

Aflatoxin Contamination of Cattle's Concentrate Feeds; A Case Study in Dar Es Salaam-tanzania

Sahan Jonathan

Ministry of Agriculture, Food Security and Cooperatives

Irene Nalumansi

Mbarara University of Science and Technology

Grace Birungi (✉ gbirungi@must.ac.ug)

Mbarara University of Science and Technology

Research Article

Keywords: Aflatoxin, Aflatoxin contamination, Dar es Salaam, Tanzania, concentrate feeds. Maximum Limit

Posted Date: July 8th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1768004/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

Aflatoxicosis associated with the consumption of aflatoxin contaminated maize and maize products has been reported in the central part of Tanzania (Chemba, Kondo, and Kiteto), however little is known about the prevalence of aflatoxin contamination in cattle's concentrate feed, yet maize constitutes a major component in the feed. The presence of aflatoxin in cattle's concentrate feed lead to reduced growth rate, and milk production and results in a carryover effect on human being resulting in human health effect. This study aimed at determining the prevalence of aflatoxins contamination, the level of awareness, knowledge, and practices associated with aflatoxin contamination, and control in cattle concentrate feeds produced and sold in Dar es Salaam, Tanzania.

Method

Eighty one samples of concentrate feed were collected from concentrate feed processors and sellers in four clusters Ilala, Kinondoni, Temeke, and Ubungu. The samples were analyzed by Enzyme linked Immuno-sorbent Assay and High-Performance Liquid Chromatography-Fluorescent Detector. The level of contamination was compared to the WHO/EAC/TBS recommended limit. Also eighty one concentrate processors and sellers were interviewed to assess the level of awareness, knowledge, and human practices associated with aflatoxin contamination and control.

Results

Sixty three 63/81 (78%) samples were contaminated with total aflatoxin (TAFs) ranging from LOD to 161.32 ppb, mean of 25.89 ± 3.3 ppb which was significantly higher than the tolerable WHO/TBS limit of 15ppb total aflatoxins ($P = 0.002$), Level of AFB1 in concentrate feed was ranged from LOD to 133.17 ppb, mean 18.87 ± 2.45 ppb and was significantly higher than the tolerable WHO/TBS limit 10 ppb for AFB1 ($P = 0.001$). The level of AFB2 was ranged from LOD to 8.21 ppb, mean 1.82 ± 0.27 ppb, AFG1 (LOD to 75.06 ppb, mean 7.75 ± 2.32 ppb) and AFG2 (LOD to 7.87 ppb, mean, 1.30 ± 0.350 ppb), among aflatoxin the level of contamination was $AFB1 > AFG1 > AFB2 > AFG2$, however, AFB2 and AFG2 had significant same level of contamination ($p = 0.518$). The samples collected from Temeke and Ilala were significant highly contaminated with aflatoxin than the WHO/TBS recommended limit (15 ppb), with TAFs of 33.63 ± 3.96 ppb, p -value = 0.00015, 30.78 ± 7.05 ppb, p -value = 0.034 respectively, while those from Kinondoni and Ubungu were not significantly higher than the recommended standard 15ppb (p -value, 0.71, 0.742) respectively. The level of awareness was 38% and not significant ($p = 0.353$) and 58/81 (72%) of respondents were not aware of the negative health effect of aflatoxins. The poor storage facility, poor handling practices, temperature, humidity and lack of awareness were the predominant factors identified to contribute a high level of aflatoxin contamination.

Conclusions

The results indicated that the concentrate feeds had aflatoxin concentrations which may pose risk to animals and/or human beings, economic losses due to increased cost of treatment and reduced cattle production. The use of improved pre and post-harvest management technologies, and awareness creation to animal feed value chain actors are important steps in the reduction of this problem.

1 Introduction

Aflatoxins are classified as naturally occurring mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasticus*, mainly affecting corn and legumes. They were first detected in 1960 after occurrences of turkey x disease in England which was later confirmed to be caused by *Aspergillus flavus* (William et al, 2004). Aflatoxin is produced under favorable temperature (20 to 41 degrees Celsius) and humidity (60–90%) conditions and affects maize, rice, figs, dried food, spices, crude vegetables oil, tree nuts like almonds, walnuts, before and after harvest (Hendrich, 2017). There are four aflatoxin compounds (aflatoxin B1, aflatoxin B2, Aflatoxin G1, and Aflatoxin G2) differentiated by the colour of their fluorescence under long wave, ultraviolet illumination (B = blue, G = green). The other aflatoxins M1 and M2 are mammalian metabolites of AFB1 and AFB2 and can be isolated from urine and milk. (Patterson et al., 1978), the structure of aflatoxins shown in Fig. 1–1.

Dietary exposure to aflatoxins (AFB1 and a mixture of B1, G1, and M1) has been associated with hepatocellular carcinoma in humans worldwide (Ferlay et al. 2004), hence their presence in food such as cereals is of concern.

Cereals are the most consumed food worldwide. In the year 2020–2021, the world cereal consumption was 2735 million tons of which 172 million tons was from developing countries. However, consumption pattern has been changing due to change in the meal menu, especially in developed countries, this includes an increase in the use of maize for animal consumption. (Olugbire. Et al., 2021). Among the cereals, maize is the most grown and consumed in Tanzania but is frequently susceptible to aflatoxin contamination (Massomo, 2020) and fumonin contamination (Bankole et al, 2003). Aflatoxin contamination in maize has been reported by several studies in concentrations exceeding WHO recommendations (Kimanya et al, 2008; Kimanya et al 2017; Kuhumba et al, 2018; Mtega et al 2020; Boni et al, 2021; Temba et, al 2021) although some were within the East African Community (EAC) standard of 10 µg/kg for total aflatoxin (Nyagi et al, 2013).

A high level of aflatoxin contamination was reported in the Arusha region ranging from 10.3 to 40.3 µg/kg (Kuhumba et al, 2018). In 2019, Aflatoxin contamination was reported in maize and groundnut samples collected from the central part of Tanzania ranging from LOD up to 198 µg/kg (mean = 25.46 µg/kg), all were above the maximum limit of 10 µg/kg tolerated in foods for human consumption in Tanzania (Magoke et al., 2019). Seetha et al, 2017, revealed a level of aflatoxin contamination in freshly harvested maize samples was 18.8 µg/kg while in stored maize was 57.2 µg/kg indicating a high exposure risk. Also Kimanya et al, 2008 revealed that 18% of the maize samples were contaminated at levels from LOD up to 158 µg kg⁻¹ (median = 24 µg kg⁻¹). Kamala et al., 2015, detected high levels of AFB₁ ranging from 3 to 1,081 µg kg⁻¹, Fumonisin B1 (FB1) ranging from 16 to 18,184 µg kg⁻¹, Fumonisin B2 (FB₂) ranging from 178 to 38,217 µg kg⁻¹ and Deoxynivalenol (DON) ranging from 68 to 2,196 µg kg⁻¹. All this research was conducted on food ready for human consumption, to date less is known about the extent of the problem caused by consumption of that food.

Nyagi et al, 2013, determined Aflatoxin contamination in maize, their results indicated mean aflatoxin of 2.94 µg/kg and all samples (n = 440) were within the East African Community (EAC) standard of 10 µg/kg for total aflatoxin, but the mean fumonisin was 5.15 mg/kg, more than double the EAC standard of 2 mg/kg, and 35% of samples exceeded this standard. The occurrence of aflatoxins in maize was also reported in Kongwa and Njombe districts, aflatoxin contamination ranged from 2.99 ± 1.43 µg/kg and 9.99 ± 0.14 for Njombe districts and 2.87 ± 0.02 µg/kg to 10.26 ± 0.46 µg/kg, Kongwa district (Mtega et al 2020). A prolonged consumption of a small dose of aflatoxin also results in a long term effect, the low level of contamination does not indicate the suitability of that food as long as it can be consumed for a long period of time.

Several studies also conducted to assess knowledge, practices and public awareness on aflatoxins issues in Tanzania showed a low level of awareness in Kongwa, Mlangwa, Manungu, Bukombe, Njombe (Magembe et al, 2010; Nyagi et al, 2013; Kimanya et al, 2016; Fundikira, 2017; Mahamudu Mohamed, 2017; Massomo et al, 2020). It was, therefore, important to determine whether the status had changed moreover information regarding aflatoxin contamination in concentrate feeds was limited.

According to Tanzania Livestock Modernization Initiative (TLMI., 2015), about 50% of all Tanzanians household keep livestock out of which 23% are involved in urban farming. The increase of urban farming increases the demand for commercial animal feed (Tanzania Livestock Master Plan, 2017/2018–2021/2022). In Tanzania, roughages and concentrates including cereal grains and related by-products, agro-industrial by-products, brewer's waste, plant protein seeds, seed cake, and animal proteins are the major animal feed resources available (Mbwambo et al., 2016), but they are susceptible to aflatoxin contamination.

Consumption of contaminated animal feed by livestock not only have the risk of contaminating human food but also affects the livestock value chain, by lowering productivity and increasing mortality rates (PACA. 2017). Limited studies on aflatoxin contamination in animal feed have been done in Tanzania. Aflatoxin contamination in animal feed collected from sunflower agro-processors in Tanzania was reported to be contaminated with aflatoxin ranging from 1.9 to 97.9 ng/g (Mnyongoyo et al, 2017), also sunflower seedcakes or sunflower-based seedcake feeds was contaminated with AFB1 ranging from LOD to 20.47 ng/g, (Mohammed et al, 2016). Kajuna et al, 2013 also reported aflatoxin B1 contamination in broiler mash (35.8 ppb), layers mash (15.1 ppb), maize bran (9.4 ppb), and sunflower seeds (31.6 ppb). The maximum tolerable limit for cattle's or dairy concentrate feeds/compound feed is 15ppb (Tanzania bureau of standards, 2004). Animal feed quality and safety including aflatoxin contamination in concentrate feed is still a big concern.

The present work intended to reveal the prevalence of aflatoxin contamination in cattle concentrate feeds processed and sold in Dar es Salaam regions where the concentrate feeds are in high demand due to increased urban cattle farming. Knowledge, awareness, and practices related to aflatoxin contamination and control were also assessed.

2 Material And Methods

2.1 Research Design

Qualitative and quantitative methodologies were used during this study. Questionnaires were distributed to respondents (concentrate feed processors and sellers/agro-dealers) to accurately gather information regarding

public awareness on the issues related to aflatoxin contamination and control. The experimental part involved (i) solvent extraction of aflatoxin from concentrate feeds (ii) purification (iii) analysis of aflatoxin using HPLC-FLD and ELISA kit. The data from questionnaires and experimental part were analyzed using SPSS version 23.

2.2 Materials

Methanol (99.8%, LOBA CHEMIE PVT.LTD), sodium chloride (99.0%, MERCK KGaA), Romer test kit for Aflatoxin (AFB1, AFB2, AFG1 and AFG2, Romer Labs Division Holding GmbH), acetonitrile 99.9% (HPLC gradient grade, LOBA CHEMIE PVT.LTD), Distilled water (HPLC gradient grade, SIGMA-ALDRICH), Methanol (Multisolvent HPLC grade, ACS ISO UV-VIS K.F, Scharlau ME0315).

High performance liquid chromatography-fluorescence detector (HPLC-1260 series Agilent Technology) for quantification of aflatoxin. Enzyme Linked Immuno Sorbent Assay kit-ELISA kit (Celer AFLA). - version no.: AVK 2.2.7.011), manufacturer (Avishkar international Pvt.Ltd) for preliminary detection of aflatoxin.

The chromatographic conditions were column temperature 30⁰ C, flow rate 1.2 mL/min, injection volume 10 µL, detector-FLD, emission 450 nm, and excitations 360 nm. The mobile phase, water: Methanol: acetonitrile (60%: 30%: 10%), C-18 column (ZORBAX RX-C18, 4.6*250mm)

2.3 Study Area

Concentrate feeds were collected from 4 clusters in Dar es Salaam 6.7924⁰S, 39.20830E (Ilala 6.9276⁰S, 39.1336⁰E, Temeke 6.869⁰S, 39.261⁰E, Kinondoni 6.7980⁰S, 39.2660⁰E and Ubungo 6.470⁰S, 39.12⁰E) which are the main districts in the region as shown in Fig. 2 – 1

Dar es Salaam is 16 m above sea level, the average annual temperature is about 26.1⁰C, and about 1114 mm/43.9 inch of precipitation fall annually (climate data.org, 2021). Dar es salaam is the most populated city in Tanzania with over 7 million people and is marked as a business city, therefore due to this fact there is a high demand for cattle and milk for consumption. It has been estimated that there is a demand supply gap of about 12.5 million litres of milk per annum in Dar es Salaam (TDL/AGRIS, 1994), the local supply meets 1/3 of the demand, hence cattle's urban farming and cattle's feed processing are of high demand compared to other regions in Tanzania.

2.4 Qualitative Analysis

The information regarding public knowledge, awareness, and practices on aflatoxin contamination and control was collected using a questionnaire and observation method of data collection. The respondents were animal feed processor and agro-dealers where the concentrate feed samples were collected and hence, the sampling size were determined by using the formula explained in **section 2.5.1**. A total number of 81 respondents were interviewed. In this study, data privacy was highly maintained to maintain business integrity for agro-dealers, and agro-processors to their customers. The questionnaire tool was pre-tested on agro-dealers and agro-processors to limit and adjust any confusion or problems associated with the tool and adjust language so that it suits the respondents.

2.5 Quantitative determination of Aflatoxin contamination in cattle feed concentrates

2.5.1. Determination of Sample Size

The sample sizes for contaminated feeds from each cluster were calculated using the method by Daniel W.W., (1999), with a sample size calculator at 95% confidence level, 5% precision rate, 0.5% prevalence rate, 1 eligibility rate, 1 valid rate, and 0.9 response rate.

The sample size:-

$$((SS = \frac{A}{e^2}), (SS_{\text{final}} = SS_{\text{theoretical}} * \frac{1}{\% \text{response}} * \frac{1}{\% \text{eligibility}} * \frac{1}{\text{valid}}), (SS = \frac{ss_{\text{initial}}}{1 + \frac{ss_{\text{initial}} - 1}{pop}}))$$

Where $A, t^2 * \% \text{prevalence} * (1 - \% \text{prevalence})$; e, precision rate; SS, Sample size, SS_{initial}, Sample size initial; t, the coefficient for the confidential interval; pop, population; SS_{theoretical}, sample size initial; % response, response rate; % eligibility, eligibility rate.

2.5.2. Sample Collection

The concentrate feed samples, were collected from feed processors and agro-vet stores in each cluster (Ubungo, Kinondoni, Ilala, and Temeke) from June to September 2021, the sample (500 g) was collected from each sampling point. The sampling plan followed the sampling standard from CXS 193–1995(Rev. 2019) - General standard for contaminants and toxins in Food and Feed published by Codex Alimentarius Commission and EAS 900:2017, Cereals and pulses – sampling published by East African Community briefly described; in a consignment of < 20 bags of concentrate feed, the sample (25 g) was drawn from every bag. In a consignment of > 20 and < 100 bags, the sample (20 g) was drawn from 25 bags which were randomly selected for sampling. In a consignment of > 100 and < 1000 bags, a sample (10 g) was drawn from 55 bags which were randomly selected for sampling. A total of 81 samples (500g) were collected from the four clusters.

2.5.3. Sample Preservation

The concentrate feed samples were collected in paper bags to prevent fungal growth during shipment and were kept at 20 degrees Celsius temperature till further analysis following the method proposed by Richard et al., 1993. All equipment used during analysis were cleaned to minimize sample contamination as recommended in CXS 193–1995, last amendment-2019, General standards for contamination and toxins in food and feed.

2.5.4. Sample Analysis

Preliminary determination of aflatoxins contamination in concentrate feed was done using Enzyme-Linked Immunosorbent Assay (ELISA) and HPLC-FLD was used as confirmatory test methods of analysis following the method of Jian et al, (2016) as described below.

2.6. Detection of Aflatoxin contamination using Enzyme-linked immunosorbent assay (ELISA)

The assay was performed in plastic microwells that had been coated with an anti-aflatoxin antibody. The sample to be analyzed was carefully mixed to make it homogeneous, then finely ground. The sample (20 g) was transferred into the Erlenmeyer flask, followed by NaCl (4 g) and mixed with the extraction solvent (100

mL) made up of methanol-water (70:30), then shaken thoroughly for 3 minutes, and filtered in a filter paper (Whatman No. 01) (Richard et al., 1993).

In the premixing wells, the enzyme-labeled aflatoxin conjugate (100 μ L) and sample (50 μ L) were mixed and 100 μ L of solution were transferred into the anti-aflatoxin microtiter plate, then incubated for ten minutes at room temperature in the dark. During the incubation, free aflatoxin in the sample and enzyme-labeled aflatoxin compete for the anti-aflatoxin antibody binding site in the solid phase. At the end of incubation, the liquid was poured into a sink and the plate was washed three times with buffer solution, any unbound enzyme conjugate and aflatoxin molecules were then removed in a washing step. A developing solution (100 mL, chromogen substrate) was added to a plate, followed by the addition of chromogen substrate (25 mL). The enzyme converted the colorless chromogen into a blue product, and 50 mL of stop solution was added and mixed thoroughly with a rotary motion for 30 seconds, the addition of stop solution reagent leads to a color change from blue to yellow. The sample was taken immediately for measurement. The absorbance was measured with a microplate reader at 450 nm.

2.7. Quantification of Aflatoxin using HPLC-FLD: - Sample preparation

Four stages were involved during sample preparation. During the first stage, the concentrate feed sample (500g) was ground into fine powder. The ground sample (25g), was transferred into the Erlenmeyer flask. To this was added methanol: water (70:30) extraction solvent (100 mL), wrapped and covered with aluminum foil, and shaken using a gyratory shaker for 30 minutes at 250 rpm, then filtered into the sample container using filter paper (Whatman no 1). During the second stage, the extract (4 mL) was diluted with distilled water (8 mL), mixed into a Teflon tube, and vortexed for 1min.

In the third cleanup stage, the ends of the immunoaffinity column were removed, the column was attached to the closed adapter and then loaded with the diluted extract which was allowed to pass through the column by gravity. The column was rinsed with distilled water (10 mL) twice, and removed from the adapter. Finally, the bonded aflatoxin was eluted from the column with HPLC grade methanol (0.5 mL) three times. Slight pressure was applied on top of the column to remove any remaining liquid. The vial was vortexed for 30 seconds, from the vortexed vial, about 0.3 mL of eluate pipetted and mixed with distilled water (0.6 mL) and acetonitrile (0.1mL), the vial vortexed for 30 seconds.

2.8. Quantification of Aflatoxin using HPLC-FLD: - Separation and Detection

The eluate was injected in the reverse phase HPLC (1200 series Agilent technology using chemstation software) with a fluorescence detector (FLD) for aflatoxin detection. Isocratic mobile phase (water: methanol: acetonitrile 60: 30: 10), was used, 10 μ L of the sample was injected, The column used in separation of aflatoxin was C-18 column (ZORBAX RX-C18, 4.6*250mm) and separation was achieved at a column temperature of 30⁰ C and flow rate of 1.2 mL/min, The detection of aflatoxin was achieved with a fluorescence detector at an emission wavelength 465 nm and an excitation wavelength of 360 nm. Separated peaks were recognized and computed by comparing with the standards (Beg et al 2006), 10 ppb mixed standards of AFB1, AFB2, AFG1, and AFG2 were used.

2.9. Quality control

Method validation was carried out according to the guidelines set by the International Conference on Harmonization by using the mixed standard solutions and certified reference materials (CRM). The contamination level for the CRM was 100 ppb of total aflatoxin (G1, G2, B1, and B2). A standard solution of Aflatoxins (B1, B1, G1, and G2) at concentration levels between 0.1 to 10ppb was used to determine the linearity.

2.10. Statistical Analysis

Data obtained from aflatoxin quantification and awareness, knowledge and practices part was subjected to statistical analysis using SPSS version 23 (SPSS, Inc., Chicago, IL, USA). A Chi-square, non-parametric method was used to test the significance of public awareness, knowledge of aflatoxin and aflatoxin control, and practices that contribute to aflatoxin contamination. A one-sample T-test was used to compare aflatoxin concentration in concentrate feed sample with the recommended standards for total aflatoxin and AFB1. Descriptive statistics were used to calculate the mean and total aflatoxins. Correlational statistics were used to compare the results from two methods (ELISA and HPLC-FLD). Regression statistics were used to calculate the correlation factor during HPLC-FLD method validation. One-way ANOVA was used to compare means differences within the cluster if they were statistically significant.

3 Results And Discussion

3.1 Determination sample size

A total of 81 key actors (feed processor 48, and agro-dealer 33) were selected for sampling of concentrate feed and for interview from four cluster which are main districts in Dar es Salaam, Temeke Ubungu, Kinondoni and Ilala. Twenty (20) key actor which were supposed to be included in sampling and interview found producing and selling other types of animal feed which were not intended for the study as described in table **3-1**.

Table 3-1: *Sample size within cluster*

Cluster	Key holder	Population	Sample size (CI 95%, 0.05%)	Actual Concentrate feed sampled	Actual Respondents
Temeke	Feed processor	14	14	10	10
	Agro-dealers	15	15	10	10
Ubungo	Feed processor	16	15	14	14
	Agro-dealers	10	10	6	6
Kinondoni	Feed processor	8	8	8	8
	Agro-dealer	10	10	6	6
Ilala	Feed processor	10	10	10	10
	Agro-dealers	20	19	17	17
Total		103	101	81	81

3.2 Method Validation

Different concentration of individual standards was used to determine linearity, the calibration curve was obtained by comparing peak area and levels of concentration and the results show that the external standard calibration can be applied for the quantitative purpose. The coefficients of correlations (r^2) were all greater than 0.999. Limit of quantification and limit of detection evaluated and the results indicated in table 3.2.

Table 3-2: Method validation

No	Linearity results			LOD	LOQ	% recovery
	I. Stds	Range (ppb)	(R^2)	Ppb	Ppb	
1	AFB1	0.25 to 15	0.99938	0.01	0.02	70-100
2	AFB2	0.25 to 15	0.99997	0.02	0.05	70-100
3	AFG1	0.25 to 15	0.99983	0.01	0.04	70-100
4	AFG2	0.25 to 15	0.99994	0.02	0.05	70-100
5	CRM	100	-	-	-	80-110

AFB1, Aflatoxin B1; AFB2, Aflatoxin B2; AFG1, Aflatoxin G1; AFG2, Aflatoxin G2; Conc., Concentration, I. stds, Individual standards.

The percentage recovery for individual standards concentration range (0.25 to 15 ppb) ranged from 70-100%, CRM >15 ppb, the recovery ranged from 80-110, four peak for aflatoxin G1, aflatoxin G2, aflatoxin B1 and aflatoxin B2, were well isolated in the HPLC system. This complies with the specific requirements of methods of analysis of aflatoxin contamination in animal feeds as recommended in CXS 193-1995, General standards for contaminants and toxins in food and feed.

3.3. Detection of Aflatoxins Contamination in Concentrate Feeds Sample

Occurrence of aflatoxin in concentrate preliminarily detected by ELISA kit, sixty three 63/81 (78 %) of all sample were found to be contaminated by aflatoxin, and 18/81 (22%) of sample were free from contamination, on which 18/20 (90 %) of sample collected from Temeke cluster were positive for aflatoxin contamination, Ubungo 13/20 (65 %), Ilala 21/27 (78 %) and Kinondoni 11/14 (79 %) (Prevalence of contamination was Temeke > Kinondoni > Ilala > Ubungo) as shown in table 3.3. This results revealed the prevalence of aflatoxin in analyzed concentrate feed and indicating threat to animal and/or human health,

The use ELISA method for preliminary/screening detection of aflatoxin contamination was due to fact that the method is fast and cheap and can analyze 96 sample simultaneously, and does not need extensive clean up (Yao et al., 2015). The use of ELISA for detection of aflatoxin has been used by different researchers including (Mohamed, 2017; Seetha et al., 2017).

Table 3-3: *Number of positive sample in four cluster (+ means positive).*

Cluster	No of + sample	% + sample	Overall no. +sample	Overall % of +sample
Temeke	18/20	90	63/81	78
Kinondoni	11/14	79		
Ilala	21/27	78		
Ubungo	13/20	65		

3.4. Quantification of Aflatoxins Contamination in Concentrate Feeds Sample

The samples were analyzed by HPLC-FLD for quantification of total aflatoxin (TAFs), AFB1, AFB2, AFG1 and AFG2, the results shown in table 3.4. One sample t-test was used to compare the mean of AFB1 and TAFs and TBS/EAC recommended limit of aflatoxin contamination (10 ppb, AFB1 and 15 ppb for total aflatoxins). The aflatoxin concentration in concentrate feed samples are shown in table. **3-4.**

Table 3-4: *Aflatoxin concentration of concentrate feed sample collected from four cluster*

SID	AFB1	AFB2	AFG1	AFG2	TAFs	S. ID	AFB1	AFB2	AFG1	AFG2	TAFs
U1	3.94	0.50	4.53	ND	8.97	T21	40.12*	2.81	3.25	ND	46.20*
U2	ND	ND	0.78	ND	0.78	T22	36.91*	2.68	3.24	ND	42.70*
U3	52.43*	3.26	75.06	6.02	136.77*	T23	38.36*	2.65	3.27	0.27	38.36*
U4	1.13	<LOD	2.45	0.31	3.89	T24	38.21*	2.67	3.14	0.20	44.22*
U5	42.07*	4.20	0.59	0.00	46.07*	T25	4.34	0.69	4.26	3.01	12.30
U6	0.70	ND	ND	ND	0.70	T26	38.91*	1.30	3.16	0.08	43.45*
U7	0.77	ND	ND	ND	0.77	T27	38.31*	2.66	3.24	ND	44.15*
U8	12.08*	1.48	3.79	0.74	18.09*	T28	3.99	0.62	4.22	2.70	11.60
U9	3.94	0.50	4.53	<LOD	8.97	T29	39.21*	2.91	3.94	ND	46.06*
U10	0.35	0.71	5.51	0.71	13.28	T30	39.94*	3.01	3.94	0.16	47.05*
U11	8.98	1.07	13.14	1.35	24.54*	T31	36.91*	2.52	2.87	ND	42.29*
U12	39.95*	2.86	3.37	ND	46.18*	T32	4.08	4.29	0.68	4.27	12.08
U13	39.68*	2.72	3.36	ND	45.76*	T33	37.52*	2.56	3.11	ND	43.19*
U14	ND	ND	ND	ND	ND	T34	38.90*	2.79	3.46	0.17	45.26*
U15	ND	ND	ND	ND	ND	T35	39.46*	2.89	3.46	0.17	45.97*
U16	ND	ND	ND	ND	ND	T36	39.57*	2.84	3.74	2.99	49.14*
U17	ND	ND	ND	ND	ND	T37	39.67*	3.22	4.20	0.23	47.32*
U18	ND	ND	ND	ND	ND	T38	3.93	0.69	3.96	2.76	11.34
U19	ND	ND	ND	ND	ND	T39	ND	ND	ND	ND	ND
U20	ND	ND	ND	ND	ND	T40	ND	ND	ND	ND	ND
I41	8.85	0.54	2.63	0.42	12.44	K68	4.51	0.62	4.68	3.00	12.80
I42	32.57	2.57	34.63	3.93	73.70*	K69	38.25*	2.73	3.90	0.00	44.88*
I43	46.99	3.86	14.29	7.87	73.01*	K70	4.34	0.75	4.23	2.63	11.95
I44	42.50*	4.27	ND	ND	46.77*	K71	4.09	0.78	4.55	2.71	12.80
I45	1.51	ND	ND	ND	1.51	K72	31.61*	5.72	24.63	4.67	66.62*
I46	5.00	5.74	ND	ND	10.74	K73	4.71	0.39	1.30	ND	6.40
I47	1.35	0.13	ND	ND	1.48	K74	1.56	ND	ND	ND	1.56
I48	133.17*	4.34	19.64	4.17	161.32*	K75	1.64	0.36	ND	ND	2.00
I49	0.77	ND	ND	ND	0.77	K76	5.36	0.57	ND	ND	5.93

I50	12.08*	1.48	3.79	0.71	18.09*	K77	46.40*	4.52	9.67	0.96	61.55*
I51	3.94	0.50	4.53	ND	8.97	K78	8.85*	0.54	2.63	0.44	12.44
I52	6.35	0.71	5.51	0.71	13.28	K79	ND	ND	ND	ND	ND
I53	8.98	1.07	13.14	1.35	24.54*	K80	ND	ND	ND	ND	ND
I54	39.95*	2.86	3.37	ND	46.18*	K81	ND	ND	ND	ND	ND
I55	39.68*	2.72	3.36	ND	45.76*	I62	ND	ND	ND	ND	ND
I56	40.12*	2.81	3.25	ND	46.18*	I63	ND	ND	ND	ND	ND
I57	36.91*	2.52	2.87	ND	42.78*	I64	ND	ND	ND	ND	ND
I58	44.78*	4.41	14.41	2.50	66.50*	I65	ND	ND	ND	ND	ND
I59	23.13*	1.30	16.95	1.77	43.15*	I66	ND	ND	ND	ND	ND
I60	17.27*	3.01	1.65	1.03	22.96*	I67	ND	ND	ND	ND	ND
I61	39.13*	8.21	21.19	2.44	70.97*						

ND, not detected; I, Ilala *Sample contaminated above the standard (10 ppb for AFB1 and 15 ppb for total aflatoxin). T, Sample from Temeke cluster; I, Ilala; U, Ubungo; K, Kinondoni.

The level of aflatoxin contamination (TAFs) observed from this study was (range LOD to 161.32 ppb, mean 25.89 ± 3.3 ppb), on which 37/81 (46%) of the sample were contaminated above the WHO/TBS recommended standard for total aflatoxin. The mean level of aflatoxin (TAFs) contamination was significant higher than the WHO/TBS recommended limit for total aflatoxin 15 ppb ($p = 0.002$), hence the concentrate feed was not suitable for animal consumption in Tanzania. The animal feed value chain in Tanzania is susceptible to aflatoxin contamination, as have been reported previously in chicken commercial feed sample in Morogoro (Kajuna et al., 2013), in sunflower cake feed in Singida (Mohammed et al., 2016), also comparable to the finding from United kingdom (D'mello et al, 1999), in Nigeria (Mgbeahurike et al, 2016), in republic of Korea (Lee Juan and Li-mien, 2006) and in Kenya (Alkahalaf et al, 2010; Shareef, 2009).

The study also revealed the predominance of AFB1 contamination (range LOD to 133.17 ppb, mean 18.79 ± 2.45 ppb) than other types of aflatoxin, AFB2 (range LOD to 8.21 ppb, mean 1.82 ± 0.27 ppb), AFG1 (range LOD to 75.06 ppb, mean 7.75 ± 2.32 ppb) and AFG2 (range LOD to 7.87 ppb, mean 1.30 ± 0.350 ppb). The level of AFB1 contamination was significantly higher than recommended WHO/TBS recommended limit 10 ppb ($p = 0.001$). This indicate that the sample were contaminated with all major types of aflatoxin, this is not comparable to the study carried out in Sudan on which only two sample were contaminated with all major types of aflatoxin (Bashir et al, 2014).

The level contamination was $AFB1 > AFG1 > AFB2 > AFG2$ which was significantly different ($p = 1 \times 10^{-13}$), this indicate high variations. Multiple comparison of contamination among types of Aflatoxins indicated that AFB2 and AFG2 contamination were not significant different ($p = 0.83$), all other comparison were significantly different ($p < 0.05$) as summarized in table 3.6. AFB1 is grouped as Hepatocellular cancer causing agent, which occur predominantly than other type's aflatoxin (Wu et al., 2011). This findings indicate threat to food safety

and animal and/or human health concern in Da re Salaam, Exposure assessment due to aflatoxin carryover from concentrate feed is necessary.

The predominance of AFB1 was also reported by Gourama and Psullerman (1995), AFB1 was also found to be common AFs contaminant in animal feed collected from South Sudan (Elizupir et al, 2009). Similarly AFB1 contamination in feed has also been previously reported by (Razac and Norian, 2014 in Iran, although less was known about aflatoxin contamination in concentrate feed in Tanzania, the result obtained could lead to economic losses due to increased cattle's treatment cost, reduce milk production and death, affecting animal feed value chain business and cattle's farming. Since aflatoxin contamination can occur at any stage from field/pre harvest stages up to a consumer level, it is important to control aflatoxin contamination with emphasis of AFB1 at all stages in the value chain to ensure safety for animals and human.

Table 3-6: Multiple comparisons of aflatoxin contamination

LSD Multiple Comparisons : Dependent Variable: - Level of Contamination						
(I) Aflatoxin contamination	(J) Aflatoxin Contamination	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
AFB1	AFB2	16.96788*	2.40295	.000	12.2403	21.6955
	AFG1	11.03753*	2.40295	.000	6.3099	15.7651
	AFG2	17.48556*	2.40295	.000	12.7580	22.2131
AFB2	AFB1	-16.96788*	2.40295	.000	-21.6955	-12.2403
	AFG1	-5.93035*	2.40295	.014	-10.6579	-1.2028
	AFG2	.51768	2.40295	.830	-4.2099	5.2453
AFG1	AFB1	-11.03753*	2.40295	.000	-15.7651	-6.3099
	AFB2	5.93035*	2.40295	.014	1.2028	10.6579
	AFG2	6.44802*	2.40295	.008	1.7204	11.1756
AFG2	AFB1	-17.48556*	2.40295	.000	-22.2131	-12.7580
	AFB2	-.51768	2.40295	.830	-5.2453	4.2099
	AFG1	-6.44802*	2.40295	.008	-11.1756	-1.7204

*. The mean difference is significant at the 0.05 level.

3.4 Comparison of Aflatoxin contamination of concentrate feed from four selected clusters (Ubungo, Ilala, Temeke and Kinondoni)

Aflatoxin contamination (TAFs) in concentrate feeds collected from Temeke ranged from LOD to 49.14 ppb, mean 33.63 ± 3.96 ppb, Ilala TAFs ranged from LOD to 161.32 ppb, mean, 30.78 ± 7.05 ppb, Ubungu TAFs ranged from LOD to 136.77 ppb, mean, 17.74 ± 3.96 ppb and Kinondoni TAFs ranged from LOD to 66.62 ppb, mean, 17.07 ± 6.14 ppb). Aflatoxin contamination from Kinondoni and Ubungu were not significant ($p=0.71$ and $p=0.74$ respectively) different from the recommended standard while aflatoxin contamination from Ilala and Temeke were significant (p -value, 0.034 and 0.00154) high than WHO/TBS recommended limit respectively, as shown in table 3.7.

Using one way ANOVA, these difference of contamination within cluster was not significant ($p=0.193$). This indicate that level of aflatoxin contamination within cluster were not statistically significant different, all cluster were equally contaminated. This results was not comparable to the study carried in Khartoum states in South Sudan on which the sample collected from different part Khartoum states showed different contamination (Elizupir et al, 2009), but comparable to study conducted in Uganda districts includes Kampala, Wakiso, Jinja, Mbale and Masindi (Nakavuma et al., 2020).

The level of AFB1 contamination was observed higher in sample collected from Temeke (range LOD to 40.12 ppb, mean 27.92 ± 3.8 ppb) and Ilala (range LOD to 133.17 ppb, mean 21.67 ± 5.47 ppb), the level of AFB1 contamination in these two cluster (Ilala and Temeke) were significant higher than the TBS/EAC standards p value, 0.043 and 0.00015 respectively, while AFB1 contamination from Ubungu and Kinondoni were below the recommended TBS/EAC standards $p > 0.05$.

The regulatory institution on food safety, TBS/EAC are concerned with total aflatoxin and aflatoxin B1, and already have set regulatory standards or limit of AFB1 (10 ppb) and TAFs (15 ppb) in concentrate feed. However, this study revealed contamination from other major types of aflatoxin AFB2 and AFG1 and AFG2 which are regards as not toxic as AFB1, The sample collected from Ubungu were contaminated by AFG1 (range LOD to 75.06 ppb, mean $.86 \pm 3.71$ ppb), AFG2 (range LOD to 6.02 ppb, mean 0.46 ± 0.3 ppb), AFB2 (range LOD to 4.2 ppb, mean 0.87 ± 0.3 ppb). In Kinondoni, AFG1 (range LOD to 21.63 ppb, mean 3.97 ± 1.76 ppb), AFG2 (range LOD to 4.67 ppb, mean 1.03 ± 0.42 ppb), AFB2 (range LOD to 5.72 ppb, mean 1.21 ± 0.48 ppb). In Ilala, AFG1 (range LOD to 34.63 ppb, mean 6.12 ± 1.69 ppb), AFG2 (range LOD to 7.78 ppb, mean 0.99 ± 0.35 ppb), AFB2 (range LOD to 8.21 ppb, mean 1.96 ± 0.41 ppb). In Temeke, AFG1 (range LOD to 4.26 ppb, mean 3.06 ± 0.29 ppb), AFG2 (range LOD to 4.27 ppb, mean 0.85 ± 0.31 ppb), AFB2 (range LOD to 4.29 ppb, mean 2.19 ± 0.27 ppb), The occurrence of AFB2, AFG1 and AFG2 in food commodities should not remain unlooked, the regulatory bodies and researcher should establish a limit for their contaminations.

Human is exposed to aflatoxin through either consumptions of aflatoxin contaminated animal product, such as milk, egg, meat and related product, the occurrence of aflatoxin in animal product is due to carry over and excretion of aflatoxin metabolite in milk or bio-accumulation in the liver (Narod et al., 2011), Aflatoxins also accumulate in the skeletal muscle, fatty tissue and blood (Adegbeye et al., 2020), in milk, porcine tissue and eggs (Beaver, et al., 1990) resulting in both short and long term health effects (Kamala et al., 2018), The occurrence of aflatoxin in concentrate feeds in this study indicate a threat to food safety and human exposure. Hence this results is a call alert to public and government of Tanzania to come up with a control measure to reduce aflatoxin contamination.

Table 3-7: *Aflatoxin concentration of concentrate feed sample from four selected clusters (Ubungo, Ilala, Temeke, Kinondoni)*

Cluster	Frequency	Aflatoxins	Mean $\pm$ SE ppb	Range ppb	t	p-value
Ubungo	65%(13/20)	AFB1	10.30 $\pm$ 3.92	52.43	0.077	0.94*
		AFB2	0.87 $\pm$ 0.3	4.2	Na	Na
		AFG1	5.86 $\pm$ 3.71	75.06	Na	Na
		AFG2	0.46 $\pm$ 0.3	6.02	Na	Na
		TAFs	17.74 $\pm$ 7.26	136.77	0.377	0.71*
Kinondoni	78.8%(11/14)	AFB1	10.81 $\pm$ 4.17	46.40	0.194	0.849*
		AFB2	1.21 $\pm$ 0.48	5.72	Na	Na
		AFG1	3.97 $\pm$ 1.76	24.63	Na	Na
		AFG2	1.03 $\pm$ 0.42	4.67	Na	Na
		TAFs	17.06 $\pm$ 6.14	66.62	0.334	0.742*
Ilala	78%(21/27)	AFB1	21.67 $\pm$ 5.47	133.17	1.876	0.043**
		AFB2	1.96 $\pm$ 0.41	8.21	Na	Na
		AFG1	6.12 $\pm$ 1.69	34.63	Na	Na
		AFG2	0.99 $\pm$ 0.35	7.87	Na	Na
		TAFs	30.78 $\pm$ 7.05	161.32	1.936	0.034**
Temeke	90%(18/20)	AFB1	27.92 $\pm$ 3.8	40.12	4.719	0.000149**
		AFB2	2.19 $\pm$ 0.27	4.29	Na	Na
		AFG1	3.06 $\pm$ 0.29	4.26	Na	Na
		AFG2	0.85 $\pm$ 0.31	4.27	Na	Na
		TAFs	33.63 $\pm$ 3.96	49.14	4.704	0.000154**

** denote P-value< 0.05. * denote P-value> 0.05, Na denote not applicable, Method used HPLC-FLD, Data analysis software IBM SPSS 2013.

HPLC-FLD have been used for aflatoxin contamination quantifications by different studies including, Seetha et al, (2017) Leszczynska et al, (2001) and Omar et al, (2020) but both ELISA and HPLC-FLD methods indicated strong positive correlation between HPLC-FLD and ELISA method on detection and quantification of aflatoxin in concentrate feed ($r > 0.99$). The two methods were also used by (Leszczynska et al, 2001; Seetha et al, 2017), for preliminary analysis and quantification of aflatoxin in grains, in peanut and peanut products (Jian et al, 2016). A strong correlation also reported by Beyeye et al, 2019, with $r > 0.99$. Nesiket et al, (2017) also reported

that both HPLC and ELISA method can be used equally to analyze mycotoxin. However, ELISA considered as screening method. This indicate that both methods can be employed in analysis of aflatoxins for detection and quantification of aflatoxin in concentrate feeds.

3.5 profile of respondent

A total of 81 respondents were interviewed, male 50/81 (61.7%) and female 31/81 (38.3%), age group of 17-34 years constituted 39.5% of the respondents, 35-64 years constituted 43.5% and >65 were 17.3%, Level of education are important parameter when analyzing the public awareness on aflatoxin contamination and control (Dosman et al, 2001). In this study, 58.0% of respondents had secondary education which not comparable to the study carried out in Mlangi, Manungu and Kongwa (Mohamed, 2017). This can be due to fact that Dar es Salaam is a major city with high quality education facility than other places in the country. The increase of concentrate feed demand in Dar es Salaam have been observed since 60/81 (74.1%) of the respondents are in concentrate feed business in less than 5 years as summarized in table 3-8.

Table 3-8: Respondent profile

Characteristics		Frequency	Percentages
Gender	Male	50	61.7
	Female	31	38.3
Age	17-34	32	39.5
	35-64	35	43.2
	>65	14	17.3
Education	None	1	1.2
	Primary	24	29.6
	Secondary	47	58.0
	Diploma	5	6.2
	Degree	4	4.9
Occupational	Agro-dealer	32	39.5
	Agro-processor	49	60.1
Duration in business	<1 year	13	16
	1 to 3 years	32	39.5
	3 to 5 years	15	18.5
	>5 years	21	25.9

3.6 Public Awareness on aflatoxin contamination and control

A total of 81 respondents were interviewed, the results revealed insignificant level of public awareness on aflatoxin contamination ($P = 0.353$), only 31/81 (38%) of respondents were knowledgeable about aflatoxin contamination and control, Also 23/81(28%) respondents (agro-processors and agro-dealers) aware about the factors that contribute into aflatoxin contamination, the level of that awareness was insignificant ($p \geq 0.657$). Aflatoxin contamination pose threat to animal and/or human being, resulting into animal and/or human health impact, but only 23/81(28%) respondent were prior informed about the impact of aflatoxin on animal and/or human health as indicated in table 3.9. This indicates a food system challenge, specifically a food safety concern.

Table 3-9: public awareness on aflatoxin contamination

Parameter	Frequency	χ^2 -test(P value)
Awareness of aflatoxin contamination.	38% (31/81)	0.353*
Awareness of factors associated with aflatoxin contamination.	28% (23/81)	0.657*
Awareness of health risk associated with aflatoxin contamination	28% (23/81)	1.00*

*denote P value > 0.05

Awareness was in the order that Ilala 41% > Temeke (40%) = Ubungo 40%, > Kinondoni cluster, 29% as summarazied in table 3-10. These differences remained unrevealed, however, awareness creation programme could be the main factor. It was also observed difference awareness among animal feed value chain actor selected for this study (agro-processors and agro-dealers), 20/29 (69%) of feed processors were aware and knowledgeable about aflatoxin contamination compared to 11/21 (52%) agro-dealers. The limited awareness and lack of knowledge on aflatoxin contamination and control contribute prevalence of aflatoxin contamination. Low level awareness on aflatoxin contamination and control have been reported to increase aflatoxin problem in Tanzania (Magembe et al, 2010; Nyagi et al, 2013; Fundikira, 2017; Massomo et al, 2020). In Uganda low level of awareness was reported to be the probable causes of aflatoxin contamination in Groundnuts flour (Kitya et al, 2019), In Kenya by (Mutiga et al., 2014; Leray et al., 2015; Sirma et al., 2015; Kiraie et al., 2016; Kiama et al., 2016). Also Nyaganga stressed the need of awareness creation to animal feed traders in Kenya. indeed, this findings indicate food system challenges in Dar es Salaam.

Table 3-10: Public awareness on aflatoxin contamination and control among clusters in Dar es Salaam

aflatoxin contamination awareness in concentrate feed

		Response		Total		response		total
		Yes	No		Value chain actor	Yes	no	
District	Ilala	11	16	27	Agro-processor	20	29	49
	Kinondoni	4	10	14	Agro-dealer	11	21	32
	Temeke	8	12	20				
	Ubungo	8	12	20				
Total		31	50	81		31	50	81

3.6.1 Human Practices on aflatoxin contamination and control

The prevalence of aflatoxin contamination in food crops is associated with pre and postharvest management as reported by (Seetha et al, 2007; Kimanya et al, 2008; Shaban et al 2015; Mahamudu, 2017; Massomo et al, 2020), also which was also observed In this study, it was observed that only 21/49 (42%) agro-processor and 20/32 (63%) agro-dealer had storage facilities for storage of concentrate and raw materials, also poor handling practices observed, 26/49 (53%) agro-processors and 15/32 (47%) agro-dealer found storing concentrate feeds unpacked and direct and stored on a ground until the buyer come to collect. The practice of storing concentrate feed in direct contact with soil increases the risk of aflatoxigenic species:-*aspergillus flavus* and *aspergillus parasticus* contamination (Horn et al., 1997).

To prevent aflatoxins producing species contamination, storage conditions should be dry and cool, and arrangement of feed consignment should allow air circulation, the roof should be water proof to maintain dry condition (Lavkor et al., 2017). It was observed that, Only 41/81 (50%) of the respondent's stored concentrate feed in a dry and cool place, this indicated that half of the concentrated feed premises sampled were not in accordance with Good manufacturing practices guidelines and HACCP guidelines. it is known that high moisture content increase the risk of aflatoxin contamination (Morenoa et al, 2009; Wu et al. 2011; Benkerroum et al, 2020) . This increased risk of aflatoxin contamination.

Poor post-harvest management influences aflatoxin contamination in cattle's concentrate feed as reported by (Matumba et al, 2015; Benkerroum et al, 2020), improper drying of raw material and feed before packing and poor handling practices (Nishimwe et al, 2019), but only ten 10/ 81 (12.4%) respondents were aware that poor storage facilities contributes to aflatoxin contamination. The majority of the respondents were not aware that poor storage facilities and handling practices contribute to aflatoxin contamination, this is the food safety alert.

Table 3-11: Practices for Aflatoxin contamination

Practices	Agro-processor		Agro dealer		Total
	Yes	No	Yes	No	
Availability of storage facility.	21	28	20	12	81
Storage of feed in a bag.	23	26	21	11	81
Storage of feed in dry condition.	24	25	17	15	81
Storage of feed in a ventilated area.	38	11	30	2	81
Feed stored in a pallet.	10	39	10	22	81
Feed arranged away from a wall.	17	32	9	23	81

The insect's damages contribute much into aflatoxin contamination by removing the physical barriers for *Aspergillus* colonization and prone commodities to aflatoxin contamination (Cotty et al, 2007; Mutiga et al, 2019; Ostry et al., 2014)). The use of damaged commodities for animal feed is a common behaviors in developing countries, This have been revealed during this study, only eight respondents 8/81 (9.9%) were aware that insects activity as a factor contributing to aflatoxin contamination, this indicate that, majority of the interviewed respondent's especially animal feed manufacturer did not sort out the damaged commodities, hence was used for processing concentrate feeds, it have been revealed that sorting out damaged kernel can reduce aflatoxin contamination up to 80% ((Park, 2002; Afolabi et al., 2006) but grains insect damage is most common problem in Tanzania (Suleiman and Rosentrater, 2015).

To control aflatoxin contamination Tanzania has set the regulations including the maximum tolerable limit, Tanzania Bureau of Standards (TBS) and East Africa states set the maximum limit of contamination (15ppb total aflatoxin, 10ppb AFB1). Nevertheless, all the respondent (100%) were not aware of the presence of regulation and standards.

Environmental factors such as temperature (optimum 25 to 35 °C, Hawkins et al., 2005; Siciliano et al., 2017); humidity (optimum, 65-95%, Ding et al., 2015); pH (3-7, Esheli et al., 2016) are causative factor for aflatoxin contamination. Humid and irrigated hot desert have reported to cause aflatoxin contamination in Tanzania (Kimanya et al, 2016), it was also concluded that harvesting during humid, rain or temperate seasons above 37.5°C in Eastern and Southwest Kenya favors *aspergillus* infestation and aflatoxin contamination (Mahuku et al., 2019). Climatic condition such as heavy rain, drought, high humidity, average temperature, floods was also reported by (Kaaya et al., 2005; Kimanya et al, 2016; Ochungo et al., 2016; Mahuku et al, 2019). However, when respondents were asked about environmental factor that contribute to aflatoxin contamination, only ten 10/81 (12.4%) of respondents indicated that temperature is the cause of aflatoxin contamination, and three respondents 3/81 (3.7%) indicated humidity. Low level of awareness on the factor which contribute to susceptibility to aflatoxin contamination increases the risk of contamination (ICRISAT, 2006; Diaz Rios and Jaffee, 2008).

As known, Dar es Salaam is most hot region characterized by average temperature 26.1°C, precipitation 1114 mm/43.9 inch, humidity 73% (climate data.org, 2021), all these environmental conditions favors aflatoxin contamination, together with low level of awareness, poor and lack of storage and poor handling observed from

revealed from this study, contributed to the prevalence of aflatoxin contamination Controlling Humidity and temperature and air circulation in storage facilities, Good agricultural practices (Nyagi et al, 2013), proper handling, storage technologies and enhanced information communication (Massomo et al, 2020), effective management of storage pests (Shaban et al, 2015), drying maize on mat or raised platforms, proper sun drying and application of insecticides (Magembe et al, 2010), in more specific drying of crops on required moisture content (grains < 12%, oils crops <10%), using good and well equipped storage facilities and public awareness creation are proposed solutions to reduce aflatoxin contamination in concentrate feeds.

4. Conclusion

The present study revealed that the prevalence of aflatoxins contamination was due to low level of awareness on causes of aflatoxin contamination and control mechanism such as use of good agricultural practices and post-harvest management practices such as good storage facility and good handling practices. Since the cattle concentrate feed showed high contamination with aflatoxin, there is a need for further study on aflatoxin contamination in milk, milk product, and other animal product produced and sold in Tanzania. Furthermore, education to farmers, processors, consumers, and other stakeholders on the causes and impact of aflatoxin contamination in concentrate feed produced and sold in Tanzania may contribute to better practices hence a reduction in aflatoxin contamination of feed.

Declarations

I, **Sahani Sylvester Jonathan** declare that this study is an original report of my research and has been written by me, and has not been submitted for any previous degree and journal for publication and will not submitted to any other journal and University for awards of any other degree.

Funding.

The financial support of inter-university Council for East Africa, KFW donors, and East African Community through the East Africa scholarship programme is highly appreciated.

Acknowledgement

An extended thanks to participants from four districts of Dar es Salaam, Agro-dealer and feed processors, Thanks also are due to Director General Dr. Athuman Yusuph Ngenya for the acceptance of my request to conduct sample analysis to Tanzania Bureau of Standard.

Conflict of interest

None

Availability of Data and Materials.

The data set generated and analyzed during this study are available from the corresponding author.

Authors Information

1. Sahan Sylivester Jonathan

MSc Chemistry Student.

Department of Chemistry, Faculty of Science

Mbarara University of Science and Technology-Uganda.

Permanent Address: Tanzania Ministry of Agriculture (MoA), National food security, Post-harvest management section, P.O.BOX 2182, Dodoma-Tanzania.

2. Grace Birungi (PhD)

Associate Professor

Department of Chemistry, Faculty of Science

Mbarara University of Science and Technology

3. Irene Nalumansi (PhD)

Senior Lecturer

Department of Chemistry, Faculty of Science

Mbarara University of Science and Technology

Author's contributions

1. Jonathan: - conceptualized the study and proposed how the study will be conducted, data collection, interpretation and performed statistical analysis, wrote the manuscript text, preparing figures and tables.
2. Birungi and I. Nalumansi guided the whole process of the study and involved in writings manuscript text, quality check and critically reviewed the manuscript.

Consent for publication

Not applicable.

Ethics approval and Consent to participate

This study was approved by Mbarara University research ethics committee (MUST-REC), ethical approval attached which includes informed consent, data collection and protocol

References

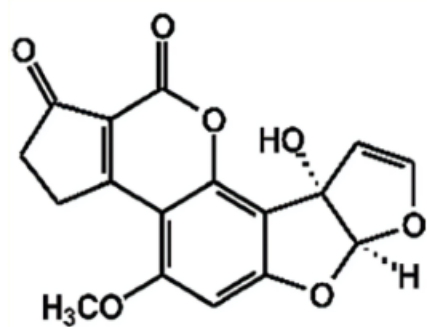
1. Adeyeye. S. A. O. (2016). Fungal mycotoxins in foods: *A review*. Cogent Food and Agriculture **2**:
2. Barkidere, S., Bora, S., Barkidere, E.G., Aydin, F., Arslan, Y., Komesli, O.T., and yildirim, E. (2012). Aflatoxin species; their health effects and determination methods in different food stuffs. Central European Journal

- of chemistry **10**: 675–685.
3. Creppy, E.E., (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Letters*. **127**: 19–28.
 4. Fallah, A.A., Jafari, T., Fallah, A., and Rahnama, M. (2009). Determination of aflatoxin M1 levels in Iranian white and cream cheese. *Food and Chemical Toxicol*, **47**:1872–1875.
 5. FAO (2008). *Animal Feed Impact on Food Safety*, Food and Agriculture Organization of the United Nations, Rome.
 6. Hendrich. S. (2017). MYcotoxins. *Food Toxicology: Current Advances and Future Challenges*, **16**: 179–198.
 7. Herzallah S.M. (2009). "Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors," *Food Chemistry*. **114**; 1141–1146.
 8. Hussein, H.S., and Brasel, J.M. (2001). Toxicity metabolism and impacts of mycotoxins on humans and animals. *Toxicity*. **167**: 101–134.
 9. Huwig, A. et al. (2001). Mycotoxindetoxication of animal feed by different adsorbents. *Toxicology Letters* **122**: 179–188
 10. Huybrechts. B. (2011). Evaluation of Immunoassay Kits for Aflatoxin Determination in Corn & Rice, CODA-CERVA, National Reference Laboratory on Mycotoxins.
 11. International Agency for Research on Cancer (WHO IARC) (2002). Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. **82**:171–274.
 12. Kaaya, N.A. and Warren, H.L. (2005). A review of past and present research on aflatoxin in Uganda. *African Journal of Food, Agriculture, Nutrition and Development* **5**: 1.
 13. Kamala, A., Ortiz, J., Kimanya, M., Haesaert, G., Donoso, S., Bendantuguka, T., and Bruno, De. M. (2015). Multiple Mycotoxin co-occurrence in maize grown in three Agro-ecological Zones of Tanzania. *Food Control* **54**: 208–215.
 14. Kang'ethe, E.K. Lang'at, K.A. (2009). Aflatoxin B1 and M1 Contamination of Animal Feeds and Milk from Urban Centers in Kenya. *African Health Sciences* **9**: 218–226.
 15. Mohammadi, H., (2011). A review of Aflatoxin M1, Milk, and Milk products In *Aflatoxins-Biochemistry and Molecular Biology; InTech*. 397–414.
 16. Munkvold, G.P. (2003). Cultural and genetic approaches to managing mycotoxins in maize. *An. Rev. Phytopattol* **41**: 99–115.
 17. Neal, G.E. Eaton, D.L. Judah, D.J. Verma, A. (1998). Metabolism and toxicity of aflatoxins M1 and B1 in human-derived in vitro systems. *Toxicol. Appl. Pharmacol*. **151**: 152–158.
 18. Ngindu, A., Kenya, P.R., Ocheng, D.M., Omondi, T.N., Ngare, W., Gatei, D., Bruce, K., Ngira J.A., Nandwa, H., Jansen, A.J., Kaviti, J.N., and Siongok, T.A. (1982). Outbreak of Acute Hepatitis caused by Aflatoxin Poisoning in Kenya. *Lancet* **320**: 1346–1348.
 19. Richard, J.L. (2007). Some major mycotoxins and their mycotoxicoses-An overview. *International Journal of Food Microbiology* **119**:
 20. Sezgin, B., Tolga, Y., Nihan, T., Mehmet, D., and Abdallah, K. (2014). Determination of trace Aflatoxin M1 levels in Milk Products consumed in Turkey by using Enzyme-linked Immunosorbent Assay. *J. Food. And.*

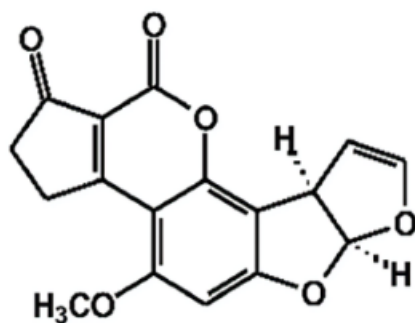
Agric. Immunol. **25**:

21. Streit, E., Schatzmayr, G., Tassis, P., Tzika, E., Marin, D., Taranu, I., Tabuc, C., Nicolau, A., Aprodu, I., Puel, O., and Oswald, I.P. (2012). Current situation of Mycotoxin Contamination and Co-occurrence in Animal feed – focus on Europe. *Toxins* **4**: 788–809.
22. Surai, P. F. Mezes. M. Melnichuk, S. D. and Fotina, T.I. (2008). Mycotoxins and animal health
23. Takino M. and Tanaka T. (2008). Determination of Aflatoxins in Food by LC-MS/MS, *Agilent Technologies*.
24. Turner, P.C., Moore, S.E., Hall, A.J., Prentice, A.M, and Wild, C. (2003) Modification of Immune function through Exposure to Dietary Aflatoxin in Gambian children. *Environ. Health. Perspectives* **111**:217–220.
25. Unusan, N. (2006). Occurrence of Aflatoxin M1 in UHT Milk in Turkey. *Food and Chemical Toxicology*. **44**: 1897–1900.
26. Verma, R.J. (2004). Aflatoxin Cause DNA Damage. *Int. J. Hum. Genet.* **4**: 231–236.
27. Womack, E.D.W., Ashli, E., Brown, A.E., and Sparks, D.L. (2014). A recent review of non-biological remediation of aflatoxins-contaminated crops. *Journal of the science of food and agriculture*. **94**: 1706–1714.
28. WHO. (2018) Mycotoxins.
29. World Population Clock – Worldometers (2015).
30. Yiannikouris, A., and Jovany J.P. (2002). Mycotoxins in feeds and their fate in animals a review animal research. **51**: 81–98.
31. Zaghini. A.G. Martelli. G. Roncad. P. Simioli. M. and Rizzi. L. (2005). Mannanoligosaccharides and aflatoxins B1 in feed for laying hens: Effects on egg quality, Aflatoxin B1 and M1 residues in eggs, aflatoxin B1 levels in Liver. *Poultry Science* **84**: 825–832.
32. Zain, M.E. (2011). Impact of Mycotoxins on Humans and Animals. *J. Saudi Chem. Soc.* **15**: 12

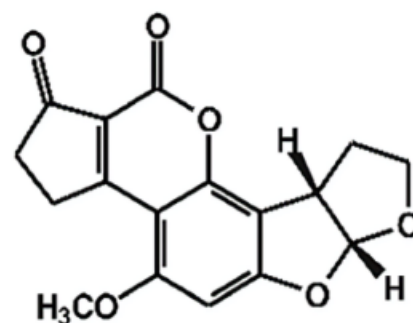
Figures



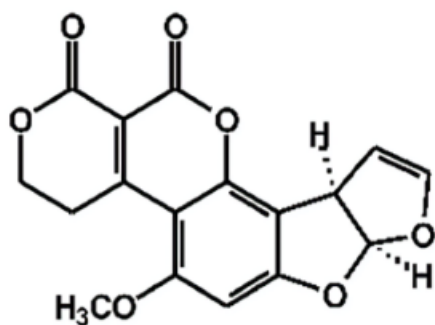
Aflatoxin M1



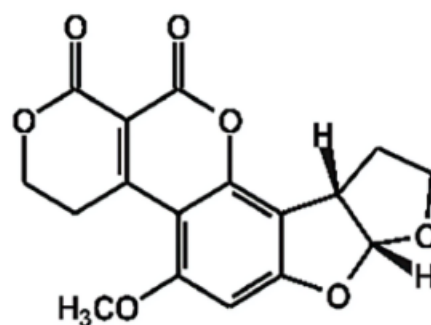
Aflatoxin B1



Aflatoxin B2



Aflatoxin G1



Aflatoxin G2

Figure 1

Chemical structure of Aflatoxin AFB1, AFB2, AFG1, AFG2 and Aflatoxin M1 (AFM1), (Zain, 2011)

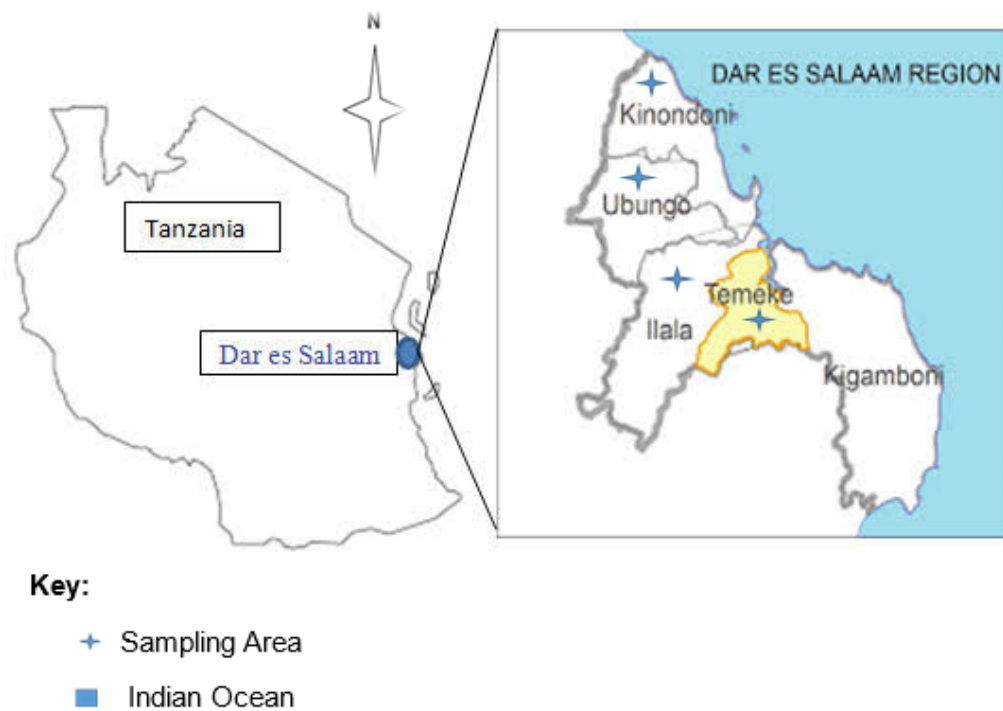


Figure 2

Map Showing Sampling Area (Modified map after Shayo eta l., 2020)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformationxx.pdf](#)