PREVALENCE OF AFLATOXIN M_1 IN PASTEURIZED AND ULTRA-HIGH TEMPERATURE (UHT) MILK MARKETED IN DAR ES SALAAM, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA

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EXTENDED ABSTRACT

A survey was conducted in year 2020/2021 to establish the levels of aflatoxin M_1 (AFM_1) in pasteurized and ultra-heat-treated milk (n=118) and awareness on mycotoxins by milk processors in Kinondoni, Temeke, Ubungo, Ilala and Kigamboni district of Dar es Salaam, the commercial capital of Tanzania. The levels of AFM_1 in pasteurized milk (n=75) and UHT milk (n=43) samples were determined by using immuno-affinity high performance liquid chromatography. AFM_1 was detected in 97% (115/118) of the heat-treated samples. Pasteurized milk and UHT milk samples were contaminated by 96% (72/75) and 100% (43/43), respectively. About 82% of the contaminated pasteurized and UHT had aflatoxin M_1 above the EU acceptable levels (0.05 µg/L) however none of the contaminated pasteurized and UHT milk sample exceed Codex limits of 0.5 µg/L. The observed contamination levels of AFM_1 in heated milk could pose a serious public health problem. Therefore, best practices including regular monitoring of AFM_1 levels in milk and milk products are crucial to protect consumers. Awareness of aflatoxin contamination of milk was assessed by using a cross-sectional descriptive statistic involving 30 milk processors. Data was collected using a structured questionnaire and statistically analysed using Statistical Package for Social Sciences (IBM SPSS® Version 27 (2020). Descriptive statistics was used to determine frequencies and percentages of social demographic, knowledge, handling and feeding practices of lactating cow. Cross tabulation was used to determine relationship between knowledge on aflatoxins with age and education level of the respondents. The majority of the respondents (83.3%) were aware of aflatoxins and none (0.0%) of the respondents were aware that milk and milk products could be contaminated with aflatoxins. It was also observed that the cattle feeding practices were poor and were a major reason for AFM_1 contamination of milk. None of the respondents were aware that feeding lactating cow with mouldy feeds could results into AFM_1 contamination of milk. It was observed that, AFM_1 analysis was not carried out in raw milk before processing in order to control AFM_1 contamination of milk and milk products. This could be due to lack of knowledge and techniques for detection and analysis of aflatoxins. This study recommended that creation of awareness of aflatoxins and use of best practices along the milk value chain was crucial in order to enhance the safety of consumers.

DECLARATION

I, Hilda Mwakosya do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

______________________________ ____________________

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The above declaration is confirmed;

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Prof. Jovin K. Mugula Date

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DEDICATION

This dissertation is lovingly dedicated to my husband, Mr. Daniel Amulike Aron for his support, encouragement and constant love and to my children Hellen, Allen and Grayson for their love and patience during my absence.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATION AND ACRONYMS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
CAC	Codex Alimentarius Commission
EU	European Union
FAO	Food and Agriculture Organization
FLD	Fluorescence Detector
GAP	Good Agricultural Practices
GDP	Gross Domestic Product
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
LOD	Limit of Detection
LOQ	Limit of Quantification
MCT	Medium-chain triglycerides
µg/L	Microgram per Liter (ppb)
RPM	Revolution per Minutes
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
UHT	Ultra High Temperature
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

1.1.1 Milk production in Tanzania

Tanzania has the third largest livestock population in Africa comprising of 25 million cattle out of which 98% are indigenous breeds (FAO, 2020). In year 2018 about 2.09 billion litres of raw milk were produced in Tanzania and milk production contributes to income, food security, nutrition and household livelihood (FAOSTAT, 2020). The sector contributes to 7.4% of total national GDP, however the annual growth rate (2.2 %) of the sector is low (FAO, 2020).

The dairy production sector in Tanzania is divided into two subsectors: traditional and improved dairy subsectors (Nell *et al.,* 2014). Traditional subsector is the most dominant and dominated by Tanzanian Shorthorn Zebu (TSHZ) cattle with production of 70% milk of which 90% is consumed at home and 10% contributes to commercial sector (Munyaneza *et al.,* 2019; URT, 2017). The improved dairy system subsector is dominated by smallholder's dairy farmers who keep mainly improved dairy cattle and contributes about 30% of all milk produced and marketed in Tanzania (URT, 2017).

1.1.2 Aflatoxin M_1 in heat treated milk

Raw milk is a valuable nutritious food. However, it is highly perishable (has a short shelf-life) and an excellent medium for the growth of microorganisms, particularly bacterial pathogens that can cause spoilage and diseases to consumers (FAO, 2021). Heat treatment of milk such as pasteurization and UHT (ultra-high temperature) allows the preservation of milk and helps to reduce food-borne illness (Melini *et al.,* 2017).

According to FAO/WHO (1982), pasteurization is defined as a heat treatment process applied to a product such as milk with the objective of minimizing numbers of harmful micro-organisms to a level at which they do not constitute a significant health hazard with minimal chemical, physical and organoleptic changes in the product. It also extends the storage time for some products by reducing the number of spoilage micro-organisms in the product (FAO/WHO,1982). Codex Alimentarius (2004), defined UHT (ultra-high temperature) treatment of milk and liquid milk products as the application of heat to a continuously flowing product using such high temperatures for short time that renders the product commercially sterile at the time of processing. When UHT treatment is combined with aseptic packaging, it results in a commercially sterile product at the heating range of 135 to 150 $^{\circ}$C for 1 s up to 4 s (Melini *et al.*,2017).

Aflatoxins are amongst the most poisonous mycotoxins and are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* fungi found in soil and that can grow in plant, human food products and feeds (WHO, 2018). The most important aflatoxins in order of toxicity are B_I, B_2, G_1, and G_2 (Ismail *et al.,* 2018; Tahira *et al.*, 2019). Aflatoxins may also be found in the milk of animals that are fed contaminated feed, in the form of aflatoxin M_1 (Langat *et al.,* 2016).

Aflatoxin M_1 (AFM_1) is the principal metabolite of aflatoxin B_1 (AFB_1), and it is formed in the liver (Langat *et al.,* 2016). Once lactating cow consume contaminated feeds with aflatoxin $B_{1,}$ it is absorbed into the gastro intestinal tract and biotransformation occurs in the liver by cytochrome P_{450} enzymes to form a 4-hydroxy metabolite known as aflatoxin M_1 a compound soluble in water and therefore it is easily excreted in milk during milking and appears within 12 hours of administration of contaminated feeds (Daou *et al.*,2020; Tahira *et al.,* 2019).

AFM_1 is a heat stable compound that can survive heat treatment such as pasteurization, UHT technique and autoclaving but also AFM_1 may be reduced but not completely destroyed by heat treatments (Mahmoodi *et al.*,2019; Tahira *et al.*,2019).

1.1.3 Ill-health effects of aflatoxin M_1

Aflatoxin M_1 is a hepato-carcinogen, classified as a group 1 carcinogen by International Agency for Research on Cancer (IARC Monograph, 2018). Exposure of humans to aflatoxin M_1 leads to several health-related problems including acute and chronic aflatoxicosis; it exerts its negative effect on health through binding to nucleic acid causing DNA damage and eventually leading to hepatotoxicity, carcinogenicity, immune suppression, cirrhosis and stunted growth in children (Singh *et al.*, 2021). .

1.1.4 Prevalence of raw and heat-treated milk with aflatoxins

Prevalence of milk with aflatoxin M_1 is due to consumption of feeds contaminated with aflatoxin B_1, which eventually contaminate milk (Langat *et al.*, 2016). Many studies have been conducted on aflatoxin M_1 contamination in raw and heat treated milk, with findings revealing levels above the Codex Alimentarius and EU limits from various countries, such as, AFM_1 contamination in pasteurized and UHT milk in Morocco (Mannani *et al.*, 2021); AFM_1 contamination in pasteurized and powdered milk products in Iran (Mahmoodi *et al.*, 2019); AFM_1 contamination in raw, pasteurized, UHT cows' milk and dairy products in Lebanon (Daou, *et al.*, 2020); AFM_1 contamination in milk and milk products in Turkey (Eker *et al.*, 2019); AFM_1 contamination in raw and processed milk in Pakistan (Tahira *et al.*, 2019) and AFM_1 contamination in milk and milk products in Kenya (Langat *et al.*, (2016).

Langat *et al.* (2016) reported that 84.32% (156/185) of the samples of raw milk, processed milk and milk products collected in Bomet county in Kenya were contaminated with aflatoxin M_1 and 43.8% of samples had contamination levels higher than the tolerance limit of 0.05 μg/l recommended by FAO and WHO. In addition, the study indicated that the level of contamination in raw milk was higher (52.0%) than in processed milk (8.6%). Mohammed (2016) reported that 83.8% (31/37) of raw cow milk samples collected randomly from different locations in Singida region, Tanzania was contaminated with AFM_1, with levels exceeding the FAO/WHO of 0.05 ng/ml.

There is scanty information on aflatoxin M_1 contamination in thermal processed cow's milk, particularly, pasteurized and UHT, in Tanzania, as well as knowledge associated with aflatoxin M_1 contamination, as previous studies mostly reported on contamination in raw cow's milk in few regions only. Therefore, the aim of this study was to carry out surveillance on aflatoxin M_1 contamination of pasteurized and UHT milk marketed in Dar es Salaam, the commercial capital of Tanzania.

1.2 Justification

Aflatoxin M_1 is a hepato-carcinogen, classified as a group 1 carcinogen by International Agency for Research on Cancer (IARC Monograph, 2018). Aflatoxin M_1 is a heat-stable and can survive pasteurization, autoclaving and thermal inactivation (Zakaria *et al.*, 2019). The contamination of milk and milk products by aflatoxin M_1 has been reported in various countries such as, AFM_1 contamination in pasteurized and UHT milk in Morocco (Mannani *et al.*, 2021); AFM_1 contamination in pasteurized and powdered milk products in Iran (Mahmoodi *et al.*, 2019); AFM_1 contamination in raw, pasteurized, UHT cows' milk and dairy products in Lebanon (Daou, *et al.*, 2020); AFM_1 contamination in milk and milk products in Turkey (Eker *et al.*, 2019); AFM_1 contamination in raw and processed milk in Pakistan (Tahira *et al.*, 2019) and AFM_1 contamination in milk and milk products in Kenya (Langat *et al.*, (2016).

Prevalence of aflatoxin M_1 in milk in Tanzania indicated that 92% of raw cow milk retailed in Dar es Salaam city was contaminated with aflatoxin M_1 (Urio *et al.*, 2006) and 83.8% of raw cow milk from households in Singida was contaminated with aflatoxin M_1 (Mohammed *et al.*, 2016). However, there is no reported information on aflatoxin M_1 contamination of pasteurized and ultra-pasteurized (UHT) milk in Tanzania, as well as the awareness of contamination. Thus, the aim of this study was to carry out surveillance on the level of contamination of aflatoxin M_1 in pasteurized and ultra-pasteurized marketed milk in Dar-es-salaam, commercial capital of Tanzania. The results of this study will provide information on level of milk contamination by aflatoxins and contribute to the efforts of food control authorities in developing strategies to ensure public safety. Also, it will serve as a basis for awareness creation for milk value chain stakeholders, including feed millers, milk processors and consumers, on the aflatoxin contamination in pasteurized milk and safety implications.

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to carryout surveillance on aflatoxin M_1 contamination in pasteurized and ultra-pasteurized (UHT) cow milk marketed in Tanzania.

1.3.2 Specific objectives

The specific objectives of this study were to:

i. determine the prevalence and levels of aflatoxin M_1 in pasteurized and UHT milk marketed in Dar es Salaam.

ii. assess the knowledge and practices of milk processors on factors associated with aflatoxins contamination in milk and milk products.

The findings of this research work were reported in two manuscripts presented as chapter two and three.

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CHAPTER TWO

PAPER ONE

2.0 PREVALENCE OF AFLATOXIN M_1 IN PASTEURIZED AND ULTRA-HIGH TEMPERATURE (UHT) MILK MARKETED IN DAR ES SALAAM, TANZANIA

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CHAPTER THREE

3.0 KNOWLEDGE, HANDLING AND FEEDING PRACTICES ASSOCIATED WITH AFLATOXIN M_1 CONTAMINATION OF MILK IN DAR ES SALAAM, TANZANIA.

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3.1 Abstract

This aim of this study was to establish the knowledge, handling and feeding practices associated with aflatoxin M_1 contamination in milk marketed in Dar es Salaam, Tanzania. A cross-sectional descriptive study involving 30 milk processors was conducted. Data was collected using a structured questionnaire and analysed using Statistical Package for Social Sciences (IBM SPSS® Version 27 (2020). Descriptive statistics was used to determine frequencies and percentages of social demographic, knowledge, handling practices of milk and feeding practices of lactating cow. Cross tabulation was used to determine relationship between knowledge of aflatoxins with age and education level of the respondents. The results indicated that the majority of the respondents (83.3%) were aware of existence of aflatoxins and none (0.0%) of the study respondents were aware of aflatoxin contamination in milk and milk products. It was also observed that feeding practices were poor and could be major contribution for AFM_1 contamination in milk.

Although respondents were aware that aflatoxins caused ill-health effects on humans and animals, but were not aware that feeding lactating cow with mouldy feeds could cause into aflatoxin contamination in milk. It was observed that, there was no AFM_1 analysis carried out in raw milk before processing to control contamination AFM_1 in milk and milk products. Therefore, knowledge creation on aflatoxin contamination and use of best feeding practices along the milk value chain is recommended in order to protect public health (safety).

Keywords: Aflatoxin M_1, Awareness, Handling, Feeding practices, Heat treated Milk, Food safety.

3.2 Introduction

Mycotoxins are toxic secondary metabolites produced mainly by certain species of moulds which contaminate variety of agricultural food stuffs as well as animal derived products such as meat, milk and eggs (Alshannaq *et al.,* 2017; Haque *et al.,* 2020; WHO, 2018). Contamination can occur at any stage in food value chain (Achaglinkame *et al.,* 2017).

It has been reported that about 25% of global food crops are contaminated with mycotoxins to the levels which may pose serious public health effects to human (Eskola *et al.,* 2020). Therefore, contamination of food products with mycotoxin is a global concern because it can result into economic losses, adverse health effects to human and domestic animals and barriers to trade (Magembe *et al.,*2016). More than 500 mycotoxins have been identified worldwide; however, there are few very toxic mycotoxins that are of public health concern specifically, aflatoxins, ochratoxins, fumonisins, patulin, zearalenone, and trichothecenes such as deoxynivalenol and T-2 toxin (Horky *et al.,* 2018; Palumbo *et al.,* 2020).

Aflatoxins are toxic secondary metabolites produced by toxigenic moulds particularly *Aspergillus* namely *A. flavus, A. parasiticus* and *A. nomius*. There are more than 20 molecules of aflatoxins and the most prominent aflatoxins are AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2 (Gurbuz *et al.,* 2019; Ismail *et al.,* 2018). AFM_1 is the 4-hydroxy derivative of AFB_1 and are found in milk and milk products of animals that are fed contaminated feeds with aflatoxin B_1 (Ayofemi, 2020; Lee *et al.,*2017; Udomkun *et al.,*2018). The concentration of aflatoxin M_1 in milk is about 0.3 to 6.2% of the concentration of aflatoxin B_1 ingested with the feed and it appears within 12 hours of administration of contaminated feeds, however the concentration level differs depending on factors such as genetics of the animals, seasonal variation, the milking process and the environmental conditions (Ketney *et al.* ,2017; Langat *et al.,* 2016; Vaz *et al.,* 2020). AFM_1 is relatively stable during milk pasteurization, storage, and processing (Mahmoodi *et al.,* 2019). The presence of AFM_1 in milk is a major risk to the public especially children who consume milk as a sole source of diet because of its toxicity and carcinogenicity effect and it has thus been re categorized by International Agency for Research on Cancer (IARC) from Group 2B to Group 1 as a proved carcinogen (IARC, 2012; Li *et al.,*2017).

Animal feeds such as maize, wheat, paddy (grains, husk or straw), green grass, oil seeds cakes and kitchen wastes are potential source of aflatoxin exposure to animals (Ayo *et al.,* 2018; Choudhary *et al.,* 2020; Tahira *et al.,* 2019). In Tanzania, studies on aflatoxin contamination in cattle feeds was reported in sunflower seeds cake by Mmongoyo *et al.,* (2017) and Mohammed *et al.,* (2016) and in maize by Kamala *et al.* (2016). Therefore, proper handling of these products is required in order to prevent exposure of aflatoxins to animals and in milk and milk products.

Several studies have been conducted on awareness and knowledge on animal feeds and milk contamination with aflatoxins worldwide. In Punjab, dairy farmers were assessed on knowledge and practices to control aflatoxin in the dairy rations (Mir *et al.*, 2020). Moreover, Kagera *et al.*, (2019) conducted a study to assess awareness, knowledge and practices on aflatoxins contamination in milk in urban and peri-urban farmers in Kasarani, Kenya.

In Tanzania, awareness and knowledge on aflatoxins contamination was conducted mainly on maize, groundnuts, animal feeds and spices (Ayo *et al.,* 2018; Magembe *et al.*, 2016; Fundikira *et al.,* 2021). There is limited information on the level of knowledge and practices affecting AFM_1 contamination of milk and milk products by milk processors in Tanzania. Therefore, the aim of this study was to assess the knowledge, handling and feeding practices by milk processors on AFM_1 contamination in milk and milk products in Tanzania. The information obtained from this study will be useful in designing measures for increasing milk processor's awareness towards reduction of AFM_1 exposure to milk and milk products.

3.3 Materials and Methods

3.3.1 Study design

A cross-sectional descriptive study was carried out between December 2020 and January 2021 in Ilala, Kinondoni, Temeke, Ubungo and Kigamboni five districts of Dar es Salaam, the commercial capital of Tanzania. This design enabled collection of data on handling, feeding practices and knowledge on aflatoxins by the study population involved in milk processing.

3.3.2 Study population

The study population was people who were involved in milk processing in five districts of Dar es Salaam. This group was selected because it was involved in milk processing. In addition, these people were more likely to respond to the study questions precisely.

3.3.3 Sampling techniques and procedures

The present study was a cross sectional study design. It examined milk processors at a specific point in time from December 2020 to January 2021. A total of 30 milk processors were interviewed from Kinondoni, Ilala, Ubungo, Temeke and Kigamboni districts in Dar es salaam, eleven milk processors were purposively obtained from list of working dairy industry in Dar es salaam registered by Tanzania Dairy Board (TDB) 2020/2021 and the other 19 milk processors were obtained by snowball technique by face-to-face interview from 11 registered milk processors.

3.3.4 Data collection tools

The data of the study was collected using quantitative methods. An interview administered questionnaire (with closed and open-ended questions) was used to study respondents who consented on participation. This questionnaire was in English and translated to Swahili.

The questionnaire administered to milk processors had forty-three questions that attempted to capture information on knowledge of aflatoxins and issues on milk handling and feeding practices. The questionnaire was pre tested on a random sample of 8 participants (milk processors) at Ilala districts to provide a clear indication on the response and relevance of the questions to the study objectives.

3.3.5 Statistical analysis

The data was analysed using Statistical Package for Social Sciences (IBM SPSS® Version 27 (2020). The data was coded appropriately and fed into SPSS version 27 to determine the frequencies. The analysis involved descriptive statistics to describe socio-demographic, knowledge, feeding and handling practices, in frequency tables. Cross tabulation (Fisher's exact test at $P < 0.05$) was used to analyse relationship between two variables such as relationship on knowledge of aflatoxins with age of milk processors and relationship on knowledge of aflatoxins with education level of milk processors.

3.4 Results and discussion

3.4.1 Demographic characteristics of the respondents

In this study 30 respondents who were involved in processing milk and milk products were interviewed from all the five districts in Dar es Salaam. The highest percentage of respondent (63.3%) belonged to the 33-43 age group, followed by 20.0% of respondents who were in (22-32) age group, then 10% respondents were in 44-54 age group and 6.7% respondents were above 55 age group. The results of this study indicated that, 66.7% of respondents were male and only 33.3% of them were female. About 37% of respondents attended college/university education while 46.7% attended secondary school education 16.7% of the respondents attended primary school education and there were no respondents who have informal education (Table 3.1).

Table 3.1: Demographic characteristics of the study respondents

Variable	Description	Frequency (N=30)	Percentage %
District	Ilala	6	20
	Kinondoni	6	20
	Temeke	6	20
	Ubungo	6	20
	Kigamboni	6	20
Gender	Male	20	66.7
	Female	10	33.3
Age categories	22-32	6	20.0
	33-43	19	63.3
	44-54	3	10.0
	Above 55	2	6.7
Education level	Informal/not attended	0	0.0
	Primary	5	16.7
	Secondary	14	46.7
	College/university	11	36.7

3.4.2 Knowledge on aflatoxins contamination

Table 3.2 indicated that all 100% of the study respondents in this study knew contaminants in milk, however none (0.0%) of the study respondents knew aflatoxin was among the contaminants in milk, 86.7% of the study respondents knew milk can be contaminated with drugs residues and 13.3% of the study respondents knew pesticide can contaminate milk.

Moreover, results of this study indicated that 83.3% of the study respondent have ever heard of the word ‘‘aflatoxins’’, whereas only 16.7% of the study respondents had never heard of the word aflatoxin. This was similar to the observation reported by Kagera *et al.,* (2019) that 80% of the respondents in Nairobi, Kenya had heard of aflatoxins; Similarly, Udomkun *et al.,* (2018) reported that 85% of farmers in eastern Democratic Republic of Congo were aware of aflatoxins. The results of this study were slightly lower than that reported by Magembe *et al.,* (2016) where 97.2% of the respondents in Kilosa District in Tanzania had heard of aflatoxins. In contrast, the results of this study were higher than the studies reported by Lee *et al.* (2017) reported that 9.4% of the respondents from six provinces of Vietnam had ever heard of aflatoxins; Ayo *et al.*, (2018) reported that 29% of livestock farmers in Meru district in Tanzania had heard of aflatoxins; Adekoya *et al.*, (2017) reported that 2% of the respondents in Nigeria had heard of aflatoxins; Mir *et al.*, (2020) reported that 42% of dairy farmers in Punjab had heard of aflatoxins; Nakavuma *et al.*, (2020) reported that 47.73% of poultry farmers and feed processors in Uganda were aware of mycotoxin; Fundikira *et al.*, (2021) reported that 3.3% of spice retailers in Dar es salaam region in Tanzania had heard of aflatoxins; Jere *et al.*, (2020) reported that 15% of food handlers in Salima district in Malawi had heard of aflatoxins and Suleiman *et al.*, (2017) reported that over 80% of the maize producers, sellers, and buyers in Tanzania were not aware of the presence of mycotoxins in foods.

The discrepancy in awareness between this particular study and other reported studies may be attributed to several factors including the nature of the study population. For example, in Tanzania in year 2016 aflatoxicosis outbreak in Chemba, Kiteto, Chamwino, Dodoma and Kondoa districts in Dodoma region (Kamala *et al.*, 2018) and in Kenya Probst *et al.* (2007) reported aflatoxicosis events which killed many people might have contributed to the increased awareness on aflatoxins.

Furthermore, none of the respondents (0.0%) in this study knew milk and milk products such as UHT and pasteurized could be contaminated with aflatoxins and all respondents (100%) were unaware on aflatoxins contamination in milk and none (0.0%) of the respondents were aware of association between aflatoxins in feeds with aflatoxins in milk. This was almost similar to the research carried out by Gizachew *et al.*, (2016) who reported that over 90% of dairy farmers in Ethiopia were unaware of aflatoxin contamination of milk. The level of contamination observed in this study was higher than that reported by Mir *et al.* (2020) in Punjab whereby 84% of dairy farmers were unaware of aflatoxin contamination of milk; Lee *et al.* (2017) reported that 79.71% respondents from six provinces of Vietnam were unaware of aflatoxin contamination in milk; Udomkun *et al.,* (2018) reported that 46% of farmers in eastern Democratic Republic of Congo were not aware of aflatoxins contamination in milk. Lack of awareness on aflatoxin contamination among milk processors in this study could be a major contributing factor to aflatoxin contamination in milk hence a threat to public health.

This study revealed moderate knowledge on ill-health effects to human and animals caused with consumption aflatoxins, where by 33.3% of the respondents were aware of the health effects to human and animals caused with consumption of aflatoxins contaminated food and 66.7% of the respondents were not aware of the health effects. This could be due to inadequate information on ill health effects caused aflatoxins. The observation made in this study similar to those observations by Magembe *et al.,* (2016) in Kilosa district in Tanzania who reported that 66.7% of respondents were not aware of the ill-health hazards caused by mycotoxins. Results on awareness level in this study were slightly higher than those reported by Mboya and Kolanisi (2014) who observed that that 58.5% of the participants in Rungwe district, Tanzania were not aware of health hazards associated with the ingestion of mycotoxins in food. In contrast, the results on awareness levels of this study were higher than the study conducted by Fundikira *et al.* (2021) who reported that none of the spice retailers in Dar es salaam, Tanzania were aware of the health effects of aflatoxins, and Ngoma *et al.* (2017) reported that only 1.4% of the parents in central part of Tanzania were aware of ill-health effects caused by aflatoxins contamination in complementary foods.

Table 3.2: Knowledge on aflatoxins by the study respondents

Variable	Description	Frequency (N=30)	Percentage %
Knowledge of contaminants in milk	Yes	30	100.0
	No	0	0.0
Contaminants in milk	Drug residue	26	86.7
	Pesticides	4	13.3
	Aflatoxin	0	0.0
Heard of aflatoxin	Yes	25	83.3
	No	5	16.7
Aware of aflatoxin contamination in milk and milk products such as UHT and pasteurized	Yes	0	0.0
	No	30	100.0
Factors for aflatoxin in milk	Cattle consume	0	0.0
	toxins in feeds	30	100.0
	Do not know		
Aware of consumption of contaminated food cause health effect to human and animals	No	20	66.7
	Yes	10	33.3

3.4.3 Milk handling practices

About 73.3% of the milk processors procured raw milk for processing from their own dairy farms and milk collectors, while 26.7% of the processors procured raw milk for processing from their own dairy farms (Table 3.3). Basing on production capacity of milk processors, 46.7% produced less than 100 l/day; 46.7% produced 100-1000 l/day; and (6.7%) produced more than 1000 l/day. It was observed that all milk processors (100%) assessed the raw milk quality parameters at reception before processing; 36.7% assessed colour, 3.3% assessed smell and 60% assessed density. However, none (0.0%) of the respondents analysed raw samples for presence of aflatoxins. This observation might be due to lack of knowledge on the carry-over of aflatoxin from feeds to animal product such as milk among milk processors. This is supported by the fact that most of the aflatoxin’s awareness in Tanzania were conducted in few products such as maize, groundnuts and animal feeds (Ayo *et al.*, 2018; Magembe *et al.*, 2016).

Table 3. 3: Handling practices of milk by the study respondents

Variable	Description	Frequency N=30	Percentage%
Where raw milk is obtained	Own dairy farm	8	26.7
	Dairy farm and milk collectors	22	73.3
Production capacity (Liters) per day	Less than 100	14	46.7
	100-1000	14	46.7
	Greater than 1000	2	6.7
Quality inspection of milk before processing	Yes	30	100.0
	No	0	0.0
Parameters for inspection	Colour	11	36.7
	Smell	1	3.3
	Density	18	60.0
Checking aflatoxin contamination in raw milk before processing	Yes	0	0.0
	No	30	100.0

3.4.4 Feeding practices of lactating cow by the study respondents

All respondents (100%) in this study raised lactating cattle as a source of milk for processing; 50% used hay, 46.7% used cereal products while 3.3% mixed feeds with other products such as wastes from the kitchen for feeding lactating cattle (Table 3.4). The results indicated that all respondents (100%) mixed lactating cattle feeds with concentrates. It was observed that kitchen wastes such as bread and mouldy left overs were used as animal feeds and cereal products like spoilt maize grains were used as cattle feed. This was also observed by Magembe *et al.* (2016) in Kilosa district, Tanzania and Mboya and Kolanisi (2014) in Rungwe district in Tanzania who reported that 18.1% and 38.4% of mouldy maize grains were used as animal feeds respectively. This observation might be a good source of aflatoxins contamination in cattle feeds and milk.

In this study 90% of the respondents did not inspect for mould growth in cattle feeds before feeding the cows and only few (10%) of respondents were carried out physical inspection by smelling, colour and appearance. This is supported by the fact that aflatoxins cannot be inspected visually only by laboratory test. The results of this study were similar to the studies reported by Jere *et al.*, (2020) whereby food handlers in Malawi inspected quality of the grains such as discoloration, damaged and off odour through physical observation and Suleiman *et al.* (2017) in Tanzania reported that quality of the grains was determined by physical observation such as broken grains, infestation and discoloration. Therefore, when these factors considered can reduce level of contamination.

It was observed that all respondents (100%) stored lactating cattle feeds for use during period of scarcity (70%), in times of lower prices (26.7%) and surplus (3.3%). About 77% of the respondents stored cattle feeds for more than one month while 23.3% of the respondents stored cattle feeds for less than one month. It was observed that 23.3% of the study respondents stored cattle feeds in quantity of less than 100 kg; 66.7% of respondents stored cattle feeds ranging from 100 to 1000 kg and 10% of respondents stored cattle feeds in quantity exceeding 1000 kg. Moreover, it was observed that 86.7% of respondents stored lactating cattle feeds on the floor while 13.3% of respondents stock piled cattle feeds. The results indicated that 80.0% of the respondents did not monitor mould growth in cattle feeds during storage while 20% of the respondents monitored mould growth on stored feeds. Only 20% of the respondents dried feeds in order to prevent mould growth while (80%) of the respondents were not aware of any means for prevention/control of mould growth in cattle feeds.

It has been reported that poor storage practices such as stock piling and other poor bulk storage practices of feeds and prolonged time in storage, influenced contamination of feed stuffs with aflatoxigenic producing fungi (Nakavuma *et al.*,2020; Makau *et al*.,2016). Therefore, presence of aflatoxins in feeds may result into aflatoxin M_1 contamination in milk upon lactating cow consumption.

Table 3.4: Feeding practices of lactating cow by the study respondents

Variable	Description	Frequency (N=30)	Percentage %
Have lactating cattle	Yes	30	100.0
	No	0	0.0
Lactating cattle feeds	Hay	15	50
	Cereal products	14	46.7
	Wastes from kitchen	1	3.3
Mixing feeds with concentrates	Yes	30	100.0
	No	0	0.0
Where concentrates purchased	Local suppler	19	63.3
	industries	10	33.3
Inspection of fungal in lactating cattle feeds	Yes	3	10
	No	27	90
Inspection Parameters	Smell	1	3.3
	Colour	2	6.7
	No inspection	27	90.0
Animal feeds storage	Yes	30	100.0
	No	0	0.0
Reason for storage	Period of scarcity	28	70.0
	Lower price	8	26.7
	Surplus	1	3.3
Storage area	Stock piling	4	13.3
	On floor	26	86.7
Storage duration	Less than month	7	23.3
	More than month	23	76.7
Storage quantity (kg)	Less than 100	7	23.3
	Between 100-1000	20	66.7
	Greater than 1000	3	10.0
Monitoring fungal growth	Yes	6	20.0
	No monitoring	24	80.0
Prevention fungal growth	No	24	80.0
	Proper drying	6	20.0

3.4.5 Relationship between knowledge of aflatoxin with age and level of education

Cross tabulation analysis was used to describe and determine the relationship between the two variables. In this study relationship between knowledge of aflatoxins with age and education level of the respondents was investigated.

Table 3.5: Relationship between knowledge on aflatoxins by education level of the study respondents

Education level of the respondents	**Have you ever heard about aflatoxins?**			***P – Value***
	Number of respondents	**Yes n (%)**	**No n (%)**	
Primary	5	0(0.0)	5 (100)	0.000*
Secondary	14	9 (64.3)	5 (35.7)	
College/University	11	11 (100)	0 (0.0)	
Total	**30**	**20 (66.7)**	**10 (33.3)**	

- Superscript denoted by * indicate significant difference at $P< 0.05$
- n is the number of the study respondents

The relationship between knowledge on aflatoxins with education level of the respondents was investigated (Table 3.5). The results obtained in this study revealed that education level was directly related to aflatoxins awareness and it was statistically significant at $P<0.05$ whereby respondents who were highly educated had better knowledge of aflatoxins compared to less educated respondents. Respondents who had attended college/university education level were more aware of aflatoxins compared to the respondents who had attended secondary school education, similarly respondents who had attended secondary school education were more aware of aflatoxins compared to the respondents who had attended primary school education. This is due to fact that people who are more educated have more access and tend to seek for more information on food safety and related issues. These findings were similar to studies done by Adekoya *et al.*, (2017); Jere *et al.,* (2020); Suleiman *et al.,* (2017) and Udomkun *et al.,* (2018) indicating relationship between education level with aflatoxins awareness reported that educated people were more knowledgeable about aflatoxins compared to less educated people.

Table 3.6: Relationship between knowledge of aflatoxins with age of the study respondents

Age of the respondents (years old)	**Number of Respondents(n)**	**Have you ever heard about aflatoxins?**		***P – Value***
		Yes n(%)	**No n(%)**	
22-32	6	2 (33.3)	4 (66.7)	0.000*
33-43	19	16 (84.2)	3 (15.7)	
44-54	3	1 (33.3)	2 (66.7)	
Above 55	2	1 (50)	1 (50)	
Total	**30**	**20 (66.7)**	**10(33.3)**	

- Superscript denoted by * indicate significant difference at $P< 0.05$
- n is the number of the study respondents

In this study, relationship between knowledge on aflatoxins with age of the respondents was investigated, (Table 3.6) indicated that 33.3% (2/6) of the respondents aged between 22-32 years were aware of aflatoxins; 84.2% (16/19) of the respondents 33-43 years old were aware of aflatoxins; 33.3% (1/3) of the respondents aged between (44-54) years old were aware of aflatoxins and 50.0% (1/2) of the respondents above 55 years old were aware of aflatoxins. The results of this study indicated younger age group had significant high knowledge on aflatoxins contamination at $P<0.05$ compared to the old age group. This could be due to the fact that younger age groups were more likely to be more educated or informed about aflatoxins. This is supported by the studies done by Ayo *et al.* (2018) and Lee *et al.* (2017) who reported that younger age groups were more aware of aflatoxins and had the ability to access information faster.

3.5 Conclusion

This study gave a highlight into the handling practices of milk, best feeding practices of lactating cow and level of knowledge of milk processors on issues of aflatoxins in milk and milk products. It was observed that, milk processors in the dairy processing had knowledge on aflatoxins and ill-health effects on humans and animals caused by consumption of foods contaminated with aflatoxins. However, none of the respondents had a knowledge of aflatoxin M_1 contamination of milk and milk products such as pasteurized and UHT milk. In addition, this study indicated that there was relationship between knowledge on aflatoxins with education level and age of the respondents.

Furthermore, none of the respondents had knowledge on factors that contribute to aflatoxin M_1 contamination in milk, that lactating cow fed with feeds contaminated with aflatoxins could excrete aflatoxin M_1 in milk. Poor feeding practices and low-quality feeds such as low-quality grains may cause contamination of milk with aflatoxin M_1. This study contributed to knowledge on aflatoxin M_1 content in milk-to-milk value chain stakeholders in Tanzania.

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CHAPTER FOUR

4.0 OVERALL CONCLUSION AND RECOMMENDATIONS

4.1 Conclusions

In this study, the level of AFM_1 in marketed pasteurized and UHT milk in Dar es salaam, Tanzania was found to be rather high and exceeding maximum level of 0.05 µg/L as per EU regulations. However, none of the contaminated sample exceeded Codex limit of 0.5 µg/L, though such contamination does not guarantee the safety of the products to the Tanzania population. Therefore, measures should be taken in order to minimize the level of AFM_1 contamination. Furthermore, samples originating from hot humid coastal climatic zones had high level of contamination of AFM_1 compared to temperate highland zones this is because hot humid coastal zones had favorable conditions for aflatoxigenic fungi to grow, therefore more effort should be taken to these areas to minimize AFM_1 contamination.

Furthermore, lack of knowledge on AFM_1 contamination in milk, poor storage practices of animal feeds and lack of knowledge on health effects caused by aflatoxins among milk processors observed in this study might be a major reason for AFM_1 contamination in milk. Therefore, the best way to deal with this problem is to reduce AFB_1 contamination in animal feedstuffs by improved processing and storage practices. At the same time, attention should be given to regular monitoring of aflatoxins in animal feed and dairy products. In addition, the governmental agencies should train the farmers, dairy companies and dairy product consumers on the potential health consequences of aflatoxins. Finally, milk and dairy products with high levels of AFM_1 must not be allowed for human consumption by the public health authorities.

4.2 Recommendations

Based on the findings of this study the followings are recommendations:

1. In order to preserve the quality and ensure the safety of milk and dairy products consumed in Tanzania, governmental authorities should set a clear strategy that aims at reducing AFM_1 contamination.

2. Regulatory authorities in Tanzania should set limits for AFM_1 in milk and milk products, since both Tanzanian standards and East African Standards not cover recommended level for AFM_1. Therefore, the findings of this study will provide a base in standard development.

3. More studies should be conducted in Tanzania to determine contamination levels of AFM_1 on milk and milk products such as cheese, yogurt, ice cream.

4. Studies to determine levels of aflatoxin B_1 in the animal feeds given to lactating cows should be conducted. Such studies will be helpful to control the health risk factors and supply of AFM_1 free milk to Tanzania population.

5. It is recommended that all stakeholders involved in milk processing should be extensively trained on aflatoxins and proper feeding quality of cattle feeds to prevent AFM_1 contamination in milk.

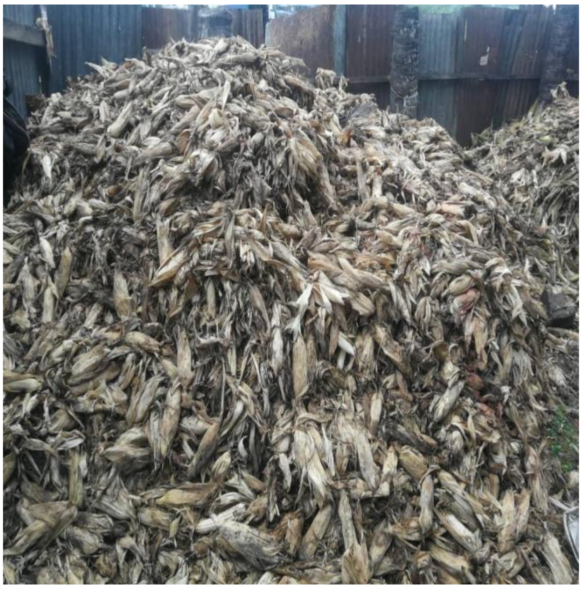
6. Knowledge on AFM_1 contamination in milk should be extended to all consumers as milk is a food product that is consumed by all age groups. Therefore, all population should be sensitized on aflatoxins ill health effects.

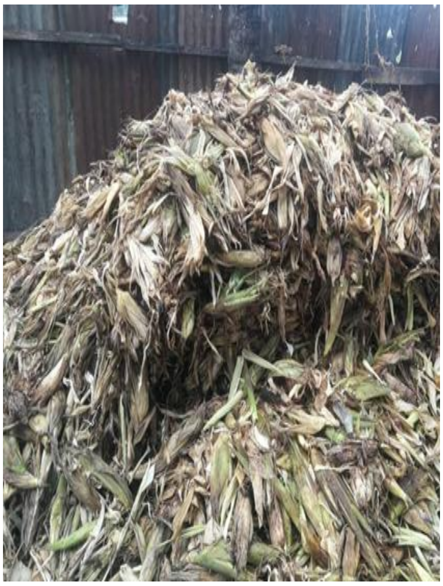
7. Regulatory authorities should reinforce extensive training to dairy farmers and livestock keepers on best practices such as best feeding practices and good storage practices of animal feeds. Sensitization of dairy farmers, milk processors and feed millers could be achieved through regulatory authorities and government agencies.

8. Intervention strategies by regulatory agencies such as Tanzania Dairy Board (TDB) through their enforcement compign should be conducted to all milk stakeholders on knowledge of aflatoxin M_1 contamination in milk and milk products and the associated health effects to human and animals.

APPENDICES

Appendix 1: Photos showing poor storage practices of cattle feeds for feeding cow





Husks and spoilt maize stored on floor were used for feeding cattle

Products from kitchen were used for feeding cattle

Appendix 2: Questionnaire to assess knowledge and practices on aflatoxin contamination in dairy products to milk processors

This questionnaire is aimed to assess milk processor's knowledge on 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 individual will not be shared to anyone.

PART A: GEOGRAPHICAL LOCATION

1. Region………………………………………………………
2. District………………………………………………………
3. Division…………………………………………………….
4. Ward…………………………………………………………
5. Village………………………………………………………

PART B: RESPONDENT DETAILS

6. Date of interview: Day……Month………Year….……...
7. Sex of respondent: ☐ Male ☐ Female
8. Age of the respondent (years)…..................
9. Level of education of the respondent
   - ☐ Primary education level
   - ☐ Secondary school education level
   - ☐ Collage/University education
   - ☐ Informal/Not attended any school
10. What type of milk product are you processing?
    - ☐ Pasteurized milk
    - ☐ Yoghurt
    - ☐ UHT milk
    - ☐ fresh milk
    - ☐ Cultured milk
    - ☐ All types of milk product

☐ Any other (Specify)…………………………………………………………

PART C: KNOWLEDGE ON AFLATOXIN

11. Do you know contaminants in milk?

☐ Yes ☐ No

12. If yes what types of contaminants in milk do you know?

☐ Aflatoxin ☐ drug residues

☐ Metals ☐ pesticides

☐ Any other (specify) ……………………………

13. Have you ever heard of aflatoxin (in local language??)?

☐ Yes ☐ No

14. If yes, where did you first hear about it?

☐ Radio ☐ TV ☐ Hospital

☐ Training ☐ Others (Specify)………

15. Do you know aflatoxin can contaminate foods and animal feeds?

☐ Yes ☐ No

16. If, yes which type of food products are most contaminated with aflatoxin

☐ Cereal products ☐ Spices

☐ Milk ☐ oil seeds

☐ Do not know ☐ Any other (specify) ………………

17. What factors contribute to aflatoxin contamination?

☐ Rodents/Insects/Molds ☐ Do not know

☐ Poor harvesting/ Storage ☐ Others (Specify) …………

☐ Moisture

18. Do you know milk and milk product such as UHT and pasteurized can be contaminated with aflatoxin?

Yes ☐ No ☐

19. If, Yes mention factor which cause milk contamination with aflatoxin

When animal consume contaminated feed Do not know

Any other (specify) ……………………………….

20. Do you know consumption of milk contaminated with aflatoxin can cause health effect to animals and human?

Yes ☐ No ☐

21. If Yes, mention any health effect of aflatoxin contamination to human?

Cancer stunted /poor growth

Do not now Any other (specify) …………………………….

PART D: MILK HANDLING PRACTICES

22. What are the sources of milk for processing?

Milk collectors ☐ Own Farm

Milk collectors and own farm ☐ any other (specify)……………

23. What is your production capacity(litres) per day?

Less than 100 ☐ Between 100 to 1000

Greater than 1000

24. Do you carry any quality inspection of milk during processing?

Yes ☐ No ☐

25. If, yes what parameters do you check

Color Moldy smell

aflatoxin

Any other (specify) ……………………………………

26. Do you check aflatoxin in milk before processing?

Yes ☐ No ☐

27. If yes, have you encountered aflatoxin problem during checking?

Yes ☐ No ☐

28. If **Yes**, how do you manage/prevent aflatoxin in milk before processing?

Rejection ☐ proceeding with next stage

Any other (specify) ……………………….

PART E: FEEDING PRACTICES FOR LACTATING COW

29. Do you have lactating cattle do you have?

Yes ☐ No ☐

30. What are the main types of feeds are you using for feeding lactating cow?

Hay ☐ cereal bran

kitchen wastes ☐ residues from breweries industries

Seed cake ☐ Any other (specify)………………….

31. Do you mix feeds with other supplements (concentrates)?

Yes ☐ No

32. If Yes, where do you obtain supplements (concentrates) for feeding lactating cow?

Local supplier ☐ Industries

Commercial processor

Any other (specify) ……………

33. Do you check fungal growth in supplements (concentrates)/feeds before feeding lactating cow?

Yes ☐ No ☐

34. If **Yes,** how do you check fungal growth of feeds/supplement?

Smelling ☐ Appearance, not moldy

Colour ☐ Any other (specify) ……….

35. During checking, have you encountered any fungal/moulds growth in supplements /feeds?

Yes ☐ No ☐

36. If **Yes**, how do you manage the problem?

Disposing ☐ Proper drying

Binder ☐ Purchase from reliable source

Nothing/proceed with feeding

Any other (specify)……………………………….

37. Do you store feeds/supplements?

Yes Yes ☐ No

38. If **Yes,** what are reasons for storing feeds/supplements?

Preparation for period of scarcity ☐ Favorable/lower prices

Others (specify) …………………………………

39. How do you store feeds/supplements?

stock piling ☐ Raised platform

On floor ☐ Any other (specify) ……………

40. What is the storage duration?

Less than 1 month ☐ more than 1 month

41. What is the storage quantity (kg)?

Less than 100 ☐ Greater than 1000

Between 100–1000 ☐

42. During storage, have you encountered any fungal/moulds growth in supplements /feeds?

Yes ☐ No ☐

43. If **Yes**, how do you manage the problem?

Fumigation ☐ Proper drying

Binder ☐ Nothing/proceed with storage

Practice first in first out Disposing

Purchase from reliable source

Any other (specify)……………………………….

Thank you* very *much.