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Occurrence of aflatoxins and associated risk factors in dairy value chain in selected districts of three agro-ecological zones in Tanzania

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OCCURRENCE OF AFLATOXINS AND ASSOCIATED RISK FACTORS IN DAIRY VALUE CHAIN IN SELECTED DISTRICTS OF THREE AGRO-ECOLOGICAL ZONES IN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Aflatoxins are natural compounds produced by specific type of fungi, which contaminate foods and animal feeds. This study assessed the occurrence of aflatoxins and associated risk factors in livestock feeds and raw cow milk through a survey of the smallholder dairy farmers (SDFs) and agrovet dealers from Mpwapwa, Serengeti, and Hai districts which represent three agroecological zones (Semi-arid, Arid and Northern Highlands) in Tanzania. The findings showed that the level aflatoxin awareness among SDFs (23.2%) and agrovet dealers (50%), respectively. The prevalence of aflatoxin B_1 (AFB_1) in livestock feeds from agrovet dealers, and SDFs was 88.5 and 86.2%, with a concentration ranging from a limit of detection (LOD) to 22.99 and 32.9 µg/kg, respectively. About 15% and 22% of feed samples from agrovet dealers and SDFs respectively were detected with AFB_1 at levels exceeding their respective regulatory limits. The prevalence of aflatoxin M_1 (AFM_1) in cow raw milk was 30.7%, and about 27.9% exceeding the European Union (EU) maximum regulatory limits of 0.05 µg/L and about 19.9% exceeding the Tanzania and East Africa maximum regulatory limits of 0.5 µg/L. The risk factors associated with AFB_1 and AFM_1 were cattle feeding systems: zero-grazing (Odds Ratio (OR) = 11.3) and mixed feeding (OR = 16.0); feed handling and storage practices: (OR=2.0). Others are agroecological zones: semi-arid zone (OR=15.2) and northern highland zone (OR=2.1) and level of education: primary (OR =16.0) and secondary (OR = 8.0) (p <0.05). This study has revealed low level of awareness on aflatoxin contamination in animal feeds and cow raw milk suggesting a potential health risk to consumers. It is therefore vital to raise awareness and strengthen other intervention for aflatoxin control in order to control aflatoxin contamination along the dairy value chain.

Keywords: Aflatoxin B_1, aflatoxin M_1, animal feeds, smallholder dairy farmers, agrovet dealers, agroecological zones

DECLARATION

I, Steven J. Kitigwa, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concomitantly submitted for degree award in any other institution.

Steven J. Kitigwa — Date: 21/08/2023

The declaration is confirmed by:

Prof. Athanasia Matemu — Date: 21/08/2023

Dr. Esther G. Kimaro — Date: 21/08/2023

Dr. Rashid A. Suleiman — Date: 21/08/2023

Dr. Yacob P. Nagagi — Date: 21/08/2023

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance for the dissertation entitled "*Occurrence of aflatoxins and associated risk factors in dairy value chain in selected district of three agro-ecological zones-Tanzania*". In partial fulfillment of the Award of the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Prof. Athanasia Matemu — Date: 21/08/2023

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DEDICATION

I dedicate my dissertation to my parents, My father Julius Kitigwa and my mother, Eva Kitigwa. Though they were less privileged to get high level of formal education, but dedicated their lives ensuring that, their children get high quality education. Now and forever, God bless you abundantly.

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LIST OF ABBREVIATIONS AND SYMBOLS

AF	Aflatoxin
AFs	Aflatoxins
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
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CAC	Codex Alimentarius Commission
CI	Confidence Interval
COMESA	Common Market for Eastern and Southern Africa
COSTECH	Tanzania Commission for Science and Technology
EA	East Africa
EAC	East African Community
EC	European Committee
ECOWAS	Economic Community of West African States
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agricultural Organization Statistics
US FDA	United State Food and Drug Administration
GDP	Gross Domestic Product
HPLC	High Performance Liquid Chromatography
IARC	International Agency Research for Cancer
KNCHREC	Kibong'oto Infectious Disease Hospital, Nelson Mandela-AIST and Cedha Health Research Ethics Committee
LiSBE	Life Sciences and Bioengineering
LOD	Limit of Detection
LOQ	Limit of Quantification
n	Sample Size
NaOH	Sodium Hydroxide
NBS	National Bureau of Statistics

NM-AIST	Nelson Mandela African Institution of Science and Technology
OR	Odd Ratio
p	p – value (probability value)
PBS	Phosphate Buffer Saline
Ppb	Parts per Billion
Ppt	Parts per Trillion
SDFs	Smallholder dairy farmers
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TSHZ	Tanzania Shorthorn Zebu
UHT	Ultra-high Temperature
URT	United Republic of Tanzania
USA	United States of America
WHO	World Health Organization
pH	Acidity or alkalinity
^{o}C	Degree centigrade
<	Less than
>	Greater than
%	Percentage
a_w	Water Activity
g	Gram
km^2	Kilometre Square
km	Kilometre
L	Litre
ml	Millilitre
mm	Millimetre
v/v	Concentration (volume/volume)
µg/kg	Microgram per Kilogram
µg/L	Microgram per Litre

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Mycotoxins are toxic compounds naturally produced by certain types of fungi such as *Aspergillus flavus*, *Fusarium graminearum*, *Fusarium verticillioides*, *Aspergillus ochraceus* and *Penicillium verrucosum* (Tolosa *et al.,* 2021). Although there are more than 400 types of mycotoxins, only a few are of public health importance. The mycotoxins of public health important include, aflatoxin, deoxynivalenol and zearalenone, fumonisins (FB_1, FB_2 and FB_3) and ochratoxin. Aflatoxins (AFs) are predominantly produced by *Aspergillus flavus*, *Aspergillus parasiticus* and Aspergillus *nomius* (Madhysatha & Marquardt, 2019). The major types include: Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). Other types of aflatoxin (AF) include aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) which are metabolites of AFB_1 AFB_2, respectively. Aflatoxin producing fungi require favourable temperature and humidity for optimal growth and toxin production (Ledo *et al.,* 2020). According to Nishimwe *et al*. (2019), *A. parasiticus* and *A. flavus* attain their optimum growth at a temperature between 29-37°C, with 0.99 water activity (a_w).

Aflatoxin contaminates food and feeds causing adverse health effects. Exposure to aflatoxins over time causes aflatoxicosis (Peles *et al.,* 2019), which can be acute or chronic depending on the dosage of aflatoxins and exposure time (Negash, 2018). Aflatoxins may affect cattle production by directly or indirectly affecting physiological function and limiting feed intake (Peles *et al.,* 2019). The predominant route by which animals can be exposed to aflatoxins is by ingesting contaminated feeds; others routes such as inhalation and dermal contact, can potentially expose them to aflatoxin to some small extent (Alvarado *et al.,* 2017). Aflatoxins affect various organs and body systems. Each type of aflatoxin (B_1, B_2, G_1, and G_2) can affect animals either individually or through a synergistic effect involving more than one type of aflatoxins (Peles *et al.,* 2019). Aflatoxin B_1 and M_1 are classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogen to humans (IARC, 2009; Ismail *et al.,* 2015). Therefore, food and feeds should be free of aflatoxin or contain contamination levels below the maximum limits set out in standards. When animals consume aflatoxins contaminated feed, Aflatoxin M_1 is excreted as a metabolic product of aflatoxin B_1 and can be found primarily in animal tissues and fluids such as milk and urine (Tolosa *et al.,* 2021). Aflatoxin M_1 is a potential human carcinogen (Alvarado *et al.,* 2017). Aflatoxin M_1 can cause hepatic cancer and is associated with child malnutrition, stunting growth and ineffective

immune system culminating into increased vulnerability to diseases in adulthood (Magoha *et al.,* 2014; Makori *et al.,* 2018; Wu, 2015; IARC, 2009). Therefore, it is crucial for the milk to be safe from aflatoxin taking into account that it cannot be destroyed by normal heat processing methods like pasteurization (Mohammed *et al.,* 2016).

Tanzania ranks third among the African countries which keep livestock, with tremendous livestock sector growth from 2 326 025 households involved in livestock keeping in 2007/08 to 2 747 910 households in 2019/20 (National Bureau of Statistics [NBS], 2020). Most of the cattle are owned by small holder dairy farmers (33.8 million) compared to large scale farmers (142 968). Milk production substantially increased to around 3.13 billion litres in 2019/20 whereby 99.4% (3.11 billion litres) were from SDFs and 0.6% (17.8 million litres) from large scale farms (National Bureau of Statistics [NBS], 2020). Due to growth in the dairy sector, feed processors and SDFs face several challenges, including feed availability and lack of feed storage facilities (Gillah *et al.,* 2012; Kavana *et al.,* 2017). This scenario cause challenges to meet demand for sufficient feeds for the cattle and assurance of its safety. Most of the animal feeds are derived from cereal crops, oilseeds, and their by-products. Studies conducted in Tanzania showed that the prevalence of aflatoxin contamination in sunflower seeds, is 15% (Mohammed *et al.,* 2018) and 10 to 80% in maize (Boni *et al.,* 2021a, 2021b; Gong *et al.,* 2020; Kamala *et al.,* 2015; Kimanya *et al.,* 2008; Mtega *et al.,* 2020; Nyangi *et al.,* 2016; Suleiman *et al.,* 2017; Suleiman & Rosentrater, 2015).

Recent studies have reported high level of aflatoxin contamination in animal feeds and milk. For instance, in Kenya high level of AFB_1 (147.86 µg/kg) was found in animal feeds (Makau *et al.,* 2016a), 39.8 µg/kg in maize bran and 54.5 µg/kg in mixed feeds in Rwanda (Nishimwe *et al.,* 2019) and 2.4 µg/kg in maize bran in Tanzania (Nyangi *et al.,* 2016). The AFB_1 levels were 9.4 g/kg in maize bran and 31.6 g/kg in sunflower seed cake (Kajuna *et al.,* 2013). Furthermore, prevalence of aflatoxin levels varies greatly according to agro-ecological zones (Dembedza *et al.,* 2019). The variations are influenced by different climatic conditions, fungal species, harvesting, handling as well as storage practices (Kamala *et al.,* 2015). The study conducted in Tanzania and Kenya reported a significant difference in AFB_1 and AFM_1 prevalence in different agroecological zones (Kamala *et al.,* 2015; Kuboka *et al.,* 2019a). The present study was conducted to assess the occurrence of aflatoxins in animal feeds and raw milk and associated risk factors along the dairy value chain in three agroecological zones of Tanzania.

1.2 Statement of the Problem

The growth of the dairy sector and the increase in milk production has resulted into high demand for animal feeds. This is due to the fact that effective milk production necessitates a consistent supply of animal feed in sufficient quantities and quality. Smallholder dairy farmers face challenges in meeting sufficient feeds for dairy cows (Lukuyu *et al.,* 2017; Nell *et al.,* 2014) as a result, the quality of animal feeds can be overlooked. A study by Nyangi *et al.* (2016) reported that sorted out cereals as bad and maize bran, which are primarily used as animal feeds or animal feed ingredients, have high aflatoxin contamination.

Studies on aflatoxin contamination in feeds and dairy products have been conducted in several countries, including Rwanda (Nishimwe *et al.,* 2019), Uganda (Kaaya & Warren, 2007) and Kenya (Kang'Ethe *et al.,* 2017; Makau *et al.,* 2016; Mutiga *et al.,* 2015) which demonstrated that dairy feeds are highly contaminated with aflatoxin. However, few studies have been conducted in Tanzania, on poultry feeds (Kajuna *et al.,* 2013), sunflower seed cakes (Mmongoyo *et al.,* 2017), maize bran (Nyangi *et al.,* 2016) and raw cow milk (Ledo *et al.,* 2020; Mohammed *et al.,* 2016; Urio *et al.,* 2006), which showed high level of aflatoxins in feeds and milk. Hence, likely to cause health effects to consumers especially young children who may be exposed to aflatoxin from the consumption of the milk. Similarly, aflatoxin contamination in animal feeds and feed ingredients has a direct cost on livestock production, resulting from reduced livestock productivity and increased the cost of maintaining health animals (Alvarado *et al.,* 2017).

Therefore, this calls for further studies to assess the status of aflatoxins contamination in dairy value chain in different agro-ecological zones, knowing that climatic conditions and animal husbandry practices differ from one place to another. The information derived from this study will provide a better understanding of the occurrence of aflatoxins contamination in dairy feeds and milk, risk factors associated with AFB_1 and AFM_1 contamination in the dairy value chain, which will facilitate implementation of strategic interventions for control of aflatoxins contamination in animal feeds and milk in Tanzania.

1.3 Rationale of the Study

Aflatoxins pose serious public health concern due to associated the health consequences in animals and humans globally. Unfortunately, more emphasis in previous researches conducted in Tanzania was given on aflatoxins in human foods, such as sunflower (Mmongoyo *et al.,* 2017; Mohammed *et al.,* 2018), groundnuts (Boni *et al.,* 2021; Gong *et al.,* 2020; Magembe *et*

al., 2016) and maize (Boni *et al.,* 2021; Mohammed *et al.,* 2018), leaving behind animal feeds and animal products which are also susceptible to contamination. This suggests a high potential risk to animals and humans from aflatoxins exposure coming from sources such as feeds and milk. The current study also assessed the occurrence and risk factors associated with AFB_1 and AFM_1 in feeds and milk. The study further assessed awareness status of key stakeholders such as SDFs, agrovet dealers and feed manufacturers on knowledge of aflatoxin contamination in feeds, milk, its health consequences and ways to prevent aflatoxin contaminations. Understanding status of AFB_1 in feeds and AFM_1 in milk along with effects of grazing systems, feed handling and storage practices across agroecological zones will facilitate in setting strategies and allocation of resources for implementation of strategies to address the problem of aflatoxin contamination along the dairy value chain.

1.4 Objectives of the Study

1.4.1 General Objective

To assess the occurrence of aflatoxins and associated risk factors in dairy value chain in the selected districts of three agro-ecological zones of Tanzania.

1.4.2 Specific Objectives

(i) To assess occurrence of aflatoxins B_1 in animal feeds from processors, agro-vet dealers and smallholder dairy farmers.

(ii) To determine occurrence of aflatoxin M_1 in raw milk from smallholder dairy farmers.

(iii) To evaluate potential risk factors associated with the occurrence of aflatoxins B_1 and aflatoxin M_1 in the dairy value chain (feeds and milk).

1.5 Research Questions

(i) What is the occurrence of aflatoxin B_1 in feeds from processors/agro-vet dealers and smallholder dairy farmers in Mpwapwa, Serengeti and Hai districts?

(ii) What is the occurrence of aflatoxin M_1 in raw milk obtained from dairy cattle kept by smallholder farmers in Mpwapwa, Serengeti and Hai districts?

(iii) What are the potential risk factors associated with the occurrence of AFB_1 in animal feeds and raw milk in Mpwapwa, Serengeti and Hai districts?

1.6 Significance of the Study

The study is expected to generate information on the prevalence and associated risk factors of aflatoxins contamination in the dairy value chain in the three agro-ecological zones of Tanzania. The findings from this study will contribute to the aflatoxin mitigation plans. Evidence on factors associated with aflatoxin contamination in animal feeds and milk will help facilitate implementation of early intervention for management of the problem along the dairy value chain. Ultimately, the interventions will lead into ensuring safety of the animal feeds, and milk produced from the animals fed on safe feed hence preventing exposure of humans and animals to aflatoxins, facilitate trade, increased productivity and improved livelihood of the SDFs.

1.7 Delineation of the Study

This study focused on assessing the occurrence of aflatoxin B_1 in dairy cattle feeds and aflatoxin M_1 in raw cow milk in Hai, Mpwapwa and Serengeti districts. Specifically, the occurrence of AFB_1 was analysed in maize bran, sunflower seedcakes and mix of sunflower seedcakes and maize bran samples obtained from SDFs and agro vet dealers; AFM_1 was analysed in cow raw milk obtained from SDFs. Also, the information on aflatoxin awareness, feeding systems, feed handling and storage practices was obtained from SDFs. This study did not cover other types of animal feeds which are not used by dairy cattle, milk from other animals, or other type of mycotoxins besides AFB_1 and AFM_1.

CHAPTER TWO

LITERATURE REVIEW

2.1 Livestock Sector in Tanzania

Livestock is an essential agricultural subsector in Tanzania. It contributes to about 6.9% of Tanzania's GDP (National Bureau of Statistics [NBS], 2017). The primary livestock in Tanzania is cattle (33.9 million), sheep (8.5 million), goats (24.5 million), pigs (3.2 million) and poultry (87.6 million) (NBS, 2020). Cattle are the dominant type of livestock in Tanzania, especially in rural and peri-urban communities. Livestock production is an important component in human development due to the provision of meat, milk, hides, draft power and manure. Therefore, the livestock sector determines households' economic and social status in many communities as it creates a substantial amount of cash revenue (Zane & Pica-Ciamarra, 2021).

One of the crucial components of the livestock sector is the dairy subsector. It provides, employment, household income and as source of animal protein (Atherstone *et al.,* 2016; NBS, 2019; Zane & Pica-Ciamarra, 2021). Livestock and the dairy sector were among the Tanzanian government's priority areas after independence. Tanzania implemented the Policy of Socialism and Self Reliance, which included the establishment of parastatal firms for the dairy sector to boost productivity and serve as development catalysts (Nell *et al.,* 2014). Additionally, in response to the requirement to control and coordinate the development of the dairy industry, the Tanzania Dairy Board (TDB) was founded in 2005 (Nell *et al.,* 2014).

According to NBS (2020), between 2018 and 2020, the total quantity of milk produced rose from 2.4 billion litres to 3.1 billion litres. Regardless of this tremendous growth, the dairy production subsector in Tanzania is constrained by several challenges, such as feed quality and availability of sufficient feed (Maleko *et al.,* 2018; Mbwambo *et al.,* 2016). Due to fluctuation in rainfall pattern high-quality feed are only available for short period during the wet season (Gillah *et al.,* 2012). In Tanzania, insufficient crop production of cereals and oilseed crops causes shortage of livestock feed concentrate due to competition between human and animal feed millers for the same raw materials (Mbwambo *et al.,* 2016). The phenomenon can lead to poor quality ingredients with a high probability of aflatoxin contamination getting entry into animal feeds (Nyangi *et al.,* 2016).

2.2 Background of Mycotoxins

Without considering the origin and etymology of the word mycotoxin, mycotoxins mean a toxin produced by a fungus (Richard, 2007). Although the toxicity effects of feeding on mouldy contaminated feeds/foods have been known for a long time, the word mycotoxin was conceptualised in 1962 following the end of an unusual disease outbreak near London, England, in which approximately 100 000 turkeys died from a mysterious disease known as Turkey X (Pickova *et al.,* 2021). It was found not only turkeys died but also ducklings and young pheasants. Following thorough investigation, a significant association was found between Brazilian groundnut meal and disease outbreaks (Negash, 2018). Findings on the nature of toxin in Brazilian groundnuts meal suggested its origin from the fungus *Aspergillus flavus*. As a result of its origin from *Aspergillus flavus*, the toxin was named aflatoxin (*Aspergillus flavus* toxin) (Pickova *et al.,* 2021). These findings sparked scientific attention and led to an era of intensive research on mycotoxins, which resulted in the discovery of numerous new mycotoxins. Although there are more than 400 types of mycotoxins, only a few are of public health importance, such as deoxynivalenol and zearalenone, fumonisins (FB_1, FB_2 and FB_3), ochratoxin and aflatoxins, with the latter predominantly produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Madhysatha & Marquardt, 2019). The major types include AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2. The AFM_1 is the metabolite resulting from AFB_1 hydroxylation in the liver (Min *et al.,* 2021). Aflatoxins chemical structures (Abdel-Azeem *et al.,* 2019) are presented in Fig. 1.

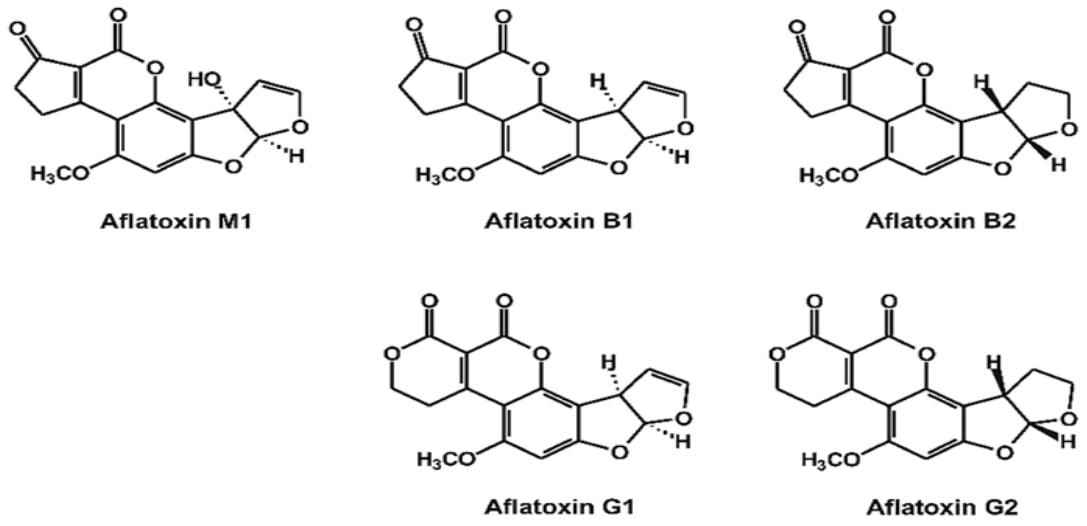

Figure 1: Structure of Aflatoxins

2.3 Prevelance of Aflatoxins

Aflatoxins are toxins produced by fungi of *Aspergillus* species cereals such as maize, groundnuts, millet, sorghum, and rice, in the field, storage, and in poor processing

environments (Omara *et al.,* 2021). Moreover, aflatoxins are also found in high concentrations in the bran and germ fractions produced during milling processes. This is due to the presence of the pericarp, the foremost part of the grain, which can easily sticky dust and colonized by fungi (Nyangi *et al.,* 2016).

Approximately more than 5.5 billion people globally have high risk for uncontrolled aflatoxin exposure (Liu & Wu, 2010). In Africa, aflatoxin has proven to be a bottleneck for farmers to meet regulatory and international standards for food safety and agricultural trade (PACA, 2014). In addition, aflatoxins cause post-harvest loss of cereal and grains by rendering them unfit for use, thus, contributing to economic loss and food insecurity. A study conducted in Western Kenya on the assessment of aflatoxins showed that, among 985 samples of maize collected, 15% had aflatoxin contamination at levels higher than the allowable limit (greater than 10 µg/kg), and 49% had levels above the limit of detection (Mutiga *et al.,* 2015).

Aflatoxin B_1 is inextricably linked with the most mycotoxins contaminants in the food chain. A survey conducted in Uganda showed that up to 83% of maize kernels from farmers in the moist zone had the highest aflatoxin (mean levels of 9.7 ppb) compared to 70% (mean levels of 7.7 ppb) and 55% of maize kernels (mean levels of 3.9 ppb) from the dry and highland zones, respectively (Kaaya & Warren, 2007; Sserumaga *et al.,* 2015). Likewise, a study in three agro-ecological zones of Tanzania showed that 45% of all maize samples were contaminated with aflatoxin, among which 26% were above the maximum limit set in national standard, which is 5 µg/kg for AFB_1 (Kamala *et al.,* 2016). High level of aflatoxin contamination in maize implies contamination of maize bran which is the main feeds ingredient. However, there is scanty information on prevalence of aflatoxin B_1 in animal feeds and aflatoxin M_1 in raw milk in Tanzania. The prevelance of AFB1 in feeds and AFM1 in milk from different regions of Tanzania is shown in Table 1.

Table 1: Prevalence of AFB_1 in feeds and AFM_1 in milk from different regions of Tanzania

Regions	Feeds	AFB_1 (µg/kg)	Source	Cow milk	AFM_1 (µg/L)	Source
Singida	Sunflower seed cake	65% (LOD - 20.47)	Mohammed *et al.* (2016)	Raw	83.8% (LOD to 2.007)	Mohammed *et al.* (2016)
	Sunflower seed cake	80% (2.0–52.8)	Mmongoyo *et al.* (2017)			
Dar es Salaam	Feeds (Maize bran, Layers feed, Sunflower cake, Broiler feed, Wheat bran)	91% (24.00 - 76.23)	Mwakosya *et al.* (2022)	Raw	92% (0.005 – 0.855)	Urio *et al.* (2006)
				UHT	100% (LOD-0.454) 96% (0.01-0.1)	Mwakosya and Mugula (2021)
				Pasteurized milk		
Morogoro	Maize bran, Sunflower cake	50% (9.4 µg/kg) 70% (31.6 µg/kg)	Kajuna *et al.* (2013)			
	Sunflower seed cake	2.7–536.0 µg/kg	Mmongoyo *et al.* (2017)			
Manyara	Maize bran Un-market maize	60% (2.4 µg/kg) 29% (1.7 µg/kg)	Nyangi *et al.* (2016)			
Arusha	Maize bran	100%	Mushi *et al.* (2018)			
Mbeya	Sunflower seed cake	(1.4–174.2)	Mmongoyo *et al.* (2017)			
Dodoma	Sunflower seed cake	(1.4– 598.4)	Mmongoyo *et al.* (2017)			
Tanga and Morogoro				Raw	63% (<0.2 µg/L) 14% (0.2-0.5 µg/L) 22% (>0.5 µg/L)	Ledo *et al.* (2020)

2.4 Health Effects of Aflatoxins

Aflatoxin exposure has detrimental effect to both humans and animals, resulting in a wide range of health consequences and substantial direct and indirect economic impacts (Sarma *et al.,* 2017). Acute and chronic aflatoxicosis can result from prolonged exposure to high and moderate quantities of aflatoxins. Aflatoxicosis symptoms include, oedema, acute liver damage, digesting issues, hemorrhage, anemia, jaundice and even death (Ahmed & Amana, 2019; Chen *et al.,* 2018); also, the teratogenic impact of chronic aflatoxicosis is linked to congenital malformation. Aflatoxins are carcinogenic and mutagenic, causing alteration in DNA and mutation in genetic code, leading to chromosomal rearrangements, breaks, loss or gain of chromosomes or changes within a gene. Species, age, sex and nutritional status affect the magnitude of toxicity due to the consumption of aflatoxin-contaminated food or feeds (Fetaih *et al.,* 2014; Negm *et al.,* 2021). The impact of aflatoxins on animals is not confined to the acute aflatoxicoses but also, chronic toxicity induced by sublethal dosages of aflatoxins over long time (Benkerroum, 2020). Chronic exposure to aflatoxin exposure has a long-term effect on livestock, causing metabolic and nutrient absorption abnormalities as well as hidden pathological changes that lower feed intake. Affecting growth that reduces weight gains hence lowers animal productivity and produce such as meat and milk (Awuchi *et al.,* 2021).

Furthermore, aflatoxin causes various types of harm to important body organs such as liver and kidney, negative effects on the endocrine and reproductive systems, as well as immune system suppression resulting in rise of disease incidences (Udomkun *et al.,* 2017).

Aflatoxins are primarily metabolized in the liver, made it the target organ where huge histological changes occur. Hepatocellular carcinoma is caused by chronic exposure to aflatoxin as evidenced in experimental animals which developed the disease after prolonged exposure of low doses of AFB_1 (Waithaka & Niyonshuti, 2022). Acute exposure may cause death in animal shortly or within few days after exposure. The clinical symptoms include, hepatic damage with increased capillary fragility, hemorrhage and delaying clotting process (Peles *et al.,* 2019). Even in chronic aflatoxicosis, majority of the effects are due to hepatic injury although the symptoms are milder (Peles *et al.,* 2019). Renal damages have also been reported as a result of prolonged exposure to aflatoxins. One among the target organs for aflatoxins is the kidneys, aflatoxin induces oxidative stress which triggers its toxicity by changing the expression of proline dehydrogenase, which lowers proline levels and causes downstream apoptotic cell death (Peles *et al.,* 2019).

Aflatoxin exposure suppresses immunity making livestock prone to various diseases caused by wide range of microorganisms such as bacteria, virus and parasitic infections, as well as reactivation of chronic infections and decreases efficacies of therapeutic and vaccines (Awuchi *et al.,* 2021; Schat & Skinner, 2022). Recent findings on animal research showed that aflatoxins negatively impact both female and male reproductive systems, inducing toxicity in egg and sperm cells of animals. The AFB_1 directly affect the male reproductive system by interfering cell differentiation process during sperm development and reducing testosterone levels. Aflatoxin B_1 decreases egg fertility in female animals by disrupting egg cell maturation through epigenetic changes, oxidative stress, excessive autophagy, and apoptosis (Udomkun *et al.,* 2017). Furthermore, aflatoxins modulate and affect the GIT in variety of ways, the most significant of which are alterations in intestine morphology, changes in the ability or activity of digestive enzymes to break down food, changes in intestine innate immunity, and changes in gut microbiota (Peles *et al.,* 2019; Sarma *et al.,* 2017). Figure 2 shows physiological functions of aflatoxins in animal cells and some healthy effects (Yang *et al.,* 2020).

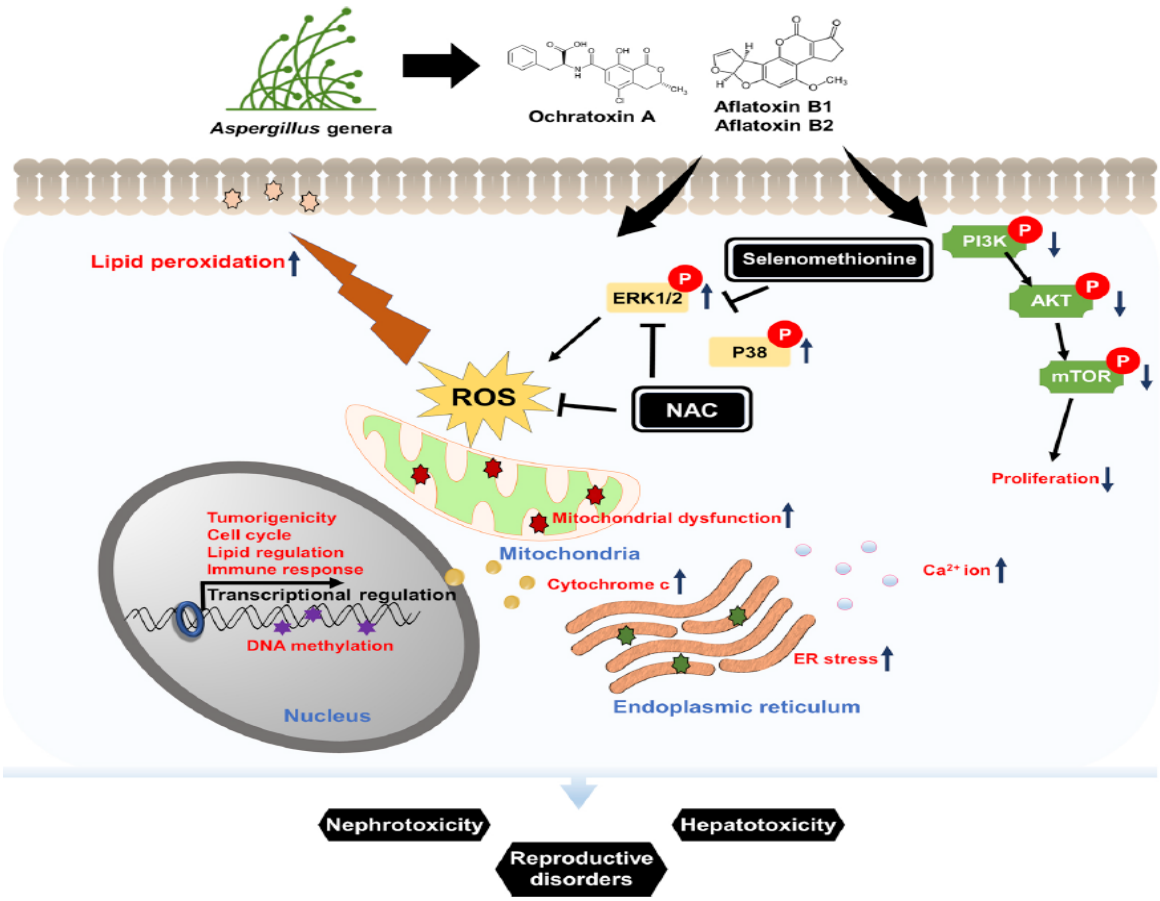

Figure 2: Physiological functions of aflatoxins in animal cells and some health effects

2.5 Overview of Aflatoxin M1 (AFM1)

AflatoxinM_1 is also known as milk toxin formed when humans and animals consume food/feed contaminated with AFB_1 as demonstrated in Fig. 3 (Kumar *et al.,* 2017). The toxin is hydroxylated in the liver by cytochrome P450 associated enzymes and excreted in faeces, urine and milk (Mahmoudi & Norian, 2014; Marchese *et al.,* 2018). The amount of AFM_1 in milk products is associated with levels of AFB_1 that dairy cows are exposed to through the feeds. Aflatoxin M_1 levels in dairy products show varying incidence throughout the world and even within the same country. Most investigations found that at least some percentage of the milk had no detectable level of AFM_1 and that even among the detectable levels, AFM_1 levels were below the European Union (EU) maximum limit of 0.05 µg/L in most countries (Saha & Wu, 2021). However, there were several countries, such as India, Turkey, Syria, Brazil, Mexico, Iran, Palestine, Pakistan, Serbia, Algeria, Nigeria, South Africa, Ethiopia, Sudan, Kenya and Tanzania, where some of the samples tested were found with AFM_1 at levels above the FDA limit of 0.5 µg/L (Saha & Wu, 2021). In one study conducted in Pakistan (Sadia *et al.,* 2012), the level of AFM_1 were reported to be extraordinarily high, up to 100 µg/L. According to in vivo and invitro studies AFM_1 exposure from contaminated milk may lead into aflatoxicosis (Peles *et al.,* 2019). Therefore, presence of AFM_1 in milk and milk product may cause public health effects and based on the fact that the toxin is heat stable, which cannot be destroyed during pasteurization (Saha & Wu, 2021).

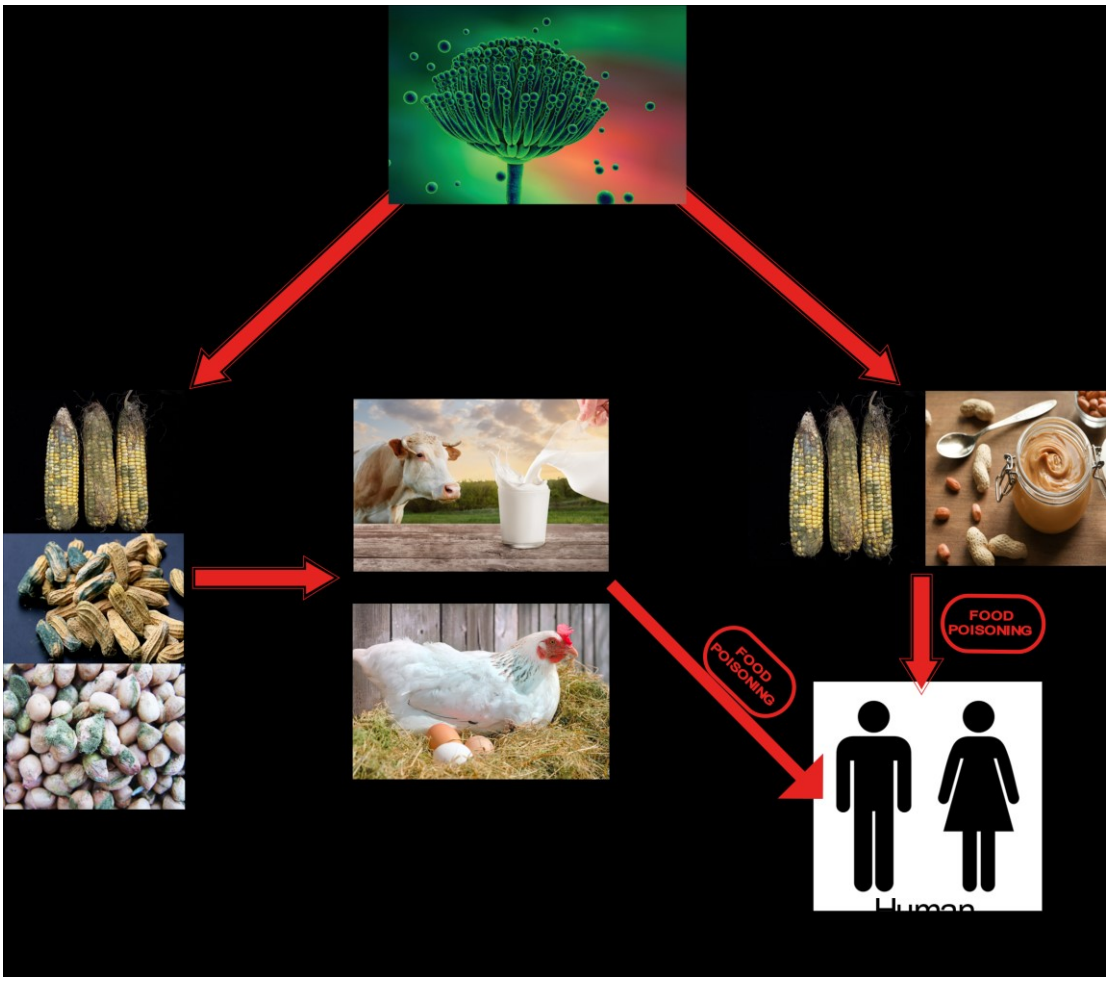

Figure 3: Aflatoxin from food and feeds to animals and humans

2.6 Aflatoxin M1 in Milk and Milk Products

Cereal grains, predominantly maize and oilseeds such as sunflower, cotton and groundnut are commonly used as animal feeds, or ingredients for animal feeds. In Tanzania most of the animals feed are concentrates and roughages. Plant protein seeds, brewer by-products, seed cake and cereal grains with its related by-products are grouped as concentrates. Conserved forage, trees, pastures; natural and planted, crop residues and shrubs are grouped as roughages (Mbwambo *et al.,* 2016). Usually, poor handling and storage conditions during production, transportation and storage are some of the main factors for aflatoxin B1 contamination in feeds (Afsah-Hejri *et al.,* 2013). When dairy cattle ingest feeds contaminated with aflatoxin B_1 (AFB_1), aflatoxin M_1 is produced after different metabolic processes in the liver (Britzi *et al.,* 2013).

The worldwide prevalence of AFM_1 as reported by Salari *et al.* (2020) in a systematic review and meta-analysis was 79.1%. The studies found that most of the samples analysed were contaminated with aflatoxin M_1 beyond the expected limit of 0.05 µg/L set by many countries (Table 2).

Table 2: Afflation M_1 beyond the expected limit of 0.05 µg/L set by many countries

Country	% of AFM1 positive sample	Range of AFM1 (µg/L)	Source
Algeria	46.43	0.096-0.557	Mohammedi-Ameur *et al.* (2020)
Egypt	20	0.01–0.086	El-Hofi and Abo El-Naga (2021)
Ethiopia	62.5	0.003–2.177	Zebib *et al.* (2022)
Nigeria	99	Mean: 0.092	Oluwatosin *et al.* (2021)
South Africa	87.1	0.01–2.85	Mulunda and Mike (2014)
Sudan	92	0.02–0.15	M El-Zubeir *et al.* (2020)
China	82.8	<0.05 to <0.5	Xiong *et al.* (2022)
India	79.1	mean.19±0.3	Thukral *et al.* (2021)
Iran	100	0.05–0.10	Movassaghghazani and Ghorbiani (2017)
Italy	12.3	0.004–0.052	De Roma *et al.* (2017)
Pakistan	71.4	0.005–0.199	Sumon *et al.* (2021)
Brazil	39	LOD–0.25	Gonçalves *et al.* (2021)
Latin America	67	0.001–23.10	Marimón *et al.* (2021)
Kenya	100	0.015–4.563	Kuboka *et al.* (2019b)
Rwanda	-	0.33–14.5	Nishimwe *et al.* (2022)

For instance, in the study conducted in India where 87 samples of milk were analysed; about 87.3% of the total samples were contaminated with aflatoxin M1 in higher concentrations ranging between 28–164 µg/L. Similar studies conducted in Thailand, Indonesia and the Philippines showed high contamination of aflatoxin M1 in milk (Jalili & Scotter, 2015). However, in many developed countries, where there are stringent regulations on aflatoxin, a low level of aflatoxin M1 contamination has been reported (Jalili & Scotter, 2015).

Numerous studies have been conducted in Africa to assess aflatoxin contamination in milk and animal feeds. For example, the study conducted in Morocco revealed that 88% of the milk samples collected were contaminated with AFM_1 levels above the Moroccan and European committee (EC) set the limit of 0.05 µg/L (Zinedine *et al.,* 2007). Also, the study conducted in five counties in Kenya to assess the prevalence of aflatoxin in feeds and cow milk showed that dairy feed concentrates samples were contaminated with AFB_1 was up to 9661 µg/kg, 39.7% of 512 analysed milk samples had levels of AFM_1 above the limit of detection, and 10.4% exceeded set limit of 0.05 µg/L (Senerwa *et al.,* 2016). Moreover, another study conducted in Tanzania (Singida and Northern region) revealed that up to 83.8% and 61.53% samples of milk and animal feed were contaminated with AFM_1 and AFB_1, respectively at levels exceeding the European Commission (EC) limit of 0.05 µg/L for AFM_1 in milk and five µg/kg for AFB_1 in dairy feed (Mohammed *et al.,* 2016). Therefore, further studies are needed in Tanzania to assess AFB_1 and AFM_1 in feeds and milk, and the risk factors associated with aflatoxins contamination in different agroecological zones.

Table 3: Occurrence of aflatoxin M_1 in raw milk in different countries

Country	% of AFM_1 positive sample	Range of AFM_1 (µg/L)	Source
Algeria	46.43	0.096-0.557	Mohammedi-Ameur *et al.* (2020)
Egypt	20	0.01–0.086	El-Hofi and Abo El-Naga (2021)
Ethiopia	62.5	0.003–2.177	Zebib *et al.* (2022)
Nigeria	99	Mean: 0.092	Oluwatosin *et al.* (2021)
South Africa	87.1	0.01–2.85	Mulunda and Mike (2014)
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China	82.8	<0.05 to <0.5	Xiong *et al.* (2022)
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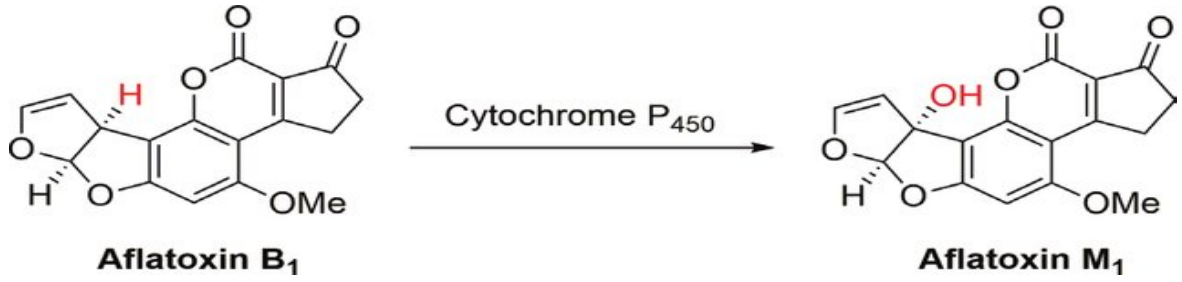
Rwanda	-	0.33–14.5	Nishimwe *et al.* (2022)

2.7 Aflatoxin M1 as a Metabolite of Aflatoxin B1

Aflatoxin M_1 is the major oxidized metabolite of AFB_1 but the metabolization is quantitatively affected by different factors. Studies which examined livestock's ability to convert AFB_1 to AFM_1, showed that dairy cows milked twice a day with less than 29 L/day and greater than 29 L/day milk yield had the ability to excrete one to two and one to six per cent of the amount of AFB_1 ingested, respectively. Days of the cow in lactation and milk yield significantly affect the carry-over of AFB_1 ingested from feeds into the milk (Britzi *et al.,* 2013). The greater carry-over rates have been observed in cows during early lactation between two to four weeks after calving, which also have greater milk yield, than cows in late lactation between 34-36 weeks after calving, when the milk yield naturally drops. Furthermore, inter species variation, animal health, hepatic biotransformation ability, feeding pace, and the integrity of the mammary alveolar cell membranes have all been demonstrated to influence the carry-over rate of AFB_1 to AFM_1 (Britzi *et al.,* 2013; Masoero *et al.,* 2007). According to a recent study conducted in Israel, the mean carry-over rate of AFB_1 to AFM_1 at steady-state, from day three to seven, was 5.8% in mid-lactation and 2.5% in late-lactation groups. Estimating the carry-over rate of AFB_1 to AFM_1 in livestock is important in determining the acceptable levels of AFB_1 intake in feed as evidence-based recommendations for setting standards on limit of maximum contamination level of AFB_1 in the feed of dairy cows (Britzi *et al.,* 2013). Figure 4 shows chemical structure changes of AFB_1 to AFM_1 when converted by cytochrome P_{450} in the liver (Du *et al.,* 2019).



Aflatoxin B_1 **Aflatoxin M_1**

Figure 4: Conversion of AFB1 to AFM1

2.8 Potential Risk Factors for Aflatoxins Contamination in Food and Feedstuffs

Animal feedstuffs can be contaminated at any point along the value chain. To ensure safety and quality of human foods and animal feeds, proper control of mycotoxins at the entire value chain from the field, pre-harvest, and post-harvest phase (processing, transportation, storage and handling stages) is fundamental (Warburton & Williams, 2014). In each stage in the value chain, aflatoxin producing fungi require special conditions such as drought and excess water or nutritional imbalance for the production of toxins. Also, other factors like water activity (aw), temperature, high humidity, the integrity of the grain, insect damage, and the quantity and type of the mycobiota are predominant factors that accelerate the growth and development of aflatoxin producing fungi in foods and feeds (Tola & Kebede, 2016), hence, the incremental concentration of aflatoxin (Kana *et al.,* 2013). In addition, according to Nyangi *et al.* (2016), the use of sorted out cereals and cereal bran for animal feeds is one of the risk factors associated with the aflatoxin contamination in feedstuffs. Other factors which can enhance aflatoxin contamination in feeds stuff include:

(i) Storage conditions: Poor storage facilities lead to contamination and growth of fungi that produce aflatoxin. For example, feeds stored on a bare floor can absorb moisture and accelerate fungi growth, leading to aflatoxin production. Therefore, proper monitoring of the relative humidity of the storage facility is required (Kaaya & Warren, 2007; Suleiman *et al.,* 2013).

(ii) Drying practices: Majority of feed processors or farmers dry feed on open bare floor/ land, mats, or polythene sheets. These practices are likely to increase the risk of fungal attack on the crops and subsequent production of aflatoxin. It is recommended that moisture content of feeds should not exceed 14% in order to reduce fungi growth and aflatoxin production (Gnonlonfin *et al.,* 2013; Mwakosya *et al.,* 2022).

(iii) Physical and insect damage: Physical and insect damage of grain hastening aflatoxin contamination by enhancing infestation of aflatoxin producing fungi. When the outer

coat of the grain is broken or softened fungi growth accelerates leading into production of aflatoxin (Neme & Mohammed, 2017).

(iv) Low awareness on measures of control of aflatoxin contamination: Level of awareness on aflatoxin and measures for control the toxin among farmers and feeds processors determine status of aflatoxin contamination in food and feed. Lack of awareness leads to bad practice during processing, transportation and storage of food crops hence, accelerating aflatoxin production. A study conducted in Tanzania (Meru district) shows that only about 25% of the interviewed respondents had heard about the term aflatoxins (Ayo *et al.,* 2018), and only 20% of respondents were aware of aflatoxin in Kilosa, Babati and Chamwino districts (Kamala *et al.,* 2016; Suleiman *et al.,* 2017).

2.9 Regulations Regarding Aflatoxins in Milk and Feedstuffs

Globally, public is concerned with the problem of aflatoxin have concerns about the contamination in feeds, and therefore countries have established the maximum tolerable limits for the toxin in feeds and milk (Table 4). Specific types of agricultural commodities, consumption pattern, age, type of animal species and the intended use are some of the factors which are taken into account in setting regulatory limits for aflatoxin and other food contaminants. Regulatory limits for aflatoxins vary across countries or continent. For example, the maximum allowed aflatoxin levels in dairy feed established by the EU and USFDA are 5 µg/kg and 10 µg/kg, respectively. The Codex Alimentarius Commission (CAC) provides the global food standards for benchmarking. Furthermore, the CAC develops codes of practice for managing aflatoxin and some of them include the CAC General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995), CAC Codes of Practice for Reduction of Aflatoxins for Milk-producing Animals (CAC/RCP 45-1997) and CAC codes of practice for good animal feeding (CAC/RCP 54-2004).

Tremendous effort had implemented in East African Community (EAC) in bringing harmonization in aflatoxin control in foods and feeds. In 2018, EAC Aflatoxin Prevention and Control Strategy and Action Plan (2017-2022) was approved. As part of this initiative, the EAC established the East African Standards Committee (EASC), which was given the responsibility to develop and issue the East African Standards (EAS). Through the efforts of the EASC, the harmonized aflatoxin standards for AFB1 in compounded cattle feed (EAS 75:2019) and AFM1 in raw cow milk (EAS 67:2019) were developed. Tanzania also aligned its standards

with the EAS, adopted the harmonized limits for AFB1 in compounded cattle feed (TZS 397:2020/EAS 75:2019) and AFM1 in raw cow milk (TZS 626:2020/EAS 67:2019).

Nevertheless, of the international and regional limit of AFB_1 and AFM_1 in feed and milk, respectively, control of aflatoxin in subsistence farming is challenging based on the fact that food crops are generally produced and consumed or used as animal feed without formal control.

Table 4: Maximum limits of AFB_1 in feeds and AFM_1 in milk

Country	Maximum limit of AFB_1 in dairy feeds (µg/kg)	Maximum limit of AFM_1 in raw milk (µg/L)	Reference
WHO/FAO	5	0.05	Kang'Ethe and Lang'A, (2009)
European Union	5	0.05	Jiang *et al.* (2021)
US FDA	10	0.5	USFDA (2005)
EAC*	5	0.5	(EAS 75:2019 and EAS 67:2019)
Kenya	5	0.5	(KS EAS 75:2019 and KS EAS 67:2019)
Uganda	5	0.5	(EAS 75:2019 and EAS 67:2019)
Rwanda	5	0.5	Nishimwe *et al.* (2019, 2022)
Tanzania	5	0.5	(TZS 397:2020 and TZS 626:2020)

East Africa Community

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in selected districts from three agro-ecological zones of Tanzania; Northern Highland Zone (Hai District in Kilimanjaro region), Arid Zone (Serengeti District in Mara region), and Semi-Arid Zone (Mpwapwa District in Dodoma region) (Fig. 5). Samples of raw milk were collected from SDFs and animal feed samples from, SDFs, feed processors and feed vendors/agro-vet dealers in the respective zones. The samples were analysed in the laboratories at the Nelson Mandela African Institution of Science and Technology (NM-AIST).

3.1.1 Description of the Study Areas

The study areas were purposely selected to represent the three agroecological zones of Tanzania based on different climatic conditions and cattle management practices. In Northern Highland Zone, Hai district was selected. The district situated within latitude 2° 50' – 3°29' S and longitude 30°30' – 37°10' E in the Kilimanjaro region of northern Tanzania. The district experiences an annual rainfall of about 1000 mm – 2000 mm, and a mean annual temperature of 23.3 ± 0.66°C. Dairy cows are mostly kept under zero-grazing and supplemented with concentrates. In Arid Zone, Serengeti district was selected, which is situated within latitude 2° 00' S and longitude 34° 50' E in the Mara region of Tanzania. The district experiences average annual precipitation between 400 mm – 600 mm and a temperature of 26°C during the summer period. The total area is 10 373 km², of which 7501 km² is occupied by Serengeti National Park, Ikorongo Game Reserve, Gurumeti Game Reserve, and open area 2872 km² is used for farming, livestock keeping and residency. In most cases free-range is practiced and cow scarcely supplemented. In Semi-Arid Zone, Mpwapwa district was selected, which is situated within latitude 6° 00' – 7°00' S and longitude 35°45' – 45°00' E in the Dodoma Region of central Tanzania, 120 km from Dodoma city centre. It has a total area of 7479 km², used extensively for agricultural activities. The district experiences average annual precipitation of 600 mm – 800 mm. It is characterized by a hot climatic condition, with cattle kept under mixed feeding systems (Mkonda, 2021; Mkonda *et al.,* 2018; NBS, 2015; United_Republic_of_Tanzania [URT], 2007).

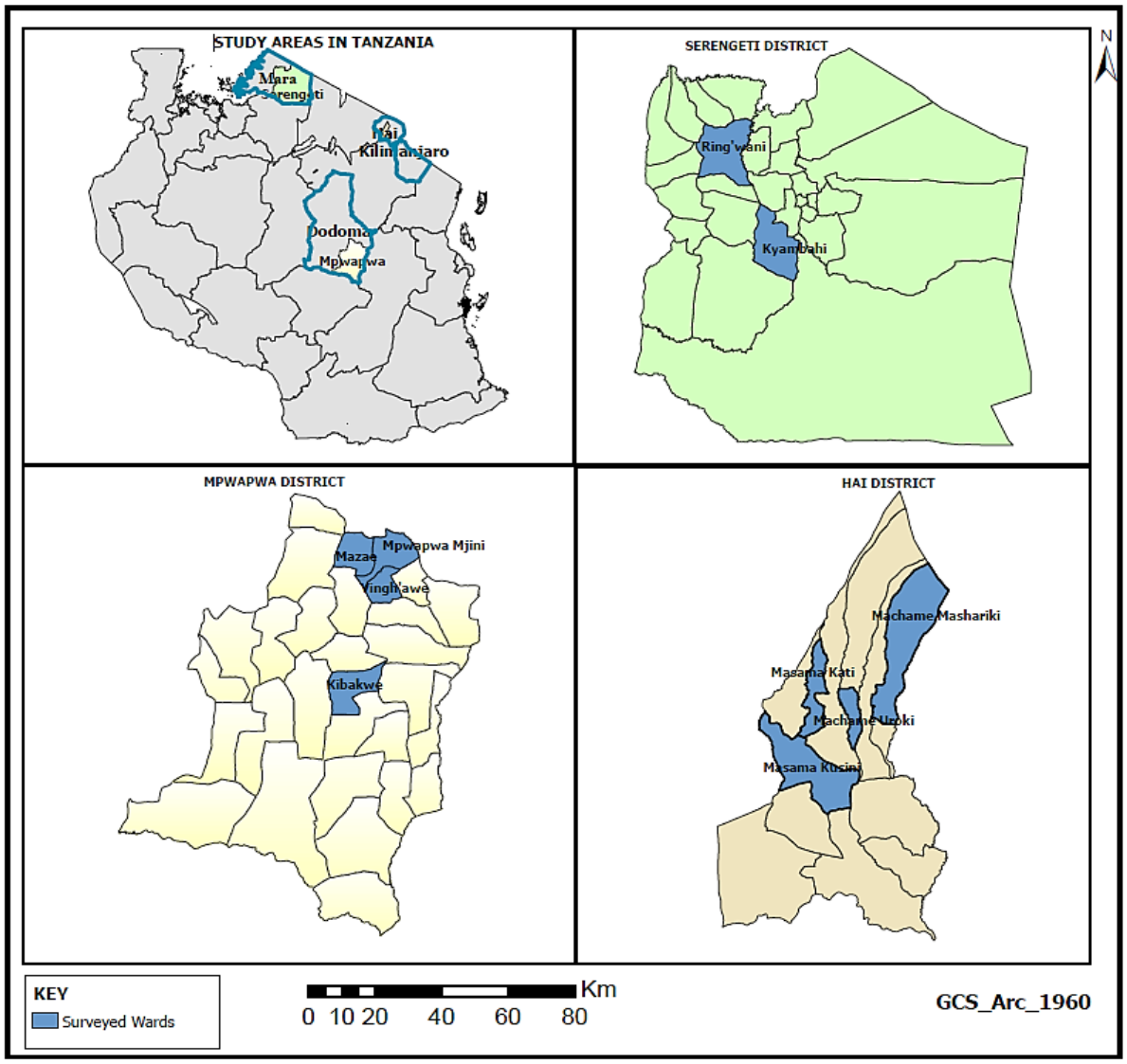


Figure 5: Location of study sites in the three agroecological zones of Tanzania

3.2 Sample Size and Sampling Design

Estimation of sample size of SDFs was done using Fischer *et al.* (1991) formula as such expressed in Equation (1). Aflatoxin contamination prevalence of 65% was used based on the recent study by Mohammed *et al.* (2016)

$$n = \frac{Z^2 p\,(q)}{d^2} \qquad (1)$$

Whereby, n = sample size, Z=1.96 at 95% confidence interval, p= 65% assumed prevalence of aflatoxin contamination, q = specificity of 65% $(1 - p)$, and d=degree of precision required (usually as a proportion, 0.05 for 5%).

$$n = \frac{1.96^2 0.65(1-0.65)}{0.05^2}$$

= 350 ≈ 400 Smallholder dairy farmers who participated in the study.

3.2.1 Sampling Procedure

Participants eligible for the study were SDFs, feed processors and feed vendors/agro-vet dealers. A total of 419 SDFs from the three districts participated in the study, i.e., approximately one third of total participants for each district. Therefore, Hai (137), Mpwapwa (147), and Serengeti (135) participants. Also, a total of 26 agrovet dealers (the ones who own or work in an end-to-end supply store for farmers, dealing in supplies of agricultural inputs, veterinary pharmaceuticals, animal feeds and or oil plants that supply sunflower seedcakes to farmers) from Mpwapwa (11), Hai (11), and Serengeti district (4) were also involved. The wards with high number of dairy farmers/ dairy cattle were purposely selected from each district (Fig. 5). At the ward level, livestock/agriculture officers were responsible for providing a list of SDFs and agrovet dealers. Systematic random sampling technique was used to select the SDFs households from the list provided, Equation 2 was used.

$$\text{Systematic Sampling Interval (i)} = \frac{\text{Size of target population (N)}}{\text{Size of the sample required (n)}} \quad (2)$$

3.3 Participant Consent and Safety

There was no potential risk associated with the study. All the information collected were used for academic/research purpose only. Participation in this study was completely voluntary; there was no negative consequence if participants decided not to participate. Participants who decided to participate were free to stop participating at any time they wanted and free not to answer any specific question. A consent form was given to each participant before the interview and sample collection in compliance with ethical requirements, KNCHREC 00037/RW/1/21 (Appendix 1)

3.4 Gender Issue

Both females and males had equal chances of participating in the study. Also, the research results gave potential clues to gender equality in female's and male's participation in the agricultural sector, particularly in the dairy value chain.

3.5 Sample Collection and Handling

3.5.1 Smallholder dairy farmers and Agrovet Dealers

A cross-sectional study design using a semi-structured questionnaire was adopted (Appendix 2). The survey data were collected from SDFs and agro vet dealers using KoBo collect app (V. 2021.2.4). Separate questionnaires were developed to capture specific information from SDFs and agrovet dealers. Information on cattle grazing and feeding systems were inquired from SDFs. Socio-demographic information, aflatoxin awareness, and information on feed storage and handling practices were inquired from both SDFs and agrovet dealers. All questionnaires were prepared in English and translated into Swahili for easy communication with respondents. The questionnaires were pre-tested in Bahi and Dodoma Municipality and the questions which were not clearly understood by the respondents were modified.

3.5.2 Collection of Raw Cow Milk and Livestock Feed Samples

Raw cow milk and livestock feed samples were collected from among the interviewed participants (depending on their availability during interviews). Systematic random sampling method were used to select one farmer to give raw milk sample from each three SDFs who filled the questionnaire. Therefore, 141 raw cow milk samples were collected: Hai (45), Mpwapwa (48), and Serengeti (48). In addition, 80 livestock feed samples (maize bran, sunflower seedcake, separately or mixed) were collected from SDFs (50 in Hai and 30 in Mpwapwa). No feed samples from SDFs were collected from the Serengeti district because cows feeding is by free-range is solely practiced. The study targeted about 30 agro vet dealers, ten from each district. A total of 26 livestock feed samples were collected from the available agro-vet dealers in all districts, Mpwapwa (11), Hai (11) and Serengeti (4). However, in Mpwapwa and Serengeti districts no feed samples were collected from dairy cow feed processors/manufacturers because of their unavailability. Only one dairy feed manufacturer was available in Hai district, no sample was collected because its results could not make a significant statistical conclusion. The milk samples from SDFs were collected in the morning using 250 mL sterile plastic amber bottles, labelled with the date of collection and household identification number. Also, about 250 g animal feed were collected in aluminium laminated paper bags. For agrovet dealers, the feed samples were taken from different bags in store at different points, top, down, middle and sides, then mixed thoroughly and about 250 g were obtained and put in aluminium laminated paper bags.

All raw milk samples were temporarily stored at -18ºC in a portable refrigerator to preserve their quality and freshness before transported to the laboratory, and stored below -25ºC until analysis. The raw milk and feed samples were collected between September and November 2021.

3.6 Laboratory Analysis

3.6.1 Chemicals and Reagents used during Laboratory Analysis

Various types of chemicals, working standards and reagent from different manufacturers were used in the laboratory analysis of AFB1 in the samples of animal feed and AFM1 in the samples of cow raw milk (Table 4).

Table 5: Chemicals and Reagents used

Chemical/Reagents	Manufacturers
Water, HPLC and Spectroscopy	Finar Limited, India
Methanol (HPLC Grade)	LOBA Chemie PVT Ltd, India
Acetonitrile 99.9% For HPLC and UV Spectroscopy	LOBA Chemie PVT Ltd, India
Phosphate buffered saline (Dulbecco A)	OXOID Ltd, England
Acetic acid glacial 99.7%	LOBA Chemie PVT Ltd, India
Sodium hydroxide	LOBA Chemie PVT Ltd, India
Trifluoroacetic acid (TFA)	LOBA Chemie PVT Ltd, India
Aflatoxin standards (B_1, B_2, G_1, G_2)	VICAM, USA
Aflatoxin M_1 Standards	VICAM, USA

3.6.2 Laboratory Equipment used during Analysis of Samples

Various instrument and equipment's from different manufacturers were used in the laboratory analysis of AFB_1 in the samples of animal feed and AFM_1 in the samples of cow raw milk (Table 5).

Table 6: Laboratory Equipment used

Equipment	Manufacturer
HPLC	Shimadzu Corporation, SIL-20A HT, Japan
HPLC Column	ThermoFisher, ODS-2 Hypersil, USA
HPLC Vials 1.5 µm	MACHERY-NAGEL GmbH & Co. KG, Germany
Microcentrifuge tubes 1.5 mL	Labsys, Canada
Whatman qualitative filter paper No. 1	Sigma Aldrich, Germany
Nylon syringe filter 0.22 µm	FilterBio, China
Syringe	Neoject, China
Aflacolumn (AFB1, AFB2, AFG1, AFG2)	VICAM, USA
Aflacolumn (AFM1)	VICAM, USA
Falcon tubes	Corning Science, Mexico S.A de C.V
Blender jar	Bionics Scientific Technologies Ltd, India
pH meter	Thermo Fisher Scientific, USA
Face mask	Xiantao Fortune Protective Prod. Co. Ltd, China
Portable freezer	Engel freezer, Sawafuji electronic Co., Ltd, Japan
Freezer	Fisher Scientific, USA
Water bath	Memmert GmbH + Co. KG, Germany
Analytical balance	OHAUS Europe GmbH, 8606 Nänikon, Switzerland
Glass Block Vacuum Manifolds	Thermo Fisher Scientific, Ireland
Pump for vacuum manifolds	WELCH, Louisiana, USA
Centrifuge machine	Eppendorf centrifuge 5810, Germany
Micropipette,	Eppendorf, Germany
Micropipette tips	Eppendorf, Germany
Macro-pipette	Eppendorf, Germany
Vortex mix	Thermo Fisher Scientific, USA

3.6.3 Validation of the Analytical Method

The precision, linearity, recovery, the limit of quantification (LOQ), the limit of detection (LOD), and selectivity were the aspect considered in validating AFB_1 and AFM_1 detection methods. The analysis of LOD and LOQ was done by the empirical method developed by (Şengül, 2016). Three independent blank samples of AFB_1 and AFM_1 were spiked by aflatoxin mixture (B_1, B_2, G_1, G_2) and AFM_1 standards at 1, 2, 5, 10, 15 µg/kg and 0.02, 0.05, 0.5, 1, 2 µg/L, respectively. The precision and recovery were evaluated by triplicate spiking of aflatoxins at levels of 1, 5, 10, 15, 20 µg/kg and 0.02, 0.05, 0.5, 1, 2 µg/L to blank feed and raw milk samples, respectively. Known negative samples of AFB_1, AFM_1, and blank reagent were used to determine selectivity by observing if there is any interference around the retention time of the target analyte. For calibration curves, the standards were prepared using standard solution of the following concentration; 0.02, 0.05, 0.5, 1, 2 µg/L for AFM_1 and 1, 5, 10, 15, 20 µg/kg for aflatoxin mixture (B_1, B_2, G_1, G_2).

3.6.4 Analysis of Aflatoxin B1 in Livestock Feed

Aflatoxin B_1 was analysed based on AOAC 2008.02-2008 method adopted by Mohammed *et al.* (2016). The feed samples were ground using a blender jar, 25 g of feed samples was weighed and placed in 250 mL Erlenmeyer flask. It was mixed with 100 mL of extraction solution, 60:40 methanol: water, vortexed using a vortex mixer for 5 minutes and filtered using Whatman paper no. 1. A total of 4 mL of the resulting extract was diluted with 8 mL Phosphate Buffer Solution (PBS), and pH adjusted to 6-8 using 0.1 M NaOH. About 12 mL of the diluted extract was passed through the immunoaffinity column (aflacolumn) at a rate of 3 mL/min. Washing was then done by passing 10 mL of HPLC grade water twice through the column at 2 drops/second. Thereafter, the immunoaffinity column was eluted by passing 1 mL HPLC grade acetonitrile through the column at a rate of 1-2 drops/second, and all the sample eluate (1 mL) were collected in a glass cuvette. The eluent was filtered using a nylon syringe filter and stored in microcentrifuge tubes. About 400 µL were taken from the eluent and mixed with 600 µL of derivatizing reagent (70:20:10 water: trifluoroacetic acid: acetic acid). The mixture was conditioned at 65°C for 15 minutes using a water bath, allowed to cool, and then injected into HPLC equipped with an RF-20A fluorescence detection system and an autosampler SIL 20AHT connected to C18 (250×4.6 mm, 5µm) column. The oven temperature was maintained at 40°C, 0.8 mL/min flow rate, and 20 µL injection volume. The mobile phase used was 60:30:10 water: methanol: acetonitrile and detector wavelength at 450 nm emission and 365 nm excitation. The concentration of the samples, ppb (µg/kg) was calculated using the Equation 3.

$$\text{ppb (or µg/kg)} = \frac{conc\ found\ \left(\frac{ng}{ml}\right) \times 1ml \times 100(ml) \times 2.5(dilution\ factor)}{4ml \times weight\ of\ the\ sample\ taken\ (g)} \quad (3)$$

3.6.5 Analysis of Aflatoxin M1 in Raw Cow Milk

Aflatoxin M_1 in raw cow milk was evaluated as per AOAC 2000.08-2004, method adopted by (Mohammed *et al.,* 2016; Shakir *et al.,* 2010). The raw milk sample was warmed to 37°C in a water bath and stirred gently to disperse fat. About 30 mL were measured into the conical vial, vortexed for 1 min, and centrifuged for 20 min at 2390 ×g. The upper-fat layer was removed, and the defatted milk filtered using Whatman paper no. 1. The acquired supernatant solution was passed through immunoaffinity columns at the rate of 1 drop/second and washed with 12 mL of water at a rate of 1 drop/second. Thereafter, the immunoaffinity column was eluted into a 15 mL glass tube by passing 1.25 mL of 3:2 v/v acetonitrile: methanol by gravity at a rate of 1 drop for every 2-3 seconds. The column was eluted again by passing 1.25 mL of HPLC water

grade by gravity and collected in the same cuvette to make 2.5 mL total volume. The eluent was well-vortexed well and filtered using a nylon syringe filter, then, 100 µL were taken and injected into HPLC for analysis. The mobile phase was 68:24:8 water: acetonitrile: methanol and detector wavelengths at 440 nm emission and 360 nm excitation.

3.7 Data Processing and Statistical Analysis

Survey data collected by the KoBo Collect app were exported to an excel file and cleaned. The R programming software (V. 4.1.3) was used for statistical analyses. Frequency tables were used to summarize the descriptive data. One-way analysis of variance (ANOVA) with Tukey's post-hoc test at 95% confidence interval and independent t-test was applied for mean differences comparison. Concentrations of AFB_1 and AFM_1 were used as the dependent variables and three districts as independent variables. The carryover effect of AFB_1 to AFM_1 was evaluated using simple linear regression. The normality and linearity assumptions were checked, and the data that were not normally distributed were converted with the log function. The probability between the dependent and explanatory variables was explained using logistic regression analysis. Chi-square statistic was used to assess significant differences between; demographic information, feeding, storage, and handling practices among districts, and occurrence of AFM_1 or AFB_1 (positive/negative). Throughout the analyses, the significance of a variable was considered at $p < 0.05$.

3.8 Ethical Clearance

Ethical clearance was obtained from Kibong'oto Infectious Disease Hospital, Nelson Mandela-AIST, and CEDHA Health Research Ethics Committee (KNCHREC) with reference number KNCHREC 00037/RW/1/21.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Socio-Demographic and Household Characteristics

4.1.1 Smallholder Dairy Farmers

The general demographic characteristics of the SDFs is shown in Table 6. Generally, women were found to be the predominant (more than half) SDFs in Hai (56.9%) and Serengeti (55.6%) except in the Mpwapwa district (41.5%). Similar results were reported by Mkama and Sulle (2019) with 75% of SDFs registered at the Njombe milk factory being women. This shows that almost there is equal opportunity in livestock keeping due to increased awareness of gender equality and women's participation in livestock production. A significant difference between age groups among the districts ($\chi^2 = 33.97$, $p<0.001$) was observed, with the majority (44.2%) falling under the age range of between 36 to 55 years. There was less participation of SDFs aged between 18 and 35 years in livestock keeping due to limited access to land, lack of capital, and aspiration for modern urban life (Lindsjö, 2019). On the other hand, the majority of the SDFs (70.1%) had primary education, and only 6.7% had college/university education ($\chi^2 = 57.63$, $p<0.001$), with farming and livestock keeping as the main occupation (89.3%) ($\chi^2=76.37$, $p<0.001$). Similar findings were observed with previous studies, which reported that a large share of small-scale farmers in Tanzania is from rural areas, mostly with primary education (Mkonda & He, 2018).

Table 7: Socio-demographic and household characteristics SDFs

Demographic characteristics	District			Total (n=419)	Test Chi – square
	Hai (n=137)	Mpwapwa (n=147)	Serengeti (n=135)		
Sex					
male	59 (43.1)	86 (58.5)	60 (44.4)	205 (48.9)	χ^2=8.36, p=0.015
female	78 (56.9)	61 (41.5)	75 (55.6)	214 (51.1)	
Age (years)					
18-35	17 (12.4)	41 (27.9)	41(30.4)	99 (23.6)	χ^2=33.97, p<0.001
36-55	55 (40.1)	64 (43.5)	66 (48.9)	185 (44.2)	
56-70	43 (31.4)	36 (24.5)	21(15.6)	100 (23.9)	
Above 70	22 (16.1)	6 (4.1)	7 (5.2)	35 (8.4)	
Level of education					
College or University	5 (3.6)	22 (15.0)	1(0.7)	28 (6.7)	χ^2=57.63, p<0.001
Secondary	25 (18.2)	15 (10.2)	16 (11.9)	56(13.4)	
Primary	105 (76.6)	83 (56.5)	105 (77.8)	294 (70.1)	
No formal education	1 (0.7)	27 (18.4)	13 (9.6)	41 (9.8)	
Occupation					
Employed and livestock keeping	4 (2.9)	19 (12.9)	0 (0.0)	23 (5.5)	χ^2=76.37, p<0.001
Farming and livestock keeping	130 (94.9)	110 (74.8)	134 (99.3)	374 (89.3)	
Livestock keeping	3 (2.2)	18 (12.2)	1 (0.7)	22 (5.3)	
Experience in keeping livestock					
Less than 5 years	11 (8.0)	34 (23.1)	23 (17.0)	68 (16.2)	χ^2=16.99, p=0.001
Between 5 to 10 years	17 (12.4)	28 (19.0)	22 (16.3)	67 (16.0)	
More than 10 years	109 (79.6)	85 (57.8)	90 (66.7)	284 (67.8)	

4.1.2 Agro-Vet Dealers

The demographic characteristics of agro-vet dealers is shown in Table 7. There was no significant difference in demographic characteristics across the districts. Generally, males were predominant in Mpwapwa (72.7%) and Serengeti (75.0%) district. Probably due to the fact that majority of Tanzanian rural women are more likely to be involved in household activities such as dairy keeping (Leavens *et al.,* 2019; Osabuohien *et al.,* 2019). In Mpwapwa district, most agrovet dealers were associated with extraction sunflower oil and selling of sunflower seedcakes to SDFs as animal feeds. Sunflower seedcakes had been reported to have high level of aflatoxins contamination (Mmongoyo *et al.,* 2017; Mohammed *et al.,* 2016, 2018; Mushi *et al.,* 2018; Rokvic *et al.,* 2020). In Mpwapwa and Hai district, the majority of agrovet dealers were youth aged 18 to 35 with secondary and college education. Most of the agrovet shop in Hai and Mpwapwa districts were owned by livestock officers and animal health graduates, similar agrovet characteristics were reported in Kenya (Auma *et al.,* 2017). Most of agrovet dealers had experience of not more than five years implying that new participants were entering and exploiting opportunities in the dairy value chain, especially youths, with agro vet dealing as their main occupation.

Table 8: Agro-vets socio-demographic and household characteristics

Demographic characteristics	Districts			Total (%)	Test
	Hai	Mpwapwa	Serengeti		
Sex					χ^2=2.09, p=0.3516
female	6(54.5)	3(27.3)	1(25.0)	10(38.5)	
male	5(45.5)	8(72.7)	3(75.0)	16(61.5)	
Age (years)					χ^2=7.43, p=0.2830
18-35	6(54.5)	7(63.6)	1(25.0)	14(52.8)	
36-55	5(45.5)	1(9.1)	2(50.0)	8(30.8)	
56-70	0(0.0)	2(18.2)	1(25.0)	3(11.5)	
Above 70	0(0.0)	1(9.1)	0(0.0)	1(3.8)	
Level of education					χ^2=11.52, p=0.1738
College or University	2(18.2)	4(36.4)	1(25.0)	7(26.9)	
No formal education	0(0.0)	0(0.0)	1(25.0)	1(3.8)	
Primary	2(18.2)	4(36.4)	2(50.0)	8(30.8)	
Secondary	6(54.5)	3(27.3)	0(0.0)	9(34.6)	
Other	1(9.1)	0(0.0)	0(0.0)	1(3.8)	
Marital status					χ^2=2.09, p=0.3516
Married	8(72.7)	5(45.5)	3(75.0)	16(61.5)	
Single	3(27.3)	6(54.5)	1(25.0)	10(38.5)	
Occupation					χ^2=8.6273, p=0.0711
Agro-vet dealer	7(63.6)	6(54.5)	2(50.0)	15(57.7)	
Employed and Agro-vet	1(9.1)	0(0.0)	2(50.0)	3(11.5)	
Others	3(27.3)	5(45.5)	0(0.0)	8(30.8)	
Experience dealing in livestock feeds					χ^2=6.21, p=0.1840
Less than 5 years	8(72.7)	9(81.8)	2(50.0)	19(73.1)	
Between 5 to 10 years	2(18.2)	0(0.0)	2(50.0)	4(15.4)	
More than 10 years	1(9.1)	2(18.2)	0(0.0)	3(11.5)	

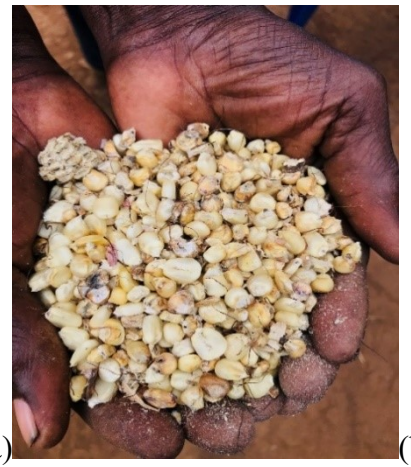
4.2 Cattle Grazing, Feeding Systems, Feed Handling, and Storage Practices

4.2.1 Smallholder Dairy Farmers

The results on the grazing system, feed handling, and storage practices are presented in Table 8. Findings showed that the grazing and feeding systems, feed handling, and storage practices were significantly different ($p < 0.05$) across the districts. For the grazing and feeding systems, free-range was solely practiced in Serengeti district, zero-grazing in Hai district, and mixed grazing and feeding practices (i.e., zero and free-range grazing) in Mpwapwa district (Table 8). As compared to other districts, it was observed that Serengeti had a designated land for free-range grazing systems. The availability of designated grazing lands enables farmers to opt for a free-range system (Kavana *et al.,* 2017; Munyaneza *et al.,* 2019; Njarui *et al.,* 2016). A mixed grazing and feeding system were also observed in Mpwapwa district, where there were adequate grazing areas, and SDFs had the capacity to store the feeds. Hai district is peri-urban and characterized by a lack of grazing areas that could allow for free-range feeding, hence, farmers mainly kept dairy cows indoors. Limited grazing land, town council bylaws, type of

breed, and fear of disease transmission are among the factors that force SDFs to opt for zero-grazing system (Gillah *et al.,* 2012; Kerario *et al.,* 2018; Laisser *et al.,* 2015).

Both forage/roughages and concentrates were the main feed for dairy cattle in Hai (83.9%) and Mpwapwa (69.4%) districts. In the Serengeti district, forage (99.3%) was the main feed used by SDFs. In all the three districts, 75.2% of the respondents reported seasonality-occasioned scarcity, expensive feed concentrates, and inadequate free-ranging land as the major challenges in securing animal feeds. Other studies in Serengeti have observed that reduction in grazing area due to farming, increased livestock population, conservation of Serengeti national parks, and climate change (Kavana *et al.,* 2017; Said *et al.,* 2021; Veldhuis *et al.,* 2019). In areas that used zero and mixed farming systems, storage of feeds for future use was among their mitigation strategies to halt feed scarcity. For instance, 29.1 to 36.2% of the respondents in the Hai district stored feeds for 1 – 12 months, whereas 53.8% of SDFs stored feeds for three (3) months, and 37.5% between 3 - 6 months in the Mpwapwa district. Although it is recommended that animal feeds are dried before storage to avoid fungal growth, majority of the SDFs (74.5%) do not dry the livestock feeds prior storage. In addition, 85.3% and 64.9% of SDFs in Mpwapwa and Hai districts use polythene bags to store feeds, which have not well dried to attain safe moisture levels. Some SDFs stored feeds in cages and racks without any covering. These practices allow moisture pick-up from the environment or moisture build-up in the plastic packages, creating favourable conditions for fungal growth, aflatoxins production, and spoilage of feeds (Negash, 2018; Patyal *et al.,* 2020). A study by Admasu *et al.* (2021) reported a higher level of AFM_1 in milk from among SDFs with no animal feed storage facilities than in those who had storage facilities, the same poor storage practices had observed also in Hai and Mpwapwa district (Fig. 6).

(a)  (b)  (c) 

(d)  (e)  (f) 

Figure 6: **Feed handling and storage practices among SDFs: (a) and (b) show sorted out maize as 'bad' intended for animal feeds, (c), (d), (e) and (f), feed handling and storage practices which can allow moisture pick-ups from environment (Images were taken during data collection)**

Table 9: The grazing and feeding system, feed handling, and storage practices

Cattle management	District			Total (n=419)	Test Chi – square
	Hai (n=137)	Mpwapwa (n=147)	Serengeti (n=135)		
Feeding practices					
Free range	0(0.0)	38(25.9)	135(100.0)	173(41.2)	χ^2=320.21, p <0.001
Zero-grazing	131(95.6)	47(31.9)	0(0.0)	178(42.4)	
Mixed grazing and feeding	06(4.4)	62(42.2)	0(0.0)	68(16.2)	
Main livestock feed					
Roughages	06(4.4)	44(29.9)	134(99.3)	216(55.1)	χ^2=254.69, p <0.001
Concentrates	15(10.9)	1(0.7)	0(0.0)	16(3.8)	
Both roughages concentrates	115(83.9)	102(69.4)	1(0.7)	187(44.6)	
Any challenge getting feeds?					
Yes	96(70.1)	115(78.2)	104(77)	315(75.2)	χ^2=2.89, p=0.234
No	41(29.9)	32(21.8)	31(23)	104(24.8)	
Do you dry the feeds?					
Yes	52(38.0)	54(36.7)	1(0.7)	107(25.5)	χ^2=64.45, p <0.001
No	85(62.0)	93(63.3)	134(99.3)	312(74.5)	
Method of drying the feeds					
Sun dried on polythene sheet	6(11.5)	12(22.2)	0(0.0)	18(16.9)	χ^2=18.36, p=0.0
Open space on the ground	46(88.5)	42(77.8)	1(100.0)	89(83.1)	
Moisture co measurement					
Non	34(65.4)	43(79.6)	1(100.0)	78(72.9)	χ^2=109.73, p <0
Others	18(34.6)	11(20.4)	0(0.0)	29(27.1)	
Do you store?					
Yes	127(93.4)	104(70.7)	1(0.7)	232(55.5)	χ^2=256.77, p <0
No	9(6.6)	43(29.3)	134(99.3)	186(44.5)	
Feeds storage duration					
< 3 months	46(36.2)	56(53.8)	1(100.0)	103(44.4)	χ^2=24.30, p<0.0
3 to 6 months	37(29.1)	39(37.5)	0(0.0)	76(32.8)	
6 months to 1 Year	43(33.9)	8(7.7)	0(0.0)	51(22.0)	
> 1 Year	1(0.8)	1(1.0)	0(0.0)	2(0.9)	
Storage material					
Woven/sisal bag	18(15.8)	4(6.5)	0(0.0)	22(12.5)	χ^2=112.5, p <0.(
Polyethene bag	74(64.9)	52(85.3)	0(0.0)	126(72.0)	
Barrel/Drum	17(14.9)	0(0.0)	0(0.0)	17(9.7)	
Heap on the ground	5(4.4)	5(8.2)	0(0.0)	10(5.7)	

4.2.2 Feed Handling, and Storage Practices of the Agro-Vet Dealers

The results on feed handling and storage practices among the agro-vet dealers are presented in Table 9. The findings showed that, there is no significant difference in feed handling, and storage practices across the districts. Most of the agro-vets, packed the feeds in plastic gunny bags (61.2%) and stored them on pallets (57.7%) for less than three months (88.5%). Some

agro vet dealers (42.3%) were not storing their feeds on pallets, and congested store allowing moisture uptake from the floor, which increases the chance for growth of fungi and production of aflatoxin (Fig. 7). Packaging and storage practices of animal feed on pallets reduce the amount of moisture pickups from the ground, and surrounding, hence, reducing fungal growth (Makau *et al.,* 2016b; Mwakosya *et al.,* 2022). Agro-vets tend to re-use packaging materials (73.1%), also, dairy cattle feeds stores were mostly cleaned once per week (46.2%), uncleaned stores and re-using of packaging materials increased the potential of aflatoxin recontamination from one batch to another (Mongkon *et al.,* 2017). Many agro-vet dealers were not certified (73.1%) and (53.8%) were never inspected by any government regulatory authorities. The frequency of inspection by government regulatory authorities, for inspected agro-vet dealers (46.2%), were once after every six months (75.0%). Uncertified and uninspected agro-vets might not adhere to good practices which protect dairy cattle feeds from aflatoxin contamination. A study in Kenya revealed high proportion of dairy cow feeds aflatoxin contamination (88.2%) from uncertified agrovet dealers (Anyango *et al.,* 2018; Makau *et al.,* 2016b). A systematic review and meta-analysis by Salari e*t al.* (2020) showed increased AFM_1 contamination in milk, which might be attributed to non-compliance with good veterinary husbandry practices. According to Makau *et al.* (2016a), in many sub-Saharan countries, there are inadequate monitoring, evaluation and enforcement measures to assure quality and safety of animal feeds and raw cow milk.

Figure 7: Storage practices (Images were taken during)

Table 10: Feed handling, and storage practices of the agro-vet dealers

Cattle management	Districts			Total (n=26)	Test Chi – square
	Hai (n=11)	Mpwapwa (n=11)	Serengeti (n=4)		
Storage duration					$\chi2$=1.06, p=0.5880
Less than 3 months	10(90.9)	9(81.8)	4(100.0)	23(88.5)	
Between 3 to 6 months	1(9.1)	2(18.2)	0(0.0)	3(11.5)	
Feeds storage					$\chi2$=10.18, p= 0.0375
On pallets	9(81.8)	6(54.5)	0(0.0)	15(57.7)	
On the ground	1(9.1)	2(18.2)	3(75.0)	6(23.1)	
Others specify	1(9.1)	3(27.3)	1(25.0)	5(19.2)	
Store cleaning					$\chi2$=11.03, p= 0.2
Once per week	4(36.4)	4(36.4)	4(100)	12(46.2)	
Once per month	4(36.4)	2(18.2)	0(0.0)	6(23.1)	
After every 3 months	1(9.1)	3(27.3)	0(0.0)	4(3.8)	
Before storing new batch	2(18.2)	2(18.2)	0(0.0)	4(15.4)	
Feed package/packing					$\chi2$=11.19, p= 0.0828
Open space on the ground	1(6.2)	0(0.0)	0(0.0)	1(2.4)	
Plastic gunny bag	11(68.8)	11(100.0)	4(80.0)	26(61.2)	
Sack bag	4(25.0)	0(0.0)	0(0.0)	4(9.4)	
Others specify	0(0.0)	0(0.0)	1(20.0)	11.5(27.1)	
Re-using packaging materials?					$\chi2$=0.933, p= 0.6272
No	4(36.4)	2(18.2)	1(25.0)	7(26.9)	
Yes	7(63.6)	9(81.8)	3(75.0)	19(73.1)	
Certified agrovet?					$\chi2$=0.009, p= 0.9956
No	8(72.7)	8(72.7)	3(75.0)	19(73.1)	
Yes	3(27.3)	3(27.3)	1(25.0)	7(26.9)	
Inspected by regulatory authorities?					$\chi2$=1.034, p= 0.5963
No	6(54.5)	5(45.5)	3(75.0)	14(53.8)	
Yes	5(45.5)	6(54.5)	1(25.0)	12(46.2)	
Frequency of inspection					$\chi2$=4.089, p= 0.6646
Once per month	1(20)	0(0.0)	0(0.0)	1(8.3)	
After every 6 months	3(60)	5(83.3)	1(100)	9(75.0)	
Once per year	1(20)	1(16.7)	0(0.0)	2(16.6)	

4.3 Awareness on Aflatoxins Contamination in Animal Feeds and Milk

4.3.1 Awareness of SDFs on Aflatoxins Contamination in Animal Feeds and Milk

The results on awareness of aflatoxin in feeds and raw milk are presented in Table 10. Generally, this study has shown that there is low level of awareness on aflatoxin among SDFs in all the three districts. Only 23.2% of the respondents reported to be aware of the toxin. Similar findings on inadequate level of awareness were reported in Rwanda (10%) (Nishimwe *et al.,* 2019), Uganda (21%) (Nakavuma *et al.,* 2020), and Tanzania (25%) (Ayo *et al.,* 2018).

Conversely, the level of aflatoxin awareness was relatively higher in Kenya (55%) (Walke *et al.,* 2014), probably due to the aflatoxin outbreak in 2004, where 317 cases were reported, which increased the concern and awareness (Probst *et al.,* 2007). Furthermore, high awareness (62%) of aflatoxin was found in the Babati district, probably due to the project of Africa Research in Sustainable Intensification for the Next Generation which was conducted there and 86% of the surveyed farmers had experience of working with other development programs (Nyangi *et al.,* 2016). Also, ongoing national initiatives and awareness campaigns for aflatoxin control, which have been implemented intensively in the country after the aflatoxicosis outbreak in Dodoma and Manyara regions which occurred in 2016, such initiatives include, Tanzania Initiative for Preventing Aflatoxin Contamination (TANIPAC), which is intensively implemented in 18 districts, including Babati district. This shows the importance of the collaborative effort of different stakeholders' involvement in increasing aflatoxin awareness which has a vital role in aflatoxin mitigation. For few SDFs who have at least heard of the word "aflatoxin", most of them got the information from radios/televisions (47.7%) and extension officers (16.9%). Likewise, Ayo *et al.* (2018) observed that mass media, village officers, and extension officers as the major routes of information transfer on aflatoxin to SDFs and other stakeholders. The majority of SDFs were not aware that aflatoxin could contaminate feed (52%), milk (72%), causes of aflatoxin (62%), control measures of aflatoxin contamination (67%), hepatoxicity due to aflatoxins (63.9%) and effects of aflatoxins on animals' milk yield and growth (78.4%) (Table 10). The low awareness on aflatoxin contamination in feeds and its fate in milk may be attributed to the fact that more emphasis on aflatoxin contamination has been put on food for human consumption, such as maize and groundnuts, compared to livestock feeds (Negash, 2018). Therefore, the limited knowledge of SDFs on aflatoxin in feeds and milk is likely to hinder implementation of intervention for addressing the problem. Previously study by Nyangi *et al.* (2016) reported that farmers tend to feed cattle on un-marketed and sorted out poor quality maize grains which are more likely to contain high levels of aflatoxin. In view of this there are chances of aflatoxin to be carried over to animal products such as milk is significant. For instance, a study in Kenya showed higher AF awareness (72%) in foods for human consumption, such as maize and groundnuts, but, 67% of the urban SDFs were not aware that milk could be contaminated with AFM1 from ingested AFB1 contaminated feeds, and neither knew how to mitigate against the AFs exposure (Hoffmann *et al.,* 2021; Kang'Ethe & Lang'A, 2009). Therefore, awareness creation on aflatoxin contamination is necessary for the SDFs since the majority of respondents (92.6%) were not aware of it.

Table 11: SDFs awareness on aflatoxin contamination of feeds and raw milk

Aflatoxins awareness	District			Total (n=419)	Test Chi – square
	Hai (n=137)	Mpwapwa (n=147)	Serengeti (n=135)		
Heard about aflatoxin?					
Yes	36(26.3)	37(25.2)	24(17.8)	97(23.2)	χ^2=3.28, p=0.194
No	101(73.7)	110(74.8)	111(82.2)	322(76.8)	
Source of information					
Village meeting/ extension officers	7(14.9)	13(23.2)	2(2.9)	22(16.9)	χ^2=14.53, p <0.001
Newspaper	5(10.6)	2(3.6)	2(2.9)	9(6.9)	
Seminar	1(2.1)	1(1.8)	0(0.0)	2(1.5)	
Radio/Tv	28(59.6)	18(32.1)	16(45.7)	62(47.7)	
Friend	1(2.1)	5(8.9)	0(0.0)	6(4.6)	
School	1(2.1)	9(16.1)	1(2.1)	11(8.5)	
Others	4(8.5)	8(14.3)	6(17.1)	18(13.8)	
Aware that aflatoxin can contaminate feeds?					
Yes	11(30.6)	25(67.6)	6(25.0)	42(43.3)	χ^2=14.53, p <0.001
No	25(69.4)	12(32.4)	18(75.0)	55(56.7)	
Can recognize aflatoxin contaminated feeds?					
Yes	12(33.3)	20(54.1)	5(20.8)	37(38.1)	χ^2=7.37, p= 0.025
No	24(66.7)	17(45.9)	19(79.2)	60(61.9)	
Knows aflatoxins causes cancer//hepatotoxicity?					
Yes	9(25.0)	17(45.9)	9(37.5)	35(36.1)	χ^2=3.49, p= 0.173
No	27(75.0)	20(54.1)	15(62.5)	62(63.9)	
Knows aflatoxin reduces livestock growth and milk yield?					
Yes	7(19.4)	11(29.7)	3(12.5)	21(21.6)	χ^2=2.71, p= 0.257
No	29(8.6)	26(70.3)	21(87.5)	76(78.4)	
Knows milk can be contaminated with aflatoxin?					
Yes	8(22.2)	10(27.0)	6(25.0)	24(24.7)	χ^2=0.22, p= 0.892
No	28(77.8)	27(73.0)	18(75.0)	73(75.3)	
Knows causes of aflatoxin contamination?					
Yes	15(41.7)	19(51.4)	3(12.5)	37(38.1)	χ^2=9.61, p= 0.008
No	21(58.3)	18(48.6)	21(87.5)	60(61.9)	
Knows control measures for aflatoxin?					
Yes	11(30.6)	19(51.4)	2(8.3)	32(33.0)	χ^2=12.34, p= 0.002
No	25(69.4)	18(48.6)	22(91.7)	65(67.0)	
Received training on aflatoxin?					
Yes	4(3.0)	8(6.0)	3(2.3)	15(7.4)	χ^2=1.57, p= 0.455
No	130(97.0)	126(94.0)	132(97.7)	388(92.6)	
Need training on aflatoxin?					
Yes	128(97.0)	95(96.0)	127(94.0)	350(95.6)	χ^2=2.35, p= 0.307
No	4(3.0)	4(4.0)	8(6.0)	16(4.4)	

The number of respondents (n) may vary within the table due to the dependent questions from previous questions during the interview

4.3.2 Awareness of Agro-Vet Dealers on Aflatoxins Contamination of Animal Feeds and Milk

The results from descriptive analysis of the agro-vet dealers' awareness on aflatoxin contamination in feeds and milk are presented in Table 11. There is no significant difference on status of aflatoxin awareness among respondents from the three districts. Awareness of the agro-vet dealer on aflatoxin was relatively higher than SDFs. About 50% of agro vet dealers were aware and have heard about aflatoxin. There was even distribution of the source of information, mostly, agro-vet dealers heard about aflatoxin through village meetings, extension officers, newspapers, seminars, radio/television, friend, school and others from the Tanzania Bureau of Standards (TBS). The observation could be due to their level of education as most of them had secondary and college education, hence more likely to be keen on matters related to aflatoxin (Anyango *et al.,* 2018). In comparison to SDFs, it was observed that a slightly high number of agro-vets were aware that aflatoxins could contaminate livestock feeds (69.2%), causes cancer (53.8%), impair milk yield and growth (61.5%) and measures for control of contamination (53.8%). Apart from the fact that most of the agro-vets had attained college level, but also, 53.8% of agro vet dealers had received training about aflatoxin and have learned about best practices for feed handling and storage and good manufacturing practices. Increased awareness on aflatoxin and a low level of aflatoxin contamination had observed among participants who received training on aflatoxin (Pretari *et al.,* 2019; Seetha *et al.,* 2017). However, 61.5% of agro vet dealers had no skills to recognize the contaminated feed and were not aware that, aflatoxin could contaminate feed and milk, hence the information gap needs to be filled.

Table 12: Agrovets awareness on aflatoxin contamination in feeds

Aflatoxin awareness	District			Total (%) (n=26)	Test Chi-square
	Hai (n=11)	Mpwapwa (n=11)	Serengeti (n=4)		
Heard about aflatoxin?					$\chi2$=0.182, p= 0.9131
Yes	5(45.5)	6(54.5)	2(50.0)	13(50.0)	
No	6(54.5)	5(45.5)	2(50.0)	13(50.0)	
Source of information					$\chi2$=15.28, p= 0.2256
Village meeting/ extension officers	1(14.3)	1(12.5)	0(0.0)	2(11.8)	
Newspaper	1(14.3)	0(0.0)	0(0.0)	1(5.9)	
Seminar	2(28.6)	0(0.0)	0(0.0)	2(11.8)	
Radio/Tv	1(14.3)	1(12.5)	1(50.0)	3(17.6)	
Friend	1(14.3)	0(0.0)	1(50.0)	2(11.8)	
School	1(14.3)	1(12.5)	0(0.0)	2(11.8)	
Others	0(0.0)	5(62.5)	0(0.0)	5(29.4)	
Knows aflatoxin can contaminate feeds?					$\chi2$=5.33, p= 0.0695
No	1(20.0)	1(16.7)	2(100.0)	4(30.8)	
Yes	4(80.0)	5(83.3)	0(0.0)	9(69.2)	
Can recognize aflatoxin contaminated feeds?					$\chi2$=2.30, p= 0.3172
No	2(40.0)	5(83.3)	1(50.0)	8(61.5)	
Yes	3(60.0)	1(16.7)	1(50.0)	5(38.5)	
Knows aflatoxins causes cancer/hepatotoxicity?					$\chi2$=2.81, p= 0.2458
No	2(40)	2(20)	2(100.0)	6(46.2)	
Yes	3(60.0)	4(80.0)	0(0.0)	7(53.8)	
Knows aflatoxin reduces livestock growth and milk yield?					$\chi2$=1.17, p= 0.5571
No	1(20.0)	3(50.0)	1(50.0)	5(38.5)	
Yes	4(80.0)	3(50.0)	1(50.0)	8(61.5)	
Knows milk can be contaminated with aflatoxin?					$\chi2$=1.59, p= 0.4510
No	3(60.0)	3(50.0)	2(100.0)	8(61.5)	
Yes	2 (40.0)	3(50.0)	0(0.0)	5(38.5)	
Knows causes of aflatoxin contamination?					$\chi2$=0.18, p= 0.9120
No	2(40.0)	2(33.3)	1(50.0)	5(38.5)	
Yes	3(60.0)	4(66.7)	1(50.0)	8(61.5)	
Knows control measures of aflatoxin?					$\chi2$=2.81, p= 0.2458
No	2(40.0)	2(33.3)	2(100.0)	6(46.2)	
Yes	3(60.0)	4(66.7)	0(0.0)	7(53.8)	
Received training on aflatoxin?					$\chi2$=2.30, p= 0.3172
No	2(40.0)	2(33.3)	2(100.0)	6(46.2)	
Yes	3(60.0)	4(66.7)	0(0.0)	7(53.8)	
Needs training on aflatoxin?					$\chi2$=2.36, p= 0.3067
No	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Yes	8(100.0)	7(100.0)	5(100.0)	19(100.0)	

4.4 Occurrence of AFB1 in Dairy Cow Feeds and AFM1 Cow Raw Milk

4.4.1 Occurrence of AFB1 in Dairy Cow Feeds

The results on the occurrence of AFB_1 in livestock feeds among the SDFs, and agro-vet dealers are presented in Table 12 and 13, respectively. Prevalence of dairy cow feed samples from

SDFs in all three districts, which were detected with AFB_1 was 86.2%, out of which 22.5% had levels above 5 µg/kg. Additionally, 88.5% of all livestock feed samples from agro-vet dealers were contaminated with AFB_1, out of which 15.38% had levels exceeded 5 µg/kg. The prevalence of samples detected with AFB_1 among SDFs were significantly differed across the districts ($p < 0.0361$), where by almost all the samples (97%) from Mpwapwa district were detected with toxin compares with the sample from Hai district (80%). However, there was no significant difference in means concentrations observed between Mpwapwa and Hai district ($p < 0.1115$). There was no significant difference observed in feed samples from agrovet dealers across the districts. However, feed samples from the Serengeti district were all positive for AFB_1, probably due to poor storage practices observed as the feeds were barely spread on the floor, although none was found with levels above 5 µg/kg.

In overall, 22.5% of the livestock feeds were contaminated with AFB_1 at levels above 5µg/kg. Only 22.5% of the feeds from SDFs and 15.38% from agro-vets had AFB_1 concentrations exceeding the maximum allowed limits (5 µg/kg) by Tanzania national standard, East Africa standard and EU standard for dairy cow feeds (Mohammed *et al.,* 2016). However, the percentage occurrence of AFB_1 in feeds (86.2%) found in this study is high compared to 65% and 80% reported in sunflower seedcakes in Singida by Mohammed *et al.* (2016) and Mmongoyo *et al.* (2017), respectively. This indicates that a significant proportion of animal feeds used by most SDFs might be contaminated by AFB_1. In an attempt to assess AFB_1 contamination levels in various animal feed sources, a study conducted in Morogoro found that 50% of maize bran and 70% of sunflower seedcakes were positive for AFB_1. Furthermore, in the most recent study conducted in Dar es salaam Tanzania in 2022, 91% of animal feed samples were detected with AFB_1 at levels ranging from 24 to 76.23 µg/kg (Mwakosya *et al.,* 2022).

Climatic condition is one of the key the factors that contribute to growth of aflatoxin producing fungi in feeds (Mmongoyo *et al.,* 2017; Nyangi *et al.,* 2016; Temba *et al.,* 2021). Relative humidity of about 70% and a temperature range of 10–40ºC are reported to favour mould growth (Ledo *et al.,* 2020) . In the semi-arid zone (Mpwapwa district) and northern highland zone (Hai district), where the climatic conditions favour aflatoxin production feed storage was commonly practiced (Table 8), this further justifies their occurrence in the two districts (Table 12).

4.4.2 Occurrence of AFM1 in Raw Milk Samples

The prevalence of AFM_1 in raw milk samples from three different agro-ecological zones is presented in Table 12. High prevalence of milk samples detected with AFM_1 was noted in Mpwapwa district (63.8%), followed by Hai district (17.8%) and Serengeti district (10.4%). Generally, 27.9% of the raw milk samples were detected with AFM_1 exceeding 0.05 µg/l (50 ppt), the permissible level for AFM_1 in raw cow milk based on the EU standards and 19.9% had detected exceeding 0.5 µg/l the permissible level for AFM_1 in raw cow milk based on the Tanzania and East Africa Standard (Turna & Wu, 2021). By district, percentage of milk samples detected with AFM_1 levels exceeding 0.05 µg/l was highest in Mpwapwa (59.6%), followed by Hai (17.8%) and lowest in Serengeti (6.2%). The prevelance and means concentrations of AFM_1 was significant difference across the districts, p = 0.001, and p = 0.0173, respectively. Low prevelance of milk samples with AFM_1 in the Serengeti district may suggest that the grazing and feeding system are likely to have significant contribution to the situation. Small holder dairy farmers in Serengeti district mostly use free ranging compared to Mpwapwa and Hai district. Free ranging does not involve the use of feed concentrates such as maize bran and sunflower seedcakes which might have aflatoxin contamination, this might associate with low prevalence of AFM_1 in Serengeti in comparison to other districts. Could significantly contribute to AFs contamination levels. A study conducted in Morogoro and Tanga reported low levels of AFM_1 in cow raw milk from free range cows (Ledo *et al.,* 2020). The results on prevalence of AFM_1 in milk found in this study are comparable to prevalence of 13.6% to 65.1% which was reported in Kenya (Kang'Ethe &Lang'A, 2009; Senerwa *et al.,* 2016). Several studies have reported high AFM_1 prevalence in other geographical locations, for instance, 83.8% in Singida (Mohammed *et al.,* 2016), 92% in Dar es salaam (Urio *et al.,* 2006), 72% in Kenya (Kang'Ethe & Lang'A, 2009), 99% and 100% in Ethiopia (Gizachew *et al.,* 2016). The variation in prevalence of AFM_1 among districts can be attributed to different agro-ecological zones and the effects of climatic conditions, different grazing and feeding systems, feed handling, storage practices (Table 8), and levels of awareness (Table 10). In this study, the carry-over effect of AFB_1 to AFM_1 was explained in a linear relationship, p = 0.0001 with an adjusted r^2 = 0.6762, which signify correlation between AFB_1 and AFM_1 in this study. However, factors such as milk yield, lactation period, species differences, animal health, hepatic biotransformation ability, feeding pace, and the integrity of the mammary alveolar cell membranes are known to influence the carry-over effects (Britzi *et al.,* 2013; Masoero *et al.,* 2007; Tolosa *et al.,* 2021).

Table 13: Occurrence of AFB_1 in feed and AFM_1 in cow raw milk

Parameters*	District							
	Hai		**Mpwapwa**		**Serengeti**	**Total**		
	AFB_1 **(n=50)**	AFM_1 **(n=45)**	AFB_1 **(n=30)**	AFM_1 **(n=48)**	AFM_1 **(n=48)**	AFB_1 **(n=80)**	AFM_1 **(n=141)**	***p* value**
Mean	2.99 [a]	0.36[a]	6.16 [a]	4.25[a]	0.026[b]	4.18	1.54	AFB_1 = 0.1115
Standard deviation	5.47	1.14	8.41	9.02	0.11	6.85	5.57	AFM_1= 0.0173
Median	0.91	0.00	2.98	0.22	0.00	1.33	0.00	
Minimum	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
Maximum	32.90	6.36	30.19	43.98	0.59	32.9	43.98	
+ve samples	40(80)[a]	8(17.8) [a]	29(96.7) [b]	30(63.8) [b]	5(10.4) [c]	69(86.2)	43(30.7)	AFB_1=0.0361
-ve samples	10(20)	37(82.2)	1(3.3)	17(36.2)	43(89.6)	11(13.8)	97(69.3)	AFM_1<0.001
$AFB_1 \geq 5$µg/kg	8(16)	-	10(33.3)	-	-	18(22.5)		
$AFB_1 \leq 5$µg/kg	42(84)	-	20(66.7)	-	-	62(77.5)		
$AFM_1 \geq 0.5$µg/L		7(15.6)		20(41.7)	1(2.08)		28(19.9)	
$AFM_1 \leq 0.5$µg/L		38(84.4)		28(58.3)	47(92)		113(80.1)	
$AFM_1 \geq 0.05$µg/L	-	8(17.8)	-	28(59.6)	3(6.2)		39(27.9)	
$AFM_1 \leq 0.05$µg/L	-	37(82.2)	-	20(40.4)	45(93.8)		101(72.1)	

* *AFB_1 (µg/kg); AFM_1 (µg/L)*

Means and positive samples followed by different superscript letters, a, b, or c between districts are significantly different ($p<0.05$).

Table 14: Occurrence of AFB_1 in feed samples from agro vet dealers

Parameters*	District			Total (n=26)	*p* value
	Hai (n=11)	Mpwapwa (n=11)	Serengeti (n=4)		
Mean	1.72	3.95	0.81	2.53	= 0.3720
Standard deviation	2.48	6.89	0.24	4.80	
Median	0.94	0.66	0.78	0.90	
Minimum	<LOD	<LOD	0.54	<LOD	
Maximum	9.06	22.99	1.13	22.99	
+ve samples	10(90.9)	9(81.8)	4(100)	23(88.5)	=0.588
-ve samples	1(9.1)	2(18.2)	0(0.0)	3(11.5)	
$AFB_1 \geq 5$ µg/kg	1(9.1)	3(27.3)	0(0.0)	4(15.38)	
$AFB_1 \leq 5$ µg/kg	10(90.9)	8(72.7)	4(100)	22(84.62)	

* *AFB_1 (µg/kg)*

4.5 Risk Factors Associated with the Occurrence of AFB1 and AFM1 in Animal Feeds and Raw Milk

The risk factors associated with AFB_1 and AFM_1 in feeds and raw milk among the SDFs are presented in Table 14 and 15, respectively. On the one hand, it was found that the occurrence of AFM_1 in raw milk was significantly influenced by the education level ($p < 0.05$) and aflatoxin awareness ($p = 0.0499$). The likelihood of AFB_1 occurrence in feed samples was 2 times (OR = 16.0, $p = 0.0066$) for SDFs with primary education compared to those with secondary education (OR = 8.0, $p = 0.0066$); those with primary education had 16 times (OR = 16.0) likelihood of their feeds being contaminated with AFB_1 compared to those with college/university education. From this study, it is evident that the level of education influences the awareness and magnitude of occurrence of aflatoxin. Other studies have found that the level of education might enlighten farmers on animal feeds and food safety as well as AFs control measures (Anyango *et al.,* 2018; Ayo *et al.,* 2018).

On the other hand, cattle management practices, including the grazing and feeding systems and the main/major livestock feeds by SDFs, significantly ($p < 0.05$) influenced the occurrence of AFB_1 in livestock feeds. Due to use of mostly feed concentrates, maize bran and sunflower seedcakes as feed supplements for zero grazing. This study observed that, in Hai and Mpwapwa districts, zero grazing practices was 11.3 times (OR = 11.3, $p = 0.0355$) and mixed feeding 16 times (OR = 16.0, $p = 0.0429$) more likely to have AFB_1 contamination compared to free-ranging. In addition, the results showed a significant ($p = 0.0441$) influence of mixed and zero-grazing practices on the occurrence of AFM_1 in raw cow milk compared to free-ranging ($p = 0.0057$). Previous studies have observed that zero-grazing and mixed grazing and feeding systems were likely to influence the occurrence of AFB_1 and AFM_1 in feeds and raw milk,

since feed concentrates are the primary source of aflatoxin contamination in the dairy value chain (Admasu *et al.,* 2021; Anyango *et al.,* 2018; Kang'Ethe & Lang'A, 2009).

In addition, the major type of feed used by SDFs indicates a relationship with the feeding systems used. While roughages were only used in free-ranging, in zero-grazing and mixed feeding the use of roughages, cut-carry, or stored roughages alongside concentrate like maize bran and sunflower seedcakes is very common (Admasu *et al.,* 2021; Patyal *et al.,* 2020). Findings from this study indicate that the use of concentrates in feeding dairy cattle was 12 times more likely (OR = 12.0) to expose the cows to AFB_1 than the use of roughages only. Also, the use of both roughages and concentrates was five times more (OR = 5.0), likely to expose the cows to AFB_1 compared to the use of roughages only. Therefore, mixing roughages and concentrates could be a good option to reduce exposure of cows to AFB1 and consequently decrease the likelihood of AFM1 in milk. Furthermore, this study has found that the occurrence of AFM_1 in raw cow milk is significantly ($p = 0.0171$) influenced by concentrates and a mixture of roughages and concentrates as well. Previous studies also reported a high level of AFB_1 in animal feeds concentrates influence the likelihood of AFM1 in milk (Mmongoyo *et al.,* 2017; Mohammed *et al.,* 2016, 2018; Nyangi *et al.,* 2016).

Feed handling practice such as drying, and the levels of moisture content in livestock feeds also influenced the occurrence of AFB_1 in the feeds and AFM_1 in milk from cows fed on the feeds. The findings show that AFM_1 contamination was two times more likely (OR = 2.0, $p = 0.0058$) in cow raw milk from SDFs that do not dry their feed properly, hence likely to have unacceptable levels of moisture content. High moisture content is among the predominant factors that promote fungal growth and aflatoxin production in foods and feeds (Mwakosya *et al.,* 2022). A recent study in Tanzania has shown a significant positive correlation between moisture content and AFB_1 in animal feeds ($r = 0.90$; $p < 0.05$) (Mwakosya *et al.,* 2022).

Furthermore, storing feeds for future use influenced the occurrence aflatoxin in feeds and raw cow milk. Stored feeds were two times (OR = 2.0) more likely to have a high level of AFB_1 in compared to un-stored feeds. Raw milk samples from SDFs who store feeds had 3.6 (OR = 3.6, $p = 0.0019$) times more likelihood of being contaminated with AFM_1 compared to those fed on un-stored feed. Noteworthy, drying feeds demonstrated lower mould counts. However, mould spores can remain in feeds after drying and later germinate and flourish if conditions become favourable (Lanyasunya *et al.,* 2005). Feedstuff stored under poor conditions such as high relative humidity, temperature, and poor ventilation, is more likely to be contaminated with

AFs (Admasu *et al.,* 2021). Therefore, it is crucial to comply with good storage practices to avoid fungal growth and aflatoxin recontamination.

Generally, this study has found a variation in the occurrence of AFB_1 and AFM_1 in the selected districts representing the three agroecological zones (Table 12). Feeds from the Mpwapwa district were 7.3 times more likely (OR = 7.3, p= 0.0658) to have AFB_1 contamination than Hai district. On the other hand, variation in the occurrence of AFB_1 and AFM_1 could not be accounted in the Serengeti district, since no animal feeds were collected. The raw cow milk collected from Mpwapwa was 7.2 times more likely (OR = 15.2, p <0.0001) to have AFM1 contamination compared to Hai district (OR = 2.1). Similarly, the milk samples from Mpwapwa were 15.2 times more likely (OR = 15.2) of being contaminated with AFM_1 compared to those from Serengeti (OR = 1.0). It is therefore sufficient to note that agroecological zones influence the likelihood of occurrence of AFs contamination. For instance, Mpwapwa, which is in the semi-arid zone of Tanzania, is characterized by semi-arid conditions with an average annual temperature of 27°C (Mengele *et al.,* 2020), with a hot and humid condition that favour fungal growth. A study in Malawi reported up to 80% of aflatoxin prevalence in hotter agroecological zones (Matumba *et al.,* 2015). The differences in occurrence of aflatoxin among agro-ecological zones (Table 12) can also be explained by the type of grazing and feeding systems, feed handling, and storage practices. The survey results (Table 8) showed that zero and mixed feeding practices were dominant in Mpwapwa and Hai districts, which corresponded to the higher levels of AFB_1 and AFM_1 compared to Serengeti, where SDFs practiced free-range feeding system. However, occupation of SDFs, their experience in keeping livestock, method of drying feeds and feed storage material were not significantly associated with the occurrence of AFB_1 in animal feeds and AFM_1 in raw cow milk.

Table 15: Logistic regression analysis for risk factors for AFB_1 occurrence

Predicted factors	Occurrence of AFB_1 in feed samples (n=80)		95%CI	
	-ve samples (%)	+ve samples (%)	Odd Ratio (OR)	*p* value
District				
Mpwapwa (Semi-arid zone)	1(9.1)	29(42.0)	7.3(1.3-136.8)	0.0658
Hai (Northern highland zone)	10(90.9)	40(58)	1.0	
Level of education				
College or University	8(34.8)	3(5.3)	1.0	
Secondary	2(8.7)	12(21.1)	8(2.14-43.9)	0.0039
Primary	13(56.5)	42(73.7)	16(2.5-155.3)	0.0066
Occupation				
Employed and livestock keeping	5(21.8)	8(14.0)	1.0(0.1-5.9)	0.965
Farming and livestock keeping	15(65.2)	44(77.2)	1.8(0.3-8.1)	0.474
Livestock keeping	3(13.0)	2(8.8)		
Experience in keeping livestock				
Less than 5 years	3(13.0)	10(14.0)	0.8(0.1-5.4)	0.8132
Between 5 to 10 years	3(13.0)	8(17.5)	1.0	
More than 10 years	17(73.9)	39(68.4)	0.7(0.1-2.6)	0.6036
Feeding practices				
Free- range	4(17.4)	1(1.8)	1.0	
Zero grazing	17(73.9)	48(84.2)	11.3(1.5-229.4)	0.0355
Mixed grazing	2(8.7)	8(14.0)	16(1.4-436.7)	0.0429
Main livestock feed				
Roughages	2(8.7)	1(1.8)		
Concentrates	1(4.3)	6(10.5)	12.0(0.6-556.6)	0.128
Both roughages and concentrates	20(87.0)	50(87.7)	5.0(0.5-111.2)	0.199
Do you dry the feeds?				
Yes	13(56.5)	24(42.1)	0.6(0.2-1.5)	0.244
No	10(43.5)	33(57.9)	1.0	
Method of drying the feeds				
Sun-dried on polythene sheet	4(30.8)	5(20.8)	1.0	
Open space on the ground	9(69.2)	19(79.2)	1.7(0.3-8.0)	0.504
Moisture content measurement				
No moisture measurements	6(46.2)	22(91.7)	0.1(0.01-0.4)	0.0058
Others	7(53.8)	2(8.3)	1.0	
Storing feeds?				
Yes	20(87.0)	54(94.7)	2.7(0.5-15.7)	0.2470
No	3(13.0)	3(5.3)	1.0	
Feeds storage duration				
Less than three months	8(38.1)	26(48.1)	1.0	
Between 3 to 6 months	5(23.8)	15(27.8)	1.8(0.5-7.5)	0.3700
Between 6 months to 1 Year	8(38.1)	13(48.1)	2.0(0.6-6.7)	0.2520
Storage material				
Barrel/Drum	2(9.5)	3(5.6)	0.8(0.1-6.9)	0.8379
Polyethene bag on ground	7(33.3)	29(53.7)	2.3(1.0-7.0)	0.1406
Polyethene bag on pallets	12(57.1)	22(40.7)		
Heard about aflatoxin				
Yes	7(30.4)	19(33.3)	1.2(0.4-3.4)	0.8023
No	16(69.6)	38(66.7)	1.0	

Table 16: Logistic regression analysis for associated risk factors for AFM_1 occurrence

Predicted factors	Occurrence of AFM1 in raw milk (n=141)		95%CI	
	-ve samples	+ve samples	Odd Ratio (OR)	*p* value
District				
Mpwapwa (Semi-arid zone)	17(36.2)	30(63.8)	15.2(5.4-50.6)	<0.0001
Hai (Northern highland zone)	37(82.2)	8(17.8)	2.1(0.7-7.3)	0.2200
Serengeti (Arid zone)	43(89.6)	5(10.4)	1.0	
Level of education				
College or University	3(3.1)	9(20.5)	1.0	
Secondary	15(15.5)	8(18.2)	0.2(0.03-0.8)	0.0020
Primary	79(81.4)	27(61.4)	0.1(0.02-0.4)	0.0303
Occupation				
Employed and livestock keeping	5(5.2)	9(20.5)	1.4(0.3-8.2)	0.6760
Farming and livestock keeping	88(90.7)	30(68.2)	0.3(0.06-1.1)	0.0647
Livestock keeping	4(4.1)	5(11.4)	1.0	
Experience in keeping animals				
Less than 5 years	17(17.5)	7(15.9)	0.9(0.3-3.0)	0.8297
Between 5 to 10 years	17(17.5)	8(18.2)	1.0	
More than 10 years	63(64.5)	29(65.9)	1.0(0.4-2.6)	0.9636
Feeding practices				
Free- range	54(55.7)	16(36.4)	0.8(0.1-2.4)	0.0057
Zero grazing	40(41.2)	21(47.7)	2.0(1.0-4.4)	0.0441
Mixed grazing	3(3.1)	7(15.9)	1.0	
Main livestock feed				
Roughages	53(54.6)	15(34.1)	1.0	
Concentrates	6(6.2)	2(4.5)	1.2(0.2-5.7)	0.3757
Both roughages and concentrates	38(39.2)	27(61.4)	2.5(1.2-5.5)	0.0171
Do you dry the feeds				
Yes	18(18.6)	17(38.6)	2.8(1.3-6.2)	0.0121
No	79(81.4)	27(61.4)	1.0	
Method of drying the feeds				
Sun-dried on polythene sheet	3(16.7)	6(35.3)	1.0	
Open space on the ground	15(83.3)	11(64.7)	0.4(0.1-1.7)	0.2160
Moisture content measurement				
No moisture measurements	18(81.8)	17(89.5)	2.0(0.3-15.0)	0.4940
Others	4(18.2)	2(10.5)	1.0	
Do you store feeds?				
Yes	47(48.5)	34(77.3)	3.6(1.7-8.5)	0.0019
No	50(51.5)	10(22.7)	1.0	
Feeds storage duration				
Less than three months	24(51.1)	19(55.9)	1.5(0.5-4.9)	0.5300
Between 3 to 6 months	12(25.5)	9(26.5)	1.4(0.4-5.3)	0.6360
Between 6 months to 1 Year	11(23.4)	6(17.6)	1.0	
Storage material				
Barrel/Drum	5(10.6)	2(5.9)	1.0	
Polyethene bag on ground	19(40.4)	16(47.1)	2.5(1.0-16.0)	0.4100
Polyethene bag on pallets	23(48.9)	16(47.1)	1.2(0.9-13.2)	0.5380
Heard about aflatoxin				
Yes	22(22.7)	17(38.6)	2.2(0.1-4.7)	0.0499
No	75(77.3)	27(61.4)	1.0	

4.6 Validation of the Analytical Method

Methods validation results are shown in Table 17. Three independent blank samples of AFB_1 and AFM_1 were spiked by AF mix (1, 2, 5, 10, 15 µg/kg) and AFM_1 (0.02, 0.05, 0.5, 1, 2 µg/L) standards, respectively for LOQ and LOD determination. The LOQ for the method was deemed satisfactory for both AFB_1 and AFM_1 since their values were below the EU maximum permissible limit for AFB_1 in dairy cow feeds (5 µg/kg) and AFM_1 for cow raw milk (0.05 µg/L). The precision and recovery were evaluated by triplicate spiking of AFs at levels of 1, 5, 10, 15, 20 µg/kg and 0.02, 0.05, 0.5, 1, 2 µg/L to blank feed and raw milk samples, respectively. The percentage recovery was calculated by dividing the detected concentration by HPLC over the spiked concentration times a hundred. Precision was done by measuring concentration of the same spiked samples three times a day (morning, afternoon and evening) for three days, and relative standard deviations (RSD%) were calculated. Selectivity of the method were confirmed, as there were no interfering peaks in the chromatogram around the retention time of each target analyte (Appendix 4)

Table 17: Recovery, precision, LOQ, LOD and linearity

Analyte	Spiked concentration range	Mean recovery (%)	Precision (%RSD)	LOD	LOQ	Linearity (R^2)
AFB1	1-20 µg/kg	97.08+/-7.4	0.77	0.530	0.630	0.99
AFM1	0.02-2 µg/L	83.06+/-4.8	0.92	0.027	0.040	0.99

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study showed that the grazing and feeding systems, feed handling, and storage practices were significantly different across Mpwapwa, Hai and Serengeti district. Free-range was solely practiced in Serengeti, zero-grazing in Hai, and mixed grazing and feeding practices (i.e., zero and free-range grazing) in Mpwapwa District. The SDFs encounter challenges in meeting sufficient and safe feeds. The majority of SDFs were not aware that aflatoxin could contaminate feed, milk and their adverse health effects. Furthermore, the study showed that most agrovets were uncertified and rarely inspected by any government regulatory authorities, which increase the likelihood of aflatoxin contamination in dairy value chain. The findings have established a high prevalence of AFB_1 (86.2%) from SDFs and (88.5%) from agrovet dealers livestock feeds, respectively and AFM_1 (30.7%). Potential risk factors associated with AFB_1 and AFM_1 contamination were cattle grazing and feeding systems, feed storage, and handling practices in the three agroecological zones. There was more likelihood of aflatoxin contamination in feeds SDFs who had a low level of education, store feeds without measuring moisture content, practices zero grazing and feed their cattle with concentrates. This finding suggests a potential health risk to animals and humans due to aflatoxin contamination in feeds and milk. Hence, a need for immediate interventions to halt AFB_1 contamination in animal feeds and consequently AFM_1 in milk.

5.2 Recommendations

The following are key recommendations derived from this study which explored the occurrence of aflatoxins and the associated risk factors in the dairy value chain. Based on comprehensive analysis and findings, the following are recommended in order to mitigate aflatoxin contamination and enhance the safety of feeds and cow milk in Tanzania.

To increase in awareness on aflatoxins in animal feeds and animal products such as milk and control measures among farmers who keep livestock with emphasize on proper feed handling and storage among others.

Government through its regulatory bodies to establish and implement a monitoring and evaluation systems, and strengthen enforcement of regulation of the animal feed. This will

facilitate to minimise exposure of cattle to aflatoxin-contaminated feeds and control AFM_1 contamination in animal products such as milk and exposure of humans to the toxin.

The government to facilitate availability of simple and cheap technologies/approaches, such as aflatoxin binders to local agrovet dealers, especially for those who takes directly feeds such as maize bran and sunflower seedcakes from milling machines, which commonly used by SDFs. Aflatoxin binders are substances added to animal feed to bind and immobilize aflatoxins, thereby reducing their bioavailability and potential harm to animals. These binders work by chemically binding to aflatoxins in the gastrointestinal tract of animals, preventing their absorption into the bloodstream and subsequent distribution to vital organs. Therefore, the binders will help in safeguarding animal health and reducing the risk of aflatoxin exposure to humans through the consumption of animal-derived products such as milk.

The government through their stakeholders to ensure availability of rapid test kit for AFM_1 in milk to enable determination of safety of the milk before is supplied to consumers.

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APPENDICES

Appendix 1: Human Informed Consent Form

HUMAN INFORMED CONSENT FORM

Student Researcher: STEVEN J. KITIGWA

Title of Project: OCCURRENCE OF AFLATOXINS AND ASSOCIATED RISK FACTORS IN RAW MILK AND DAIRY FEEDS IN THREE AGRO-ECOLOGICAL ZONES OF TANZANIA.

I am asking for your voluntary participation in my research study. Please read the following information about the research study. If you would like to participate, please sign in the appropriate area below

Purpose of the Research:

To assess the prevalence of Aflatoxin and associated risk factors in raw milk and dairy feeds in three agro-ecological zones of Tanzania.

If you participate, you would be asked to:

Answer some question that related to, aflatoxin awareness, feeds manufacturing and handling practices, feed storage practices and animal husbandry practices. Also, you will be requested to collect a small sample of raw milk/feeds volume for laboratory assessment of Aflatoxin B1/M1 contamination, along with assessing questionnaire for the analysis of risk factors.

The time required for participation: about 15-25 minutes

Potential Risks of the study:

There is no potential risk in this study

Benefits:

General, participant and society will benefit by being aware of Aflatoxin contamination and understanding the risk factors associated. Also, the results will show the occurrence of Aflatoxin B1 in feeds and Aflatoxin M1 in raw milk for the district selected.

All of the records will be stored securely and confidentially.

Voluntary Participation:

Participation in this study is completely voluntary if you decide not to participate there will not be any negative consequence. Please be aware that if you decide to participate, you may stop participating at any time and you may decide not to answer any specific question.

By signing this form, I am attesting that I have read and understood the information above and I freely give my consent/assent to participate or permission for my child to participate.

Adult Informed Consent or Minor Assent Date Reviewed and signed

……

Signature

……

Parent/Guardian Permission Name Date Reviewed and signed

……

If you have any questions about this study, feel free to contact:

Student Researcher: Steven Kitigwa

Phone number: +255788558267 / +255713947749

Email: kitigwas@nm-aist.ac.tz

Appendix 2: Questionnaire for smallholder dairy farmers

OCCURRENCE OF AFLATOXINS AND ASSOCIATED RISK FACTORS IN DAIRY VALUE CHAIN IN THREE AGRO-ECOLOGICAL ZONES OF TANZANIA.

Study Participant Questionnaire

(Smallholder dairy farmers)

GENERAL INFORMATION			
District:	Ward:	Village:	Code:
Household No:		Participant Code:	GPS Code:
Date of visit:			

A. DEMOGRAPHIC INFORMATION			
S/N	Questions/Item	Choices	Response
1.	Age of respondent	1. 18 – 35 Years	
		2. 36 – 55 Years	
		3. 56 – 70 Years	
		6. Above 70	
2.	Sex	Male	
		Female	
3.	Marital status	Single	
		Married	
		Divorced	
		Widow	
4.	Level of education	No formal education	
		Primary	
		Secondary	
		College or university	
		Others (specify)	
5.	What is your main occupation?	Livestock keeping	
		Farming and livestock keeping	
		Employed and livestock keeping	
		Others (specify)	
6.	For how long have you been involved in livestock keeping?	1.Less than 5 Years	
		2.Between 5 to 10 Years	
		3. More than 10 ten years	
B. FEEDING SYSTEM/PRACTICES			
7.	What is your role in cattle management? (Multiple options)	1 Person looking after cattle	
		2.Owner of cattle	
		3.Occasionally look after cattle	
		4.Am not involved in cattle management	
8.	How many cattle do you keep?		
9.	How many milking cows do you keep?		
10.	How do you keep your livestock?	1. Free ranging	
		2.Zero grazing	
		3.Grazing and supplementation	
		4.Others, please explain	
11.	Why do you use such a feeding method? Explain		
12.	What types of livestock feed do you use? (Select all applicable)	1.Roughages	
		2.Concentrates	
		Both roughages and concentrates	
		Others, please explain	

17.	Do you experience any challenge(s) in getting adequate feeds for your animals?	1.Yes	
		2.No	
18	If Yes, what are the challenges? Explain		
C. FEED HANDLING AND STORAGE PRACTICES			
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 (specify)	
20.	Do you measure moisture content after drying?	1.No moisture measurements	
		2. Moisture meter	
		3.Others (specify)	
21.	How long do you store the livestock feeds?	Less than six months	
		More than six months but less than one year	
		More than one year	
22.	How do you store the livestock feeds?	1. Woven/sisal bag (gunia)	
		2.Polyethyne bag (mifuko ya salfeti)	
		3. Barrel/Drum (pipa)	
		4.Plastic bucket	
		5. Heap on the ground	
		6.Others (specify)	
23.	How do you prepare the concentrates for your livestock		
D. AFLATOXIN AWARENESS			
24.	Have you ever heard the word aflatoxin?	1. Yes	
		2. No	
25.	Where did you hear it from?	1.Village meeting/ extension officers	
		2. Newspaper	
		3.Seminar	
		4.Radio/Tv	
		5.Friend	
		6.School	
		7.Others (specify)	
26.	Do you know that aflatoxins can contaminate livestock feeds?	1. Yes	
		2. No	
27.	Can you recognise aflatoxin contaminated feeds	1.Yes	
		2. No	
28.	If, yes, how? Please, specify		
29.		1.Yes	

	Do you know that aflatoxin can cause liver cancer in both human and livestock?	2.No	
30.	Do you know that aflatoxins can reduce livestock growth and milk yields?	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 the causes of aflatoxin contamination?	1. Yes	
		2. No	
33	If Yes, mention them,	1.	
		2.	
		3.	
		4.	
		5.	
34.	Do you know any control measure for aflatoxin contamination of livestock feeds?	1.Yes	
		2.No	
35.	If yes, which control measures do you apply?		
36.	Have you ever received any training on aflatoxins?	1.Yes	
		2.No	
37.	What was the training about?	1.Good storage practices	
		2.General awareness about aflatoxins	
		3.Use of new storage techniques (e.g., hermetic storage)	
		4.GAP	
		5.Others (specify)	

Appendix 3: Questionnaire for Agro Vet Dealers

OCCURRENCE OF AFLATOXINS AND ASSOCIATED RISK FACTORS DAIRY VALUE CHAIN IN THREE AGRO-ECOLOGICAL ZONES OF TANZANIA

Study Participant Questionnaire (Agrovet dealers)

GENERAL INFORMATION			
District:	Ward:	Village:	Code:
Household No:		Participant Code:	GPS Code:
Date of visit:			

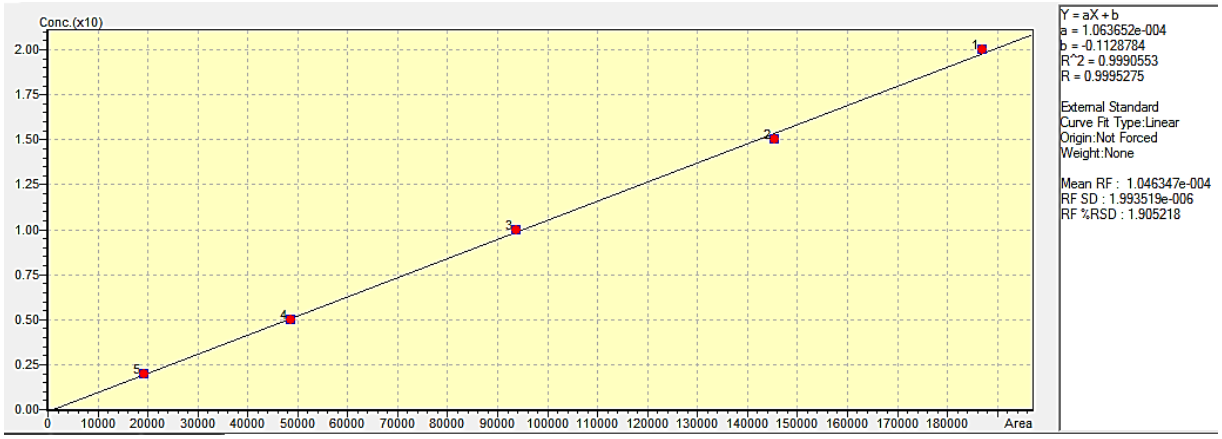
S/N	Questions/Item	Choices	Response
1.	Age of respondent	1. 18 – 35 Years	
		2. 36 – 55 Years	
		3. 56 – 70 Years	
		4. Above 70	
2.	Sex	1.Male	
		2.Female	
3.	Marital status	1.Single	
		2.Married	
		3.Divorced	
		4.Widow	
4.	Level of education	1.No formal education	
		2.Primary	
		3.Secondary	
		4.College or university	
		5.Others (specify)	
5.	What is your main occupation?	1.Employed	
		2.Agrovet dealer	
		3. Employed and agrovet dealer	
		4.Others (specify)	
6.	For how long have you been involved as livestock feed dealer?	1.Les than 5 years	
		2.For 5 to 10 Year	
		3.For more than 10 years	
B. MANUFACTURING, HANDLING AND STORAGE PRACTICES			
7.	Which animal feeds do you sell?	1.	
		2.	
		3.	
		4.	
		5.	
8.	How long you store the livestock feeds?	1.Less than six months	
		2.More than six months but less than one year	
		3.More than one year	
9.	How do you store the livestock feeds?	1.On the ground	
		2.Pallets	
		3.Others specify	
10.	How often do you clean your feed store?	1. Everyday	
		2. Before storing new batch	
		3. Rarely I do clean	
11.	How do you pack the livestock feeds?	1. Sack bag (gunia)	
		2. Plastic gunny bag (mifuko ya salfeti)	
		3. Barrel/Drum (pipa)	

		4. Open space on the ground	
		5.Hermatic bags	
		6. Others specify	
12.	Are you re-using the packaging materials?	1. Yes	
		2. No	
13.	Are you a certified livestock feed dealer/manufacturer?	1. Yes	
		2. No	
14	Have you ever inspected by regulatory authorities? monitoring agencies visit your place?		
15	How often quality monitoring agencies visit your place?	1.Twice per year	
		2.Once per year	
		3.Others specify	
C. AFLATOXIN AWARENESS			
16	Have you ever heard the word aflatoxin?	1. Yes	
		2. No	
17	Where did you hear it from?	1.Village meeting/ extension officers	
		2. Newspaper	
		3.Seminar	
		4.Radio/Tv	
		5.Friend	
		6.School	
		7.Others (specify)	
18	Do you know that aflatoxins can contaminate livestock feeds?	1. Yes	
		2. No	
19.	Can you recognise aflatoxin contaminated feeds	1.Yes	
		2. No	
20.	If, yes, how? Please, specify		
21.	Do you know that aflatoxin can cause liver cancer in both human and livestock?	1.Yes	
		2.No	
22.	Do you know that aflatoxins can reduce livestock growth and milk yields?	1. Yes	
		2. No	
23.	Do you know that aflatoxin can be transferred from contaminated feeds consumed by your livestock to milk?	1. Yes	
		2. No	
24.	Do you know the causes of aflatoxin contamination??	1. Yes	
		2. No	

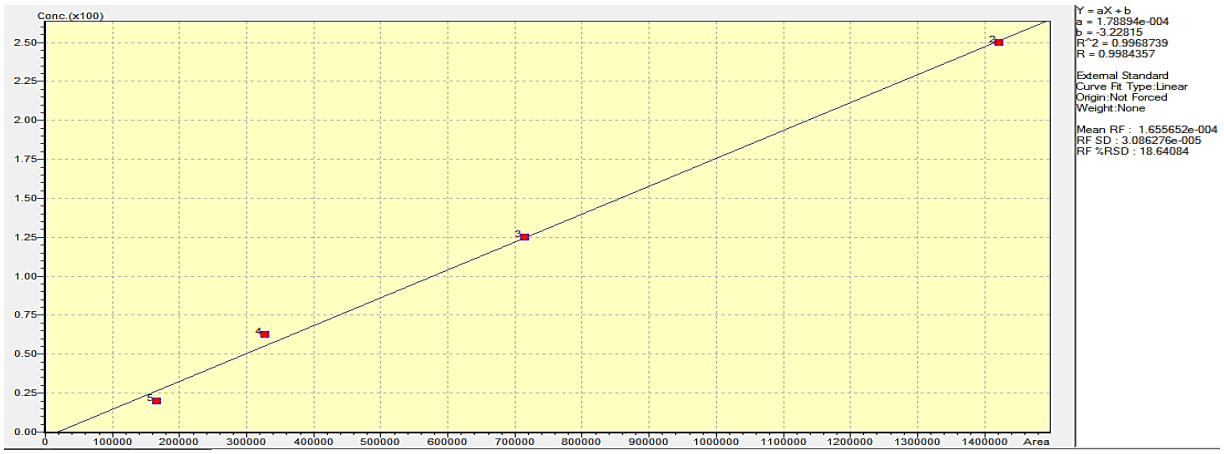
25.	If Yes, mention them,	1.	
		2.	
		3.	
		4.	
		5.	
26.	Do you know any control measure for aflatoxin contamination of livestock feeds?	1.Yes	
		2.No	
27.	If yes, which control measures do you apply?		
28.	Have you ever received any training on aflatoxins?	1.Yes	
		2.No	
29.	What was the training about	1.General awareness of aflatoxin problem Proper storage practise	
		2.Use of new storage technique (hermatic storage)	
		3.GAP	
		4.Others (specify)	

Appendix 4: Validation of analytical method

(a)

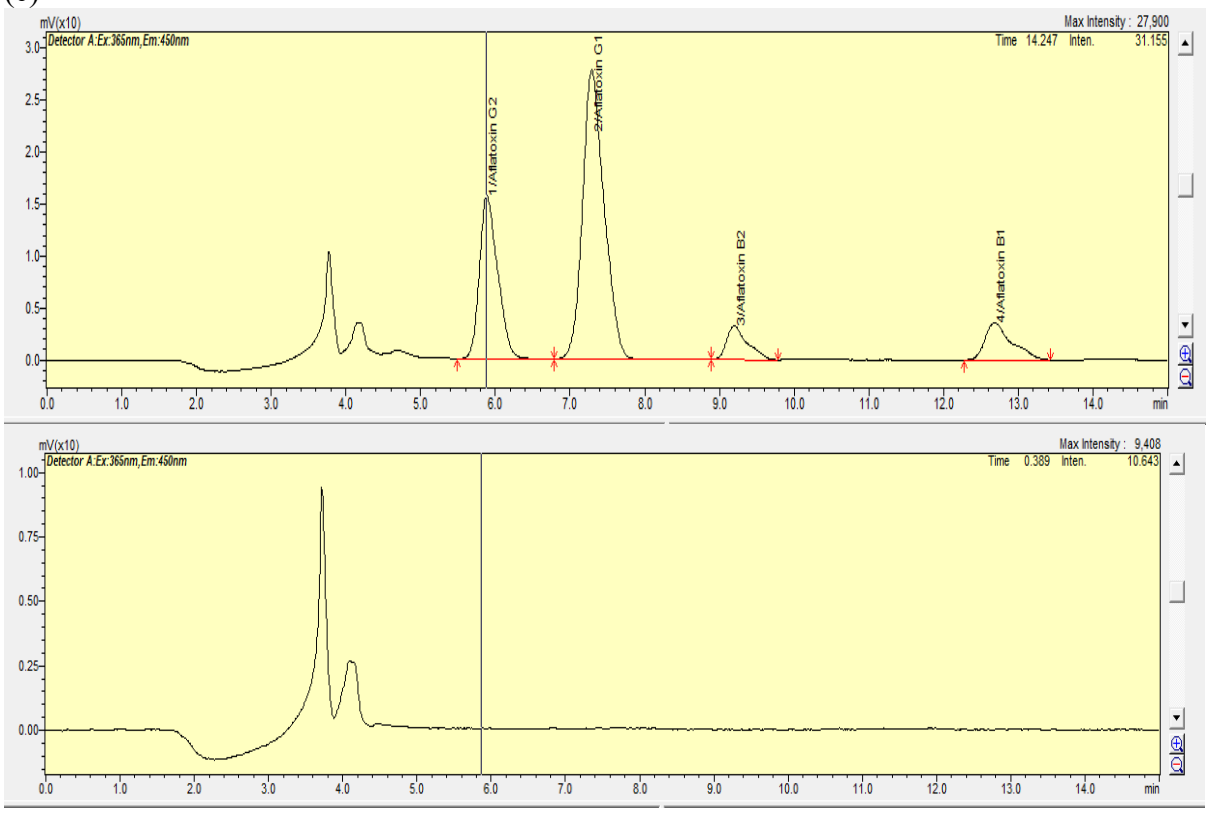


(b)



Calibration curve of AFB_1 (a) and AFM_1 (b)

(c)



Chromatogram shows no interfering peaks in the retention time of each target analyte (c)

RESEARCH OUTPUTS

(i) Publication

Kitigwa, S. J., Kimaro, E. G., Nagagi, Y. P., Kussaga, J. B., Suleiman, R. A., & Matemu, A. (2023). Occurrence and associated risk factors of aflatoxin contamination in animal feeds and raw milk from three agroecological zones of Tanzania. *World Mycotoxin Journal,* 16(2), 149-163.

(ii) Poster Presentation