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Occurrence, Distribution and Variations of Aflatoxins in Chicken Organs with Respect to Chicken Age

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Abstract

Aflatoxins, toxic secondary metabolites produced naturally by fungi, pose a serious threat to food safety by contaminating a wide range of agricultural crops and animal products. While previous studies have examined aflatoxin levels in poultry feed and selected tissues, this study presents a comprehensive and previously unexplored systematic analysis of aflatoxin distribution across multiple chicken organs (meat muscles, heart, liver, gizzard, and crop) in relation to chicken age and feed contamination. A total of 75 organ samples and 6 feed samples were analyzed using high-performance liquid chromatography coupled with florescence detector. The analysis revealed that 65.3% of organ samples were contaminated by total aflatoxins with concentrations varying differently across organs. Notably, the crop exhibited the highest aflatoxin levels (4.33-12.74 ng/g), followed by the liver (2.41-7.98 ng/g), gizzard (1.53-5.53 ng/g), heart (1.78-3.02 ng/g) and meat (0.95-2.64 ng/g). Importantly, while grower feed showed high contamination (98.08 ± 0.76 ng/g), all edible organ (meat muscles, heart, liver and gizzard) samples remained below regulatory limits (5 ng/g for AFB_1 and 10 ng/g for total aflatoxins). The study offers critical insights into organ specific aflatoxin bioaccumulation patterns in chickens, an area that has been underexplored. The identification of high risk organs such as the crop and liver underscores potential food safety concerns. These findings are significant for public health risk assessment and highlight the need for targeted monitoring and regulatory actions to minimize dietary exposure to aflatoxins.

Keywords: Aflatoxins; Broilers; Chicken products; Food safety; HPLC analysis

Introduction

Aflatoxins are toxic secondary metabolites produced by fungal species, namely, *Aspergillus parasiticus* and *Aspergillus flavus,* which are commonly found in soil and decaying vegetation (Bankole and Adebanjo 2003). Among the 18 known types of aflatoxins, the four most prevalent are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). Aflatoxin B_1 is the most prevalent, comprising 60-80% of total aflatoxins, and it is the most harmful due to its direct link to liver cancer (Negash 2018). Since their discovery, aflatoxins have been recognized globally for causing various health problems in both livestock and humans (Ali 2019). They have been directly associated with liver cancer (hepatocellular carcinoma) through their bioactivation in the body (Globocan 2020). Liver cancer is one of the leading cancers in Africa, with aflatoxins suspected to cause 30% of all cases (Ndom 2019, Lyimo et al 2020). According to the global cancer estimates in the United Republic of Tanzania, liver cancer ranks the eighth among the top ten leading cancers in Tanzania (Globocan 2020). Different studies have reported significant aflatoxin contamination in foodstuffs (Magoha et al. 2016, Mohamed 2017, Mtega

et al. 2020) and on animal feeds (Muja et al. 2024). However, despite of the studies which have been conducted, only few studies have been on animal products such as milk and chicken eggs (Mohammed et al. 2016, Muja et al. 2024, Mwakosyaa et al. 2022) leaving a need for more investigation on aflatoxin accumulation in chicken products.

Tanzania has witnessed a significant increase in chicken meat consumption in recent years (Ringo and Lekule 2020). The introduction of exotic chicken breed has further promoted this trend, particularly in urban areas due to their relatively lower price and shorter raising period compared to indigenous chicken breed (Poultry Policy Briefs 2019). However, the risk of aflatoxin exposure is notably higher in exotic chicken, as their diets often rely on crops leftovers such as maize bran, cotton seed hulls and sunflower seed hulls, which are highly prone to mycotoxin contamination (Binder et al. 2007, Keutchatang et al. 2022). Furthermore, of the two categories of exotic chickens; broilers and layers, broilers are more susceptible to aflatoxin exposure due to their significantly higher daily feed intake, which is two to three times greater than that of layers. A study in Punjab, Pakistan by Iqbal et al. (2014) on aflatoxin levels in broilers, layers, and domestic chicken meat and liver, revealed higher concentrations in broilers compared to layers and domestic chicken. In Tanzania, data on aflatoxin contamination in broiler meat and organs, particularly in major urban centers like Dar es Salaam, are scarce. Despite findings by Muja et al. (2024) on the occurrence and variations of total aflatoxins and aflatoxin B_1 in different types of chicken feeds marketed in Dar es Salaam, Tanzania, revealed high contamination with aflatoxins, with levels beyond the recommended tolerable limits, there has been no comprehensive assessment of how this contamination translates into aflatoxin residues in chicken tissues and organs (Muja et al. 2024). Given the high consumption of broiler meat and the potential health risks associated with aflatoxin exposure, this knowledge gap is concerning. This study therefore provides a comprehensive and previously unexplored analysis of the occurrence, distribution, and variation of aflatoxins in different chicken organs (meat muscles, heart, liver, gizzard, and crop) in relation to chicken age and feed contamination. The findings aim to inform food safety risk assessments and guide interventions to protect public health in Tanzania and similar contexts.

Materials and Methods

Sample collection

A total of 30 broiler chicks, aged two weeks and their feed were purchased for the study. Broilers were selected due to their widespread commercial production and high consumption rates in Tanzania, particularly in urban areas. Their rapid growth rate and higher daily feed intake two to three times more than layers make them more vulnerable to aflatoxin exposure, providing a relevant model for assessing potential food safety risks. Aflatoxin levels were analysed in both the feed being consuming at the time of purchase (starter pellets) and the feed the broilers were transitioning to (grower mash). The chicks were then raised under standard conditions to the age of seven weeks. Starting at three weeks of age, three broilers were randomly selected each week for organ analysis. Following humane slaughter, five specific organs (meat muscles, crop, gizzard, liver and heart) were collected from chicken. These organs were chosen based on their relevance to human consumption and food safety risk. Meat muscles, liver, gizzard, and heart are commonly consumed in Tanzania, unlike organs such as kidneys or intestines which are less frequently eaten. The crop, although not typically consumed, was included due to its potential to retain high levels of aflatoxins from ingested feed, making it a valuable indicator of exposure and accumulation. Aflatoxin levels were analyzed in these organs weekly, from three to seven weeks. In total, 75 chicken organ samples and six feed samples were collected and analyzed in triplicate throughout the study. All organ samples were individually ground, packed in plastic bags, stored in a cool box at −4 °C, and transported to the Tanzania Bureau of Standards (TBS) laboratories for aflatoxin analysis.

Chemicals and reagents

The chemicals used for aflatoxin analysis included aflatoxin standards (AFB_1, AFB_2, AFG_1 and AFG_2) and Aflacolumns (Immunoaffinity columns), both purchased from Romer labs, Austria. High-performance liquid chromatography (HPLC) grade solvents, including methanol, acetonitrile, and hexane were purchased from Sigma- Aldrich Inc., while sodium chloride was purchased from Merck, Darmstadt, Germany.

Experimental procedures

Extraction of chicken organs samples

Approximately 25 g of ground chicken organs were measured separately using an analytical balance, followed by addition of 2 g sodium chloride and 100 mL methanol: water (70:30 v/v) on each. The mixture was shaken in a gyratory shaker for 30 minutes and filtered. From each filtrate, 10 mL was mixed with 5 mL of hexane and vigorously shaken in a separating funnel until distinct layers formed. The aqueous layer was collected for further analysis, while the lipid layer was discarded.

Extraction of aflatoxins from chicken feeds

Approximately 25 g of ground chicken feed samples (starter and grower mash) were measured using an analytical balance. Each sample was then mixed with 100 mL of an extracting solvent (methanol: water, 70:30 v/v) and shaken in a gyratory shaker for 30 minutes. The mixture was then filtered using Whatman No. 1 filter paper.

Dilution and sample clean-up

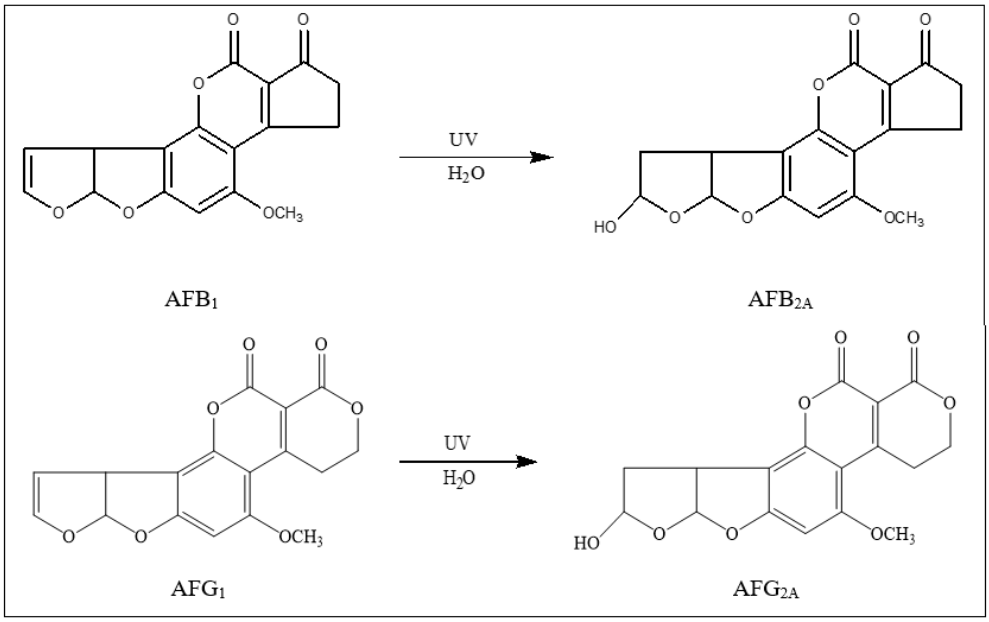
A 4 mL portion of each filtrate was transferred into Teflon tubes, followed by the addition of 8 mL of distilled water. The mixtures were vortexed for 30 seconds and then loaded onto aflacolumns, allowed to elute by gravity. The columns were washed by rinsing the Teflon tubes with 10 mL distilled water.

Sample elution

After the clean-up process, the adsorbed aflatoxins were eluted using 1.5 mL of methanol, and the eluates were collected in vials. A 300 µL portion of each eluate was then transferred to separate vials and mixed with 700 µL of the mobile phase (water: acetonitrile, 6:1 v/v)). The mixtures were vortexed for 30 minutes before being injected into an HPLC-FLD for aflatoxin analysis using post-column derivatization.

Derivatization

The native fluorescence of AFB_2 and AFG_2 is stronger compared to that of AFB_1 and AFG_1. To enhance their detectability and improve fluorescence, AFB_1 and AFG_1 were derivatized into AFB_{2A} and AFG_{2A} through post-column derivatization as shown in Scheme 1.



Scheme 1: Derivatization Reactions of AFB_1 and AFG_1 into AFB_{2A} and AFG_{2A} to Improve their Detection in HPLC (Wacoo 2014).

HPLC analysis

A 50 µL volume of eluates and aflatoxin standards was injected into a reverse phase HPLC for aflatoxin detection. The mobile phase consisted of water, methanol, and acetonitrile in a 6:3:1 v/v ratio. Separation was carried out using a C_{18} column at 30 °C with a flow rate of 1.2 mL/min. Aflatoxin detection was achieved at an excitation wavelength of 360 nm and.an emission wavelength of 465 nm. Distinct peaks were observed, and aflatoxin concentrations were computed accordingly.

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

The performance characteristics of the analytical method were evaluated in terms of recovery test, limit of detection (LOD) and limit of quantification (LOQ). For the recovery test, chicken meat muscles, liver and feed samples were spiked in triplicates with known concentrations (2 ng/g) of aflatoxins standards (AFB_1, AFB_2, AFG_1, and AFG_2). Both spiked and unspiked samples were analyzed, and the results were used to calculate the percentage recovery, which ranged from 70.3 to 98.8%.

The LOD and LOQ values were determined by analysing a series of aflatoxin standards at concentrations ranging from 1 to 10 ng/mL. The peak areas obtained from these standards were used to construct calibration curves, which were then utilized to calculate the LOD and LOQ values (Ribani et al. 2007). The LOD values for AFB_1, AFB_2, AFG_1, and AFG_2 ranged from 0.101 to 0.122 ng/mL, while the LOQ values ranged from 0.120 to 0.169 ng/mL.

Statistical analysis

A one-way Analysis of Variance (ANOVA) was conducted to determine whether there were statistically significant differences in aflatoxin concentrations among different chicken organs (meat muscles, heart, liver, gizzard, and crop) across the sampling weeks using MaxStat Lite software. The organs were treated as the independent variable, while aflatoxin concentration served as the dependent variable. Data were first tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. A significance level of $p < 0.05$ was considered statistically significant. All quantitative results were expressed as mean ± standard deviation (SD).

Results and Discussion

Levels of aflatoxins in chicken feed

Broiler feeds (starter and grower mash) were analyzed to determine the levels of aflatoxin, and the results are presented in Table 1.

Table 1: Levels of AFB_1, AFB_2, AFG_1, AFG_2 and TAF in Chicken Feeds

Feed Type	n	AFB_1 (ng/g)	AFB_2 (ng/g)	AFG_1 (ng/g)	AFG_2 (ng/g)	TAF (ng/g)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Starter pellets	3	ND	ND	ND	ND	ND
Grower mash	3	62.3 ± 6.08	9.1 ± 0.77	21.75 ± 0.46	4.93 ± 0.34	98.08 ± 0.76

Levels of aflatoxins in the broiler's starter pellets were below the detection limit, suggesting that this feed was likely prepared from fresh ingredients uncontaminated ingredients that had not been stored for prolonged periods. Additionally, the pellet form of the feed might have contributed to the absence of detectable aflatoxins, as the pelleting process typically involves exposure to high temperatures (approximately 80 °C) forced drying. These conditions are known to reduce fungal growth and subsequent mycotoxin production (Binder et al., 2007).

The grower mash feed was found to have detectable levels of aflatoxins contamination. This feed contained all four major groups of aflatoxins, with AFB_1 comprising approximately 63.5% of the total aflatoxin concentration (98.08 ± 0.76 ng/g). These findings are consistent with previous studies reported AFB_1 as the predominant form of aflatoxins in contaminated agricultural

products, constituting about 60−80% of total aflatoxins (Binder et al. 2007, Rawal et al. 2010, Negash 2018). The prevalence of Aflatoxin B_1 (AFB_1) in poultry feed, as well as other agricultural products, reflects a consistent global trend of contamination that has been documented in several regions, including Nigeria, and Kenya. For instance, in Nigeria, Mgbeahuruike et al. (2020) reported AFB_1 as the predominant aflatoxin in poultry feed samples, with levels frequently exceeding regulatory limits, thus indicating a severe contamination issue within that region. In Kenya, research conducted by Mutegi et al. (2013) corroborated these findings, showing that AFB_1 was the most frequently detected aflatoxin in both feed and maize samples. This particular study highlighted the widespread contamination of agricultural products in Kenya, stressing the need for strict monitoring and management of aflatoxin levels in food supplies. The grower mash feed tested in this study exhibited AFB_1 concentrations above the regulatory limit of 10 ng/g (FAO/WHO 2017). The contamination of the grower mash feed could have resulted from poor storage conditions observed at the vendor's store, where feeds were stored in sacks placed directly on the floor. Keeping sacks on the floor facilitates the penetration of moisture and heat into the feed, conditions that favor fungal growth. Additionally, poultry feeds are prepared using ingredients such as maize bran, cotton seeds hulls, and sunflower seeds hulls which are normally highly contaminated with aflatoxins (Muja et al. 2024). Due to this, poultry feed is at a high risk of aflatoxin contamination. Studies conducted on poultry feeds in Tanzania (Mushi et al. 2018, Muja 2020, Mwakosya et al. 2022) have reported high levels of aflatoxins in feeds. For instance, Muja (2020) conducted a study to determine levels of aflatoxins on marketed feeds in Dar es Salaam, detecting total aflatoxins contamination ranging from 6.61 to 45.94 ng/g in cotton hulls-based feed, 3.93 to 150.48 ng/g in sunflower seed hulls-based feed and 8.65 to 245.47 ng/g in maize bran-based feed. Another study by Mushi and co-workers (2018) in Arusha reported the presence of AFB_1 ranging from 1.1 to 80.1 ng/g in poultry feeds.

Accumulation of different types of aflatoxins and total aflatoxins (TAF) in chicken organs with respect to age

Chicken organs were analyzed for aflatoxins contamination weekly from the age of 3 to 7 weeks. Levels of aflatoxins in chicken organs showed increased accumulation with prolonged exposure to the same type of feed (grower mash feed) used from the age of 2 to 7 weeks, as shown in Tables 2− 6. The retention of aflatoxins varied among organs; some organs such as crop, and liver, showed a notable increase in aflatoxins while others, like gizzard, meat muscles and heart, exhibited slower increase.

Aflatoxins were detected in liver samples from chicken aged 4 to 7 weeks, as summarized in Table 2. No detectable levels of aflatoxins were observed in liver samples from chickens of 3 weeks old, likely due to the short time period for broilers to accumulate detectable levels of aflatoxins in their body tissues. All groups of aflatoxins were present, with AFB_1 showing the highest concentration across all weeks. However, the observed aflatoxin concentrations were within the acceptable limits of 10 ng/g for total aflatoxins and 5 ng/g for AFB_1 (PACA 2016, FAO/WHO 2017).

Table 2: Variations of Aflatoxins Concentration (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) Observed in the Liver at Different Chicken Age Intervals

Chicken age (weeks)	n	AFB_1 (ng/g) Mean ± SD	AFB_2 (ng/g) Mean ± SD	AFG_1 (ng/g) Mean ± SD	AFG_2 (ng/g) Mean ± SD	TAF (ng/g) Mean ± SD
3	3	ND	ND	ND	ND	ND
4	3	1.09 ± 0.15	0.67 ± 0.13	0.65 ± 0.13	ND	2.41 ± 0.25
5	3	1.71 ± 0.11	0.83 ± 0.73	0.25 ± 0.25	0.38 ± 0.18	3.16 ± 0.67
6	3	1.91 ± 0.09	0.76 ± 0.41	1.66 ± 0.27	0.30 ± 0.19	5.31 ± 1.72
7	3	4.24 ± 0.33	1.44 ± 0.75	2.25 ± 0.64	0.71 ± 0.18	7.98 ± 1.09

Levels of AFB_1 in the liver were observed to increase continuously with time, as depicted in Figure 1. The highest concentration (4.24 ± 0.33 ng/g) of AFB_1 was observed in the seventh week, while the lowest concentration (1.09 ± 0.15 ng/g) was recorded in the fourth week. For edible organs, the liver exhibited the highest concentrations of AFB_1, highlighting its ability to accumulate aflatoxins with prolonged exposure via aflatoxin-contaminated feedstuff.

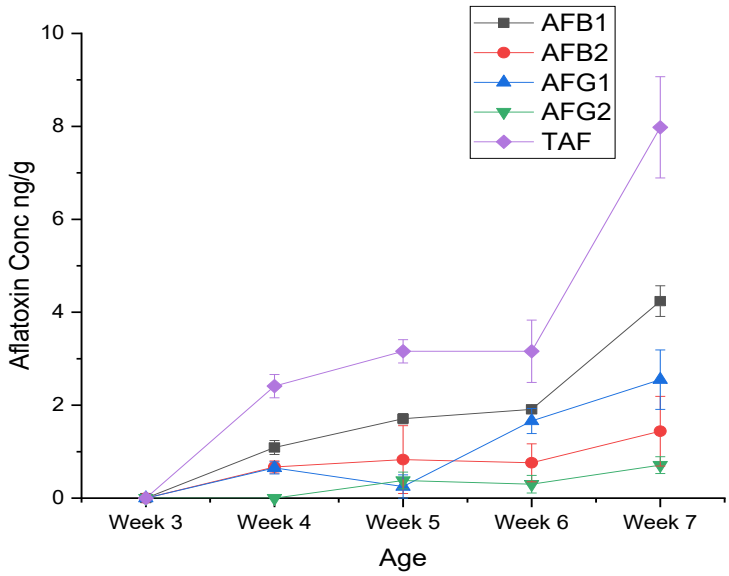


Figure 1: Accumulation and Variations of AFB_1, AFB_2 AFG_1 and AFG_2 and Total Aflatoxins (TAF) in the Liver with Respect to Age

The results obtained in this study are consistent with the findings reported by Hussain et al. (2010), which demonstrated an increase in aflatoxin B_1 (AFB_1) levels in poultry liver with prolonged exposure to contaminated feed. According to Yiannikouris and Jouany (2002), aflatoxins normally undergo biotransformation in the liver, where a portion of their metabolites and residues become fixed in the tissues, while the rest are excreted via urine and feces. Given that the liver is the primary organ for detoxification and metabolic transformation, it often retains the highest levels of aflatoxin residues. These residues can also be transported to other organs through systemic circulation. Since older chicken have higher feed intake than young ones, this increases their risk of aflatoxins accumulation than young chickens. Furthermore, as chicken age, lipid accumulation in tissues often increases, proving more binding sites for lipophilic compounds like aflatoxin, further enhancing the potential for bioaccumulation. The findings of this study further corroborated by those of El-Desouky et al. (2014) in Egypt, who reported AFB_1 contamination in 45% of liver samples, 32% of gizzard samples, and 25% of heart samples sold in retail markets, with the liver exhibiting the highest concentration levels. Similarly, Sineque et al. (2017) in Mozambique reported relatively high levels of AFB_1 in 39% of 100 broiler liver samples and 13.8% of 80 gizzard samples collected from both home-grown and industrial poultry production sectors in Maputo. Both studies highlighted the liver as the most contaminated organ, aligning closely with the organ specific aflatoxin distribution observed in this study.

AFB_2 was detected in chicken organs starting from the fourth week, with the highest concentration (1.44 ± 0.75 ng/g) observed in the seventh week (Table 2). However, AFB_2 did not show a substantial increase across consecutive weeks, with a slight decrease

noted in the sixth week (Figure 1). AFG_1 was observed in liver samples from the fourth to the seventh week. Levels of AFG_1 showed a progressive increase with time, except for a slight decrease in the fifth week (Figure 1). The gradual increase observed in other weeks indicates that AFG_1 accumulated in the liver at a similar pace to AFB_1. AFG_2 was detected starting from the fifth week onwards, with the highest concentration (0.71 ± 0.18 ng/g) observed in the seventh week. Levels of AFG_2 fluctuated throughout the study period, indicating lower accumulation compared to other groups of aflatoxins (Figure 1). The high concentration of TAF observed was primarily contributed by levels of individual groups such as AFB_1 and AFG_1. Furthermore, the liver exhibited the highest concentration of total aflatoxins compared to other edible organs such as meat muscles, heart, and gizzard, reflecting its role as the target organ for aflatoxins detoxification (Bbosa et al. 2013).

A one-way analysis of variance (ANOVA) was applied to determine if the observed differences in mean total aflatoxin concentrations (TAF) between the liver and other organs were statistically significant. The results indicated that there was no significant difference in aflatoxins accumulation in several organs, as the observed *p* values of the crop ($p = 0.16$), heart ($p = 0.08$) and gizzard ($p = 0.25$) were all greater than 0.05. However, meat muscles showed a statistical significantly difference in aflatoxin accumulation with a *p* value less than 0.05 ($p = 0.04$), indicating that, the rate of aflatoxins accumulation varied significantly between the liver and meat muscles. This difference can be attributed to the liver's role as the primary organ responsible for detoxifying contaminants (Chen et al. 2023). Upon ingestion, aflatoxins are transported to the liver for biotransformation, resulting in higher levels of aflatoxins present in the liver compared to meat muscle. in higher aflatoxin levels in the liver compared to meat muscles. Additionally, the liver's robust blood supply, further increases its exposure to aflatoxins.

A similar trend was observed in crop samples, where aflatoxins were detected from the fourth to the seventh week, as presented in Table 3. The highest total aflatoxins concentration (12.74 ± 2.40 ng/g) was observed in the seventh week, exceeding the maximum acceptable limit of 10 ng/g for TAF. Additionally, the concentration of AFB_1 on the seventh week (6.62 ± 1.20 ng/g) also surpassed the maximum acceptable limit of 5 ng/g (PACA 2016, FAO/WHO 2017).

Table 3: Variations of Aflatoxins Concentration (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) Observed in the Crop at Different Chicken Age Intervals

Chicken Age (weeks)	n	AFB_1 (ng/g)	AFB_2 (ng/g)	AFG_1 (ng/g)	AFG_2 (ng/g)	TAF (ng/g)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
3	3	ND	ND	ND	ND	ND
4	3	2.19 ± 0.38	0.66 ± 0.06	1.31 ± 0.43	0.18 ± 0.18	4.33 ± 0.62
5	3	2.23 ± 0.88	1.79 ± 0.64	1.36 ± 0.03	1.00 ± 0.11	6.48 ± 0.24
6	3	4.69 ± 0.74	0.88 ± 0.13	3.28 ± 0.23	0.59 ± 0.38	9.42 ± 1.46
7	3	6.62 ± 1.20	2.51 ± 0.61	2.91 ± 1.55	1.66 ± 0.06	12.74 ± 2.40

AFB_1 exhibited higher accumulation in the crop compared to the other aflatoxin groups, as shown in Figure 2. The concentration of AFB_1 in the crop ranged from 2.19 to 6.62 ng/g. These higher concentrations of AFB_1 observed in the crops across all weeks correlate well with feeds results, which showed a high concentration of 62.3 ± 6.08 ng/g for AFB_1. AFB_2 was detected consistently from the fourth week to the seventh week. Its concentrations varied across these weeks and did not exhibit a clear accumulation trend, as shown in Figure 2. AFG_1 was also observed from the fourth week throughout to the seventh week. Similar to AFB_2, AFG_1 did not show a distinct accumulation trend; there was a slightly increase in concentration from the fourth to the fifth week and a decrease in concentration on the seventh week, as shown in Figure 2. AFG_2

was detected from the fourth week to the seventh week as well. The concentrations of AFG_2 remained consistently low throughout this period, likely influenced by its low concentration observed in the feed. Consequently, AFG_2 did not display a clear accumulation trend, as illustrated in Figure 2.

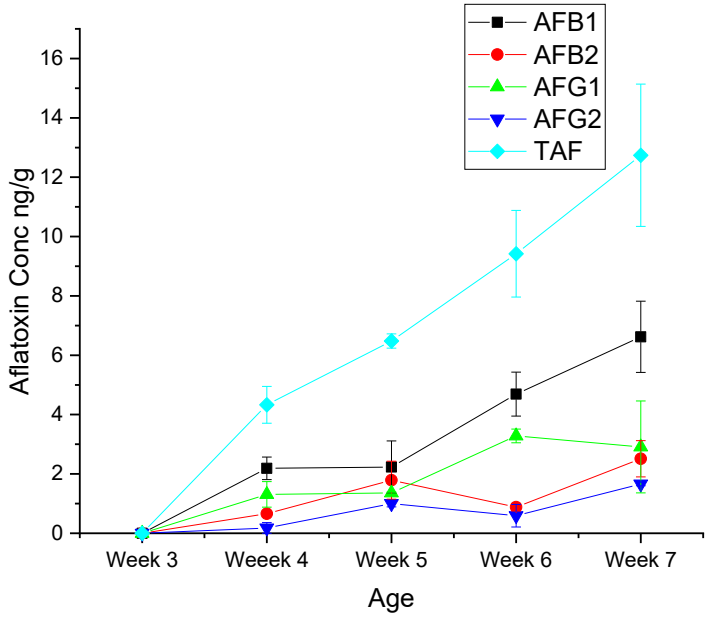


Figure 2: Accumulation and Variations of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) in the Crop with Respect to Chicken Age

Total aflatoxin recorded in the crop over four weeks showed a consistent increase, indicating accumulation. The total aflatoxins exhibit a clear accumulation trend, reaching its peak in the seventh week with a concentration of 12.74 ± 2.4 ng/g. Based on the observation over these weeks, it is evident that aflatoxins accumulate in the crop more than other organs. This might be due to direct absorption of aflatoxins from feed into crop tissues, as aflatoxins are highly liposoluble compounds (Bbosa et al. 2013). The crop stores feed up to 12 hours, allowing ample time for maximum aflatoxins absorption.

Single factor ANOVA was applied to determine if the observed variations in mean aflatoxins concentration (TAF) between crop and other organs were significant. The results revealed a significant difference in aflatoxins accumulation in the heart (p = 0.02), meat muscles (p = 0.01), and gizzard (p = 0.04). This indicated that the rate of aflatoxins accumulation in the crop is indeed higher compared to these organs. However, the results indicated no significant difference in aflatoxins accumulation in the liver (p = 0.17), likely because the crop and liver both interact directly with aflatoxins through digestion in the crop and detoxification in the liver. Gizzard samples were found to have detectable levels of aflatoxins from the fourth week to the seventh, as presented in Table 4. Gizzard was the third most contaminated organ and the second most contaminated edible organ. The observed concentration of both total aflatoxin and AFB_1 for all weeks were below the acceptable limits of 10 ng/g for total aflatoxin and 5 ng/g for AFB_1, respectively.

Table 4: Variations of Aflatoxins Concentration (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) Observed in the Gizzard at Different Chicken Age Intervals

Chicken Age (weeks)	n	AFB_1 (ng/g) Mean ± SD	AFB_2 (ng/g) Mean ± SD	AFG_1 (ng/g) Mean ± SD	AFG_2 (ng/g) Mean ± SD	TAF (ng/g) Mean ± SD
3	3	ND	ND	ND	ND	ND
4	3	0.58 ± 0.21	0.16 ± 016	0.55 ± 0.09	0.24 ± 0.03	1.53 ± 0.12
5	3	1.29 ± 0.23	ND	0.69 ± 0.54	0.12 ± 0.12	2.10 ± 0.61
6	3	1.56 ± 0.54	0.64 ± 0.44	0.92 ± 0.22	0.12 ± 0.04	3.24 ± 0.60
7	3	1.91 ± 0.14	0.51 ± 0.36	2.71 ± 0.71	0.41 ± 0.13	5.53 ± 1.05

Levels of AFB_1 increased consistently over the four weeks, indicating its accumulation in the gizzard as shown in Figure 3. A study conducted by El-Desouky et al. (2014) also identified the gizzard as the second most contaminated edible organ after liver. According to El-Desouky et al. (2014), 32% of 60 gizzard sample were contaminated with AFB_1, with a maximum concentration of 0.92 ng/g.

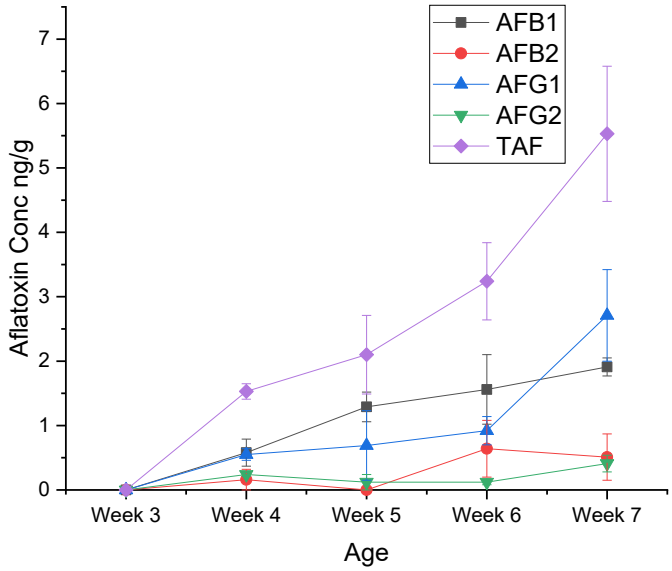


Figure 3: Accumulation and Variations of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) in the Gizzard with Respect to Chicken Age

AFB_2 was detected only in the fourth, sixth and seventh weeks. Its concentrations fluctuated, indicating no accumulation in this organ, as shown in Figure 3. AFG_1 was observed every week from the fourth to the seventh week, with levels increasing consistently throughout these four weeks, as depicted in Figure 3. AFG_2 was present at low concentrations in all four consecutive weeks as presented in Figure 3. However, the highest concentration of AFG_2 (0.41 ± 0.13 ng/g) was observed in the seventh week.

Total aflatoxins observed over the four consecutive weeks showed a consistent increase, as shown in Figure 3. Higher concentrations of aflatoxins were found in the gizzard, likely due to its role in the feed digestion. A single factor analysis of variance (ANOVA) was performed to compare TAF between gizzard and other organs. Statistically, there was no significant difference in the mean of TAF accumulated in the liver ($p = 0.33$), heart ($p = 0.25$) and meat muscles ($p = 0.14$). This suggests that the rate of aflatoxin accumulation in gizzard is similar to these organs. However, the differences in the mean TAF for gizzard were found to be

significant compared to those observed in the crop ($p = 0.04$) with the p value less than 0.05. In heart samples, aflatoxins were detected starting from the fifth week onwards. Only a few groups of aflatoxins were detected, and at low concentrations, as presented in Table 5. In addition, the total aflatoxins concentration and AFB_1 observed in all weeks were within the tolerable limits.

Table 5: Variations of Aflatoxins Concentration (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) Observed in the Heart at Different Chicken Age Intervals

Chicken Age (weeks)	n	AFB_1 (ng/g) Mean ± SD	AFB_2 (ng/g) Mean ± SD	AFG_1 (ng/g) Mean ± SD	AFG_2 (ng/g) Mean ± SD	TAF (ng/g) Mean ± SD
3	3	ND	ND	ND	ND	ND
4	3	ND	ND	ND	ND	ND
5	3	0.97 ± 0.08	0.36 ± 0.12	0.36 ± 0.11	ND	1.78 ± 0.40
6	3	1.14 ± 0.47	ND	0.59 ± 0.35	ND	2.09 ± 0.31
7	3	1.96 ± 0.66	0.45± 0.12	1.26 ± 0.22	ND	3.02 ± 0.89

AFB_1 was detected from the fifth week throughout to the seventh week. The levels of AFB_1 increased every week, indicating its accumulating in the heart, as presented in Figure 4. However, the concentrations of AFB_1 in the heart were relatively lower than those observed in the other organs, except for meat muscles. AFB_2 was observed only in the fifth and the seventh week, with fluctuating levels, indicating that it was not accumulating, Figure 4. AFG_1 was observed in the heart samples from the fifth to the seventh week. In each of these weeks, the concentration of AFG_1 increased, indicating accumulation, as depicted in Figure 4. AFG_2 was not detected at all in all weeks, which is probably due to its very low concentration observed in chicken feed. Total aflatoxins observed from the fifth to the seventh week showed a consistent increase, suggesting accumulation of aflatoxins in the heart. However, the rate of accumulation in the heart was lower compared to that of crop, liver, and gizzard, but higher than in meat muscles.

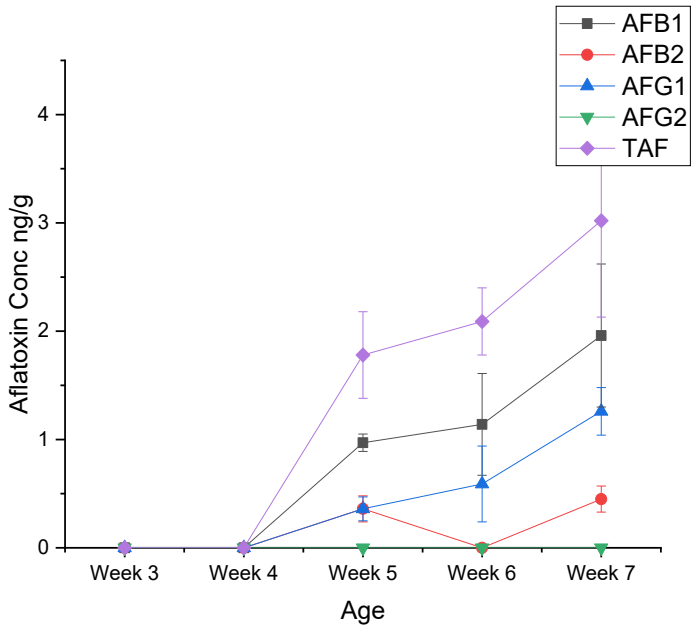


Figure 4: Accumulation and Variations of Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) in the Heart with Respect to Chicken Age

The low concentration of aflatoxins in the heart might be due to its lack of direct involvement in feed digestion and detoxification. Heart comes into contact with aflatoxins through the blood circulation system, which accounts for its low level of aflatoxins contamination. A single factor ANOVA was conducted to determine if the variations in mean TAF observed in heart was significantly different from the ones observed in other organs. The results revealed that TAF accumulation in the heart was significantly different from that in the crop ($p = 0.01$) but not significant different from that in meat muscles ($p = 0.64$), liver ($p = 0.08$) and gizzard ($p = 0.65$).

Broiler meat muscles were analyzed in different weeks to determine their aflatoxin levels. Aflatoxins were detected from the fifth to the seventh week, as presented in Table 6. Only AFB_1 and AFG_1 were detected during these weeks, likely due to their high concentration observed in the feed. The observed concentrations of AFB_1 and total aflatoxins (TAF) in broiler meat were significantly below the permissible levels set by TMDA/TBS and the World Health Organization (WHO), with maximum limit of 10 ng/g for TFA and 5 ng/g for AFB_1 in food (WHO 2018).

Table 6: Variations of Aflatoxins Concentration (AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) Observed in the Meat muscles at Different Chicken Age Intervals

Chicken Age (weeks)	n	AFB_1 (ng/g) Mean ± SD	AFB_2 (ng/g) Mean ± SD	AFG_1 (ng/g) Mean ± SD	AFG_2 (ng/g) Mean ± SD	TAF (ng/g) Mean ± SD
3	3	ND	ND	ND	ND	ND
4	3	ND	ND	ND	ND	ND
5	3	0.61 ± 0.13	ND	0.34 ± 0.12	ND	0.95 ± 0.50
6	3	1.42 ± 0.14	ND	0.36 ± 0.21	ND	1.68 ± 0.22
7	3	1.48 ± 0.43	ND	0.96 ± 0.12	ND	2.64 ± 0.41

AFB_1 was observed in broilers meat muscles from the fifth to the seventh week. In all weeks where AFB_1 was observed, there was a significant increase in concentration except for the seventh week, where there was a slight increase, as presented in Figure 5. However, the accumulation trend showed that AFB_1 was accumulating in the meat muscles more slowly than in any other organ. A similar observation of lower AFB_1 accumulation in meat muscles compared to other organs was reported by Herzallah (2013). The concentration of AFG_1 observed in the fifth and sixth weeks were almost identical, resulting in a constant accumulation trend, but they increased in the seventh week. Total aflatoxins increased from the fifth week to the seventh, indicating that aflatoxins were accumulating in the meat muscles. The accumulation of aflatoxins in this organ was lower than other organs because meat muscles do not have direct interaction with aflatoxins. Instead, muscles come into contact with aflatoxins through blood circulation system.

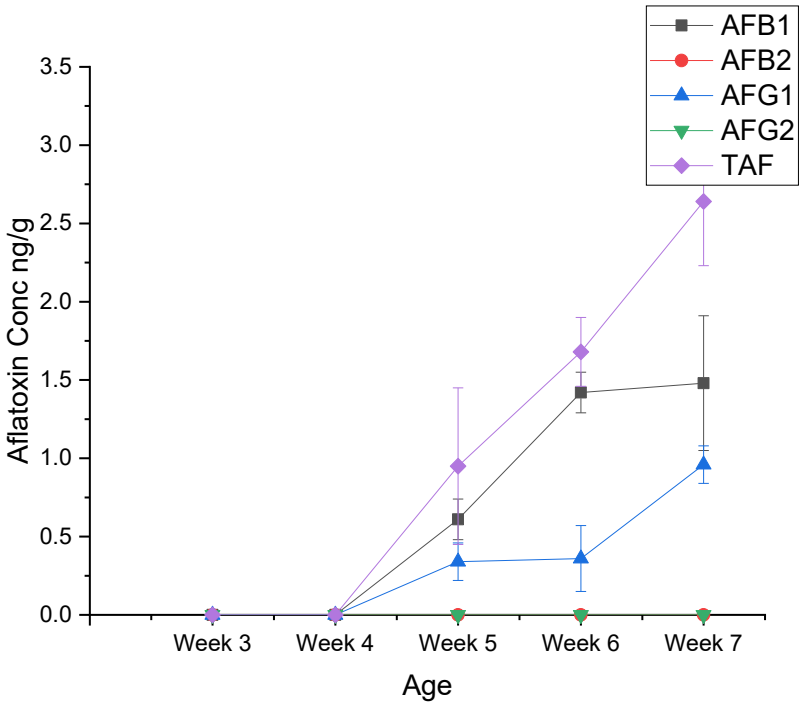


Figure 5: Accumulation and Variations of AFB_1, AFB_2, AFG_1 and AFG_2) and Total Aflatoxins (TAF) in the Meat muscles with Respect to Chicken Age

Single factor ANOVA was carried out, and results revealed that TAF accumulation in meat muscles was significantly different from the one observed in liver (p = 0.04) and crop (p = 0.01), as these two organs have direct interaction with feed while muscles do not. However, TAF accumulation in meat muscles was not significantly different from that in the heart (p = 0.65) and gizzard (p = 0.25) with p values greater than 0.05.

Generally, the observed levels of aflatoxins did not exceed the acceptable limits, for edible organs (meat muscles, gizzard, heart and liver) but exceeded in crop on the seventh week. Furthermore, aflatoxins concentrations were very low in all organs at the standard marketable age (5 weeks). However, this does not guarantee the safety of poultry products, thus, precautions should be taken when consuming chicken. It is therefore recommended to consume meat muscles rather than internal organs such as gizzard, liver, and heart as it was observed to be the least contaminated organ in all weeks. Furthermore, the livestock ministry should conduct routine inspections of livestock feeds sold by vendors to ensure their quality and safety. Experts should assess whether the feeds are stored properly and provide vendors with guidance on best storage practices. Additionally, regulatory bodies in Tanzania should consistently monitor aflatoxin levels in chicken meat and other poultry products to protect public health.

Conclusion

The aflatoxin concentrations detected in various chicken organs (crop, liver, gizzard, heart and meat muscles) at different age intervals indicate a tendency for these toxins to accumulate in poultry products. The observed levels of aflatoxins are alarming, highlighting the need for continuous monitoring of aflatoxins in poultry products and feeds. Therefore, it is imperative for both official and scientific organizations to intervene in order to ensure daily monitoring to prevent aflatoxin levels from exceeding permissible limits. The comprehensive analysis of multiple organs in this study underscores the critical importance of this

research in offering a wide-range and detailed understanding of aflatoxin distribution and accumulation in poultry. This emphasizes the necessity of food safety across various consumable parts of chickens.

Ethical Consideration

All animal handling and experimental procedures were carried out in accordance with internationally accepted animal welfare standards. The chickens were housed in welfare-friendly conditions and received humane care throughout the study period. Appropriate measures were taken to minimize stress, discomfort, and harm, ensuring the ethical treatment of animals during the entire research process.

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