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Multi-mycotoxin occurrence and their risk to poultry health in semi-intensive broiler farms in Kenya

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ABSTRACT

Scarcity of feed ingredients, unregulated feed mills, and limited monitoring of mycotoxin levels in feed increase the risk of mycotoxin exposure for poultry in sub-Saharan Africa. This study examined mycotoxins in feed from 122 Kenyan broiler farms and an association between on-farm feed handling practices and mycotoxin levels. Using a validated multi-mycotoxin liquid chromatography-tandem mass spectrometry method (LC-MS/MS), all feed samples contained at least one mycotoxin and 93 % (n=113) had >3 mycotoxins. The most prevalent EU-regulated mycotoxins detected were fumonisins (93 %; 79.2 – 1285.3 µg/kg), deoxynivalenol (88 %; 96.6 – 2131.2 µg/kg), aflatoxins (34 %; 4.6 – 87.8 µg/kg), and ochratoxin A (4 %; range 14.90 – 59.20 µg/kg). Deoxynivalenol, fumonisins, and zearalenone frequently co-occurred in the feed samples. Among the surveyed farms, 33 % (n= 40) were at risk of subclinical exposure to deoxynivalenol, while 14 % and 7 % faced similar risks from total aflatoxins and fumonisins, respectively. Univariate analysis found no significant associations between farm-specific feed handling practices and mycotoxin levels in feed. This study found a high co-occurrence of mycotoxin at low to moderate concentrations in compound broiler feed from the selected farms. While these levels pose a potential risk, no direct link to broiler health outcomes was found. Our findings highlight the need for further research to explore the effects of subclinical mycotoxin exposure on broilers and to develop context-specific mycotoxin level guidelines for the region.

Introduction

Feed costs constitute a significant portion of input expenses in broiler farming as diet plays a pivotal role in the growth performance of birds (Abdollahi et al., 2018; Aftab et al., 2018; FAO, 2022). In commercial broiler systems in Kenya, farmers primarily depend on compound commercial feed purchased from animal feed retail shops (FAO, 2018a; Onono et al., 2018; Onono, 2022). Broiler farmers in Kenya generally follow a two-phase feeding regimen, starting with starter mash or crumbs from day 1 to week 3, followed by finisher mash, pellets or crumbs from weeks 4 to 6, when birds are sold. Some farms follow a three-phase regimen by incorporating a grower feed during a middle phase before transitioning to the finisher feed (Onono, 2022).

The Kenyan feed industry is relatively well-developed, with the Association of Kenya Feed Manufacturers (AKEFEMA) overseeing 70 % of the compound feed distributed nationwide. AKEFEMA includes over 100 members, comprising raw material suppliers, animal feed manufacturers, and producers of mineral supplements (Abro et al., 2020; Karuri, 2023). Various regulations, including the Standards Act (Cap 496) and the Fertilizer and Animal Foodstuff Act (Cap 345), provide a legal framework to ensure feed safety, quality, and fair trade. However, the industry faces significant challenges, such as the rise of unregulated millers and a heavy reliance on imported raw materials, which increases the risk of substandard feed and ingredients through counterfeiting or

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adulteration (ABS TCM, 2013; Abro et al., 2020; Bartz, 2022; Karuri, 2023). Furthermore, effective monitoring is hindered by the limited number of accredited feed analysis laboratories in the country (ABS TCM, 2013; Karuri, 2023).

A major challenge to poultry feed safety is the presence of mycotoxin-producing fungi in feed ingredients and finished feed products, with their proliferation often increased in sub-Saharan Africa due to favorable environmental conditions (Kana et al., 2013; Ochieng et al., 2021). Mycotoxins are naturally occurring secondary metabolites of fungi or mould that are toxic to animals and humans even at low concentrations (Peng et al., 2018). The main mycotoxins of concern are produced by fungi belonging to the genera *Fusarium, Aspergillus, Cladosporium, Alternaria, Claviceps*, and *Penicillium* (Peng et al., 2018; Ochieng et al., 2021). Mycotoxins are common in poultry feed due to the ubiquitous occurrence of fungi producing several metabolites (Battilani et al., 2020; Kemboi et al., 2020; Kagot et al., 2022). Co-occurrence can result in synergistic, additive or antagonistic effects, varying the toxicological impacts on target species (Battilani et al., 2020; Ochieng et al., 2023).

While various mycotoxins have been detected in the region, most legislations and research efforts focus on aflatoxins, particularly aflatoxin B_1 (AFB_1), a potent hepatocarcinogen in humans and animals (Alonso-Jauregui et al., 2021; Kemboi et al., 2020; Mengesha et al., 2024; Nakavuma et al., 2020; Ochieng et al., 2021). Kenya and the East African Community set maximum limits for total aflatoxins at 50 μg/kg and AFB_1 at 20 μg/kg for adult poultry and 10 μg/kg for young poultry (Sirma et al., 2018). In poultry, high aflatoxin levels are linked to impaired growth, immunosuppression, increased disease susceptibility, pathological lesions, and mortality (Antonissen et al., 2015; Battilani et al., 2020; Ochieng et al., 2021).

Growing awareness of the harmful effects of various mycotoxins has led to increased multi-mycotoxin studies in the region, highlighting the presence of fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEN), and nivalenol (NIV) (Changwa et al., 2021; Kemboi et al., 2020; Kagot et al., 2022; Mwihia et al., 2020). The European Union sets permissible limits for poultry feed at 5000 μg/kg for DON, 250 μg/kg for ZEN, 100 μg/kg for ochratoxin A (OTA), and 20,000 μg/kg for FUMs. In contrast, the USA limits are slightly higher at 10,000 μg/kg for DON and 30,000 μg/kg for FUMs (Dohlman, 2007; Ochieng et al., 2021; The Commission of the European Communities, 2006). Mycotoxins, even at levels below legislated limits, can adversely affect broiler health (Kolawole et al., 2020). For example, low doses of naturally contaminated dietary *Fusarium* toxins, below the EU regulatory limits, have been shown to impair production performance and intestinal health, cause immunosuppression, and lead to vaccine failure, increasing susceptibility to infectious diseases (Kolawole et al., 2020; Lucke et al., 2017; Santos and van Eerden, 2021).

Linking mycotoxin contamination to pathological symptoms in small-scale intensive broiler farms in Kenya is challenging due to the short feed storage duration and the short production cycles of broilers (4-6 weeks). Additionally, mycotoxin testing is infrequent due to limited access to accredited laboratories and high analysis costs (Kibugu et al., 2022). Therefore, the region's monitoring and data on mycotoxin prevalence in poultry feed remains scarce. This study aims to assess the occurrence of mycotoxins in commercial broiler finisher feeds collected from small-scale farmers (200-2000 birds) in Kenya, identify factors influencing their presence, and model their potential health risk to poultry.

Materials and methods

Study site and survey

The study was conducted in three peri-urban counties in Kenya: Kiambu, Machakos and Kajiado county. These counties were purposively selected due to their high population of broiler farmers and their proximity to Nairobi County, a major market for poultry and poultry products (KNBS, 2019). One hundred and twenty-nine farms were proportionately selected from a sampling pool of 1711 farms: 80 (5 %) in Machakos, 170 (10 %) in Kajiado and 1461 (85 %) in Kiambu County; and visited at the end of the broiler production cycle. The farms selected were based on an inclusion criterion of having between 200 and 2000 birds, which was representative of broiler farms in these counties. These farms are categorized as medium-scale intensive broiler farms that fall within sectors two and three of the FAO classification of poultry production systems, having between low and moderate biosecurity measures (FAO, 2018b). Data collection, through face-to-face interviews, was carried out by trained enumerators using an Open Data Kit (ODK) tool developed at the International Livestock Research Institute (ILRI) which provided information regarding their farming and feed storage practices, and broiler health indicators like mortality and disease symptoms (Muloi et al., 2024).

Collection of feed samples

In each farm, one commercial feed sample of approximately 300 – 500 g was collected. The representative sample was collected from different sections of the commercial feed bag (top, middle, and bottom of the open bag) found in the feed storeroom or from different poultry feeders in the poultry house if the feed storeroom was empty. These samples were then stored in plastic specimen bags and transported to the lab in cooler boxes before being stored at -80 °C until grinding. Feed samples were collected from 124 farms, with five farms excluded as feed had been exhausted at sampling due to flock depopulation. Two more farms were excluded from the analysis as they only had starter feed, leaving 122 samples for analysis.

Sample preparation

The boiler finisher feed samples, (90 % pellets, 10 % mash) were milled into smaller particles using the Romer Series II Mill (Romer Labs, Inc.; Getzersdorf, Austria). Thereafter, 25 g subsamples in duplicate, were stored at -20 °C until further analysis.

Multi-mycotoxin analysis

A validated LC-MS/MS method, using a Waters® Acquity UPLC system linked to a Waters® Quattro Premier XE™ tandem-quadrupole mass spectrometer (Waters, Milford, MA) was used for the sample analysis, with the Quanlynx application used for data processing as described by (Monbaliu et al., 2010). This method was used to detect and quantify mycotoxins both under EU legislation including AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), DON, OTA, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), fumonisin B_3 (FB_3), ZEN, T-2 toxin (T-2), and HT-2 toxin (HT-2), and those with no legislation like 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), NIV, neosolaniol (NEO), fusarenone-X (F-X), diacetoxyscirpenol (DAS), alternariol monomethyl ether (AME), alternariol (AOH), sterigmatocystine (STERIG), roquefortine-C (ROQ-C), and enniatin B (ENNB).

Briefly, 5.00 g (±0.05 g) of each sample was extracted with 20 mL of Acetonitrile/water/acetic acid (79/20/1), agitated for 1 h and then centrifuged for 15 min at 4000 rpm (3291 g). C_{18}-solid phase extraction (SPE) columns (Phenomenex, Utrecht, Holland) were preconditioned by passing 2 × 5 mL acetonitrile/water/acetic acid (79/20/1) before filtering the supernatant into 25 mL volumetric flasks which were then adjusted using the same extraction solvent. This filtrate was then defatted using hexane and then 10 mL of this was mixed with 20mL of acetonitrile/acetic acid (99/1) and purified using Multisep® 226 columns (Biopure, Romer Labs, Inc.; Getzersdorf, Austria). Another 6 mL of the hexane-defatted extract was passed through a glass microfiber filter paper (47 mm) (Whatman, Cytiva) to produce a filtered extract.

Subsequently, 2 mL of this filtered extract was added to the cleaned-up eluent from the Multisep® 226 columns and placed in a water bath (40 °C) to dry under a gentle nitrogen flow. The resulting residue was redissolved in injection solvent before the LC-MS/MS analysis (Monbaliu et al., 2010). Mycotoxins were then quantified using the Quanlynx application, with those with values below the decision limit (CCα) regarded as absent.

Risk characterization of mycotoxin exposure

Further analysis was carried out to determine the relevance of our findings and whether the mycotoxin concentrations found in the poultry feed could result in adverse health effects for the broilers. Current legislation in many African countries, including Kenya, focuses on aflatoxins, with non-aflatoxin mycotoxins rarely investigated, even in accredited laboratories (Kibugu et al., 2022; Ochieng et al., 2021). Therefore, the European Food Safety Authority (EFSA) scientific opinions, which provide a risk assessment of selected mycotoxins and their adverse health effects on different animal species, including broilers, were utilized for these toxins. Through these publications, the Lowest Observed Adverse Effect Level (LOAEL) for the individual mycotoxins was stated as 2.5 mg/kg feed for the sum of FUM (FB_1, FB_2, and FB_3) (EFSA CONTAM Panel et al., 2022), 1.9 mg/kg feed for DON and its acetylated forms (3-ADON and 15-ADON) (EFSA CONTAM Panel et al., 2023b), 0.1 mg/kg feed for OTA(EFSA CONTAM Panel et al., 2023a), 3 mg/kg feed for NIV (EFSA CONTAM Panel, 2013), and 200 mg/kg for ZEN, a mycotoxin to which broilers are regarded as resistant (EFSA CONTAM Panel et al., 2017). No LOAEL was provided for the risk of aflatoxins exposure in broilers, with the publications focusing on humans and dairy-producing animals, hence we used the EAC guideline of the maximum limit for total aflatoxins, 50 μg/kg for the risk classification of this toxin (EFSA CONTAM Panel et al., 2020). Although the LOAEL for OTA is provided, this was excluded from the analysis due to its low prevalence in the samples.

In classifying the risks, a Reference Point (RP) was determined by applying an uncertainty factor of 3, as done by the EFSA CONTAM panels when determining the No Adverse Effect Level (NOAEL). The risk posed by each mycotoxin in a farm was then classified as 1; low risk where the concentration was less than the RP (0.3 × LOAEL), 2; or medium risk where the concentration was between the RP and LOAEL and 3; high risk where the concentration was above the LOAEL. For samples where the mycotoxins of interest were absent, the risk was classified as 0.

Data analysis

Data analysis was performed using R version: 4.3.1. Descriptive statistics included calculating the summary statistics of the different mycotoxins and plotting of histograms, bar charts and the heat map.

The probability of co-occurrence was calculated using the mycotoxin occurrence data, using the co-occur function in R which is used to calculate pairwise co-occurrence patterns in community ecology datasets (Griffith et al., 2016). Additionally, a heatmap visualizing the pairwise associations summarizing positive, negative and random associations was plotted. The co-occurrence network diagram was then developed using igraph package in R with those mycotoxins that had over 40 % probability of co-occurrence (Csardi and Nepusz, 2006; Csárdi et al., 2025).

Categorical data representing the farm practices including the storage area, feed bag elevation, cleaning frequency, and cleaning techniques were described in proportions and frequencies. Thereafter, these were analyzed as possible drivers of the mycotoxin concentrations or 'risk levels' in the finisher feed samples through univariable analysis.

Results

Mycotoxin detection and quantification

All samples had at least one mycotoxin, 93 % (n=113) had > 3 mycotoxins, and 2 % (n=3) had at least 10 mycotoxins (Fig. 1a). We detected 17 of the 23 targeted mycotoxins. Total aflatoxins were detected in 34 % of the samples, ranging from 4.6 to 87.8 μg/kg, with one sample exceeding the Kenyan regulatory limit. AFB_1 was the most prevalent aflatoxin, found in 25 % of samples (range 8.7–57.6 μg/kg), with 4 % (n=5) exceeding 20 μg/kg (range 20.1–57.6 μg/kg) and one surpassing the maximum limit of 50 μg/kg. Other aflatoxins identified included AFB_2 in 20 % of samples (range 4.6–13.2 μg/kg), AFG_2 in 5 % (range 4.7–5.8 μg/kg), and AFG_1 in 2 % (range 7.0–12.1 μg/kg).

Other mycotoxins detected included FB_1+FB_2 (93 %; range 79.2 - 1285.3 μg/kg), OTA (4 %; range 14.9 - 59.2 μg/kg), and DON (88 %; 96.6 - 2131.2 μg/kg), which were all present in acceptable concentrations. Generally, FUM were also highly prevalent in the feed samples, with FB_1 (87 %; range 79.2 - 896.8 μg/kg), FB_2 (62 %; range 97.6 - 388.5 μg/kg) and FB_3 (47 %; range 53.1 - 176.6 μg/kg) being detected. Additional *Fusarium* mycotoxins, including ZEN and NIV, were also quantified (Table 1, Supplementary Table 1).

Mycotoxin Co-occurrence

The co-occurrence matrix revealed that the mycotoxin co-occurrence patterns were mostly random (Fig. 2a, Supplementary Table 2). However, there was a negative association between 15-ADON and the mycotoxins STERIG, AFB_2 and FB_3, and between OTA and DON. FB_2 had positive associations with AFB_1, 3-ADON, FB_3, ZEN, AFB_2, and STERIG. Expectedly, there were positive associations between DON and its acetylated forms 3-ADON and 15-ADON, and between different FUM pairs FB_1 +FB_2, FB_1 + FB_3, and FB_2 +FB_3. The mycotoxin AFB_1 was interestingly shown to have positive associations with not just other aflatoxins but also STERIG and FB_3.

Network analysis carried out in the mycotoxins that had > 40 % probability of co-occurring resulted in the selection of five mycotoxins (DON, FB_1, FB_2, FB_3, and ZEN) that had the highest occurrence in the samples, and high co-occurrence. The resulting seven pairs were as follows: DON—ZEN, DON—FB_1, DON—FB_2, DON—FB_3, ZEN—FB_1, FB_1—FB_2, and FB_1—FB_3 (Fig. 2b). Three mycotoxins, FB_3, FB_2 and ZEN, were each linked to two nodes, where each node represents a mycotoxin. FB_1 and DON were connected to four nodes, showing they had high co-occurrence with all the mycotoxins in the network (Fig. 2b). From this diagram, DON, FB_1 and FB_2 are the most likely found to co-occur.

Risk Characterization of Mycotoxins

Five groups of mycotoxins were analyzed for putative risk to poultry health, based on the LOAELs and chosen Reference Point value. For both NIV and ZEN, all the levels found were at risk level 1, i.e. less than a third of the LOAEL set for these toxins (Fig. 3, Supplementary Table 3). A descriptive analysis of the farm indicated that the impact of total DONs was likely to be the most common, with 33 % of farms (n= 40 samples) being at real risk of subclinical exposure to harmful levels of this mycotoxin following the recent EFSA guidelines. Moreover, 14 % of farms (n =17 samples) faced possible risk due to aflatoxin exposure, with 7 % (n= 8 samples) facing a risk due to fumonisin exposure.

Association of farm variables with mycotoxin risk levels

Of the farmers interviewed, 51 % (n=62) indicated that they stored the feed in the chicken coop, with only 46 % (n=54) having a separate room used as a storage space for their feed. Additionally, about 63 % (n=73) of them kept the feed elevated on pallets whereas the rest stored feed in direct contact with the ground. Moreover, 45 % (n=44) of the

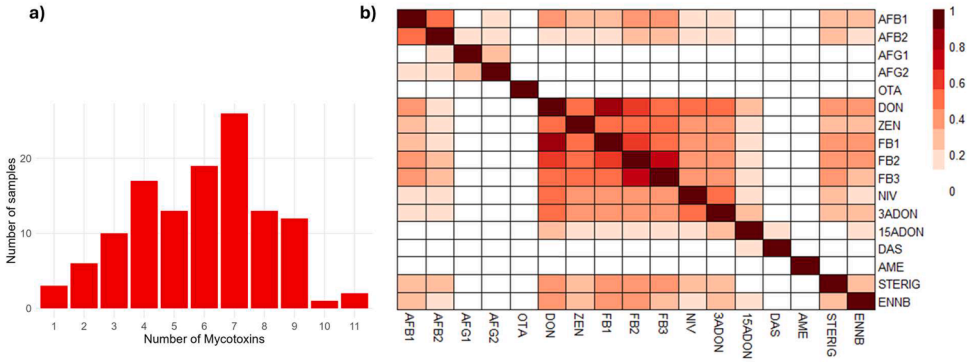

Fig. 1. The co-occurrence of mycotoxins in feed samples collected from broiler farms in Kajiado, Kiambu and Machakos counties, Kenya. a) Histogram showing the frequency of co-occurrence of samples with mycotoxins, x-axis; the number of mycotoxins in the sample, y-axis; frequency of samples. b) heatmap showing the observed two-way co-occurrence of common mycotoxins investigated in the study.
AFB1—aflatoxin B1, AFB2—aflatoxin B2, AFG1—aflatoxin G1, AFG2—aflatoxin G2, OTA – ochratoxin A, DON – deoxynivalenol, ZEN – zearalenone, FB1- fumonisin B1, FB2 – fumonisin B2, FB3 – fumonisin B3, NIV – nivalenol, 3-ADON – 3-acetyl deoxynivalenol, 15-ADON – 15-acetyl deoxynivalenol, DAS – diacetoxyscirpenol, AME – alternariol methylether, STERIG – sterigmatocystine, ENNB – enniatin B.

Table 1
The occurrence of EU-regulated and unregulated mycotoxins in finisher feed collected from broiler farms in Kenya in December 2022

Mycotoxin	%Positive samples	Decision limit (CCα) (μg/kg)	Minimum in positive samples (μg/kg)	1st Quartile of positive samples (μg/kg)	Median of positive samples (μg/kg)	3rd Quartile of positive samples (μg/kg)	Maximum in positive samples (μg/kg)	Average concentration in positives ± SD (μg/kg)
AFB_1	25 %	8.7	8.7	10.2	13.5	17.1	57.6	15.8± 9.6
AFB_2	20 %	2.8	4.6	4.9	5.1	6.4	13.2	6.4 ± 2.6
AFG_1	2 %	3.1	7.0	8.6	10.2	11.2	12.1	9.8± 2.6
AFG_2	5 %	3.3	4.7	4.9	5.0	5.5	5.8	5.5 ± 0.5
OTA	4 %	10.9	14.9	15.8	39.9	53.9	59.2	36.7 ± 20.8
DON	88 %	94.4	96.6	167.0	374.1	649.7	2131.2	442.6 ± 326.8
ZEN	47 %	25.2	30.9	82.8	118.2	193.2	776.5	164.8 ± 147.9
FB_1	87 %	79.2	79.2	148.9	228.9	318.9	896.8	252.7 ± 144.3
FB_2	62 %	93.1	97.6	130.8	153.4	205.0	388.5	172.7 ± 61.1
FB_3	47 %	51.4	53.1	63.6	80.2	101.2	176.6	85.49 ± 26.9
NIV	42 %	61.2	61.5	74.0	91.6	141.4	404.0	122.0 ± 75.8
3-ADON	41 %	15.9	16.5	26.7	31.6	38.0	62.4	32.3 ± 9.9
15-ADON	22 %	13.8	21.9	40.1	64.7	87.1	126.9	66.9 ± 32.9
DAS	9 %	0.9	1.00	1.15	1.5	1.55	3.9	1.60 ± 0.8
AME	2 %	43.9	64.8	66.9	68.9	71.0	73.0	68.9 ± 5.8
STERIG	40 %	11.2	11.3	14.6	24.9	26.8	32.8	21.6 ± 6.6
ENNB	44 %	13.6	13.7	39.4	61.0	126.8	515.1	97.1 ± 92.3

AFB_1– aflatoxin B_1, AFB_2 – aflatoxin B_2, AFG_1 – aflatoxin G_1, AFG_2 – aflatoxin G_2, OTA – ochratoxin A, DON – deoxynivalenol, ZEN – zearalenone, FB_1– fumonisin B_1, FB_2 – fumonisin B_2, FB_3 – fumonisin B_3, NIV – nivalenol, 3-ADON – 3-acetyl deoxynivalenol, 15-ADON – 15-acetyl deoxynivalenol, DAS – diacetoxyscirpenol, AME – alternariol monomethyl ether, STERIG – sterigmatocystine, ENNB – enniatin B.

Fig. 2. Figures showing the resulting co-occurrence in the mycotoxins. a) Co-occurrence matrix showing the random, positive, or negative probabilities of co-occurrence in select mycotoxins analyzed. Further information is found in Supplementary Table 2. b) Network diagram showing the co-occurrence between mycotoxin pairs that had > 0.4 probability of co-occurring. The nodes represent the mycotoxins, with the size of the node corresponding to the number of connections. The weight of the edges, which connect the nodes were normalized to represent the observed co-occurrence from the current samples.
From left to right: OTA – ochratoxin A, 15-ADON – 15-acetyl deoxynivalenol, AFG1—aflatoxin G1, AFG2—aflatoxin G2, DON – deoxynivalenol, NIV – nivalenol, STERIG – sterigmatocystine, AFB2—aflatoxin B2, ZEN – zearalenone, FB3 – fumonisin B3, 3-ADON – 3-acetyl deoxynivalenol, AFB1—aflatoxin B1, FB2 – fumonisin B2.

farmers stated that they cleaned the feed storage space weekly, 37 % (*n*=38) said monthly and less than 1 % shared that they cleaned the storage quarterly, while the rest admitted that they never cleaned the feed store (Supplementary Figure 1; Supplementary Table 4). Over a third of the farms (*n*=47) only swept the storage area, with 28 % (n=34) of the farmers using a disinfectant when cleaning.

At least 40 % (*n*=50) of the farmers reported that their birds were showing some disease symptoms at the time of the visit, with the most common symptom being water-belly (ascites) (32 %, *n*=15). Additionally, 10 % (*n*=5) of these farms described gut pathologies such as

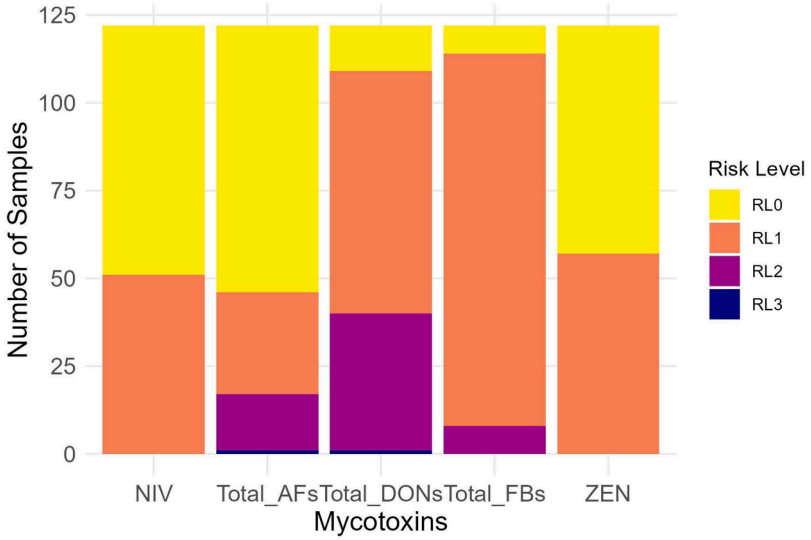

Fig. 3. Stacked bar graph showing the distribution of risk-level classes for the samples regarding five mycotoxin groups; Total AFs, Total DONs, Total FBs, NIV, and ZEN. Risk Value 0- Mycotoxin not detected, 1- Mycotoxin level < 0.3 × LOAEL, 2- 0.3 × LOAEL < Mycotoxin Level < LOAEL, 3- Mycotoxin Level > LOAEL. Total_AFs; AFB1 +AFB2 + AFG1 +AFG2, Total_DONs; DON + 3-ADON + 15-ADON, Total_FBs; FB1 + FB2 + FB3. Where AFB1—aflatoxin B1, AFB2—aflatoxin B2, AFG1—aflatoxin G1, AFG2—aflatoxin G2, DON – deoxynivalenol, 3-ADON – 3-acetyldeoxynivalenol, 15-ADON – 15-acetyldeoxynivalenol, FB1- fumonisin B1, FB2 – fumonisin B2, FB3 – fumonisin B3, NIV – nivalenol, ZEN – zearalenone.

bloody, yellow, white, or green diarrhoea. Other symptoms described by at least one farmer included rales (6 % (*n*=3) of cases), retarded growth (10 % (*n*=5) of cases), drooping wings and/or legs (6 % (*n*=3) of cases), decreased appetite (2 % (*n*=1) of cases), sneezing (8 % (n=4) of cases), weakness (4 % (n=2) of cases) and sudden death (12 % (n=6) of cases). Our univariate logistic regression evaluating the relationship between various farm-level demographics and feed mycotoxin contamination did not reveal a statistically significant association (Supplementary Tables 3, 4).

Discussion

This study assessed the risk of mycotoxin exposure to broilers in smallholder farms in Kenya. While tropical conditions are known to promote fungal growth and mycotoxin occurrence, their impact on poultry health remains unclear and is explored in this research (Aboagye-Nuamah et al., 2021; Chilaka et al., 2022; Gruber-Dorninger et al., 2019). The mycotoxins detected align with those reported in previous studies on animal feed in the region (Kemboi et al., 2020; Ochieng et al., 2021). Except for aflatoxin B_1, all quantified mycotoxins were within EU regulatory limits.

The co-occurrence of mycotoxins observed in this study, agreed with earlier findings, including a global study that indicated that at least 64 % of feed samples were co-contaminated with two or more toxins, with African compound feed frequently containing combinations of DON, FBs, and ZEN (Gruber-Dorninger et al., 2019). The positive associations between STERIG and AFs can be attributed to their production by fungal species, primarily within the genus *Aspergillus*. STERIG also shares a biosynthetic pathway with aflatoxins as a precursor of AFB_1 and AFG_1. In highly aflatoxigenic fungi, STERIG is rapidly converted to *O*-methylsterigmatocystin, leading to aflatoxin formation. However, in species that are unable to support this conversion, STERIG accumulation occurs (Nieto et al., 2018; Zingales et al., 2020).

The high incidence of *Fusarium* mycotoxins in these samples suggests the proliferation of field toxigenic fungi that introduced these mycotoxins into feed ingredients before harvest and processing (Tola and Kebede, 2016). The presence of mycotoxins such as OTA, AFs, and some FUMs can be attributed to storage fungi, primarily from the Penicillium and Aspergillus genera (Daou et al., 2021; Tola and Kebede, 2016). The relatively low concentrations of mycotoxins from both field and storage fungi indicate adherence to good harvest and storage practices, reducing contamination. This highlights the importance of a comprehensive approach to controlling mycotoxins in poultry feed, involving both end-user and upstream mitigation strategies targeting toxigenic fungi within the commercial feed production chain. Implementing a Hazard Analysis Critical Control Point (HACCP) system, as recommended by the Codex Alimentarius Commission, is essential for stakeholders in feed production and utilization to prevent and manage mycotoxin contamination (FAO, 2001; Stoev, 2013).

The farms included were located in two counties with distinct agroecological zones. Kiambu County, located in the central highlands, experiences high annual rainfall and mild temperatures. Machakos and Kajiado are in the semi-arid uplands, characterized by lower annual rainfall and a higher risk for aridity and prolonged drought (Elnour et al., 2023; The World Bank Group, 2021). Although environmental factors such as temperature and humidity have been extensively discussed in relation to feed quality, we did not investigate these environmental factors and our results showed no significant difference in the commercial feed stored on these farms (Casu et al., 2024; Garcia-Cela and Gasperini, 2024; Ng'ang'a and Niyonshuti, 2022). We investigated possible associations between feed management practices, including the use of a designated feed storage room, the frequency of cleaning, and the cleaning methods used. While these practices have linked to fungal accumulation, and consequently, mycotoxin contamination, no clear association was observed between these practices and mycotoxin risk in the study farms (Golob, 2007; Matumba et al., 2021). A possible explanation for these findings is the high feed turnover among broiler farmers in the region, resulting in short-term storage that may help mitigate mycotoxin accumulation.

Our findings show that DON and FUM exposure in some farms could pose a health risk to the broilers. Exposure to DON has been previously linked to impacted intestinal health and predisposition to infectious diseases which can be accompanied by decreased body weight gain (Antonissen et al., 2015; EFSA CONTAM Panel et al., 2023b; Ochieng et al., 2021; Riahi et al., 2020). Similarly, subclinical exposure to *Fusarium* mycotoxins like DON and its acetylated form 15-ADON, FUMs and ZEN has also been shown to result in enhanced coccidiosis or delayed recovery of coccidia-challenged broilers (Girgis et al., 2010; Grenier et al., 2016).

Likewise, aflatoxin detected in 34 % of our samples could indicate

risk to broiler health as aflatoxin-contaminated diets have been linked to negative impacts on the metabolism and growth, hepatic histopathological changes, decreased body weight gain and even mortality in broilers (Aikore et al., 2019; Ochieng et al., 2023; Saminathan et al., 2018; Tessari et al., 2010). Evidence demonstrates that the immunosuppression caused by exposure to aflatoxins results in vaccination failure, poor antibody titers and even disease outbreaks that cause economic losses to the farmers (Murugesan et al., 2015; Ochieng et al., 2021). Moreover, bioaccumulation of aflatoxins in broiler tissues including the liver, kidneys, and some cases, muscle, has possible negative impacts on the consumers of broilers (Chang et al., 2020; Hussain et al., 2016; Ochieng et al., 2023). However, determining the exact cause of the disease symptoms witnessed in these farms, including gut challenges, can be complicated when there is a co-occurrence of these mycotoxins, as was the case in our samples. Additionally, there is not enough definitive evidence to link this mycotoxin exposure to the poor health symptoms observed. This statement is especially true in farm settings where other factors, including low biosecurity measures, adherence to vaccination programs, and welfare and management practices like stocking density and litter quality could play a role in broiler health (Dosković et al., 2019; EFSA AHAW Panel et al., 2023).

Recent studies indicating that lower mycotoxin concentrations could have adverse health effects on broilers have led to reviews by EFSA and other regulatory bodies to update their maximum limits (Grenier et al., 2016; Kolawole et al., 2020; Lucke et al., 2017, 2021; Ochieng et al., 2023; Riahi et al., 2020). These reviews, which rely on various scientific *in vivo* and *in vitro* studies, guided our limits and LOAEL values to assess the health risks posed by mycotoxins. However, these do not consider the difference between poultry production systems in LMICs and those in the study areas, including compound feed composition, rearing systems, farm management, and biosecurity, which could also impact flock health. This highlights the need to have local regulations and limits that can be directly applied to broiler production in the region.

One limitation of the study was that feed samples were collected once in the production cycle, and no animal specimen was collected for correlation between mycotoxins in feed, presence in animal tissues, and possible health effects in the broilers. Although the sample collection was designed to be representative of the on-farm mycotoxin exposure, mycotoxins are often not homogenously distributed as fungi grow in hotspots and not equally in feed and feed ingredients, which could under- or over-represent the actual mycotoxin exposure (Golob, 2007).

This study aimed to assess mycotoxin occurrence in commercial broiler finisher feeds collected in farms, identify factors influencing their occurrence, and model their potential health risk to poultry. The samples were contaminated with low to moderate levels of mycotoxins, with only AFB_1 being found above the EAC and EU regulation limits. DON was the most prevalent mycotoxin, followed by other *Fusarium* mycotoxins including FBs and ZEN. There was a high co-occurrence of mycotoxins, with 93 % of the feed samples containing more than three mycotoxins. Consequently, this complicates the analysis of the effect of these toxins on broiler health due to possible antagonistic, synergistic, or additive outcomes these toxins could have. There is a need for further studies that can demonstrate the impact of subclinical levels of mycotoxins on broilers and how to mitigate their toxic effects.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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