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The Occurrence and Variations of Total Aflatoxins and Aflatoxin B_1 in Different Types of Chicken Feeds Marketed in Dar es Salaam, Tanzania

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Abstract

This paper reports on the occurrence and variation of total aflatoxins (TAF) and aflatoxin B_1 (AFB_1) in different types of chicken feeds collected from various locations in Dar es Salaam. A total of 63 chicken feed samples based on cotton seed hulls, sunflower seed hulls, maize bran and mixed feeds were analysed using HPLC-FLD. It was revealed that all samples were contaminated with both TAF and AFB_1, varying significantly with respect to their types. Specifically, 52.9% of the cotton seed hulls and 64.7% of maize bran samples exceeded the set tolerable limit of 5 ng g^{-1} for AFB_1. All sunflower seed hull samples were contaminated, showing varying mean concentrations of TAF across the sites: Manzese (150.48 ng g^{-1}), Mbagala (56.20 ng g^{-1}), Bunju (49.08 ng g^{-1}) and Kigamboni (33.83 ng g^{-1}). Remarkably, all chicken feed samples from the poultry farms were contaminated with levels beyond the recommended international maximum tolerable limit of 20 ng g^{-1} of TAF for feeds with levels decreasing from Farm C (77.71 ng g^{-1}), Farm A (48.19 ng g^{-1}), Farm E (38.95 ng g^{-1}) and Farm F (24.48 ng g^{-1}). These findings call for urgent stringent quality control measures to mitigate aflatoxin contamination in chicken feeds, thereby safeguarding animal health and preventing potential health risks to humans.

Keywords: Aflatoxins; High Performance Liquid Chromatography; Poultry feeds; Contamination; Mycotoxins

Introduction

Aflatoxins are poisonous substances (mycotoxins) produced by fungi that grow naturally in almost all agricultural commodities such as cereal crops, spices, oil seeds, black pepper, dried fruit and peanuts (Gurav and Medhe 2018, Dors et al. 2011). The main species of fungi that produce aflatoxins are *Aspergillus parasiticus* and *Aspergillus flavus*, primarily found in humid and warm regions of the world. Aflatoxin-producing fungi grow in different crops and products when exposed to favourable environmental conditions such as high temperature and humidity (Pratiwi et al. 2015). It has been reported that over 5 billion people and animals in developing countries worldwide are at risks of exposure to aflatoxins by consuming contaminated food and feed (Williams et al. 2004). Aflatoxins occur in four major groups: aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Additionally, aflatoxin M_1 and aflatoxin M_2 are metabolites of AFB_1 and AFB_2, respectively, reported from the milk of animals fed on contaminated feed (Negash 2018). Aflatoxins are known to cause health problems and even death to animals, birds, fish, and human beings, as well as an economic burden by damaging agricultural

commodities annually (WHO 2000). Among the main classes of aflatoxins, aflatoxin B_1 is known to be the most potent, potentially lethal and carcinogenic agent (Fitzmaurice et al. 2015, Mwakosya et al. 2022). Information on the levels and control of both aflatoxins B_1 and total aflatoxins in different agricultural products and animal feeds is essential to safeguard animals' health (Bintvihok and Kositcharoenkul 2006). Typically, aflatoxins affect animals' growth, immune functioning, metabolic activities, and the decline of egg production in chicken (Negash 2018).

Animals, including chickens, are exposed to aflatoxins through direct ingestion of contaminated feed and by inhalation of aflatoxin dust from factories and industries (Agag 2004). Once inside the animal body, aflatoxins are metabolized and transferred to various products such as eggs, meat, milk and blood tissues. Consequently, humans are indirectly exposed to aflatoxins through the consumption of contaminated animal products. In the human body, aflatoxins can lead to a range of health issues, including cancer, fatty liver, immunosuppression, cardiovascular and renal disorders, abdominal discomfort, and impaired growth and development (Mwakosya et al. 2022, Kyalo et al. 2023, Murokore et al. 2023). Aflatoxin B_1, in particular, is known to induce cancer in human organs such as the liver, kidneys, breast, and small intestine, and to impair the physiological functions of the brain, lungs, and kidneys (Murokore et al. 2023). To limit human exposure to aflatoxins, it's crucial to identify and manage all potential routes of exposure. Given that aflatoxins are considered inevitable contaminants in the food chain, various regulatory bodies have established maximum allowable concentrations to reduce exposure in both animals and humans. In an effort to curtail exposure, Tanzania's regulatory body, the Bureau of Standards (TBS), has set the maximum permissible levels in food at 10 ng g^{-1} for total aflatoxins (TAF) and 5 ng g^{-1} for aflatoxin B_1 (AFB_1), aligning with standards from the European Commission (European Commission 2006). Furthermore, the Food and Drug Administration (FDA) in the United States determined that the upper limit for total aflatoxins (TAF) in all foods, including animal feed, should be 20 ng g^{-1} (Tanah 2010). Table 1 shows the acceptable limits established by various countries.

Table 1: The Established Tolerable Limits of Aflatoxins for Human Intake in Different Countries

Country	**AFB_1 (ng g^{-1})**	**TAF (ng g^{-1})**
European countries	5	10
United Arab Emirates	5	10
Tanzania	5	10
Kenya	5	20
India	5	30
Nigeria	5	20
South Africa	5	10
Iran	5	10
Republic of Korea	10	15
Zimbabwe	5	20
United states	5	20
Sri Lanka	5	30
Mexico	5	20

Source: (FAO 2017, IITA 2015)

Chicken consumption is highly prevalent in Tanzania. As of 2020, the chicken population in the country totaled 83,280,000, with 38,770,000 being indigenous breeds and 44,510,000 exotic breeds (Ringo and Lekule 2020). Therefore, monitoring the presence of aflatoxins in poultry feed is crucial due to its potential contamination. In Tanzania, key

agricultural products like maize, millet, sunflower seeds, and cotton seeds, along with their byproducts, are commonly used in poultry feed formulations. These items are often susceptible to contamination by aflatoxin-producing fungi.

Previous studies have highlighted this issue; for instance, Mohammed et al. (2016) reported that 61.5% of sunflower cake samples used in animal feeds contained aflatoxin B_1 levels above the permissible limit. Similarly, Kajuna et al. (2013) reported aflatoxin contamination in maize bran, while Nyangi et al. (2016) noted contamination in sunflower seed cake. Furthermore, Mmongoyo et al. (2017) reported that 17% of sunflower cake samples surpassed the international safety threshold of 20 ng g^{-1} for total aflatoxins. Given the frequent occurrence of acute aflatoxicosis in the region (Kamala et al. 2018), this study focused on examining the levels and variations of aflatoxin B_1 and total aflatoxins in chicken feeds sourced from various agrovet stores and poultry farms in Dar es Salaam. The study highlights the critical need for stringent monitoring and control of aflatoxin levels in poultry feeds in Tanzania, which can significantly reduce the health risks to both poultry and humans consuming contaminated animal products. Addressing these contamination issues aims not only to safeguard public health but also to alleviate the economic impact caused by aflatoxins on the agricultural sector.

Materials and Methods

Sample Collection

A total of sixty-three (63) samples were collected for this study, including fifty-one (51) samples of chicken feed made from cotton seed hulls (n=17), sunflower seed hulls (n=17), and maize bran (n=17). These samples were randomly gathered from six agrovet locations in Dar es Salaam, namely: Bunju, Manzese, Kigamboni, Gongo la Mboto, Kitunda, and Mbagala. Additionally, twelve (12) samples were sourced from six unnamed poultry farms, in compliance with ethical standards: Farm A (Ukonga), Farm B (Chamazi), Farm C (Gongo la Mboto), Farm D (Kawe), Farm E (Tegeta), and Farm F (Kigamboni). 25 eggs samples (n=5) were also collected from farms A, B, C, E, and F, where feeds showed high contamination levels. All samples were sealed in polyethylene bags to prevent moisture absorption and transported to the Tanzania Bureau of Standards (TBS) laboratories for analysis.

Chemicals and Reagents

Chemicals utilized in this study included acetonitrile (HPLC grade) obtained from Sigma-Aldrich Inc, and methanol (HPLC grade) sourced from Fisher Scientific, UK. The immunoaffinity columns, branded as Aflacolumns, and aflatoxin standards, specifically aflatoxin B_1, B_2, G_1, and G_2, were procured from Romer Labs, Austria. Water used was also of HPLC grade. The mobile phase for the stock solution of aflatoxins was prepared using a mixture of acetonitrile, methanol, and water.

Extraction of Aflatoxins from Chicken Feed Samples

To extract aflatoxins from chicken feed samples composed of cotton seed hulls, sunflower seed hulls, and maize bran-based feeds, the samples were first pulverized using a blender. Subsequently, 25 g of each ground sample was transferred to an Erlenmeyer flask. To this, 100 mL of a methanol-water mixture (70:30 v/v) was added as the extraction solvent. The flask was then sealed with aluminium foil, and the contents were agitated on a gyratory shaker at 250 rpm for 30 minutes. The mixture was then filtered through Whatman No.1 filter paper.

Extraction of Aflatoxins from Eggs

The eggshells were cracked to access the yolks, which were then vigorously stirred to ensure homogeneity. Approximately 25 mL of this homogenized sample was measured using a measuring cylinder and transferred into an Erlenmeyer flask. This was followed by the addition of 100 mL of an extracting solvent consisting of methanol and water (70:30 v/v) with added sodium hydroxide to reduce oil content. The flask was then sealed with aluminium foil and placed on a gyratory shaker, where it was agitated for 30 minutes

at 250 rpm. Following this, the mixture was filtered through Whatman No.1 filter paper.

Clean-Up of Sample Extracts

The extracted samples were cleaned using immunoaffinity columns. Prior to sample loading, the columns were pre-rinsed twice with 10 mL of distilled water each time. To ensure all residual water was removed, the columns were subjected to a vacuum clean-up process and then disconnected from the adapter. Each column was eluted thrice with 0.5 mL of 100% methanol (HPLC grade), collecting the eluate in amber vials to achieve a total volume of 1.5 mL. Any residual oil droplets in the eluted samples were filtered out prior to analysis. The prepared samples were then analysed using a High-Performance Liquid Chromatography (HPLC) system equipped with a fluorescence detector (HPLC-FD), utilizing post-column derivatization techniques.

HPLC Conditions

For the detection of aflatoxins, a reversed-phase High-Performance Liquid Chromatography (HPLC) system equipped with a fluorescence detector (FLD) was utilized. The mobile phase composition was a mixture of water, methanol, and acetonitrile in a ratio of 6:3:1 v/v. Aflatoxin separation occurred on a C_{18} column, maintained at a temperature of 30°C with a flow rate of 1.2 mL/min. Detection of aflatoxins was achieved using the fluorescence detector set to an emission wavelength of 465 nm and an excitation wavelength of 360 nm. Peaks were identified and confirmed by comparing their retention times with those of standard aflatoxin samples.

Recovery Test

The accuracy of the method was verified through a recovery test. Blank samples of feed and eggs, confirmed to be aflatoxin-free through prior analysis, were spiked with 5 ng/mL of aflatoxin standards AFB_2, AFG_1, AFG_2, and AFB_1. Thereafter, the recovery test was conducted in duplicate, adhering to standard analytical procedures to determine the concentration of aflatoxins. The results obtained were then used to compute percentage recovery using Equation 1, confirming the accuracy of the method.

$$\% \text{ Recovery} = \left(\frac{r-b}{s}\right) \times 100\% \qquad 1$$

where r = the recovered amount, b = blank concentration and s = the spiked amount

Method Calibration

Calibration curves were generated using standard solutions with concentrations of 1.0, 2.5, 5.0, and 10.0 ng/mL. The regression equations for aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 were derived by plotting the peak areas against the concentrations. These equations were then used to calculate the concentration of each specific aflatoxin in the samples.

Detection Limit and Quantification Limit

The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined using equations 2 and 3, respectively (Armbruster and Pry 2008).

LOD = Mean concentration of the blank + 3.3SD…2

LOQ = Mean concentration of the blank + 10SD… 3

where SD = Standard Deviation of the lowest concentration.

Statistical Data Analysis

Statistical analysis was performed for the data collected from the study to determine if there were significant differences in the data from various sampling locations and/or among different feed types, using MaxStat Lite software. Specifically, a one-way analysis of variance (ANOVA) was used to compare the average levels of aflatoxin contamination in chicken feed from agrovet stores and poultry feed from farms.

Results and Discussion

Calibration of the Method

Solutions containing mixtures of aflatoxin standards (G_1, G_2, B_1, and B_2), were prepared and analysed at concentrations of 1, 2.5, 5, and 10 ng/mL. This procedure was employed to create a four-point calibration curve, as illustrated in Figure 1. The HPLC system was uniformly conditioned across all tests. This calibration curve was used to verify the linearity and accuracy of aflatoxin quantification.

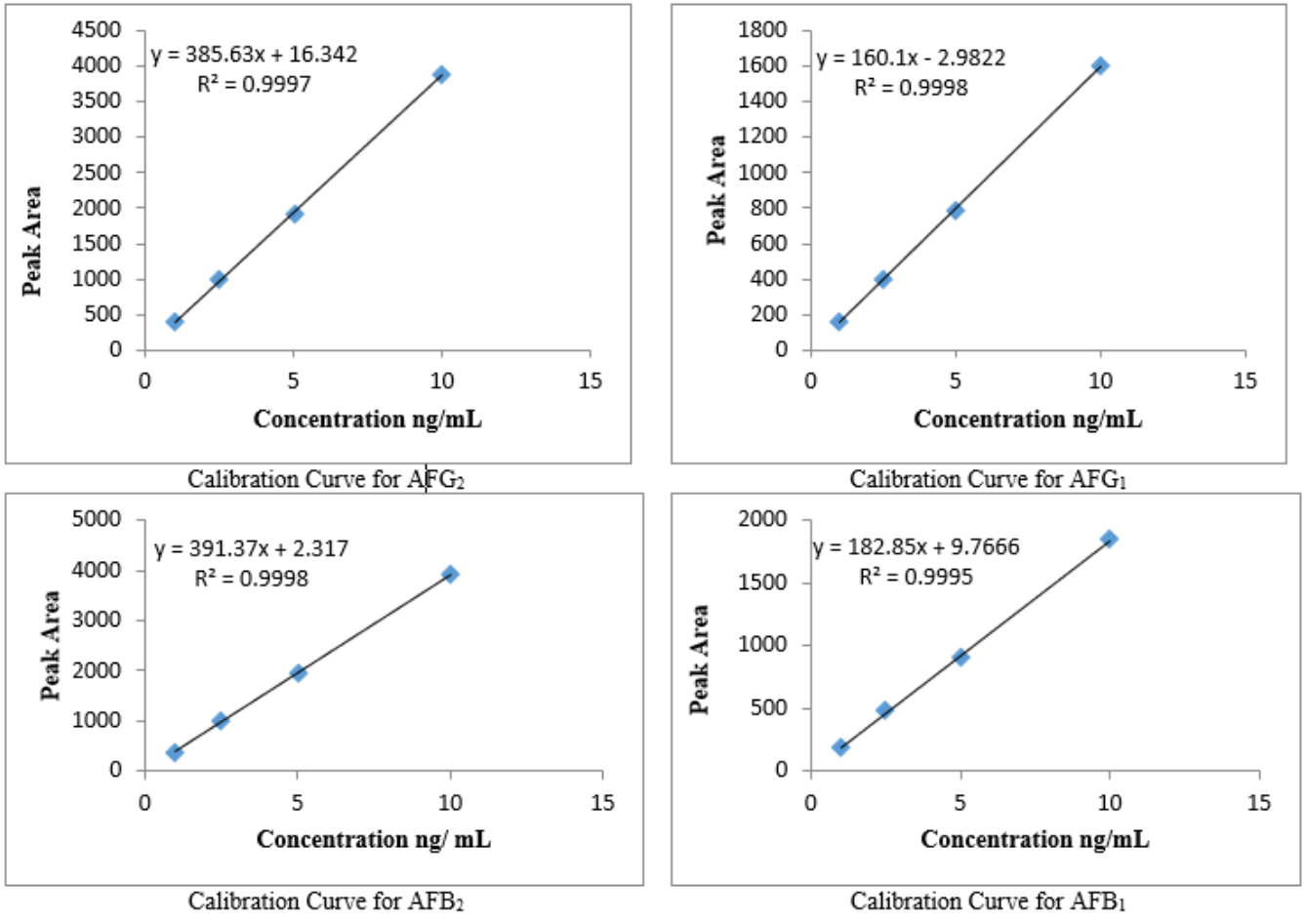

Figure 1: Calibration curves for AFB_1 AFG_1, AFB_2 and AFG_2

Determination of Limit of Detection, Limit of Quantitation and Percentage Recovery

The analytical method was evaluated for its capability to quantify total aflatoxins and aflatoxin B_1. The accuracy of the method was verified by conducting a recovery test, and the percentage recovery for aflatoxin-spiked samples was calculated using equation 1. For all types of collected chicken feeds, the recovery percentages ranged between 70.4% and 106.6%, and for eggs, between 70.2% and 83.2% (Tables 2 and 3). These values are within the acceptable range of 70% to 120% (Shah et al. 2000).

Table 2: Percentage Recoveries of Aflatoxins in Selected Chicken Feed Samples

Aflatoxins	Unspiked Concentration. (ng g^{-1})	Spiked Concentration (ng g^{-1})	Detected Concentration (ng g^{-1})	% Recovery
AFG_2	0.00	5	5.33	106.60
AFG_1	0.00	5	3.52	70.40
AFB_2	0.00	5	5.09	101.80
AFB_1	0.00	5	5.01	100.20

Table 3: Percentage Recoveries of Aflatoxins in Selected Eggs Samples

Aflatoxins	Unspiked Concentration (ng g^{-1})	Spiked Concentration (ng g^{-1})	Detected Concentration (ng g^{-1})	% Recovery
AFG_2	0.00	5	3.95	79.0
AFG_1	0.00	5	3.51	70.2
AFB_2	0.00	5	3.86	77.2
AFB_1	0.00	5	4.16	83.2

The sensitivity and accuracy of analytical techniques are assessed by the Limit of Detection (LOD) and Limit of Quantification (LOQ). For AFB_1, AFB_2, AFG_1, and AFG_2, the LOD values were recorded between 0.13 and 0.16 ppb, while the LOQ values varied from 0.16 to 0.29 ppb, as indicated in Table 4. These results confirm that our methods are suitable for detecting and quantifying analytes at low concentrations. The method's capacity to precisely detect minimal analyte levels is reflected in the low LOD and LOQ values, as discussed by Taleuzzaman (2018).

Table 4: Limit of Detection and Limit of Quantitation for Each Aflatoxin

Aflatoxins	LOD (ng/mL)	LOQ (ng/mL)
AFG_2	0.125	0.163
AFG_1	0.133	0.214
AFB_2	0.131	0.177
AFB_1	0.160	0.292

Variations of Aflatoxin B_1 in Different Types of Chicken Feeds Between the Sampled Locations in Dar es Salaam

All 63 chicken feed samples from agrovet shops and poultry farms were found to be highly contaminated with total aflatoxins (TAF) and aflatoxin B_1 (AFB_1). The concentrations of AFB_1 ranged from 2.30 to 29.21 ng g^{-1} and TAF from 6.61 to 45.94 ng g^{-1} in cotton seed hulls across the sampled agrovet shops. In sunflower seed hulls, AFB_1 concentrations varied from 2.31 to 132.62 ng g^{-1} and TAF from 3.93 to 150.48 ng g^{-1}. In maize bran, the ranges were 3.42 to 146.03 ng g^{-1} for AFB_1 and 8.65 to 245.47 ng g^{-1} for TAF, as depicted in Figures 2 and 3. Specifically, the maize bran samples from Manzese (146.03 ng g^{-1}), Bunju (142.63 ng g^{-1}), and Gongo la Mboto (129.76 ng g^{-1}) showed the highest AFB_1 contamination. For

TAF, the highest contaminations were noted in maize bran from Bunju (245.47 ng g^{-1}), Manzese (170.87 ng g^{-1}), Gongo la Mboto (153.10 ng g^{-1}), and Mbagala (90.89 ng g^{-1}). These findings indicate that maize bran and sunflower seed hulls are particularly prone to aflatoxin contamination and should be carefully treated when used in chicken and animal feed preparation. The results from this study align with those reported by Mwakosya et al. (2022). The variation in aflatoxin levels in poultry feed ingredients such as maize bran, sunflower seed hulls, and cotton seed hulls may be associated, to some extent, with the differing agro-ecological origins of these materials, as pointed out by Nsiah et al. (2023).

For maize bran chicken feed ingredients, all analysed samples showed varying levels of aflatoxin contamination. The levels of contamination of AFB_1 in these samples ranged from 3.42 to 146.03 ng g^{-1}. The mean concentration of AFB_1 varied across sampling sites (Figure 2). The mean concentration of the total aflatoxins (TAF) ranged from 8.65 to 245.47 ng g^{-1}. Notably, maize bran samples from Kitunda and Kigamboni exhibited lower contamination levels compared to other locations, followed by Mbagala (MB). The low levels of contamination observed at Kitunda (KT) and Kigamboni (KG) samples can be attributed to short storage time, driven by high demand, as well as good storage conditions and facilities observed at these sites. Furthermore, samples from Kitunda site were observed to be dry and most of its agrovet shops maintained a hygienic environment. Conversely, samples from Bunju (BJ) recorded highest levels of contamination, followed by Manzese (MN) and Gongo la Mboto (GM). This heightened contamination of maize bran-based feed sample could be linked to substandard storage practices observed in agrovet shops around these areas, where feeds were often stored in bags outside the shops, increasing moisture content. Furthermore, numerous human activities in these areas contributed to damp conditions conducive to fungal growth. The maize bran-based feed samples from Bunju were particularly moist, insect-damaged, and appeared rotten at the time of sampling, conditions that promote the proliferation of aflatoxin-producing fungi (Terezinha et al. 2013, Rajarajan and Rajasekaran 2013). The levels of AFB_1 detected in this study, ranging from 3.42 to 146.03 ng g^{-1}, are significantly higher than those reported in maize bran-based poultry feed in Tanzania (Kajuna et al. 2013), which ranged from below Detection Limit to 64 ng g^{-1}.

All samples of sunflower seed hulls tested positive for aflatoxins, including AFB_1 and TAF. The mean concentration of AFB_1 in the analyzed sunflower seed hulls varied considerably, ranging from 2.31 to 132.62 ng g^{-1}. The levels of aflatoxin B_1 contamination differed across various locations (Figure 2), with samples from Manzese, Bunju, Kigamboni, and Mbagala showing notably high levels of contamination.

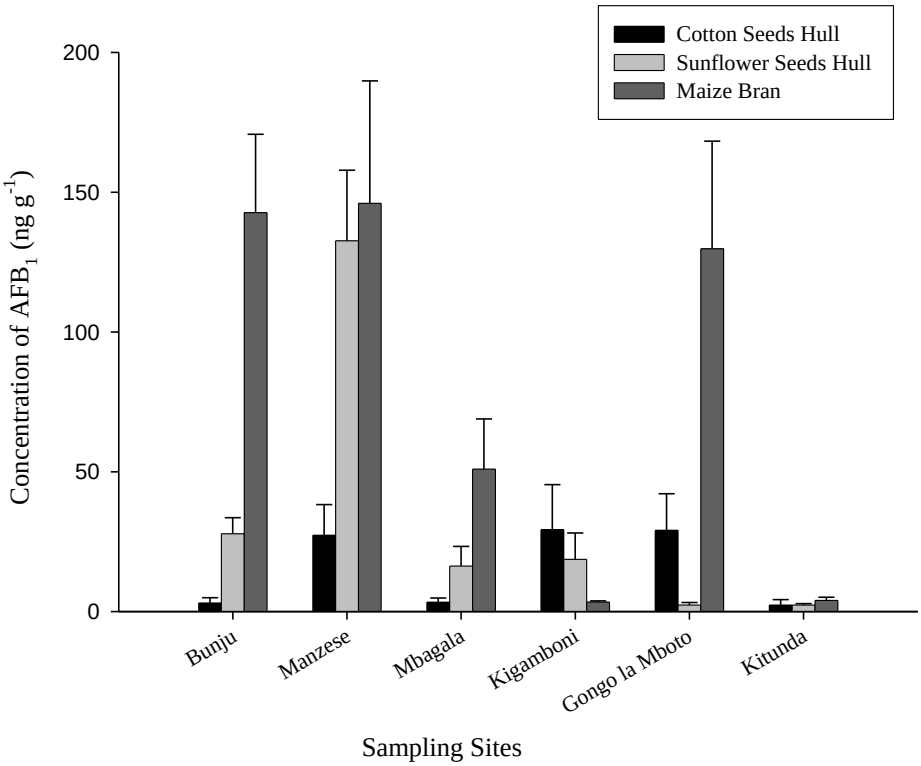

Figure 2: Variations of AFB_1 in cotton seeds hull, sunflower seeds hull and maize bran among the agrovet shops in Dar es Salaam.

Variations of Total Aflatoxins in Different Types of Chicken Feeds between the Sampled Locations in Dar es Salaam

Total aflatoxin (TAF) levels in sunflower seed hulls samples ranged from 3.93 to 150.48 ng g^{-1} across all six sampling sites. The highest TAF concentrations were recorded at Manzese (150.48 ng g^{-1}), followed by Mbagala (56.20 ng g^{-1}), Bunju (49.08 ng g^{-1}), and Kigamboni (33.83 ng g^{-1}). The higher contamination levels at these sites likely resulted from their proximity to numerous human activities, contributing to increased humidity and consequently fostering fungal growth as reported by Pratiwi et al. (2015). Poor hygiene at these sites also likely played a role in the high contamination levels. In some of the shops, sunflower seed hulls were stored directly on the floor, exposing them to moisture and making the feed prone to fungal attack. Conversely, lower levels of aflatoxins observed at Kitunda might be attributed to good storage practices and ventilation of agrovet stores and shops. At these sampling sites, feed sacks were kept on dry wood and adequately covered. In addition, high turnover due to strong demand also helped to keep contamination low. The mean concentration of AFB_1 in sunflower animal feed found in this study ranged from 2.31 to 132.62 ng g^{-1}, which is higher than findings reported by Mohammed et al. (2016) in Tanzania, where the mean concentration ranged from 2.184 to 20.465 ng g^{-1}. Similarly, the study conducted by Kajuna et al. (2013) in Morogoro, Tanzania, reported contamination levels ranging from below detection to 66 ng g^{-1}, which is lower than the concentrations reported in this study. Further, a study conducted in Pakistan (Chohan et al. 2016) reported contamination in sunflower seed hulls ranged from 12.39 to 39.21 ng g^{-1},

which is lower compared to the concentration levels reported in this study.

The susceptibility of maize bran to aflatoxin contamination can be attributed to the frequent infestation of maize by aflatoxin-producing fungi during pre-harvest, harvest, and postharvest stages. Furthermore, during the de-hulling process, maize is exposed to moisture through water spraying, thus, creating favorable conditions for the growth of aflatoxins producing fungi in the bran. The moisture influx leads to elevated moisture content in maize bran, providing an ideal environment for fungi proliferation. Sunflower seed hulls, which ranked second in contamination after maize bran in this study (Figures 2 and 3), can similarly be explained by the presence of oil residues rich in fatty acids in the sunflower seed hulls, creating a conducive environment for fungal growth. Notably, the variation in aflatoxin levels among chicken feed ingredients highlights the influence of handling conditions. It should further be noted that in Tanzania, maize bran and sunflower seed hulls are prominently used in poultry feed production due to their cost-effectiveness and widespread availability (Nishimwe et al. 2019, Mwakosya et al. 2022). This explains the possible high levels of aflatoxins in the manufactured poultry feeds from maize bran and sunflower seed hulls. Consequently, the high levels of aflatoxins in manufactured poultry feeds derived from these ingredients align with previous findings reported by Kajuna et al. (2013) and Nyangi et al. (2016). Overall, the significant contamination of raw materials with aflatoxins for chicken feeds signals a considerable risk of acute aflatoxicosis within the Tanzanian community.

It was observed, however, that cotton seeds hull had relatively low contamination of both AFB_1 and TAF compared to maize bran and sunflower seed hulls. A total of 17 samples of cotton seeds hull collected from the six sites in Dar es Salaam (Bunju, Manzese, Mbagala, Kigamboni, Gongo la Mboto and Kitunda) were all contaminated with aflatoxin B_1. The mean concentration of total aflatoxins in cotton seed hull was relatively high for samples collected from Manzese, Kigamboni and Gongo la Mboto. The relatively higher contamination levels were likely due to poor storage conditions observed at the sites. Most of the agrovet shops stored their feed in sacks and buckets kept on the floor, which might have facilitated the growth of aflatoxins producing fungi, due to moisture formed at the bottom part of sacks and buckets. Discussions with the shop owners revealed that most of the chicken feed ingredient stocks take a long time to be cleared because they are bought in bulky during low price period and stored for extended periods without proper drying. Furthermore, levels of AFB_1 were lower for samples collected from Bunju, Mbagala and Kitunda, which might be attributed to the better storage condition observed at these sites. Most of the agrovet shops from Kitunda were not close to other human activities, and the demand for chicken feed ingredients at Kitunda is very high. This minimizes mold growth and, consequently, aflatoxin production and contamination. Levels of AFB_1 found in the present study, ranging from 2.30 to 29.21 ng g^{-1}, were lower compared with the levels in cotton seed hulls feed ingredient, which ranged from 6.92 to 185.97 ng g^{-1}, similar to the results reported by Chohan et al. (2016) in Pakistan.

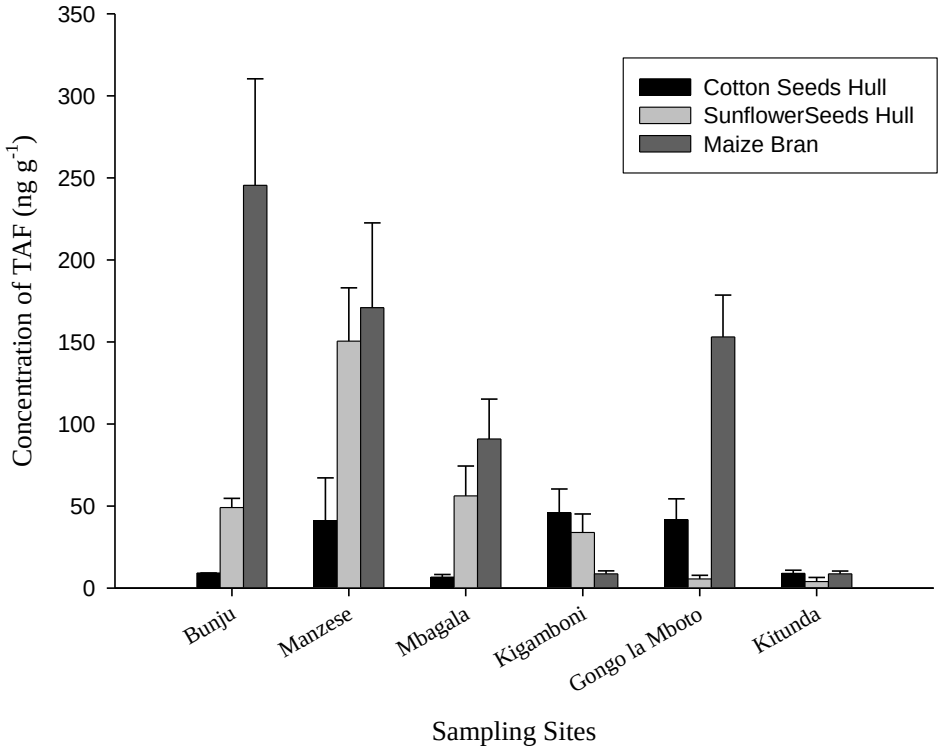

Figure 3: Variations of total aflatoxins (TAF) in cotton seeds hull, sunflower seeds hull and maize bran among the agrovet shops in Dar es Salaam

The analysis of chicken feeds collected from poultry farms revealed that all feeds were contaminated with AFB_1 and total aflatoxins at alarming levels, as shown in Table 5. The results indicated that chicken feeds collected from six poultry farms in Dar es Salaam were contaminated by AFB_1, ranging from 1.99 to 30.25 ng g^{-1} and with TAF ranging from 5.01 to 77.71 ng g^{-1}. Notably, 66.67% of the samples exceeded the tolerable limits of 20 ng g^{-1} total aflatoxin for feed ingredients.

Table 5: Levels and Variation of Aflatoxin B_1 and Total Aflatoxins among the Sampled Poultry Farms in Dar es Salaam.

		AFB_1	TAF
		(ng g^{-1})	(ng g^{-1})
Sites	n	Mean ± SD	Mean ± SD
Farm A	2	30.25±1.94	48.19±9.59
Farm B	2	5.46±0.45	9.65±0.28
Farm C	2	11.01±8.78	77.71±14.83
Farm D	2	1.99±0.25	5.01±1.94
Farm E	2	28.21±5.74	38.95±3.20
Farm F	2	10.13±9.49	24.48±4.47

All samples from poultry farms were highly contaminated with aflatoxins, indicating that feeds from agrovet shops and/or manufacturers in Dar es Salaam are not safe for chicken consumptions. Total aflatoxin levels beyond the recommended maximum

tolerable limit were observed in feeds collected from Farm C (77.71 ng g^{-1}), Farm A (48.19 ng g^{-1}), Farm E (38.95 ng g^{-1}), and Farm F (24.48 ng g^{-1}). The high levels of aflatoxin contamination were likely due to the contamination of raw materials used in feed preparation. Keutchatang et al. (2022) reported that conditions in which feeds are produced or stored can promote the toxin production by aflatoxins producing fungi such as *Aspergillus*. During sampling, it was observed that the bags and sacks containing chicken feeds from Farm A and Farm C were laid on the floor outside the house, and some raw materials used as ingredients, such as maize bran, animal bone and animal blood were in poor condition and poorly stored. Feeds from Farm E and Farm F were wet and rotten, which might explain the high levels of contaminations in these feed samples. Additionally, chicken feeds collected from Farm A and Farm C were infested with insects, with some insect observed roaming on the feeds during sampling, contributing to contamination. In contrast, the feeds at Farm B and Farm D were in good condition, resulting in low contamination levels. Feeds from Farm D were dry during sampling, and the chicken house was clean and well ventilated compared to all other sites. The presence of aflatoxins contamination in all sampled poultry feeds from the selected farms in Dar es Salaam raises concerns about the high probability of aflatoxins transfer into poultry products such as chicken meat and eggs, which may pose human health risks.

Despite of the high levels of aflatoxin contamination in chicken feeds from the poultry farms, the eggs collected from the respective farms were not contaminated. This lack of contamination in egg samples might be due to the limited exposure time of laying hens to the contaminated feeds. For aflatoxin residue to appear in eggs, the laying hens must be exposed to highly contaminated feed continuously for a longer period. Similar studies that reported aflatoxins contamination in eggs, the laying hens were fed continuously with feed containing higher levels of contamination than those detected in this study (Herzallah, 2013, Salwa et al. 2009). A study conducted by Herzallah (2013) at Karak, Jordan, on aflatoxins residue in eggs and flesh of laying hens fed on contaminated feed, revealed that the aflatoxins residue observed in eggs was 0.66 ng g^{-1} for hens fed on contaminated feed with an aflatoxin concentration of 894.12 ng g^{-1} continuously for six weeks. The levels of aflatoxins in the feed fed to laying hens were sixty times higher than the concentration of aflatoxins found in chicken feeds in the present study. Another study conducted by Salwa et al. (2009) in Cairo, Egypt, revealed that levels of aflatoxins in eggs varied depending on the concentration of aflatoxins in feed fed to the laying hens continuously for sixty days. Hens fed with feed containing 25 ng g^{-1} of aflatoxins continuously for sixty days were reported to have 0.04 ng g^{-1} of aflatoxin B_1 in their eggs. Other groups of hens were fed on contaminated feed with concentration of 50 and 100 ng g^{-1} for sixty days, resulting in aflatoxins residue in eggs of 0.05 and 0.07 ng g^{-1}, respectively. Therefore, the mean concentration of aflatoxins detected in feed in this study and the duration of exposure of laying hens to these feeds was probably not sufficient to cause the carryover of aflatoxin residues in eggs.

Conclusion

The data reported in this study indicates that all samples of poultry feed ingredients and feeds were contaminated with aflatoxins. Poor handling and long-term storage of feed in stores seems to be the contributing factors to the contamination of aflatoxins in animal feed. Aflatoxins were not detected in egg samples; implying that the carryover of aflatoxin residues in eggs was below the limit of detection (LOD). Although aflatoxin contamination was not detected in eggs, this does not conclude that contaminated feed had no effect on chicken products. Aflatoxin residues can accumulate in other parts of the chicken body such as liver, bones, and meat. It is therefore recommended that further studies be conducted to investigate the levels of aflatoxin in chicken organs such as liver, meat, and bones. Furthermore, it is necessary to create awareness among shopkeepers and

farmers regarding the health risk associated with aflatoxins contamination of foodstuff.

Declaration of Conflict of Interest

The authors declare that there is no competing conflict of interest regarding this publication.

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