Aflatoxin Contamination in Animal Feeds and Fresh Milk in Kondoa District, Tanzania

Abstract:
The occurrence of aflatoxins in animal feeds and milk is common around the World as most livestock keepers are not aware of aflatoxins.
Aims: This study was conducted to assess storage and feeding practices, and assessment of aflatoxin B1 and M1 contamination in animal feeds and fresh milk samples in Kondoa.
Study design: The study involved a survey using semi-structured questionnaires and Laboratory analysis of aflatoxin B1 and aflatoxin M1in animal feeds and fresh milk samples collected from among smallholder dairy farmers.
Methodology: Purposive selection of120 respondents among smallholder dairy farmers in six wards purposively selected due to having larger number of dairy cattle was conducted. Likewise, 20 samples of animal feeds and 25 composite milk samples from among 120 interviewed farmers were analysed for aflatoxin B1 and M1 using High-Performance Liquid Chromatography (HPLC).
Results: The results indicates that, aflatoxin B1 in feeds was 55%, with mean concentration of 8.6 ±5.9 µg/kgranging from the lowest limit of detection (LOD) to 21.1µg/kg with 5% samples above the maximum level stipulated by the Tanzania Bureau of Standards (TBS) and European Union (EU) of 20 µg/kg. Likewise, about 44% of the milk samples revealed to contain traces of aflatoxin M1 with a mean amount of 0.07 ±0.08 µg/L, ranging from the limit of detection (<LOD) to 0.23 µg/L, and 12% exceeding the TBS and EU standards of 0.05 µg/kg.
Conclusion: There is need to scale up aflatoxin awareness campaigns and regular monitoring throughout the food system in Tanzania.

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

Graphical abstract:

KeyContribution:
The feed storage and feeding practices had low association to aflatoxin awareness. In other hand, the despite intervention to create awareness of aflatoxin among farmers, there is still aflatoxin detected in feeds and milk hence recommends monitoring of any strategy implemented to prevent aflatoxin contamination.

1.0 Introduction

Aflatoxins refer to the class of mycotoxins produced by *Aspergillus flavus* or *Aspergillus parasiticus.* Production of aflatoxins occurs when crops, specifically maize, or their processed by-products (maize bran) and groundnuts, are exposed to favorable environments of high temperature and moisture content. Other Aspergillus species, such as *A. nominus, A. ochraceoroseus, A. bombycis, A.*

fumigitus, and A. pseudotamari, are said to be little-identified causes of aflatoxin[1]. About 20 different types of aflatoxins have been identified in different studies. However, the most common types of aflatoxins are Aflatoxins B1, B2, G1, and G2. The metabolic products after aflatoxin B1 and B2; aflatoxins M1 and M2, are commonly found in animal products such as eggs, meat, and milk when livestock consume aflatoxin-contaminated feeds. Aflatoxin B1 (AFB1) and its metabolite, aflatoxin M1 (AFM1), are the most potent of all aflatoxins and are classified as Type 1 carcinogenic by the International Agency for Research on Cancer (IARC) in humans, due to their ability to cause cancer.

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

The occurrence of AFB1 in animal feeds and AFM1 in milk has been reported in different parts of the world. However, aflatoxins are said to be highly prevalent in areas around latitudes 40° north and south of the equator. The study by [4] found that developing countries in the tropics are at higher risk of aflatoxin contamination than others due to the presence of favorable conditions of temperature and humidity that lead to aflatoxin production. Therefore, milk is likely to be contaminated with aflatoxins as a result of livestock consuming AFB1-contaminated feeds. Milk acts as a good source of AFM1 contamination when consumed directly or in dairy products. The study [5]found that people in urban and peri-urban areas consume twice the amount consumed by the rural population, thus making them more exposed to AFM1.

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

Aflatoxin levels in grain foods and feeds are subject to strict regulations in more than 60 countries worldwide. The Tanzania Bureau of Standards (TBS) has set the maximum tolerable level of aflatoxin B1 in foods as 5 µg/kg, while 10 µg/kg is the maximum level for total aflatoxins count [10]. However, AFB1 in feeds intended for dairy cattle is limited to 20 µg/kg, while AFM1 levels in milk are limited to 0.05 µg/L according to TBS, Codex standard, and United States Food and Drugs Authority (USFDA). Studies conducted in some parts of Tanzania including Dar es Salaam, Morogoro, and Singida revealed higher levels of AFM1 in milk ranging from the limit of detection (LOD) to 2.007 ng/ml, as compared to the maximum daily intake of 1 ng/kg body weight/day for an adult [11][12][13]. The current study was conducted to assess the contamination of AFB1 in animal feeds and the occurrence of AFM1 in fresh milk, and to recommend the safety status of feeds and milk in the Kondoa district and what should be done to minimize aflatoxin contamination throughout Tanzania in general.

2.0 Materials and Methods

2.1 Description of the study area

Kondoa is one of the districts in Dodoma region, currently divided into two administrative councils: the Kondoa town council (916.13 sqkm) and the Kondoa district council (4640.87 sqkm). It is located at latitude 0°40'54" South and longitude 350°47'0" East, approximately 169.9 km from Dodoma city, and bordered by Manyara region to the north, Chemba district to the south, Bahi district to the west, and Chamwino district to the east. The district's soils are of low fertility, base-exchange capacity, bulk density, and water-retention capacity. The vegetation consists of bushland with isolated bush grasslands. The climate is a wet savannah, characterized by a long dry season from late April to December and a short wet season from early December to April. Average rainfall ranges from 400

mm in the plateau to 1000 mm in the highlands. Major food crops grown include sorghum, millet, and maize, while major cash crops are maize, groundnuts, sunflower, castor seeds, and sesame.

2.2 Study site selection

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

2.3 Sample size

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

2.4 Study design

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

2.5 Methods

2.5.1 Assessment of storage and feeding practices among smallholder dairy farmers

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

2.5.2 Determination of aflatoxin B1 in animal feeds

A: Sample sources

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

B: Sample preparation

During laboratory analysis, 100 g of each feed sample was thoroughly ground, and then 25 g sampled from different sites within the bag was taken for AFB1 extraction. The samples were treated with a methanol-deionized water mixture prepared by mixing 5 g of sodium chloride and 300 ml of a methanol-water mixture at a ratio of 80:20 (v/v). The mixture was shaken for 30 minutes at 120 RPM to obtain a uniform mixture. The solution was filtered through filter paper, and 20 ml of the filtrate was diluted with 130 ml of deionized water. Then, 75 ml of the dilute sample extract was passed through the Aflatest 1AC column at a rate of 2-3 ml/min. The columns were washed with 15 ml of water. To elute AFB1 from the column, 0.5 ml of HPLC-grade solvent was added, followed by 0.75 ml of the same solvent one minute afterward. Finally, 3 ml of HPLC-grade water was added to the elevator, and 20 µL of the final solution was injected into the HPLC for aflatoxin B1 analysis following the procedures described by [15].

C: HPLC analysis

Aflatoxin quantities from the sample were determined using HPLC coupled with a fluorescence detector (FLD) (Model Agilent ChemStation Technology, Series 1260 INFINITY SERIES). The column C18 (4.6×150 mm, 5 µm), Eclipse XDB-C18, was used to separate groups of aflatoxins (AFB1, AFB2, AFG1 and AFG2). The column temperature was set at 30 °C, the flow rate at 1.0 mL/min, and the

mobile phase was Water: Methanol: Acetonitrile (60:30:10 v/v). The fluorescence was excited at a wavelength of 360 nm, and the emission wavelength was set at 460 nm. The retention for AFB1 took effect after 16-17 minutes.

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

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

2.5.3 Determination of aflatoxin M1 in fresh milk
A: Sample taking and preparation
One hundred and fifty milliliters (150 ml) of milk were collected in sterile condition from each cow, with a maximum of three samples collected from three cows in one household. The samples were immediately stored in an ice box to ensure cold-chain storage. All samples collected from one ward were stored in a deep freezer at -20°C until all samples from all villages within the ward had been collected. Upon completion of milk samples collection, the samples were packed into an ice box and transported to the Tanzania Bureau of Standards (TBS) laboratory in Dar-es-salaam, where they were kept frozen at -20°C before AFM1 analysis. After one month, the samples were removed from the freezer and prepared for AFM1 analysis.

B: Procedures
Milk samples were thawed in water at 45°C for five minutes. Milk samples from the same farmer were mixed to obtain composite samples. One hundred milliliters (100 ml) of each sample were placed into test tubes for cream separation by centrifugation at 4000 rpm. After cream separation, the milk samples were filtered to ensure that no cream remained. Then, 30 ml of skim milk was loaded into the tubes, which were finally connected to the aflatoxins column for extraction. The samples were allowed to pass through the column with an affinity to bind all aflatoxins present while milk passed down the vacuum manifold. The column was then rinsed with distilled water three times to ensure that no milk samples were left in the tube. Finally, the AFM1 particles bound in the column were eluted by passing pure methanol through the aflatoxins column down the microtubes. Ultrafiltration using a syringe connected to a microfilter was conducted to obtain the clearest supernatants ready to be injected into the HPLC machine for detection and quantification.

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

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

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

3.0 Results

3.1 Animal feed storage practices

Chi-square test results on farmers' routine practices and the association to the level of awareness of factors which may cause potential aflatoxin contamination during various feed practices during storage were as indicated in Table 1.

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

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

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

3.2 Feeding practices among smallholder dairy farmers

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

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

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

give them	1kg to 2kg	40(95.2)	2(4.8)
	3kg to 5kg	26(92.9)	2(7.1)
	None	40(80.0)	10(20.0)

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

3.3 Aflatoxin levels in animal feeds and fresh milk

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

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

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

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

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

Laboratory analysis			Sample Detection and Quantification				Descriptive statistics	
Aflatoxins (AF)	**Sample tested (n)**	**Limit of Detection (LOD) (µg/kg)**	**Limit of Quantification (LOQ) (µg/L)**	**Aflatoxin Positive sample (%)**	**Samples exceeded EU standards (%)**	**The sample exceeded Codex std (%)**	**Mean ±STD (µg/kg or L)**	**Range (µg/kg or L)**
AFB1	20	0.2	1.5	55	5	5	8.6 ±5.9	2.0 - 21.1
AFM1	25	0.005	0.01	44	12	0	0.07±0.08	0.01- 0.23

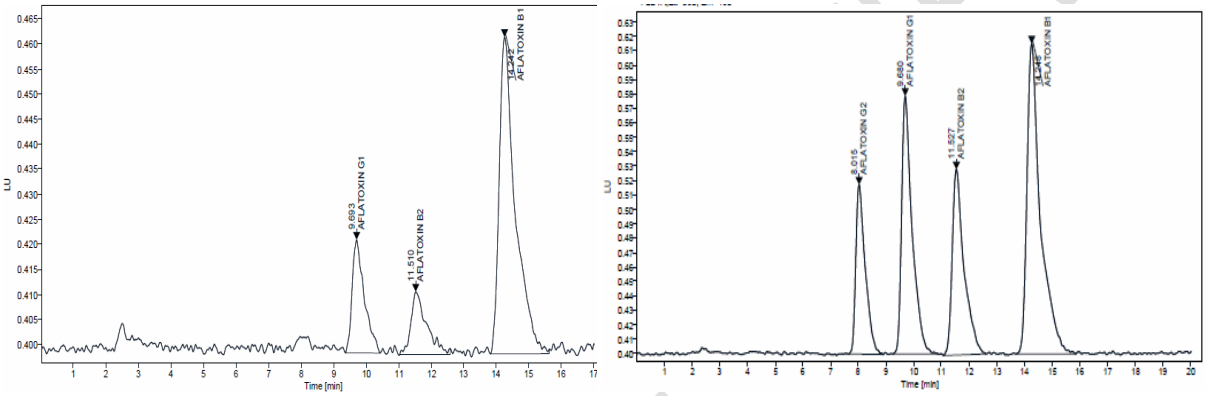

Figure 1: The elution pattern of the highest aflatoxin B1 in a feed sample (left) and a standard sample (right) at 360 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model.

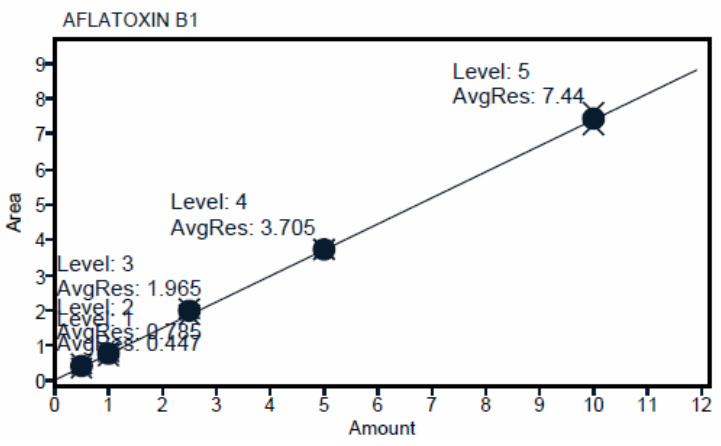

Figure 2: Calibration curve of aflatoxin B1 standard at 360 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model.

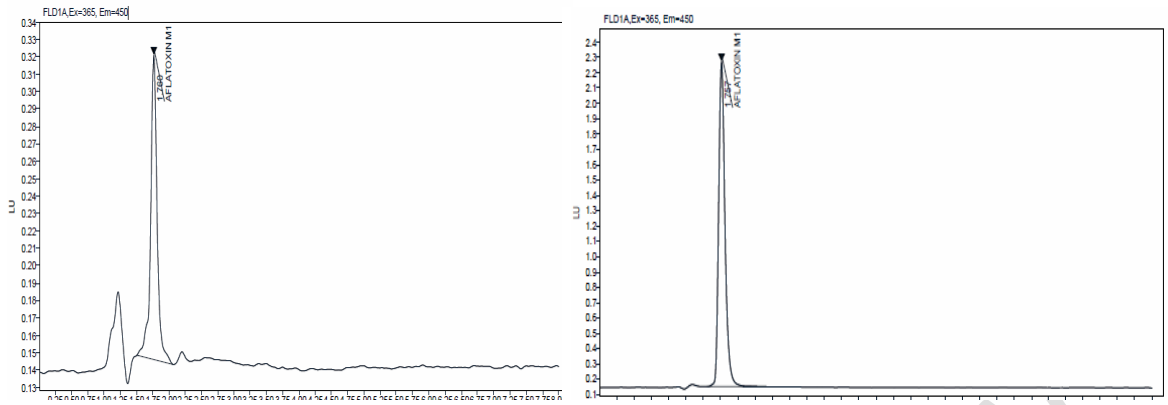

Figure 3: Elution pattern of the maximum concentration of aflatoxin M1 in milk sample (left) vs standard sample (right) at 365 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model.

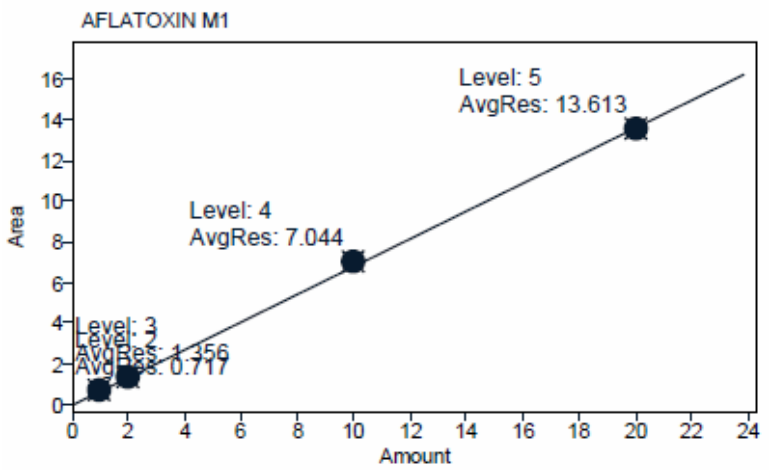

Figure 4. Calibration curve of aflatoxin M1 standard at 365 nm wavelength analyzed by Agilent ChemStation Technology, Series 1260 HPLC Model.

4. Discussion

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

Occurrence of Aflatoxin B1 contamination in animal feeds was detected in 55% of all samples collected with a mean amount of 8.6 ±5.9 µg/kgranging from the lowest limit of detection (LOD) to

21.1µg/kg. However, only 5% exceeded the recommended safe limit for dairy cow feeds of 20 µg/kg. The results reveal low contamination of aflatoxin B1 with lower mean concentration when compared to the studies conducted in most tropical African countries such as Tanzania, Rwanda, Uganda, and the Democratic Republic of Congo. Some Asian countries with higher contamination of AFB1 include Iran, Pakistan, and India in regions where farmers had little, or no knowledge of aflatoxin. These results build on the existing evidence that, when smallholder dairy farmers have knowledge of aflatoxins they are more willing to participate in the reduction and or prevention of aflatoxin contamination in food systems [18]. The findings are in line with [19]who postulated that a lack of awareness among farmers increased the risks of aflatoxins to both humans and livestock.

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

The successful prevention of aflatoxin in food systems hasspreading positive effects to the stakeholders along the dairy value chain.Some of these benefits include an increased growth rate of livestock, improved productivity, an improved immune system, and reduced veterinary costs [22]. Likewise, achieving the prevention of aflatoxin removes restrictions for international trade, specifically the exportation of animal feeds, ingredients for animal feed formulation, and processed dairy products. This increases the livelihood of smallholder dairy farmers and the contribution of the livestock sector to the Gross Domestic Product (GDP). The prevention of aflatoxin in the food system also provides consumer health protection against liver cancer, hepatic diseases, and stunted growth. The study by [23]concluded that the prevention of aflatoxin contamination enhances food security, alleviates malnutrition, and strengthens economic sustainability.

5. Conclusion and recommendation

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

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

Ethical Approval:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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

Data Availability Statement: Data generated in this study is open accessed through the online link at https://www./ethics.

References

[1] A. Jallow, H. Xie, X. Tang, Z. Qi, and P. Li, "Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control," *Compr. Rev. Food Sci. Food Saf.*, vol. 20, no. 3, pp. 2332–2381, 2021, doi: 10.1111/1541-4337.12734.

[2] H. Beitollahi *et al.*, "Recent advances in the aptamer-based electrochemical biosensors for detecting aflatoxin b1 and its pertinent metabolite aflatoxin m1," *Sensors (Switzerland)*, vol. 20, no. 11, pp. 1–17, 2020, doi: 10.3390/s20113256.

[3] G. Ilievska, B. Stojanovska-Dimzoska, D. Koceva, G. Stojković, A. Angeleska, and E. Dimitrieska-Stojković, "Dietary Exposure and Health Risk Assessment of Aflatoxin M1 in Dairy Products Consumed by Population of North Macedonia," *J. Food Qual. Hazards Control*, vol. 9, no. 1, pp. 14–22, 2022, doi: 10.18502/jfqhc.9.1.9686.

[4] H. Strosnider *et al.*, "Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries," *Environ. Health Perspect.*, vol. 114, no. 12, pp. 1898–1903, 2006, doi: 10.1289/ehp.9302.

[5] I. Kagera *et al.*, "Status of aflatoxin contamination in cow milk produced in smallholder dairy farms in urban and peri-urban areas of Nairobi County: a case study of Kasarani sub county, Kenya," *Infect. Ecol. Epidemiol.*, vol. 9, no. 1, Jan. 2019, doi: 10.1080/20008686.2018.1547095.

[6] L. Min *et al.*, "An overview of aflatoxin B1 biotransformation and aflatoxin M1 secretion in lactating dairy cows," *Anim. Nutr.*, vol. 7, no. 1, pp. 42–48, 2021, doi: 10.1016/j.aninu.2020.11.002.

[7] M. Gashaw, "Review on Mycotoxins in Feeds: Implications to Livestock and human health," *E3 J. Agric. Res. Dev.*, vol. 5, no. 3, pp. 137–0144, 2015, [Online]. Available: http://www.e3journals.org

[8] T. N. Kiama *et al.*, "Kenya dairy farmer perception of moulds and mycotoxins and implications for exposure to aflatoxins: A gendered analysis," *African J. Food, Agric. Nutr. Dev.*, vol. 16, no. 3, pp. 11106–11125, 2016, doi: 10.18697/ajfand.75.ILRI10.

[9] S. Yin, L. Niu, and Y. Liu, "Recent Progress on Techniques in the Detection of Aflatoxin B1 in Edible Oil: A Mini Review," *Molecules*, vol. 27, no. 19, pp. 1–35, 2022, doi: 10.3390/molecules27196141.

[10] A. Kamala *et al.*, "Local post-harvest practices associated with aflatoxin and fumonisin contamination of maize in three agro ecological zones of Tanzania," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 33, no. 3, pp. 551–559, 2016, doi: 10.1080/19440049.2016.1138546.

[11] S. Mohammedi-Ameur, M. Dahmane, C. Brera, M. Kardjadj, and M. H. Ben-Mahdi, "Occurrence and seasonal variation of aflatoxin M1 in raw cow milk collected from different regions of Algeria," *Vet. World*, vol. 13, no. 3, pp. 433–439, Mar. 2020, doi: 10.14202/vetworld.2020.433-439.

[12] A. W. Mwakosya, S. M. Limbu, N. Majaliwa, X. Zou, J. Shi, and O. Kibazohi, "Aflatoxin B1 variations in animal feeds along the supply chain in Tanzania and its possible reduction by

heat treatment," *Food Agric. Immunol.*, vol. 33, no. 1, pp. 192–206, 2022, doi: 10.1080/09540105.2022.2045908.

[13] S. J. Kitigwa, E. G. Kimaro, Y. P. Nagagi, J. B. Kussaga, R. A. Suleiman, and A. Matemu, "Occurrence and associated risk factors of aflatoxin contamination in animal feeds and raw milk from three agroecological zones of Tanzania," *World Mycotoxin J.*, vol. 16, no. 2, pp. 149–163, May 2023, doi: 10.3920/wmj2022.2801.

[14] * Kang'ethe Ek and Lang', "Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya," 2009.

[15] A. Vaz, A. C. C. Silva, and P. Rodrigues, "Detection Methods for Aflatoxin M1 in Dairy Products," pp. 1–16, 2020.

[16] M. U. Beg, K. R. Beg, L. N. Ali, and T. Saeed, "Mycotoxins in Poultry Feed in Kuwait," vol. 602, no. April 2002, pp. 594–602, 2006, doi: 10.1007/s00244-005-2094-0.

[17] P. Jolly *et al.*, "Determinants of aflatoxin levels in Ghanaians : Sociodemographic factors , knowledge of aflatoxin and food handling and consumption practices," vol. 209, pp. 345–358, 2006, doi: 10.1016/j.ijheh.2006.02.002.

[18] E. M. Ayo, A. Matemu, G. H. Laswai, and M. E. Kimanya, "Socioeconomic Characteristics Influencing Level of Awareness of Aflatoxin Contamination of Feeds among Livestock Farmers in Meru District of Tanzania," *Scientifica (Cairo).*, vol. 2018, 2018, doi: 10.1155/2018/3485967.

[19] D. Negash, "A Review of Aflatoxin: Occurrence, Prevention, and Gapsin Both Food and Feed Safety," *J. Appl. Microbiol. Res.*, vol. 1, no. 1, pp. 1–35, 2018.

[20] J. F. Lindahl, I. N. Kagera, and D. Grace, "Aflatoxin M1 levels in different marketed milk products in Nairobi, Kenya," *Mycotoxin Res.*, vol. 34, no. 4, pp. 289–295, Nov. 2018, doi: 10.1007/s12550-018-0323-4.

[21] S. Marchese, A. Polo, A. Ariano, S. Velotto, and S. Costantini, "Involvement in Cancer Development," pp. 1–19, 2018, doi: 10.3390/toxins10060214.

[22] A. Kebede, "Review on Aflatoxin and its Impacts on Livestock," *J. Dairy Vet. Sci.*, vol. 6, no. 2, pp. 1–7, 2018, doi: 10.19080/jdvs.2018.06.555685.

[23] P. Udomkun, A. N. Wiredu, M. Nagle, J. Müller, B. Vanlauwe, and R. Bandyopadhyay, "Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application – A review," *Food Control*, vol. 76, pp. 127–138, 2017, doi: 10.1016/j.foodcont.2017.01.008.