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Mycoflora, aflatoxin, and fumonisin levels present in layer feed in Nairobi County

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Abstract

Layer feeds in Africa largely comprise cereals that are susceptible to mycotoxin contamination. Mycotoxins pose a health and production hazard in the poultry industry. This study investigated the mycoflora associated with layer feeds and mycotoxin content. 50 samples each of chick mash, grower mash, and layer mash were sampled from five regions in Nairobi County where urban poultry farming is carried out. Dilution plate technique was used to isolate fungi in Potato Dextrose Agar (PDA) and identified using morphological and molecular methods. Total aflatoxins (AF) and total fumonisins (FUM) levels were screened using Enzyme-Linked Immunosorbent Assay (ELISA). *Aspergillus* (91%), *Penicillium* (77%), *Fusarium* (13%), *Mucor* (11%), *Rhizopus* (9%), *Cladosporium* (4%), and *Cochliobolus* (1%) were isolated. AF was detected in 65% of feed samples (0.22 μg/kg - 157.89 μg/kg) while FUM in 91% of feed samples (210 μg/kg - 15173 μg/kg). Sixty-two samples (42%) had AF levels greater than the Kenya Bureau of Standards (KEBS) recommended limit of 10 μg/kg in poultry feed. No sample contained FUM levels higher than the European Union (EU) recommended limit of 20000 μg/kg. Our results highlight AF contamination in farms in the County, raising concern to poultry and public health due to AF residues in eggs.

Keywords: *Aflatoxins, Fumonisins, Layer feeds*

1. Introduction

Poultry farming is the rearing of domesticated birds such as chickens, ostriches, and turkeys for meat and eggs. As of 2006, Kenya had an approximated poultry population of 37.3 million birds, where 31.4 million were free-ranging indigenous birds, 3.1 million were layers, 2.1 million were broilers, while other poultry species were 0.7 million (Omiti and Okuthe, 2009). The sector contributes roughly 55% to the livestock sector and 30% of the agricultural Gross Domestic Product (GDP), or 7.8% of the total national GDP offering employment to roughly two million people either directly or indirectly (Omiti and Okuthe, 2009).

Nairobi County houses Nairobi, the capital city of Kenya. Over the years, the County population has been on the rise, majorly attributed to natural increase given its young age structure and in-migration (Otiso, 2017).

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According to the 2019 census, Nairobi County was cited as the most populated County (KPHC, 2019). This rise in populations has resulted in increased food demand in the County. To meet this demand, farmers have ventured into chicken farming to serve as a source of income and nutrition. Increased food demand coupled with growing land constraints and dietary shift towards white meat are the factors that have steered the growth of urban poultry farming in the County (Omondi, 2018).

The success of urban poultry farming largely relies on the feed manufacturing industry. However, feed manufacturing industries compete with humans for feed materials (Thuita *et al*., 2019). Therefore, the production of good quality and affordable feeds lie in the availability of the ingredients for human use and feed manufacture (Macharia *et al*., 2020; and Thuita *et al*., 2019). Good quality feeds are expensive, and in a bid to cut costs, some farmers prefer to make their feeds or end up purchasing low-quality feeds (Adeyeye, 2016). Feed alone accounts for 65-70% of total production costs; therefore is key to prosperous poultry farming (Farrell, 2013; and Macharia *et al*., 2020).

Low quality feeds face the risk of being contaminated with hazardous components beyond the permitted limit. One of these compounds is mycotoxins. Mycotoxins are low molecular weight compounds produced during secondary metabolism of various toxigenic fungi, mainly of the genera *Aspergillus*, *Fusarium*, and *Penicillium* (Iheshiulor *et al*., 2014). Mycotoxin contamination in poultry feeds can occur at any stage, from grain production, processing to storage (Jean *et al*., 2013). Their presence in feed is of great concern due to its adverse effects on animal health and the possible carry-over effect of each toxin to humans through their byproducts (Pinotti *et al*., 2016; and Rom *et al*., 2017). All poultry species are sensitive to mycotoxins, with their sensitivity depending on the type, age, and category of the poultry species, nutritional status, duration, and quantity of mycotoxin ingestion (Iheshiulor *et al*., 2014; and Resanovic *et al*., 2009). Major mycotoxins include aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxin, patulin, trichothecenes, and zearalenone (Adeyeye, 2016).

Aflatoxins and fumonisins commonly occur in tropical climates where environmental conditions favor fungal infection by *Aspergillus* and *Fusarium* and have been reported to be prevalent in major dietary products in Africa (Adeyeye, 2016; Darwish *et al*., 2014; and Kagot *et al*., 2019). They are also the two major mycotoxins detected in Kenya's cereal grains (Kang'Ethe *et al*., 2017). Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and are divided into B and G groups depending on their green and blue fluorescence under UV light when absorbed to solid particles (Hossein and Gürbüz, 2015). Fumonisins are primarily produced by *F. verticilloides* and *F. proliferatum* and are divided into four series A, B, C, and P with the B series, mainly Fumonisin B1 (FB1), Fumonisin B2 (FB2), and Fumonisin B3 (FB3) being the most abundant naturally occurring fumonisins (Kamle *et al*., 2019; and Kouzi *et al*., 2015).

Approximately 3,432 households are reported to carry out layer farming in Nairobi County (KNBS, 2019). Eggs are rich in micronutrients such as carbohydrates, fats, fatty acids, protein and amino acids, vitamins (D and B12), and minerals (Onono *et al*., 2018). Aflatoxins in layer hens result in decreased weight gain, decreased growth rate, decreased feed conversion efficiency, reduced egg production, and egg quality, reduced hatchability, reduced reproductive performance, changes in organ weights, kidney disorders, leg and bone problems, pigmentation problems, immortality and immune suppression (Hossein and Gürbüz, 2015; Iheshiulor *et al*., 2014; Lizárraga-Paulín *et al*., 2011; Murugesan *et al*., 2015; and Sharma *et al*., 2018). Aflatoxins residues may become concentrated in meat and eggs meant for human consumption (Akande *et al*., 2006). Aflatoxins are carcinogenic, teratogenic, and mutagenic hence difficult to prevent or detoxify them (Sarma *et al*., 2017). Fumonisins suppress the immune system, cause deficiency in nutrients such as folic acid, and the modification of sphingomyelin metabolism (Iheshiulor *et al*., 2014; Murugesan *et al*., 2018; Patil *et al*., 2014; and Kumi *et al*., 2019). The Kenya Bureau of Standards (KEBS) has set the limit of aflatoxins in poultry feed to 10 μg/kg; however, there is no regulation on fumonisins in poultry feed (Kibugu *et al*., 2019).

In Kenya, little information is available regarding the quality of layer feeds available in the market concerning fungal counts and mycotoxin contamination. Therefore, this study investigated the mycoflora, aflatoxin, and fumonisin levels present in layer feeds collected from five regions in Nairobi County where urban poultry farming is carried out.

2. Materials and methods

2.1. Study setting

This study was carried out in Nairobi County, which houses Kenya's capital city, Nairobi. The County has an estimated population of 4.3 million, covering a land area of 695 square kilometers (Otiso, 2017; and KNBS, 2019). The county receives a mean annual rainfall of 879 mm with a mean maximum temperature of 23.6 °C and lies between 1,600 and 1,850 meters above sea level on the southeastern edge of Kenya's agricultural heartland at 1016'S latitude and 36048'E longitude (Obiero and Onyando, 2013; and Otiso, 2017). Five regions, selected as sample sites (Dagoretti South, Kasarani, Langata, Roysambu, and Ruai) were identified by the Ministry of Agriculture, Livestock, and Fisheries as the areas where active small scale poultry production is carried out in Nairobi County.

2.2. Sample collection

Thirty farmers from each of the five regions were randomly selected with Ministry of Agriculture, Livestock, and Fisheries extension officers. The selected farmers were then informed about the purpose of the study. One hundred grams of feed samples were collected using a closed spear driven through each sacks' top and sides and placed in sterile bags, sealed, and stored at 4 °C. A total of 150 feed samples (50 chick mash, 50 grower mash, and 50 layer mash were collected between January 2019 and February 2019 from the five regions.

2.3. Determination of fungal contamination of feeds using the serial dilution method

For each feed sample, three serial dilutions, 10^{-1}, 10^{-2}, 10^{-3}, were made. From each dilution, 1 ml was plated on Potato Dextrose Agar (PDA) in triplicates using the spread plate method. The plates were incubated at 29 °C for 5-7 days and examined daily for fungal growth and sporulation. On day 7, the colonies were counted on the plate and expressed in colony-forming units per gram of the unit sample (CFU / g). CFU was calculated using the formula:

$$= \frac{Number\ of\ colonies}{Volume\ used} \times Dilution\ factor$$

Pure cultures of the fungal growth were obtained by sub-culturing onto freshly prepared PDA and incubated at 29 °C for 5-7 days.

2.4. Identification of fungal isolates using morphological methods

Filamentous fungi were identified at the genus level according to macro and microscopic features following Pitt and Hocking (2009). Fungal isolates identified to species level were *Penicillium spp* according to Pitt and Hocking (2009), *Aspergillus spp*. according to Klich (2002) and *Fusarium spp.* according to Booth (1971). The isolation frequency (*Fr*) and Relative density (*Rd*) of each fungal genera isolated was calculated using the formula:

$$Fr(\%) = \frac{Number\ of\ samples\ with\ a\ genus\ or\ species}{Total\ number\ of\ samples} \times 100$$

$$Rd(\%) = \frac{Number\ of\ colonies\ of\ a\ genus\ or\ species}{Total\ number\ of\ fungi\ isolated} \times 100$$

2.5. Molecular characterization of fungal isolates

To confirm morphological identification of the fungi isolated from feed samples, molecular analysis was done.

2.6. DNA extraction from fungal isolates

Mycelium (0.1-0.2 g) was scraped using a sterile scalpel from PDA, put into 1.5 ml Eppendorf tubes, weighed, and then placed in a mortar. Liquid Nitrogen or white quartz sand was added and ground using a pestle until the tissue was fine. Exactly 500 μl of extraction buffer [100 mM Tris- HCl (pH 8.0), 20 mM Na2 EDTA, 0.5 M NaCl and 1% sodium dodecylsulfate] was added to the mortar so that the mixture became saturated. An equal

amount of phenol-chloroform isoamyl alcohol (24: 24: 1) was added, and grounding continued vigorously with a pestle until a thick paste was formed. 1ml of the paste was transferred into a new labeled 1.5 ml Eppendorf tube, capped, and centrifuged at 16,000 rpm for 10 min at 4 °C. Tissue debris and the sand pelleted to the bottom of the tube. Using a micropipette, 500 μl of the supernatant was transferred into a new Eppendorf tube. Afterwards, 3 M sodium acetate and 600 μl of ice-cold isopropanol were added and incubated at -20 °C overnight to precipitate the DNA. The tubes were then centrifuged at a speed of 12,000 rpm for 10 min to pellet the DNA at room temperature. The isopropanol was discarded, taking care not to pour out the pellet. The pellet was washed by adding 500 μl of 70% ethanol and spinning it at a speed of 12,000 rpm for 10 min at room temperature. The pellets' final wash was done by adding 500 μl absolute ethanol and spinning at 12,000 rpm for 10 min at room temperature. The absolute ethanol was discarded, taking care not to dislodge the pellet. The pellet was then air-dried by inverting the tubes on a clean paper towel for one hour at room temperature. The pellet was re-suspended in 100 μl of TE buffer (10 mM Tris-HCl, 1 mM Na2EDTA, pH 8.0) and 20 μl of RNAse (20 ug / ml) enzyme was added to remove any RNA contamination. Quality and quantity of DNA were done by subjecting 7 µl of the preparation to 1% agarose gel electrophoresis.

2.7. PCR amplification of DNA extracted from fungal isolates

Polymerase chain reaction (PCR) analysis of the internal transcribed spacers (ITS1 and ITS2) and 5.8S gene were amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTT ATTGATATGC-3') regions of the ribosomal DNA gene cluster. PCR amplification was performed on 25 μl of a reaction mixture containing $MgCl_2$-free reaction buffer, 50 mM $MgCl_2$, 10 uM dNTP mix, 10 μM of each primer, 5 U / uL Taq DNA polymerase, and 5 ng / uL of template DNA. PCR was carried out as follows: (1) one step at 94 °C for 3 min; (2) 30 cycles of the following three steps: 1 min at 94°C, 1 min at 57°C, 1 min at 72 °C, and (3) one final 10-min step at 72°C. The PCR products were separated by 1.2% agarose gel electrophoresis in a Tris-base, acetic acid, and EDTA buffer and stained with Ethidium bromide. PCR product purification was done using QIAquick PCR Purification Kit (Qiagen), and sequencing performed at Inqaba Ltd (Cape Town, Republic of South Africa). The obtained DNA sequences were trimmed using CLC Genomics Workbench version 5.5.2 and then compared with the sequences deposited in the NCBI GenBank via nucleotide Blast (http: / / www.ncbi.nlm.nih.gov /).

2.8. Aflatoxin extraction and quantification using ELISA technique

Analysis of Total Aflatoxin (AF) was done using Helica Total Aflatoxin Low Matrix Enzyme-Linked Immunosorbent Assay (ELISA) kit following the manufacturer's instructions. By measurement, 2 g of the ground feed sample was transferred to 200 ml of 80% acetonitrile in a beaker and mixed thoroughly for 10 min. The sample was centrifuged at 3,500 rpm to pellet the particulate matter and the supernatant obtained. The aliquot of the extract was reconstituted in wash buffer and a ratio of 1:10. Using a micropipette, 200 μl of the sample diluent was dispensed into each mixing well. With a new pipette tip for each, 100 μl of each standard and prepared sample was added to the appropriate mixing well containing the diluent and mixed by priming the pipette at least three times. Thereafter, 100 μl of the contents from each mixing well was transferred to a corresponding antibody-coated microtiter well and incubated at room temperature for 30 minutes. The microwells were washed five times by filling each with PBS-Tween wash buffer and decanting the wash into a discard basin. The microwells were tapped faced down on a layer of absorbent paper to remove residual wash. 100 μl of Aflatoxin HRP conjugate was added to each antibody-coated well and incubated at room temperature for 30 minutes. The microwells were washed once again, and 100 μl of substrate solution was added to each microwell and incubated at room temperature for 10 min. Finally, 100 μl of the stop solution was added to each microwell and the optical density for each microwell read with a microtitre plate reader using a 450 nm filter and the results recorded.

2.9. Fumonisin extraction and quantification using ELISA technique

Analysis of fumonisin was done using AgraQuant® Total Fumonisin Assay 0.25 / 5.0 kit (Romer Labs ®) following manufacturers' instruction. Briefly, 20 grams of the finely ground feed sample was placed in a clean beaker and 100 ml of 70% methanol was added and mixed thoroughly for 3 min. The sample was allowed to settle and filtered using Whatman filter paper to obtain the filtrate. Then 200 μl of the conjugate was dispensed into the green bordered dilution well. Using a fresh pipette tip for each, 100 μl of each standard or sample was

dispensed into the dilution well containing the 200 µl conjugate and mixed well by priming the pipette. Afterwards, 100 µl of the contents from each dilution well was transferred into a corresponding antibody-coated microwell and incubated at room temperature for 10 min. Contents of each microwell were emptied into a waste container and washed by filling each well with distilled water five times. The microwells were tapped faced down on a layer of absorbent paper to remove residual wash. Using a micropipette, 100 µl of the substrate was added into each microwell and incubated at room temperature for 5 min. Finally, 100 µl of the stop solution was then added and the optical density for each microwell read with a microtitre plate reader using a 450nm filter and the results recorded.

2.10. Statistical analysis

Statistical analyses were performed using R version 3.5.2 with 95% accuracy. Poisson negative binomial distribution was run to predict the Number of fungal counts based on region and feed type in the five regions while two-way Analysis of Variance (ANOVA) with interaction effect was used to compare aflatoxin and fumonisin concentration between the feed types and the five regions.

3. Results

3.1. Fungal contamination of feeds

All the feed samples had positive growth for at least one type of fungus. Chick mash had a CFU of $6.11 \times 10^{-2} \pm 4.51$ while growers mash had a mean total CFU of $5.80 \times 10^{-2} \pm 4.69$ and layers mash had a mean total CFU of $4.73 \times 10^{-2} \pm 3.866$. Feeds from Kasarani had the highest mean total CFU of $7.16 \times 10^{-2} \pm 4.68$ whereas feeds from Roysambu recorded the lowest mean CFU of $4.20 \times 10^{-2} \pm 3.22$. The mean total CFU of feeds from Langata, Ruai and Dagoretti South were $5.76 \times 10^{-2} \pm 4.43$, $5.13 \times 10^{-2} \pm 3.62$ and $5.49 \times 10^{-2} \pm 5.28$ respectively Mycological quality of the feeds were determined according to Gimeno where samples can be qualified as good (count range <3.10 - 4 CFU / g), regular (count range $3.10^{-4} - 7.10^{-4}$ CFU / g), and bad (>7.10^{-4} CFU / g) (Greco *et al*., 2014). According to this criterion, all the feed samples regardless of the feed type were qualified as good. A negative binomial Poisson regression was run to predict the Number of fungal counts based on region and feed type. Region and feed type had a significant effect on the Number of fungal counts in Kasarani, Langata and Dagoretti south ($p < 0.05$), while in Roysambu and Ruiru, there was no significant effect on the Number of fungal counts ($p > 0.05$).

3.2. Mycoflora of poultry feeds sampled from Nairobi County

Seven fungal genera were isolated from layer feeds, three of which, *Aspergillus, Fusarium* and *Penicillium,* are known to be mycotoxigenic. *Aspergillus spp* (90.67%) were the most frequently isolated fungi closely followed by *Penicillium spp* (77.33%) while the least isolated was *Cochliobolus spp* as presented in Table 1. From the genus *Aspergillus*, *A.flavus*, *A.niger*, *A.fumigatus*, *A. parasiticus*, *A. tamari, A. clavatus* and *A. ruber* were isolated. *P. aethiopicum* and *P. crustosum* were isolated from the genus *Penicillium* while *F. verticilloides* from the genus

Table 1: Fungal genera present in poultry layer feed in Nairobi County

Fungal genera	No. of isolates*	Frequency of isolation (%)	Relative density (%)
Aspergillus spp	136	90.67	44.01
Penicillium spp	116	77.33	37.54
Fusarium spp	20	13.33	6.47
Mucor spp	16	10.67	5.18
Rhizopus spp	14	9.33	4.53
Cladosporium spp	6	4	1.94
Cochliobolus spp	1	0.007	0.32

Note: * Total number of samples was 150.

Fusarium. From the genus *Mucor, M. circinelloides* and *M. fragilis, Rhizopus microsporus* from the genus *Rhizopus, Cladosporium pseudocladosporoides* from the genus *Cladosporium* and *Cochliobolus heterostrophus* from the genus *Cochliobolus spp.*

3.3. Aflatoxin contamination of poultry feeds sampled from Nairobi County, Kenya

Ninety eight samples (65%) had quantifiable levels of AF while fifty two samples (35%) had no detectable levels of AF. Sixty three samples (42%) exceeded the KEBS of 10 μg / kg maximum permitted limit of AF in finished poultry feed (Kibugu *et al*., 2019). Total AF contamination of all the feed types ranged from 0.22 μg / kg to 157.89 μg / kg. The mean of total positives in chick mash, grower mash and layers mash were 56.00 ± 50.1 μg / kg, 49.10 ± 46.0 μg / kg and 35.30 ± 42.6 μg / kg respectively (Table 2). Ruai recorded the highest AF mean of 53.3 ± 50.8 μg / kg whereas the lowest AF mean of 40.2 ± 41.8 μg / kg was observed in Langata (Table 3). Two-way Analysis of Variance revealed that total AF levels were not significantly different among the feed types (F = 1.813, df = 2, p = 0.170) and regions (F = 0.350, df = 4, p = 0.843) in Nairobi County. However, a significant interaction effect (F = 2.116, df = 8, p =0.043) was observed between the region and the feed type on the total AF levels in the feed samples.

Table 2: Aflatoxin levels of layer feed grouped by feed type in Nairobi County, Kenya

Feed type	Occurrence (n) %	>KEBS limit* (n) %	Range of Total AF (ug/kg)	Mean ± SD of (ug/kg)
Chick mash (*n* = 50)	32 (64)	21 (42)	0.74-157.89	56.00 ± 50.1
Grower mash (*n* = 50)	33(66)	24 (48)	0.8-154.30	49.10 ± 46.0
Layers mash (*n* = 50)	33(66)	18 (36)	0.22-145.74	35.30 ± 42.6

Note: AF= Aflatoxin; KEBS (Kenya Bureau of Standards) maximum permitted limit of 10 μg/kg of finished poultry feeds; *n* = Total Number of samples; and SD = Standard Deviation.

Table 3: Aflatoxin levels of layer feeds grouped by regions in Nairobi County, Kenya

Region	Occurrence (n) %	>KEBS limit* (n) %	Range of Total AF (ug/kg)	Mean ± SD of (ug/kg)
Dagoretti south (*n* = 30)	20 (67)	16 (53)	2.2-157.89	44.3 ± 42.4
Kasarani (*n* = 30)	15 (50)	9 (30)	1.18-145.74	52.4 ± 55.8
Langata(*n* = 30)	20 (67)	12 (40)	1.18-145.74	40.2 ± 41.8
(Roysambu (*n* = 30)	24 (80)	13 (43)	0.22-129.0	45.3 ± 47.2
Ruai (*n* = 30)	19 (63)	13 (43)	0.8-154.30	53.3 ± 50.8

Note: AF= Aflatoxin; KEBS (Kenya Bureau of Standards) maximum permitted limit of 10 μg/kg of finished poultry feeds; *n* = Total number of samples; and SD = Standard Deviation.

3.4. Fumonisin contamination of poultry layer feed sampled from Nairobi County, Kenya

One hundred and thirty six samples (91%) had detectable levels of FUM, while 14 samples (9%) had no detectable levels of FUM. No sample had FUM levels higher than the recommended European Union (EU) guidance limit of 20000 μg / kg (FAO, 2003). Total FUM contamination ranged from 210 μg / kg to 15173 μg / kg. The mean of total positives FUM contamination in chick mash, grower mash and layers mash were 2020 μg / kg, 1620 μg / kg, 1460 μg / kg, respectively (Table 4). Ruai recorded the highest total FUM mean of 2450 μg / kg while Kasarani had the lowest total FUM mean of 1110 μg / kg (Table 5). Two-way Analysis of Variance revealed that total FUM levels were not significantly different among the feed types (F = 0.169, df = 2, p = 0.844) but were significantly different among the regions (F = 5.524, df = 4, p = 0.000) in Nairobi County. There was no

significant interaction effect observed between the region and the feed type on the total FUM levels in the feed samples, ($F = 1.427$, df = 8, $p = 0.193$).

Table 5: Fumonisin levels of layer feeds grouped by feed type in Nairobi County, Kenya

Feed type	Occurrence (n) %	Range of total FUM (mg/kg)	Mean ± SD (mg/kg)
Chick mash (*n* = 50)	44 (88)	0.21-15.2	2.02 ± 15.2
Grower mash (*n* = 50)	48 (96)	0.22-13.0	1.62 ± 13.0
Layer mash (*n* = 50)	44 (88)	0.24-12.3	1.46 ± 12.3

Note: FUM = Fumonisin; *n* = Total number of samples; and SD = Standard Deviation.

Table 6: Fumonisin levels of layer feeds grouped by regions in Nairobi County, Kenya

Region	Occurrence (n) %	Range of total FUM (mg/kg)	Mean ± SD (mg/kg)
Dagoretti south (*n* = 30)	30 (100)	0.26-4.25	1.40 ± 0.868
Kasarani (*n* = 30)	26 (87)	0.32-4.85	1.11 ± 1.11
Langata(*n* = 30)	22 (73)	0.21-13.0	1.45 ± 2.79
Roysambu (*n* = 30)	29 (97)	0.22-12.3	1.94 ± 2.49
Ruai(*n* = 30)	29 (97)	0.24-15.2	2.45 ± 3.21

Note: FUM = Fumonisin; *n* = Total number of samples; and SD = Standard Deviation.

4. Discussion

4.1. Fungal contamination of layer feeds in Nairobi County, Kenya

The mean CFU of chick mash was 6.11×10^{-2}, grower mash 5.80×10^{-2} and layer mash 4.73×10^{-2}. Fungal count act as indicators of the hygienic quality and safety of the feed (Krnjaja *et al*., 2008; and Dalcero *et al*., 1998). Chick mash was the most contaminated as compared to grower and layers mash. This could be because of longer storage of chick mash by the agrovets and manufacturers due to less demand by farmers. The longer the storage period of farm products, the higher the fungal load and toxin contamination of those products (Salisu and Almajir, 2020; and Mokubedi *et al*., 2019). Poultry feeds can become contaminated either directly or indirectly through contact with soil, rodent, birds, dust, human carrier, sewage or water during processing and storage (Sultana *et al*., 2017). Fungal growth results in losses in volume, quality of feed ingredients and subsequently feeds made from them (Okoli *et al*., 2006). Similar results were observed by Ghaemmaghami *et al*. (2016) who reported CFU between 1 and 1.2×10^{-3} with a mean of 0.302×10^{-3} in finished feed. Mean mold count of 7.0×10^{-2} was reported Cegielska-Radziejewska *et al*. (2013) with growers mash reporting a higher mean CFU of 3.2×10^{-2}. The average CFU in mashed feeds was 15×10^{-3} while that of pelleted feeds was 11×10^{-2} with the fungal load being higher in the finisher feed as compared to the starter feed (Ghaemmaghami *et al*., 2018). Poultry feed mixtures from Slovakia were found to have a mean count of 1.8×10^{-3} (Labuda and Tanèinová, 2006) while Shareef (2010) reported an average count of 7.2×10^{-5} CFU / g from finished feed in Nineveh. Studies regarding microbiological quality of feed materials used between 2009 to 2012 in Poland revealed mycological counts of up to 10^{-8} in cereal grains (Kukier *et al*., 2013). Counts of 2.18×10^{-3}, 5.13×10^{-3} and 3.27×10^{-3} were observed in dichloran rose bengal chloramphenicol agar (DRBC), dichloran 18% glycerol agar (DG18) and dichloran chloramphenicol peptone agar (DCPA) respectively by Oliveira *et al*. (2006). Queiroz *et al*. (2013) on the other hand reported fungal counts in the range 1.0×10^{-3} to 2.3×10^{-6} in DRBC and 1.0×10^{-2} to 2.9×10^{-6} in DG18 media. Fungal counts ranging from 6.6×10^{-3} to 6.3×10^{-5} CFU / g were seen in DRBC medium from poultry feeds collected from Rio Cuarto, Argentina (Dalcero *et al*., 1998) while counts of 1-2.41×10^{-5} were reported by Krnjaja *et al*. (2017). Mold counts higher than 1×10^{-5} were reported by Rosa *et al*. (2006)

while mean mold counts in the range of 3.0×10^{-4} to 9.6×10^{-5} were reported from four different brand of poultry feeds each constituting starter, grower, layer and finisher feed types (Ukaegbu-Obi *et al*., 2017). 2.0×10^{-5}, 7.0×10^{-5} and 1×10^{-6} were the counts observed in Umuahia, Osisioma feed distributors and poultry farms respectively in Abia state, Nigeria by Okechukwu *et al*. (2019) while in Abeokuta fungal counts between 4×10^{-3} and 42×10^{-3} were observed by Kehinde *et al*. (2014).

4.2. Mycoflora of poultry layer feeds in Nairobi County, Kenya

Aspergillus spp, *Penicillium spp*, *Fusarium spp*, *Mucor spp*, *Rhizopus spp*, *Cladosporium spp*, *and Cochiliobulus spp* were isolated from the collected feed samples. Similar results were obtained by Ariyo *et al*. (2013), Greco *et al*. (2014), Cegielska-Radziejewska *et al*. (2013), Ibrahim *et al*. (2017); and Ukaegbu-Obi *et al*. (2017). *Aspergillus* (90.67%), *Penicillium* (77.33%) and *Fusarium* (13.33%) were the most prevalent fungi isolated. This results are in accordance with Embaby *et al*. (2015), Shareef (2010), Saleemi *et al*. (2010), Sivakumar *et al*. (2014) and Okechukwu *et al*. (2019). Poultry feed have been found to be the more predominant animal feed infected with mycotoxigenic fungi (Sivakumar *et al*., 2014). *Aspergillus* and *Penicillium* are considered storage fungi while *Fusarium* species are often classified as field fungi (Kotinagu *et al*., 2015). An estimated 20-45% of world cereal production are contaminated with storage fungi (Harcarova *et al*., 2018). Field fungi require 70%-90% relative humidity, temperatures between 20 °C and 25 °C, water activity (a_w) greater than 0.85 for active growth, and 0.99 a_w for optimal growth of whereas most of *Aspergillus* and *Penicillium* species require a minimum a_w of 0.75-0.85 and grow well at 0.93-0.98 a_w (Agriopoulou *et al*., 2020). *Aspergillus spp* can adapt to temperatures of 30 °C-40 °C while *Penicillium spp* exhibits optimal growth at temperatures between 25 °C-30 °C (Agriopoulou *et al*., 2020). *Aspergillus spp* has been found to be predominant in cereals and other ingredients used in poultry feed production (Ariyo *et al*., 2013). Nairobi is located towards the south of the equator with the warmest average daily maximum temperature of 27.5 °C occurring during January to March and coolest daily maximum temperature of 22.5 °C occurring between June to August (UOC, 2017). It receives a mean annual rainfall of 879 mm with rainfall occurring in two seasons, long rains occurring from March to May recording around 310 mm and short rains occurring from November to December recording around 200 mm (Obiero and Onyando, 2013; and UOC, 2017).

Contrast to our results, Algabr *et al*. (2018), Ghaemmaghami *et al*. (2016) and Krnjaja *et al*. (2017) reported *Fusarium* as being the dominant fungal genera followed by *Aspergillus* and *Penicillium* while Greco *et al*. (2014) also found *Fusarium* to be the most dominant mycotoxigenic fungi but followed by *Eurotium*, *Penicillium* and finally *Aspergillus*. Dalcero *et al*. (1998) and Kubizna *et al*. (2011) identified *Aspergillus* and *Fusarium* as dominant genera isolated in poultry feeds. Many studies have depicted *Aspergillus* and *Penicillium* as the frequent fungal genera contaminating poultry feeds (Aliyu *et al*., 2012; Ariyo *et al*., 2013; Heperkan and Alperden, 1988; Labuda and Tanèinová, 2006; Oliveira *et al*., 2006; and Shareef, 2010). *Aspergillus*, *Cladosporium*, and *Penicillium* were the frequently isolated from feeds intended for ornamental birds in Brazil (Queiroz *et al*., 2013). *Mucor, Rhizopus* and Yeast were found to be the most common fungi occurring in commercial poultry feed in Imo state, Nigeria (Okoli *et al*., 2006) whereas other studies reported *Rhizopus* as the most prevalent fungi in feed samples (Cegielska-Radziejewska *et al*., 2013; Osho *et al*., 2007; and Uwaezuoke and Ogbulie, 2010).

4.3. Aflatoxin and Fumonisin contamination of poultry layer feeds in Nairobi County, Kenya

Fumonisins was detected in 91% of the samples, while aflatoxin was detected in 64% of the feed samples. Fifty eight percent (58%) of the samples had AF levels within the KEBS recommended limit of 10 μg / kg, while no sample had fumonisin levels greater than the EU guidance limit of 20000 μg / kg (FAO, 2003). Similar range of aflatoxin was reported in feeds collected from the southern, central, northern and east coast regions of Peninsular Malaysia (Wan *et al*., 2017). Mokubedi *et al*. (2019) reported Fumonisin B1(FB1) as the most dominant mycotoxin recovered from feed samples from South Africa with a mean 1075.6 μg / kg with a maximum concentration of 7125.3 μg / kg and a mean AF 0.5 μg / kg with a maximum concentration of 3.7 μg / kg. Fumonisin was also the most frequently isolated mycotoxin from poultry feed and feed ingredients in Nigeria reporting a mean of 1014 μg / kg and a mean AF of 74 μg / kg (Akinmusire *et al*., 2019). Fumonisin was detected in all feed samples from Argentina with a median of 1.750 μg / kg while aflatoxin was detected in 90% of the samples with a median of 2.685 μg / kg (Greco *et al*., 2014). Queiroz *et al*. (2013) reported fumonisin contamination in 95% of total samples with levels from 92 to 668 μg / kg and aflatoxins contamination in 40% of total samples with levels between 12 and 902 μg / kg.

Aflatoxins, however, were the most frequently isolated from grain and feed commodities from Kenya, Nigeria and Ghana and this was attributed to their warmer climates (Rodrigues *et al*., 2011). Ranges between 16 and 1930 μg / kg of FB1 with a mean of 468 μg / kg were observed in poultry feeds from Cameroon with a mean AF of 40 μg / kg (Abia *et al*., 2013). Kana *et al*. (2013) found 87% of both broiler and layer feeds were contaminated with aflatoxin recording a mean AF 11.1 μg / kg and 6.6 μg / kg respectively. However, Shareef, (2010) reported aflatoxins and ochratoxins as the most frequently occurring mycotoxins closely followed by fumonisins with means of 179.1 μg / kg, 159.4 μg / kg, and 127 μg / kg respectively.

Mycotoxins are more widespread in developing countries due to improper agricultural, storage and processing practices (Velmurugu, 2009). Storage temperatures between 25 °C and 30 °C coupled with 97% relative humidity greatly favor the production of toxins (Sivakumar *et al*., 2014). Aflatoxins are the most common and toxic mycotoxins in poultry feed and majorly contaminate maize which is a chief component of the feed (Salisu and Almajir, 2020). Stress caused by dry conditions, wounding by insects, rain prior to or during harvesting, delayed harvest and late irrigation are factors associated with increased aflatoxin levels in grains (Milani, 2013). Fumonisin B1 (FB1) and Fumonisin B2 (FB2) production in maize by *F.proliferatum* and *F. verticilloides* is greatly favored at 0.956 a_w and 0.968 a_w with temperatures between 25 °C and 30 °C (Marin *et al*., 1995). The detection of both aflatoxin and fumonisins in our study could be attributed to the isolation of toxigenic *A. flavus* and *F. verticilloides*. Among the three different types of feeds, chick mash once again exhibited the highest levels of aflatoxins and fumonisins. This may still be attributed to the lower demand of feed as compared to the other two feed types, thus leading to longer storage. Kehinde *et al*., (2014) reported ranges between 13.5 to 95.1 μg / kg in feeds in Abeokuta, Nigeria while Gherbawy *et al*. (2019) reported range between 0.90-60 μg / kg in feeds in the western region of Saudi Arabia. Kobashigawa *et al*. (2019) reported a mean AF of 3.8 ± 4.8 μg / kg and a mean FUM of 1,310 ± 1,050 μg / kg in finished feed stored in farms with 42 samples equal or above the limit of quantification. Kajuna *et al*. (2013) found 68% of feed samples from Morogoro, Tanzania were contaminated with Aflatoxin B1 (AFB1) with layers mash recording a mean AFB1 level of 15.1 ± 22.2 μg / kg. Low ranges of aflatoxin and fumonisin 0.02-22 ppb and 0.5-4.6 ppm respectively were reported by Agyei-henaku *et al*. (2019).

5. Conclusion

Our results confirm aflatoxin contamination in layer feeds in small scale layer farms in Nairobi County. This not only negatively impact the productivity of layer hens but pose health concern to the public at large as their residues may be transferred to the eggs and meat meant for human consumption. Aflatoxins are potent carcinogens and have been linked to hepatocellular carcinoma in human beings. Mitigation measures thus need to be put in place to minimize and control contamination of layer feeds with mycotoxins. To increase in demand for commodities used in production of animal feeds has been on the rise due to high demand of livestock production. This has led to increased awareness of animal feed safety. Animal feeds play a crucial role in farm animal to human food chain, therefore, infectious and non-infectious hazards existent in feed pose a threat to human health. One of the major hazards are mycotoxins which are commonly found in cereals used in the production of animal feeds. Mycotoxins are more prevalent in tropical and subtropical climates and pose a serious threat to feed supply chain, animal and human health. The detection of both aflatoxin and fumonisin in the feed samples is of major concern in the poultry industry. Therefore, there is need for tougher implementation of regulation to ensure food and feed safety. Mitigation measures also need to be enforced to effectively manage mycotoxin contamination.

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