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Carry Over Effect of Aflatoxin from Broiler Feed to Broiler Meat and Organs in Chicken raised in Nairobi City County, Kenya

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Abstract:
Background*: Aflatoxin is a threat and a food safety concern particularly in developing countries due to the climatic conditions that favor the growth of the aflatoxin fungi. Consequently, this is a major risk to feed ingredients used in the manufacture of animal feed and subsequently a great risk to human consumers due to the detrimental effects of these toxins. Since there are no documented studies on carry over effect of aflatoxin in broilers in Kenya, a study to establish the carry over effect of aflatoxin in broiler chicken was carried out in Nairobi City County.*
Materials and Methods*: The study employed a longitudinal study design where by broiler chicken were followed for a period of six weeks. The broilers were sampled from six farms in six sub counties within Nairobi City County hence, a total of 42 birds were sampled.The samples obtained were meat (muscle, liver and gizzard), feed and water and were analyzed using the LC-MS/MS technique to determine the Aflatoxin levels.The carry over effect in this study was determined statistically. STATA version 12 was used to analyze the data. Tukey Kramer post hoc test was used for comparison of means and statistical significance was determined at 5%.*
Results*:There was a statistical significant difference ($p<0.05$) in the carry over ratio of aflatoxin per week. The highest carry over ratio of >10% was observed in the liver, followed by the gizzard and the least was in the muscle.The highest transfer ratio was observed in week 5 and 6 in the liver and in week 6 in the muscle. The carry over ratio in the muscle was below 1%.*
Conclusion:*The study concludes that it is less safe to consume the liver and gizzard as the transfer is high however it is safer to consume the muscle as the transfer is low. There is need to constantly monitor aflatoxin levels in feed and feed ingredients hence prevent carry over into animal tissues consequently reducing risk to humans.*
Key Word*:Aflatoxin, Carry Over, Metabolism*

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I. Introduction

There is a growing concern globally on unsafe food emanating from biological, physical, or chemical hazards resulting in more than 200 known illnesses starting from diarrhea to cancers (1). Although aflatoxin adulteration mostly affects developing countries, there is insufficient documented evidence therefore the burden in SSA is underestimated (2). Humans are at risk of the effects of aflatoxin as they are carried over into blood tissue, gizzard, breast muscles, liver and eggs of poultry therefore becoming a risk to human consumers (3). Studies have also shown that aflatoxins have genotoxic, teratogenic and hepato carcinogenic effects on humans (4).All mycotoxins inclusive of aflatoxins are metabolized in the gastrointestinal tract, liver or kidneys depending on their chemical structure.The metabolism of AFB1 is performed through an oxidation reaction process by a group of CYP450 isoenzymes. There are different types of metabolizing enzymes used in the metabolic reaction in various animal species. For instance, in poultry CYP2A6, CYP3A37, CYP1A5 and

CYP1A1 isoenzymes are responsible for the metabolism of AFB1(5)(6). In humans, CYP3A4 in the liver and CYP2A13 in the lung are responsible for the metabolism of AFB1 to AFBO. AFB1 is responsible for hepatocellular carcinoma in humans (7)(8). Among the animal species, rabbits are highly susceptible to the hazardous properties of AFB1. Chicken are highly sensitive to aflatoxins and broilers are considered to be more susceptible to aflatoxin exposure than layers(9)while fish and swine are fairly susceptible(10). Cattle and sheep are the most resilient of all the animal species to AFB1(10). Studies have also demonstrated that younger animals are more susceptible to AFB1 contaminant than older persons (6).

Aflatoxin transfer into poultry meat and eggs gives rise to adverse consequences on human health (11). Food security is one of Kenya's big 4 agenda in the attainment of the country's vision 2030, therefore food safety is paramount (12).

Whereas there is need for robust food safety policy to address food safety concerns, the current policies on food safety are incoherent and do not clearly address food safety gap in the country (13). Studies on Aflatoxin in Kenya have mostly majored on cereals and their products (14) and studies on the 'carry over' of aflatoxin in poultry meat are limited. It is from this background that this study was conducted.

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 South.Nairobi is the capital city of Kenya and is one of Africa's strategic financial, business, transport, communications, non-governmental organizations and diplomatic capital. Nairobi city county population is about 4.397 million (2019 census). Nairobi unlike other towns in Kenya has been found to be the ultimate destination for poultry countrywide, and is also the main entry and transfer point for poultry within the East African Community (15).

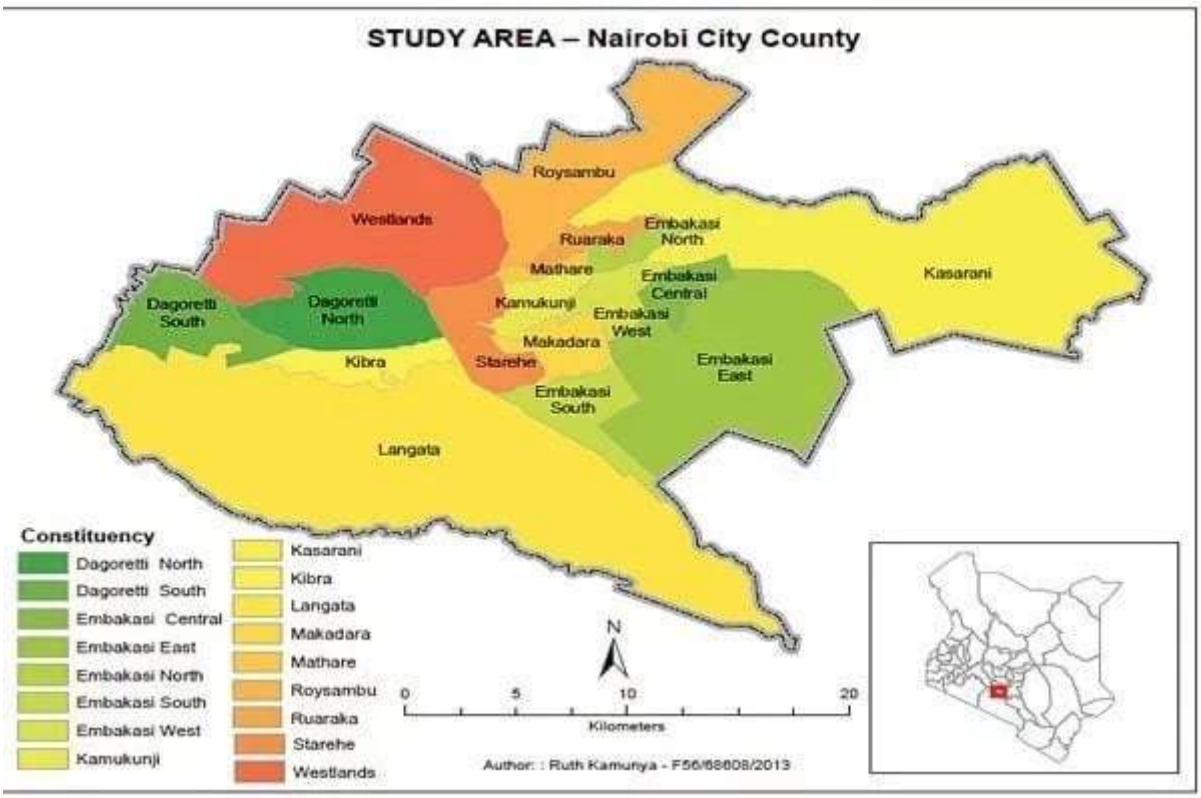

Figure 1: Map of Nairobi City County, Kenya (Source: Ruth Kamunya, 2013)

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 was conducted. In total six farms were selected. The follow up was done for a period of six weeks and samples(meat, feed and water) were collected from each farm from week 0 (day old chick) to week 6 hence a total of 42 birds were sampled. The sample was arrived at using Wan and Wan (2017) (16) sample size calculation in animal studies.The aflatoxin levels obtained from meat (muscle, liver and gizzard), feed and water were used to determine the carry over ratio.

Carry over ratiocalculation: The carry over effect in this study was determined statistically using a carry-over ratio or transfer ratio (17)(18)(19). The carry over ratio or transfer ratio was calculated using the following formula;

$$\frac{\text{Difference in Aflatoxin levels between weeks in meat (muscle, liver and gizzard)}}{\text{Mean Aflatoxin levels in (feed +water)}} \quad \text{Equation 1}$$

Statistical analysis

STATA version 12 was used to analyze the data. The data was subjected to two-way ANOVA to establish differences in means between the various meat parts sampled weekly. Comparison of means 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

This section entails presentation of results on the carry over effect of aflatoxin from broiler feed into broiler meat. The carry over effect in the present study was determined statistically by use of carry over ratio or transfer ratio as described in the methodology section.

There was significant difference ($p<0.05$) in the carry over ratio of AFB1 between the gizzard, liver and muscle per week as shown in Table 1.

Table 1: AFB1 Carry over ratio per meat part

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 1	0^{a}	0.017 ± 0.01^{abc}	0^{a}
WEEK 2	0.021 ± 0.01^{abcd}	0.058 ± 0.01^{de}	0^{a}
WEEK 3	0.036 ± 0.01^{abcd}	0.038 ± 0.01^{abcde}	0^{a}
WEEK 4	0.041 ± 0.01^{bcde}	0.075 ± 0.01^{ef}	0.01 ± 0.01^{ab}
WEEK 5	0.056 ± 0.01^{cde}	0.106 ± 0.01^{fg}	0.013 ± 0.01^{ab}
WEEK 6	0.037 ± 0.01^{abcde}	0.134 ± 0.01^{g}	0.009 ± 0.01^{ab}
P value	**<0.0001**	**<0.0001**	**<0.0001**

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SE (Values in the table can also be expressed as %)

The highest carry over ratio of AFB1 was observed in the liver, followed by the gizzard and the least was in the muscle. In the liver, the highest transfer ratio was observed in week 5 (10.6%) and week 6 (13.6%). There was no transfer in the gizzard in week 1 and in the muscle in week 1, 2 and 3. There was a decrease in transfer in the liver in week 2 to week 3 after which there was a steady increase as shown in Figure 2.

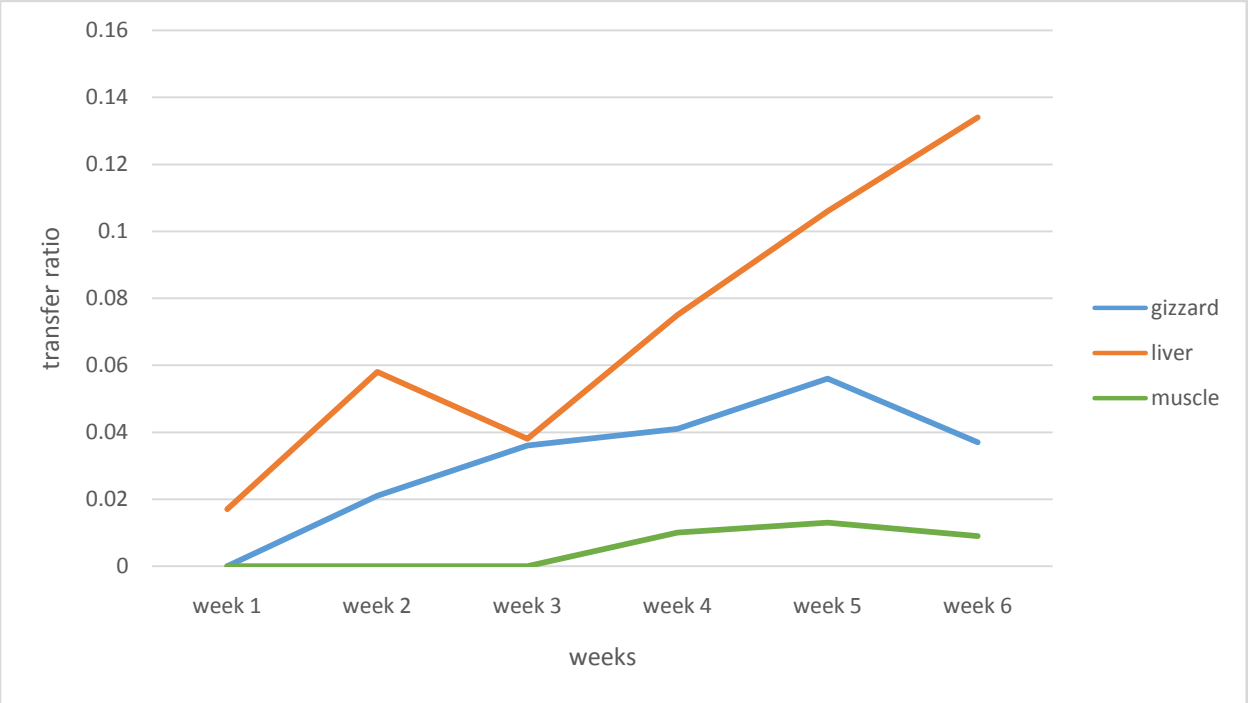

Figure 2: AFB1 carry over ratio trend per week per meat part

There was significant difference ($p<0.05$) in the carry over ratio of AFB2 between the gizzard, liver and muscle per week as shown in Table 2.

Table 2: AFB2 carry over ratio per meat part

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 1	0^{a}	0^{a}	0^{a}
WEEK 2	0^{a}	0.036 ± 0.01^{ab}	0^{a}
WEEK 3	0.012 ± 0.01^{a}	0.084 ± 0.01^{abc}	0^{a}
WEEK 4	0.074 ± 0.01^{abc}	0.151 ± 0.01^{abc}	0^{a}
WEEK 5	0.264 ± 0.01^{cd}	0.219 ± 0.01^{bc}	0^{a}
WEEK 6	0.117 ± 0.01^{abc}	0.454 ± 0.01^{d}	0^{a}
P value	**<0.0001**	**<0.0001**	**<0.0001**

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SE (Values in the table can also be expressed as %)

The highest carry over ratio of AFB2 was observed in the liver, followed by the gizzard and the least was in the muscle. In the liver, the highest transfer ratio was reported in week 5 (21.9%) and week 6 (45.4%) and in the gizzard in week 5 (26.4%). There was no transfer in the gizzard in week 1 and 2, in the liver in week 1 and in the muscle in week from week 1 to week 6. In the gizzard there was a decrease in the transfer in week 5 and 6 as shown in Figure 3.

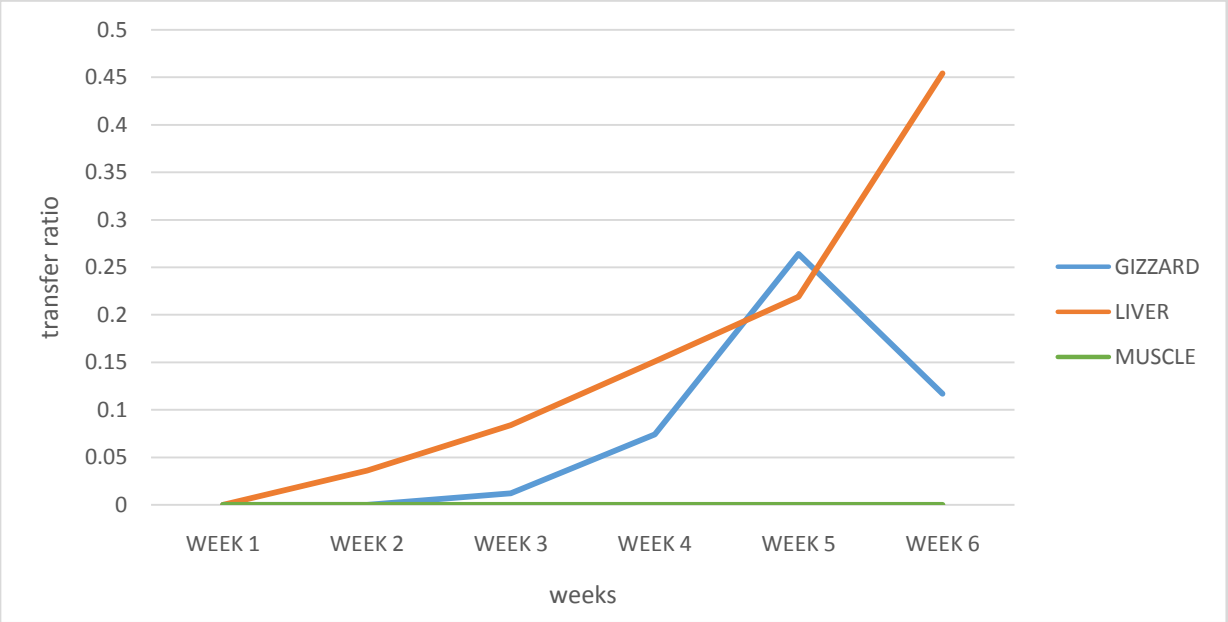

Figure 3: AFB2 carry over ratio trend per week per meat part

There was significant difference ($p<0.05$) in the carry over ratio of AFG1 between the gizzard, liver and muscle per week as shown in Table 3. The highest carry over ratio was observed in the liver, followed by the gizzard and the least was in the muscle. In the liver, the highest transfer ratio was observed in week 5 (21.6%) and week 6 (14.4%).

There was no AFG1 transfer in the gizzard in week 1and in the muscle in week from week 1 to week 4. There was a decrease in transfer in the gizzard from week 4 to week 6 as shown in Figure 4.

Table 3: AFG1 Carry over ratio per meat part

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 1	0^{a}	0.005 ± 0.02^{a}	0^{a}
WEEK 2	0.005^{a}	0.065 ± 0.02^{abc}	0^{a}
WEEK 3	0.033 ± 0.02^{ab}	0.04 ± 0.02^{ab}	0^{a}
WEEK 4	0.073 ± 0.02^{abc}	0.08 ± 0.02^{abc}	0^{a}
WEEK 5	0.049 ± 0.02^{ab}	0.144 ± 0.02^{cd}	0.004 ± 0.02^{a}
WEEK 6	0.011 ± 0.02^{bc}	0.216 ± 0.02^{d}	0.021 ± 0.02^{a}
P value	**<0.0001**	**<0.0001**	**<0.0001**

KEY: Means with different superscript letters in each column and row are statistically significant at p<0.05 ±SE (Values in the table can also be expressed as %)

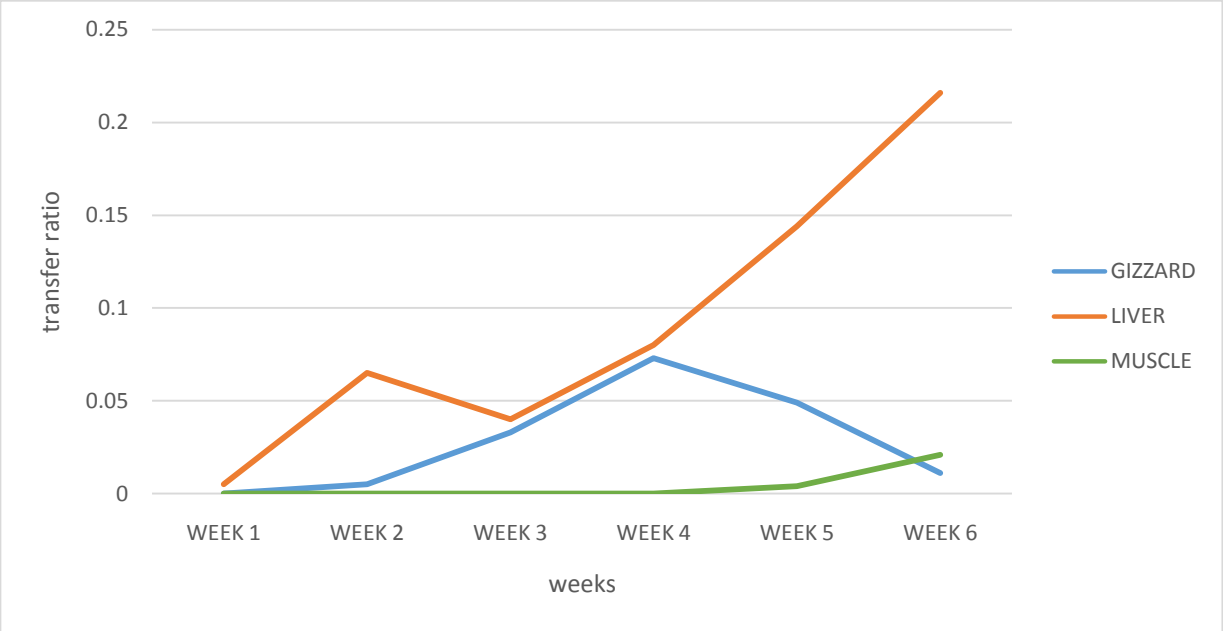

Figure 4: AFG1 Carry over ratio trend per week per meat part

There was significant difference (p<0.05) in the carry over ratio of AFG2 between the gizzard, liver and muscle per week as shown in Table 4. The highest carry over ratio was observed in the liver, followed by the gizzard and the least was in the muscle.

Table 4: AFG2 Carry over ratio per meat part

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 1	0^{a}	0^{a}	0^{a}
WEEK 2	0^{a}	0^{a}	0^{a}
WEEK 3	0^{a}	0^{a}	0^{a}
WEEK 4	0^{a}	0.046 ± 0.05^{ab}	0^{a}
WEEK 5	0^{a}	0.526 ± 0.05^{d}	0^{a}
WEEK 6	0.267 ± 0.05^{bc}	0.41 ± 0.05^{cd}	0^{a}
P value	**<0.0001**	**<0.0001**	**<0.0001**

KEY: Means with different superscript letters in each column and row are statistically significant at p<0.05 ±SE (Values in the table can also be expressed as %)

In the liver, the highest transfer ratio of AFG2 was observed in week 5 (52.6%) and 6 (41%) and in the gizzard in week 6 (26.7%). There was no transfer in the gizzard in from week 1 to week 5, in the liver week 1 to week 3 and in the muscle from week 1 to week 6. There was a decrease in transfer in the liver in week 5 and 6 as shown in Figure 5.

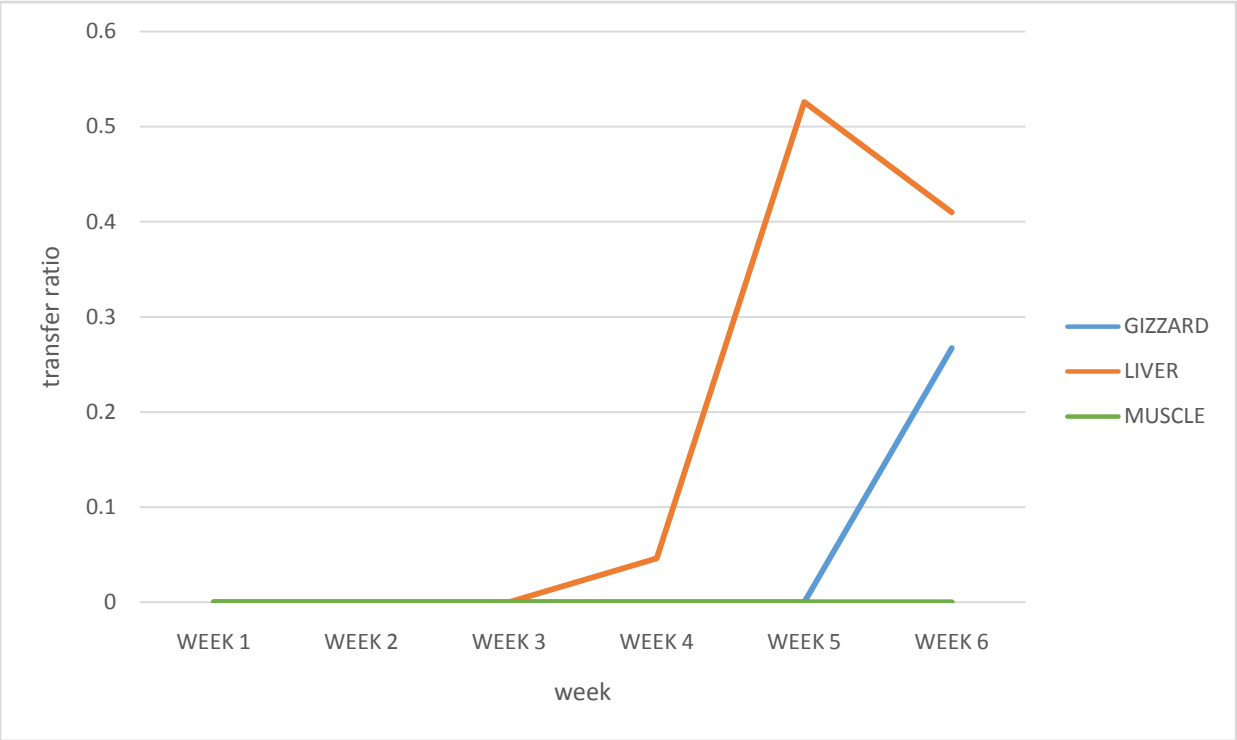

Figure 5: AFG2 carry over ratio trend per week per meat part

There was significant difference ($p<0.05$) in the carry over ratio of Total Aflatoxin between the gizzard, liver and muscle per week as shown in Table 5.

Table 5: Total Aflatoxin Carry over ratio per meat part

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 1	0^{a}	0.011 ± 0.01^{ab}	0^{a}
WEEK 2	0.013 ± 0.01^{ab}	0.051 ± 0.01^{bc}	0^{a}
WEEK 3	0.029 ± 0.01^{abc}	0.038 ± 0.01^{abc}	0^{a}
WEEK 4	0.044 ± 0.01^{abc}	0.066 ± 0.01^{c}	0.006 ± 0.01^{ab}
WEEK 5	0.062 ± 0.01^{c}	0.126 ± 0.01^{d}	0.009 ± 0.01^{ab}
WEEK 6	0.062 ± 0.01^{c}	0.166 ± 0.01^{d}	0.009 ± 0.01^{ab}
P value	**<0.0001**	**<0.0001**	**<0.0001**

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SE (Values in the table can also be expressed as %)

The highest Total Aflatoxin carry over ratio of was observed in the liver, followed by the gizzard and the least was in the muscle. There was a decrease in transfer in the liver in week 2 to week 3 after which there was a steady increase as shown in Figure 6. The highest transfer ratio was observed in the liver in week 5 (12.6%) and 6 (16.6%) and in the gizzard in week 5 (6.2%) and 6 (6.2%). There was no transfer in the gizzard in week 1 and in the muscle from week 1 to week 3.

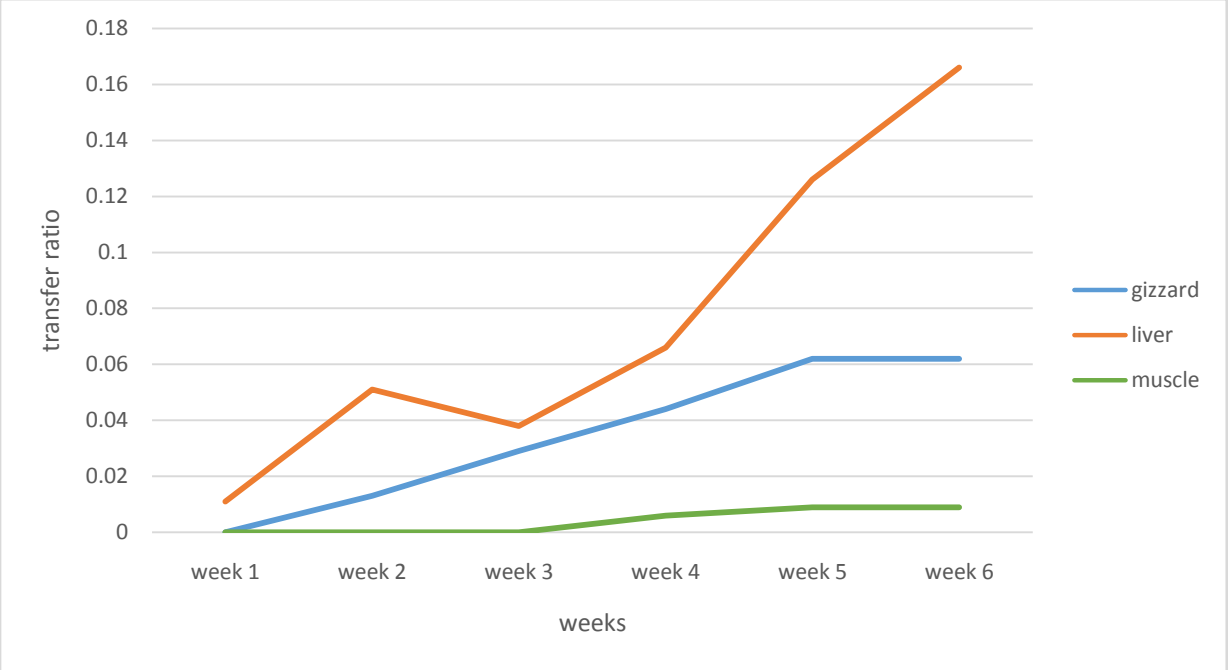

Figure 6: Total Aflatoxin transfer ratio trend per week per meat part

IV. Discussion

Mycotoxin contamination of cereals and feed has been reported worldwide (20). Studies have demonstrated that the occurrence of mycotoxins in food of animal origin has been associated with the contamination of animal feed (21); (22)(23) this results in the transfer of the toxins into animal products.Carryover ratios/transfer ratios signifies a way to demonstrate the bio-accumulation capability of toxins into specific tissues (24).

Studies on carry over especially in animals are limited as only the fundamentals of mycotoxin activity have been reported (21). Additionally, standardized parameters for the calculation of carry-over ratios are non-existent and trials are unmatched due to the diverse study designs employed (25).

A study by Agag (2004) observed the 'carry over' effect of AFB1 from layer feed to eggs at dietary levels of 100-400 µg/kg AFB1 (26). The result was that 0.2 to 3.3 µg/kg of AFB1 was found in eggs and aflatoxin ratio in feed and tissue was found to be minimal ranging from 500:1 to 14,000:1 aside from the liver especially in comparison to what was found in milk (70:1). On the other hand, a study by Zaghiniet al (2005) displayed no quantifiable deposit of AFB1 or its metabolites in eggs. These inconsistent findings could be attributed to the presence of oligosaccharides in naturally occurring aflatoxins in adulterated feeds at varied levels of toxicity (27).

Studies have demonstrated that in broilers and layers, AFB1 residues varies from no detection to 3.0µg/kg in the liver of birds fed on 250-3310 µg/kg of AFB1 over specified periods of time (28). However, there was no significant increase in aflatoxin deposits in the liver of the birds until 1800 µg/kg of aflatoxin adulterated feed was fortified with aflatoxin concentration of 1200 µg/kg with no binding agent (29). Younger birds have significant increase in aflatoxin residues in the liver compared to non-exposed birds. To add on, birds at 3 weeks of age that were fed on 1800 µg/kg of aflatoxins displayed quantifiable levels of AFB1 in the liver. In Kenya limited studies have been done on this.

Carry over ratios or transfer ratios differ for instance in the muscles, the values are below 0.01 (1%) (21), this agrees with the results of the current study as the transfer ratio for AFB1 in week 1, 2, 3, 4 and 6 and AFG1 from week 1 to week 5 and AFB2 from week 1 to week 6 and AFG2 from week 1 to week 6 and Total Aflatoxin from week 1 to week 6 in the muscle were below 1%. However, AFG1 in week 6 was 0.021(2.1%) and AFB1 in week 5 was 0.013 (1.3%) which was above 0.01(1%). Owing to its detoxification role, higher carry-over ratios are found in the liver (21) this agrees with the results of the current study where the highest carry over ratio were observed in the liver in week 5 and 6 in all the analogues for instance; AFB1 was 0.106 (10.6%) and 0.134 (13.4%) in week 5 and 6 respectively, AFB2 was 0.219 (21.9%) and 0.456 (45.6%) in week 5 and 6 respectively, AFG1 was 0.144 (14.4%) and 0.216 (21.6%) for week 5 and 6, AFG2 was 0.526 (52.6%) and 0.41 (41%) in week 5 and 6 respectively and Total Aflatoxin was 0.126 (12.6%) and 0.166 (16.6%) in week 5 and 6 respectively. Upon intake by the host organism (human or animal), these toxins enter the blood stream where they can be found in detectable levels. Völkel et al (2011) (25) reported that carry-over ratios not only

vary only across different mycotoxins groups and animal species, but also across different tissues sampled from a single host. This agrees with the results of the current study where the carry over ratios or transfer ratios were different in the gizzard, liver and muscle. Furthermore, the highest carry over ratio was observed in the liver followed by the gizzard and the least was in the muscle. Studies have also shown that when an animal feeds on contaminated feed, enzymatic and microbial transformations are set in motion giving rise to the formation of gut metabolites. The metabolites are absorbed in the bloodstream and later excreted through urine and feces, but their residues are lodged in organs and muscles (30).

V. Conclusion

The highest carry over ratio was observed in the liver this is consistent with other studies, followed by the gizzard and the least was in the muscle. Higher transfer ratios were observed in the liver and gizzard in week 5 and 6. There was no transfer of AFB2 and AFG2 in the muscle. Carry over ratio in the muscle was below 0.01 (1%) this agrees with other studies. The study concludes that it is less safe to eat the liver and gizzard as the transfer is high. There is need for continuous monitoring and surveillance of aflatoxin in feed by regulatory bodies and national and county government to prevent carry over in meat. The findings from this study will act as a baseline for the determination of carry-over/transfer ratios of aflatoxin in other food animals.

More studies on the carry over effect of aflatoxin need to be carried out in other species of poultry and other food animals and in various localities as information is limited.

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