QUANTIFICATION, CHARACTERIZATION AND CARRY OVER EFFECT OF AFLATOXIN IN BROILER CHICKEN RAISED IN NAIROBI CITY COUNTY, KENYA

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JUNE, 2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my late mum it was her wish that I attain this degree, I wish she was here to see how proud I have made her.

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TABLE OF CONTENTS

DECLARATION ..... **i**
DEDICATION ..... **ii**
ACKNOWLEDGEMENT ..... **iii**
TABLE OF CONTENTS ..... **iv**
LIST OF APPENDICES ..... **ix**
ABBREVIATIONS AND ACRONYMS ..... **x**
DEFINITION OF OPERATIONAL TERMS ..... **xi**
ABSTRACT ..... **xii**
CHAPTER ONE: INTRODUCTION **1**
1.1 Background 1
1.2 Statement of the problem 3
1.3 Justification 5
1.4 Research questions 7
1.5 Objectives 7
1.5.1 Broad objective 7
1.5.2 Specific objectives 7
1.6 Significance of the study 8
1.7 Limitation 8
1.8 Delimitation 9
1.9 Conceptual framework 9
CHAPTER TWO: LITERATURE REVIEW **11**
2.0 Introduction 11
2.1 Classification and structure of Aflatoxin 11
2.2 Occurrence of aflatoxin globally 12
2.3 Aflatoxin in Kenya 13
2.4. Regional Distribution of Aflatoxins in Kenya 15
2.5. Food stuff contaminated with aflatoxin in Kenya 16
2.6 Aflatoxin metabolism and effects 16
2.7 Chicken consumption and feed production in Kenya 18
2.8 Factors influencing the occurrence of aflatoxin in animal feeds 19
2.9 Effects on human to Aflatoxin exposure 21

2.10 Effects of aflatoxin in poultry....23
2.11 Food safety....25
2.12 'Carry- over' of aflatoxins in chicken....28
2.13 Farmers knowledge on aflatoxin adulteration....29
2.14 Aflatoxin regulations in feedstuff....31
2.15 Detection and Quantification of Aflatoxin levels....32
2.16 Management techniques of Aflatoxin....34
2.16.1 Preharvest Management....34
2.16.2 Postharvest Management strategies....36
2.17 Gaps in literature....38
CHAPTER THREE: METHODOLOGY....39
3.1 Research design....39
3.2 Variables....39
3.3 Location of study....39
3.4 Sampling technique....40
3.4.1 Cross sectional design sampling....40
3.4.1.1 Sample size determination for Cross sectional design....41
3.4.2 Longitudinal design sampling....42
3.4.2.1 Sample size determination for Longitudinal design....42
3.5 Inclusion and exclusion criteria....45
3.5.1 Inclusion criteria....45
3.5.2 Exclusion criteria....45
3.6 Research instruments....45
3.8 Sample collection....46
3.9 Aflatoxin analysis....47
3.9.1 Instrument Set up....47
3.9.2 Sample preparation for Aflatoxin analysis of samples....48
3.10 Carry over ratio/Tranfer ratio....51
3.11 Data collection techniques and process....51
3.12 Pre test....51
3.12.1 Validity....51
3.12.2 Reliability....52
3.13 Data analysis....52

3.14 Logistic and ethical consideration 53
CHAPTER FOUR: RESULTS 54
4.1 Knowledge on Aflatoxin by farmers 54
4.2 Detection, Quantification and Characterization of aflatoxin levels in feed 63
4.3 Detection, Quantification and Characterization of aflatoxin levels in broiler meat 67
4.4 Carry over effect of Aflatoxin from broiler feed to broiler meat 85
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION 92
5.1 Discussion 92
5.1.1 Knowledge on Aflatoxin by farmers 92
5.1.1.1 Channels through which farmers heard about aflatoxins 93
5.1.1.3 Farmers' ability to detect feed contamination and action taken with feeds found contaminated with fungal toxins 95
5.1.1.4 Farmers' knowledge on detoxification of contaminated feed 96
5.1.1.5 Farmers' knowledge on feed management practices 97
5.1.2 Detection, Quantification and Characterization of aflatoxin levels in feed 98
5.1.3 Detection, Quantification and Characterization of aflatoxin levels in meat 101
5.1.4 Carry over effect of aflatoxin from broiler feed into broiler meat 104
5.2 CONCLUSION 106
5.2.1 Knowledge on Aflatoxin by farmers 106
5.2.2 Detection, Quantification and Charaterization of aflatoxin levels in feed 106
5.2.3 Detection, Quantification and Charaterization of aflatoxin levels in broiler meat 106
5.2.4 Carry-over effect of aflatoxin from broiler feed to broiler meat 106
5.3 RECOMMENDATIONS 107
5.3.1 Knowledge on Aflatoxin by Farmers 107
5.3.2 Detection, Quantification and Characterization of aflatoxin levels in feed 107
5.3.4 Recommendation for objective 4 on carry-over of aflatoxin from broiler feed into broiler meat 108
5.4 Areas of further research 108
REFERENCES 109
APPENDICES 147

LIST OF TABLES

Table 3.1: Sampling frame for number of farms sampled per ward. 41
Table 4.1: Sociodemographic characteristics of the respondents in the study area 54
Table 4.2: Knowledge of farmers on Aflatoxin based on various parameters 55
Table 4.3: Association between sociodemographic characteristics and knowledge of aflatoxin ..57
Table 4.4: Knowledge on signs to suspect presence of fungal toxins in feed 57
Table 4.5: Association between sociodemographic characteristics and knowledge on signs to suspect aflatoxin contamination..... 58
Table 4.6: Means through which farmers heard about aflatoxin 58
Table 4. 7: Association between knowledge and sources of information on Aflatoxin 59
Table 4.8: Focus Group Discussion on farmers' knowledge on Aflatoxin 62
Table 4.9: Aflatoxin levels (ppb) for broiler starter feed per farm 63
Table 4.10: Aflatoxin levels (ppb) for broiler finisher per farm..... 64
Table 4.11: Aflatoxin levels (ppb) in broiler starter and broiler finisher per Aflatoxin type 65
Table 4.12: Aflatoxin levels (ppb) for water samples per farm..... 66
Table 4.13: Aflatoxin levels (ppb) in water per Aflatoxin type 66
Table 4. 14: Aflatoxin B1 levels (ppb) in broiler meat parts sampled weekly..... 67
Table 4.15: Aflatoxin B1 levels (ppb) in meat samples per farm..... 69
Table 4.16: Aflatoxin B2 levels (ppb) in broiler meat parts sampled weekly..... 70
Table 4.17: Aflatoxin B2 levels in meat samples per farm 71
Table 4.18: Aflatoxin G1 levels (ppb) in broiler meat parts samples weekly 73
Table 4.19: Aflatoxin G1 levels in meat samples per farm..... 75
Table 4.20: Aflatoxin G2 levels (ppb) in broiler meat parts samples weekly 76
Table 4.21: Aflatoxin G2 levels in meat samples per farm..... 78
Table 4. 22: Aflatoxin M1 levels (ppb) in broiler meat parts samples weekly 79
Table 4.23: Aflatoxin M1 levels in meat samples per farm 80
Table 4.24: Total Aflatoxin levels (ppb) in broiler meat parts samples weekly..... 81
Table 4. 25: Total Aflatoxin (ppb) levels in meat samples per farm 83
Table 4. 26: AFB1 Carry over ratio per meat part..... 85
Table 4.27: AFB2 carry over ratio per meat part..... 86
Table 4.28: AFG1 Carry over ratio per meat part..... 88
Table 4.29: AFG2 Carry over ratio per meat part..... 89
Table 4.30: Total Aflatoxin Carry over ratio per meat part 90

LIST OF FIGURES

Figure 1. 1: Conceptual framework 10
Figure 2. 1: Aflatoxin chemical structures 11
Figure 2.2: Aflatoxin metabolism 17
Figure 4.1: Proportion of farmers with knowledge on aflatoxin in the study area 55
Figure 4.2: Length of time farmers heard about aflatoxin 59
Figure 4.3: Place where broiler feed is stored 60
Figure 4.4: Broiler feed storage surface 60
Figure 4. 5: Frequency of purchase of broiler feed 61
Figure 4. 6: State of broiler feed 61
Figure 4.7: Aflatoxin B1 levels in meat parts per week 68
Figure 4.8: Aflatoxin B2 levels in meat parts per week 70
Figure 4.9: Aflatoxin G1 levels in meat parts per week 73
Figure 4. 10: Aflatoxin G2 levels in meat parts per week 76
Figure 4. 11: Aflatoxin M1 levels in meat parts per week 79
Figure 4. 12: Total Aflatoxin levels in meat parts per week 82
Figure 4. 13: AFB1 carry over ratio trend per week per meat part 86
Figure 4. 14: AFB2 carry over ratio trend per week per meat part 87
Figure 4. 15: AFG1 Carry over ratio trend per week per meat part 88
Figure 4. 16: AFG2 carry over ratio trend per week per meat part 89
Figure 4. 17: Total Aflatoxin transfer ratio trend per week per meat part 91

LIST OF APPENDICES

Appendix 1: Consent Form 147
Appendix 2a: Questionnaire 149
Appendix 2b: Focus Group Discussion Guide for farmers 152
Appendix 3: Assent form 153
Appendix 4: Ethical Approval by Kenyatta University Ethical Review Committee 154
Appendix 5: NACOSTI Research License 156
Appendix 6: Kenyatta University Graduate School Approval 158
Appendix 7: Authorization for Reasearch from CDVS Nairobi City County 159
Appendix 8: Aflatoxin Standards 160
Appendix 9a: Calibration standards for meat samples and LOD and LOQ for meat samples 161
Appendix 9b: Calibration curves for meat samples 162
Appendix 10a: Calibration standards for feed samples and LOD and LOQ for feed samples 163
Appendix 10b: Calibration curves for feed samples 164
Appendix 11a: Calibration standards for water samples and LOD and LOQ for water samples 165
Appendix 11b: Calibration curves for water samples 166
Appendix 12: Map of Nairobi City County 167

ABBREVIATIONS AND ACRONYMS

ABS-TCM	-	African Breeders Services Total Cattle Management
AF	-	Aflatoxin
ANOVA	-	Analysis of Variance
B1	-	Aflatoxin B1 (AFB1)
B2	-	Aflatoxin B2 (AFB2)
CYP	-	Cytochrome P
DVS	-	Directorate of Veterinary Services
ELISA	-	Enzyme Linked Immunosorbent Assay
EU	-	European Union
FGD	-	Focus Group Discussion
G1	-	Aflatoxin G1 (AFG1)
G2	-	Aflatoxin G2 (AFG2)
HPLC	-	High Performance Liquid Chromatography
KMT	-	Kenya Market Trust
LC/MS-MS	-	Liquid Chromatography tandem Mass Spectrometry
M1	-	Aflatoxin M1 (AFM1)
M2	-	Aflatoxin M2 (AFM2)
RTA	-	Right Track Africa
SSA	-	Sub Saharan Africa
SPSS	-	Statistical Package for Social Scientists
STATA	-	Students and Data

DEFINITION OF OPERATIONAL TERMS

Aflatoxin – a group of toxic and carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (WHO/FDA, 2018)

Bioaccumulation - refers to the build- up of toxic elements in the body of an organism (WHO/FAO, 2018).

Carry over - transfer of toxin into meat and eggs after metabolism in the GIT, liver of kidneys depending on their chemical structure (Filazi *et al*., 2017)

Characterization - technique used in distinguishing aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* (WHO/FDA, 2018)

Establishment - any facility that rears broiler chicken, be it farm or household

Genotoxic - Toxic (damaging) to DNA. Substances that are genotoxic may bind directly to DNA or act indirectly leading to DNA damage by affecting enzymes involved in DNA replication, thereby causing mutations which may or may not lead to cancer or birth defects (inheritable damage). Genotoxic substances are not necessarily carcinogenic (Alberto *et al*., 2017).

Hepato Cellular Carcinoma - is the most common type of primary liver cancer. Hepatocellular Carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B or hepatitis C infection.

Isoenzymes - Isozymes (also known as isoenzymes) are enzymes that differ in in amino acid sequence but catalyze the same chemical reaction.

Metabolism - incorporation of the products of digestion into the cell metabolism (Filazi *et al.,* 2017)

Teratogenic - Any agent that can disturb the development of an embryo or fetus. Teratogens may cause a birth defect in the child. Or a teratogen may halt the pregnancy outright.

Total Aflatoxin - Sum of Aflatoxin (B1+B2+G1+G2).

ABSTRACT

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 a major risk to feed ingredients used in the manufacture of animal feed and subsequently is a great risk to human consumers due to the detrimental effects of these toxins as they are regarded as Type 1 carcinogens. For that reason, a study to establish the carry over effect of aflatoxin in broiler chicken was carried out in Nairobi City County as there is limited data. The specific objectives of the study were to quantify and characterize aflatoxin levels in broiler feed and broiler meat, to determine carry over effect from broiler feed into broiler meat and to assess the knowledge of farmers on aflatoxin in Nairobi City County. The findings will provide a scientific basis for the endorsement of regulations that are key in the decision making process and policy formulation of food and feed with respect to Aflatoxins.The study utilized a cross sequential study design which included both cross sectional and longitudinal study. The longitudinal study was done for six weeks which corresponded to the period of raising broilers from day old chicks to slaughter. Detection and quantification of aflatoxin levels in broiler feed and meat was done using the (LC/MS-MS) technique. A structured questionnaire on knowledge of aflatoxin was administered and multistage randomn sampling was used. Two FGD comprising of twelve members each among farmers in two sub counties were conducted. A pretest of the questionnaire was carried out in Kiambu County. SPSS version 26 was used for quantitative analysis of questionairres and STATA version 12 was used to carry out one way and two-way ANOVA for laboratory analysis. NVIVO soft ware was used for analysis of data from FGDs. 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 of the study show that majority of the farmers (58.2%) had knowledge on aflatoxin. There was a significant association ($p<0.05$) between socio demographic characteristics of farmers and knowledge on aflatoxin. Aflatoxin levels in broiler starter were; B1(17.26±3.07), B2 (2.44±0.84), G1 (8.87±2.41), G2 (0.9±0.44) and Total AF (29.47±6.13). Aflatoxin levels in broiler finisher were B1 (17.17±3.09), B2 (2.68±1.18), G1 (9.25±2.7), G2 (1±0.45) and Total AF (30.1±6.88). 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. 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%. This study concluded that the farmers had adequate knowledge on aflatoxin occurrence in feeds and methods to reduce the occurrence, but had no knowledge on carry over effect. Although there were appreciable amounts of aflatoxin in the broiler feeds and broiler meat, the carry over effect was low in the muscle but higher in the liver and gizzard. The study recommends that there is need constant monitoring of Aflatoxin levels in poultry feed & products by KEBS & national & county government and application of stringent allowable limits in feed and feed ingredients.

CHAPTER ONE: INTRODUCTION

1.1 Background

Aflatoxins are a group of extremely lethal, carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Sana *et al.,* 2019). The US Food and Drug Administration (FDA) terms aflatoxins as an inevitable food contaminant that regularly contaminates agricultural products worldwide and largely in developing countries (WHO/FDA, 2018). 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) (Omara *et al.,* 2020). 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 (Sahib *et al*., 2020). In Kenya, aflatoxins are prevalent and the 2004 epidemic of acute aflatoxicosis was the highest ever recorded in mycotoxin history (Omara *et al.,* 2021).

About 600 million (1 in 10 people globally) suffer from food borne infections leading to about 420, 000 deaths annually resulting in the loss of 33 million Disability Adjusted Life Years (DALYs). A considerable fraction of this burden is heavily felt in the African continent where unsafe food is responsible for about 91 million cases of food borne illnesses yearly and out of these 137,000 die prematurely (WHO, 2019).

Aflatoxin adulteration of poultry feed and raw feed ingredients is a serious concern globally (Mahbuba *et al*., 2018). Close to 5 billion people in developing countries are at risk of chronic exposure to aflatoxins (Udomkun *et al*., 2018). The effects exhibited in poultry include; reduced feed consumption rate, feed conversion efficiency and reproductive performance, decreased growth rate and heightened risk of morbidity and fatality (Naseem

et al., 2018a, Bhatti *et al*., 2018). Humans are also at risk of the effects of aflatoxin as aflatoxins are carried over into blood tissue, gizzard, breasts, liver and eggs of poultry therefore becoming a risk to human consumers (AL- Ruwaili *et al.,* 2018). Studies have also shown that aflatoxins have genotoxic, teratogenic and hepato carcinogenic effects on humans (Naseem *et al*., 2018b). Poultry is considered to be the most susceptible of all the animal species to the effects of aflatoxins (Pinotti *et al*., 2016).

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 (Ebeed *et al.,* 2016).

There has been a rising increase in the specialization of commercial broiler farming in urban towns in Kenya due to the ready market availability compared to up-county where indigenous chicken farming dominates (Maud *et al*., 2017). The population of commercial chicken in Kenya is about 8 million and this huge population relies on manufactured poultry feed. It is also estimated that close to 500,000 tonnes of animal feed is manufactured yearly of which approximately 70% belongs to poultry (Altherstone *et al*., 2016).

The ingredients used in the production of poultry feed is susceptible to mycotoxin adulteration due to the ecological, climatic and storage conditions in developing countries (Mahbuba *et al*., 2018). To add on, socioeconomic factors such as informal marketing systems, poor means of transport, absence of necessary tools, materials and equipment, lack of information and knowledge on pre and post-harvest management and lastly weak

governmental regulations and legislations also contribute to the aflatoxin burden (Kamika *et al*., 2016).

In addition, aflatoxin levels in food and feed is constantly monitored in developed countries through various chromatographic and immune-enzymatic methods (Iqbal *et al*., 2014), however, this close monitoring is non existent in many developing nations. Furthermore, main key players i.e. farmers that could have a substantial role in the control of aflatoxins have inadequate knowledge on the causes, effects and control measures of aflatoxins (Sirma *et al*., 2015). Consequently, they are not keen on incurring the costs of controlling aflatoxin adulteration owing to that fact that most of their dealings are in informal markets without strict regulations (Sirma *et al*., 2015). This is attributed to the lack of knowledge and alternatives for disposal of adulterated cereal at the household level and ultimately it is fed to domestic animals (Kiama *et al.*, 2016).

Due to the detrimental effects of these toxins, many countries have instigated regulations on animal feeds and food items (WHO/FAO, 2018). Therefore, quantification of aflatoxin levels in meat after ingestion of the toxin through feed is critical to public health (Sahib *et al.*, 2020). It is along these lines that this study was carried out.

1.2 Statement of the problem

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 (Valery *et al*., 2018). Studies have reported that high levels of Aflatoxin in feed samples leads to high levels of Aflatoxin in animal products (Faten *et al.,* 2016). Studies have also reported that it is difficult to have fungal toxin free feeds under normal

conditions, hence impossible to eliminate. Contamination of food with aflatoxins and its impact in Kenya has been calamitous and has been evidenced by the periodic reported incidences of acute aflatoxicosis as well as alarming levels of chronic exposure in the Kenyan population (Gong *et al.*, 2012). Alarmingly high proportions of food commodities that surpass the Kenyan regulatory threshold of 10 μg/kg set for total aflatoxins and 5 μg/kg set for aflatoxin B_1 have been reported in Kenya (KEBS, 2018a). High levels of Aflatoxin have been found in maize, peanuts and in animal feed in Kenya. These levels, coupled with the regular consumption of substantial rations of maize products across diverse age groups (Kang'ethe *et al.,* 2017), provides insights into the high chronic aflatoxins exposure rates in the country, of about 67% of the population (Githang'a and Awuor, 2016). Additionally, Aflatoxin levels whose values stretch to four-digit (ppb) have on many instances resulted in death.

The poultry industry in Kenya 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 (Ochieng *et al.,* 2021). To add on, cereal traders utilize trade loopholes to divert aflatoxin contaminated cereal into animal feed manufacturing companies and hence these posses a risk to poultry and human health (East African Community, 2018a).

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 (Mccarron *et al*., 2015). However, illegal food processing practices in Nairobi City County have have been reported with reference to the use of unsafe meat preservatives by meat vendors (Githika, 2018).

In Nairobi City County, little is known or documented about the carry over effect of aflatoxin in broiler meat. Aflatoxin residue levels in broiler meat consumed among the residents is also not known, as an investigation on microbial contamination of broiler meat in Nairobi only found high pathogen infestation in the meat (Odwar *et al*., 2014) and aflatoxin levels were not determined. In addition, it is not known whether the farmers' have sufficient knowledge on proper feed management practices and the carry over effect of Aflatoxin.

1.3 Justification

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 (WHO, 2020). Although aflatoxin adulteration mostly affects developing countries, there is insufficient documented evidence therefore the burden in SSA is underestimated (Grace *et al*., 2015). Poultry are considered to be sensitive to the effects of aflatoxins and broilers are further considered to be more susceptible to aflatoxin exposure (Althersone *et al*., 2016). In Kenya, there is a shift from consumption of red meat to white meat this is according to a study done by the Kenya Market Trust (KMT, 2019). Majority of the Kenyan population has related the rise in the incidence of Non-Communicable Diseases such as cancer, cardiovascular diseases, autoimmune conditions like gout and arthritis to the intake of red meat. Besides this, doctors and nutritionists are advising their clients to cut down or entirely stop the consumption of red meat and substitute with white meat (KMT, 2019). A study in Kenya demonstrated that farmers perceived the consumption of moldy food by humans to be unsafe but considered meat from animals fed on moldy feed to be harmless (Kiama *et al*., 2016).

Nairobi County unlike other counties serves as the major harbor for broiler market across the country and beyond (Mccarron *et al*., 2015). The consumption of broiler meat in Nairobi City County is projected to rise to 30.5 thousand metric tonnes by the year 2030 and thus to cater for this escalating demand, broiler and feed production is expected to rise (Maud *et al*., 2017).

The recent revelations of contamination of specific maize flour and peanut butter brands with aflatoxin (MOH, 2019) in Kenya has unearthed the loopholes in the food safety systems and food regulatory bodies (Mutegi *et al.*, 2018), and has shed light on the quality of food we consume as a country. Food security is one of Kenya's big 4 agenda in the attainment of the country's vision 2030, therefore food safety is paramount (GOK, 2018). 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 (Food Safety Policy, 2021). Studies on Aflatoxin in Kenya have mostly majored on cereals and their products (Okoth, 2016) and studies on the 'carry over' of aflatoxin in poultry meat are limited and specifically in Nairobi City County. To add on, there has been heavy bias of data collected on Aflatoxin from the eastern region of Kenya compared to other parts of the country, elicited by the acute aflatoxicosis cases reported from the region as well as the presence of pre-disposing factors (Mutegi *et al.*, 2018) hence why the present study was conducted in Nairobi City County. It is from this background that this study was conducted and recommendations given to the relevant authorities.

1.4 Research questions

1. What is the farmers' knowledge on aflatoxin in Nairobi City County, Kenya?
2. What are the levels of aflatoxin quantified and characterized in broiler feed in Nairobi City County, Kenya?
3. What are the levels of aflatoxin quantified and characterized in broiler meat in Nairobi City County, Kenya?
4. What is the carry over effect of aflatoxins from broiler feed into broiler meat in Nairobi City County, Kenya?

1.5 Objectives

1.5.1 Broad objective

To quantify, characterize and to determine the 'Carry Over' effect of aflatoxin in broiler chicken raised in Nairobi City County, Kenya.

1.5.2 Specific objectives

1. To assess the knowledge of farmers on aflatoxin in Nairobi City County, Kenya.
2. To quantify and characterize aflatoxin levels in broiler feed in Nairobi City County, Kenya.
3. To quantify and characterize aflatoxin levels in broiler meat in Nairobi City County, Kenya.
4. To determine the carry over effect of aflatoxins from broiler feed into broiler meat in Nairobi City County, Kenya.

1.6 Significance of the study

Aflatoxins pose a major risk to the health of both humans and animals. Grounded on this knowledge, the study has obtained information that will be valuable in creating awareness on the occurrence, levels and distribution of aflatoxin in broiler feed and broiler meat. The study will also form a scientific basis for the endorsement of regulations that are key in the decision making process to instigate permissible limits of aflatoxin in feed. The results of this study will also be beneficial to the Ministry of Agriculture through the department of livestock and Ministry of Health Division of Public Health in that; farmers will be sensitized on the strategies to be applied to prevent the occurrence of aflatoxin in feed and consequently in broiler meat. The results will be shared with feed manufacturers and regulatory bodies, policy makers and other stakeholders and advice given on appropriate control strategies. The study will also be beneficial to the community in that the community will be enlightened on the subject of aflatoxin in meat and this will be instrumental in aiding the community to make the right choices and adopt the appropriate strategies in the prevention of aflatoxin in feed and consequently in broiler meat.

1.7 Limitation

The study did not consider seasonal and geographical variation and its effect on aflatoxin levels and was only limited to broiler chicken; studies that will include the stated factors should be carried out.

1.8 Delimitation

The study sought to establish the 'carry over' effect of aflatoxin from broiler feed into broiler meat per week (age of chicken) and as well as illustrating the bio accumulation of aflatoxin levels per week in the broiler meat parts (gizzard, liver and muscle).

1.9 Conceptual framework

The conceptual framework for this study is as shown in figure 1.1 below. Sociodemographic characteristics of the farmers such as age, sex, marital status and level of education will have an influence on the farmers' knowledge with respect to storage of feed and on the signs to detect feed contamination. This will inturn influence the production choice the farmer will make with regards to the type and choice of feed the farmer will purchase. If the farmers do not apply proper feed management practices, this will result in *Aspergillus* growth in the feed hence toxin formation in the feed leading to feed contamination. Once the toxin is ingested by the animal (broiler), it undergoes toxicokinetics; digestion, absorption and assimilation of the toxin in the body of the animal. These toxins are then 'carried over' or transferred to meat and the outcome will be that there will be bio-accumulation of the toxin in the animal hence, the broiler meat will be contaminated with aflatoxin posing a risk to humans through consumption of the contaminated meat.

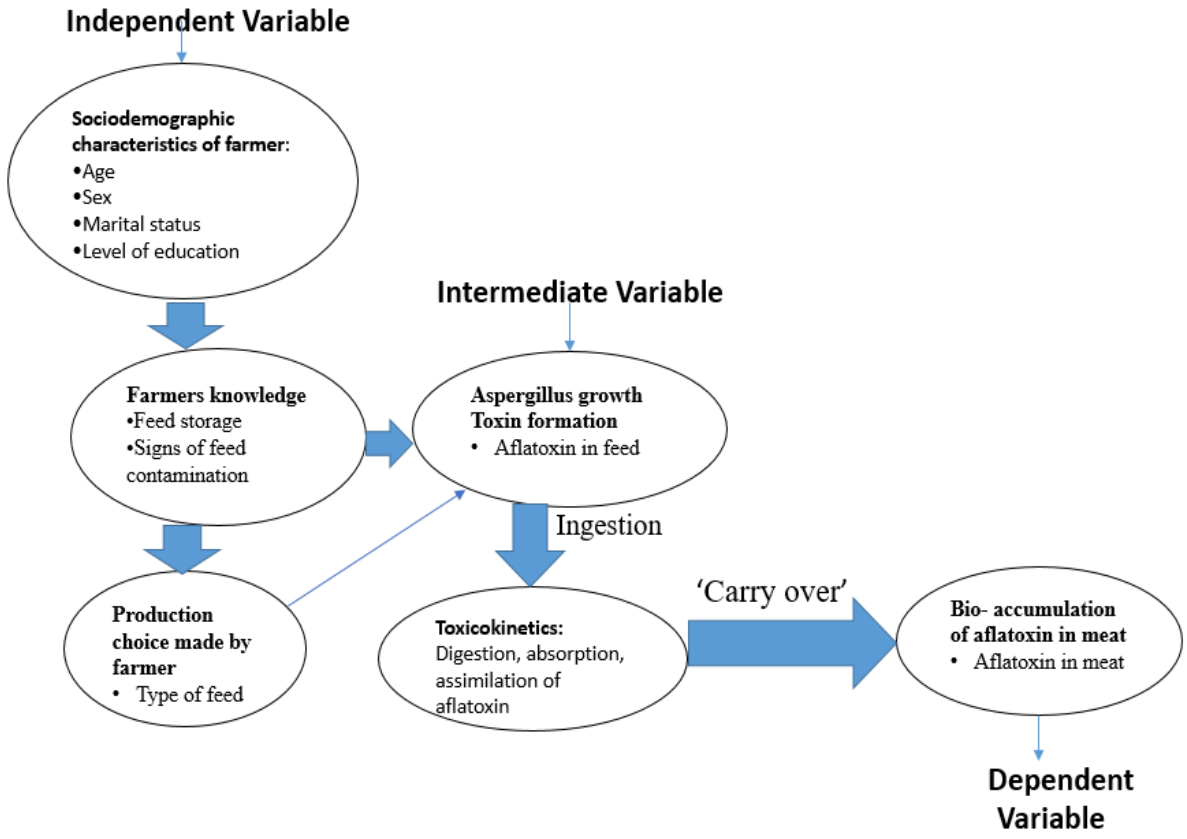

Figure 1.1: **Conceptual framework**

Source: (Peles *et al.*, 2019) and modified

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter has literature touching on aflatoxin, it highlights the factors influencing growth of aflatoxin in feed, effects of aflatoxin in poultry and humans and the 'carry over' effect. It also touches on awareness of farmers on aflatoxin and food safety policy in Kenya on aflatoxin.

2.1 Classification and structure of Aflatoxin

The term aflatoxin is a blend of three words, a, for *Aspergillus genus*, fla, for the species Flavus and toxin meaning poison. The invention of aflatoxin dates back to 1960 where it caused the death of a thousand turkeys in the United Kingdom that fed on adulterated peanut, and continues to pose a risk to the poultry business to date (Diaz and Murcia, 2011). Aflatoxins (AFs) are a class of mycotoxins that are produced by Aspergillus species i.e. *A. Flavus, A. parasiticus and A. nominus.* Secondary metabolites of AF can adulterate foodstuff particularly maize, peanuts and cottonseed. Aflatoxins comprise of AF; B1, B2, G1, G2, M1, and M2 (Sumit *et al.*, 2010).

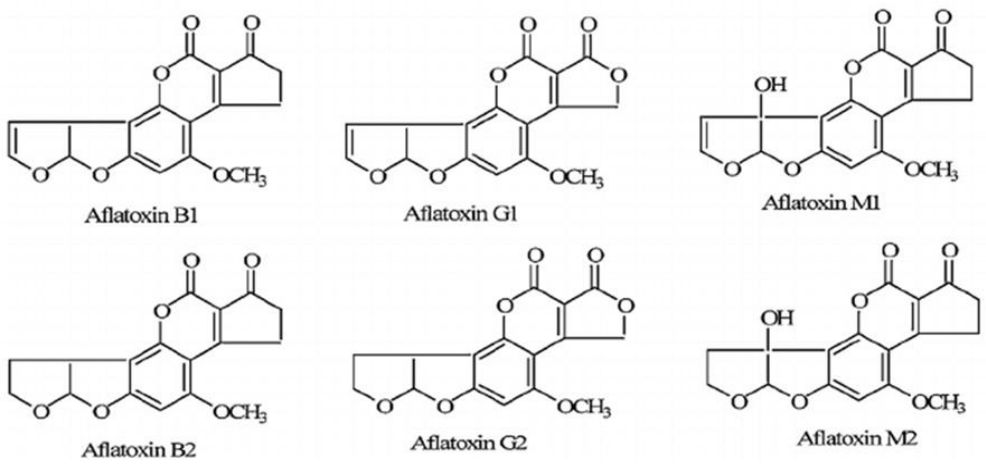

Figure 2.1: **Aflatoxin chemical structures** (Source: Zain, 2011)

AFs are chemically polysubstituted compounds with analogous chemical structures (Omara *et al.,* 2020). There exist over 20 different categories of AFs but aflatoxin B_1 (AFB_1), aflatoxin G_1 (AFG_1), aflatoxin M_1 (AFM_1), aflatoxin B_2 (AFB_2), and aflatoxin G_2 (AFG_2) have proven to be toxicologically significant (Wu *et al.,* 2009). B-type AFs are pentanone byproducts that exhibit strong blue fluorescence under UV light. The G-series of AFs are six-membered lactones that display yellow-green under UV light and hence the B and G nomenclature (Bennett and Klich, 2003). Aflatoxin G_2 and B_2 are analogues of G_1 and B_1 which the 8,9-double bond is absent in the furan ring. Aflatoxins M_1 and M_2 are metabolic byproducts of B_1 and B_2 that display blue-violet fluorescence under UV light and are commonly tested in urine and milk of animals that have fed on AFB_1 adulterated feed. In order of increasing toxicity; $AFG_2 < AFB_2 < AFG_1 < AFM_1 < AFB_1$ (Wu *et al.,* 2009).

2.2 Occurrence of aflatoxin globally

Aflatoxins occur globally in various foods and feeds particularly in cereals. Contamination with aflatoxin can occur in the farm, during storage, during distribution and in the production cycle. In processed animal feed, the adulteration of one constituent will cause the adulteration of the whole lot (Galo *et al.*, 2015). In addition, the inclusion of feedstuff adulterated with aflatoxin generating fungi can cause the degeneration of the other feed consignments and acts as a channel though which feeds in the industrial environment become adulterated and this becomes hard to eradicate. This decline in quality has a substantial impact on the worldwide market and the universal exchange of animal feed and feed constituents (Kovalsky *et al*., 2016). The occurrence of mycotoxin in processed feed poses adverse effects to the health of humans and animals owing to the synergistic effects among the toxins (Kovalsky *et al*., 2016).

The worldwide manufacture of animal feed got to 964 million tonnes in 2014 (FEFAC, 2015). Cereals specifically maize are extensively utilized as carbohydrate sources in animal feed for diverse groups of animals. These raw ingredients constitute 50-80% of animal food in America and Europe. USA and Brazil are the key corn exporter countries while Japan and Mexico are the principal importer countries (ITC, 2017). For instance, most of the constituents that are used in Malaysia for the manufacture of animal feed such as cereals, soybean meal, corn gluten meal and soybean meal are imports from Thailand, China, India, Argentina, USA, Australia and Canada (ITC, 2017). Mycotoxin adulteration of feeds due to improper storage during manufacture and distribution is common (Afsah-Hejri *et al*., 2013). In Costa Rica, animal feed manufacture is centered on corn products and in 2015 over 764, 254 tonnes of corn products were imported (PROCOMER, 2017).

2.3 Aflatoxin in Kenya

In Kenya, aflatoxins are largely produced by *Aspergillus parasiticus* and *A. flavus* (Oloo *et al.,* 2019; Mitema *et al.,* 2019; Monda *et al.,* 2020; Islam *et al.,* 2018; Okoth *et al.,* 2018; Menza *et al.,* 2018). *A. flavus* is a worldwide fungus well-known to produce AFB_1 and AFB_2 together with aspergillic, cyclopiazonic, and kojic acids (Varga *et al.,* 2009). *A. parasiticus* produces both AFs B and G and kojic and aspergillic acids (Baquiao *et al.,* 2013). *A. niger*, *A. terreus*, and *A. versicolor* have been found in soils and mill dust in Eastern Kenya (Muthomi *et al.,* 2009). Additionally, the presence of *A. alliaceus, A. tamarii*, and *A. caelatus* in Kenya has been replicated (Mutegi *et al.,* 2012; Ndung'u *et al.,* 2013; Muriithi, 2014). A genetic profiling study found that *A. minisclerotigenes* in Eastern Kenya presented a higher AF biosynthesis capability than *A. flavus* (Oloo *et al.,* 2019). Although both the L- and S-strain morphologies of *Aspergillus flavi* have been found in Kenya, epidemiological studies have discovered that aflatoxicoses linked with maize

consumption in Kenya have been due to the novel S-morphology fungus formerly associated with the 2004–2006 aflatoxicosis outbreak in Kenya (Probst *et al.,* 2012). By and large, *A. flavus* is regarded as the major producer of AFs in agricultural goods with an optimum growth temperature of 25°C and a minimum water activity of 0.75, although AF biosynthesis begins at 10–12°C (Lizarraga- Paulin *et al.*, 2011).

Kenya is endowed with an erratic tropical climate occasioned by seasonal drought, high humidity, and elevated temperatures before the harvesting season (Ochungo *et al.,* 2016). The country's climate is hot along the coast, moderate inland, and dry in the north and northeast. The country has varied weather patterns whereby between March and June there are extended rains and from October to December there are short rains. The country has four distinct climatic zones and is further sub divided into agroecological zones based on the rainfall and temperature conditions suitable for various staple crops. The Central Highlands and Rift Valley regions are endowed with rich soils, rainfall of up to 3000 mm annually and temperatures between 21°C to 26°C. Conversely, the western region is hot and wet throughout the year. Rainfall in this region is more than 1000 mm annually with temperatures between 27°C to 29°C. The Northern and eastern regions are relatively hot with annual rains below 510 mm with elevated temperatures of upto 39°C in some regions (Ochungo *et al.,* 2016). Poor post harvest management of cereals for instance utilization of propylene storage bags, drying of cereals on bare grounds, insect invasion, improper storage facilities (stores with leaking roofs), poor transportation, and poor management of crops as well as recurring poverty have proven to be the predisposing factors for aflatoxin adulteration of foods in Kenya (Obonyo and Salano, 2018; Kiarie *et al.,* 2016; Koskei *et al.,* 2020). Contamination has also been associated with planting of maize in ecologically predisposed regions of the country (Mutiga *et al.,* 2015; Mwhihia *et al.,* 2018). To add on

biophysical factors such as soil, plant genetic make up and vulnerability to fungal growth coupled with sociodemographic factors such as low education levels, inadequate sensitization and gender disparity have contributed to the prevalence of AFs in Kenya (Leroy, 2015, Kiama *et al.*, 2016, Mutiga *et al.*, 2014; Sirma *et al.*, 2015).

Studies on AFs have shown that AFB_1 is by far the most studied AF in Kenya, followed by AFM_1 (Mutegi *et al.*, 2018) hence numerous studies have reported on the levels of AFB_1, AFM_1, or total AFs. Due to the increase in the cases of aflatoxin witnessed in the country, numerous studies have been conducted and the results have dispalyed alarming levels of aflatoxin and this is nerve-wrecking (Nabwire *et al.*, 2020, Kibugu *et al.*, 2019).

2.4. Regional Distribution of Aflatoxins in Kenya

Kenya was among the first AF hotspot countries ever documented (Asplin and Carnaghan, 1961; Stevens *et al.*, 1960) together with Uganda and Brazil (Benkerroum, 2019). Kenya is categorized into 7 agroecological zones; humid, subhumid, transitional, temperate, semiarid, arid, and per-arid (Jaetzold and Schmidt, 2009). AF has been detected in samples from all the 7 zones (IFPRI, 2010; Gachara *et al.*, 2018; Sirma *et al.*, 2014). This has been ascribed to the resemblance in the agronomic and pre-, peri-, and postharvest management and the interregional marketing of foods (Mutegi *et al.*, 2009; Okoth, 2016; Kipkoech *et al.*, 2007; Kirimi *et al.*, 2011). The eastern region of the country is more predisposed to aflatoxin contamination as the region posseses the highly toxigenic *Aspergillus* spp, and has remained the hub of all aflatoxicoses reported in Kenya (Monda *et al.*, 2020). This region experiences hotter and drier climatic conditions compared to the Western region. Due to this, it is classified as a semihumid to semiarid region whereas the Western region is categorized as a subhumid to semihumid agroecological zone (Gachara *et al.*, 2018). Environmental conditions have proven to have an influence on the capability of the

Aspergillus fungi to contaminate, inhabit, and survive in crops as well as produce mycotoxins. Additionally, variations in such environments also have an effect on the quantities as well as the composition of aflatoxin-producing fungi (Bandyopadhyay *et al.*, 2016). The prevalence of AFs in Eastern Kenya is in agreement with earlier findings that mycotoxin contamination is multifactorial, but climate is the most significant factor (Milani, 2013).

2.5. Food stuff contaminated with aflatoxin in Kenya

Aflatoxins in Kenya adulterate staple foods such as maize (*Zea mays* L.) and its products (*Busaa, chan'gaa, githeri, irio, muthokoi,uji,* and *ugali)* (Mutahi, 2019; Okoth *et al.*, 2012; Murithi, 2014; Obonyo and Salano, 2018; Kiarie *et al.*, 2016; Nabwire *et al.*, 2020; Obade *et al.*, 2016), sorghum (*Sorghum bicolor* L.) (Kiarie *et al.*, 2016; Obade *et al.*, 2016; Sirma *et al.*, 2015) millet (*Eleusine coracana*) (Sirma *et al.*, 2015), pigeon peas (Obade *et al.*, 2016; Mutungi *et al.*, 2008), peanuts and its products (Ndung'u *et al.*, 2013; Mutegi et al., 2013; Obade *et al.*, 2016), cassava, rice, and dried silverfish (*Rastrineobola argentea*, locally called *omena*) (Obade *et al.*, 2016; Orony *et al.*, 2015), animal feeds (Mwihia *et al.*, 2018; Nyanganga, 2014), dairy products (milk, yoghurt, and *Lala*) (Kiarie *et al.*, 2016; Lindahl *et al.*, 2018), and herbal products (Keter *et al.*, 2017). Studies on AFs in Kenya have focused majorly on maize, peanuts, animal feeds, and dairy products particularly milk (Okoth, 2016). In spite of their existence in foods, food processing techniques cannot entirely destroy AFs in precontaminated foodstuffs due to their thermoresistance nature (Medina *et al.*, 2017).

2.6 Aflatoxin metabolism and effects

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 as shown in figure 2.2 below (Monson *et al*., 2015; Yarru *et al*., 2009). 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 (Bbosa *et al*., 2013; Dohnal *et al*., 2014). Among the animal species, rabbits are highly susceptible to the hazardous properties of AFB1 succeeded by ducks and turkeys. Chicken are highly sensitive to the contaminant while fish and swine are fairly susceptible. Cattle and sheep are the most resilient of all the animal species to the contaminant (Lozano, 2006). Studies have also demonstrated that younger animals are more susceptible to AFB1 contaminant than older persons (Yarru *et al*., 2009).

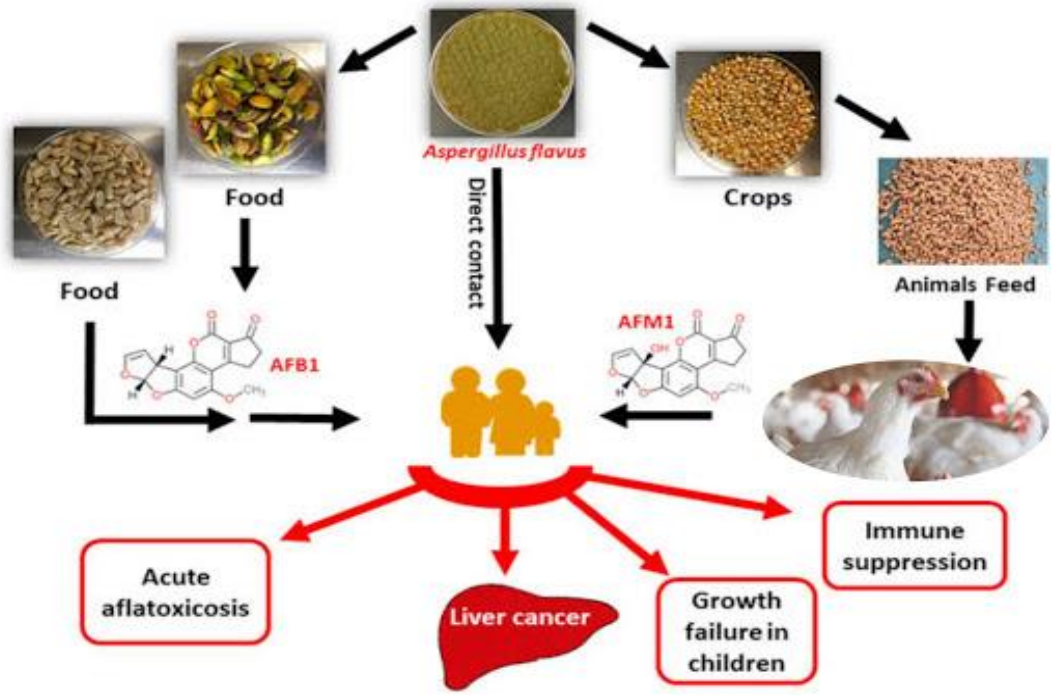

Figure 2.2: Aflatoxin metabolism Source: Raza, 2018

Aflatoxicosis can present acute or chronic effects in animals as shown in figure 2.2 above. Acute aflatoxicosis leads to death while chronic aflatoxicosis leads to cancer, toxicity and

immunosupression. The principal target organ is the liver. AFB1 is carcinogenic (IARC, 2015) and this is as a result of bioactivation of cytochrome P450 in the liver and by the production of AFB1-8, 9-exposide (AFBO) whereby AFBO facilitates the carcinogenic and toxic activities in the liver (Wu *et al*., 2009). Susceptibility to aflatoxins is dependent on species, age, gender and nutrition however there are individual differences in the degree of stimulation of the mycotoxins in different species.

2.7 Chicken consumption and feed production in Kenya

In Kenya, the bulk of the consumers of chicken are from high income (96%), followed by middle income (88%) and lastly (82%) in the low income segment (KMT, 2019). According to the division of veterinary services, in the year 2016, 9,503,536 poultry was slaughtered in the country (DVS, 2017). An estimate of about 500,000 tonnes of animal feed is manufactured yearly in Kenya of which about 70% is poultry feed (Atherstone *et al*., 2016). These animal feeds are manufactured by numerous recognized feed millers' mainly in major cities and they utilize both local and imported ingredients. Corn, wheat and their by-products such as bran, germ and pollard are the chief raw materials used in the feed industry as energy sources while the main raw materials utilized as protein sources are sunflower, soy bean and cotton seed meals (RTA & Nutrimix limited, 2016).

A 100% of cotton seed meal, wheat grain and maize germ and 75-80% of maize grain, maize germ and wheat pollard and lastly about 50-70% of wheat bran, sunflower, maize germ, bran and soy bean meals are manufactured in the country (RTA & Nutrimix limited, 2016). Conversely, 75% of soy bean and sunflower cake and 57% of cotton cake is imported. The animal feeds are fortified in small amounts according to the animal requirements with vitamin and mineral premixes and amino acids of which are 100%

imported (RTA & Nutrimix Limited, 2016). Other raw materials that are used in feed manufacture include; sorghum, molasses, rice polish, copra/coconut meal and rice bran for energy and *Rastineobola argentea* (omena), fish meal, peanuts and canola cake and meal. The raw materials used in the production of feed in Kenya are imported chiefly from Tanzania, Uganda and South Africa while the feed fortifications are imported from China (RTA &Nutrimix Limited, 2016).

Poultry feed manufacture and costs are the main bottlenecks faced by international and local manufacturers owing to the competition for feed ingredients shared by humans and animals (Abidin *et al*., 2011). A country's feed industry relies heavily on by-products from other industries, breweries and food processors (ABS-TCM, 2013). Adulterants can come from man-made organic chemical sources like pesticides, or from environmental sources of adulteration, or from naturally occurring plant toxins (phytotoxins) and fungi (mycotoxins) (Spragg & Watts, 2013). Animal feeds that are susceptible to mycotoxin adulteration precisely aflatoxins include maize, cottonseed, copra and peanuts. To add on, if the feeds are stored under improper conditions, the concentrates, ingredients and the supplements are prone to mycotoxin contamination (Grace *et al*., 2015).

2.8 Factors influencing the occurrence of aflatoxin in animal feeds

Mycotoxin levels in animal feed should be observed from the field to the table to ensure the safety of the feed to humans and animals. The adulteration of cereals and other agrarian supplies utilized in animal feed could happen in the farm in the pre-harvest phase or in the post-harvest phase. In the pre harvest phase, the occurrence of aflatoxin forming fungi could be influenced by several factors for instance plant genetics (Fountain *et al*., 2014). In the developing and harvesting phase, toxin development is fostered by agrarian practices such as chemical control of fungi and pests, the adoption of open pollinated strains

(Warburton, 2014), the interaction with aflatoxin forming fungi or spores, climatic conditions during planting and developing and lastly entomological destruction.

Humidity and temperature contribute a major proportion in the development of fungi and in the formation of aflatoxins. Mycotoxin forming fungi require high level of moistness between (20.0-25.0g/100g) for the formation of aflatoxin in the pre-harvest phase in the farm than fungi that develops in storage (Bryden, 2012). The agrarian practices that increase the risk of development and contamination of aflatoxin comprise of the species of crops that are planted, the planting date, crop alternation and cultivation (Magan *et al*., 2003). The occurrence of aflatoxin forming fungi like the *Aspergillus parasiticus* or *Aspergillus flavus* in crops or the farm milieu is not an indication that that the crops have been contaminated with the toxin. The development of aflatoxins requires pressure influences such as nutritional difference, famine or moisture (Tola, 2016). Climate is a major influence in the development of fungi and in the formation of aflatoxin in crops in the farm and during storage (Tola, 2016).

The development of fungi in cereals and animal feed during post-harvest, during distribution and during storage is stimulated by environmental factors such as temperature, moistness, and water movement, the state of the grain, entomological destruction and the amount and variety of mycobiota (Tola, 2016). High moisture content in cereals and feeds during distribution and storage escalates the development of aflatoxin (Kana *et al*., 2013). Other factors that also influence the development of aflatoxin include geographic origin, transportation route, storage area, storage period and specific climatic conditions. Based on this, factors such as geographical location, temperature, moistness and length of storage should be taken into consideration when linking aflatoxin assessment of raw feeds and processed feeds (Guerre, 2016).

Cereals are not sole constituents of animal feed but similarly the by-products of these cereals are majorly utilized to feed animals (Fafiolu *et al*., 2015; Pinotti *et al*., 2016). Mycotoxins are resilient to a bulk of food processing procedures. Food processing procedures such as milling, manufacture of ethanol, beer preparation affects aflatoxin dispersal and concentration (Norgaard *et al*., 2012; Almeida *et al*., 2012; Adeola, 2014). Cereal by products are commonly used in enriching animal feed such as rice milling where a number of by products for instance rice hulls; rice bran, chipped rice and rice polishing are utilized as constituents of animal feed (Pinotti *et al*., 2016). Another example is in the manufacture of cheese whereby AFM1 is present in whey which is normally used to feed younger animals or is utilized as a feed ingredient independently (Chavarria *et al*., 2017).

2.9 Effects on human to Aflatoxin exposure

Ever since the advent of AFs, Kenya has experienced devastating and fatal effects of exposure to AFs (Kilonzo *et al.,* 2014; Mehl and Cotty, 2010; Okioma, 2008). Exposure to AFs is primarily through consumption of adulterated food. Consumption of foods of AF levels of more than 6000 mg/kg in food results in liver degeneration leading to hepatic failure and can be fatal after 1-2 weeks of exposure (acute aflatoxicosis) (Groopman *et al.,* 1988). Aflatoxicosis effects include oedema, convulsions, vomiting, jaundice, abdominal pain, sudden liver failure, and lastly death (Mwanda *et al.,* 2005). Epidemiologocal studies have shown that acute toxicity cases linked with exposure to high levels of AF are not experienced in most countries, but are dominant in high-risk localities such as Makueni County of Kenya (Nabwire *et al.,* 2020). In humans, acute toxicity due to exposure to elevated dietary levels of AFs of between (2,000–6,000 μg/day) in adulterated maize was recorded in Western India in 1974 with a case mortality rate of 10% (Krishnamachari *et al.,* 1975; Tandon *et al.,* 1977). In Taiwan, 26 members of 3 families were casualities of

ingestion of 200 μg/kg of AFs in mouldy rice and three of the victims lost their lives (Pitt, 1989). In Uganda, a 15-year-old boy also succumbed to death after consuming cassava contaminated with AFs containing 1,700 μg/kg levels of AFs, leaving behind a brother and a sister who survived by a whisker (Serck-Hanssen, 1970). In recent times, ingestion of AF-adulterated maize in Tanzania resulted in aflatoxicosis in humans with a case fatality rate of 50% (Kamala *et al.,* 2018).

In Kenya, the debut of aflatoxicosis was in 1960 which resulted in the death of 16,000 ducklings (Peers and Linsell, 1973). In 1981, Kenya experienced its first ever severe reported epidemic of human aflatoxicosis (Ngindu *et al.,* 1982). It was observed that 7 days after ingestion of adulterated maize grains having 3.2–12 mg/kg of AFB_1, signs of abdominal discomfort, anorexia, general malaise, and low-grade fever were displayed in 20 patients between the ages of 2.5 and 45 years of age. Twelve out of the 20 patients developed liver failure, all of whom succumbed to death between 1–12 days after being admitted to hospital. The greatest cartastrophic episode of human aflatoxicosis in history ever recorded was in Kenya in the year 2004 with over 317 confirmed cases of which 125 were fatal (Probst *et al.,* 2007). This epidemic occured in the Eastern Province of the country with a case mortality rate of 39% and out of 308 cases for whom age data was existing, 68 (22%) were <5 years, 90 (29%) were between 5–14 years, and 150 (49%) were >15 years. Children below 14 years representing 51% of the children population were considered to have higher risk to aflatoxicosis. The case fatality rate was considerably higher in Makueni County than in Kitui County (Azziz-Baumgartner *et al.,* 2005; Daniel et al., 2011; Ngindu *et al.,* 1982; Kang'ethe *et al.,* 2017; CDC, 2004).

Since 2004, epidemics among subsistence farmers have recurred yearly in the Eastern Province and the enormity of exposure to AFs could be higher than reported due to the lack

of robust surveillance systems (Obonyo and Salano, 2018; Kilonzo *et al.*, 2014). Evidence from numerous studies on AF exposure in humans have revealed that low level chronic consumption of AFs is more destructive than one-time high level consumption as it is associated to the on set of liver cancer (Kucukcakan and Hayrulai, 2015; Marin *et al.*, 2013; Petruzziello, 2018; Wild and Gong, 2010; Magnussen and Parsi, 2013). During the aflatoxicosis epidemic that was witnessed in 2010 in Kenya, the levels of AFB_1 documented in Kenya were among the highest ever reported globally (IFPRI, 2013).

Exposure to aflatoxins in Kenya commences from infancy due to lactation as breast milk is the first source of food for infants this is according to a study conducted in Makueni and Nandi counties. The study established that a high proportion of mothers tested positive for aflatoxin M1 (Kang'ethe *et al.,* 2017). The proportion ranged from 56.7% in Nandi County to 86.7% in Makueni County (Kang'ethe *et al.,* 2017) to add on, elevated levels of aflatoxin M1 were found in urine samples from children below 2.5 years (mean of 1.182 µg/kg and 0.857 µg/kg aflatoxin M1 in Makueni and Nandi counties, respectively).

2.10 Effects of aflatoxin in poultry

Aflatoxin contamination in poultry can lead to detrimental consequences such as liver toxicity, teratogenicity, carcinogenicity, mutagenicity, hematological issues and immunosuppression (Oguz *et al.*, 2000). 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 (Monson *et al*., 2015). Aflatoxin contamination in poultry causes suppression of the immune response and this causes an impairment of the T cell manufacture, reduced

phagocytosis and apopstosis in the thymus and spleen (Rawal *et al*., 2010; Peng *et al*., 2015).

Aflatoxin contaminant exposure in poultry causes grave risks to the health of the birds by escalating susceptibility to infections or by decreasing vaccination effectiveness. Epidemiological studies have demonstrated that a great correlation exists between epidemics of new castle disease and aflatoxin adulteration in broiler chicken (Yanus *et al*., 2009). Yang *et al* found out that broilers fed with 36.9-95.2 µg kg-1 of AFB1 toxin displayed altered serum biochemical parameters, compromised liver antioxidant operations and severe lesions in the liver tissues (Yang *et al*., 2012).

Focal necrosis of liver cells, biliary hyperplasia, Kupffer cell hypertrophy, microvesicular fatty disintegration and apopstosis was also witnessed. Severe effects in broilers such as paralysis, lying down and growth retardation was also witnessed. Other effects exhibited in broilers include yellowing of the hepatic, numerous hemorrhages and a distinctive reticular presentation of the capsular surface (Hussain *et al*., 2016).

In very severe cases, the kidneys are distended and filled with urates. In addition, unusual fatty tissue accretion and hepatic lesions resulting into hepatomegaly with loss of normal color (dark brown), with hemorrhage in the left lobule with no gallbladder enlargement, was observed in chicken that consumed feeds adulterated with aflatoxin (Hussain *et al*., 2016). In chicks that were fed with aflatoxin contaminated feed, they displayed a reduction in liver size, increased liver paleness, nodular appearance without bleeding and gallbladder enlargement. Studies have also demonstrated diverse presentation of clinical symptoms in poultry fed on AFB1 contaminated feed of between 400-800 µg kg-1 such as stress, ruffled

feathers, watery stool, dehydration, reduced feed intake, nervous impairment such as torticollis and death (Hussain *et al*., 2016).

In chicken, the presence of 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 (Buzala *et al*., 2017). Aflatoxins alter the hypothalamic regulation of neuropeptides that are responsible for the feeding behavior pattern and are responsible for low body weight and reduced weight gain experienced (Trebak *et al*., 2015). Evidence has shown that the consequences of AFB1 on the assimilation of nutrients give varied outcomes. They can alter various roles of the GIT for instance reduced surface area for nutrient assimilation, variation of nutrient carriers, absence of buffer function and enhancing constant enteritis (Grenier, 2013).

It is however unknown how the gastric lesions impair development and affect feed efficacy in poultry. A study done by Kalpana *et al* reported that enrofloxacin and ciprofloxacin deposits in hepatic, kidney, skin and fat was present in mycotoxin-exposed broiler chicken whereas in the unexposed broiler chicken it was only present in the liver. This therefore points out that sub chronic AFB1 exposure significantly affects the deposit levels of enrofloxacin in the tissue of broiler chicken (Kalpana *et al*., 2012).

2.11 Food safety

Food safety is a worldwide concern not only to policymakers but also to the general population. Food safety hazards are a serious public health concern globally; however, there is low level of awareness on socioeconomic costs of unsafe food and the benefits of preventive measures in developing countries (Henson *et al.,* 2018). Food is regarded as

safe when there is assurance that no damage will accrue from its consumption (Ogutu, 2016). Food safety contains the involvement of all players in the food value chain and it is paramount to apply a comprehensive food value chain approach in food safety control strategies (WHO, 2018).

Applaudable strides have been made towards AF control in Kenya through nationwide sensitization (Kange'the, 2011; Atehnkeng and Mutegi, 2018). The regional mycotoxin facility at the Kenya Agricultural and Livestock Research Organization (KALRO) in Katumani provides training to people from both the public and private sectors.

Following the deadly aflatoxicosis between 1970 and 1980s in which dogs were fed on adulterated rations and died, KEBS developed a standard for dog feeds in 1985 (Omara *et al*., 2021). Standards for maize grain, and other grains, and their products that have been in existence have also been reviewed. For instance, total AFs were originally at 20 μg/kg; this has been reviewed to 10 μg/kg, with 5 μg/kg as the limit for AFB_1 (KEBS, 2018). Over 25 standards designed to regulate AFs have been drafted and are in full utilization and include key parameters such as moisture, mouldy grains, pest damage, filth, broken kernels/seeds, foreign matter, and discoloured grains. Most of these standards have been harmonized with the East African Standards by the Eastern Africa Grain Council (EAGC) in partnership with KEBS through the Eastern Africa Grain Institute with its headquarters in Kenya in Nairobi (Stronger, 2018). Between the year 2015 and 2018, the two corporations have trained maize exporters, traders, farmer-based organizations, and warehouse handlers on understanding the integrated East African maize standard (EAS 2:2013), food standardization, comparison of East African standards with international standards, standard maize sampling methods, maize grading, mycotoxins, and the existing techniques for mycotoxin analysis (Stronger, 2018).

Following the unveiling of the EAGC in 2006, EAGC has continued to be in the forefront in the fight against AFs in the entire of East Africa. The council has developed advanced prevention strategies to lessen the incidence of AFs and these are; (1) synchronization of AF prevention methods and refining the regulatory standards, (2) unveiling of AF control training programs, (3) supplying humidity meters and water-resistant sheets for drying, fumigation, and storing grains, (4) subcontracting portable kits for detecting and quantifying AFs, (5) farmer-oriented evaluation of AFs prevalence, (6) partnering with East African Community to extend AF testing and surveillance to maize, and (7) developing approaches for the Partnership for Aflatoxin Control in Africa (PACA) strategy 2013–2022 and reviewing EAC AFs communication strategy (Stronger, 2018). To add on, AF monitoring and capacity has been heightened through the PACA (Partnership for Aflatoxin Control in Africa) and Africa Aflatoxin Information Management System (Africa-AIMS) in seven member countries: Kenya, Malawi, Nigeria, Senegal, Tanzania, The Gambia, and Uganda.

Kenya Agricultural and Livestock Research Organization in collaboration with the International Institute of Tropical Agriculture (IITA) in 2018 came up with a farmer-oriented manual for the control of AFs in maize and peanuts (Atehnkeng and Mutegi, 2018). The manual gives an over-all synopsis of AFs pre-harvest and post harvest management strategies and agricultural practices that promote AF development. It was specifically developed to offer insight on the best practices for preventing AF contamination in maize and peanuts and to increase the value of these dietary staples.

Additionally, there are several projects concerning mycotoxin epidemic that are ongoing in the country for instance; the Aflacontrol Project and Purchasing for Progress (P4P) Programme. The Aflacontrol Project endevous to reduce the destruction of AFs in maize

and peanut value chains and is supported by International Food Policy Research Institute (IFPRI). Additionaly, the project endevous to intensify the understanding of the economic and health effects of AF adulteration and pinpoint and support cost-effective approaches and technologies that are accessible to decrease adulteration of foods and feeds. The project, is sponsored by the Bill and Melinda Gates Foundation in partnership with the International Maize and Wheat Improvement Center (CIYMMT), University of Pennsylvania (USA), United States Uniformed Health Services, Kenya Agricultural Research Institute (KARI), and Agricultural Cooperative Development Initiative (ACDI-VOCA). Trials of this project have been conducted in Mbere (Embu), Makueni, Homa Bay, Kisii, and Rongo at the household level (Kang'ethe, 2011). To date, the project has conducted numerous policy briefs and held several launches and countrywide workshops to disseminate information on AFs. These are directed at the Ministries of Agriculture and Ministry of Health Division of Public Health, who are the key players in averting AFs. Conversely, the Purchasing for Progress Programme which is spearheaded by the World Food Programme procures maize from local farmers with strict adherence to AF limit guidelines in cereals. The cereals are purchased at fair prices, encouraging the farmers to comply with good pre-, peri-, and postharvest management practices (Kang'ethe, 2011). Numerous collaborations are presently running in the country with FAO and CDC to alleviate AFs in Kenya. These have been discussed at great lengths in a review by Mutegi et al (Mutegi *et al.*, 2018).

2.12 'Carry- over' of aflatoxins in chicken

All mycotoxins inclusive of aflatoxins are metabolized in the gastrointestinal tract, liver or kidneys depending on their chemical structure. Their transmission into poultry meat and eggs gives rise to adverse consequences on human health (Filazi *et al.*, 2017). Agag

observed the 'carry over' effect of AFB1 from layer feed to eggs at dietary levels of 100-400 µg/kg AFB1 (Agag, 2004). 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, Zaghini *et al.* displayed no quantifiable deposit of AFB1 or its metabolites in eggs. These conflicting findings could be attributed to the presence of oligosaccharides in naturally occurring aflatoxins in adulterated feeds at varied levels of toxicity (Zaghini *et al*., 2005).

Studies have demonstrated that in broilers and layers, AFB1 residues varies from no detection to 3.0µg/kg in the hepatic of birds fed on 250-3310 µg/kg of AFB1 over specified periods of time (Hussain *et al*., 2010). However, there is 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 (Fowler *et al*., 2015). 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 their hepatic. In Kenya limited studies have been done on this.

2.13 Farmers knowledge on aflatoxin adulteration

By and large, farmers and the general public in developing countries know little concerning aflatoxins and their related health impacts (Unneverhr, 2013; Waliyah *et al*., 2008). In Kenya, farmers perceived the consumption of moldy food by humans to be unsafe, but on the other hand they considered meat from animals fed with moldy feeds to be harmless (Kiama *et al*., 2016). This illustrates that the issue of aflatoxin adulteration of feeds is

unknown. Studies conducted in various regions of the world demonstrate that the level of awareness of aflatoxin is low. Part of the documented levels are for instance, 25% in Vietnam (Lee *et al*., 2017), 6% in Zimbabwe (Loreen, 2015), 12% in Ethiopia (Gizachew *et al*., 2015) and 20% in Tanzania (Kamala *et al*., 2016; Ngoma *et al*., 2017).

Knowledge of aflatoxins and the other mycotoxins have been proven to differ with several socio demographic characteristics. For example, in Kenya, women were found to be more knowledgeable of the dangers of mycotoxins and were careful to moldy feeds than men (Kiama *et al*., 2016). In Vietnam, young farmers (at age of 21-29) were found to be more knowledgeable of aflatoxins in crops than the older population (Lee *et al*., 2017). In Tanzania, studies have established that education level has a positive effect on aflatoxin awareness (Ngoma *et al*., 2017; Magembe *et al*., 2017). In Ghana, it was established that the field of study mainly life sciences has a positive impact on aflatoxin awareness (Awuah *et al*., 2008).

In Ethiopia, farmers were found to be less knowledgeable on aflatoxins than persons in other occupations (Ephrem *et al*., 2014). Information on knowledge of aflatoxin among farmers in Kenya and other countries is scarce. To add on, the existing reports are more inclined towards awareness of aflatoxins in food crops such as groundnuts and maize than feeds. Furthermore, existing reports focusing on awareness of aflatoxins in feeds are deficient of crucial facts that would be essential in the mitigation of challenged linked to aflatoxin presence in feeds (Ayo *et al*., 2018). Additionally, the reports are sketchy in indicating the burden in specific localities. There is scarcity of information concerning knowledge of aflatoxin adulteration of feeds among farmers even in aflatoxin prone regions. This stalemate can lead to the transmission of unknown levels of aflatoxin to humans and animals and consequently ruin the health of the public. Farmers' knowledge

in resolving a farming problem may be regarded as the initial step towards identification and modeling mitigation measures (Ayo *et al*., 2018). Thus, knowledge of aflatoxins in feeds among poultry farmers is of paramount importance in designing plans to minimize risks of aflatoxin exposure.

2.14 Aflatoxin regulations in feedstuff

Owing to the synergistic effect of AFB1 there are no existing precise safe limits for aflatoxin occurrence. Typically, there should be zero level of aflatoxin in feed (WHO/FAO, 2018). The FDA and EU recommended acceptable limits of AFs in poultry feed to be 20 µg/kg for FDA and 10 µg for EU and WHO 5ppb. It is therefore recommended that aflatoxin adulterated feeds be fed to poultry at the least probable limit for the shortest period of time (WHO/FAO, 2018). The permissible level for human consumption is way lower than the animal feed level, with 4µg/kg for overall aflatoxins and 2µg/kg for AFB1 due to health effects.

In Africa, six out of the 54 countries and 1 region (East African Community, (EAC) have standards for AFs in poultry feeds. South Africa is the only country with regulation guidelines for Ochratoxin A (OTA), Fumonisin B (FBs), Deoxynivalenol (DON) and Aflatoxins (AFs) in poultry feed (Njobeh *et al.*, 2012). The East African Community has set the maximum recommended limit for total AFs at 50 µg/kg and AFB1 at 20 µg/kg for poultry feeds (Sirma *et al.*, 2018). Most African regional and national mycotoxin regulatory limits are set and enforced due to trade and the need to comply with export regulations (Sirma *et al.,* 2018; Nishimwe *et al.,* 2019). The European Union (EU) has set limits for mycotoxins in feeds for different animal species for AFB1, Fumonisin B (FBs), Deoxynivalenol (DON), Zearalenon (ZEN), and Ochratoxin A (OTA) in poultry feeds

(Kolawole *et al.,* 2020). The Canadian Food Inspection Agency (CFIA) has set limits for both Ochratoxin A (OTA) and Trichothecene (T-2) in poultry feed at higher levels than that of the EU (Xue *et al.,* 2010). The United States of America has also set higher limits Deoxynivalenol (DON) and Fumonisin B (FBs) in poultry feeds than those set by the European Union (Placinta *et al.,* 1999).

2.15 Detection and Quantification of Aflatoxin levels

Detection and quantification of AFs is crucial to its alleviation because its presence in samples is normally skewed (Turner *et al.,* 2009). The initial stage for precise detection and quantification of AFs is sampling, i.e. sampling/subsampling is the greatest cause of error in AFs analysis (Whitaker, 2003). Because of this reason, a representative sample should be picked from the larger lot. For the over 50 KEBS registered laboratories for monitoring mycotoxins in foods in Kenya, Gafta methods (No. 130, 24:1) and EAS 79 are used as the main sampling procedures. Meanwhile, some clients do their own sampling and the testing laboratories do not query the purpose for the analysis, or where and how the samples were obtained. To add on, data from such analyses are more than often priviledged and are not used for evidence-based decision making by policymakers (Kang'ethe, 2011).

An emerging threat in the analysis of food toxins in Africa, Asia, America, and Europe is the "disguised mycotoxins" as they are more than often not recognized and detected by conventional analytical techniques (Kamle *et al.,* 2019). Disguised (matrix-associated) mycotoxins are biosynthesized by toxigenic fungi and later undergo biomodification by plant enzymes during the contagious stages. They can be lodged in the vacuoles in soluble form or bound to macromolecules and therefore go on undetected (Berthiller *et al.,* 2013). Regrettably, these modified toxins can hydrolyze and degenerate into their toxic form in the course of processing or digestion (Nagl *et al.,* 2014; Broekaertet *et al.,* 2015). One way

to bypass this analytical barrier is to hydrolyze the modified forms (using enzymes, alkaline, or acidic pretreatments) (Dall'Asta *et al.,* 2009; Vidal *et al.,* 2018; Beloglazova *et al.,* 2013) into their free forms which can be detected (Dall'Asta *et al.,* 2009; Dall'Asta *et al.,* 2008). Data on disguised AFs is scanty as they normaly go undetected and quantification is usually conducted on free AFs in matrices.

Generally, AF studies in Kenya utilize laboratory-based enzyme-linked immunosorbent assays (ELISAs), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), fluorimetry, liquid chromatography-tandem mass spectrometry (LC-MS/MS), tandem quadrupole mass spectrometry (TQMS), and ultra-high-pressure liquid chromatography (UHPLC) and lateral flow immunochromatography (LFI) has also been used. There has been a change in the techniques used for AF analysis, as demonstrated by progression from non-differential TLC in 1973 to the relatively fast and differential UHPLC to the triple quadruple mass spectrometry (UHPLC-TTQS) in 2017–2020 (Omara *et al.*, 2021). Generally, the most utilized method is ELISA which has gone through numerous advancements in recent years. This can be attributed to it being cheap, easy to use, and highly sensitive for routine analysis of food products, requires minimal sample clean up, and poses no significant health hazards as it uses enzyme labels (Omara *et al*., 2021). To add on, simultaneous analysis of numerous samples on a 96-well assay platform is possible and hence has a high sample output with low sample volume requirement which is quite advantageous (Wacoo *et al.,* 2014). Additionaly, ELISA has lower detection limits than most instrumental techniques that are used for AF detection (Wacoo *et al.,* 2014).

Conversely, the limitation of earlier standard methods is that they are not suitable for rapid and real-time tests in food and feed sample analysis as they are quite engaging and require some level expertise to operate them. Rapid and robust techniques such as polymerase

chain reaction (PCR) and non-destructive techniques based on fluorescence/near-infrared spectroscopy (FS/NIRS) and hyperspectral imaging (HSI) have been established for fast and easy detection of AFs (Tao *et al.,* 2018). Several studies in Kenya (Okoth *et al.,* 2012; Oloo *et al.,* 2019; Mitema *et al.,* 2019; Monda *et al.,* 2020; Islam *et al.,* 2018; Gachara *et al.,* 2018; Castelino *et al.,* 2015) have used PCR in their analysis. It is worth noting that, at the manufacturing level, food and feed processing industries monitor cereal total AFs levels using single-step lateral flow immunoassays utilizing Reveal Q+ test strips that are formed and read on AccuScan Gold readers (Guguyu, 2017). Bright greenish-yellow fluorescence (BGYF) or the black light test, which can accurately detect goods that are assumed to be adulterated with AFs, is available in Kenya (Murithi, 2014). This test is fairly cheap and easy to use particularly in detecting AFs in maize where the kernels are observed under an ultraviolet lamp at 365 nm for a specific bright greenish-yellow fluorescence, which indicates a probability of presence of aflatoxigenic fungi or presence of mycotoxin (Yao *et al.,* 2010). This test can be embraced by regulatory agencies for AFs surveillance.

2.16 Management techniques of Aflatoxin

2.16.1 Preharvest Management

Approaches such as cultivation of food crops that are disease, famine, and pest resistant or less prone to fungal attack could be implemented. This strategy is the most effective in the reduction of effects of AFs-producing fungi (Brown *et al.,* 2013). A study by Menza *et al* found out that Valencia red (a peanut variety) was least adulterated with AFs and had higher oil content than the Uganda local variety and the Homa Bay local variety (Menza *et al.,* 2016). Food oils and microbs are possible inhibitors of AF biosynthesis (Williams *et al.,* 2004) as they interfere with the signal transduction regulatory chains in AF gene expression thereby inhibiting AF biosynthetic cytosolic enzymes and down regulating

fungal genes of the oxidative stress defence system (Ranasinghe *et al.,* 2002). Furthermore, host and parasite macro and micromolecular operations whose aim is to evade AF effects through the use of a cross species RNA interference have been tried in maize and peanuts (Ssekandi, 2018; Raruanget *et al.,* 2020). Ubiquitin (UBI), Controlled Ovarian Hyperstimulation (COH), 26s genetic markers, Adenosine Triphosphate (ATP), Polyphosphate kinase (PPK), Imipenemase (IMP), ATP-Binding Cassette (ABC), and *Aspergillus flavus* gene (aflM) were recommended as the viable genes for RNAi silencing of *A. flavus in vivo* (Ssekandi, 2018; Raruanget *et al.,* 2020). This may however be hampered by the existing policy on genetically modified organisms in the country.

Timely harvest of mature food crops as well as careful disposal of broken kernels or cobs are recommended AF mitigation strategies (Hell *et al.,* 2008). Sorting, winnowing, and dehulling can reduce AF levels in cereals by 40–80% (Whitaker, 2003; Fandohan *et al.,* 2005). Sorting is more appropriate for groundnuts (Hell *et al.,* 2008; Park, 2002; Turner *et al.,* 2005; Nde'de *et al.,* 2012). Soaking or cooking in *magadi soda*, malting, and roasting are other approaches reported to reduce food AF levels (Mutungi *et al.,* 2008; Fandohan *et al.,* 2005; Omara *et al.,* 2019; Makokha *et al.,* 2002). *Magadi soda* and wood ash are used by the Kalenjin of the Rift Valley region, Nyanza and Western region to increase the tastiness of food improve and to decrease cooking time as well as phytates and increase the availability of niacin (Muindi *et al.,* 2006).

Pest control is another strategy used in AF management. This can be achieved by using ash for maize (Avantaggiato *et al.,* 2003; Munkvold, 2003) and essential oils which are broad-spectrum bio insecticides (Omara *et al.,* 2018; Kirima *et al.,* 2020; Bankole, 1997; Adegoke *et al.,* 2000). Competitive exclusion is another AF control strategy that has not been fully explored. Crop modification from toxigenic strains to non-toxigenic strains is possible and

Kenya has already approved a potential biocontrol product (Aflasafe KE01) (Mutahi, 2019; Atehnkeng and Mutegi, 2018). A classic example is a product composed of a rhizosphere-competent nonaflatoxigenic *Aspergillus* strain having competitive saprophytic potential (Abbas *et al.*, 2017; Dorner, 2009). For peanuts, a non-toxigenic *A. flavus* strain (NRRL 21882) is commercialized as Afla-Guard® in the US (Dorner, 2005). Moreover, pseudomonads and *Trichoderma* spp that inhabit rhizospheres of several plants have been used to target the toxigenic *A. flavus.* In recent times, various *Streptomyces*, *Pseudomonas*, and *Trichoderma* spp have been isolated, assessed, and confirmed to possess antagonistic effects towards *A. flavus* (Anjaiah *et al.*, 2006). Nonetheless, their effectiveness should be investigated more in Kenya like other African and Asian countries since they are reported to have AF reduction rate of 79% (Harini *et al.*, 2011).

2.16.2 Postharvest Management strategies

Proper drying of cereals to moisture levels between 12 and 14% preferably 12.5% or below is advised. Newly harvested crops should be shelled and cleaned before storage to reduce pest invasion that may facilitate the growth of moulds (Kaaya and Kyamuhangire, 2006). Additionaly, proper ventilation of storage utilities is necessary to avoid crops reaching temperatures of between 25°C and 32°C and humidity of 65% favourable for the growth of moulds (Villers, 2014). Moisture of 12–13% and temperatures below 18°C do not facilitate the growth of *Aspergillus* fungi (Summer and Dee, 2012).

Following good agricultural, storage, and manufacturing practices together with the use of advanced agricultural technologies can decrease AF adulteration (Kamle *et al.*, 2019).

Numerous methods of detoxifying feed or food products having mycotoxins exist and among them is the utilization of mycotoxin adsorbents, in feed and enzymatic or microbial decontamination. The addition of various types of binders particularly clay mineral has

been vastly used in the feed and farm business to counter the harsh mycotoxic properties of the toxin in animal feed however they are not effective (Murugesan *et al*., 2015). Dos Anjos et al examined the effectiveness of three different aflatoxin binders; bentoline clay, diatomaceous earth and tumeric powder in broiler chicks fed on aflatoxin adulterated feed (Dos Anjos *et al*., 2015). The researchers found out that poultry fed on tumeric (minus aflatoxins) exhibited reduced weight increase compared to the control poultry. The poultry fed on AFB1 with the binder bentonite clay did not display a reduction in feed consumption and feed gain was displayed in the poultry fed with AFB1 alone. Poultry fed on diet consisting of AFB1 with diatomaceous and turmeric binders exhibited poor growth compared to those fed on AFB1 alone.

The toxic effects and lesions in the hepatic were not countered by any of the binder actions (Dos Anjos *et al*., 2015). An excellent review of experimental studies illustrated varied decontamination strategies in poultry feed was written by Oguz *et al* (Oguz *et al*., 2000). Denli et al observed that the supplementation with AflaDetox® considerably counteracted the toxic effects of AFB1. The researchers further suggested that the inclusion of AflaDetox (1,2 and 5g $kg^{-1}$of feed) to feed having AFB1 considerably enhanced performance, countered the serum biochemical and histopathological variations, decreased the relative weight of the hepatic and spleen (Denli *et al*., 2009). Mineral adsorbents are effective in countering aflatoxins but are non-specific and can bind other molecules such as vitamins and other nutrients (Ismail *et al*., 2018). Organic elements like humic acids have the capacity to counter mycotoxins, yeast and yeast extracts and consequently the aflatoxin effects. The management strategies proposed each have their merits and demerits. Therefore, biological control methods together with physicochemical methods would be the best approach to take to manage the plague of AFs in Kenya.

2.17 Gaps in literature

There are limited documented studies on the ‘carry over’ effect of aflatoxin from broiler feed to broiler meat in Africa and specifically in Kenya therefore the magnitude of the burden is not clearly defined. To add on, studies on the knowledge of farmers on aflatoxin are scanty and the reports available are sketchy in indicating the burden in specific localities.

CHAPTER THREE: METHODOLOGY

3.1 Research design

The study used a cross sequential study design which is a study design that combines both cross sectional and longitudinal study design. The cross sectional aspect of the study was done through administering of questionnaires and conducting of focus group discussions (qualitative approach). The longitudinal part of the study was done through a follow up of the broilers for a period of six weeks. According to Greene, the triangulation of both designs provides the desired validation of results from different methods (Greene, 2015).

3.2 Variables

The independent variable in this study was the sociodemographic characteristics of farmers i.e. Age, sex, marital status and level of education. The intermediate variable in the study was the presence of Aflatoxin in broiler feed. If the feeds are contaminated with the toxin and farmers do not have knowledge on prevention of aflatoxin in feed, the outcome will be that aflatoxin will be 'carried over' to the meat therefore the dependent variable or the outcome variable in this study was the presence of aflatoxin in broiler meat.

3.3 Location of study

The study was conducted in Nairobi City County in Westlands, Kasarani, Embakasi Central, Embakasi East, Dagoreti North and Dagoreti South sub counties as shown in (Appendix 12). Nairobi is the capital city of Kenya and is one of Africa's strategic financial, business, transport, communications, non-governmental organizations and diplomatic capital. It is also referred to as the 'safari capital of the world' owing to the globally recognized wildlife and tourism industry. Nairobi county population is about 4.397 million (KNBS, 2019) therefore chicken production is expected to rise to meet this growing demand. 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 (Mccarron *et al*., 2015).

3.4 Sampling technique

Since the study adopted a dual study design of both longitudinal and cross sectional design, the sampling criteria was as illustrated in the following sections.

3.4.1 Cross sectional design sampling

The study used a multistage cluster random sampling technique (two stage). Thirty percent of clusters give a good representative sample this is according to Mugenda and Mugenda (Mugenda & Mugenda, 2003), and this method was used to randomly select the sub counties and wards respectively. Nairobi City County has 17 sub counties and each sub county has 5 wards. Using the 30% rule on multistage cluster random sampling, six sub counties were randomly selected. In each sub county, two wards were randomly selected using the 30% rule on cluster sampling where the questionnaires were administred.

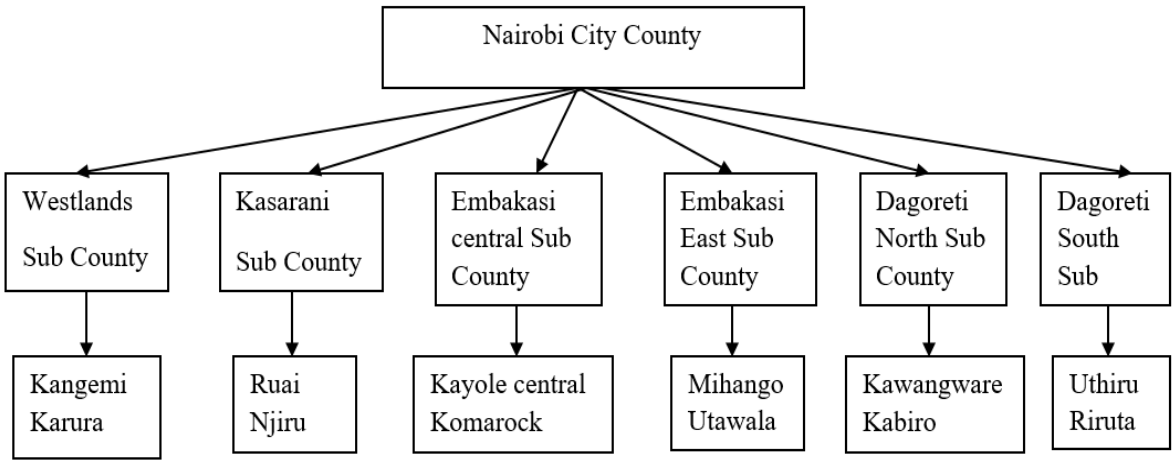

Figure 3.1: Multistage cluster sampling criteria for cross sectional study design
Source: Nairobi City County profile

The sub county livestock officers were instrumental in the identification of farms where broiler chicken were reared within the wards sampled. Systematic random sampling was used to select the farms where the questionnaires were administered and every 3rd farm was sampled (as shown in section 3.4.1.1) until the desired sample size was attained. The table below shows how the sample was attained in the six sub counties this was done proportionately.

Randomnization was achieved by use of computer generated random numbers in the selection of the sub counties and farms where the questionaires were administered. This is in line with ARRIVE (Animal Research Reporting for Invivo studies) guidelines 2.0 of 2010 on randomnization (Percie *et al.,* 2020).

Table 3.1: Sampling frame: Number of farmers sampled per sub-county in the study on quantification, characterization and carry over effect of Aflatoxin in broiler chicken raised in Nairobi City County, Kenya.

Sub county	Total number of farmers	Number of farmers sampled
Westlands	100	40
Kasarani	200	80
Embakasi Central	75	30
Embakasi East	100	40
Dagoreti South	75	30
Dagoreti North	50	20
Total	**600**	**240**

3.4.1.1 Sample size determination for Cross sectional design

Since the population is less than 10,000, Yamane *et al* formula (Yamane *et al.,* 2002) was used to determine the sample size as shown below.

$$n = \frac{N}{1 + Ne^2}$$

Where n- estimated sample size N- Estimated population size e- Margin of error (0.05)

$$n = \frac{N}{1 + Ne^2} = \frac{600}{1 + 600x0.05^2} = 240$$

240 was the number of farmers sampled from each ward in the six sampled sub counties within Nairobi City County as shown in Table 3.1 above.

Systematic random sampling formula; $K = N/n$ 600÷240= 2.5 rounded off to 3

3.4.2 Longitudinal design sampling

Random sampling was used to identify one farm in each of the six sub counties where the follow up study (longitudinal) was conducted. Randomnization was achieved by use of computer generated random numbers in the selection of the farms, this is in line with ARRIVE (Animal Research Reporting for Invivo studies) guidelines 2.0 of 2010 on randomnization (Percie *et al.,* 2020). In total six farms were selected. The follow up (longitudinal study) was done for a period of six weeks and samples were collected each farm from week 0 (day old chick) to week 6 and a total of 42 birds were sampled. This is further illustrated in section 3.4.2.1 below.

3.4.2.1 Sample size determination for Longitudinal design

Fitts (2011) explains that in order to determine the number of animals that should be used for a study, it should be noted that a relatively small sample size decreases the chances of getting significant results in the study and relatively large sample size will lead to unnecessary wastage of the resources and animals (Fitts, 2011).

The study was evaluating the aflatoxin carry over effect from week zero (day one) to week six hence it was the model fitted best in One Way ANOVA. In that case, the sample size was calculated using Wan and Wan (2017) for determination of Minimum and Maximum Sample Sizes for Group comparison—one-way ANOVA (Wan & Wan, 2017). The group was the number of broiler chichen in week 0 to week 6.

For one-way ANOVA, the between-subject error DF (that is, the within-subject) (Arifin & Zahiriddin, 2017) is calculated as;

DF = N – k (which is the total number of subjects (animals) – total number of treatments (groups) N= kn hence

Error DF= kn – k = k (n – 1),

Where N = total number of subjects, k = number of groups, and n = number of subjects per group. By rearranging the formula, n is given as:

$$n = \frac{DF}{k} + 1$$

Conventionally, the value of Error DF should lie between 10 and 20 (Charan & Kantharia, 2013. 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 (Jaykaran and Kantharia, 2016). Therefore, any sample size which keeps the DF between 10 and 20 should be regarded as sufficient to give significant results (Charan & Kantharia, 2013).

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 studies where it entails sacrificing of animals, (n multiplies by k) therefore 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. Oftenly animal testing is conducted with very small sample sizes e.g. 10 or less animals per group (Bonapersona *et al.,* 2021).

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). This sample size determination is in line with ARRIVE guidelines 2.0 of 2010 where it states that for the determination of sample size one needs to specify the number of experimental units in each group and to indicate total number of animals used (Percie *et al.*, 2020), this was the case for the present study. 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 (2004) advise that the mean of varying outcomes in a sample is the best representation of the sample than an individual score in the sample (Frank & Althoen, 2004). For that reason, each of the six chickens was selected from the six farms than six chickens collected from one farm which may create unprecedented bias.

The study did not have a control group and this was within the ARRIVE guidelines 2.0 of 2010 whereby it states that a control group is not necessary so long as the rationale is clearly explained and if the study is longitudinal (Percie *et al.*, 2020). The study was observatory in that the bio accumulation was observed weekly and the comparison in this study was based on the MRLs (Minimum Recommended Limits) of aflatoxin in food.

3.5 Inclusion and exclusion criteria

3.5.1 Inclusion criteria

Farms where broiler chicken were reared and farmers who gave consent were included.

3.5.2 Exclusion criteria

Broiler farms reported to have had an outbreak of disease.

3.6 Research instruments

A structured questionnaire testing the knowledge of farmers on Aflatoxin was administered. The study employed a tool that was used in a study on knowledge of aflatoxin in feeds by farmers to measure knowledge (Ayo *et al.*, 2018). Two focus group discussions comprising of twelve members each in two sub counties were conducted. The Focus Group Discussions mainly concentrated on farmers' knowledge on aflatoxin in feed.

3.7 Laboratory analysis of samples

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

3.8 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. These three meat parts were selected because, the liver is the site where metabolism takes place, the gizzard is where digestion takes place and the muscle is the part most people consume however it has no metabolic function. Studies have shown that in areas where metabolic functions occur, they accumulate higher toxin levels. Tissues and organs perform specific functions and accumulate toxins differently. Higher toxin levels are found in the liver followed by gizzard and the least in the muscle Okoye *et al.*, 2015.

Sampling was done from week (0, 1, 2, 3, 4, 5, and 6). 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 ziplock bags and clearly labeled indicating the farm, week, and date collected. The samples from feeds were collected each week alongside the meat samples for the entire study period and were put in well labeled airtight containers. Water samples too were collected each week from the drinkers and were put in 50ml water bottles and were well labeled. In week 0 water samples were collected from the zero collection point (taps). All the samples obtained from the farms were kept in the cooler box then taken

to the lab. The samples obtained i.e. meat, feed and water were stored in the freezer at - 20 degrees celsius in the lab (Sahib *et al*., 2020) to prevent further production of metabolite and microoragnisms until the time of analysis (Ifie *et al.*, 2022).

3.9 Aflatoxin analysis

Each of the samples collected for meat, water and feed underwent extraction, clean up and preconcentration and instrumental analysis. Analysis of all the samples was done in triplicate. The samples were analysed in an accredited laboratory. The principle investigator worked together with the laboratory technicians specifically trained to handle the LC-MS/MS machine.

3.9.1 Instrument Set up

3.9.1.1 Caliberation 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. The caliberation standards and curves are shown in (Appendix 9 ,10 and 11).

3.9.1.2 Limit of Detection

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 analysed as shown in (Appendix 9a ,10a and 11a).

3.9.1.3 Limit of Quantification

Limit of Quantification (LOQ) The limit of detection (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 analysed as shown in (Appendix 9a ,10a and 11a).

3.9.2 Sample preparation for Aflatoxin analysis of samples

3.9.2.1 Reagents and equipment used in water sample analysis

The chemicals and reagents used were acetonitrile; HPLC grade; purity ≥99.9%, formic acid; purity ≥99.9%, ammonium formate; purity ≥99.9% and LC-MS/MS HPLC grade water (bottled). Chemicals and reagents used for the analysis were from Sigma-Aldrich.

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

3.9.2.1.1 Sample extraction procedure for water samples

Water samples were first thawed then water samples with pH 7 were first passed directly over the AflaTest column, then the column was eluted with methanol. Water samples with pH less than 7 were diluted with PBS (Phosphate buffer solution) first before passing over the AflaTest column then the column was eluted with methanol at a rate of 1–2 drops per second. The eluate was then 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 seconds, the 2 layers were allowed to separate. The lower aqueous layer was removed with the help of a separating funnel and filtered through a 0.45mm syringe filter prior to injection into the LC column. Method adopted from Bucheli et al (Bucheli *et al.*, 2008).

3.9.2.2 Reagents and equipment used in feed sample analysis

The chemicals and reagents used were acetonitrile; HPLC grade water; purity≥99.9%, formic acid; purity ≥ 99.9%, ammonium formate; purity ≥99.9% and LC-MS/MS HPLC grade water (bottled).

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

3.9.2.2.1 Sample extraction procedure for feed samples

Feed samples were first thawed then they were weighed. A ground sample weighing 10.0g±0.3 was placed in a 100 ml screw bottle flask, 4.0 ml of HPLC grade water and 76 ml of acetonitrile (84:16) was added to the ground sample and was shook for 45 minutes in a reciprocal shaker thereafter the sample was handshaken for 15 seconds. The sample was then filtered through a flutted paper into a 100 ml beaker and then passed through a syringe filter of 0.45µM. Thereafter, 200µL of the filtrate was pipetted into a 1ml vial, 100µL of 100 ppb Aflatoxin M1 was added and diluted with 32.5 mM formic acid and was shaken before injecting to LC-MS/MS. Method adopted from Kongkapan et al (Kongkapan *et al.*, 2016).

3.9.2.3 Reagents and equipment used in meat samples 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 Equipments 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.

3.9.2.3.1 Sample extraction procedure for meat samples

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 immune-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 (Iqbal *et al.*, 2014).

3.10 Carry over ratio/Tranfer ratio

The carry over effect in the study was determined statistically using a carry over ratio or transfer ratio (Driesen *et al.*, 2021; Jondreville *et al.*, 2017; Takaki *et al.*, 2015). The carry over ratio or transfer ratio was calculated using the following formula;

Difference in Aflatoxin levels between weeks in meat ÷ Mean Aflatoxin levels in (feed +water)

3.11 Data collection techniques and process

A questionnaire measuring the knowledge of farmers on aflatoxin was administered in the six randonmly selected sub counties, within Nairobi City County. Focus group discussions each comprising of twelve members were carried out among famers in two randomly selected sub counties.

3.12 Pre test

A pretest of the questionnaire was conducted in Kiambu County whereby about 24 questionnaires which comprise of 10% of the sample size were pretested. According to Mugenda & Mugenda, 10% - 30% of the sample size is sufficient to give accurate results in a pre test (Mugenda & Mugenda, 2003). Adjustments to confirm precision and totality were prepared post pre-test.

3.12.1 Validity

Validity is described as an assessment of the degree of accuracy of an outcome or an assessment of how well the designed tool will give accurate results. To ensure validity of

the data collection tool (questionnaire), the tool was subjected to expert validation to determine if the tool would measure what they were designed to measure as recommended (Bolarinwa, 2015).

3.12.2 Reliability

Reliability is described as an assessment of the consistency of the results in a study. Cronbach's alpha technique was used to determine the reliability of the questionnaire where a value greater than 0.7 is considered reliable. In this study, Cronbach alpha was 0.71 as shown below hence, the tool was reliable as recommended from previous studies (Tavakol & Dennick, 2011; Bolarinwa, 2015).

Research Tool	Cronbach's Alpha coefficient	Number of items
Questionairre	0.71	26

3.13 Data analysis

Statistical Package for Social Sciences (SPSS) version 26 was used to analyse the quantitative data from questionaires. The data was subjected to descriptive analysis to determine proportions and chi square test was used to determine association between variables. STATA version 12 was used to analyse quantitative data from the laboratory analysis. The data was subjected to two-way ANOVA to establish differences in aflatoxin levels in the meat samples that were sampled weekly. Data was subjected to one-way ANOVA to determine the aflatoxin levels in feed and water samples that were sampled weekly in the various farms. Paired t-test was used to compare mean differences between variables. Post ANOVA test was done using Tukey Kramer post hoc test. The level of significance was determined at 5%. Data on carry over was subjected to two-way ANOVA

to establish the difference in mean levels per each meat part sampled weekly. Qualitative analysis was done using NVIVO software and the data was subjected to thematic analysis. Data was presented using charts and tables.

3.14 Logistic and ethical consideration

Approval to carry out the study was obtained from Kenyatta University graduate school (Appendix 6). Ethical approval was obtained from Kenyatta University Ethical and review committee (Appendix 4). 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. A research permit to carry out the study was obtained from National commission for Science, Technology and innovation (NACOSTI) (Appendix 5). Authorization was also obtained from the Ministry of Agriculture, Division of Veterinary Services before commencement of the study (Appendix 7). Consent was sought from each participant on voluntary basis before participating in the study (Appendix 1). 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 (Appendix 3). 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.

CHAPTER FOUR: RESULTS

4.1 Knowledge on Aflatoxin by farmers

The sociodemographic characteristics of farmers as shown in (Table 4.1) below show that most farmers were ≤50 years old (34.7%). The study also shows that most of the farmers were female (63.2%). The study further indicates that majority of the farmers were married (88.3%). The farmers' level of education from the study shows that most of the farmers had secondary level of education (67.8%).

Table 4.1: Sociodemographic characteristics of the respondents in the study area

Variable	Category	Frequency n (%)
Age of participants	21-24	9(3.8)
	25-29	8(3.3)
	30-34	13(5.4)
	35-39	41(17.2)
	40-44	42(17.6)
	45-49	43(18)
	>= 50	83(34.7)
Sex	Male	88(36.8)
	Female	151(63.2)
Marital status	Married	211(88.3)
	Divorced	5(2.1)
	Single	23(9.6)
level of education	Primary	29(12.1)
	Secondary	162(67.8)
	Tertiary	48(20.1)

Figure 4.1 below shows that the farmers who had knowledge on aflatoxin were (58.2%). The knowledge of aflatoxin was the mean of responses from the respondents based on various knowledge parameters asked.

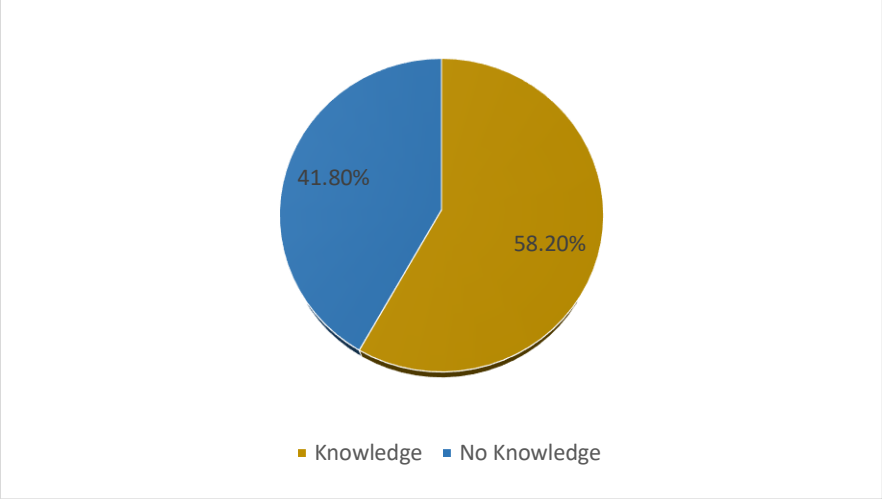

Figure 4.1: Proportion of farmers with knowledge on aflatoxin in the study area

Table 4.2 below shows various responses from farmers on knowledge of aflatoxin from various parameters. Farmers were tested on the knowledge of aflatoxin based of various parameters.

Table 4.2: Knowledge of farmers on Aflatoxin based on various parameters

Knowledge parameter	Response	Proportion n (%)
Period of rearing chicken	<1 year	15(6.6)
	1-5	100(43.9)
	6-10	83(36.4)
	>10	30(13.2)
Number of broilers kept in the farm	<500	170 (75.6)
	501-1000	35 (15.6)
	1001-2000	12 (5.3)
	>2000	8 (3.6)
Possibility of fungal toxins to be found in feed	Yes	192 (80.3)
	No	47 (19.7)
Possibility of fungal toxins to be transferred from feed to poultry	Yes	190(79.5)
	No	49 (20.5)
Possibility that fungal toxins in feed can affect poultry health	Yes	192 (80.3)
	No	47 (19.7)
Heard about aflatoxins	Yes	225 (94.5)

	No	13(5.5)
Ability to detect molds in feed	Yes	201 (84.1)
	No	38 (15.9)
Action taken with feeds found contaminated with aflatoxin	Dispose	30 (13)
	Continue feeding	137(59.6)
	Alternative use	63 (27.4)
Possibility of detoxifying fungal toxins in feed	Yes	20 (8.4)
	No	217 (91.6)
Are broilers fed on any other feed other than the commercial feed	Yes	11(4.6)
	No	227 (95.4)

The results indicate that majority of the farmers reared broilers between 1-5 years (43.9%) and most of them kept <500 broilers (75.6%) in their farms. Majority of the farmers agreed that there is a possibility of fungal toxins to be found in feed (80.3%), to be transferred from feed to the broiler (79.5%) and that the fungal toxins can affect the poultry health (80.3%). The results further indicate that majority of the farmers (94.5%) had heard about aflatoxins and most of them were able to detect molds in feed (84.1%). The results also show that in the event that the feeds are contaminated with aflatoxin, majority of the farmers (59.6%) reported that they will continue feeding the broilers with the adulterated feed. The results further show that most of the famers (91.6%) had no knowledge on the possibility of detoxifying fungal toxins in feed. Those who knew (8.4%) about the possibility of detoxifying fungal toxins in feed stated the following methods can be used; boiling, sun drying, grinding, good storage, heating, mixing with toxin binder and sieving.

The results also show that most of the farmers (95.4%) did not feed their broilers with any other feed other than the commercially processed feed. To add on, (4.6%) of the farmers reported that they fed the broilers on other feeds together with the commercially processed

feed and they further added that they fed the broilers on greens, kitchen refuse, left overs, *ugali*, grounded maize, bran and maize.

Table 4.3: Association between sociodemographic characteristics and knowledge of aflatoxin

Variable	Category	Yes	No	Chi square (X^2)	P value	Remark
Sex	Male	67 (28.8%)	20(8.6%)			
	Female	123(52.8%)	23(9.9%)	**1.896**	**0.169**	Not significant
Marital status	Married	165(70.8%)	40(17.2%)			
	Divorced	4(1.7%)	1(0.4%)		**0.446**	Not significant
	Single	21(9%)	2(0.9%)	**1.616**		
Education level	Primary	17(7.3%)	9(3.9%)			
	Secondary	133(57.1%)	27(11.6%)		**0.035**	Significant
	Tertiary	40(17.2%)	7(3%)	**5.174**		
Age	21-24	3(1.3%)	4(1.7%)			
	25-29	6(2.6%)	2(0.9%)			
	30-34	11(4.7%)	2(0.9%)			
	35-39	35(15%)	5(2.1%)		**0.047**	Significant
	40-44	31(13.3%)	11(4.7%)			
	45-49	36(15.5%)	5(2.1%)			
	≥50	68(29.2%)	14(6%)	**11.055**		

Statistical analysis revealed that there was a significant association ($p<0.05$) between age and level of education with knowledge of aflatoxins as shown in Table 4.3 above.

Table 4.4: Knowledge on signs to suspect presence of fungal toxins in feed

Response	Abnormal colour	Abnormal consistency	Bad odor	Insect/larva presence	Impaired animal health/death
Yes	53 (22.3%)	195 (85.1%)	187 (79.6%)	101(43.5%)	124 (54.6%)
No	185 (77.7%)	34 (14.9%)	48 (20.4%)	131(56.5%)	98 (45.4%)

Table 4.4 above shows that majority of the farmers were knowledgable on the signs used to suspect fungal contamination in feed as (85.1%) of the farmers were able to identify abnormal consistency, (79.6%) bad odor, (43.5%) presence of insect/larva and (54.6%) impaired animal health /deaths. However, majority of the farmers (77.7%) did not know how to identify abnormal colour in feed.

Table 4.5: Association between sociodemographic characteristics and knowledge on signs to suspect aflatoxin contamination

Variable	Category	Yes	No	X^2	P value	Remark
Sex	Male	71(31.8%)	12(5.4%)			
	Female	115(51.6%)	25(11.2%)	**0.435**	**0.510**	Not significant
Marital status	Married	164(73.5%)	32(14.3%)			
	Divorced	2(0.9%)	2(0.9%)		**0.179**	Not
	Single	20(9%)	3(1.3%)	**3.445**		significant
Education level	Primary	19(8.5%)	8(3.6%)			
	Secondary	126(56.5%)	25(11.2%)		**0.043**	Significant
	Tertiary	41(18.4)	4(1.8%)	**5.246**		
Age	21-24	7(3.1%)	1(0.4%)			
	25-29	7(3.1%)	1(0.4%)			
	30-34	8(3.6%)	4(1.8%)			Not
	35-39	33(14.8%)	7(3.1%)		**0.697**	significant
	40-44	33(14.8%)	4(1.4%)			
	45-49	33(14.8%)	8(3.6%)	**3.850**		
	≥50	65(29.1%)	12(5.4%)			

Table 4.5 above shows that there was a significant association ($p<0.05$) between level of education and knowledge on signs to suspect aflatoxin contamination in broiler feed.

Table 4.6: Means through which farmers heard about aflatoxin

Response	Reading	Mass media TV/Radio	Seminars	Friends/neighbours
Yes	28(11.8%)	225(94.5%)	185(78.1%)	71(30%)
No	209(82.2%)	13(5.5%)	52(21.9%)	166(70%)

Table 4.6 above shows that most of the farmers heard about aflatoxin through mass media (94.5%), followed by seminars (78.1%) then through friends/neighbours (30%) and lastly (11.8%) was through reading.

Table 4.7: Association between knowledge and sources of information on Aflatoxin

Variable	Yes	No	X^2	P value	Remark
Reading	28(12.1%)	203 (87.9%)	**2.768**	**0.046**	Significant
Mass media	140(60.6%)	91(39.4%)	**1.121**	**0.290**	Not significant
Seminars	52(22.5%)	179 (77.5%)	**9.661**	**0.002**	Significant
Friends &neighbours	69(29.9%)	162 (70.1%)	**0.182**	**0.669**	Not significant

There was a significant association ($p<0.05$) between knowledge of aflatoxins among farmers and the media through which farmers heard about aflatoxin. Reading and seminars were found to be significant as shown in Table 4.7 above.

Figure 4.2 below shows that most farmers (78%) had heard about aflatoxin between 1-6 months ago while few farmers (1%) had heard about aflatoxin 13-18 months ago and >=19 months ago.

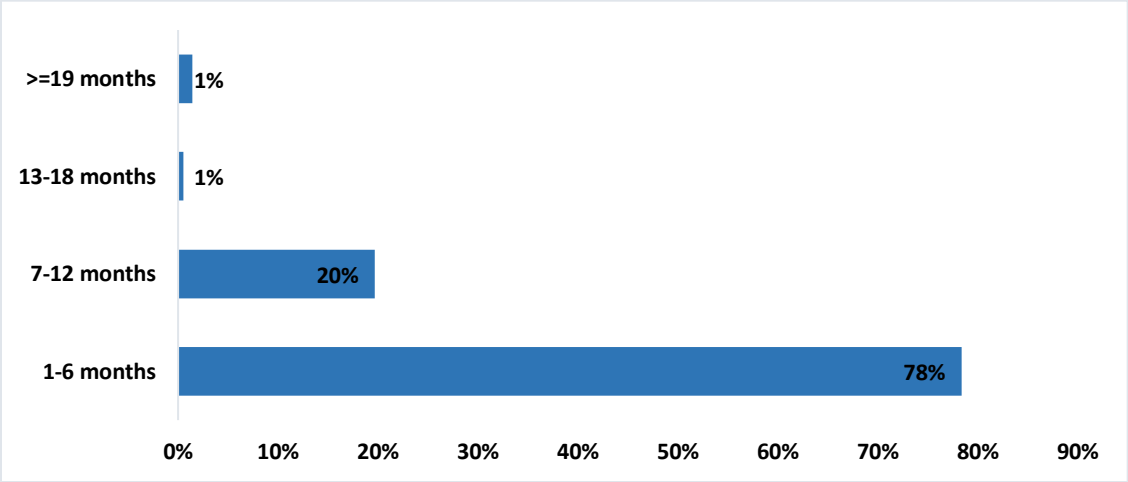

Figure 4.2: Length of time farmers heard about aflatoxin

On the farmers' feed storage practices, the study observed that most of the farmers (90%) stored their broiler feed in well ventilated stores whereas (10%) stored their broiler feed in the open as shown in figure 4.3 below.

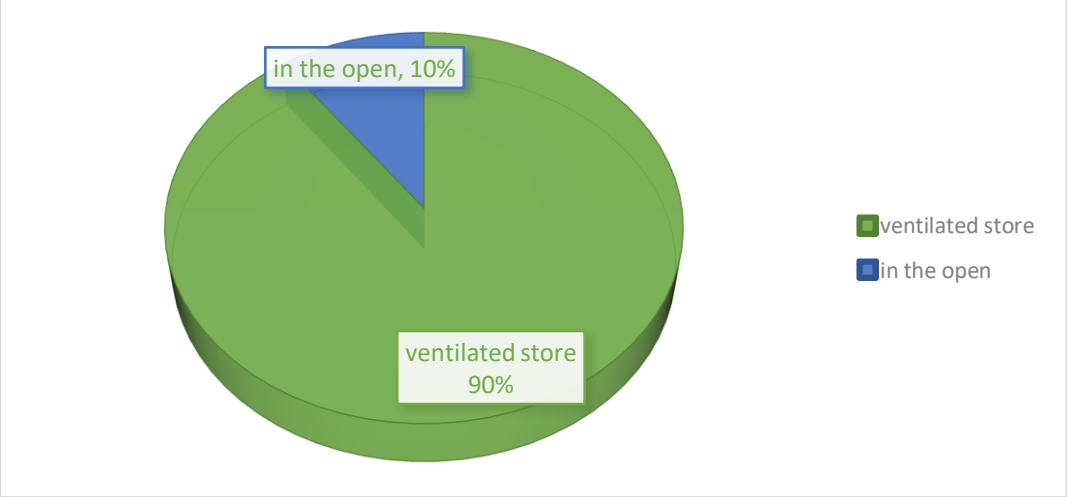

Figure 4.3: Place where broiler feed is stored

On feed placement methods used by the farmers, the study observed that majority of the farmers (80%) placed their broiler feed on raised ground whereas (20%) of the farmers placed their broiler feed on the floor as shown in figure 4.4 below.

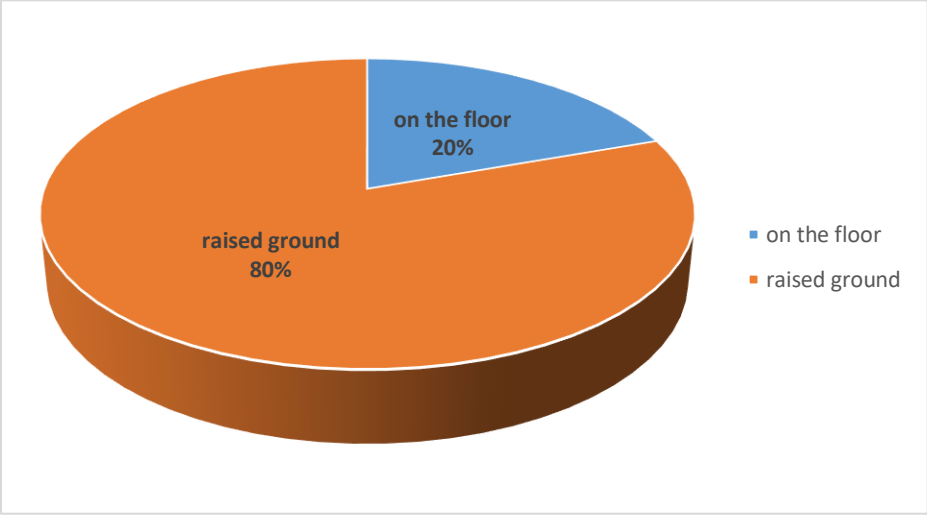

Figure 4.4: Broiler feed storage surface

Figure 4.5 below indicates that (76%) of the farmers bought broiler feed on a weekly basis, (10%) of the farmers bought broiler feed on a monthly basis, (8%) bought fortnightly and lastly (6%) bought broiler feed on a daily basis.

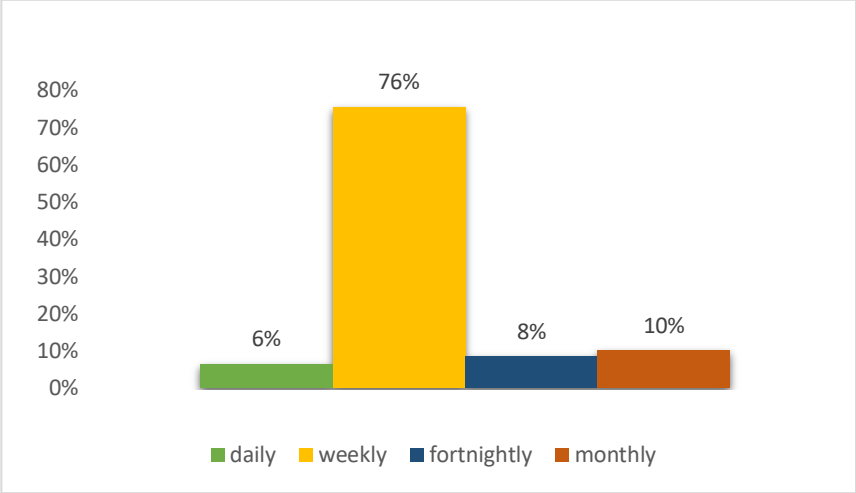

Figure 4.5: Frequency of purchase of broiler feed

Broiler feeds were inspected the study observed that (84%) of the feed was in good condition (non moldy and loose), (15%) was moldy and loose and lastly (1%) was moldy and compact (cake like) as shown in figure 4.6 below.

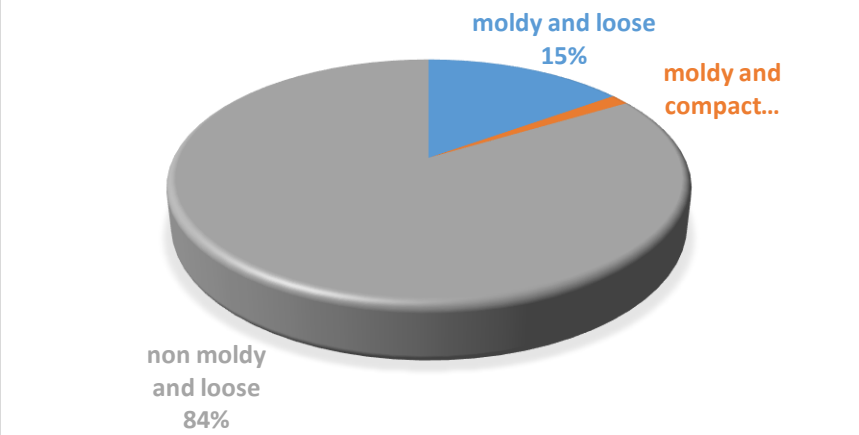

Figure 4.6: State of broiler feed

Findings from the Focus Group Discussion show that the respondents had knowledge on the presence of aflatoxin in food and feed, on signs of feed contaminated with fungal toxin

and on prevention and control of aflatoxin. On the contrary the respondents did not have knowledge on aflatoxin carry over, on measures taken with feed found contaminated with fungal toxins/aflatoxin and on detoxification of contaminated feed. This is shown in Table 4.8 below.

Table 4.8: Focus Group Discussion on farmers' knowledge on Aflatoxin

Parameter	**FGD 1**	**FGD 2**	**Total**
Knowledge on presence of aflatoxin in food and feed	√	√	**2**
Knowledge on causes of aflatoxin in feed	√	**x**	**1**
Knowledge on aflatoxin carry over	**x**	**x**	**0**
Knowledge on signs of feed contaminated with fungaltoxin	√	√	**2**
Knowledge on health impacts of aflatoxin	√	**x**	**1**
Knowledge on measures taken with feed found contaminated with aflatoxin/fungal toxins	**x**	**x**	**0**
Knowledge on prevention and control of aflatoxin	√	√	**2**
Knowledge on detoxification of contaminated feed	**x**	**x**	**0**
Knowledge on how to place and store feed	√	√	**1**

KEY: √ Had knowledge **x** Had no knowledge

4.2 Detection, Quantification and Characterization of aflatoxin levels in feed

There was no statistical significant difference ($p>0.05$) in AFB1, AFB2, AFG1, AFG2 and Total Aflatoxin levels in broiler starter feed in all the farms as shown in Table 4.9 below. However, there was significant difference ($p<0.05$) in AFG1 levels in farm 3 and farm 4.

Table 4.9: Aflatoxin levels (ppb) for broiler starter feed per farm

FARM	B1	B2	G1	G2	TOTAL AF
FARM 1	14.94±2.38^{a}	2.62±0.59^{a}	8.35±2.44^{ab}	0.88±0.69^{a}	26.79±5.96^{a}
FARM 2	17.96±2.99^{a}	2.72±0.36^{a}	10.0±0.48^{ab}	1.01±0.17^{a}	31.69±3.05^{a}
FARM 3	14.87±4.29^{a}	1.95±1.72^{a}	5.65±3.34^{a}	0.47±0.55^{a}	22.95±9.61^{a}
FARM 4	19.51±0.71^{a}	2.71±0.26^{a}	10.22±0.54^{b}	0.99±0.15^{a}	33.42±2.37^{a}
FARM 5	19.47±1.16^{a}	2.65±0.6^{a}	10.06±0.91^{ab}	1.21±0.26^{a}	33.38±1.21^{a}
FARM 6	16.80±2.94^{a}	1.96±0.8^{a}	8.97±1.99^{ab}	0.87±0.42^{a}	28.59±5.36^{a}
P value	**0.0782**	**0.6067**	**0.0397**	**0.2931**	**0.0784**
STANDARDS: KEBS	**B1 10ppb**	**Total Aflatoxin 20ppb**		**EU B1 20ppb**	
EAC	**B1 20ppb**	**Total Aflatoxin 50ppb**		**WHO/FAO B1 20ppb**	

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SD

AFB1 levels were above the KEBS limit in all the farms however it was below the EAC, EU and WHO/FAO limit. Total Aflatoxin levels in all the farms were above the KEBS limit but below the EAC limit. Farm 2, 4 and 5 had high levels of AFG1. Farm 3 had the least level of AFB2 while farm 6 had the least level of AFG2 as shown in Table 4.10 above.

There was statistically significant difference ($p<0.05$) in broiler finisher feeds in AFB1 levels in farm 2 and farm 5 whereby high levels of AFB1 were reported in farm 5 as shown in Table 4.10 below. Additionally, there was no significant difference ($p>0.05$) in AFB2, AFG1, AFG2 and Total Aflatoxin in all the farms.

Table 4.10: Aflatoxin levels (ppb) for broiler finisher per farm

FARM	B1	B2	G1	G2	TOTAL AF
FARM 1	15.56±1.60^{ab}	3±1.49^{a}	8.45±2.35^{a}	0.79±0.36^{a}	27.8±5.44^{a}
FARM 2	12.91±1.69^{a}	1.51±0.53^{a}	5.33±1^{a}	0.58±0.61^{a}	20.34±3.79^{a}
FARM 3	17.09±2.8^{ab}	3.73±2.15^{a}	10.27±4.2^{a}	1.31±0.66^{a}	32.4±9.78^{a}
FARM 4	18.56±3.31^{ab}	2.6±0.46^{a}	10.86±1.36^{a}	1.05±0.39^{a}	33.08±5.5^{a}
FARM 5	20.44±1.76^{b}	2.76±0.73^{a}	10.67±1.49^{a}	1.08±0.23^{a}	34.95±3.61^{a}
FARM 6	18.49±1.19^{ab}	2.45±0.31^{a}	9.92±0.88^{a}	1.2±0.23^{a}	32.06±2.46^{a}
P VALUE	**0.0166**	**0.3650**	**0.0711**	**0.4138**	**0.0731**

STANDARDS: KEBS B1 10ppb Total Aflatoxin 20ppb EU B1 20ppb
EAC B1 20ppb Total Aflatoxin 50ppb WHO/FAO B1 20ppb

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SD

AFB1 levels in all the farms were above the KEBS limit but below the EAC, EU and WHO/FAO limit except farm 5 which was slightly above the EAC, EU and WHO/FAO limit. Total Aflatoxins in all the farms were above the KEBS limit but below the EAC limit. High levels of AFG1 were detected in farm 3, 4 and 5. Low levels of AFB2 and AFG2 were detected in farm 2. This is illustrated in Table 4.10 above.

There was no statistical significant difference ($p>0.05$) in aflatoxin levels in broiler starter and broiler finisher as shown in Table 4.11 below.

Table 4.11: Aflatoxin levels (ppb) in broiler starter and broiler finisher per Aflatoxin type

Aflatoxin type	Broiler starter	Broiler finisher	T statistic	P value
B1	17.26±3.07	17.17±3.09	0.0869	0.9312
B2	2.44±0.84	2.68±1.18	0.7735	0.2219
G1	8.87±2.41	9.25±2.7	0.4751	0.3186
G2	0.9±0.44	1±0.45	0.7257	0.2361
TOTAL AF	29.47±6.13	30.1±6.88	0.3153	0.3771

STANDARDS: KEBS B1 10ppb Total Aflatoxin 20ppb EU B1 20ppb
EAC B1 20ppb Total Aflatoxin 50ppb WHO/FAO B1 20ppb

KEY: $p<0.05$ ±SD

AFB1 levels in both broiler starter and broiler finisher were above the KEBS limit but were below the EAC, EU and WHO/FAO limit. Total Aflatoxin levels were above the KEBS limit but below the EAC limit. Broiler finisher had high levels of AFB2, AFG1, AFG2 and Total Aflatoxin than broiler starter whereas broiler starter had slightly higher levels of AFB1 than broiler finisher. This is shown in Table 4.11 above.

There was a statistical significant difference ($p<0.05$) in AFB1 levels in water used in farm 3, 4 and 5 where high levels were detected in farm 3 as shown in Table 4.12 below. Furthermore, there was statistical significance difference ($p<0.05$) in AFB2 levels in water used in farm 1 and all the other farms whereby AFB2 levels were not detected in farm 1. There was however no statistical significance difference in AFG1, AFG2 and Total Aflatoxin in water used across all the farms.

Table 4.12: Aflatoxin levels (ppb) for water samples per farm

FARM	B1	B2	G1	G2	TOTAL AF
FARM 1	0.12±0.02^{ab}	ND	0.06±0.04^{a}	ND	0.18±0.04^{a}
FARM 2	0.11±0.02^{ab}	0.05±0.06^{ab}	0.03±0.04^{a}	ND	0.19±0.1^{a}
FARM 3	0.14±0.04^{b}	0.12±0.09^{a}	0.03±0.04^{a}	ND	0.28±0.08^{a}
FARM 4	0.04±0.04^{c}	0.1±0.05^{a}	0.12±0.15^{a}	ND	0.26±0.1^{a}
FARM 5	0.09±0.02^{a}	0.09±0.06^{ab}	0.06±0.04^{a}	0.01±0.04^{a}	0.25±0.1^{a}
FARM 6	0.11±0.01^{ab}	0.05±0.06^{ab}	0.06±0.04^{a}	0.01±0.04^{a}	0.24±0.05^{a}
P value	**0.0001**	**0.0109**	**0.2387**	**0.5570**	**0.2302**
STANDARD:	**EU B1 2ppb**		**Total Aflatoxin 4ppb**		
	WHO/FAO B1 5ppb		**Total Aflatoxin 10ppb**		

KEY: Means with different superscript letters in each column and row are statistically significant at $p<0.05$ ±SD ND -not detected

AFB1 levels in water in all the farms were below the EU and WHO/FAO limit. Similarly, Total Aflatoxin levels in all the farms were below the EU and WHO/FAO limit. High levels of AFG1 were detected in farm 4. AFG2 levels were not detected in farm 1, 2, 3 and 4.

Table 4.13: Aflatoxin levels (ppb) in water per Aflatoxin type

Aflatoxin type	AF levels
B1	0.1±0.04
B2	0.07±0.07
G1	0.06±0.07
G2	0.004±0.02
Total Aflatoxin	0.23±0.09
STANDARDS:	**EU B1 2ppb** **Total Aflatoxin 4ppb**
	WHO/FAO B1 5ppb **Total Aflatoxin 10ppb**

KEY: ±SD

Table 4.13 above shows that aflatoxin levels in water in all the analogues (B1, B2, G1, G2 and Total Aflatoxin) were below the EU and WHO/FAO limits. Total Aflatoxin levels were the highest while AFG2 levels were the least.

4.3 Detection, Quantification and Characterization of aflatoxin levels in broiler meat

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 4.14 below.

Table 4. 14: 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

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 4.14 above. The levels of aflatoxin B1 in all the chicken parts increased with time as shown in figure 4.7 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 4.7) 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.

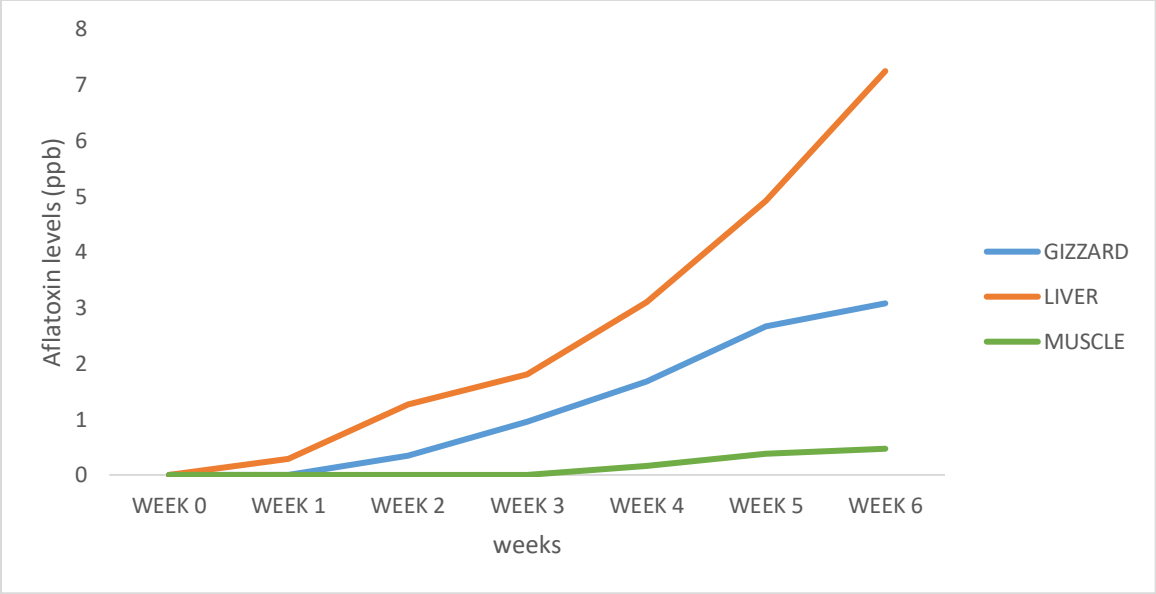

Figure 4.7: Aflatoxin B1 levels in meat parts per week

Aflatoxin B1 levels in the gizzard were not detected in week 0 and week 1 in all the farms. AFB1 levels in the gizzard increased weekly in all farms. The highest levels were observed in week 6 in all the farms. AFB1 levels in the gizzard were below the WHO/FAO limit in all the farms however they were above the EU limit in week 4 in farm 5 as well as in week 5 and 6 in all the farms. There was a statistical significant difference ($p<0.05$) in AFB1 levels in the liver per week in all the farms as shown in Table 4.15 below. AFB1 levels were not detected in the liver in week 0 in all the farms. The levels of aflatoxin increased weekly in all the farms. The highest levels of AFB1 were detected in week 6 in all the farms as well as in farm 5 week 5. AFB1 levels were above the EU limit in week 4, 5 and 6 in all the farms as well as in week 3 in farm 5. The levels were above the WHO/FAO limit in week 6 in all the farms as well as in week as well as in week 5 in farm 2 and farm 5. AFB1 levels in the muscle were only detected in week 4, 5 and 6 in all the farm however in week 4 in farm 1 and farm 6 AFB1 were not detected. AFB1 levels in the muscle were all below the EU and WHO/FAO standards. This is shown in Table 4.15 below.

Table 4.15: Aflatoxin B1 levels (ppb) in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	0.56 ± 0.01^{b}	1.05 ± 0.01^{c}	1.22 ± 0.01^{d}	2.66 ± 0.01^{e}	3.04 ± 0.01^{j}
FARM 2	ND	ND	0.48 ± 0.01^{b}	1.14 ± 0.01^{c}	1.68 ± 0.01^{e}	2.34 ± 0.01^{f}	2.82 ± 0.01^{hi}
FARM 3	ND	ND	0.34 ± 0.01^{b}	1.36 ± 0.01^{d}	2.03 ± 0.01^{f}	2.21 ± 0.01^{g}	2.45 ± 0.01^{h}
FARM 4	ND	ND	0.4 ± 0.01^{b}	0.86 ± 0.01^{c}	1.35 ± 0.01^{d}	2.76 ± 0.01^{e}	3.51 ± 0.01^{h}
FARM 5	ND	ND	ND	0.4 ± 0.01^{b}	2.07 ± 0.01^{g}	3.02 ± 0.01^{i}	4.58 ± 0.01^{j}
FARM 6	ND	ND	0.34 ± 0.01^{b}	0.85 ± 0.01^{d}	1.71 ± 0.01^{f}	2.34 ± 0.01^{h}	2.82 ± 0.01^{c}
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	0.43 ± 0.01^{b}	1.17 ± 0.01^{c}	1.77 ± 0.01^{fg}	2.85 ± 0.01^{h}	4.33 ± 0.01^{i}	6.34 ± 0.01^{j}
FARM 2	ND	0.27 ± 0.01^{bc}	1.53 ± 0.01^{e}	2.02 ± 0.01^{d}	3.28 ± 0.01^{h}	5.2 ± 0.01^{i}	7.05 ± 0.01^{k}
FARM 3	ND	0.33 ± 0.01^{c}	1.72 ± 0.01^{f}	1.83 ± 0.01^{g}	2.21 ± 0.01^{h}	3.05 ± 0.01^{i}	6.37 ± 0.01^{j}
FARM 4	ND	0.24 ± 0.01^{b}	0.98 ± 0.01^{c}	1.41 ± 0.01^{e}	2.01 ± 0.01^{d}	4.36 ± 0.01^{i}	8.5 ± 0.01^{j}
FARM 5	ND	0.21 ± 0.01^{b}	0.75 ± 0.01^{c}	2.3 ± 0.01^{d}	4.2 ± 0.01^{e}	6.02 ± 0.01^{f}	8.16 ± 0.01^{g}
FARM 6	ND	0.29 ± 0.01^{bc}	1.05 ± 0.01^{e}	1.95 ± 0.01^{d}	3.2 ± 0.01^{h}	4.76 ± 0.01^{i}	7.07 ± 0.01^{k}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	0.25 ± 0.01^{c}	0.4 ± 0.01^{b}
FARM 2	ND	ND	ND	ND	0.23 ± 0.01^{b}	0.33 ± 0.01^{d}	0.35 ± 0.01^{e}
FARM 3	ND	ND	ND	ND	0.21 ± 0.01^{c}	0.32 ± 0.01^{d}	0.55 ± 0.01^{e}
FARM 4	ND	ND	ND	ND	0.21 ± 0.01^{c}	0.36 ± 0.01^{e}	0.57 ± 0.01^{f}
FARM 5	ND	ND	ND	ND	0.3 ± 0.01^{c}	0.41 ± 0.01^{b}	0.45 ± 0.01^{d}
FARM 6	ND	ND	ND	ND	ND	0.4 ± 0.01^{b}	0.7 ± 0.01^{c}
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

STANDARDS: EU 2ppb WHO/FAO 5ppb

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

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 4.16 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 4.8) below.

Table 4.16: 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

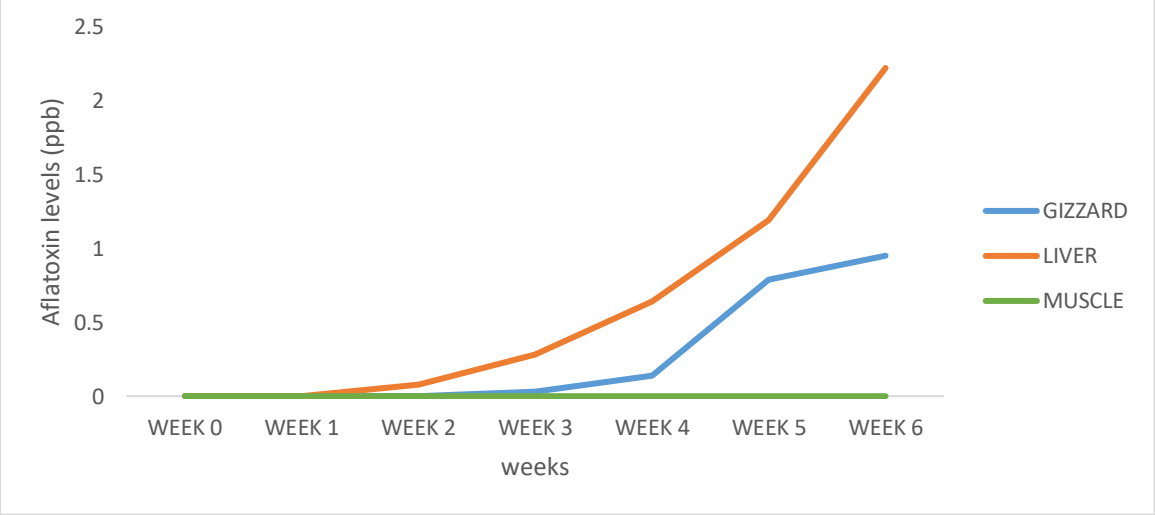

Figure 4.8: **Aflatoxin B2 levels in meat parts per week**

There was a statistical significant difference ($p<0.05$) in aflatoxin B2 levels in gizzard, liver and muscles of broiler chicken between weeks in all the farms as shown in Table 4.18 above. The AFB2 levels in the gizzard were not detected in week 0, 1, 2 and 3 in all the farms as well as in week 4 in farm 1, 2 and 5. AFB2 levels increased weekly in all the farms and high levels were detected in week 6 in all the farms. In the liver AFB2 levels were not detected in week 0 and week 1in all the farms as well as in week 2 in farm 1, 2, 4 and 5. AFB2 levels in the liver increased weekly in all the farms and the highest levels were detected in week 6 in all the farms. AFB2 levels were not detected in the muscle in all the farms as shown in Table 4.17 below.

Table 4.17: Aflatoxin B2 levels in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	0.31 ± 0.01^{b}	0.92 ± 0.01^{bc}
FARM 2	ND	ND	ND	ND	ND	0.91 ± 0.01^{bc}	0.96 ± 0.01^{c}
FARM 3	ND	ND	ND	ND	0.4 ± 0.01^{d}	0.72 ± 0.01^{e}	1.03 ± 0.01^{f}
FARM 4	ND	ND	ND	ND	0.13 ± 0.01^{b}	0.96 ± 0.01^{c}	1.11 ± 0.01^{bf}
FARM 5	ND	ND	ND	ND	ND	0.82 ± 0.01^{e}	1.21 ± 0.01^{f}
FARM 6	ND	ND	ND	ND	0.41 ± 0.01^{d}	0.63 ± 0.01^{f}	0.86 ± 0.01^{ce}
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	0.43 ± 0.01^{b}	0.53 ± 0.01^{c}	0.95 ± 0.01^{d}	2.4 ± 0.01^{e}
FARM 2	ND	ND	ND	0.36 ± 0.01^{f}	0.61 ± 0.01^{g}	1.39 ± 0.01^{h}	2.83 ± 0.01^{k}
FARM 3	ND	ND	0.26 ± 0.01^{bc}	0.27 ± 0.01^{b}	0.58 ± 0.01^{g}	0.72 ± 0.01^{h}	2.6 ± 0.01^{i}
FARM 4	ND	ND	ND	ND	0.25 ± 0.01^{bc}	1.32 ± 0.01^{i}	2.09 ± 0.01^{j}
FARM 5	ND	ND	ND	0.3 ± 0.01^{e}	0.92 ± 0.01^{d}	1.31 ± 0.01^{i}	2.05 ± 0.01^{j}
FARM 6	ND	ND	0.21 ± 0.01^{c}	0.34 ± 0.0^{ef}	0.96 ± 0.01^{dh}	1 ± 0.01^{h}	2.87 ± 0.01^{k}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	ND
FARM 2	ND	ND	ND	ND	ND	ND	ND
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	ND	ND
FARM 5	ND	ND	ND	ND	ND	ND	ND
FARM 6	ND	ND	ND	ND	ND	ND	ND
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<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

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 4.18 below.

Table 4.18: Aflatoxin G1 levels (ppb) in broiler meat parts samples weekly

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 0	ND	ND	ND
WEEK 1	ND	0.04 ± 0.15^{a}	ND
WEEK 2	0.04 ± 0.15^{a}	0.58 ± 0.15^{abc}	ND
WEEK 3	0.31 ± 0.15^{ab}	0.84 ± 0.15^{bc}	ND
WEEK4	0.84 ± 0.15^{bc}	1.32 ± 0.15^{cd}	ND
WEEK 5	1.26 ± 0.15^{cd}	2.58 ± 0.15^{e}	0.04 ± 0.15^{a}
WEEK6	1.96 ± 0.15^{de}	4.16 ± 0.15^{f}	0.23 ± 0.15^{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

AFG1 levels increased weekly in all the meat parts as shown in figure 4.9 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 4.18 above.

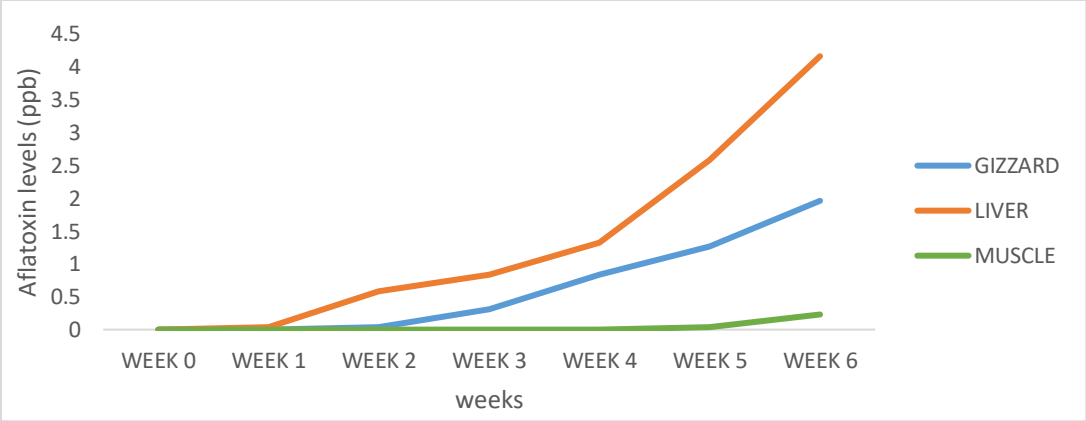

Figure 4.9: Aflatoxin G1 levels in meat parts per week

There was statistical significance difference ($p<0.05$) in AFG1 levels in the liver between the weeks in all the farms and the levels increased weekly as shown in Table 4.19 below. AFG1 levels were not detected in the gizzard in week 0 and week 1 in all the farms as shown in Table 4.20 below. In week 2 farm 1 was the only farm that detected AFG1 levels. In farm 4 and 5 AFG1 levels were detected from week 4 to week 6. AFG1 levels were not detected in week 0 in all the farms. High levels of AFG1 were detected in week 6 in all the farms. In the muscle AFG1 levels were only detected in week 6 in all the farms. It is important to note that there was no statistical significant difference ($p<0.05$) in AFG1 levels in the muscle across all the farms in week 6.

Table 4.19: Aflatoxin G1 levels in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	0.22 ± 0.01^{d}	0.26 ± 0.01^{d}	0.35 ± 0.01^{b}	0.72 ± 0.01^{e}	2.4 ± 0.01^{f}
FARM 2	ND	ND	ND	0.35 ± 0.01^{b}	0.75 ± 0.01^{e}	1.45 ± 0.01^{f}	1.55 ± 0.01^{c}
FARM 3	ND	ND	ND	0.41 ± 0.01^{b}	0.93 ± 0.01^{d}	1.03 ± 0.01^{e}	1.51 ± 0.01^{c}
FARM 4	ND	ND	ND	ND	0.52 ± 0.01^{b}	2.05 ± 0.01^{c}	1.16 ± 0.01^{f}
FARM 5	ND	ND	ND	ND	1.2 ± 0.01^{f}	1.31 ± 0.01^{g}	3.16 ± 0.01^{h}
FARM 6	ND	ND	ND	0.35 ± 0.01^{b}	1.26 ± 0.01^{d}	1.52 ± 0.01^{c}	1.98 ± 0.01^{e}
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	0.25 ± 0.01^{c}	0.26 ± 0.01^{c}	0.88 ± 0.01^{fg}	1.01 ± 0.01^{d}	2.03 ± 0.01^{b}	4.62 ± 0.01^{e}
FARM 2	ND	ND	0.53 ± 0.01^{b}	0.86 ± 0.01^{f}	1.38 ± 0.01^{g}	2.76 ± 0.01^{h}	5.01 ± 0.01^{i}
FARM 3	ND	ND	0.8 ± 0.01^{b}	0.91 ± 0.01^{gh}	1.02 ± 0.01^{di}	1.18 ± 0.01^{j}	4.11 ± 0.01^{k}
FARM 4	ND	ND	0.35 ± 0.01^{e}	0.67 ± 0.01^{c}	1.06 ± 0.01^{i}	2.01 ± 0.01^{b}	3.51 ± 0.01^{d}
FARM 5	ND	ND	0.25 ± 0.01^{c}	0.92 ± 0.01^{h}	2.01 ± 0.01^{b}	3.91 ± 0.01^{d}	4.35 ± 0.01^{f}
FARM 6	ND	ND	0.37 ± 0.01^{e}	1.01 ± 0.01^{d}	2.04 ± 0.01^{b}	2.51 ± 0.01^{c}	4.51 ± 0.01^{f}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	0.21 ± 0.01^{b}
FARM 2	ND	ND	ND	ND	ND	ND	0.22 ± 0.01^{b}
FARM 3	ND	ND	ND	ND	ND	ND	0.2 ± 0.01^{b}
FARM 4	ND	ND	ND	ND	ND	ND	0.21 ± 0.01^{b}
FARM 5	ND	ND	ND	ND	ND	ND	0.3 ± 0.01^{b}
FARM 6	ND	ND	ND	ND	ND	ND	0.26 ± 0.01^{b}
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<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

There was statistical significance difference ($p<0.05$) in AFG2 levels in the liver, gizzard and muscle between weeks as shown in Table 4.20 below.

Table 4.20: 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 ± 0.04^{a}	ND
WEEK 5	ND	0.50 ± 0.04^{b}	ND
WEEK6	0.29 ± 0.04^{b}	0.83 ± 0.04^{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

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 tbale 4.20 above. 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 4.10) below.

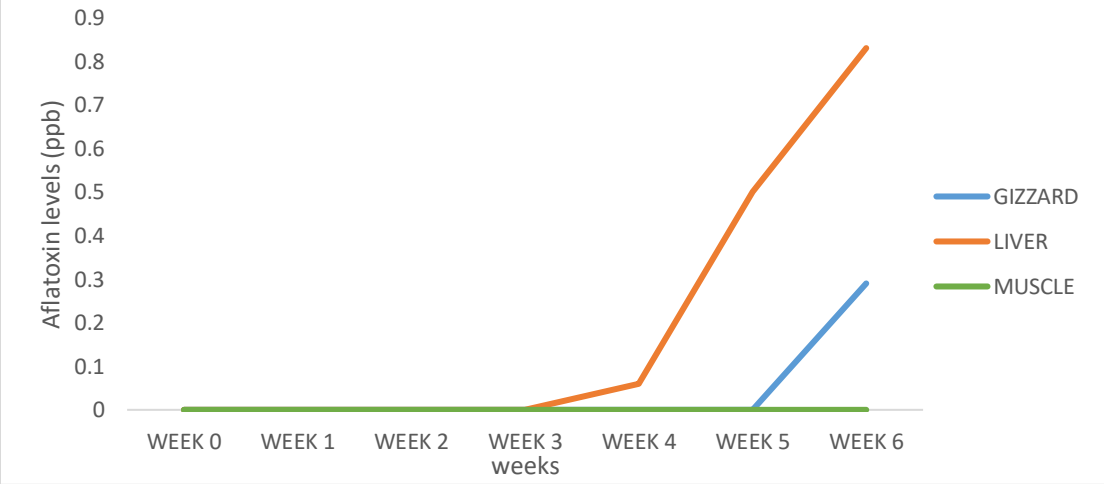

Figure 4. 10: Aflatoxin G2 levels in meat parts per week

There was a statistical significant difference ($p<0.05$) in aflatoxin G2 levels in gizzard, liver and muscles of broiler chicken between weeks in all the farms as shown in Table 4.21 below. AFG2 levels in the gizzard were only detected in week 6 and in farm 1, 4, 5 and 6 as shown in Table 4.22 above. In farm 2 and 3 AFG2 levels were not detected in all the weeks. There was no statistical significant difference ($p<0.05$) in AFG2 levels in the gizzard across all the farms in week 6. In the liver AFG2 levels were not detected in week 0 to week 4 in all the farms except in farm 5 where AFG2 levels were detected in week 4. In farm 3 AFG2 levels were only detected in week 6. AFG2 levels were detected in all the farms in week 6 and farm 5 had the highest level in week 6. In the muscle AFG2 was not detected in all the weeks and in all the farms.

Table 4.21: Aflatoxin G2 levels in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	0.28 ± 0.01^{b}
FARM 2	ND	ND	ND	ND	ND	ND	ND
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	ND	0.29 ± 0.01^{b}
FARM 5	ND	ND	ND	ND	ND	ND	0.89 ± 0.01^{b}
FARM 6	ND	ND	ND	ND	ND	ND	0.25 ± 0.01^{b}
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	0.31 ± 0.01^{bc}	0.72 ± 0.01^{d}
FARM 2	ND	ND	ND	ND	ND	0.67 ± 0.01^{c}	0.77 ± 0.01^{b}
FARM 3	ND	ND	ND	ND	ND	ND	0.97 ± 0.01^{b}
FARM 4	ND	ND	ND	ND	ND	0.37 ± 0.01^{b}	1.03 ± 0.01^{c}
FARM 5	ND	ND	ND	ND	0.33 ± 0.01^{c}	0.73 ± 0.01^{d}	1.11 ± 0.01^{e}
FARM 6	ND	ND	ND	ND	ND	0.28 ± 0.01^{b}	0.45 ± 0.01^{c}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	ND
FARM 2	ND	ND	ND	ND	ND	ND	ND
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	ND	ND
FARM 5	ND	ND	ND	ND	ND	ND	ND
FARM 6	ND	ND	ND	ND	ND	ND	ND
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<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

Table 4.22: 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 ± 0.01^{b}	ND
WEEK6	ND	0.1 ± 0.01^{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

There was a statistical significance difference ($p<0.05$) in AFM1 levels in the liver in week 5 and 6 as shown in Table 4.22 above. 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 4.11 below.

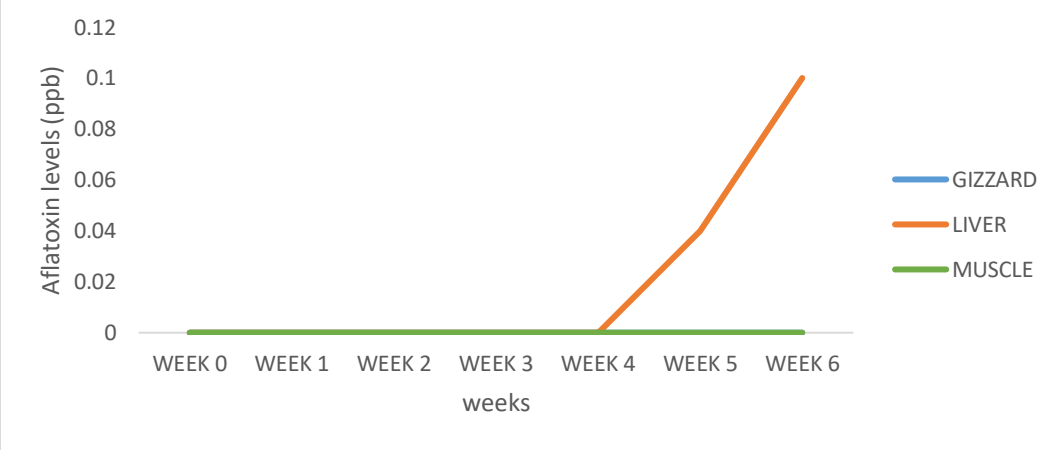

Figure 4. 11: Aflatoxin M1 levels in meat parts per week

AFM1 levels were not detected in the gizzard and in the muscle in all the farms in all the weeks as shown in Table 4.23 below. In the liver AFM1 levels were detected in week 5 in farm 4 and 6 and in week 6 in all the farms except farm 3.

Table 4.23: Aflatoxin M1 levels in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	ND
FARM 2	ND	ND	ND	ND	ND	ND	ND
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	ND	ND
FARM 5	ND	ND	ND	ND	ND	ND	ND
FARM 6	ND	ND	ND	ND	ND	ND	ND
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	0.11 ± 0.01^{b}
FARM 2	ND	ND	ND	ND	ND	ND	0.12 ± 0.01^{b}
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	0.1 ± 0.01^{c}	0.12 ± 0.01^{b}
FARM 5	ND	ND	ND	ND	ND	ND	0.12 ± 0.01^{b}
FARM 6	ND	ND	ND	ND	ND	0.12 ± 0.01^{b}	0.13 ± 0.01^{c}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	ND	ND
FARM 2	ND	ND	ND	ND	ND	ND	ND
FARM 3	ND	ND	ND	ND	ND	ND	ND
FARM 4	ND	ND	ND	ND	ND	ND	ND
FARM 5	ND	ND	ND	ND	ND	ND	ND
FARM 6	ND	ND	ND	ND	ND	ND	ND
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<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

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 4.24 below.

Table 4.24: Total Aflatoxin levels (ppb) in broiler meat parts samples weekly

WEEK	GIZZARD	LIVER	MUSCLE
WEEK 0	ND	ND	ND
WEEK 1	ND	0.33 ± 0.32^{ab}	ND
WEEK 2	0.4 ± 0.32^{ab}	1.93 ± 0.32^{bc}	ND
WEEK 3	1.28 ± 0.32^{abc}	2.94 ± 0.32^{c}	ND
WEEK 4	2.65 ± 0.32^{c}	5.11 ± 0.32^{d}	0.16 ± 0.32^{a}
WEEK 5	4.71 ± 0.32^{d}	9.18 ± 0.32^{e}	0.42 ± 0.32^{ab}
WEEK 6	6.28 ± 0.32^{d}	14.46 ± 0.32^{f}	0.7 ± 0.32^{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

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 4.12) 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 4.24 above. 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.

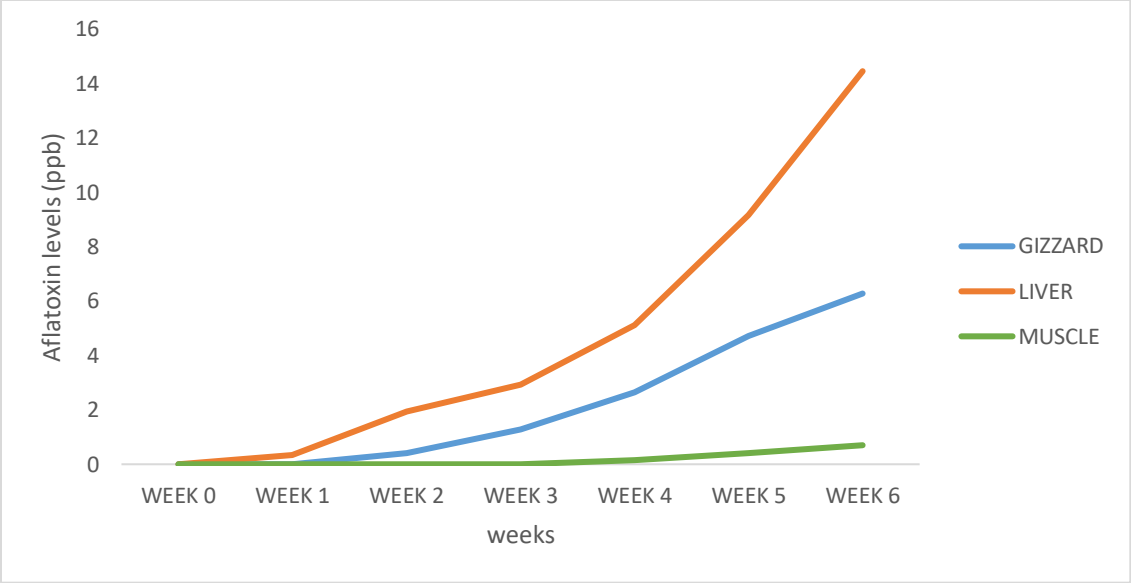

Figure 4.12: Total Aflatoxin levels in meat parts per week

There was a statistical significant difference ($p<0.05$) in Total Aflatoxin levels in the liver per week in all the farms as shown in Table 4.25 below. Total Aflatoxin levels were not detected in the liver in week 0 in all the farms. The levels of Total aflatoxin increased weekly in all the farms.

Table 4.25: Total Aflatoxin (ppb) levels in meat samples per farm

GIZZARD							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	0.82 ± 0.02^{b}	1.78 ± 0.02^{d}	1.57 ± 0.02^{f}	3.19 ± 0.02^{g}	6.65 ± 0.02^{j}
FARM 2	ND	ND	0.48 ± 0.02^{c}	1.5 ± 0.02^{f}	2.43 ± 0.02^{g}	4.5 ± 0.02^{h}	5.32 ± 0.02^{i}
FARM 3	ND	ND	0.34 ± 0.02^{b}	1.77 ± 0.02^{d}	3.36 ± 0.02^{e}	3.96 ± 0.02^{f}	4.98 ± 0.02^{g}
FARM 4	ND	ND	0.4 ± 0.02^{bc}	0.99 ± 0.02^{e}	1.87 ± 0.02^{d}	5.16 ± 0.02^{i}	6.66 ± 0.02^{j}
FARM 5	ND	ND	ND	0.4 ± 0.02^{bc}	3.28 ± 0.02^{eg}	5.16 ± 0.02^{i}	9.83 ± 0.02^{j}
FARM 6	ND	ND	0.34 ± 0.02^{b}	1.19 ± 0.02^{c}	3.37 ± 0.02^{e}	4.62 ± 0.02^{h}	5.68 ± 0.02^{i}
LIVER							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	0.68 ± 0.02^{b}	2.05 ± 0.02^{d}	3.21 ± 0.02^{e}	3.63 ± 0.02^{g}	7.62 ± 0.02^{i}	14.09 ± 0.02^{h}
FARM 2	ND	0.27 ± 0.02^{bc}	2.06 ± 0.02^{d}	3.24 ± 0.02^{ef}	5.27 ± 0.02^{g}	10.11 ± 0.02^{h}	15.55 ± 0.02^{i}
FARM 3	ND	0.33 ± 0.02^{c}	2.78 ± 0.02^{d}	3.02 ± 0.02^{e}	4.82 ± 0.02^{f}	3.95 ± 0.02^{g}	14.05 ± 0.02^{h}
FARM 4	ND	0.24 ± 0.02^{bc}	1.33 ± 0.02^{e}	2.07 ± 0.02^{d}	3.33 ± 0.02^{f}	8.08 ± 0.02^{g}	15.14 ± 0.02^{h}
FARM 5	ND	0.21 ± 0.02^{b}	1 ± 0.02^{c}	3.53 ± 0.02^{g}	7.46 ± 0.02^{h}	11.97 ± 0.02^{i}	15.67 ± 0.02^{j}
FARM 6	ND	0.29 ± 0.02^{bc}	1.62 ± 0.02^{d}	3.3 ± 0.02^{ef}	6.2 ± 0.02^{g}	8.56 ± 0.02^{h}	14.89 ± 0.02^{i}
MUSCLE							
FARM	**WEEK 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**	**WEEK 6**
FARM 1	ND	ND	ND	ND	ND	0.25 ± 0.02^{b}	0.61 ± 0.02^{c}
FARM 2	ND	ND	ND	ND	0.23 ± 0.02^{b}	0.33 ± 0.02^{c}	0.57 ± 0.02^{d}
FARM 3	ND	ND	ND	ND	0.21 ± 0.02^{b}	0.52 ± 0.02^{c}	0.55 ± 0.02^{d}
FARM 4	ND	ND	ND	ND	0.21 ± 0.02^{b}	0.36 ± 0.02^{c}	0.78 ± 0.02^{d}
FARM 5	ND	ND	ND	ND	0.3 ± 0.02^{b}	0.63 ± 0.02^{c}	0.75 ± 0.02^{d}
FARM 6	ND	ND	ND	ND	ND	0.4 ± 0.02^{b}	0.96 ± 0.02^{c}
P value:	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

STANDARDS EU 4ppb WHO/FAO 10 ppb

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

Total Aflatoxin levels in the gizzard were not detected in week 0 and week 1 in all the farms. Total Aflatoxin levels in the gizzard increased weekly in all farms. The highest levels were observed in week 6 in all the farms. Total Aflatoxin levels in the gizzard were all below the WHO/FAO limit in all the farms however they were above the EU limit in week 6 in all the farms 5 as well as in week 5 in farm 2, 5 and 6.

The highest levels of Total Aflatoxin were detected in week 6 in all the farms as well as in farm 2 week 5 and farm 5 week 5. Total Aflatoxin levels were above the EU limit in week 5 and 6 in all the farms as well as in week 4 in farm 2, 3, 5 and 6. The levels were also above the WHO/FAO limit in week 6 in all the farms as well as in week as well as in week 5 in farm 2 and farm 5.

Total Aflatoxin levels in the muscle were only detected in week 4, 5 and 6 in all the farms however in week 4 in farm 1 and farm 6, Total Aflatoxin levels were not detected. Total Aflatoxin levels in the muscle were all below the EU and WHO/FAO standards. Total Aflatoxin levels were detected in the muscle in week 4, 5 and 6 in all the farms except in week 4 in farm 1 and 6 where Total Aflatoxin levels were not detected.

4.4 Carry over effect of Aflatoxin from broiler feed to broiler meat

This section entails presentation of results on objective 4 of the study 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 in section 3.10.

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 4.26 below.

Table 4.26: 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 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 4.13 below.

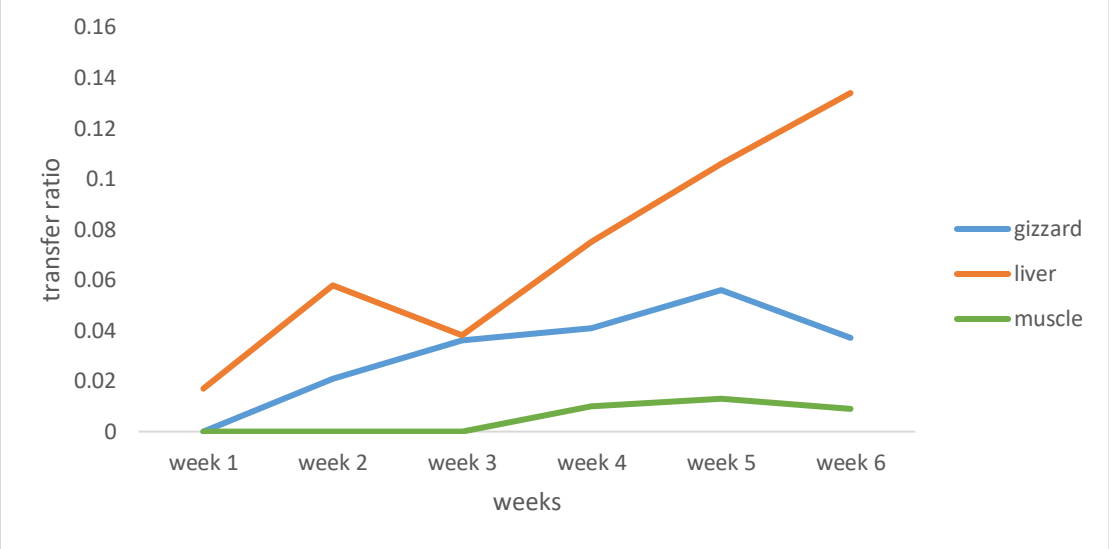

Figure 4. 13: **AFB1 carry over ratio trend per week per meat part**

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 4.27 below.

Table 4.27: 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 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 4.14) below.

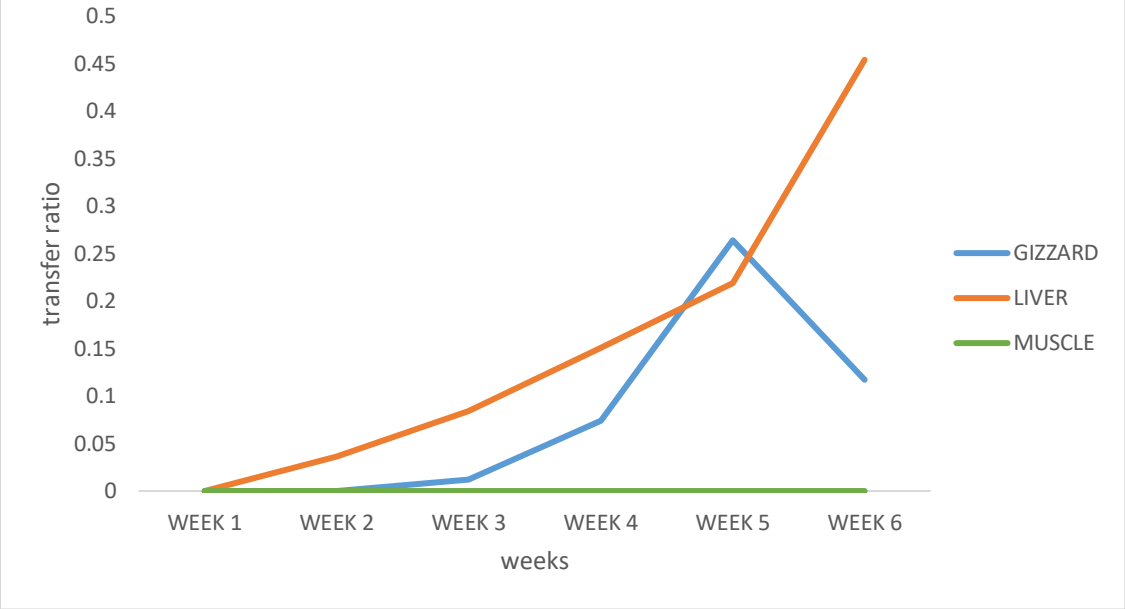

Figure 4.14: AFB2 carry over ratio trend per week per meat part

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 4.28 below. 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 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 4.15 below.

Table 4.28: 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 %)

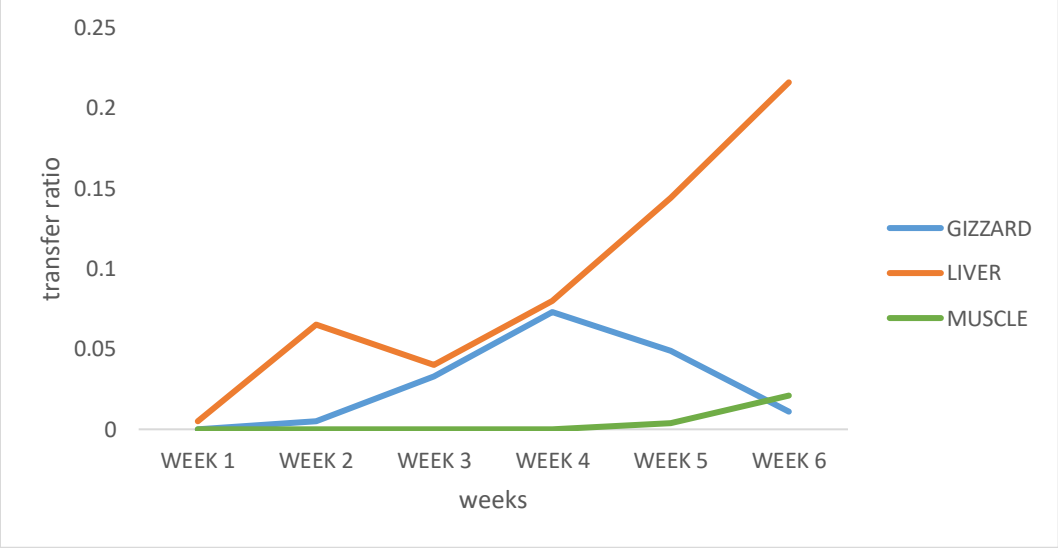

Figure 4.15: **AFG1 Carry over ratio trend per week per meat part**

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 4.29 below. The highest carry over ratio was observed in the liver, followed by the gizzard and the least was in the muscle.

Table 4.29: 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 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 4.16 below.

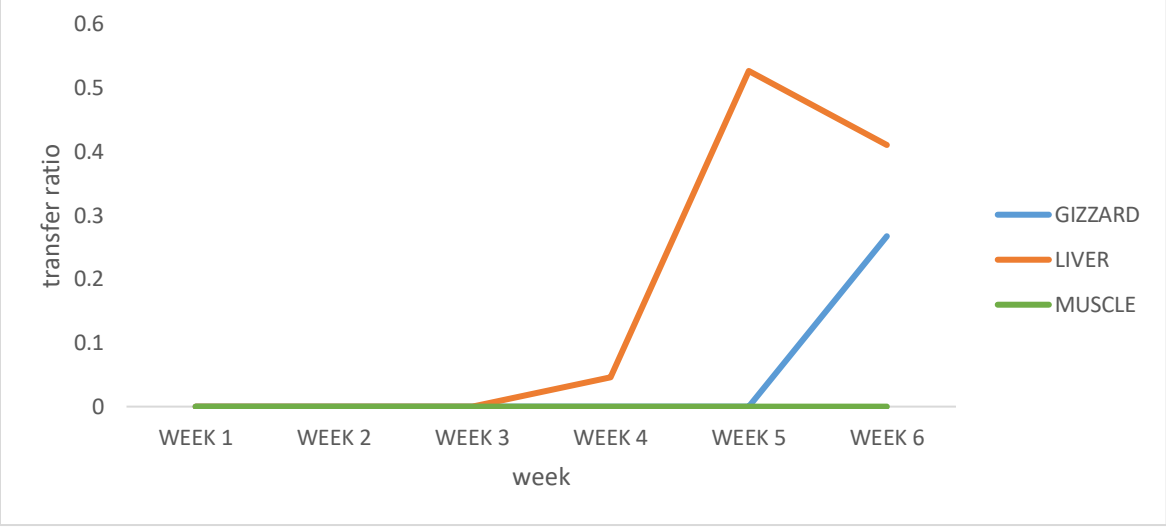

Figure 4.16: AFG2 carry over ratio trend per week per meat part

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 4.30 below.

Table 4.30: 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 carry over ratio 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 4.17. 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.

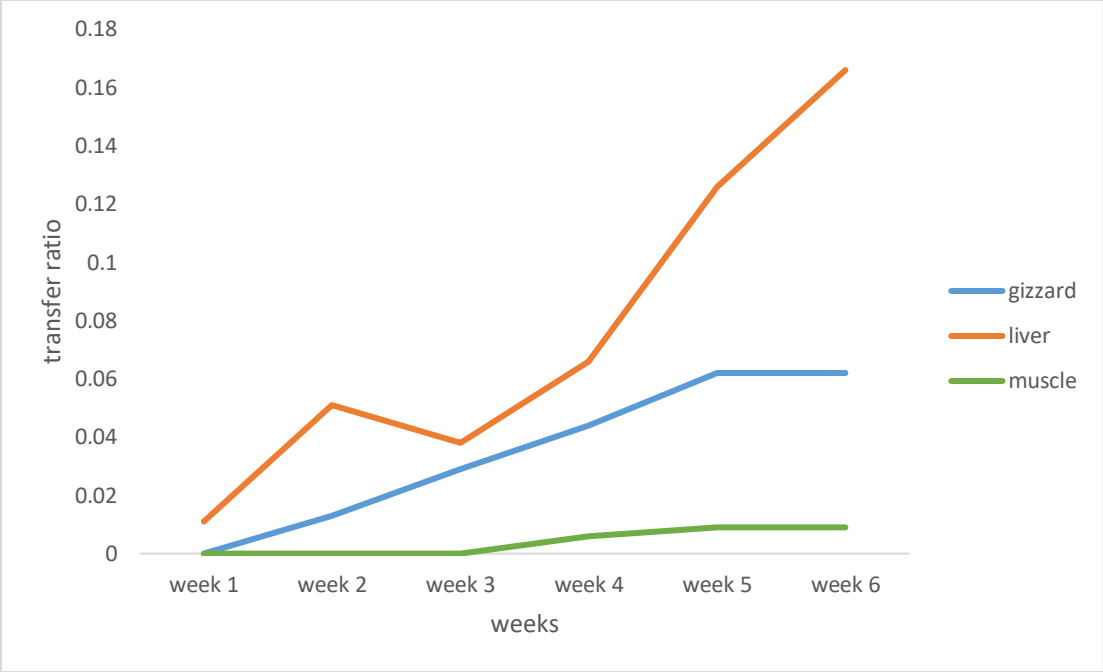

Figure 4. 17: Total Aflatoxin transfer ratio trend per week per meat part

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This section entails discussion on the findings of this study based on the study objectives.

5.1.1 Knowledge on Aflatoxin by farmers

Studies have reported that farmers' in developing countries know little about aflatoxin (Unneverhr, 2013; Waliyah *et al*., 2008) however in the present study, the proportion of farmers who had knowledge on aflatoxin was relatively high. The proportion of farmers who had knowledge on aflatoxin in the current study was (58.2%), this was slightly higher than the value reported by Nakavuma et al (52.9%) (Nakavuma *et al.,* 2020). Makau et al (Makau *et al.,* 2016) reported 38.5% of farmers had knowledge on aflatoxin while Marechera and Ndwiga (2014) reported (92.5%) of farmers had knowledge on aflatoxin in the lower eastern part of Kenya (Marechera and Ndwiga, 2014). This high level in Eastern Kenya was because the region has suffered numerous aflatoxin epidemics in humans in earlier years and the area is an aflatoxin endemic region and also numerous studies have been conducted in the area and farmer awareness has been increased due to numerous seminars and workshops conducted in the area. Studies have reported that the knowledge of aflatoxins and the other mycotoxins vary with several socio demographic characteristics (Kiama *et al*., 2016). In Ethiopia, farmers were found to be less knowledgable compared to persons in other proffessions (Ephrem *et al*., 2014). Most respondents interviewed in the present study had attained secondary level of education with only a few who had attained tertiary education level. However, this was inconsistent with study by Nyangaga whereby the larger proportion of the farmers had attained tertiary level of education (Nyangaga, 2014). Studies from various countries have reported that the level of education has an

impact on aflatoxin awareness. In Tanzania, studies have revealed that education level has a positive effect on knowledge of aflatoxin (Ngoma *et al*., 2017; Magembe *et al*., 2017).

5.1.1.1 Channels through which farmers heard about aflatoxins

The findings of the present study on the proportion of farmers who had heard about aflatoxin was (94.5%), this was higher than the findings from Marechera and Ndwiga who reported (93%) (Marechera and Ndwiga, 2014) and Kamala et al who reported (20%) (Kamala *et al.,* 2016). This high percentage from the present study is due to the fact that aflatoxin is well known to a majority of the population. *'We know about Aflatoxins since we have heard about it through various media channels incuding social media where cases of maize and flour contamination have been reported'* this was from FGD. Likewise, majority of farmers from the current study had heard about aflatoxin between 1-6 months this indicates that there has been an increase in the awareness of aflatoxin over time in the study area; this agrees with a study done by Ayo et al (Ayo *et al.,* 2018) where he reported similar findings. Furthermore, most of the farmers heard about aflatoxin through mass media this suggests that mass media is the best channel to convey information regarding aflatoxin to farmers and the general population. On the contrary, the percentage of farmers who heard about aflatoxin through reading was the least and this suggests that there could be inadequacy of written materials on aflatoxins, low reading drive by farmers, or the materials are too advanced for the farmers. This was in agreement with the findings reported by Ayo et al (Ayo *et al.,* 2018).

5.1.1.2 Knowledge of farmers on contamination of feeds with aflatoxin

The current study reported that a higher proportion of the farmers were knowledgeable on the occurrence of fungal toxins in feed this was consistent with the findings reported by Ayo et al in Tanzania (Ayo *et al.,* 2018). *'We know about the various ways in which animal*

feed can be contaminated with Aflatoxin and poor storage is a key factor,' this was reported from FGD. However, this was inconsistent from studies from various settings that reported that farmers have low awareness on the concept of aflatoxins (Loreen & Moses, 2015; Gizachew *et al.,* 2015; Kamala *et al.,* 2016). Most farmers from the present study had knowledge on the possibility of fungal toxins in feed affecting poultry/ animal health and experimental results from various studies support this phenomenon (Grace, 2013; Sohooa *et al.,* 2015). Acute levels (high) of toxins are lethal within a short period of time while chronic levels (low) lead to death after a relatively long period of time causing immunosuppression, increase in susceptibility and opportunistic diseases. Fungal toxin adulteration of feeds is also linked to animal death due to impaired health and consequently leads to low production performance (Grace *et al.,* 2015).

Additionally, majority of the farmers from the present study reported that there is likelihood of fungal toxins to be transferred (carried over) from feed to the tissues of poultry / animals, however this disagrees with a study done in Tanzania where majority of the farmers reported that the transfer of toxins from feed to poultry/animal is not possible (Ayo *et al.,* 2018). The results of the study in Tanzania were consistent with a report by Kiama et al (Kiama *et al.,* 2016) on the perception of dairy farmers in Kenya, which revelaed that consumption of mouldy food by humans is unsafe but consuming products from animals fed on mouldy feeds is harmless. Other reports by Grace and Okoth disagrees with this perception (Grace, 2015; Okoth, 2016). Their findings proved that fungal toxins ingested in feeds by animals are assimilated into body tissues and transferred into foods of animal origin and are also toxic to human consumers.

The findings of this study illustrate that farmers were knowledgable on the signs of feed contamination with fungal toxins and are able to identify various signs that indicate

contamination such as abnormal consistency, bad odor, presence of insects/larvae and impaired animal health/ death. These indicators and signs were also found in an on-farm study by Golob on approaches to control mould and fungal toxin development in feeds (Golob, 2007). Golob reported that these suggestive signs are instrumental in identifying mouldy feeds that are suspected to be contaminated with aflatoxins. Nonetheless, it is important to note that lack of these signs does not warrant that the feeds are safe. Numerous studies have reported that it is impossible to have fungal toxin free feeds under normal conditions. Results from these studies have shown that feed discoloration and off-smell are useful indicative factors to suspect feed adulteration and probably presence of aflatoxins and other fungal toxins. Furthermore, majority of the farmers from the current study reported that they did not know any indicator to suggest presence of these toxins in feeds. Studies have reported that the lack of ability to suspect and identify feed degeneration and adulteration using fast rapid tests, could lead to exposure to aflatoxin adulteration of feeds thus posing a risk to human consumers (Golob, 2007).

5.1.1.3 Farmers' ability to detect feed contamination and action taken with feeds found contaminated with fungal toxins

A high proportion of the respondents from the present study reported that they were able to detect presence of molds in feed this agrees with the findings by Ayo et al in Tanzania who that reported similar findings (Ayo *et al.,* 2018). These results are in agreement with an on farm study by Golob (Golob, 2007) where he reported that although fungal toxins in feeds are not visible, moulds growing on feeds are visible. The farmers stated that moulds often change colour and have an effect on the appearance of the feed on which they are developing (Golob, 2007).

Findings from the present study show that most of the farmers will continue feeding their poultry/animals with feed found to be contaminated with fungal toxins. *'Broiler feeds are very expensive and the consumption rate also is high hence due to this fact and also because of the lack of knowledge on the action taken with feeds found to be contaminated with aflatoxins, we will continue feeding the broilers on these feeds as we fear making losses'* this was reported from FGD. This was contrary to the finding by Golob where he reported that feeds contaminated with moulds produce an unpleasant/off smell. It is clear that the manifestation of mould in feeds is a good indicator of the likelihood of contamination of feed with fungal toxins which is instrumental in aiding the farmer to make decisions on disposing the feeds (Golob, 2007). It is therefore clear that from the current study, farmers were unaware on the action taken with contaminated feed. An inspection of broiler feed through observation in the current study revealed that a higher proportion of the farmers had feeds that were in good condition that is non moldy and loose. This implies that majority of the farmers were keen on good feed storage practices.

5.1.1.4 Farmers' knowledge on detoxification of contaminated feed

Majority of farmers from the present study had no knowledge on the possibility of detoxifying fungal toxins in feed while fewer respondents had knowledge on the possibillity of detoxifying feed found aldurated with aflatoxins. Those who knew further stated the following methods can be used; boiling, sun drying, grinding, good storage, heating and sieving. Studies have shown that farmers lack knowledge and options for disposal of adulterated cereal at the household level and ultimately it is fed to domestic animals (Kiama *et al.,* 2016). It is clear that farmers in the present study are not knowledgable on the methods of detoxifying aflatoxins in feed and this was also evident from FGD; *'We have never heard about the use toxin binders and we do not know what*

they are and what they do'.The findings of the current study were inconsistent with the study done by Ayo et al where the study reported that, the proportion of farmers who had knowledge on the possibility of detoxifying fungal toxins in feed were higher than those who did not have the knowledge (Ayo *et al.,* 2018). The methods that the farmers from the study by Ayo et al reported to use in detoxifying feeds suspected to be contaminated with fungal toxins are use of soda-ash, plant ashes, charcoal, salt, and some herbs. Ashes have been used in treating animal feeds for other uses such as decreasing ant nutritional factors in monogastric animals and fibre digestibility improvement (Kyarisiima et al., 2004; Laswai *et al.,* 2007). Some compounds in form of antioxidants from plants sources have counteractive effect against the oxidative stress induced by aflatoxin in animal body after absorption (Abdulmajeed, 2011).

It is therefore evident that the knowledge on the methods of detoxifying aflatoxin in adulterated feeds is lacking and much needs to be done to bridge this gap as contaminated animal feeds are a major source of exposure to the human consumers (Ráduly *et al.,* 2020).

5.1.1.5 Farmers' knowledge on feed management practices

Most farmers from the present study had knowledge on mycotoxin prevention strategies for instance good storage practices as this was evident in the following areas; majority of the famers stored their feeds in well ventilated stores and majority of them placed the poultry feed on raised ground. *'We know about the various methods that are used to prevent aflatoxin occurrence in feeds and placing the feeds on raised ground and storing feeds in well ventilated stores are some of the methods used'* this was from FGD. Studies have shown that improper storage practices for instance stack piling of feeds and storing feeds on bare floor and other poor bulk management practices of feeds, including extended time in storage, predisposes feeds to adulteration with aflatoxin forming fungi (Cheat *et al*.,

2016; Makau *et al.,* 2016). In the present study most of the farmers bought their feeds weekly hence most feeds do not stay for long in storage.

5.1.2 Detection, Quantification and Characterization of aflatoxin levels in feed

Feed adulteration with mycotoxins due to growth of moulds is a challenge to farmers globally (Moretti *et al.,* 2017). Aflatoxins are not prevalent at the pre-harvest stage as other mycotoxins this is because aflatoxins are regarded as storage moulds (Afolabi *et al.,* 2019; Leggieri *et al.,* 2020). Aflatoxin adulteration in the animal feed chain is not given much attention in developing countries yet it contributes to exposure of human consumers to adulterated products (Akande *et al.,* 2006; Ráduly *et al.,* 2020).

Besides AFB1, other AFs, including AFB2, AFG1, AFG2, and aflatoxin M1 (AFM1), have also been detected in poultry feeds and feed ingredients (Akinmusine *et al.,* 2018; Kemboi *et al.,* 2020; Mokubedi *et al.,* 2019; Rodrigues *et al.,* 2011). The presence of AFM1 in feeds and feed ingredients in developing countries has been associated with the production of traces of AFM1 by most strains of aflatoxigenic *Aspergilli spp* (Ezekiel *et al.,* 2012) although in the current study AFM1 levels were not detected in feed.

Worldwide, different studies have reported varying levels of aflatoxin in feed. A study by Nemati et al (Nemati *et al.,* 2010) from Northwestern region of Iran, reported the average level of AF adulteration in broiler feed at (11.6 µg/kg) in a different study done by Ifie et al in Nigeria found AF levels of (21 µg/kg) in broiler finisher (Ifie *et al.,* 2022). This was inconsistent with the findings of the current study where AFB1 levels of broiler finisher was (17.17 ppb). AF levels in feed in the current study were slightly higher than the levels from Guyana, where the average level of AF in poultry feeds was between 3.81 to 27.38 µg/kg (Morrison *et al.,* 2017). Higher levels of (24.–185.25 µg/kg) of AF were also reported in various types of chicken feed from large-scale and small-scale manufacturers

in Uganda (Nakavuma *et al.*, 2020). Aboagye-Nuamah et al (Aboagye-Nuamah *et al*., 2021) also found higher AF levels of between (11.83–88.37 µg/kg) in poultry feed samples from Ghana compared to the findings of the current study. Differences in the levels of AF can be ascribed to the variations in geographic location, weather, farming and storage practices. Prevention of Aflatoxin in feed ingredients can be done by embracing good farm management practices like the use of drought resistant crops; timely harvesting before physiological maturity; drying to moisture content of 13%; and proper storage (Xu *et al.*, 2021). A study carried out in Kenya on aflatoxin levels in commercial poultry feed found that all the poultry feed samples were adulterated with AFs, ninety-five percent (95%) of the samples exceeded 10 ppb and while 35% exceeded 100 ppb and AFs levels ranged from 5.13 -1123 ppb (Okoth and Kola, 2012). In a study by Mahbuba et al where he studied aflatoxin levels in broiler starter and broiler finisher, he found that broiler finisher had lower levels compared to broiler starter (Mahbuba *et al.*, 2018) however in the present study broiler finisher had higher levels compared to broiler starter. The quality of finished feed largely depends on the quality of raw feed ingredients. Adulterated, low quality raw feed ingredients eventually leads to low graded finished feed which is toxic to both poultry and human consumers. Beg et al reported low levels of AFs in broiler starter feed and broiler finisher feed with broiler starter levels at 0.48 ppb level (range 0 to 3.26 ppb) and broiler finisher at 0.39 ppb level (range 0 to 1.05 ppb) (Beg *et al.*, 2006), this disagrees with the findings of the current study. In the present study AFB1, AFB2, AFG1 and AFG2 were detected in all the feed samples and this is similar to study by Mgbeahuruike in Nigeria where all these analaogues were present in broiler feed but at different levels (Mgbeahuruike, 2016). In a study in Nakuru Kenya, the total aflatoxin mean level for the broiler starter and broiler finisher feed samples were 19.37 ± 2.45 and

19.86 ± 2.21 µg/kg respectively (Thuita *et al.,* 2019) these levels were lower than those of the present study where the total aflatoxin levels for broiler starter and broiler finisher were 29.47±6.13 and 30.1±6.88 respectively. In a different study by Muhammad et al, the mean total aflatoxin levels in broiler finisher and broiler starter was (50.38 ppb) and (49.52 ppb) respectively (Muhammad *et al.,* 2022) these were higher than the levels obtained from the present study.

5.1.2.1 Detection, Quantification and Characterization of Aflatoxin levels in water

The occurrence of mycotoxins in food has been researched extensively as well as its effects in human health and yet, data about its distribution in the environment is limited (Mata *et al.,* 2014). Different fungi that are able to produce mycotoxins have been detected in water. The methods applied in the analysis of mycotoxins in water samples must be specific and sensitive as these toxins are found in trace levels and have varied physicochemical characteristics and an extensive range of polarities. A study by Mata et al on aflatoxin concentration in bottled water, found that aflatoxin B2 was the most frequently detected mycotoxin present in 11 of the analysed samples with a maximum level of 0.48±0.05 ppb followed by aflatoxin B1 and aflatoxin G1 with maximum concentrations of 0.70±0.06 ppb and 0.60±0.02 ppb respectively, these were lower than findings of the present study. Mycotoxin levels detected in the samples were all below the European maximum limits for mycotoxins in foodstuffs (Mata *et al.,* 2014).

In tap water, due to treatment with chlorine, some mycotoxins are unstable and are not easily detectable (Mata *et al.,* 2014). Existing AF standards show limits in food but no limits are currently applied in water. Therefore, the results on water in the present study are measured against the limits applied for food.

A study by Picardo et al on aflatoxins in freshwater, found the following levels of AF; aflatoxin B2 (AFLB2) was the highest detected AF with a maximum level of 0.48 ng/L followed by aflatoxin B1 (AFL B1) and aflatoxin G1 (AFL G1 (Picardo *et al.,* 2018). A huge gap exists with studies on aflatoxin in water and more studies need to be carried out to bridge this gap. The results from the present study show that Total Aflatoxin levels were the highest (0.23ppb) followed by AFB1 (0.1ppb) followed by AFB2 (0.07), followed by AFG1 (0.06ppb) and the least was AFG2 (0.004ppb). The results of the present study are inconsistent with the findings by Mata et al and Picardo et al where in both studies AFB2 levels were the highest. In the present study Total aflatoxin levels were the highest. On the other hand, the findings of the current study were consistent with both of these studies in that the aflatoxin levels were below the recommended limits. The presence of aflatoxin in water in the current study was attributed to the contamination of the water by the broilers during feeding.

5.1.3 Detection, Quantification and Characterization of aflatoxin levels in meat

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 (Adegbeye *et al.,* 2020; Li *et al.,* 2021). 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 (Meerpoel *et al.,* 2020; Emmanuel *et al.,* 2020), chronic exposure to these low levels has a significant impact on the health of human consumers (Sineque *et al.,* 2017; Tardieu *et al.,* 2021). To add on AFB1 is the most toxic mycotoxin found in meat.

A study by Sahib et al 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 (Sahib *et al.,* 2020) this is consistent 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 (Begum *et al.,* 2001: Bintyihok *et al.,* 2002; Bintyihok *et al.,* 2006: Arulmozhi *et al.,* 2002) this is because the liver is the principal target organ of Aflatoxins.

Additionally, from the same study by Sahib et al 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 (Sahib *et al.,* 2020). The findings from the study by Sahib et al support previous findings that AFM1 accumulated more in the liver than in muscles (Begum *et al.,* 2001: Bintyihok *et al.,* 2002; Bintyihok *et al.,* 2006: Arulmozhi *et al.,* 2002) however in the present study, AFM1 was not detected in the muscle this was attributed to the absence of AFM1 in feed. Aflatoxin M1 is formed from AFB1 during its metabolism. Previous studies from animal models revealed that AFM1 has hepatotoxic and carcinogenic properties (Anfossi *et al.,* 2008). 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 (Zhang *et al.,* 2015).

Studies have shown that elevated levels of AFB1 in feed samples lead to high levels of AFB1 in liver and muscle respectively (Faten *et al.,* 2016; Eleftheriadou *et al.,* 2004; Begum *et al.,* 2001; Saqer, 2013); Zahid *et al.,* 2010). Sineque et al reported that tissue residues of aflatoxin were higher in the liver than in the gizzard (Sineque *et al.,* 2017). This

is consistent with the present study whereby AF levels were higher in the liver compared to the gizzard.

Iqbal et al (Iqbal *et al.,* 2014) 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 (El-Desouky *et al.,* 2014) 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) (Emmanuel *et al.*, 2020). 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 respectvely (Olatoye *et al.,* 2020) 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 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 (Faten *et al.,* 2016) 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 Resanović (Resanović, 2000) and Saeed et al (Saeed *et al*., 2003) 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 (Herzallah, 2013) and Darwish et al (Darwish *et al*., 2016) 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 agrees with the results of the current study.

5.1.4 Carry over effect of aflatoxin from broiler feed into broiler meat

Mycotoxin contamination of cereals and feed has been reported worldwide (Binder *et al.,* 2007). Studies have demonstrated that the occurrence of mycotoxins in food of animal origin has been associated with the contamination of animal feed (Pleadin *et al.,* 2021; Pleadin *et al.,* 2013; Persi *et al.,* 2014) this results in the transfer of the toxins into animal products. Carry over ratios/transfer ratios signifies a way to demonstrate the bio-accumulation capability of toxins into specific tissues (Amutova *et al.,* 2020).

Studies on carry over in products of animal origin are limited as only the fundamentals of mycotoxin activity have been reported (Pleadin *et al.,* 2021). Additionally, standardised parameters for the calculation of carry-over ratios are non existent and trials are unmatched due to the diverse study designs employed (Volkel *et al.,* 2011).

Carry over ratios or transfer ratios differ for instance in the muscles, the values are below 0.01 (1%) (Pleadin *et al.,* 2021), 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 (Pleadin *et al.,* 2021)

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 repectively. Upon intake by the host organisn (human or animal), these toxins enter the blood stream where they can be found in detectable levels. Völkel et al (Volkel *et al.*, 2011) 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 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 (Adegbeye *et al.,* 2021).

5.2 CONCLUSION

This section entails conclusion of the study based on specific objectives of the study.

5.2.1 Knowledge on Aflatoxin by farmers

The study concludes that there was a significant association ($p<0.05$) between sociodemographic characteristics of farmers and knowledge on Aflatoxin.

5.2.2 Detection, Quantification and Charaterization of aflatoxin levels in feed

The study concludes that; the levels from analysis of feed and water samples showed that higher Aflatoxin levels were found in feed than in water hence higher transfer of Aflatoxin was attributed to feed as aflatoxin levels in water were found in trace levels.

5.2.3 Detection, Quantification and Charaterization of aflatoxin levels in broiler meat

The study concludes that it is safer to eat the muscle as levels are below the MRLs. Levels of Aflatoxins in all the meat parts analyzed were highest in week 6 and Aflatoxin levels increased weekly due to bio accumulation.

5.2.4 Carry-over effect of aflatoxin from broiler feed to broiler meat

The study concludes that the highest carry over ratio was observed in the liver followed by the gizzard and the least was in the muscle.

5.3 RECOMMENDATIONS

5.3.1 Knowledge on Aflatoxin by Farmers

Based on the study findings although majority of the farmers had knowledge on aflatoxins, results from FGD show that there is need for continous awareness particularly on feed management. Therefore, it is recommended that;

- There is need for continous sensitization of farmers on aflatoxins particularly in feed management through extension services by the Ministry of Agriculture and Ministry of Health Division of Public Health, to safeguard public from exposure to aflatoxin contamination through meat.

5.3.2 Detection, Quantification and Characterization of aflatoxin levels in feed

Based on the study findings, aflatoxin levels in feed for both broiler starter and broiler finisher were above KEBS limit. It is recommended that;

- There is need for continous surveillance and monitoring of aflatoxin levels in feed and feed ingredients through various laboratory and rapid detection techniques by the national and county government and regulatory bodies (KEBS) and to extend the capacity of aflatoxin testing of feed to farmers.

5.3.3 Detection, Quantification and Characterization of aflatoxin levels in meat

Based on the study findings, aflatoxin levels in the liver and gizzard were above the EU and WHO/FAO limit in week 4, 5 and 6. It is recommended that;

- 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 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.

5.3.4 Recommendation for objective 4 on carry-over of aflatoxin from broiler feed into broiler meat

Based on the study findings, the highest carry over ratio 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. It is recommended that;

- There is need for constant 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.

5.4 Areas of further research

More studies on the carry over effect of aflatoxin in broiler meat 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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APPENDICES

Appendix 1: Consent Form

My name is Ruth Kirinyet I am a PhD student from Kenyatta University. I am conducting a study on 'Quantification, Characterization and Carry-over effect in broilers raised in Nairobi City County, Kenya.

Procedures to be followed

Participation in this study is voluntary and will require that I ask you some questions concerning aflatoxin in broilers. I will record the information from you in a questionnaire or an interview guide. You may ask questions related to the study at any time. You may refuse to respond to any questions and you may stop an interview at any time.

Risks

There are no risks whatsoever for participating in this study.

Benefits

If you participate in this study you will help us understand the gaps and what needs to be done to address the issue of aflatoxin since it is a Public Health concern and also the Ministry of Agriculture and Health.

Rewards

The study has no direct/immediate rewards such as payment

Confidentiality

All the information gathered from the interviews will be treated with the uttermost confidentiality that it deserves and your name will not appear anywhere in the questionnaire. The questionnaire will also be kept safe under lock and key and privacy will be maintained.

Participant's statement

The above information on my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study will be entirely voluntary. I understand that my records will be kept private and that I can stop participation at any time.

Name of the participant ……

---------- ----------

Signature Date

Investigators statement

I, the undersigned, have explained to the volunteer in a language s/he understands the procedures to be followed in the study and the benefits involved.

Name of the interviewer: _____

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Interviewer signature/ Thumb print Date

Contact information

For any questions or clarifications, you can contact the following:

THE CHAIRPERSON

Kenyatta University Ethical Review Committee on chairman.kuerc@ku.ac.ke

Dr. P. Warutere on 0721993833 or warutere.peterson@ku.ac.ke

Dr. P. Nguhiu on 0722737711 or nguhiu.purity@ku.ac.ke

Dr. P. Ojola on 0722939090 or odeny.patroba@ku.ac.ke

Appendix 2a: Questionnaire

Question	Socio demographic characteristics	
1.	Age	
2.	Sex	Male () Female ( )
3.	Marital status	Married () Divorced () Single ( )
4.	Completed level of education	Primary () Secondary () Tertiary () None ( )
5.	Period of rearing poultry	
	Awareness on fungal toxins contamination in feeds	
6.	Is there a possibility of fungal toxins to be found in feeds?	Yes ( ) No ( ) Don't know ( )
7.	Is there a possibility that the fungal toxins in feeds can affect poultry's health?	Yes ( ) No ( ) Don't know ( )
8.	Is there a possibility that fungal toxins can be transferred from feed to poultry?	Yes ( ) No ( ) Don't know ( )
9.	What are the signs to suspect presence of fungal toxins in feed? **(Multiple response)**	Abnormal color Yes ( ) No ( ) Don't know ( ) Abnormal consistence Yes ( ) No ( ) Don't know ( ) Bad odor (rotten/soil smell) Yes ( ) No ( )

		Don't know ( ) Insect/larva presence Yes ( ) No ( ) Don't know ( ) Impaired animal health /deaths Yes ( ) No ( ) Don't know ( )
10.	Are you able to detect molds in feed?	Yes ( ) No ( ) Don't know ( )
11.	What do you do to feeds that are found to be contaminated with fungal toxins i.e. molds?	Continue feeding ( ) Dispose ( ) Look for alternative use ( )
12.	Is there a possibility of detoxifying aflatoxins in feed?	Yes ( ) No ( ) Don't know ( )
13.	If Yes in question …13 What is the method	1 2 Don't know ( )
14.	Have you ever heard about aflatoxins?	Yes ( ) No ( ) Don't know ( )
15.	How did you get to hear about aflatoxins? If yes	Reading ( ) Mass media ( ) (TV/radio) Seminars ( ) Friends/neighbors ( )
16.	How long ago did you get to hear about aflatoxins?	
17.	Where do you store the poultry feed?-	Store ventilated ( ) (ask to see) In the open ( ) (ask to see storage area)

18.	Where do you place the poultry feed during storage? **Ask to see**	On the floor ( ) Raised ground ( )
19.	How frequently do you buy chicken feeds	Daily ( ) Weekly ( ) Fortnightly ( ) Monthly ( )
20.	Do you feed your broilers with any other feed other than the commercially processed feed?	Yes ( ) (If yes which one? No ( ) Don't know ( )
21.	Ask to see the state of the broiler feed and record the condition	Moldy and loose ( ) Moldy and Compact ( ) Non moldy and loose ( )

Appendix 2b: Focus Group Discussion Guide for farmers

1. Have you heard about aflatoxins and in your view what are they?
2. How does feed/food get contaminated with aflatoxin?
3. Once you realise that feed is contaminated with aflatoxin/fingal toxin, what action do you take?
4. What are some of the ways you can prevent aflatoxin in feed?
5. What are the signs that show that broiler has been infected with aflatoxin?
6. How should feed be stored to prevent fungal growth/aflatoxin contamination?

Appendix 3: Consent form for longitudinal study

TITLE: QUANTIFICATION, CHARACTERIZATION AND CARRY OVER EFFECT OF AFLATOXIN IN BROILER CHICKEN RAISED IN NAIROBI CITY COUNTY, KENYA

I…… do hereby consent/do not consent that my farm will be used in the said study to collect samples needed in the said study.

Ms Ruth Kirinyet has explained to me clearly and in detail the requirements of the study. I do understand that participation in this study is voluntary and I can choose to drop out of the study at any time without any victimization. There are no risks involved whatsoever in participating in the study. Each time the samples are collected, I will be compensated (payment) until the study period is completed. I am informed that the findings of the samples collected after analysis will be kept confidential.

Farm owner signature Date

…… ……

I the undersigned, have explained the relevant details of this study to the farm owner in a language that s/he understands and the procedures to be followed and the benefits involved.

Name of researcher ……

Signature ……

Date…….

Appendix 4: Ethical Approval by Kenyatta University Ethical Review Committee

KENYATTA UNIVERSITY
DIRECTORATE OF ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

P. O. Box 43844,

Website: www.ku.ac.ke
Our Ref: KU/ERC/APPROVAL/VOL.1

Tel: 8710901/12

Date: 24th November, 2020

Ruth Chepkosgei Kirinyet
P.0 Box 43844-00100
NAIROBI

Dear Ms. Kirinyet,

RE: APPLICATION NUMBER: PKU/2163/II307 AFLATOXIN CHARACTERIZATION AND CARRY OVER FROM BROILER FEED INTO BROILER MEAT IN CHICKEN REARED IN NAIROBI CITY COUNTY, KENYA

This is to inform you that ***KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE*** has approved version 4 of the study protocol together with the attached consent forms dated 12.09.2020. Your application approval number is **PKU/2163/II307**. The approval period is 24th ***November, 2020 TO 24th November, 2021.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY DIRCTORATE OF ETHICS REVIEW COMMITTEE***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITYDIRECTORATE OF ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be

Appendix 4: Continued

reported to ***KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE*** within 72 hours

v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN ETHICS REVIEW COMMITTEE P.O. BOX 43844 - 00100 NAIROBI

24 NOV 2020

Prof. Judith Kimiywe

DIRECTOR- KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.

Appendix 5: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **377490**

Date of Issue: **09/December/2020**

RESEARCH LICENSE

This is to Certify that Ms.. Ruth Chepkosgei kirinyet of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: AFFLATOXIN CHARACTERIZATION AND CARRY OVER FROM BROILER FEED INTO BROILER MEAT IN CHICKEN REARED IN NAIROBI CITY COUNTY, KENYA. for the period ending : 09/December/2021.

License No: **NACOSTI/P/20/8037**

377490

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix 5: Continued

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is Guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014

CONDITIONS

1. The License is valid for the proposed research, location and specified period
2. The License any rights thereunder are non-transferable
3. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research
4. Excavation, filming and collection of specimens are subject to further necessary clearence from relevant Government Agencies
5. The License does not give authority to tranfer research materials
6. NACOSTI may monitor and evaluate the licensed research project
7. The Licensee shall submit one hard copy and upload a soft copy of their final report (thesis) within one year of completion of the research
8. NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice

National Commission for Science, Technology and Innovation
off Waiyaki Way, Upper Kabete,
P. O. Box 30623, 00100 Nairobi, KENYA
Land line: 020 4007000, 020 2241349, 020 3310571, 020 8001077
Mobile: 0713 788 787 / 0735 404 245
E-mail: dg@nacosti.go.ke / registry@nacosti.go.ke
Website: www.nacosti.go.ke

Appendix 6: Kenyatta University Graduate School Approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School **DATE:** 27th August, 2020

TO: Ms. Ruth C. Kirinyet
C/o Department of Environmental & Occupational Health

REF: Q97/38209/2017

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 1st July, 2020, approved your Research Proposal for the Ph.D. Degree entitled, **"Aflatoxin Characterization and Carry Over from Broiler Feed into Broiler Meat in Chicken Reared in Nairobi City County, Kenya."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D studies.

Thank you.

EDWIN OBUNGU
FOR: DEAN, GRADUATE SCHOOL

CC. Registrar (Academic) Att. Mr. Richard Chweya

Chairman, Environmental & Occupational Health Department
Supervisors:

1. Dr. Peterson Warutere
   C/o Environmental & Occupational Health Department
   Kenyatta University
2. Dr. Purity Nguliu
   C/o Animal Science Department
   Kenyatta University
3. Dr. Patrick Ojola
   Biochemistry, Microbiology & Biotechnology Dept.
   Kenyatta University

Appendix 7: Authorization for Reasearch from CDVS Nairobi City County

NAIROBI CITY COUNTY

Telephone: 020 3536843
Fax 020 3523948

County Director of Veterinary Services,
Nyayo House (14th floor)
P. O. Box 40851-00100
NAIROBI. KENYA

24th February, 2021.

AGRICULTURE, LIVESTOCK AND FISHERIES

Ref: NBI/EDUC/VOL.I/18/175

Ruth C. Kirinyet.
Kenyatta University.

RE: AUTHORITY TO CARRY OUT RESEARCH WORK IN NAIROBI COUNTY.

Your letter dated 24/02/2021 refers. The department of Veterinary Services has accepted your request to conduct research on Aflatoxin Characterisation in Broilers in Nairobi as per the research licence from NACOSTI Ref: No. 377490 dated 09/12/2020. This authority allows you to do your work within four (4) months from the date of this letter.

The result of your work is expected to be shared with the department afterwards.

COUNTY DIRECTOR OF VETERINARY SERVICES
P. O. BOX 40851 - 00100
NAIROBI

Dr. Kabatha J.M.M.
FOR: COUNTY DIRECTOR OF VETERINARY SERVICES
NAIROBI CITY COUNTY

Appendix 8: Aflatoxin Standards

Standard	Type of sample	Aflatoxin B1	Total Aflatoxin
EU	FEED	20ppb	-
	MEAT	2ppb	4ppb
US/FDA	FEED	20ppb	-
	MEAT	-	-
WHO/FAO	FEED		
	MEAT	5ppb	10ppb
EAC	FEED	20ppb	50ppb
	MEAT	-	-
KEBS	FEED	10ppb	20ppb
	MEAT	-	-

Appendix 9a: Calibration standards for meat samples and LOD and LOQ for meat samples

AFLATOXIN B1	
Cal Stds levels(ug/kg)	Abundance
0	0
10	10000
25	30000
50	50000
75	70000
100	90000

AFLATOXIN B2	
Cal. Stds (ug/kg)	Abundance
0	0
10	10000
25	30000
50	50000
75	70000

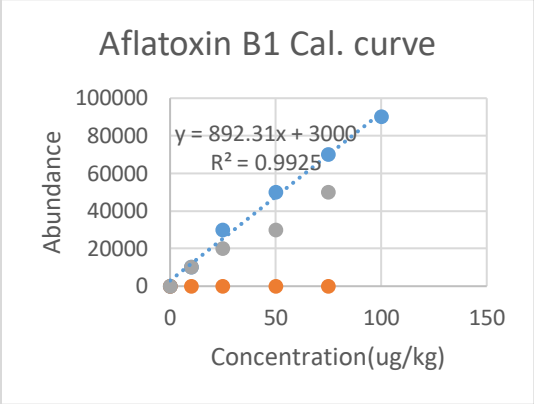
AFLATOXIN G1	
Cal. Stds(ug/kg)	Abundance
0	0
10	10000
30	30000
50	50000
80	70000

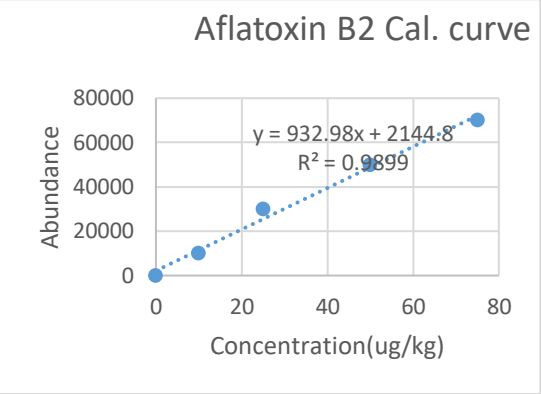
AFLATOXIN M1	
Cal. Stds(ug/kg)	Abundance
0	0
5	10000
10	20000
20	30000
30	50000

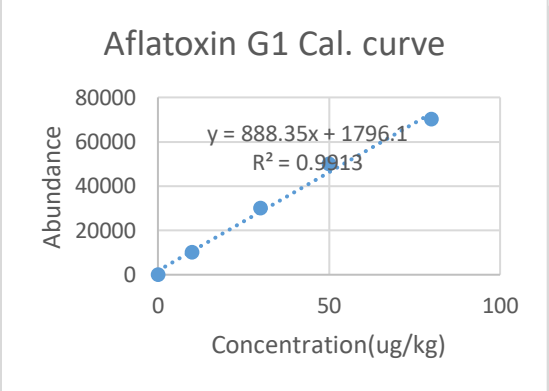
AFLATOXIN G2	
Cal stds levels(ug/kg)	Abundance
0	0
5	5000
15	10000
30	30000
50	50000
75	70000

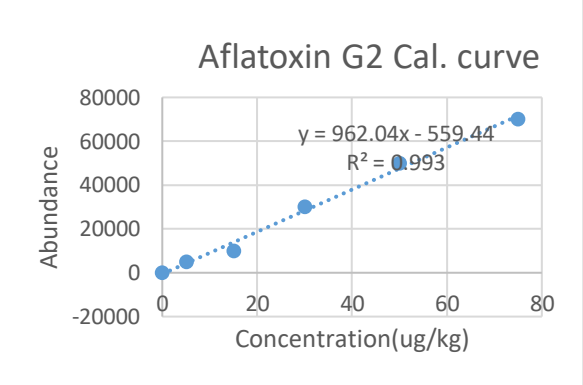
Linearity, LODs and LOQs of the five Mycotoxins			
Mycotoxins	R2	LOD(ug/kg)	LOQ(ug/kg)
Aflatoxin B1	0.9925	0.09	0.2
Aflatoxin B2	0.9899	0.15	0.23
Aflatoxin G1	0.9913	0.09	0.2
Aflatoxin G2	0.993	0.15	0.23
Aflatoxin M1	0.986	0.02	0.1

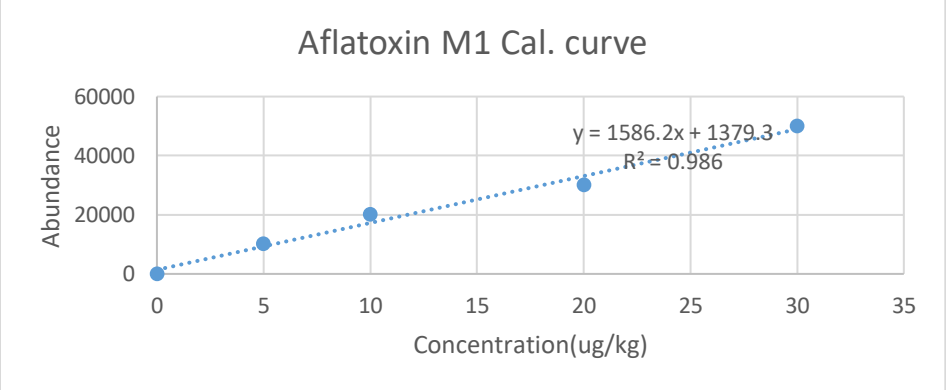
Appendix 9b: Calibration curves for meat samples











Appendix 10a: Calibration standards for feed samples and LOD and LOQ for feed samples

AFLATOXIN B1	
Cal Stds levels(ug/kg)	Abundance
0	0
5	20000
15	40000
25	60000
50	100000
100	200000

AFLATOXIN B2	
Cal. Stds (ug/kg)	Abundance
0	0
5	10000
10	20000
25	50000
35	70000
50	90000

AFLATOXIN G1	
Cal. Stds(ug/kg)	Abundance
0	0
5	20000
15	40000
25	60000
50	100000
100	200000

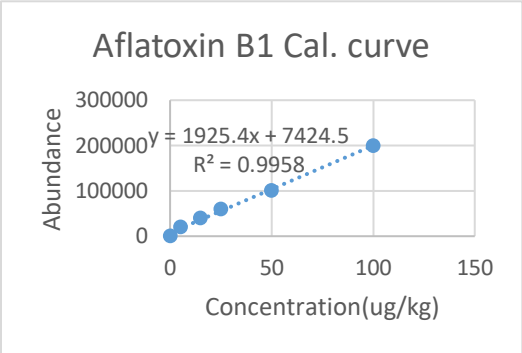
AFLATOXIN G2	
Cal stds levels(ug/kg)	Abundance
0	0
10	10000
25	30000
50	50000
75	70000
100	90000

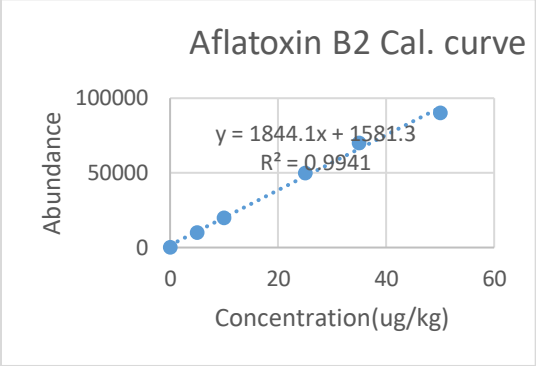
AFLATOXIN M1	
Cal. Stds(ug/kg)	Abundance
0	0
15	10000
30	20000
50	30000
70	50000
100	70000

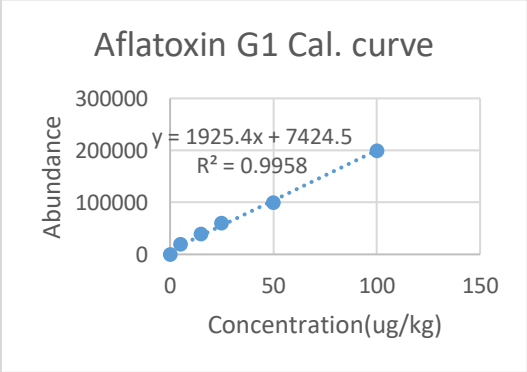
Linearity, LODs and LOQs of the five Mycotoxins

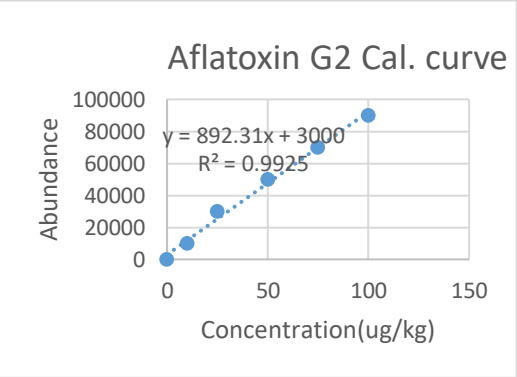
Mycotoxins	R2	LOD(ug/kg)	LOQ(ug/kg)
Aflatoxin B1	0.9958	0.2	1
Aflatoxin B2	0.9941	0.06	1
Aflatoxin G1	0.9958	0.2	1
Aflatoxin G2	0.9925	0.5	2
Aflatoxin M1	0.9935	0.1	0.3

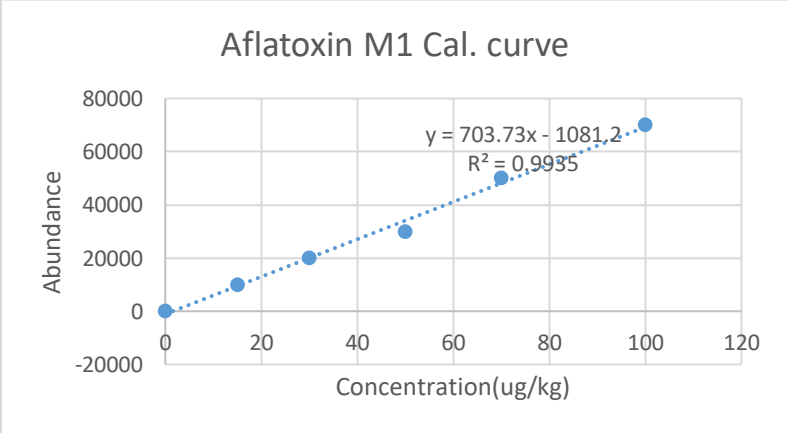
Appendix 10b: Calibration curves for feed samples











Appendix 11a: Calibration standards for water samples and LOD and LOQ for water samples

AFLATOXIN B1	
Cal. Stds levels(ug/l)	Abundance
0	0
1	10000
3	30000
5	45000
7	70000
10	90000

AFLATOXIN B2	
Cal. Stds (ug/l)	Abundance
0	0
1	5000
3	15000
5	30000
7	50000
10	70000

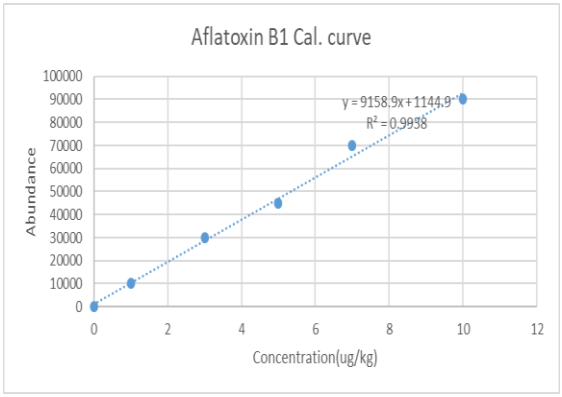
AFLATOXIN G1	
Cal. Stds(ug/l)	Abundance
0	0
1	1000
3	3000
5	7000
7	10000
10	15000

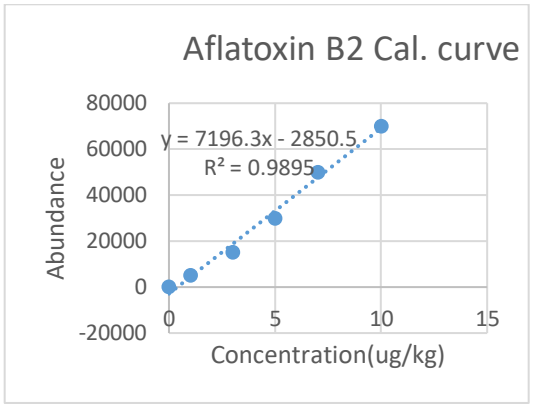
AFLATOXIN G2	
Cal. stds levels(ug/l)	Abundance
0	0
1	700
3	2000
5	3500
7	5000
10	8000

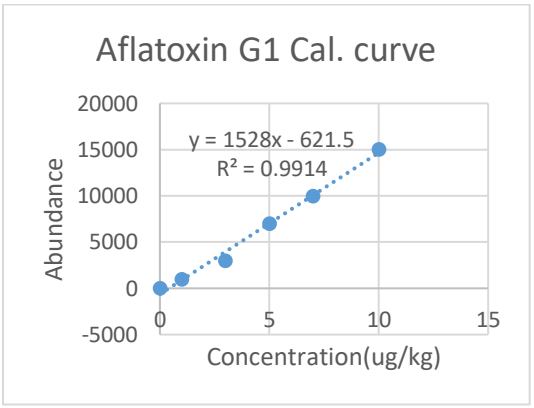
AFLATOXIN M1	
Cal. Stds(ug/l)	Abundance
0	0
0.2	600
0.5	2000
1	4000
1.5	6000
2	8000

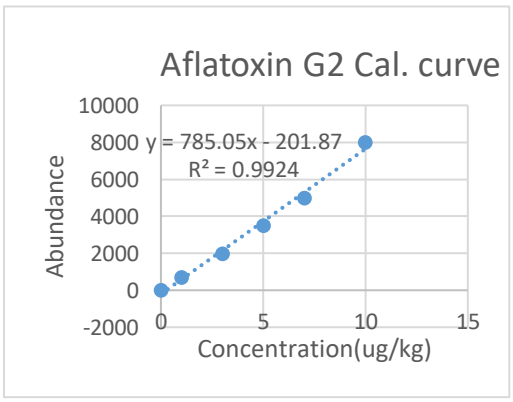
Linearity, LODs and LOQs of the five Mycotoxins			
Mycotoxins	R2	LOD(ug/l)	LOQ(ug/l)
Aflatoxin B1	0.9938	0.02	0.05
Aflatoxin B2	0.9895	0.05	0.1
Aflatoxin G1	0.9914	0.03	0.07
Aflatoxin G2	0.9924	0.09	0.2
Aflatoxin M1	0.9994	0.01	0.04

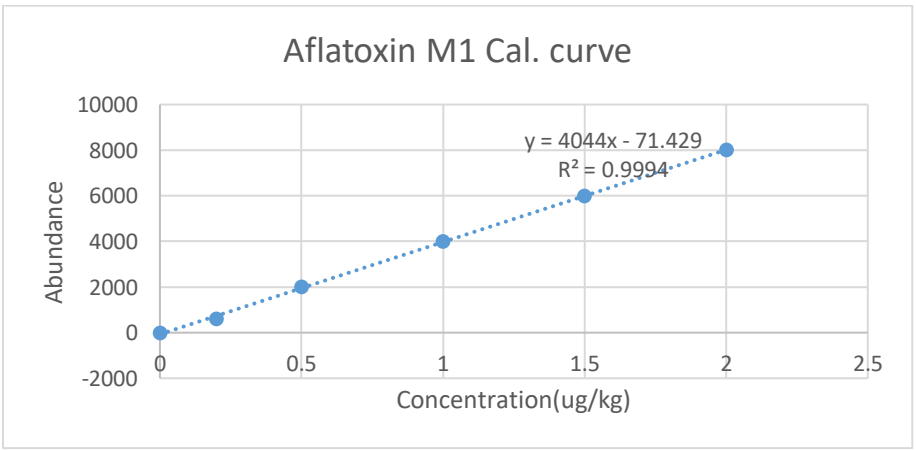
Appendix 11b: Calibration curves for water samples











Appendix 12: Map of Nairobi City County