars, radio and television, at school and from friends. The association of the source of information and the awareness of aflatoxins among the councils in Kondoa district is as shown in Table 2.

On the other hand, the association between nominal and ordinal

variables used for assessing awareness among smallholder dairy farmers in Kondoa district is shown in Table 3.

**3.2.2. Effects of aflatoxin-contaminated feeds**

Table 4 showed that 31.8% of the surveyed farmers were not aware that aflatoxin causes liver cancer when humans and animals were exposed to aflatoxin-contaminated food/feeds. Regarding the possible transmission of the aflatoxin effects to milk, 36.7% of respondents did not understand that aflatoxin can be transferred to milk and milk products.

**3.2.3. Factors influencing aflatoxin contamination and control measures for prevention**

The results from this study show that 57.5% of farmers understood at least one factor influencing aflatoxin contamination whereas 42.5% were not aware (Table 5). The most suggested reasons were poor feed storage practices (15%), high moisture content (10.8%) and poor harvesting practices (2.5%), whereas 29.2% were able to state both three factors as the cause of aflatoxins contamination. On the other hand, 42.5% were not aware of the factors for aflatoxins. Also, on the control

**Table 2**  
Association between source of information and awareness of aflatoxin among smallholder dairy farmers at Kondoa (Kondoa District Council & Kondoa Town Council).

Variable	Response	Kondoa DC	Kondoa TC	Chi-square	p-value
Where have you heard it from	Village meeting/extension officers	42 (66.7%)	21 (33.3%)	23.205	0.001**
	Newspaper	3 (100.0%)	0 (0.0%)		
	Seminar	0 (0.0%)	2 (100.0%)		
	Radio/TV	2 (13.3%)	13 (86.7%)		
	Friend	1 (20.0%)	4 (80.0%)		
	School	0 (0.0%)	0 (0.0%)		
	Both of the above	8 (44.4%)	10 (55.6%)		
	None of the above	5 (35.7%)	9 (64.3%)		

Statistically significant at \* p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns = not significant.

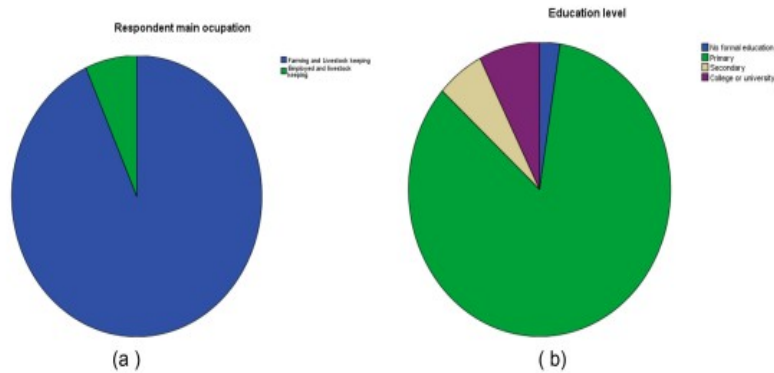


Fig. 3. Respondents' main occupation (a) and their education level presented in (b) above.

A.R. Onemo et al.

**Table 3**  
Association between variables and the general awareness of aflatoxin among farmers in Kondoa DC & TC.

Variables	Response	District Council	Town Council	Chi-squares	P values
		Frequency	Frequency		
Have you heard of the word aflatoxins before?	Yes	56(52.8%)	50 (47.2%)	1.450	0.229 <sup>ns</sup>
	No	5 (35.7%)	9 (64.3%)		
Do aflatoxins cause liver cancer in humans and animals	Yes	52 (63.4%)	30 (36.6%)	16.400	0.000 <sup>***</sup>
	No	9 (23.7%)	29 (76.3%)		
Can aflatoxins harm your animals	Yes	50 (61.0%)	32 (39.0%)	10.658	0.001 <sup>**</sup>
	No	11 (28.9%)	27 (71.1%)		
Have you ever trained on aflatoxins?	Yes	46 (65.7%)	24 (34.3%)	14.885	0.000 <sup>***</sup>
	No	15 (30.0%)	35 (70.0%)		
What was the training about	Proper storage	4 (80.0%)	1 (20.0%)	28.330	0.000 <sup>***</sup>
	New storage technique	2 (100.0%)	0 (0.0)		
	G.A.P	19 (95.0%)	1 (5.0%)		
	Both	22 (48.9%)	23 (51.1%)		
	None	14 (29.2%)	34 (70.8%)		

Statistically significant at \*  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns = not significant.

measures, 55.8% of farmers were aware of aflatoxins control measures. Some farmers reported good agriculture practices (G.A.P) (28.3%), good storage practices (20.8%), and good harvesting practices (5.8%) as control measures for aflatoxins contamination. However, 44.2% of respondents were not aware of any control measures. On the possibility of aflatoxins detoxification and rendering feed safe, 94.2% reported no possible method to detoxify the aflatoxins contaminated feeds while 5.8% of farmers said aflatoxins feeds can be detoxified.

#### 3.2.4. Possible training on aflatoxins awareness and control measures application

During this study, 58.3% of farmers reported having received short term training on aflatoxins, the causes and control measures application whereas 41.7% of farmers had not received any training on aflatoxins (Table 5). The training covered proper storage practices, the use of new storage techniques (hermetic storage), and Good Agriculture Practices (G.A.P) all aimed at mitigating aflatoxins contamination from food systems.

## 4. Discussion

### 4.1. Gender responsibility

This study involved 120 smallholder dairy farmers, the majority were males and about a quarter of the respondents were females. These results suggest that males are the main participants in managing dairy cattle as compared to females hence highly responsible for overall management activities. The gender issue and division of responsibilities are observed in this study. Females were mainly responsible for taking care of their families while males around 51–60 years old were responsible for the overall management of the family and division of responsibility among members of the family. These findings are contrary

**Table 4**  
Association between respondent awareness of aflatoxins in animal feeds/milk with location.

Variables	Response	District Council	Town Council	Chi-squares	P values
		Frequency	Frequency		
Do you know that aflatoxins can contaminate animal feeds	Yes	50 (53.8%)	43 (46.2%)	1.420	0.223 <sup>ns</sup>
	No	11 (40.7%)	16 (59.3%)		
Can you recognize signs of feed contaminated with aflatoxins?	Yes	44 (52.4%)	40 (47.6%)	0.268	0.604 <sup>ns</sup>
	No	17 (47.2%)	19 (52.8%)		
Can aflatoxins be transferred to milk from consumed contaminated feeds	Yes	48 (63.2%)	28 (36.8%)	12.597	0.001 <sup>**</sup>
	No	13 (29.5%)	31 (70.5%)		
Do you know the factors influencing aflatoxin contamination	Yes	45 (65.2%)	24 (34.8%)	13.440	0.000 <sup>***</sup>
	No	16 (31.4%)	35 (68.6%)		
Do you know the control measures	Yes	44 (55.7%)	23 (34.3%)	13.364	0.000 <sup>***</sup>
	No	17 (32.1%)	26 (67.9%)		
Can aflatoxins be detoxified and render the feeds safe?	Yes	5 (71.4%)	2 (28.6%)	1.262	0.261 <sup>ns</sup>
	No	56 (49.6%)	57 (50.4%)		

Statistically significant at \*  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns = not significant.

to study by Ref. [11] from other districts of Tanzania such as in Arumeru where in addition to family rearing done by females, they are more responsible as compared to males. In Kondoa, men are generally involved with routine management practices required for dairy cattle. This may be the reason why men were more voluntarily willing to respond to the questionnaires as compared to females. In contrast to the findings by Ref. [3] in Morogoro rural and Kilosa districts, women have limited access to and right over resources that they can utilize to improve their livelihood as compared to men. Likewise, the findings differ from the study by Ref. [3] who found that most women are the key participants and decision-makers in Kenya dairy enterprises.

### 4.2. Demographic information among smallholder dairy farmers

Demographic information includes the socio-economic and cultural behaviors of the given society. This study has demonstrated the relationship between marital status (single, married, divorced, or widow) and some aflatoxin variables. It was found that the majority of respondents were married as compared to others. This implies that the dairy enterprise needs collaborative efforts and division of responsibilities for which men feel to participate in dairy cattle production while women perform family caring in addition to farming activities. It is probably hard for a single person to be involved with cattle production as compared to couples who can perform a division of work based on age and sex groups. The awareness of aflatoxins differs among them as a result of social-cultural behavior [14].

Likewise, in this study majority of respondents performed mixed farming managing both livestock and crop production activities, while others were employed people performing livestock keeping as their additional source of income. These support findings that livestock keeping is performed by people of different professional backgrounds and age groups. Unless a community awareness program is conducted,

**Table 5**  
Association between some variables and the awareness of aflatoxin contamination and possible control measures.

Variables	Response	District Council	Town Council	Chi-squares	P values
		Frequency	Frequency		
Source of information on aflatoxins	Village meeting/extension officers	42 (66.7%)	21(33.3%)	23.205	0.001**
	Newspaper	3 (100.0%)	0 (0.0%)		
	Seminar	0 (0.0%)	2 (100.0%)		
	Radio/TV	2 (13.3%)	13 (86.7%)		
	Friend	1 (20.0%)	4 (80.0%)		
	School	0 (0.0%)	0 (0.0%)		
	Both of the above	8 (44.4%)	10 (55.6%)		
	None of the above	5 (35.7%)	9 (64.3%)		
Mention signs for aflatoxins-contaminated feeds.	Moisture/moldy	19 (38.8%)	30(61.2%)	10.246	0.069 <sup>ns</sup>
	Yellowish	11 (57.9%)	8 (42.1%)		
	Blackish	5 (100.0%)	0 (0.0%)		
	Greenish	1 (100.0%)	0 (0.0%)		
	Both of the above	8 (66.7%)	4 (33.3%)		
	None of the above	17 (50.0%)	17(50.0%)		
Mention factors influencing aflatoxins contamination	High moisture content	10 (76.9%)	3 (23.1%)	14.355	0.006*
	Poor harvesting practices	2 (66.7%)	1 (33.3%)		
	Poor storage practices	11 (61.1%)	7 (38.9%)		
	Both of the above	22 (62.9%)	13 (37.1%)		
	None of the above	16 (31.4%)	35 (68.6%)		

Statistically significant at \*  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns = not significant.

uneducated and primarily educated farmers normally have insufficient information on aflatoxins and their impacts as compared to educated/employed farmers [11].

Determination of chi-square correlation test results on the age of respondents, marital status, educational level and duration which they have spent in livestock keeping revealed no association with awareness of aflatoxins in animal feeds and milk in Kondo. This is contrary to the findings by Ref. [11] which revealed evidence of association between education level and awareness of aflatoxins. However, the sex and occupation of the respondents revealed strong evidence of association with aflatoxins awareness among respondents. This may be because males are considered as the head of the households hence a higher chance to participate in coordinated training and seminars on aflatoxins than females in Kondo. Likewise, most of the respondents' occupations were both farmers and livestock keepers while a small proportion were employed and performed livestock keeping as an alternative source of income. This has shown a significant association with awareness of aflatoxins among smallholder farmers in Kondo ( $p = 0.039$ , and  $p = 0.025$  for sex and occupation respectively) at a 5% level of significance. The employed respondent seems to have a higher chance of awareness of aflatoxins and their effects in animal feeds and milk due to their professionalism which exposed them to science subjects like chemistry and biology where microbiology is the part of their study. This agrees with the findings by Ref. [15] that educated household members are more knowledgeable about aflatoxins in the Democratic Republic of Congo (DRC).

#### 4.3. Awareness of aflatoxins

Community education programs on aflatoxins information have been found to improve awareness among smallholder dairy farmers in various areas that previously had low levels of aflatoxins awareness. In Kondo, the majority (88.3%) of smallholder dairy farmers were aware of aflatoxins and heard it through mainly village meetings coordinated and organized by extension officers in their respective wards or villages. Other methods such as seminars, mass media communication, and sharing information with friends have been shown to contribute to aflatoxins awareness. This is almost similar to the studies by Refs. [10,16] in Uganda and Pakistan, respectively. These studies revealed an increase in awareness above 80% in the communities that had a historical background of aflatoxin outbreaks. In addition to that, awareness of aflatoxins contamination in animals and milk was found to be higher

when compared to the previous area where the study was conducted.

Although more respondents heard of aflatoxins as shown in Table 2, about half of them were not aware of the factors influencing aflatoxins contamination. This may be caused by different means by which they heard aflatoxins such as radio and television, newspaper or from friends. Although mass media communication may organize sufficient information to present, the community's attention to presentation in mass media may be very low. This may lead to insufficient information for the audience about aflatoxins awareness in general. The report by Ref. [17] pointed out that, aflatoxins message structured and presented in mass communication on aflatoxins makes it difficult to understand the terminologies used as the audience is mainly smallholder dairy farmers who are primarily educated hence low follow-up and no audience response. Nathaniel [17] proposed training supplemented by audio-visual method and computer-animated sequences as powerful means to create aflatoxins awareness. Association between some variables and awareness of aflatoxins was shown in Table 2 implying that, response to whether they heard of aflatoxins revealed no significant association to aflatoxins awareness. The awareness of aflatoxins' effects such as causing cancer, harming animals, training to aflatoxins, and the content of training shows association with awareness of aflatoxins in general.

#### 4.4. Awareness, causes and effects of aflatoxins contamination

Understanding the factors favoring aflatoxins production and contamination in animal feeds is the key step to the application of the appropriate measures for control. In this study, more than 50% of the smallholder farmers were able to mention at least one factor influencing aflatoxins contamination. Of this, the majority mentioned high moisture/moldy contents, and poor harvesting and storage practices as the major factors influencing aflatoxins production and hence contamination. In addition to that, more than three-quarters of the respondents understood the possible contamination in feeds and milk, the aflatoxins possibility to harm animals, the signs to recognize aflatoxins contaminated feeds and factors that favours aflatoxin production, and their control measures. The majority listed moldy/high moisture content food as the sign to identify contaminated feeds. Other signs included a change in the color of feeds to yellowish, blackish, and greenish which constitute 71.3% of the respondents who were able to mention at least one sign of aflatoxins contamination. These findings emphasize the role of public education campaigns in awareness creation.

A.R. Onesmo et al.

The effects of aflatoxins are classified as acute and chronic depending on the amount of toxin contamination and duration by which animal or human beings were exposed. This study reported more than two-thirds of surveyed respondents knew that aflatoxins can harm humans and animals leading to liver cancer, retarded growth, and immune suppression. Also, more than sixty percent of farmers were found to understand the possible transmission of aflatoxins to milk when animals consumed aflatoxins-contaminated feeds. However, surveyed farmers were not able to differentiate between the short-term and long-term effects of aflatoxicosis. This may cause failure to accurately differentiate some of the acute effects on animals such as vomiting, depression, hemorrhage, jaundice, general body weakness, and lack of appetite which are more similar to other diseases hence inaccurate diagnosis that contributes to increased veterinary costs due to ineffective treatments [18].

#### 4.5. Aflatoxins detoxification

Various studies have suggested some physical, chemical, and biological methods for aflatoxins detoxification. These include Fluorescence-based automatic sorting, Ozone treatment, microbial decontamination and cold plasma treatment [19]. In this study, 94.2% of all respondent farmers were not aware of any feed detoxification methods. Even a few respondents who agreed on the possibility of detoxification were not able to mention any method. This implies that people are not aware of any method that can be applied to feeds and render them safe from aflatoxins. In contrast to the study by Ayo [11] and Nakavuma [16] farmers suggested methods like the use of binders, soda ash, charcoal, salt and some herbs as means of aflatoxins detoxification. However, it was pointed that some methods have less to do with detoxification rather than preservatives to increase shelf life. The study by Ref. [13] is urban and peri-urban dairy farmers in Kenya revealed the use of aflatoxin binders such as NovaSil binders and benzonite clays which are rich in Montmorillonite had positive effects of reducing aflatoxin and hence leads to production of milk which with reduced amount of AFM1 when farmers are trained on the application of the binders in prepared feeds supplements. Therefore, farmers are advised to use the aflatoxin binders in feeds to control aflatoxin occurrence in milk. It was also pointed out that, training on application of Good Agriculture Practice is an efficient method to reduce aflatoxins in food system especially when designed training considered local applicability [20].

#### 4.6. Role of training in mitigating aflatoxins

Public education campaigns have been reported as a means to raise aflatoxins awareness among farmers. When training is planned, designed and coordinated appropriately with community mobilization and awareness of aflatoxins, it has been shown to raise the involvement of people in aflatoxins mitigation strategies. In this study, the majority of smallholder dairy (70%) have already received training on aflatoxins. This training covered Good Agriculture Practices (G.A.P), good harvesting practices and good storage practices using new storage techniques as a key means to mitigate aflatoxins in food and feed. Because Kondoa is among districts with a historical background of aflatoxins cases, various programs previously implemented have helped to increase awareness among farmers. It was expected that various interventions would create awareness of aflatoxins in cereal crops rather than animal feeds and milk. This study has proved the effectiveness of various intervention contributions to raise awareness of aflatoxins, its effects on human, and livestock health and products, and factors favoring production and control measures in both humans and livestock. On the other hand, these findings are in line with the study by Yunus [10] which reported that 95% of farmers in Pakistan were not aware of aflatoxins, but after conducting awareness training, the understanding increased to 98.3%. Also, the study by Ref. [20] found the majority of

farmers were not aware of aflatoxins, how animals get aflatoxins, and how it is transferred to milk. Recent findings [10] in Pakistani and [21] in Kenya reported an increase in the level of aflatoxins awareness to about 80% after conducting several training and awareness campaign programs.

## 5. Conclusion and recommendations

The majority of livestock farmers in Kondoa are aware of aflatoxins in cereal crops, animal feeds and milk. The awareness program intervention in central regions of Tanzania including Dodoma and Singida which was earlier organized and implemented by extension officers through village meetings with smallholder farmers is among the most important reasons for this achievement. Even though the majority have heard about aflatoxins, a few of them still have no sufficient information on aflatoxins causes, effects and control measures. It is emphasized that, more public awareness programs must be conducted preferably through village meetings and or seminars by making aflatoxins the permanent agenda. Members who represent the households must share information about aflatoxins with others at the family level. It is further recommended to establish and support more interventions in cereal and sunflower crop producers in various regions of Tanzania. Training programs supplemented with audio-visual and computer-automated series must be prepared and applied. Also, the simple designed mobile messages with summarized information on aflatoxins awareness to effectively communicate to the public is recommended. This will enable the mobile users to receive various information covering subtopics in aflatoxins that will leads to improved awareness of aflatoxins among the society in general.

#### Funding

This study was funded by the Ministry of Agriculture under Tanzania Initiative for Prevention of Aflatoxins Contamination (TANIPAC) project.

#### CRediT authorship contribution statement

A.R. Onesmo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. A.N. Frida: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. N.W. Alex: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

#### Acknowledgments

The authors acknowledge the potential support from various institutes and departments including the TANIPAC project for its financial support, my supervisors in Department of Food Science and Agro-processing, Tanzania Livestock Research Institute (TALIRI), extension staffs form Department of Livestock and Fisheries Development at Kondoa DC & TC, and Tanzania Bureau of Standards leads to the fulfillment of this study. Last but not least, much appreciations are due

A.R. Onemo et al.

to the farmers who participated in the study, without whom, this study could not be feasible.

#### References

- [1] Y. Jiang, I.M. Ogunade, D. Vyas, A.T. Adesogan, Aflatoxin in dairy cows: Toxicity, occurrence in feedstuffs and milk and dietary mitigation strategies, *Toxins* 13 (4) (2021), <https://doi.org/10.3390/toxins13040283>.
- [2] A. Jallow, H. Xie, X. Tang, Z. Qi, P. Li, Worldwide aflatoxin contamination of agricultural products and foods: from occurrence to control, *Compr. Rev. Food Sci. Food Saf.* 20 (3) (2021) 2332–2381, <https://doi.org/10.1111/1541-4337.12734>.
- [3] T.N. Kiama, et al., Kenya dairy farmer perception of moulds and mycotoxins and implications for exposure to aflatoxins: a gendered analysis, *Afr. J. Food Nutr. Sci.* 16 (3) (2016) 11106–11125, <https://doi.org/10.18697/ajfand.75.11R110>.
- [4] J. Fink-Gremmels, Mycotoxins in cattle feeds and carry-over to dairy milk: a review, *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* 25 (2) (2008) 172–180, <https://doi.org/10.1080/02652030701823142>.
- [5] M.J. Mughal, X. Peng, A.A. Kamboh, Y. Zhou, J. Fang, Aflatoxin B1 Induced systemic toxicity in poultry and Rescue effects of selenium and zinc, *Humana Press Inc. Biol. Trace Elem. Res.* 178 (2) (2017) 292–300, <https://doi.org/10.1007/s12011-016-0923-9>, Aug. 01
- [6] P. Wang, et al., Spatial and temporal patterns of consumption of animal-source foods in Tanzania, *Agribusiness* 38 (2) (2022) 328–348, <https://doi.org/10.1002/agb.23728>.
- [7] I. P. T. Report, Tanzania Smallholder Dairy Value Chain Development : Situation Analysis and Trends.
- [8] S.J. Kitigwa, E.G. Kimaro, Y.P. Nagagi, J.B. Kussaga, R.A. Suleiman, A. Matemu, Occurrence and associated risk factors of aflatoxin contamination in animal feeds and raw milk from three agroecological zones of Tanzania, *World Mycotoxin J.* 16 (2) (May 2023) 149–163, <https://doi.org/10.3920/wmj.2022.2891>.
- [9] R. Zamora-analberia and F. Granados-chinchilla, "We Are IntechOpen , the World ' S Leading Publisher of Open Access Books Built by Scientists , for Scientists TOP 1 %".
- [10] A.W. Yunus, J.F. Lindahl, Z. Anwar, A. Ullah, M.N.M. Ibrahim, Farmer's knowledge and suggested approaches for controlling aflatoxin contamination of raw milk in Pakistan, *Front. Microbiol.* 13 (October) (2022) 1–10, <https://doi.org/10.3389/fmicb.2022.980105>.
- [11] E.M. Ayo, A. Matema, G.H. Laswal, M.E. Kimanya, Socioeconomic characteristics influencing level of awareness of aflatoxin contamination of feeds among livestock farmers in meru district of Tanzania, *Scientifica (Calto)*. 2018 (2018), <https://doi.org/10.1155/2018/3485967>.
- [12] A. Kamala, et al., Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro ecological zones of Tanzania, *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* 33 (3) (2016) 551–559, <https://doi.org/10.1080/19440049.2016.1138546>.
- [13] G. Anyango, et al., Effectiveness of training and use of novastil binder in mitigating aflatoxins in cow milk produced in smallholder farms in urban and periurban areas of Kenya, *Toxins* 13 (4) (2021), <https://doi.org/10.3390/toxins13040281>.
- [14] M. Sabran, et al., Socio-demographic and socio-economic determinants of adults' knowledge on fungal and aflatoxin contamination in the diets. *Asian Pacific Journal of Tropical Biomedicine, Asian Pac. J. Trop. Biomed.* (2012) 1835–1841 [Online]. Available: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb).
- [15] P. Udoukuu, T.W.N. L. N. Charity, M. Bernard, V. Ranjith, Incidence and Farmers' Knowledge of Aflatoxin Contamination and Control in Eastern Democratic Republic of Congo, 2018, pp. 1607–1620, <https://doi.org/10.1002/lns3.735>, no. April.
- [16] J.L. Nakavuma, A. Kirabo, P. Bogere, M.M. Nabulime, A.N. Kaaya, B. Gnononfin, Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda, *Int. J. Food Contam.* 7 (1) (2020) 1–10, <https://doi.org/10.1186/s40550-020-00079-2>.
- [17] N. Nathaniels, Communicating aflatoxin messages: recent Tanzanian experience, July, *Int. Inst. Trop. Agric.* (2014) 1–41 [Online]. Available: International Institute of Tropical Agriculture July, 2014.
- [18] F. Peles, et al., Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock, *Front. Microbiol.* 10 (2019), <https://doi.org/10.3389/fmicb.2019.02381>, Frontiers Media S.A., Dec. 11.
- [19] H. Marshall, et al., Novel decontamination approaches and their potential application for post-harvest aflatoxin control, *Trends Food Sci. Technol.* 106 (November) (2020) 489–496, <https://doi.org/10.1016/j.tfs.2020.11.001>.
- [20] G.N. Marete, et al., Effects of the use of good agricultural practices on aflatoxin levels in maize grown in nandi county, Kenya, *Science* 2 (4) (2020) 1–16, <https://doi.org/10.3390/sci2040085>.
- [21] I. Kagera, et al., Status of aflatoxin contamination in cow milk produced in smallholder dairy farms in urban and peri-urban areas of Nairobi County : a case study of Kasarani sub county , Kenya, *Infect. Ecol. Epidemiol.* 9 (1) (2019), <https://doi.org/10.1080/2008686.2018.1547095>.

CHAPTER THREE  
PAPER II



*European Journal of Nutrition & Food Safety*

Volume 15, Issue 12, Page 10-21, 2023; Article no.EJNFS.109989  
ISSN: 2347-5641

## Aflatoxins Contamination in Animal Feeds and Fresh Milk in Kondoa District, Tanzania

Onesmo A. Robert <sup>a\*</sup>, Frida A. Nyamete <sup>a</sup>,  
Mwemezi L. Kabululu <sup>b</sup> and Alex W. Ngungulu <sup>a</sup>

<sup>a</sup> Sokoine University of Agriculture (SUA), P.O. Box 3006, Morogoro, Tanzania.

<sup>b</sup> Tanzania Livestock Research Institute (TALIRI), P.O. Box 202, Mpwapwa - Dodoma, Tanzania.

### Authors' contributions

This work was carried out in collaboration among all authors. Authors OAR, FAN and AWN helped in conceptualization and designing methodology. Authors OAR and MLK did software analysis. Authors FAN, AWN and MLK did data investigation, curation and validation. Authors OAR and MLK did formal analysis. Authors OAR, FAN, AWN and MLK did resources and wrote, reviewed and edited the manuscript and data visualization. Author OAR wrote original draft of the manuscript. Authors FAN and AWN supervised the study. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/EJNFS/2023v15i121384

### Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/109989>

Original Research Article

Received: 27/09/2023

Accepted: 04/12/2023

Published: 11/12/2023

### ABSTRACT

**Abstract:** The occurrence of aflatoxins in animal feeds and milk is common around the World as most livestock keepers are not aware of aflatoxins.

**Aims:** This study was conducted to assess storage and feeding practices, and assessment of aflatoxin B1 and M1 contamination in animal feeds and fresh milk samples in Kondoa.

**Study Design:** The study involved a survey using semi-structured questionnaires and Laboratory analysis of aflatoxin B1 and aflatoxin M1 in animal feeds and fresh milk samples collected from among smallholder dairy farmers.

**Methodology:** Purposive selection of 120 respondents among smallholder dairy farmers in six

\*Corresponding author: Email: onesmoandrew288@gmail.com;

wards purposively selected due to having larger number of dairy cattle was conducted. Likewise, 20 samples of animal feeds and 25 composite milk samples from among 120 interviewed farmers were analysed for aflatoxin B1 and M1 using High-Performance Liquid Chromatography (HPLC).

**Results:** The results indicates that, aflatoxin B1 in feeds was 55%, with mean concentration of  $8.6 \pm 5.9 \mu\text{g/kg}$  ranging from the lowest limit of detection (LOD) to  $21.1 \mu\text{g/kg}$  with 5% samples above the maximum level stipulated by the Tanzania Bureau of Standards (TBS) and European Union (EU) of  $20 \mu\text{g/kg}$ . Likewise, about 44% of the milk samples revealed to contain traces of aflatoxin M1 with a mean amount of  $0.07 \pm 0.08 \mu\text{g/L}$ , ranging from the limit of detection (<LOD) to  $0.23 \mu\text{g/L}$ , and 12% exceeding the TBS and EU standards of  $0.05 \mu\text{g/kg}$ .

**Conclusion:** There is need to scale up aflatoxin awareness campaigns and regular monitoring throughout the food system in Tanzania.

#### GRAPHICAL ABSTRACT:



**Keywords:** Aflatoxin B1; Aflatoxin M1; Animal feeds; Fresh milk; Occurrence; Smallholder dairy farmers; and High-Performance Liquid Chromatography (HPLC).

## 1. INTRODUCTION

Aflatoxins refer to the class of mycotoxins produced by *Aspergillus flavus* or *Aspergillus parasiticus*. Production of aflatoxins occurs when crops, specifically maize, or their processed by-products (maize bran) and groundnuts, are exposed to favorable environments of high temperature and moisture content. Other *Aspergillus* species, such as *A. nominus*, *A. ochraceoroseus*, *A. bombycis*, *A. fumigatus*, and *A. pseudotamari*, are said to be little-identified causes of aflatoxin [1]. About 20 different types of aflatoxins have been identified in different studies. However, the most common types of aflatoxins are Aflatoxins B1, B2, G1, and G2. The metabolic products after aflatoxin B1 and B2; aflatoxins M1 and M2, are commonly found in animal products such as eggs, meat, and milk when livestock consume aflatoxin-contaminated feeds. Aflatoxin B1 (AFB1) and its metabolite, aflatoxin M1 (AFM1), are the most potent of all aflatoxins and are classified as Type 1 carcinogenic by the International Agency for

Research on Cancer (IARC) in humans, due to their ability to cause cancer.

Aflatoxins contaminate animal feeds such as maize bran, sunflower seed cakes, silage, and hay, typically before and after harvest, and during storage. When these feeds are consumed by livestock, they are metabolized in the digestive system and then in the liver, where further metabolism takes place, and excreted in milk/meat/eggs in the form of AFM1. Studies show that about 0.5-5% of the AFB1 consumed in feeds is converted to AFM1 in milk [2]. The toxicity of AFM1 is less potent compared to that of AFB1 in foods, despite both being classified as class 1 carcinogenic toxins [3].

The occurrence of AFB1 in animal feeds and AFM1 in milk has been reported in different parts of the world. However, aflatoxins are said to be highly prevalent in areas around latitudes  $40^\circ$  north and south of the equator. The study by Strosnider [4] found that developing countries in the tropics are at higher risk of aflatoxin

contamination than others due to the presence of favorable conditions of temperature and humidity that lead to aflatoxin production. Therefore, milk is likely to be contaminated with aflatoxins as a result of livestock consuming AFB1-contaminated feeds. Milk acts as a good source of AFM1 contamination when consumed directly or in dairy products. The study Kagera [5] found that people in urban and peri-urban areas consume twice the amount consumed by the rural population, thus making them more exposed to AFM1.

The effects of aflatoxins in human and livestock depend on various factors including age, genetic, physiology, and the environment. Research has shown that young animals are more susceptible to aflatoxins than adults because their livers are not yet fully developed to handle detoxification and other metabolic processes [6]. Aflatoxins can reduce productivity and reproductive performance by impairing nutritional and physiological functions, as well as overall health [7]. In addition to these physiological impairments, aflatoxins can lead to loss of animal life, decreased livestock production, disposal of contaminated feeds and products, and increased costs for veterinary care due to acute and chronic illnesses in livestock. Long-term exposure to aflatoxins can also result in chronic health effects, such as liver damage, for both cows and humans [8]. In humans, the breakdown of aflatoxins can contribute to the development of chronic diseases or severe cancers, leading to increased morbidity and mortality rates [9].

Aflatoxin levels in grain foods and feeds are subject to strict regulations in more than 60 countries worldwide. The Tanzania Bureau of Standards (TBS) has set the maximum tolerable level of aflatoxin B1 in foods as 5 µg/kg, while 10 µg/kg is the maximum level for total aflatoxins count [10]. However, AFB1 in feeds intended for dairy cattle is limited to 20 µg/kg, while AFM1 levels in milk are limited to 0.05 µg/L according to TBS, Codex standard, and United States Food and Drug Authority (USFDA). Studies conducted in some parts of Tanzania including Dar es Salaam, Morogoro, and Singida revealed higher levels of AFM1 in milk ranging from the limit of detection (LOD) to 2.007 ng/ml, as compared to the maximum daily intake of 1 ng/kg body weight/day for an adult [11-13]. The current study was conducted to assess the contamination of AFB1 in animal feeds and the occurrence of AFM1 in fresh milk, and to recommend the safety status of feeds and milk in

the Kondoa district and what should be done to minimize aflatoxin contamination throughout Tanzania in general.

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area

Kondoa is one of the districts in Dodoma region, currently divided into two administrative councils: the Kondoa town council (916.13 sqkm) and the Kondoa district council (4640.87 sqkm). It is located at latitude 0°40'54" South and longitude 35°47'0" East, approximately 169.9 km from Dodoma city, and bordered by Manyara region to the north, Chemba district to the south, Bahi district to the west, and Chamwino district to the east. The district's soils are of low fertility, base-exchange capacity, bulk density, and water-retention capacity. The vegetation consists of bushland with isolated bush grasslands. The climate is a wet savannah, characterized by a long dry season from late April to December and a short wet season from early December to April. Average rainfall ranges from 400 mm in the plateau to 1000 mm in the highlands. Major food crops grown include sorghum, millet, and maize, while major cash crops are maize, groundnuts, sunflower, castor seeds, and sesame.

### 2.2 Study Site Selection

With the help of the District Livestock and Fisheries Officers (DLFO), a total of six wards were selected to participate in this study, three from each council (Kondoa Town Council and Kondoa District Council). The wards included Kondoa mjini, Kolo, Borisa, Soera, Kikiro, and Haubi; and were purposively selected based on their agriculture and livestock-keeping activities, with the largest population of smallholder dairy farmers.

### 2.3 Sample Size

A total of 120 smallholder dairy farmers from six wards with larger number of dairy cattle were selected for survey questionnaire to assess feed storage practices and feeding practices. Twenty (20) dairy farmers were selected from each ward. Determination of AFB1 and AFM1 were conducted on feed and milk samples collected from 25 farmers. Four smallholder dairy farmers with at least one milking cow were selected randomly from each ward, except for the Kondoa mjini ward, where five farmers were selected under the same criteria.

## 2.4 Study Design

This study involved a quantitative methodology for determining aflatoxins in animal feed and milk. Samples of feed and fresh milk were collected from 25 households selected from among the 120 households who participated in the survey for analysis of AFB1 and AFM1. Fresh milk samples were collected from the farmers from which feed samples had been taken. Samples were taken to ensure the representation of farmers who practice zero grazing, grazing and supplementation, and pure grazing management systems [14].

## 2.5 Methods

### 2.5.1 Assessment of storage and feeding practices among smallholder dairy farmers

This was conducted through a survey using a semi-structured questionnaire to assess animal feed storage and feeding practices, hence determining association between feed storage and feeding practices with awareness of aflatoxins among smallholder dairy farmers in the district. To assess feed storage practices, face-to-face interview on feed drying methods, duration of storage, feed storage, and preparation methods were conducted. Likewise, the assessment of feeding practices interview included management system, the predominant source of concentrate, frequency and amount of feeding concentrates surveyed.

### 2.5.2 Determination of aflatoxin B1 in animal feeds

#### A: Sample sources

Feed samples of 250 g were taken from each household participating in this study. The samples were packed in plastic, transparent, sealed bags and stored appropriately before being transported to the TBS food chemistry laboratory for AFB1 analysis.

#### B: Sample preparation

During laboratory analysis, 100 g of each feed sample was thoroughly ground, and then 25 g sampled from different sites within the bag was taken for AFB1 extraction. The samples were treated with a methanol-deionized water mixture prepared by mixing 5 g of sodium chloride and 300 ml of a methanol-water mixture at a ratio of

80:20 (v/v). The mixture was shaken for 30 minutes at 120 RPM to obtain a uniform mixture. The solution was filtered through filter paper, and 20 ml of the filtrate was diluted with 130 ml of deionized water. Then, 75 ml of the dilute sample extract was passed through the Aflatest 1AC column at a rate of 2-3 ml/min. The columns were washed with 15 ml of water. To elute AFB1 from the column, 0.5 ml of HPLC-grade solvent was added, followed by 0.75 ml of the same solvent one minute afterward. Finally, 3 ml of HPLC-grade water was added to the elevator, and 20 µL of the final solution was injected into the HPLC for aflatoxin B1 analysis following the procedures described by Vaz and Silva [15].

#### C: HPLC analysis

Aflatoxin quantities from the sample were determined using HPLC coupled with a fluorescence detector (FLD) (Model Agilent ChemStation Technology, Series 1260 INFINITY SERIES). The column C18 (4.6×150 mm, 5 µm), Eclipse XDB-C18, was used to separate groups of aflatoxins (AFB1, AFB2, AFG1 and AFG2). The column temperature was set at 30 °C, the flow rate at 1.0 mL/min, and the mobile phase was Water: Methanol: Acetonitrile (60:30:10 v/v). The fluorescence was excited at a wavelength of 360 nm, and the emission wavelength was set at 460 nm. The retention for AFB1 took effect after 16-17 minutes.

#### D: Quantification

Aflatoxins quantification of the fully separated AFB1 was performed by comparison with standard aflatoxin B1 using the formula and checking the linearity [16]. The concentration of AFB1 in micrograms per kilogram (µg/kg) was determined by;

$$= \frac{\text{Standard peak height} \times \text{Sample peak height} \times \text{Final volume of sample spotted}}{\text{Standard peak height}}$$

### 2.5.3 Determination of aflatoxin M1 in fresh milk

#### A: Sample taking and preparation

One hundred and fifty milliliters (150 ml) of milk were collected in sterile condition from each cow, with a maximum of three samples collected from three cows in one household. The samples were immediately stored in an ice box to ensure cold-chain storage. All samples collected from one ward were stored in a deep freezer at -20°C until all samples from all villages within the ward had

been collected. Upon completion of milk samples collection, the samples were packed into an ice box and transported to the Tanzania Bureau of Standards (TBS) laboratory in Dar-es-salaam, where they were kept frozen at -20°C before AFM1 analysis. After one month, the samples were removed from the freezer and prepared for AFM1 analysis.

#### B: Procedures

Milk samples were thawed in water at 45°C for five minutes. Milk samples from the same farmer were mixed to obtain composite samples. One hundred milliliters (100 ml) of each sample were placed into test tubes for cream separation by centrifugation at 4000 rpm. After cream separation, the milk samples were filtered to ensure that no cream remained. Then, 30 ml of skim milk was loaded into the tubes, which were finally connected to the aflatoxins column for extraction. The samples were allowed to pass through the column with an affinity to bind all aflatoxins present while milk passed down the vacuum manifold. The column was then rinsed with distilled water three times to ensure that no milk samples were left in the tube. Finally, the

AFM1 particles bound in the column were eluted by passing pure methanol through the aflatoxins column down the microtubes. Ultrafiltration using a syringe connected to a microfilter was conducted to obtain the clearest supernatants ready to be injected into the HPLC machine for detection and quantification.

#### C: HPLC Analysis

Aflatoxins M1 quantities from the samples were determined using HPLC coupled with a fluorescence detector (FLD) (Model Agilent ChemStation Technology, Series 1260 INFINITY SERIES). The fluorescent was excited at a wavelength of 365 nm while the emission wavelength was set at 450 nm, taking 16-17 minutes to retain AFM1 in the sample.

#### D: Quantification

Quantification of AFM1 was performed by loading microtubes into the HPLC set, programmed for quantification ranging from 0.02µg/L to 0.75µg/L. Data from all samples were recorded in Table 1.

**Table 1. Association between feed storage practices and awareness of aflatoxins in animal feeds and milk**

Variables	Response categories	Have you heard of the word aflatoxin before?		Chi-square	p-value
		Yes Frequency (%)	No Freq (%)		
How do you dry your feeds	Using solar	1(50.0)	1(50.0)	4.748	0.19 <sup>ns</sup>
	Sundried on polythene sheet	45(93.8)	3(6.2)		
	Open space on the ground	46(85.2)	8(14.8)		
	None	14(87.5)	2(12.5)		
How long do you store the feeds?	Less than six month	105(88.2)	14(11.8)	0.133	0.72 <sup>ns</sup>
	More than six months but less than one year	1(100.0)	0(0.0)		
How do you store feeds	Woven/sisal bag	21(100.0)	0(0.0)	5.117	0.08 <sup>ns</sup>
	Polythene bag	72(87.8)	10(12.2)		
	Heap on the ground	13(76.5)	4(23.5)		
How do you prepare concentrates	Manual mixing	59(90.8)	6(9.2)	1.026	0.60 <sup>ns</sup>
	Machine mixing	1(100.0)	0(0.0)		
	None	46(85.2)	8(14.8)		

Statistically significant at \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns = not significant

## 2.6 Statistical Analysis

Data were entered into an Excel sheet, and the saved file was imported to be analyzed using the Statistical Package for Social Science (SPSS) version 26 of 2019. Qualitative data were summarized using frequency distribution tables, while quantitative data were summarized using descriptive statistics, where mean, standard deviation (SD), standard error of the mean, range, and interquartile range were determined. Chi-square statistic was used to assess the statistical association between storage and feeding variables and awareness of aflatoxins.

## 3. RESULTS

### 3.1 Animal Feed Storage Practices

Chi-square test results on farmers' routine practices and the association to the level of awareness of factors which may cause potential aflatoxin contamination during various feed

practices during storage were as indicated in Table 1.

### 3.2 Feeding Practices among Small Holder Dairy Farmers

The determination of a chi-square test to establish an association between routine feeding practices and the awareness of aflatoxin in animal feeds and milk yielded chi-square values and p-values, as shown in the Table 2.

### 3.3 Aflatoxin Levels in Animal Feeds and Fresh Milk

The animal feeds and milk samples collected from smallholder dairy farmers were analyzed for aflatoxin B1 and aflatoxin M1 by using High-Performance Liquid Chromatography (HPLC). The results for the presence and amount of aflatoxin contaminated in each sample were tabulated in Table 3.

**Table 2. Association between feeding practices with awareness of aflatoxin contamination in animal feeds**

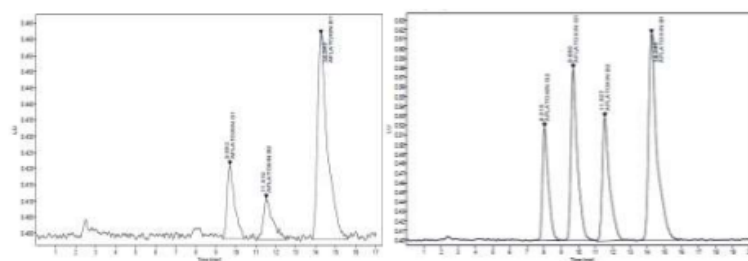
Variables	Response categories	YES Frequency (%)	NO Frequency(%)	Chi-square	p-value
How do you feed your animals	Free grazing	0(0)	0(0)	2.829	0.24 <sup>ns</sup>
	Zero grazing	35(94.6)	2(5.4)		
	Grazing and supplementation	60(87.0)	9(13.0)		
	Both zero grazing, grazing, and supplementation	11(78.6)	3(21.4)		
Predominant type of animal feed you are using	Roughages	100(87.7)	14(12.3)	.834	0.36 <sup>ns</sup>
	Mixed feeds	6(100.0)	0(.00)		
Where do you get the concentrates	Commercial	15(100.0)	0(.00)	6.342	0.4 <sup>ns</sup>
	Compounded on farm	57(91.9)	5(8.1)		
	None	34(79.1)	9(20.9)		
What type of concentrates do you feed your animals	Maize bran	5(100.0)	0(0)	7.189	0.07 <sup>ns</sup>
	Both	18(100.0)	0(0)		
	Maize bran and sunflower seedcake	44(91.7)	4(8.3)		
	None	39(79.6)	10(20.4)		
How many times are you feeding concentrate	Dairy	40(97.6)	1(2.4)	7.363	0.12 <sup>ns</sup>
	Once per week	12(92.3)	1(7.7)		
	Three times per week	14(87.5)	2(12.5)		
	Five times per week	1(100.0)	0(0.0)		
	None	39(79.6)	10(20.4)		
How much do you give them	Less than 1kg	0(0)	0(0.0)	5.868	0.05 <sup>ns</sup>
	1kg to 2kg	40(95.2)	2(4.8)		
	3kg to 5kg	26(92.9)	2(7.1)		
	None	40(80.0)	10(20.0)		

Statistically significant at \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, ns=not significant

The summary of aflatoxin contamination results for both feeds and fresh milk sample was analyzed and the measure of central tendency; mean, standard deviation and range of the quantities of aflatoxin within the samples were deduced and recorded in Table 4.

**Table 3. Aflatoxin B1 in animal feeds and aflatoxin M1 in fresh milk samples collected from smallholder dairy farmers in Kondoa district, Tanzania**

Feed Sample ID	Amount of aflatoxin B1 in feeds ( $\mu\text{g}/\text{kg}$ )	Milk sample ID	Amount of aflatoxin M1 in milk ( $\mu\text{g}/\text{L}$ )
TT14252	Not detected	TT14613	0.05
TT14253	Not detected	TT14614	Not detected
TT14254	Not detected	TT14615	Not detected
TT14255	Not detected	TT14616	Not detected
TT14256	Not detected	TT14617	Not detected
TT14257	2	TT14618	Not detected
TT14258	Not detected	TT14619	Not detected
TT14259	Not detected	TT14620	Not detected
TT14260	3.7	TT14621	0.02
TT14261	Not detected	TT14622	Not detected
TT14262	15.6	TT14623	Not detected
TT14263	21.1	TT14624	0.03
TT14264	11.9	TT14625	Not detected
TT14265	11.3	TT14626	0.04
TT14266	8.5	TT14627	0.04
TT14267	5.2	TT14628	0.23
TT14268	6.2	TT14629	Not detected
TT14269	2.8	TT14630	0.03
TT14270	6.3	TT14631	0.08
TT14271	Not detected	TT14632	0.22
		TT14633	Not detected
		TT14634	Not detected
		TT14635	0.01
		TT14636	0.01
		TT14637	Not detected



**Fig. 1. The elution pattern of the highest aflatoxin B1 in a feed sample (left) and a standard sample (right) at 360 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model**

Table 4. Aflatoxin B1 (AFB1) in animal feeds and aflatoxin M1 (AFM1) in milk, and the measure of central tendency

Laboratory analysis		Sample Detection and Quantification						Descriptive statistics	
Aflatoxins (AF)	Sample tested (n)	Limit of Detection (LOD) ( $\mu\text{g/kg}$ )	Limit of Quantification (LOQ) ( $\mu\text{g/L}$ )	of Aflatoxin sample (%)	Positive Samples exceeded TBS/EU standards (%)	The sample exceeded Codex std (%)	Mean $\pm$ STD ( $\mu\text{g/kg}$ or L.)	Range ( $\mu\text{g/kg}$ or L.)	
AFB1	20	0.2	1.5	55	5	5	8.8 $\pm$ 5.9	2.0 - 21.1	
AFM1	25	0.005	0.01	44	12	0	0.07 $\pm$ 0.08	0.01-0.23	

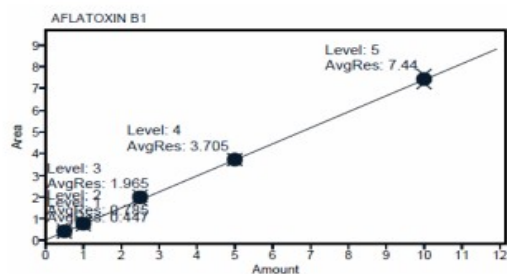


Fig. 2. Calibration curve of aflatoxin B1 standard at 360 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model

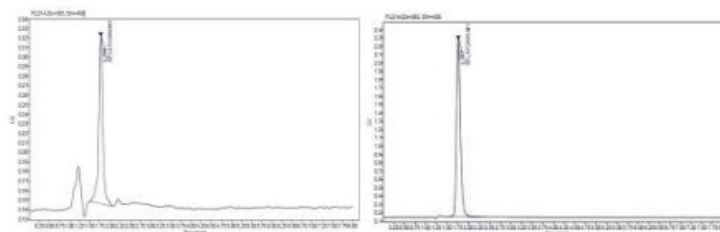


Fig. 3. Elution pattern of the maximum concentration of aflatoxin M1 in milk sample (left) vs standard sample (right) at 365 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model

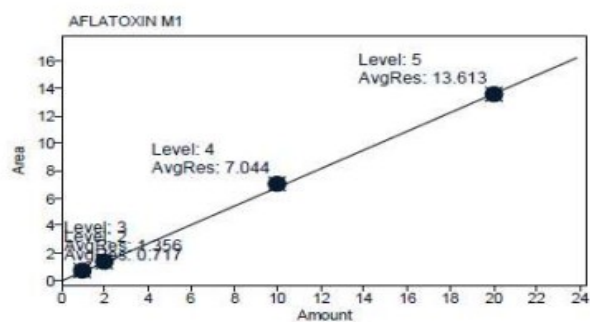


Fig. 4. Calibration curve of aflatoxin M1 standard at 365 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model

#### 4. DISCUSSION

Aflatoxin contamination in animal feeds or milk causes chronic to acute health effects in human and animals. However, variations of aflatoxin occurrence differ from place to place due to the method of detection, the difference in forage and feed quality, climatic and geographic conditions, farming system adopted, and feed storage practices [11]. This study has assessed the association between storage practices and feeding practices to awareness of aflatoxin. It was found that feed storage and feeding practices have an insignificance association with awareness of aflatoxin, contrary to the study by Mohammedi-Ameur et al [11] and Kitigwa et al [13]. This may be attributed to the feeding system where most of the farmers adopted natural grazing without supplementation. In this system, roughages are a dominant source of feed for their cows. Only a small proportion, specifically those found in the village and the majority in the urban area, manage dairy cows under zero grazing where concentrate supplementation is their routine feeding practice. The majority of rural dairy farmers rarely supplement their cows. However, when they do supplement, they usually use ingredients that they have prepared at home after harvesting crops. This is because they are aware of the dangers of aflatoxins, and they want to ensure that their animal feed is prepared properly to minimize the risk of fungal contamination and aflatoxin production. Furthermore, these farmers only prepare a small amount of concentrate that can be stored for less than a week. By following all of these practices, they greatly reduce the likelihood of aflatoxin production. This is similar to the study by Jolly [17] who found that the levels of aflatoxins varied with differences in feeding patterns, agricultural practices, and awareness of aflatoxins in general.

Occurrence of Aflatoxin B1 contamination in animal feeds was detected in 55% of all samples collected with a mean amount of  $8.6 \pm 5.9$   $\mu\text{g}/\text{kg}$  ranging from the lowest limit of detection (LOD) to 21.1  $\mu\text{g}/\text{kg}$ . However, only 5% exceeded the recommended safe limit for dairy cow feeds of 20  $\mu\text{g}/\text{kg}$ . The results reveal low contamination of aflatoxin B1 with lower mean concentration when compared to the studies conducted in most tropical African countries such as Tanzania, Rwanda, Uganda, and the Democratic Republic of Congo. Some Asian countries with higher contamination of AFB1 include Iran, Pakistan, and India in regions where farmers had little, or

no knowledge of aflatoxin. These results build on the existing evidence that, when smallholder dairy farmers have knowledge of aflatoxins they are more willing to participate in the reduction and or prevention of aflatoxin contamination in food systems [18]. The findings are in line with [19] who postulated that a lack of awareness among farmers increased the risks of aflatoxins to both humans and livestock.

On the other hand, 44% of the milk samples collected from Kondoa contained detectable traces of AFM1, with a mean amount of  $0.07 \pm 0.08$   $\mu\text{g}/\text{L}$  ranging from the limit of detection (<LOD) to 0.23  $\mu\text{g}/\text{L}$ . Only 12% of all samples tested exceeded the minimum allowable limit of aflatoxins based on TBS and EU standards (0.05  $\mu\text{g}/\text{L}$ ). No sample exceeded the WHO, USFDA, and Codex Alimentarius limits (0.5  $\mu\text{g}/\text{kg}$ ). Contrary to the findings by Kang'ethe and Lang' [14] Negash,[20] in Kenya and Mwakosya et al [12], Kitigwa et al [13] in Tanzania, which revealed high occurrences of aflatoxin M1 (above 80%) with higher mean concentrations in milk samples collected from various stakeholders. These findings should be taken into account to justify the role of training programs on awareness of aflatoxins and their prevention among smallholder dairy farmers. The farmers' awareness of aflatoxins, feed storage, feeding practices, and feeding system adopted may have contributed to the low levels of occurrence of AFB1 and AFM1 in feeds and milk, respectively. Although the levels of aflatoxins in feeds and milk were very low, it is worth preventing contamination. This is because both AFB1 and AFM1 are classified as Group 1 carcinogenic toxins, and even at low exposure, they can lead to cancer [21]. That is why it is very important to adhere to the principles of prevention and elimination of aflatoxin throughout the food system to ensure safety of both livestock, their products and the consumer health.

The successful prevention of aflatoxin in food systems has spreading positive effects to the stakeholders along the dairy value chain. Some of these benefits include an increased growth rate of livestock, improved productivity, an improved immune system, and reduced veterinary costs [22]. Likewise, achieving the prevention of aflatoxin removes restrictions for international trade, specifically the exportation of animal feeds, ingredients for animal feed formulation, and processed dairy products. This increases the livelihood of smallholder dairy farmers and the contribution of the livestock

sector to the Gross Domestic Product (GDP). The prevention of aflatoxin in the food system also provides consumer health protection against liver cancer, hepatic diseases, and stunted growth. The study by Udomkun et al [23] concluded that the prevention of aflatoxin contamination enhances food security, alleviates malnutrition, and strengthens economic sustainability.

## 5. CONCLUSION AND RECOMMENDATIONS

Aflatoxin contamination has been found to cause health hazard to animal and human when consumed feeds and food contains a trace of aflatoxins. Large amount of aflatoxin exposure results in acute toxicity while low amount of exposure cause chronic toxic effects. The results from this study reveal contamination of aflatoxin B1 in feeds and aflatoxin M1 in milk despite intervention programs to create awareness and prevention of aflatoxin. This leads to the production of feeds and milk of public health concern that can lead to cancer in long-term consumption of contaminated milk. The IARC classified aflatoxin B1 and M1 as class one carcinogens due to their greater contribution to cause cancer. Therefore regular monitoring of feed storage, feed preparation, and feeding practices along with awareness creation programs from the village meeting to the household level to prevent aflatoxin contamination in food systems is highly recommended.

## CONSENT

Informed consent was obtained from all smallholder dairy farmers' prior participation on responding to semi-structured questionnaires in the study.

## ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Research Committee of Sokoine University of Agriculture and also by the Tanzania Livestock Research Institute (TALIRI) for studies involving livestock resource and products.

## ACKNOWLEDGEMENTS

In this section, you can acknowledge any support given which is not covered by the author

contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Jallow A, Xie H, Tang X, Qi Z, Li P, Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control," *Compr. Rev. Food Sci. Food Saf.* 2021;20(3):2332-2381 DOI: 10.1111/1541-4337.12734
2. Beitollahi H. et al, Recent advances in the aptamer-based electrochemical biosensors for detecting aflatoxin b1 and its pertinent metabolite aflatoxin m1, *Sensors (Switzerland)*. 2020;20(11):1-17 DOI: 10.3390/s20113256
3. Ilievska G, Stojanovska-Dimzoska B, Kocева D, Stojković G, Angeleska A, Dimitrieska-Stojković E, Dietary exposure and health risk assessment of aflatoxin m1 in dairy products consumed by population of North Macedonia, *J. Food Qual. Hazards Control*. 2022;9(1):14-22 DOI: 10.18502/jfqc.9.1.9686.
4. Strosnider H. et al., Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries, *Environ. Health Perspect.* 2006;114(12):1898-1903, DOI: 10.1289/ehp.9302
5. Kagera I. et al., Status of aflatoxin contamination in cow milk produced in smallholder dairy farms in urban and peri-urban areas of Nairobi County: a case study of Kasarani sub county, Kenya, *Infect. Ecol. Epidemiol.* 2019;9(1). DOI: 10.1080/20008686.2018.1547095
6. Min L. et al. An overview of aflatoxin B1 biotransformation and aflatoxin M1 secretion in lactating dairy cows, *Anim. Nutr.* 2021;7(1):42-48. DOI: 10.1016/j.aninu.2020.11.002
7. Gashaw M, Review on Mycotoxins in Feeds: Implications to Livestock and human health, *E3 J. Agric. Res. Dev.* 2015;5(3):137-0144 Available: <http://www.e3journals.org>
8. Kiama TN. et al, Kenya dairy farmer perception of moulds and mycotoxins and

- implications for exposure to aflatoxins: A gendered analysis, *African J. Food, Agric. Nutr. Dev.* 2016;16(3):11106–11125  
DOI: 10.18697/ajfand.75.ILR110
9. Yin S, Niu L, Liu Y., Recent progress on techniques in the detection of aflatoxin b1 in edible oil: A mini review, *Molecules.* 2022;27(19):1–35  
DOI: 10.3390/molecules27196141
  10. Kamala A. et al., Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro ecological zones of Tanzania," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* 2016;33(3):551–559  
DOI: 10.1080/19440049.2016.1138546
  11. Mohammedi-Ameur S, Dahmane M, Brera C, Kardjadj M, Ben-Mahdi MH, Occurrence and seasonal variation of aflatoxin M1 in raw cow milk collected from different regions of Algeria, *Vet. World.* 2020;13(3):433–439  
DOI: 10.14202/vetworld.2020.433-439.
  12. Mwakosya AW, Limbu SM, Majaliwa N, Zou X, Shi J, Kibazohi O, Aflatoxin B1 variations in animal feeds along the supply chain in Tanzania and its possible reduction by heat treatment, *Food Agric. Immunol.* 2022;33(1):192–206  
DOI: 10.1080/09540105.2022.2045908
  13. Kitigwa SJ, Kimaro EG, Nagagi YP, Kussaga JB, Suleiman RA, Matemu A, Occurrence and associated risk factors of aflatoxin contamination in animal feeds and raw milk from three agroecological zones of Tanzania, *World Mycotoxin J.* 2023;16(2):149–163.  
DOI: 10.3920/wmj2022.2801
  14. \* Kang'ethe Ek and Lang', Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya; 2009.
  15. Vaz A, Silva ACC, Rodrigues P, Detection methods for aflatoxin m1 in dairy products. 2020;1-16.
  16. Beg MU, Beg KR, Ali LN, Saeed T, Mycotoxins in Poultry Feed in Kuwait, 2006;602:594–602  
DOI: 10.1007/s00244-005-2094-0
  17. Jolly P. et al. Determinants of aflatoxin levels in Ghanaians: Sociodemographic factors, knowledge of aflatoxin and food handling and consumption practices. 2006;209:345–358,  
DOI: 10.1016/j.ijheh.2006.02.002
  18. Ayo EM, Matemu A, Laswai GH, Kimanya ME, Socioeconomic characteristics influencing level of awareness of aflatoxin contamination of feeds among livestock farmers in Meru District of Tanzania, *Scientifica (Cairo)*; 2018  
DOI: 10.1155/2018/3485967
  19. Negash D, A Review of Aflatoxin: Occurrence, Prevention, and Gapsin Both Food and Feed Safety, *J. Appl. Microbiol. Res.* 2018;1(1):1-35.
  20. Lindahl JF, Kagera IN, Grace D, Aflatoxin M1 levels in different marketed milk products in Nairobi, Kenya, *Mycotoxin Res.* 2018;34(4):289–295  
DOI: 10.1007/s12550-018-0323-4
  21. Marchese S, Polo A, A. Ariano S, Velotto, Costantini S, Involvement in cancer development. 2018;1-19.  
DOI: 10.3390/toxins10060214.
  22. Kebede A, Review on aflatoxin and its impacts on livestock, *J. Dairy Vet. Sci.* 2018;6(2):1-7.  
DOI: 10.19080/jdvs.2018.06.555685
  23. Udomkun P, Wiredu AN, Nagle M, Müller J, Vanlauwe B, Bandyopadhyay R, Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application – A review, *Food Control.* 2017;76:127–138  
DOI: 10.1016/j.foodcont.2017.01.008

© 2023 Robert et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:  
The peer review history for this paper can be accessed here:  
<https://www.sdiarticle5.com/review-history/109989>

## CHAPTER FOUR

### 4.0 GENERAL DISCUSSION

#### 4.1 Demographic Information

This study has considered gender, age group, occupation, and experience among 120 smallholder dairy farmers. The majority of participants were male, aged between 40 and 51 years old. It was found that the majority had a primary education, compared to college or university-educated farmers. This is common in many African communities, as the agriculture sector employs more than 75% of the working population. Therefore it is not surprising to have a small number of smallholder farmers with a college or university education. This is because the highly educated individual are generally few in number and become even fewer among the educated who are interested in livestock farming. Most of them engage in livestock keeping as an alternative source of income following employment in government institutions or non-government organizations. Most of these highly educated farmers usually practice zero-grazing, employing labor to bring pastures and supplements to their animals in sheds. The demographic information indicates that males are highly involved in livestock keeping compared to females. This is common in many African communities, where the head of the family is the dominant owner of everything and is responsible for coordinating responsibilities among different age groups and sexes within their households.

#### 4.2 Awareness of Aflatoxins

The findings of this study has demonstrated that, more smallholder dairy farmers in Kondoa are aware of aflatoxin. Majority of the respondents in both Kondoa district council and Kondoa town council had already heard of aflatoxins from various sources. Those who knew that aflatoxins can contaminate animal feeds and potentially causing harmful effects on animals and humans were more than 65%. This is a good indication that majority of smallholder dairy farmers in Kondoa were aware of aflatoxins as compared to studies previous studies conducted by Yunus *et al.* (2022), Nakavuma *et al.* (2020), Boni *et al.* (2021) and Kiama *et al.* (2016) in Pakistan, Uganda, Tanzania and Kenya, respectively where awareness of

aflatoxin was very low. These results suggest the contribution of training and other awareness programs organised and implemented by various stakeholders in collaboration with extension officers through village meetings, where awareness of aflatoxins is an important agenda have raised awareness of aflatoxin among small holder's dairy farmers. Moreover, other sources of information such as seminars, radio, television presentations, and newspapers were reported to contribute in raising awareness of aflatoxins. This suggests need for planning, organizing and coordination of more awareness programs in other areas to increase create awareness of aflatoxins among people of all age groups and hence increase participation of farmers and other stakeholders to reduce aflatoxins contamination along the food systems.

Furthermore, the study by Yunus *et al.* (2022) revealed that when people are aware of aflatoxins, they are more willing to participate in control practices and or to pay a premium price for feeds and livestock products that are aflatoxins-free. The awareness of aflatoxins in animal feeds and milk among Kondoa smallholder dairy farmers has contributed to their performance in all matters related to aflatoxins contamination and control practices. However, the HPLC analysis of animal feeds and raw milk sample collected from among smallholder's dairy farmers revealed contamination of the sample with aflatoxin. This indicates that there were still some people who are not aware of aflatoxins and much more others having insufficient knowledge on aflatoxins causes, effects and control strategies. Therefore, there is need to increase awareness among smallholder farmers through regular monitoring and supervision of the routine practices such as feed storage and feeding practices. This can be achieved by ensuring that all extension staffs have relevant skills and knowledge for aflatoxins prevention along the dairy value chain in order to ensure good practices in matters related to animal feeds processing, transportation and storage.

#### **4.3 Food Storage Practices**

Food storage practices are among the key determinants of aflatoxins production and contamination in animal feeds and their products. Feeds must be stored appropriately in a good temperature and dry

environment to prevent mold growth during and after harvest, as well as during storage. This study has revealed that most smallholder dairy farmers, especially in rural areas, utilize post-harvest residue to feed their cows. These residues are collected and stored in pellets, some under sheds. Most of these feeds were used for three to six months to supplement their livestock after grazing. Similarly, around 50% of smallholder dairy farmers purchase ingredients for concentrate supplementation at milling and pressing machines for maize bran and sunflower seed cakes. These ingredients are normally dried and stored in polythene bags to prevent mold growth. These farmers usually purchase a small amount that can sustain supplementation for a week, mixing it with available ingredients to supplement their cows during milking, mostly 2 kg of concentrates. Due to the high cost of these supplements, most farmers do not supplement their livestock and instead rely solely on grazing in natural pastures. The findings of this study are in line with the study conducted by Kiama *et al.* (2016), which suggests that feed storage practices, when accompanied by sorting and discarding likely aflatoxins-affected feeds, proper drying, and good agricultural practices, have the potential to reduce aflatoxins contamination in livestock feeds and hence in milk and other livestock products.



**Figure 1:** Hay storage practice at one of the smallholder dairy farmer (SHDFs) at Kondoa town

#### **4.4 Feeding practices**

Aflatoxins contamination is also determined by the feeding management adopted by farmers. In this study, smallholder dairy farmers in Kondoa district adopted different management systems, including zero-grazing, mixed grazing, and pure grazing. The choice of management system was determined by factors such as fluctuations in feed availability during the rainy and dry seasons, distant grazing areas, the need to participate in other production activities apart from livestock keeping and the cost of different management options. The majority of farmers adopted pure grazing on natural pastures, where they take their animals to grazing grounds without supplementation and then return them home. Pure grazing is the cheapest and easiest form of cattle management, requiring only

one laborer to take care of a herd of cattle and resulting in low investment costs. However, this method requires animals to walk long distances in search of pasture and water, which can reduce productivity. On the other hand, since this method relies solely on pastures, there is a low chance of contamination with aflatoxins, as standing hay and other pasture sources are rarely contaminated with aflatoxins. Due to agricultural practices, no farmers adopted free-ranging, as this could lead to conflicts among farmers. Regardless of the dairy management system adopted, farmers should be aware of aflatoxins and control measures. This will influence farmer's voluntary participation in preventing aflatoxins contamination in animal feeds and milk.

Concentrate supplementation practices were most commonly performed by smallholder dairy farmers under zero-grazing. This was especially prevalent among urban and peri-urban farmers in Kondoa, where grazing areas are limited. The majority of farmers prepared feeds at home and were able to identify feeds that were potentially contaminated with aflatoxins by noting changes in color. Most of them stated that aflatoxins-contaminated feeds were usually moldy, blackish-greenish, or yellowish. This helped them to isolate feeds that perceived to be contaminated. This practice helped to ensure safety by reducing aflatoxins contamination in animal feeds and hence in milk.

#### **4.5 Occurrence of aflatoxins in Animal Feeds and Fresh Milk**

The laboratory analysis of feed and milk samples for aflatoxin B1 and aflatoxin M1 showed an association with the level of aflatoxins awareness among smallholder dairy farmers in Kondoa which is the result of stakeholder's awareness programs such as TANIPAC. The results of the feed analysis revealed that 55% of the samples contained detectable traces of aflatoxin B1, with only 5% of the samples exceeding the recommended limit of 20ug/Kg for animal feeds intended for dairy cattle feeding, according to TBS, EU, WHO, *Codex alimentarius*, and UFDA. This resulted in a low level of aflatoxin M1 in the milk, as aflatoxins are metabolized in the body of animals. It was found that 44% of the milk samples contained detectable traces of aflatoxins M1, with only 12% of the samples

exceeding the safe limit of 0.05ug/Kg for milk consumption, according to TBS and EU. However, according to WHO, *Codex alimentarius*, and UFDA, the maximum level of aflatoxin M1 in milk for consumption is 0.5ug/Kg and no milk samples exceeded this limit. These results indicate a low level of aflatoxin B1 and M1 present in feeds and milk from Kondoa district compared to other studies conducted by Nakavuma *et al.* (2020), Gladys *et al.*(2018), Mwakyosa *et al.* (2022), Kitigwa *et al.* (2023), Kiama *et al.* (2018), Kangethe, and Lang'a.

The low level of aflatoxins in feeds and milk samples in Kondoa indicates that the majority of smallholder dairy farmers are aware of aflatoxins in animal feeds and milk. This was evident through an assessment of feed storage practices and feeding practices, which implies that most farmers understand good storage and feeding practices. This suggests that when training programs on aflatoxins awareness are planned and implemented among agricultural communities, there is a high chance of success in imparting knowledge and skills for reducing aflatoxins contamination through good agricultural practices such as good harvesting, and storage practices. These practices will help produce feeds with little to no aflatoxins contamination, ensuring the production of safe milk for public consumption. Similar findings were concluded by Yunus *et al.* (2022), stating that when awareness of aflatoxins is promoted among farmers, they become willing to participate in aflatoxins decontamination. This reduces and prevents aflatoxins contamination throughout the food system.

## **5.0 Conclusion and Recommendations**

Awareness of aflatoxins is a prerequisite strategy for preventing aflatoxins contamination in the food system. The findings in this study revealed that smallholder dairy farmers in Kondoa district were aware of aflatoxins. However, farmers need to have sufficient information on the causes, effects and control measures which can be improved through well planned and organized seminar and training specifically for improving awareness of aflatoxin among smallholders farmers. The results reflect stakeholder interventions, such as coordinated training programs aimed at increasing aflatoxins awareness. Although these training programs were specifically

designed for agricultural crop producers, smallholder dairy farmers also benefited from them, as they practiced mixed farming and used crop residues as feed ingredients. The HPLC analysis of aflatoxin in feed and raw milk samples revealed the low occurrence of aflatoxins.

However, it is highly recommended to improve awareness of aflatoxins and monitor its occurrence to ensure that the awareness level is below the permissible level safe for livestock and human consumption. It is worth to understand that even at low levels, aflatoxins in food and feeds can potentially cause cancer through chronic effects. Program interventions, including properly designed training programs, seminars, mass media communication, radio and television broadcasts, and newspapers, must be established across all regions in Tanzania to improve awareness of aflatoxin in general and build capacity of smallholder dairy farmers to understand various method that can be applied to prevent aflatoxins contamination and hence ensure food safety for public consumption.

### References

- Association of American Feed Control Officials (AAFCO), (2017). Annual Meeting. Committee Reports. August 10–12, 2017.
- Balina, A., Kebede, A., & Tamiru, Y. (2018). Review on aflatoxin and its impacts on livestock. *Journal of Dairy and Veterinary Sciences*, 6(2), e555685.
- Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W., & Kyegombe, D. B. (2013). Review of the biological and health effects of aflatoxins on body organs and body systems. *Aflatoxins-recent advances and future prospects*, 12, 239-265.
- Ceniti, C., Costanzo, N., Spina, A. A., Rodolfi, M., Tilocca, B., Piras, C., & Morittu, V. M. (2021). Fungal contamination and Aflatoxin B1 detected in hay for dairy cows in South Italy. *Frontiers in Nutrition*, 641.
- Daniel, J. H., Lewis, L. W., Redwood, Y. A., Kieszak, S., Breiman, R. F., Flanders, W. D., & McGeehin, M. A. (2011). Comprehensive assessment of maize aflatoxin levels in Eastern Kenya, 2005–2007. *Environmental Health Perspectives*, 119(12), 1794-1799.

- Daniel, W. W., & Cross, C. L. (2018). *Biostatistics: a foundation for analysis in the health sciences*. Wiley.
- Dunshea, F. R., Oluboyede, K., DiGiacomo, K., Leury, B. J., & Cottrell, J. J. (2019). Betaine improves milk yield in grazing dairy cows supplemented with concentrates at high temperatures. *Animals*, 9(2), 57.
- Gashaw, M. (2016). Review on Mycotoxins in Feeds: Implications to Livestock and human health. *J. Agric. Res. Dev*, 5, 137-144.
- Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food control*, 59, 773-779.
- Hilda, F. M., & Jovin, K. M. (2021). Prevalence of aflatoxin M1 in pasteurized and ultra-high temperature (UHT) milk marketed in Dar es Salaam, Tanzania. *African Journal of Microbiology Research*, 15(9), 461-466.
- Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive reviews in food science and food safety*, 20(3), 2332-2381.
- Kamala, A., Kimanya, M., Haesaert, G., Tiisekwa, B., Madege, R., Degraeve, S., & De Meulenaer, B. (2016). Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agroecological zones of Tanzania. *Food Additives & Contaminants: Part A*, 33(3), 551-559.
- Kajuna, F. F., Temba, B. A., & Mosha, R. D. (2013). Surveillance of aflatoxin B1 contamination in chicken commercial feeds in Morogoro, Tanzania. *Livestock Research for Rural Development*, 25(3), 51.
- Kang'ethe, E. K., & Lang'a, K. (2009). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. *African Health Sciences*, 9(4).
- Kitigwa, S. J., Kimaro, E. G., Nagagi, Y. P., Kussaga, J. B., Suleiman, R. A., & Matem, A. (2022). Occurrence and associated risk factors of aflatoxin B1 and aflatoxin M1 contamination in feed and raw milk in three agroecological zones of Tanzania. *Tanzania Journal of Health Research*, 23.

- Kurwijila, L. R. (2010). An overview of dairy development in Tanzania. *Smallholder dairy production and marketing-Opportunities and constraints*, 174.
- Lalah, J. O., Omwoma, S., & Orony, D. A. (2019). Aflatoxin B1: Chemistry, environmental and diet sources and potential exposure in humans in Kenya. *Aflatoxin B1 Occurrence, Detection and Toxicological Effects*.
- Ledo, J., Hettinga, K. A., Kussaga, J. B., & Luning, P. A. (2020). Implications of differences in safety and hygiene control practices for microbial safety and aflatoxin M1 in an emerging dairy chain: The case of Tanzania. *Food Control*, 118, 107453.
- Magembe, K. S., Mwatawala, M. W., & Mamiro, D. P. (2016). Mycotoxin contamination in stored maize and groundnuts based on storage practices and conditions in subhumid tropical Africa: The case of Kilosa District, Tanzania. *Journal of food protection*, 79(12), 2160-2166.
- Mahato, D. K., Lee, K. E., Kamle, M., Devi, S., Dewangan, K. N., Kumar, P., & Kang, S. G. (2019). Aflatoxins in food and feed: an overview on prevalence, detection and control strategies. *Frontiers in microbiology*, 10, 2266.
- Makau, C. M. (2017). *The prevalence of Brucella abortus and mycotoxin contamination of animal feeds and implications on milk safety in rural and peri-urban small-holder dairy systems in Nakuru County, Kenya* (Doctoral dissertation, Egerton University).
- Min, L., Fink-Gremmels, J., Li, D., Tong, X., Tang, J., Nan, X., & Wang, G. (2021). An overview of aflatoxin B1 biotransformation and aflatoxin M1 secretion in lactating dairy cows. *Animal Nutrition*, 7(1), 42-48.
- Mohammed, S., Munissi, J. J., & Nyandoro, S. S. (2016). Aflatoxin M1 in raw milk and aflatoxin B1 in feed from household cows in Singida, Tanzania. *Food Additives & Contaminants: Part B*, 9(2), 85-90.
- Mughal, M. J., Peng, X., Kamboh, A. A., Zhou, Y., & Fang, J. (2017). Aflatoxin B1 induced systemic toxicity in poultry and rescue effects of selenium and zinc. *Biological trace element research*, 178(2), 292-300.

- Mwakosya, H. F., & Mugula, J. K. (2021). Prevalence of aflatoxin M1 in pasteurized and ultra-high temperature (UHT) milk marketed in Dar es Salaam, Tanzania. *African Journal of Microbiology Research*, 15(9), 461-466.
- Navale, V., Vamkudoth, K. R., Ajmera, S., & Dhuri, V. (2021). Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology reports*, 8, 1008-1030.
- Negash, D. (2018). A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. *Journal of Applied Microbiological Research*, 1(1), 35-43.
- Nleya, N., Adetunji, M. C., & Mwanza, M. (2018). Current status of mycotoxin contamination of food commodities in Zimbabwe. *Toxins*, 10(5), 89.
- Peles, F., Sipos, P., Gyóri, Z., Pfliegler, W. P., Giacometti, F., Serraino, A., & Pócsi, I. (2019). Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock. *Frontiers in microbiology*, 10, 2861.
- Puga-Torres, B., Ron, L., & Gómez, C. (2022). Dietary aflatoxin B1-related risk factors for the presence of aflatoxin M1 in raw milk of cows from Ecuador. *Open Veterinary Journal*, 12(1), 129-137.
- Rodríguez-Blanco, M., Ramos, A. J., Prim, M., Sanchis, V., & Marín, S. (2020). Usefulness of the analytical control of aflatoxins in feedstuffs for dairy cows for the prevention of aflatoxin M1 in milk. *Mycotoxin research*, 36(1), 11-22.
- Schenck, J., Djurle, A., Funck Jensen, D., Müller, C., O'Brien, M., & Spörndly, R. (2019). Filamentous fungi in wrapped forages were determined with different sampling and culturing methods. *Grass and Forage Science*, 74(1), 29-41.
- Senerwa, D. M., Sirma, A. J., Mtimet, N., Kang'ethe, E. K., Grace, D., & Lindahl, J. F. (2016). Prevalence of aflatoxin in feeds and cow milk from five counties in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, 16(3), 11004-11021.
- Silva, S. B. D., Barbosa, J. R., Silva Martins, L. H. D., Moraes, V. S. V., Bichara, C. M. G., Sousa, F. R. S., & Lopes, A. S. (2021). Microbial Degradation of Aflatoxin. In *Recent Advances in Microbial Degradation* (pp. 1-18). Springer, Singapore.

- Tatfo Keutchatang, F. D. P., Tchuenchieu, A. K., Nguégwouo, E., Mouafo, H. T., Bouelet Ntsama, I. S., Kansci, G., & Medoua, G. N. (2022). Occurrence of Total Aflatoxins, Aflatoxin B1, and Ochratoxin A in Chicken and Eggs in Some Cameroon Urban Areas and Population Dietary Exposure. *Journal of Environmental and Public Health*, 2022.
- U.S. Food and Drug Administration (2019). Action Levels for Aflatoxins in Animal Food, Compliance Policy Guide.
- Vaz, A., Cabral Silva, A. C., Rodrigues, P., & Venâncio, A. (2020). Detection methods for aflatoxin M1 in dairy products. *Microorganisms*, 8(2), 246.
- Wang, P., Tran, N., Enahoro, D., Chan, C. Y., Shikuku, K. M., Rich, K. M., & Thilsted, S. H. (2022). Spatial and temporal patterns of consumption of animal-source foods in Tanzania. *Agribusiness*, 38(2), 328-348.
- Yin, S., Niu, L., & Liu, Y. (2022). Recent Progress on Techniques in the Detection of Aflatoxin B1 in Edible Oil: A Mini Review. *Molecules*, 27(19), 6141.

## APPENDICES

### **Appendix 1: Questionnaires**

*SMALLHOLDER LIVESTOCK FARMERS QUESTIONNAIRES.*

#### **PREVALENCE OF AFLATOXIN IN ANIMAL FEEDS AND FRESH MILK: THE CASE OF KONDOA; IN DODOMA REGION.**

##### **Instructions**

This questionnaire is aimed to assess milk producers' knowledge of factors and practices associated with aflatoxin contamination in milk. It will take less than thirty minutes to complete this questionnaire. Please note that your answer will be considered completely confidential and your name will not be included in any reports about these results. Also, your answer as an individual will not be shared with anyone.

##### **General objective**

The general objective of this study will be to determine the levels of aflatoxin contamination in animal feeds and fresh milk.

##### **1.3.2 Specific objectives: -**

- i. To assess livestock farmers' awareness to aflatoxin and milk safety, their harvesting and feed storage practices.
- ii. To examine aflatoxins B1 and M1 levels in animal feeds and raw milk respectively.



<b>A. DEMOGRAPHIC INFORMATION</b>			
<b>S/N</b>	<b>Questions/Item</b>	<b>Choices</b>	<b>Response</b>
1.	Age of respondent	1. 18 – 30 Years	
		2. 31 – 40 Years	
		3. 41 – 50 Years	
		4. 51 – 60 Years	
		5. 61 – 70 Years	
		6. Above 70	
2.	Sex	1. Male	
		2. Female	
3.	Marital status	1. Single	
		2. Married	
		3. Divorced	
		4. Widow	
4.	What is the level of education attained?	1. No formal education	
		2. Primary	
		3. Secondary	
		4. College or university	
		5. Others ( <i>specify</i> )	
5.	What is your main occupation?	1. Livestock keeping	
		2. Farming and livestock keeping	
		3. Employed and livestock keeping	
		4. Others ( <i>specify</i> )	
6.	For how long have you involved in livestock keeping?	1. Less than 5 Years	
		2. Between 5 to 10 Years	
		3. More than 10 ten years	
<b>B. FEEDING SYSTEM/PRACTICES</b>			
7.	What is your role in cattle management? ( <i>multiple options</i> )	1. Principle person looking after cattle	
		2. Owner	
		3. Occasionally look after cattle	
		4. Do not look after the cattle	

8.	How many cattle do you currently have in the herd?	
9.	How many dairy cows do you currently have in the herd?	
10.	How do you feed your animals?	1. Free ranging
		2. Zero grazing
		3. Grazing and supplementation
11.	Why are you using that system to feed your animal? <i>Explain</i>	
12.	What is the predominant type of animal feed you are using	1. Roughages
		2. Concentrates
		3. Mixed feeds
13.	If you provide concentrates, where do you get the concentrates	1. Commercial
		2. Compounded on-the-farm
14.	What type of concentrates do you feed your animals?	1.
		2.
		3.
		4.
		5.
15.	How many times are you using feed concentrates?	1. Daily
		2. Once per week
		3. Three times per week
		4. Five times per week
16.	How much do you give them?	1. Less than 1kg
		2. 1kg to 2 kg
		3. 3kg to 5 kg
		4. More than 5kg
17.	Are you facing any challenge of getting suffice feeds for your animals?	1. Yes
		2. No
18.	If Yes, what are those challenges? <i>Explain</i>	
<b>C. FEED HANDLING AND STORAGE PRACTICES</b>		
19.	How do you dry your feeds?	1. Using Solar dryer
		2. Sun dried on polythene sheet
		3. Open space on the ground

		4.Others ( <i>specify</i> )	
20.	How do you measure moisture content after drying?	1.No moisture measurements	
		2. Moisture meter	
		3.Others ( <i>specify</i> )	
21.	How long do you store the feeds?	1. Less than six months	
		2. More than six months but less than one year	
		3. More than one year	
22.	How do you store feeds?	1. Woven/sisal bag (gunia)	
		2.Polyethyne bag (mifuko ya safeti)	
		3. Barrel/Drum (pipa)	
		4.Plastic bucket	
		5. Heap on the ground	
		6.Others ( <i>specify</i> )	
23.	How do you prepare concentrates		
<b>D. AFLATOXIN AWARENESS</b>			
24.	Have you heard of the word aflatoxin before?	1. Yes	
		2. No	
25.	Where have you heard it from?	1.Village meeting/ extension officers	
		2. Newspaper	
		3.Seminar	
		4.Radio/Tv	
		5.Friend	
		6.School	
		7.Others ( <i>specify</i> )	
26.	Do you know aflatoxins can cause contamination in animal feeds?	1. Yes	
		2. No	
27.	Can you recognize any sign(s) which shows the feed is contaminated with aflatoxins?	1.Yes	
		2. No	
28.	If, yes, which signs, <i>Specify</i>		

29.	Do you know that aflatoxin can cause liver cancer in human and animals?	1.Yes	
		2.No	
30.	Do you know that aflatoxins can harm your animals?	1. Yes	
		2. No	
31.	Do you know that aflatoxin can be transferred from contaminated feeds consumed by animals to milk?	1. Yes	
		2. No	
32.	Do you know factors influencing aflatoxin contamination?	1. Yes	
		2. No	
33.	If <b>Yes</b> , mention them,	1.	
		2.	
		3.	
		4.	
		5.	
34.	Do you know that any control measure for aflatoxin contamination in animal feeds?	1.Yes	
		2.No	
35.	If yes, which control measure do you apply to prevent aflatoxin contamination in feeds?		
36.	Aflatoxins in feeds can be detoxified to render the feeds safe?	1.Yes	
		2.No	
37.	Have you ever received any training on aflatoxins?	1.Yes	
		2.No	
38.	What was the training about?	1.Proper storage practise	
		2.Use of new storage technique (hermatic storage)	
		3.GAP	
		4.Others ( <i>specify</i> )	

**THANK YOU FOR PARTICIPATING.**

## Appendix 2: Research Permit

## CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA



UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND  
TECHNOLOGY.SOKOINE UNIVERSITY OF AGRICULTURE  
OFFICE OF THE VICE-CHANCELLORP.O Box 3000, CHUO KIKUU, MOROGORO, TANZANIA.  
Phone: +255 (023) 2640006/7/8/9, Direct Line: +255 (023) 2640015,  
E-mail: [vc@sua.ac.tz](mailto:vc@sua.ac.tz), Website: <https://www.sua.ac.tz>

Please refer to:

Our Ref: SUA/ADM/R.1/8/963

Date: 22<sup>nd</sup> December, 2022

Permanent Secretary,  
President's Office,  
Regional Administration and Local Government,  
P.O. Box 1923, Mji wa Serikali,  
41185 DODOMA.  
Email: [ps@tamisemi.go.tz](mailto:ps@tamisemi.go.tz)

**RE: UNIVERSITY STAFF, STUDENTS AND RESEARCHERS CLEARANCE**

The Sokoine University of Agriculture was established by University Act No. 7 of 2005 and SUA Charter, 2007 which became operational on 1<sup>st</sup> January 2007 repealing Act No. 6 of 1984. One of the mission objectives of the University is to generate and apply knowledge through research. For this reason the staff and researchers undertake research activities from time to time.

2. To facilitate the research function, the Vice Chancellor of the Sokoine University of Agriculture (SUA) is empowered to issue research clearance to staff, students, research associate and researchers of SUA on behalf of the Tanzania Commission for Science and Technology.

3. The purpose of this letter is to introduce to you **Mr. Onesmo Andrew Robert** a bonafide **MSc. (Food Quality and Safety Assurance)** student with Registration number **MFQ/D/2021/0002** of SUA. By this letter **Mr. Onesmo Andrew Robert** has been granted clearance to conduct research in the country. The title of the research in question is **"PREVALENCE OF AFLATOXIN IN ANIMAL FEEDS AND MILK: THE CASE OF KONDOA IN DODOMA REGION."**



THE UNITED REPUBLIC OF TANZANIA  
MINISTRY OF LIVESTOCK AND FISHERIES  
TANZANIA LIVESTOCK RESEARCH INSTITUTE (TALIRI)

Director General, Tanzania Livestock Research Institute, Block No. 39,  
P. O. Box, 834, Dodoma, Email: [dg@taliri.go.tz](mailto:dg@taliri.go.tz) or [hq@taliri.go.tz](mailto:hq@taliri.go.tz)



REF NO. TLR/RCC.23/003

RESEARCH CLEARANCE CERTIFICATE

1. This certificate is hereby presented to:

Mr. Onesmo Andrew Robert.

Title of the proposed research:

Prevalence of Aflatoxins in Animal Feeds and Fresh Milk: The case of Kondoa in Dodoma Region.

2. General objective of the Research:

Determination of the levels of Aflatoxin contamination in Animal Feeds and Fresh Milk.

3. Study areas:

This research will be conducted in 1 Region of Tanzania - Dodoma.

4. Starting date: 27<sup>th</sup> January, 2023

5. Ending date: 26<sup>th</sup> January, 2024

DIRECTOR GENERAL  
Tanzania Livestock Research Institute  
(TALIRI)

Signature of the Director General

Date

27/01/2023



### **Kuhusu Tasnifu Hii**

Utafiti huu umefanyika kupima uelewa wa jamii za wafugaji wadogo wa ng'ombe wa maziwa wilayani Kondo juu ya sumukuvu katika vyakula vya mifugo na maziwa pamoja na kubaini viwango vya sumukuvu B1 na sumukuvu M1 katika vyakula vya mifugo na maziwa. Jumla ya washiriki 120 wenye ng'ombe wanayekamuliwa walishiriki. Katika uchunguzi wa kimaabara sampuli 20 za vyakula na sampuli 25 maziwa kutoka miongoni mwa wafugaji 120 zilifanyiwa uchunguzi wa viwango vya sumukuvu kwa kutumia "*High Performance Liquid Chromatography (HPLC)*". Matokeo ya utafiti huu yameonesha kwamba asilimia 88 ya wafugaji wamewahi kusikua kuhusu sumukuvu kupitia mikutano ya vijiji, semina zilizoandaliwa, magazetini, redioni na katika televisheni. Aidha matokeo ya uchambuzi wa maabara kwa sampuli za vyakula vya mifugo na maziwa ziliokusanywa kutoka kwa wafugaji wilayani Kondo yamebaini kiwango cha asilimia 55 za sampuli za vyakula zinavimelea vya sumukuvu na asilimia tano (5%) zilikuwa juu ya kiwango himilivu kwa matumizi ya ng'ombe wa maziwa cha 20 $\mu$ g/kg. Uchambuzi wa sampuli za maziwa umebaini asilimia 44 ya sampuli zinavimelea vya sumukuvu ambapo asilimia 12 zipo juu ya kiwango kinachoshauriwa na Shirika la Viwango la Tanzania, EU, and WHO kwa maziwa yanayokusudiwa kutumiwa na binadamu cha 0.05 $\mu$ g/L. Hivyo, ni muhimu kwa wataalamu wa mifugo kutoa elimu juu ya sumukuvu na kufanya ukaguzi kwa wafugaji wanaowasimamia ili kuzuia sumukuvu katika vyakula na maziwa.