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# Aflatoxin Bioaccumulation in Broiler Chicken Meat and Organs from Selected Farms in Nairobi City County, Kenya

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***Abstract:***
***Background****: Food safety is a worldwide concern not only to policymakers but also to the general population. Food is regarded as safe when there is assurance that no damage will accrue from its consumption. Aflatoxins pose a major risk to the health of both humans and animals. Studies on residue levels in broiler chicken are limited in Kenya hence grounded on this knowledge, this study sought to determine aflatoxin residue levels in broiler meat and organs in Nairobi City County.*
***Materials and Methods****: The study employed a longitudinal study design for a period of six weeks. A total of 42 broilers were sampled. Samples were analyzed using the LC-MS/MS technique. Data was analyzed using STATA version 12. Tukey Kramer post hoc test was used for comparison of means and statistical significance was determined at 5%. Ethical approval was sought from relevant authorities before commencement of the study and consent was sought from the participants before taking part in the study.*
***Results****: Results from the study show that there was a significant association ($p<0.05$) in AFB1 and Total Aflatoxin levels in the gizzard, liver and muscle per week. AFB1 levels in the gizzard were below the WHO/FAO limit of 5 ppb however they were above the EU limit of 2ppb in week 5 and 6. In the liver AFB1 levels were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6.In the muscle AFB1 levels were all below the WHO/FAO and EU limit. Total Aflatoxin levels in the liver were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6. In the muscle Total Aflatoxin levels were all below the WHO/FAO and EU limit.*
***Conclusion:****The results of the study suggest that there were appreciable levels of aflatoxin in the liver and gizzard hence there is need for continuous surveillance and monitoring of aflatoxin levels in feed by regulatory bodies, county and national government to prevent carry over in meat.*
***Key Word****:Aflatoxin, Bioaccumulation*

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## I. Introduction

Aflatoxins exist in as many as 20 analogues but those that are toxicologically significant are B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), MI (AFM1) and M2 (AFB2) (1). In order of increasing toxicity; $AFG_2 < AFB_2 < AFG_1 < AFM_1 < AFB_1$(2). Among all the analogues, AFB1 occurs in cultures, in food and feed products and is considered as the most toxigenic fraction as it is associated with hepatocellular carcinoma (3). Aflatoxins are considered as fatal carcinogens and the global prevalence of hepatocellular carcinoma due to aflatoxin adulteration is 25% majorly in developing countries, owing to the improper post-harvest management and the frequent ingestion of aflatoxin adulterated food (4). Studies have reported that high levels of Aflatoxin in feed samples leads to high levels of Aflatoxin in animal products (5). Studies have also shown that aflatoxins have genotoxic, teratogenic and hepato carcinogenic effects on humans (6). Poultry is considered to be the most

susceptible of all the animal species to the effects of aflatoxins (7). Poultry have demonstrated to be highly sensitive to low levels of AFB1 exposure. In order of susceptibility; ducks are more susceptible than turkeys, turkeys are more susceptible than quails and finally quails are more susceptible than chicken (8). Aflatoxin contamination in poultry causes suppression of the immune response and this leads to impairment of the T cell manufacture, reduced phagocytosis and apopstosis in the thymus and spleen (9)(10).

In chicken, the occurrence of prolonged prothrombin time (PT) is a pointer of aflatoxin contamination and the exposure illustrates a direct correlation between aflatoxin dose and the exposure time. PT is evidence of the action of blood clotting factors V, VII, IX, X, prothrombin and fibrinogen and this serves as diagnostic evidence of liver lesions in poultry (11).

Chicken meat is not only tasty, inexpensive, fast and easy to prepare but also provides a distinct well balanced source of minerals, vitamins, proteins and healthy fats for all ages. Its high quality, low calorie content and ease of digestibility make it valuable in many therapeutic diets for adults (12). Aflatoxin levels in food and feed is constantly monitored in developed countries through various chromatographic and immune-enzymatic methods (13), however, this close monitoring is non-existent in many developing nations. The poultry industry is grappling with feed insecurity due to high cost of feeds and feed safety due to regular adulteration of feeds with mycotoxins particularly in sub Saharan Africa (14). To add on, cereal traders utilize trade loopholes to divert aflatoxin contaminated cereal into animal feed manufacturing companies and hence this is possess a risk to poultry and human health (15). In Nairobi City County, little is known or documented on Aflatoxin residue levels in broiler meat consumed by the residents. An investigation on microbial contamination of broiler meat in Nairobi only found high pathogen infestation in the meat (16) however aflatoxin levels in meat is unknown. Due to the detrimental effects of these toxins, many countries have instigated regulations on animal feeds and food items (17). Therefore, quantification of aflatoxin levels in meat after ingestion of the toxin through feed is critical to public health (3). It is along these lines that this study was carried out.

## II. Material and Methods

**Study Design:** longitudinal study design.

**Study Location**: The study was carried out in Nairobi City County in six sub counties namely; Westlands, Kasarani, Embakasi Central, Embakasi East, Dagoreti North and Dagoreti.

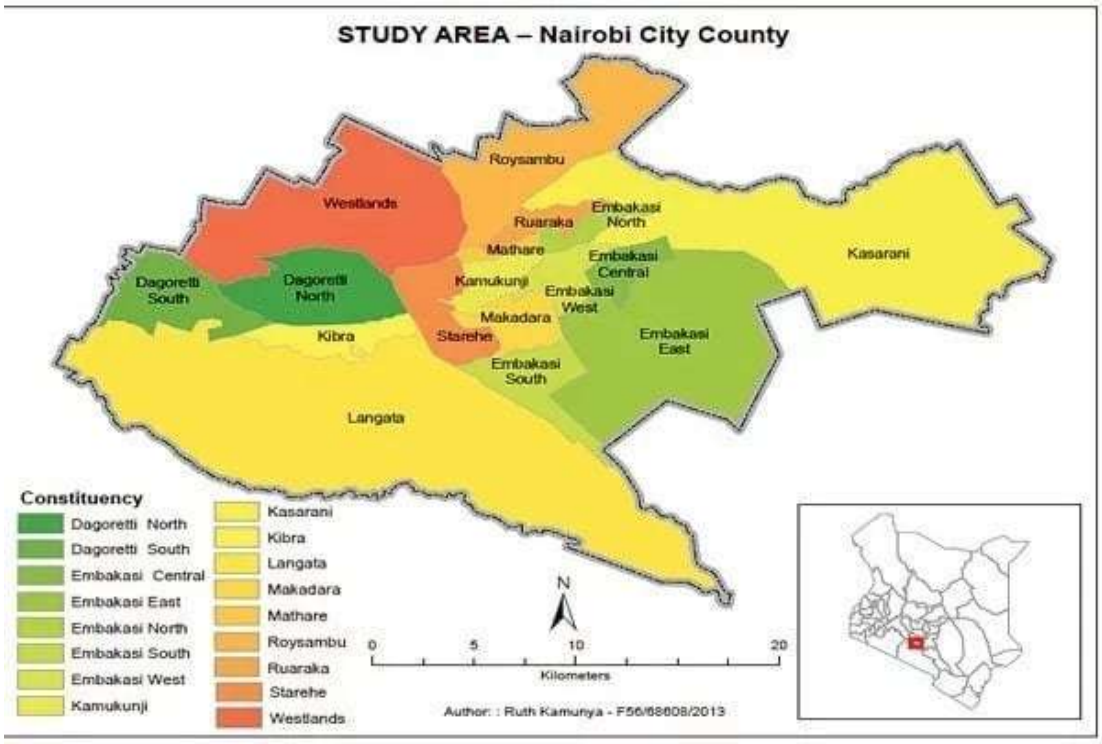


**Figure 1: Map of Nairobi City County (Source: Ruth Kamunya, 2013)**

**Study Duration:** April 2021 to June 2021.

**Sample size:** Random sampling was used to identify one farm in each of the six sub counties where the follow up study (longitudinal) was conducted. In total six farms were selected. The follow up (longitudinal study) was done for a period of six weeks and samples were collected in each farm from week 0 (day old chick) to week 6 and a total of 42 birds were sampled.

**Sample size calculation:** The sample size was calculated using Wan and Wan (2017) formula for determination of Minimum and Maximum Sample Sizes for Group comparison—one-way ANOVA (18). The group was the number of broiler chicken from week 0 to week 6. Conventionally, the value of Error DF should lie between 10 and 20 (19). This method is applicable to all animal experiments. If the DF is less than 10, addition of more animals will increase the chances of obtaining more significant results, but if the DF is more than 20, addition more animals will not increase the chances of obtaining significant results but will lead to unnecessary wastage of resources and animals (19). Therefore, any sample size which keeps the DF between 10 and 20 should be regarded as sufficient to give significant results (19).

In this study, k (number of groups) was seven (week 0 to week 6) and hence the **minimum** number of chicken per group was

$$n = \frac{DF}{k} + 1 = \frac{10}{7} + 1 = 1.4 + 1 = 2.4 \ \ rounded\ up\ to\ 3$$

The **maximum** number of chicken per group was;

$$n = \frac{DF}{k} + 1 = \frac{20}{7} + 1 = 3 + 1 = 4$$

In total, the minimum and maximum numbers of animals required are:

Minimum N = Minimum n x k =3x7=21

Maximum N = Maximum n x k =4x7=28

A total of 42 animals were sampled in the study which was above the minimum and maximum limit of 10 and 20 respectively as shown above. Owing to expected attrition in the study, the number of animals (n) in the seven groups was increased from maximum of four to six.

Therefore, N = n x k =6x7=42; Where n is total number of subjects (animals) per group and k is the total number of groups (weeks).

Variability within various groups (farms) was expected since the study did not have control of all the variables. Such variations included the feeds and the type of broiler reared in the farm among other expected variations. Frank and Althoen (1994) advise that the mean of varying outcomes in a sample is the best representation of the sample than an individual score in the sample (20).

**Sample collection**: Samples were taken from carcasses of the broilers after they were slaughtered humanely. The broilers to be sampled were randomly picked from their establishment each week. The birds were weighed each week (live weight) and their weight recorded before they were slaughtered. The samples that were obtained were the muscle (breast and leg), liver and gizzard. Sampling was done from week (0, 1, 2, 3, 4, 5, and 6). In total 126 samples were collected (42 muscle, 42 gizzards and 42 livers). In week 0 (day old chick) the broiler chicks were sampled for analysis before they were fed and this was the baseline. All the samples collected were put in zip lock bags and clearly labeled indicating the farm, week, and date collected. All the samples obtained from the farms were kept in the cooler box then taken to the lab. The samples obtained were stored in the freezer at - 20 degrees Celsius in the lab (3) to prevent further production of metabolite and microorganisms until the time of analysis (21).

**Inclusion criteria:**

1. Farms where broiler chicken are reared
2. Those who gave consent

**Exclusion criteria:**

1. Farms reported to have an outbreak of disease

**Procedure methodology**

**Analysis of meat samples**

Detection and quantification of aflatoxin levels in meat samples was done using the Liquid Chromatography technique with triple quadruple mass detector (LC-MS/MS Agilent 6460). In an accredited ISO 17025:2017 certified laboratory.

**Calibration curves**

Standard calibration curves were established for each aflatoxin analogue (B1, B2, G1, G2 and M1) to determine the linearity of the LC-MS/MS system. The linearity of the method was tested by running AF standard in the range of

0.0–100 µg/kg (0, 5, 10, 15, 25, 30, 50, 75 and 100 µg/kg), and a correlation coefficient ($R^2$) of >0.9500 for each analogue was obtained.

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) is the lowest concentration level that the analytical process can reliably detect. Each of the five Aflatoxin analogues (B1, B2, G1, G2 and M1) the LOD was determined for each sample matrix analyzed. Limit of Quantification (LOQ) The limit of quantification (LOQ) is the lowest concentration level that the analytical process can reliably quantify. Each of the five Aflatoxin analogues (B1, B2, G1, G2 and M1) the LOQ was determined for each sample matrix analyzed.

**Chemicals and Equipment used for analysis**

The chemicals and reagents used were acetonitrile; HPLC grade; purity≥99.9%, Formic acid; purity ≥ 99.9%, ammonium formate; purity ≥99.9%, LC-MS/MS HPLC grade water (bottled), sodium chloride, phosphate buffer, tween-20.8 and nitrogen gas.

Materials and Equipment used were Agilent 1260 coupled with mass spectrometry Agilent 6460, 100 ml beaker, 100 ml measuring cylinder, 10 ml volumetric flask, 24 cm flutted filter, 0.45µM syringe filter, 100 ml screw bottle flask, reciprocating shaker, electronic digital balance (accuracy 0.0001g), top weighing balance, 10 ml syringes, powderless gloves, pasteur pipette, micro pipette (1ml), micro pipette (0.2ml), vortex mixture and immuno affinity column.

**Sample extraction procedure**

From each tissue sample (gizzard, liver and muscle), 25g of the sample was obtained, thawed and minced in a high speed mixer, 5g of NaCl was added and blended with 100 mL of a methanol-water mixture (80:20) for 3 min at 6000 revolutions per minute. The mixture was then filtered through a paper filter, an aliquot of 10 mL of the filtrate (equivalent to 2g of the tissue sample) was diluted with 40 mL of phosphate-buffered saline and with 0.1% of Tween-20.8. The mixture was then applied to an immuno-affinity column and passed at a flow rate of 1–2 drops per second by a pressure of (30 mmHg) on the SPE-10 Manifold apparatus. The immune-affinity columns were washed with 20 mL of distilled water. Finally, aflatoxins were eluted with 1.0 mL of methanol, at a rate of 1–2 drops per second. The eluate was collected in a glass vial and dried near to dryness under a gentle stream of nitrogen. The extracted solution was evaporated near to dryness using nitrogen in a screw cap vial and re-dissolved in 200 mL of hexane. After adding 1.95 mL of a mixture of deionized water and acetonitrile (9:1) and vortexing for 30 s, the 2 layers were allowed to separate. The lower aqueous layer was removed with the help of a separating funnel and filtered through a 0.45 lm syringe filter prior to injection into the LC column. Method adopted from Iqbal et al (13).

**Statistical analysis**

STATA version 12 was used to analyze quantitative data from the laboratory analysis. The data was subjected to two-way ANOVA to establish differences in means in aflatoxin levels in the meat samples that were sampled weekly. Post ANOVA test was done using Tukey Kramer post hoc test. The level of significance was determined at 5%. Data was presented in tables and graphs.

## III. Result

**1. Aflatoxin B1 levels in broiler meat and organs**

There was a statistical significant difference($p<0.05$) in aflatoxin B1 levels in gizzard, liver and muscles of broiler chicken between weeks as shown in Table 1 below. The highest levels of Aflatoxin B1 were found in the liver in week 6 while the least levels were found in the gizzard in week 0 and week 1, in the liver in week 0 and in the muscles in week 0, 1, 2 and 3 as shown in Table 1 below. The levels of aflatoxin B1 in all the chicken parts increased with time as shown in figure 1 below. This observation could be attributed to bio accumulation of the aflatoxins. Furthermore, high levels of aflatoxin B1 were found in the liver followed by the gizzard and the least levels were in the muscles as shown in (figure 2) below. AFB1levels in the gizzard were below the WHO/FAO limits however they were above the EU limit in week 5 and 6. In the liver AFB1 levels were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6. In the muscle AFB1 levels were all below the WHO/FAO and EU limit.

**Table 1: Aflatoxin B1 levels (ppb) in broiler meat parts sampled weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | 0.29 ±0.16$^{ab}$ | ND |
| WEEK 2 | 0.35± 0.16$^{ab}$ | 1.27±0.16$^{cde}$ | ND |
| WEEK 3 | 0.95 ± 0.16$^{bcd}$ | 1.81±0.16$^{e}$ | ND |
| WEEK 4 | 1.68 ± 0.16$^{de}$ | 3.10±0.16$^{f}$ | 0.16±0.16$^{ab}$ |
| WEEK 5 | 2.67 ± 0.16$^{f}$ | 4.92±0.16$^{g}$ | 0.38±0.16$^{ab}$ |
| WEEK 6 | 3.08 ± 0.16$^{g}$ | 7.25±0.16$^{h}$ | 0.47±0.16$^{bc}$ |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |
| **STANDARDS:** | **EU 2ppb** | **WHO/FAO 5ppb** | |

**KEY:** Means with different superscript letters in each column and row are statistically significant at p<0.05 ±SE
ND- not detected Week 0- Day old chick

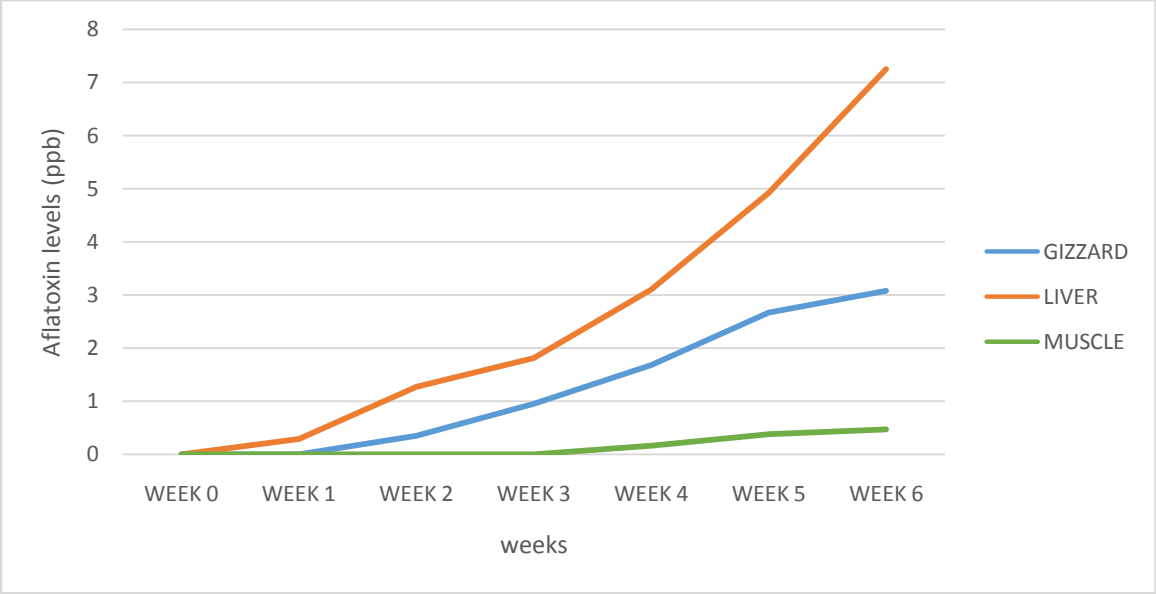


**Figure 2: Aflatoxin B1 levels in meat parts per week**

**2. Aflatoxin B2 levels in broiler meat and organs**

There was a statistical significant($p<0.05$) difference in aflatoxin B2 levels in gizzard, liver and muscles of broiler chicken between weeks as shown in Table 2 below. AFB2 levels were not detected in all the meat parts in week 0 and week 1. AFB2 levels were not detected in the muscle entirely. The AFB2 levels increased weekly in both the gizzard and liver however in the muscle AFB2 was not detected as shown in (figure 3) below.

**Table 2: Aflatoxin B2 levels (ppb) in broiler meat parts sampled weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | ND | ND |
| WEEK 2 | ND | 0.08±0.08$^{a}$ | ND |
| WEEK 3 | 0.03 ±0.08$^{a}$ | 0.28±0.08$^{ad}$ | ND |
| WEEK4 | 0.14±0.08$^{a}$ | 0.64±0.08$^{bd}$ | ND |
| WEEK 5 | 0.79±0.08$^{bc}$ | 1.19±0.08$^{c}$ | ND |
| WEEK6 | 0.95±0.08$^{bc}$ | 2.22±0.08$^{e}$ | ND |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |

**KEY:** Means with different superscript letters in each column and row are significantly different at p<0.05 ±SE
ND-not detected Week 0- Day old chick

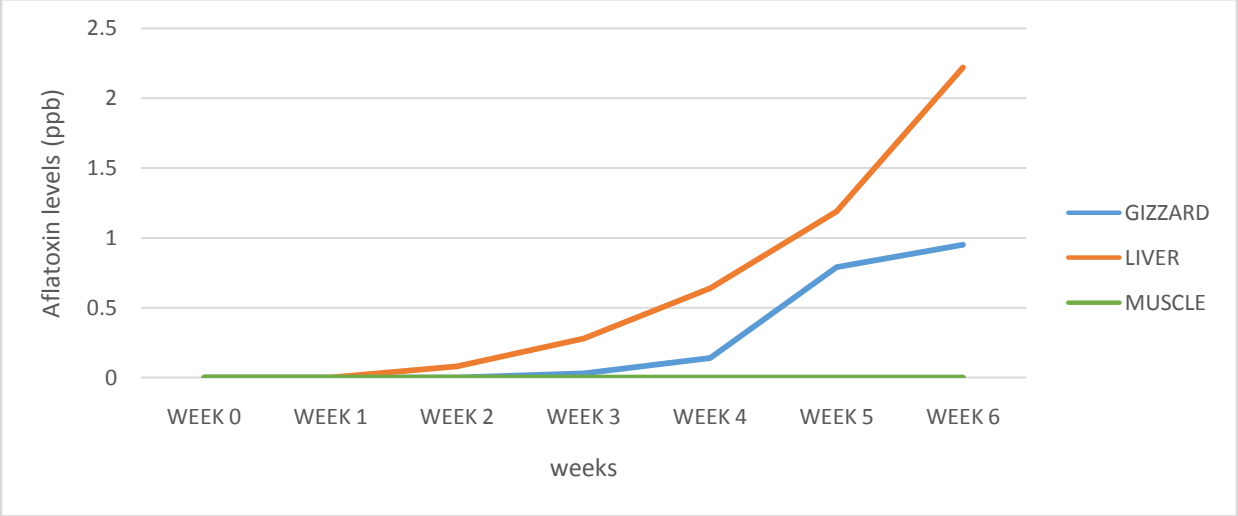


**Figure 3: Aflatoxin B2 levels in meat parts per week**

### 3. Aflatoxin G1 levels in broiler meat and organs

There was a statistical significant difference ($p<0.05$) in aflatoxin G1 levels in gizzard, liver and muscles of broiler chicken between weeks as shown in Table 3 below. AFG1 levels increased weekly in all the meat parts as shown in figure 4 below. AFG1 levels were not detected in all the meat parts in week 0 and in week 1and in the gizzard between week 0 and week 4 in the muscle. High levels of AFG1 was detected in the liver as shown in Table 3 below.

**Table 3: Aflatoxin G1 levels (ppb) in broiler meat parts samples weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | $0.04\pm0.15^{a}$ | ND |
| WEEK 2 | $0.04\pm0.15^{a}$ | $0.58\pm0.15^{abc}$ | ND |
| WEEK 3 | $0.31\pm0.15^{ab}$ | $0.84\pm0.15^{bc}$ | ND |
| WEEK4 | $0.84\pm0.15^{bc}$ | $1.32\pm0.15^{cd}$ | ND |
| WEEK 5 | $1.26\pm0.15^{cd}$ | $2.58\pm0.15^{e}$ | $0.04\pm0.15^{a}$ |
| WEEK6 | $1.96\pm0.15^{de}$ | $4.16\pm0.15^{f}$ | $0.23\pm0.15^{ab}$ |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |

**KEY:** Means with different superscript letters in each column and row are significantly different at $p<0.05$ ±SE
ND-not detected Week 0- Day old chick

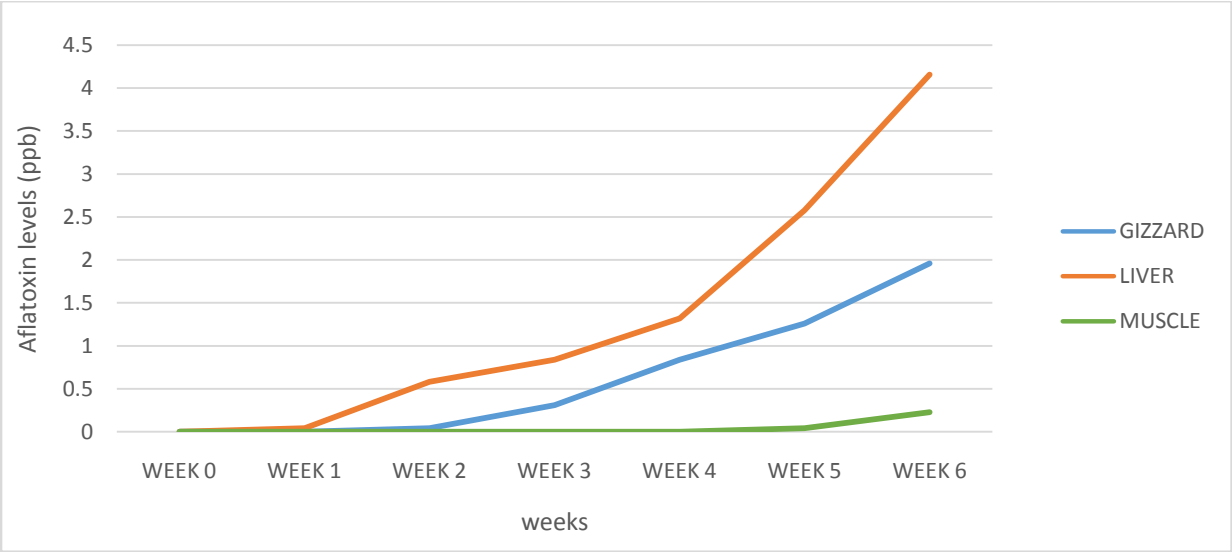


**Figure 4: Aflatoxin G1 levels in meat parts per week**

## 4. Aflatoxin G2 levels in broiler meat and organs

There was statistical significance difference ($p<0.05$) in AFG2 levels in the liver, gizzard and muscle between weeks as shown in Table 4 below. AFG2 levels were not detected in all the meat parts between week 0 and week 3. AFG2 levels were not detected in the muscle in all the weeks as shown in table 4 below. AFG2 levels were detected in the gizzard in week 6 only and in the liver in week 4, 5 and 6. There was steady increase in AFG2 levels in the liver from week 3 to week 6 as shown in (figure 5) below.

**Table 4: Aflatoxin G2 levels (ppb) in broiler meat parts samples weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | ND | ND |
| WEEK 2 | ND | ND | ND |
| WEEK 3 | ND | ND | ND |
| WEEK4 | ND | $0.06\pm0.04^{a}$ | ND |
| WEEK 5 | ND | $0.50\pm0.04^{b}$ | ND |
| WEEK6 | $0.29\pm0.04^{b}$ | $0.83\pm0.04^{c}$ | ND |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |

**KEY:** Means with different superscript letters in each column and row are significantly different at $p<0.05$ ±SE
ND-not detected Week 0- Day old chick

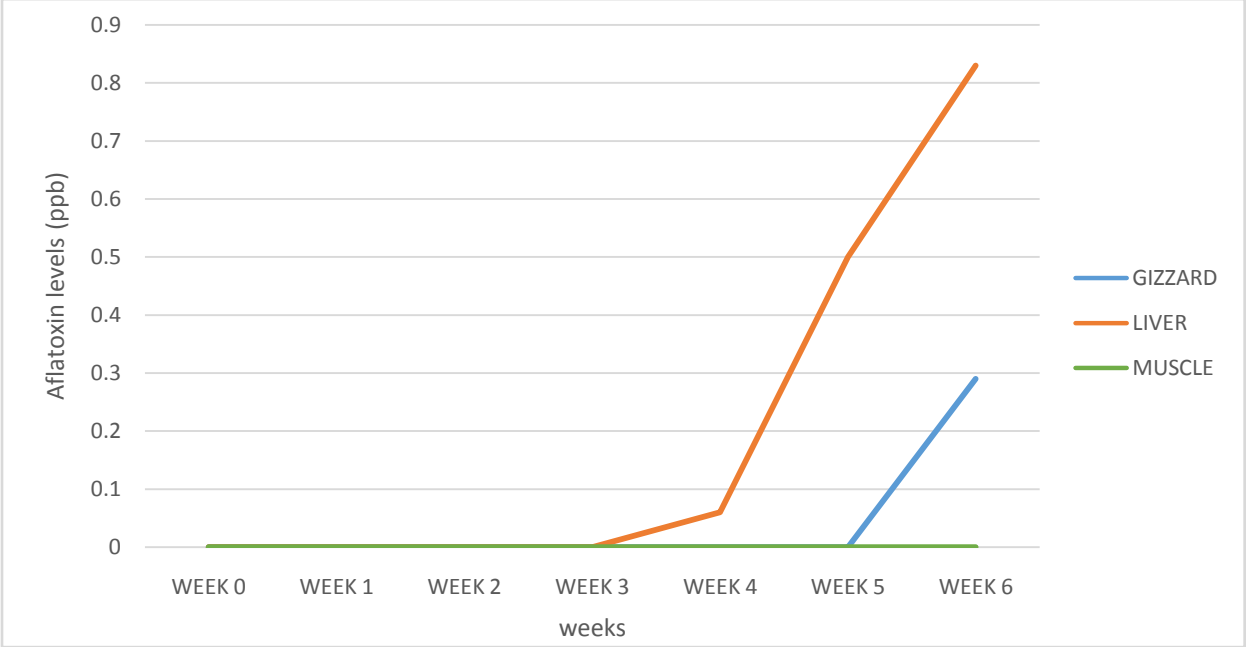


**Figure 5: Aflatoxin G2 levels in meat parts per week**

## 5. Aflatoxin M1 levels in broiler meat and organs

There was a statistical significance difference ($p<0.05$) in AFM1 levels in the liver in week 5 and 6 as shown in Table 5 below. AFM1 levels were not detected in the gizzard and muscle but was detected in the liver in week 5 and 6 as shown in figure 6 below.

**Table 5: Aflatoxin M1 levels (ppb) in broiler meat parts samples weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | ND | ND |
| WEEK 2 | ND | ND | ND |
| WEEK 3 | ND | ND | ND |
| WEEK4 | ND | ND | ND |
| WEEK 5 | ND | $0.04\pm0.01^{b}$ | ND |
| WEEK6 | ND | $0.1\pm0.01^{c}$ | ND |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |

**KEY:** Means with different superscript letters in each column and row are significantly different at $p<0.05$ ±SE
ND-not detected Week 0- Day old chick

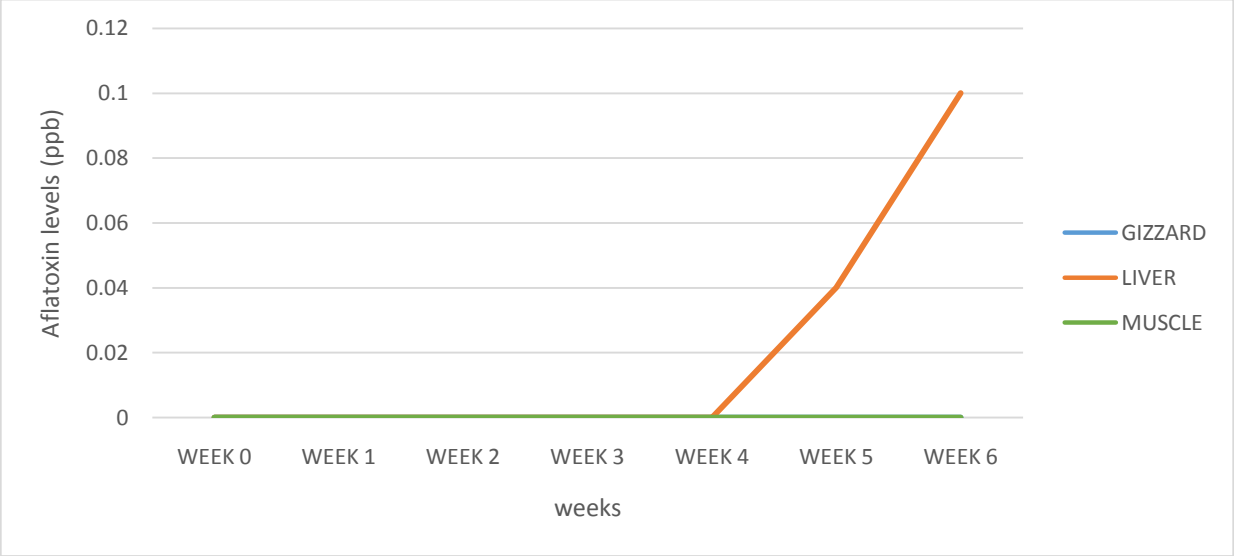


**Figure 6: Aflatoxin M1 levels in meat parts per week**

## 6. Total Aflatoxin levels in broiler meat and organs

There was a statistical significant difference ($p<0.05$) in Total Aflatoxin levels in gizzard, liver and muscles of broiler chicken between the weeks as shown in Table 6 below. Total Aflatoxin levels were not detected in the gizzard in week 0 and week 1, in the liver in week 0 and in the muscle in week 0, 1, 2 and 3. Total Aflatoxin levels in all the meat parts increased weekly as shown in (Figure 7) below. High levels of Total Aflatoxin were found in the liver followed by the gizzard and the least values were in the muscles This is illustrated in Table 6 below. Total Aflatoxin levels in the gizzard were below the WHO/FAO limits however they were above the EU limit in week 5 and 6. In the liver Total Aflatoxin levels were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6. In the muscle Total Aflatoxin levels were all below the WHO/FAO and EU limit.

**Table 6: Total Aflatoxin levels (ppb) in broiler meat parts samples weekly**

| WEEK | GIZZARD | LIVER | MUSCLE |
|---|---|---|---|
| WEEK 0 | ND | ND | ND |
| WEEK 1 | ND | $0.33\pm0.32^{ab}$ | ND |
| WEEK 2 | $0.4\pm0.32^{ab}$ | $1.93\pm0.32^{bc}$ | ND |
| WEEK 3 | $1.28\pm0.32^{abc}$ | $2.94\pm0.32^{c}$ | ND |
| WEEK 4 | $2.65\pm0.32^{c}$ | $5.11\pm0.32^{d}$ | $0.16\pm0.32^{a}$ |
| WEEK 5 | $4.71\pm0.32^{d}$ | $9.18\pm0.32^{e}$ | $0.42\pm0.32^{ab}$ |
| WEEK 6 | $6.28\pm0.32^{d}$ | $14.46\pm0.32^{f}$ | $0.7\pm0.32^{ab}$ |
| **P value** | **<0.0001** | **<0.0001** | **<0.0001** |
| **STANDARDS:** | **EU 4ppb** | **WHO/FAO 10ppb** | |

**KEY:** Means with different superscript letters in each column and row are significantly different at $p<0.05$ ±SE
ND-not detected Week 0- Day old chick

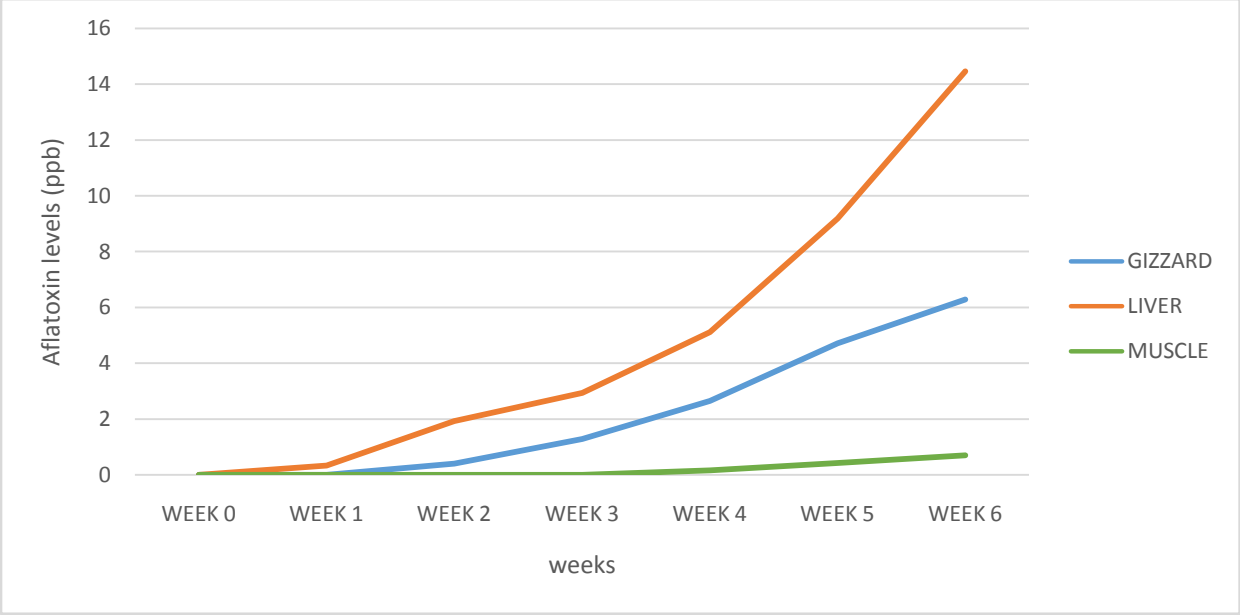


**Figure 7: Total Aflatoxin levels in meat parts per week**

## IV. Discussion

Aflatoxin residues are majorly found in eggs, milk and meat and ingestion by humans is the main route of exposure of mycotoxin leading to a myriad of harmful effects (22)(23).AFB1 is the most toxigenic analogue as it is linked to hepatocellular carcinoma and several studies have found that meat and other animal products are only minor contributors to human dietary mycotoxin exposure (24)(25), chronic exposure to these low levels has a significant impact on the health of human consumers (25). To add on AFB1 is the most toxic mycotoxin found in meat.

A study by Sahib et al (2020) on carry-over of AFB1 from feed to broiler meat found that the residual level of AFB1 in the liver of broilers was comparatively higher than that in the muscles (3) this agrees with the results of the current study. The results from the present study are in agreement with earlier studies where the level of AFB1 residues in the liver were higher than those in muscles (26)(27)(28)(29)this is because the liver is the principal target organ of Aflatoxins.

Additionally, from the same study by Sahib et al (2020) AFM1 residues were detected only in the liver and muscles of broilers fed with 200 ppb of AFB1 in feed whereas at 100 ppb AFB1 in feed, residues of M1 were only detected in the liver but not in the muscle, this therefore implies that a higher level of AFB1 in feed translates into a higher level of AFM1 in the liver and muscle consequently (3). The findings from the study by Sahib et al support previous findings that AFM1 accumulated more in the liver than in muscles (26)(28)(27)(29) however in the present study, AFM1 was not detected in the muscle. Aflatoxin M1 is formed from AFB1 during its metabolism. Previous studies from animal models revealed that AFM1 has hepatotoxic and carcinogenic properties (30). Studies have shown that the toxicity of AFB1 in animals is comparable to or slightly higher than that of AFM1. On the other hand, the carcinogenicity of AFB1 is almost one or two times greater than that of AFM1 (31).

Studies have shown that elevated levels of AFB1 in feed samples lead to high levels of AFB1 in liver and muscle respectively (5)(32)(26)(33)(34). Sineque et al (2017) reported that tissue residues of aflatoxin were higher in the liver than in the gizzard (25). This is agrees with the findings of the present study whereby AF levels were higher in the liver compared to the gizzard.

Iqbal et al (2014)(13) from Pakistan, reported that 35% of chicken meat samples were positive for aflatoxins, with a maximum level of AFB1 and total aflatoxins in the livers at 2.98 ± 0.76 and 3.23 ± 0.82 µg/kg respectively however in the present study the maximum AFB1 levels in the liver was (7.25±0.32) observed in week 6 and Total Aflatoxin was (14.46±0.32) this was higher than that of the study from Pakistan. El-Desouky et al (2014)(35) from Egypt, found AFB1 in the gizzard with a cummulative maximum level of 2.24 µg/kg. In the present study the maximum level of AFB1 in the gizzard was 6.28±0.32 which was higher than that of the study in Egypt. In a study in Mozambique, AFB1 was detected in 39% of liver samples (mean level: 1.7 µg/kg) and about 14% of gizzard samples (mean level: 1.1 µg/kg) (25). A study by Olatoye et al in Nigeria found the mean AFB1 level in the

muscle, gizzard and liver in broilers at 4 weeks to be 0.07 ± 0.02, 0.18 ± 0.05 and 0.13 ± 0.02 respectively (36) these levels were lower than that of the present study as the levels at week 4 were 0.16±0.16, 1.68 ± 0.16 and 3.10±0.16 respectively.

Faten et al (2016) found AFB1 6.5±1.03, AFG1 41±1.4, AFB2 1.7 ±0.6, AFG2 0.7 ±0.3 and Total Aflatoxin 8.9± 1.5 in the muscle, these findings were higher than the findings of the present study at week 6. To add on he found AFB1 17.3+/-3.3, AFG1 13.5+/-2.1µg/kg, AFB2 7.6+/-4.8 µg/kg, AFG2 1.5+/-0.9 and Total Aflatoxin 22.8+/-4.1 in the liver (5) these levels were higher than those of the current study. The results showed that AF levels in the liver were higher compared to the muscle which agrees with the results of the current study. These results were in agreement with those reported by Saeed et al (2003)(37)who reported that although aflatoxins residues are found in the liver, muscles, stomach, kidneys and adipose tissue, the liver is the harbor site of aflatoxin residues. In the same line, the results agreed with those found by Herzallah (2013)(38) and Darwish et al (2016)(39) as they found high levels of AFB1 and total aflatoxins in the liver than in the gizzard, while the least levels were in the muscle these findings agree with the findings of the present study.

## V. Conclusion

The results from the analysis of broiler meat showed that the level of aflatoxin in the gizzard, liver and muscle increased weekly due to bioaccumulation. Aflatoxin levels in day old chicks (week 0) were not detected in the gizzard, liver and muscle. The levels of aflatoxin in all the meat parts analyzed were highest in week 6.

AFB1 levels in the gizzard were below the WHO/FAO limits however they were above the EU limit in week 5 and 6. In the liver AFB1 levels were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6. In the muscle AFB1 levels were all below the WHO/FAO and EU limit. Total Aflatoxin levels were above the EU limit in week 4, 5 and 6 and above the WHO/FAO limit in week 6. In the muscle Total Aflatoxin levels were all below the WHO/FAO and EU limit.

AFB2 levels were not detected in the gizzard, liver and muscle in week 0 and week 1 and were not detected in the muscle entirely. AFG1 levels were not detected in the muscle from week 0 to week 4. AFG2 levels were not detected in the muscle and was detected in the gizzard in week 6 only and in the liver in week 4, 5 and 6. AFM1 levels were not detected in the gizzard and muscle but was detected in the liver in week 5 and 6. Total Aflatoxin levels were not detected in the gizzard in week 0 and week 1, in the liver in week 0 and in the muscle in week 0, 1, 2 and 3. The findings of the present study suggest that it is safer to eat the muscle since the levels are below the recommended limits.

There is need for constant monitoring of aflatoxin levels in poultry feed and poultry products meant for human consumption by regulatory bodies i.e. KEBS (Kenya Bureau of Standards) and national and county government and application of hazard analysis critical control point (HACCP) in feed manufacturing, storage and broiler production and more stringent allowable limits in feed by regulatory bodies should be instigated.

## Ethical Consideration

Approval to carry out the study was obtained from Kenyatta University graduate school. Ethical approval was obtained from Kenyatta University Ethical and review committee Approval number (PKU/2163/II307). A research permit to carry out the study was obtained from National commission for Science, Technology and innovation (NACOSTI) license number (NACOSTI/P/20/8037. Authorization was also obtained from the Ministry of Agriculture, Division of Veterinary Services before commencement of the study. The broilers were maintained in animal welfare friendly housing and were treated humanely. The information on the presence of aflatoxin in broiler meat in specific farms was kept confidential. In the farms where broilers were sampled for lab analysis, consent was sought from the farm owners and the nature and details of the study was clearly explained to the farm owners. The scope, the benefits and the risks of the study was thoroughly illustrated to the participants. Participation in the study was on voluntary basis and respondents chose to or not to take part in the study.

## Conflicts of interest

The authors declare that there was no conflict of interest.

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