

Analysis of Aflatoxin Levels in Broiler Chicken Feed from Selected Farms in Nairobi City County, Kenya

ABSTRACT

Aflatoxin levels in animal feed should be observed from the farm to the table to ensure the safety of the feed to animals and humans. The contamination of cereals and other agricultural supplies used in animal feed production could happen in the farm in the pre-harvest phase or in the post-harvest phase. The study sought to determine Aflatoxin levels in broiler feed from selected farms in Nairobi City County. A total of 42 feed samples were collected. Samples were analyzed using the LCMS/MS technique. Results from the study show that Aflatoxin levels in broiler starter were; B1(17.26±3.07 ppb), B2 (2.44±0.84 ppb), G1 (8.87±2.41 ppb), G2 (0.9±0.44 ppb) and Total AF (29.47±6.13 ppb). Aflatoxin levels in broiler finisher were B1 (17.17±3.09 ppb), B2 (2.68±1.18 ppb), G1 (9.25±2.7 ppb), G2 (1±0.45 ppb) and Total AF (30.1±6.88 ppb). Results from analysis of feed samples showed that 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. There is need to enhance the capacity of feed surveillance and monitoring in the country through various laboratory analysis techniques among various agencies in the feed value chain to ensure feed safety.

Key words: Aflatoxin, contamination, detection, detoxification

Abbreviations: AF (Aflatoxin), EAC (East African Community), EU (European union), KEBS (Kenya Bureau of Standards), WHO/FAO (World Health Organization/Food and Agriculture Organization)

1. INTRODUCTION

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 (1). 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 through 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 (2). 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 (2).

In Kenya, aflatoxins are largely produced by *Aspergillus parasiticus* and *A. flavus* (3) (4) (5) (6) (7) (8). *A. flavus* is a worldwide fungus well-known to produce AFB₁ and AFB₂ together with aspergillilic, cyclopiazonic, and kojic acids (9). *A. parasiticus* produces both AFs B and G and kojic and aspergillilic acids (10). 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 (11). 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 (12).

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 (13) (14) (15). Contamination has also

been associated with planting of maize in ecologically predisposed regions of the country (16) (17). 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 (18) (19) (20) (21).

Studies have reported that high levels of Aflatoxin in feed samples leads to high levels of Aflatoxin in animal products (22). Studies have also reported that fungal toxins are predominant in feeds hence have become difficult to eliminate. Nairobi County unlike other counties serves as the major harbor for broiler market across the country and beyond (23). The consumption of broiler meat in Nairobi 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 (24).

There is scanty information on the levels of aflatoxin in broiler feed in Nairobi City County therefore this study sought to determine the aflatoxin levels in broiler feed hence bridge this gap. 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.

2. METHODOLOGY

2.1 Study area

The study was conducted in Nairobi City County, Kenya in selected farms in Westlands, Kasarani, Embakasi Central, Embakasi East, Dagoreti North and Dagoreti South sub counties.

2.2 Sample size determination

Multistage cluster random sampling (two stage) was used to randomly select the farms to be sampled. One farm in each sub county was randomly selected hence a total of six farms were sampled. The farms where samples were collected were the ones where broilers were reared (only from broiler farmers) and sample size was determined using Wan and Wan (2017) resource equation formula (25) as shown below;

$$n = k \times n \quad \text{(Equation 1)}$$

Where; k is total number of subjects (feed samples) n is total number of treatments (weeks)

Hence; $n = 6 \times 7 = 42$

Samples were collected for a period of six weeks (week 0 to week 6) from six farms as this corresponded to the number of weeks it takes for a broiler to be ready for consumption. Hence a total of 42 feed samples were collected.

2.3 Laboratory analysis of feed samples

Detection and quantification of aflatoxin in feed and water 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.

2.4 Sample collection

Feed samples were collected and were put in well labeled airtight containers. All the feed samples obtained from the farms were kept in the cooler box then taken to the lab. The feed samples were stored in the freezer at - 20 degrees Celsius in the lab (26) to prevent further production of metabolite and microorganisms until the time of analysis (27).

2.5 Aflatoxin analysis

Each of the feed samples collected underwent extraction, clean up, preconcentration and instrumental analysis. Analysis of all the samples was done in triplicate. The samples were analyzed in an ISO 17025:2017 certified laboratory.

2.6 Instrument Set up

2.6.1 Calibration curves

Standard calibration curves were established for each aflatoxin analogue (B1, B2, G1, G2 and M1) to determine the linearity of the LC-MS/MS system. The linearity of the method was tested by running AF standard in the range of 0.0–100 µg/kg (0, 5, 10, 15, 25, 30, 50, 75 and 100 µg/kg), and a correlation coefficient (R^2) of >0.9500 for each analogue was obtained.

2.6.2 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 analyzed.

2.6.3 Limit of Quantification

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

2.7 Sample preparation for Aflatoxin analysis of samples

2.7.1 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 **Equipment** used were; Agilent 1260 coupled with mass spectrometry, Agilent 6460, 100 ml beaker, 100 ml measuring cylinder, 10 ml size volumetric flask, fluted 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.

2.7.2 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 hand shaken for 15 seconds. The sample was then filtered through a fluted 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 (28).

2.8 Data analysis and presentation

STATA version 12 was used to analyze quantitative data from the laboratory analysis. Data was subjected to one-way ANOVA to compare variation between means of levels of Aflatoxin in feed samples that were collected. 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 was presented in tables.

3. RESULTS

There was no significant statistical difference ($p>0.05$) of the mean levels of AFB1, AFB2, AFG1, AFG2 and Total Aflatoxin levels in broiler starter feed in all the farms as shown in Table 1 below.

AFB1 levels were above the KEBS (Kenya Bureau of Standards) 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 1 below.

Table 1: 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

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 2 below. Additionally, there was no significant difference ($p>0.05$) in AFB2, AFG1, AFG2 and Total Aflatoxin in all the farms.

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 2 below.

Table 2: 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

There was no statistical significant difference (p>0.05) in aflatoxin levels in broiler starter and broiler finisher as shown in Table 3 below.

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 3 below.

Table 3: 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

4. DISCUSSION

Feed adulteration with mycotoxins due to growth of molds is a challenge to farmers globally (29). Aflatoxins are not prevalent at the pre-harvest stage as other mycotoxins this is because aflatoxin producers are regarded as storage molds (30) (31). 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 (32) (33).

Besides AFB1, other AFs, including AFB2, AFG1 and AFG2, have also been detected in poultry feeds and feed ingredients (34) (35) (36) (37) (38) in the current study all these analogues were detected in feed.

Worldwide, different studies have reported varying levels of aflatoxin in feed. A study by Nemati et al (39) from North western region of Iran, reported the average level of AF adulteration in broiler feed at (11.6 ppb) in a different study done by Ifie et al in Nigeria found AF levels of (21 ppb) in broiler finisher

(27). This was consistent 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 ppb (40). Higher levels of (24.–185.25 ppb) of AF were also reported in various types of chicken feed from large-scale and small-scale manufacturers in Uganda (41). Aboagye-Nuamah et al (42) also found higher AF levels of between (11.83–88.37 ppb) 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 (43). A study carried out in Kenya on aflatoxin levels in commercial poultry feed by Okoth and Kola 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 (44) . 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 (45) 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 grade 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) (46) , 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 analogues were present in broiler feed but at different levels (47). In a study in Nakuru Kenya by Thuita et al, the total aflatoxin mean level for the broiler starter and broiler finisher feed samples were 19.37 ± 2.45 and 19.86 ± 2.21 ppb respectively (48) 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 ppb 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 (49) these were higher than the levels obtained from the present study.

5. CONCLUSION AND RECOMMENDATION

Results from analysis of feed samples showed that 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.

The study recommends that there is need for continuous 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.

ETHICAL APPROVAL AND CONSENT

Approval to carry out the study was obtained from Kenyatta University graduate school. Ethical approval was obtained from Kenyatta University Ethical and review committee Approval number (PKU/2163/II307). A research permit to carry out the study was obtained from National commission for Science, Technology and innovation (NACOSTI) license number (NACOSTI/P/20/8037. Authorization was also obtained from the Ministry of Agriculture, Division of Veterinary Services before commencement of the study. In the farms where feed samples were obtained for lab analysis, assent was sought from the farm owners and the nature and details of the study was clearly explained to the farm owners. The scope, the benefits and the risks of the study was thoroughly illustrated to the participants. Participation in the study was on voluntary basis and respondents chose to or not to take part in the study.

REFERENCES

1. Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF. Review on Mycotoxin Issues in Ruminants: Occurrence in Forages, Effects of Mycotoxin Ingestion on Health Status and Animal Performance and Practical Strategies to Counteract Their Negative Effects. *Toxins (Basel)* [Internet]. 2015 Aug 12 [cited 2023 Feb 20];7(8):3057–111. Available from: <https://publres.unicatt.it/en/publications/review-on-mycotoxin-issues-in-ruminants-occurrence-in-forages-eff-8>
2. Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T, Schatzmayr G, et al. Co-Occurrence of Regulated, Masked and Emerging Mycotoxins and Secondary Metabolites in Finished Feed and Maize—An Extensive Survey. *Toxins (Basel)* [Internet]. 2016 Dec 6 [cited 2023 Feb 20];8(12). Available from: [/pmc/articles/PMC5198557/](https://pubmed.ncbi.nlm.nih.gov/3198557/)
3. Oloo RD, Okoth S, Wachira P, Mutiga S, Ochieng P, Kago L, et al. Genetic profiling of aspergillus isolates with varying aflatoxin production potential from different maize-growing regions of kenya. *Toxins (Basel)*. 2019 Aug 1;11(8).
4. Mitema A, Okoth S, Rafudeen SM. The development of a qPCR assay to measure aspergillus flavus biomass in maize and the use of a Biocontrol Strategy to Limit Aflatoxin Production. *Toxins (Basel)*. 2019;11(3):11070384.
5. Monda E, Masanga J, Alakonya A. Variation in Occurrence and Aflatoxigenicity of *Aspergillus flavus* from Two Climatically Varied Regions in Kenya. *Toxins* 2020, Vol 12, Page 34 [Internet]. 2020 Jan 6 [cited 2023 Feb 20];12(1):34. Available from: <https://www.mdpi.com/2072-6651/12/1/34/htm>
6. Ismail A, Gonçalves BL, de Neeff D V., Ponzilacqua B, Coppa CFSC, Hintzsche H, et al. Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Res Int* [Internet]. 2018 Nov 1 [cited 2023 Feb 20];113:74–85. Available from: <https://pubmed.ncbi.nlm.nih.gov/30195548/>
7. Okoth S, De Boevre M, Vidal A, Di Mavungu JD, Landschoot S, Kyallo M, et al. Genetic and toxigenic variability within *Aspergillus flavus* population isolated from maize in two diverse environments in Kenya. *Front Microbiol* [Internet]. 2018 Jan 26 [cited 2023 Feb 20];9(JAN):57. Available from: [/pmc/articles/PMC5790802/](https://pubmed.ncbi.nlm.nih.gov/3198557/)
8. Nelson MC, Margaret MW, Nelson MC, Margaret MW. Occurrence of Aflatoxigenic *Aspergillus* Species in Peanut Varieties in Busia and Kisii Central Districts, Kenya. *Open J Med Microbiol* [Internet]. 2018 Oct 20 [cited 2023 Feb 20];8(4):98–108. Available from: <http://www.scirp.org/journal/PaperInformation.aspx?PaperID=88341>
9. Varga J, Frisvad JC, Samson RA. A reappraisal of fungi producing aflatoxins. <https://doi.org/10.3920/WMJ20081094> [Internet]. 2009 Apr 28 [cited 2023 Feb 20];2(3):263–77. Available from: <https://www.wageningenacademic.com/doi/10.3920/WMJ2008.1094>
10. Baquião AC, De Oliveira MMM, Reis TA, Zorzete P, Diniz Atayde D, Correa B. Polyphasic approach to the identification of *Aspergillus* section *Flavi* isolated from Brazil nuts. *Food Chem*. 2013 Aug 15;139(1–4):1127–32.
11. Lizárraga-Paulin G. E, Moreno-Martinez E, P. S. Aflatoxins and Their Impact on Human and Animal Health: An Emerging Problem. *Aflatoxins - Biochem Mol Biol*. 2011 Oct 3;
12. Atherstone C, Grace D, Lindahl JF, Kang'ethe EK, Nelson F. Assessing the impact of aflatoxin consumption on animal health and productivity. *African J Food, Agric Nutr Dev* [Internet]. 2016 Aug 12 [cited 2023 Feb 28];16(3):10949–66. Available from: <https://www.ajol.info/index.php/ajfand/article/view/141923>
13. Obonyo MA, Salano EN. Perennial and seasonal contamination of maize by aflatoxins in eastern

- kenya. *Int J Food Contam* [Internet]. 2018 Aug 31 [cited 2023 Feb 20];5(1):1–5. Available from: <https://foodsafetyandrisk.biomedcentral.com/articles/10.1186/s40550-018-0069-y>
14. Kiarie GM, Dominguez-Salas P, Kang'ethe SK, Grace D, Lindahl J. Aflatoxin exposure among young children in urban low-income areas of Nairobi and association with child growth. *African J Food, Agric Nutr Dev* [Internet]. 2016 Aug 12 [cited 2023 Feb 20];16(3):10967–90. Available from: <https://www.ajol.info/index.php/ajfand/article/view/141924>
 15. Koskei P, Bii CC, Musotsi P, Muturi Karanja S. Postharvest Storage Practices of Maize in Rift Valley and Lower Eastern Regions of Kenya: A Cross-Sectional Study. *Int J Microbiol*. 2020;2020.
 16. Mutiga SK, Hoffmann V, Harvey JW, Milgroom MG, Nelson RJ. Assessment of Aflatoxin and Fumonisin Contamination of Maize in Western Kenya. *Phytopathology* [Internet]. 2015 Sep 1 [cited 2023 Feb 20];105(9):1250–61. Available from: <https://pubmed.ncbi.nlm.nih.gov/25894319/>
 17. Mwhia EW, Mbuthia PG, Eriksen GS, Gathumbi JK, Maina JG, Mutoloki S, et al. Occurrence and Levels of Aflatoxins in Fish Feeds and Their Potential Effects on Fish in Nyeri, Kenya. *Toxins* 2018, Vol 10, Page 543 [Internet]. 2018 Dec 17 [cited 2023 Feb 20];10(12):543. Available from: <https://www.mdpi.com/2072-6651/10/12/543/htm>
 18. Leroy JL, Wang JS, Jones K. Serum aflatoxin B1-lysine adduct level in adult women from Eastern Province in Kenya depends on household socio-economic status: A cross sectional study. *Soc Sci Med*. 2015 Dec 1;146:104–10.
 19. Kiama TN, Lindahl JF, Sirma AJ, Senerwa DM, Waithanji EM, Ochungo PA, Poole EJ KE and DG. Kenya Dairy Farmer Perception Of Moulds And Mycotoxins And Implications For Exposure To Aflatoxins: A Gendered Analysis. *KENYA DAIRY FARMER Percept Mould MYCOTOXINS Implic Expo TO AFLATOXINS A GENDERED Anal*. 2016;1–7.
 20. Mutiga SK, Were V, Hoffmann V, Harvey JW, Milgroom MG, Nelson RJ. Extent and drivers of mycotoxin contamination: Inferences from a survey of Kenyan maize mills. *Phytopathology* [Internet]. 2014 Nov 1 [cited 2023 Feb 20];104(11):1221–31. Available from: <http://dx.doi.org/10.1094/PHYTO-01-14-0006-R>
 21. Sirma AJ, Ouko EO, Murithi G, Mburugu C, Mapenay I, Ombui J, et al. Prevalence of aflatoxin contamination in cereals from Nandi county, Kenya. *Int J Agric Sci Vet Med* [Internet]. 2015;3(3):1–9. Available from: www.ijasvm.com
 22. Hasanen F, Mohammed M, H. M, Hassan W, Amro F. Aflatoxins residues in chicken and turkey tissues. *Benha Vet Med J*. 2016;31(2):130–5.
 23. McCarron M, Munyua P, Cheng PY, Manga T, Wanjohi C, Moen A, et al. Understanding the poultry trade network in Kenya: Implications for regional disease prevention and control. *Prev Vet Med* [Internet]. 2015 Jul 1 [cited 2023 Feb 23];120(3–4):321–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/26002998/>
 24. Carron M, Alarcon P, Karani M, Muinde P, Akoko J, Onono J, et al. The broiler meat system in Nairobi, Kenya: Using a value chain framework to understand animal and product flows, governance and sanitary risks. *Prev Vet Med*. 2017 Nov 1;147:90–9.
 25. Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. *Malays J Med Sci* [Internet]. 2017 Sep 1 [cited 2023 Feb 27];24(5):101–5. Available from: <https://pubmed.ncbi.nlm.nih.gov/29386977/>
 26. Alam S, Khan NA, Muhammad A, Jan I, Hashmi MS, Khan A, et al. Carryover of aflatoxin B1 from feed to broilers' tissues and its effect on chicken performance. *Fresenius Environ Bull*. 2020;29(1):214–21.
 27. Ifie I, Igwebuike CG, Imasuen P, Akalamudo W, Oghenebrorhie O, Akpodiete JO, et al. Assessment of aflatoxin and heavy metals levels in maize and poultry feeds from Delta State,

- Nigeria. *Int J Environ Sci Technol* [Internet]. 2022 Dec 1 [cited 2023 Feb 20];19(12):12551–60. Available from: <https://link.springer.com/article/10.1007/s13762-022-03996-1>
28. Kongkapan J, Poapolathep S, Isariyodom S, Kumagai S, Poapolathep A. Simultaneous detection of multiple mycotoxins in broiler feeds using a liquid chromatography-tandem-mass spectrometry. *J Vet Med Sci* [Internet]. 2016 Mar 1 [cited 2023 Mar 2];78(2):259. Available from: </pmc/articles/PMC4785115/>
 29. Moretti A, Logrieco AF, Susca A. Mycotoxins: An underhand food problem. *Methods Mol Biol*. 2017;1542(58):3–12.
 30. Afolabi CG, Ezekiel CN, Ogunbiyi AE, Oluwadairo OJ, Sulyok M, Krska R. Fungi and mycotoxins in cowpea (*Vigna unguiculata* L) on Nigerian markets. *Food Addit Contam Part B, Surveill* [Internet]. 2019 Nov 18 [cited 2023 Feb 20];13(1):52–8. Available from: <https://europepmc.org/article/med/31739763>
 31. Leggeri MC, Lanubile A, Dall'Asta C, Pietri A, Battilani P. The impact of seasonal weather variation on mycotoxins: maize crop in 2014 in northern Italy as a case study. <https://doi.org/10.3920/WMJ20192475> [Internet]. 2019 Nov 26 [cited 2023 Feb 20];13(1):25–36. Available from: <https://www.wageningenacademic.com/doi/10.3920/WMJ2019.2475>
 32. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE. Nutritional and health implications of mycotoxins in animal feeds: A review. *Pakistan J Nutr*. 2006;5(5):398–403.
 33. Ráduly Z, Szabó L, Madar A, Pócsi I, Csernoch L. Toxicological and Medical Aspects of Aspergillus-Derived Mycotoxins Entering the Feed and Food Chain. *Front Microbiol*. 2020 Jan 9;10:2908.
 34. Akinmusire OO, El-Yuguda AD, Musa JA, Oyedele OA, Sulyok M, Somorin YM, et al. Mycotoxins in poultry feed and feed ingredients in Nigeria. *Mycotoxin Res* [Internet]. 2019 May 1 [cited 2023 Feb 20];35(2):149–55. Available from: <https://link.springer.com/article/10.1007/s12550-018-0337-y>
 35. Kemboi DC, Ochieng PE, Antonissen G, Croubels S, Scippo ML, Okoth S, et al. Multi-Mycotoxin Occurrence in Dairy Cattle and Poultry Feeds and Feed Ingredients from Machakos Town, Kenya. *Toxins (Basel)* [Internet]. 2020 Dec 1 [cited 2023 Feb 20];12(12):762. Available from: </pmc/articles/PMC7761711/>
 36. Mokubedi SM, Phoku JZ, Changwa RN, Gbashi S, Njobeh PB. Analysis of mycotoxins contamination in poultry feeds manufactured in selected provinces of South Africa using UHPLC-MS/MS. *Toxins (Basel)*. 2019;11(8):1–17.
 37. Rodrigues I, Handl J, Binder EM. Mycotoxin occurrence in commodities, feeds and feed ingredients sourced in the middle East And Africa. *Food Addit Contam Part B Surveill*. 2011;4(3):168–79.
 38. Ezekiel CN, Bandyopadhyay R, Sulyok M, Warth B, Krska R. Fungal and bacterial metabolites in commercial poultry feed from Nigeria. *Food Addit Contam - Part A*. 2012;29(8):1288–99.
 39. Ghaffarian Bahraman A, Mohammadi S, Jafari A, Ghani-Dehkordid J, Arabnezhad MR, Rahmdel S, et al. Occurrence of Aflatoxin M1 in Milks of Five Animal Species in Iran: A Systematic Review and Meta-analysis. *Food Rev Int*. 2020;36(7):692–712.
 40. Morrison DM, Ledoux DR, Chester LFB, Samuels CAN. A limited survey of aflatoxins in poultry feed and feed ingredients in Guyana. *Vet Sci*. 2017;4(4).
 41. Nakavuma JL, Kirabo A, Bogere P, Nabulime MM, Kaaya AN, Gnonlonfin B. Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. *Int J Food Contam*. 2020;7(1):1–10.
 42. Aboagye-Nuamah F, Kwoseh CK, Maier DE. Toxigenic mycoflora, aflatoxin and fumonisin

- contamination of poultry feeds in Ghana. *Toxicon*. 2021 Jul 30;198:164–70.
43. Xu F, Baker RC, Whitaker TB, Luo H, Zhao Y, Stevenson A, et al. Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination. *World Mycotoxin J*. 2022;15(2):171–86.
 44. Okoth SA, Kola MA. Market samples as a source of chronic aflatoxin exposure in Kenya. *Afr J Health Sci*. 2012;20(1):56–61.
 45. Lubna MA, Debnath M, Hossaini F. Detection of Aflatoxin in Poultry Feed and Feed Materials through Immuno Based Assay from Different Poultry Farms and Feed Factories in Bangladesh. *Bangladesh J Microbiol* [Internet]. 2018 Jan 15 [cited 2023 Feb 21];35(1):75–8. Available from: <https://www.banglajol.info/index.php/BJM/article/view/39807>
 46. Beg MU, Al-Mutairi M, Beg KR, Al-Mazeedi HM, Ali LN, Saeed T. Mycotoxins in poultry feed in Kuwait. *Arch Environ Contam Toxicol* [Internet]. 2006 May [cited 2023 Feb 21];50(4):594–602. Available from: <https://pubmed.ncbi.nlm.nih.gov/16435083/>
 47. Anthony CM, Emmanuela IN, Onwumere OSI. A survey of the aflatoxin level and molecular identification of fungal contaminants in poultry feed mills from different geopolitical zones of Nigeria. *African J Biotechnol*. 2020 Aug 31;19(8):500–7.
 48. Thuita F N, Tuitoek J K KAM and OMA. Prevalence of aflatoxins contamination in commercial broiler feeds in Kenya. *Preval aflatoxins Contam Commer broiler Feed Kenya* [Internet]. 2019 [cited 2023 Feb 21];31. Available from: <http://www.lrrd.org/lrrd31/1/jtuit31003.html>
 49. Naveed M, Haleem KS, Ghazanfar S, Tauseef I, Bano N, Adetunji CO, et al. Quantitative Estimation of Aflatoxin Level in Poultry Feed in Selected Poultry Farms. *Biomed Res Int* [Internet]. 2022 [cited 2023 Feb 21];2022. Available from: <https://pubmed.ncbi.nlm.nih.gov/35141333/>