Original Research Article

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 thatAflatoxin 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 thatAFB1 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 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 (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 AFB_1 and AFB_2 together with aspergillic, cyclopiazonic, and kojic acids (9). *A. parasiticus* produces both AFs B and G and kojic and aspergillic 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 it is difficult to have fungal toxin free feeds under normal conditions, hence impossible 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 quantify and to characterize aflatoxin levels in broiler feed hence bridge this gap.

Comment [H1]: This sentence does not have a clear meaning

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 were the ones where samples of broilers were taken and sample size was determined using Wan and Wan (2017) resource equation formula (25) as shown below;

Comment [H2]: Re-phrase this sentence to give the right meaning. Did you take samples of broilers or broiler feeds?

$n = k \times n$ (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).

Comment [H3]: Please remove this it has been stated in the sentence above that the samples were taken to the lab.

2.5 Aflatoxin analysis

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

Comment [H4]: Remove and replace with a (,) comma

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 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 analyzed.

Comment [H5]: Quantification

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 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, powderlessgloves, pasteur pipette, micro pipette (1ml), micro pipette (0.2ml) and vortex mixture.

Comment [H6]: Remove (s) equipment has no singular or plural

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 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 (28).

Comment [H7]: Separate “Hand shaken”

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 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.

Comment [H8]: Please write in full the first time

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 aflatoxins 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).

Comment [H9]: Not the aflatoxins but the aflatoxin producers

Besides AFB1, other AFs, including AFB2, AFG1, AFG2, and aflatoxin M1 (AFM1), have also been detected in poultry feeds and feed ingredients (34)(35)(36)(37). 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*Aspergillispp*(38) although in the current study AFM1 levels were not detected in feed.

Comment [H10]: Examine your source to confirm how true this information is . AFM1 is usually produced in animal products like milk as a result of the consumption of AFB1. AFM1 should not be detected in feeds

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 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) (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.

Comment [H11]: Grade not graded

5. CONCLUSION AND RECOMMENDATION

Results from analysis of feed samples showed thatAFB1 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 AFB1than 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.

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Comment [H12]: Adjust reference number 11 to begin with the name and not the initials.

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