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Research Application Summary

Mycotoxins detected in chicken products fed with contaminated poultry feeds from smallholder farmers in Western Kenya

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Abstract

Poultry production among smallholder farmers in Western Kenya is a major enterprise, providing food, income and employment; however, birds are maintained with minimum inputs; scavenge feeds which could potentially be contaminated with mycotoxins. Feed quality impacts on growth performance, productivity, well-being, health and is key to profitability and sustainability in farming. Objectives of this study were to identify common fungal genera in indigenous chicken feeds and determine mycotoxin contamination of feeds, eggs and chicken tissues. A purposive multi-stage sampling survey design was used to collect 260 feed samples from 180 smallholder indigenous poultry farmers in Siaya, Busia and Kakamega Counties – Kenya. A total of 235 were analysed for both fungal and mycotoxin contamination. Additionally, 60 eggs and 240 chicken tissues (liver, kidney, breast and thigh muscles – n = 60 each) samples were collected. Molecular tools were used to identify fungal isolates from feeds while Competitive Enzyme-linked Immunosorbent assay was used to determine mycotoxin levels. Dominant mycotoxigenic fungal genera included A*spergillus* (32%), *Penicillium* (29%) and *Fusarium* (12%).Commercial feeds were significantly ($p<0.05$) contaminated (44.25ppb) with aflatoxin. Posho-mill waste, sorghum, maize and sorghum mixture, maize bran and maize were significantly ($p<0.05$) contaminated with fumonisin. For ochratoxin A, no feed exceeded regulatory limit of 100ppb.Eggs had traces of aflatoxin while liver had the highest (4.19±0.75) followed by breast muscle (3.57±0.59); but did not exceed regulatory limits of 10 ppb. Tissues sampled from free range production system had slightly higher levels of aflatoxin than semi-free range. Smallholder poultry farmers from Western Kenya use locally available feed resources which are contaminated with mycotoxins. Preventive measures and enhanced surveillance need to be instituted to mitigate the challenge and farmers sensitized on these measures.

Key words: Food safety, fungi isolates, indigenous chicken feeds, Mycotoxin contamination, Western Kenya

Résumé

La production de volaille chez les petits exploitants agricoles de l'Ouest du Kenya est une entreprise majeure, fournissant de la nourriture, des revenus et des emplois. Cependant, les oiseaux sont entretenus avec un minimum d'intrants ; récupérez des aliments potentiellement contaminés par des mycotoxines. La qualité des aliments a un impact sur les performances de croissance, la productivité, le bien-être, la santé et est essentielle à la rentabilité et à la durabilité de l'élevage. Les objectifs de cette étude étaient d'identifier les genres fongiques communs dans les aliments

pour poulets indigènes et de déterminer la contamination par les mycotoxines des aliments, des œufs et des tissus de poulet. Une conception d'enquête d'échantillonnage à plusieurs étapes a été utilisée pour collecter 260 échantillons d'aliments auprès de 180 petits éleveurs de volaille indigènes dans les comtés de Siaya, Busia et Kakamega - Kenya. Un total de 235 ont été analysés pour la contamination fongique et mycotoxine. De plus, 60 œufs et 240 échantillons de tissus de poulet (muscles du foie, des reins, de la poitrine et de la cuisse – n = 60 chacun) ont été prélevés. Des outils moléculaires ont été utilisés pour identifier les isolats fongiques dans les aliments tandis que le dosage immuno-enzymatique compétitif a été utilisé pour déterminer les niveaux de mycotoxines. Les genres de champignons mycotoxigènes dominants comprenaient Aspergillus (32 %), Penicillium (29 %) et Fusarium (12 %). Les aliments commerciaux étaient significativement ($p<0,05$) contaminés (44,25 ppb) par l'aflatoxine. Les déchets du moulin, le sorgho, le maïs et le mélange de sorgho, le son de maïs et le maïs étaient significativement ($p<0,05$) contaminés par les fumonisines. Pour l'ochratoxine A, aucun aliment ne dépassait la limite réglementaire de 100 ppb. Les œufs avaient des traces d'aflatoxine tandis que le foie en avait le plus ($4,19 \pm 0,75$) suivi du muscle de la poitrine ($3,57 \pm 0,59$); mais n'a pas dépassé les limites réglementaires de 10 ppb. Les tissus prélevés dans le système de production en plein air avaient des niveaux d'aflatoxine légèrement plus élevés que ceux en semi-libre. Les petits aviculteurs de l'Ouest du Kenya utilisent des ressources alimentaires disponibles localement qui sont contaminées par des mycotoxines. Des mesures préventives et une surveillance renforcée doivent être instituées pour atténuer le défi et les agriculteurs doivent être sensibilisés sur ces mesures.

Mots clés: Sécurité alimentaire, isolats de champignons, aliments indigènes pour poulets, contamination par les mycotoxines, Ouest du Kenya

Introduction

Food quality and safety are key issues in food and nutrition security and should be addressed at all levels along the value chain. Factors determining safety and quality include source, handling practices, processing methods and storage facilities and duration. Research findings have shown that post-harvest grain management practices can result into contamination and is exacerbated by high humidity, lack of aeration in storage areas and prolonged storage duration (Kaaya *et al.,* 2005; Lewis *et al.,* 2005; Wagacha and Muthomi, 2008). Consuming feed contaminated withmycotoxin can result into negative health consequences, not only on animal performance but also human. Impacts of consuming low doses of mycotoxins over prolonged period can be far reaching (Githanga *et al.,* 2019). Smallholder indigenous chicken farmers depend primarily on plant-based feeds which may be inadequate and of poor quality (Ochieng *et al.,* 2013; Pleadin, 2015). Animal feeds may contain pathogenic fungal species that compromise quality by reducing nutrient content, dry matter, causing sour flavour and most significantly by mycotoxin production (Iheshiulor *et al.,* 2011). Some of these toxins (aflatoxins and fumonisins) and fungi responsible for their production have been reported in cereals from several parts of Kenya (Josephat *et al.,* 2015; Mutiga *et al.,* 2015; Awuor *et al.,* 2020). Important mycotoxins commonly found in food and feed include aflatoxins, ochratoxin, fumonisins, trichothecenes and zearalenone (Milicevic *et al.,* 2010).

In developing nations, Kenya included, food security, safety and poverty eradication are key targets in Vision 2030. Indigenous chicken production has been perceived as one way to alleviate these challenges as it acts a source of food and income to smallholder farmers (Munyasi *et al.,* 2009). Advantages of chicken production include quick returns to investment and relatively simple management practices with numerous market outlets for products (Say, 1987; FAO, 1997). Small

holder farmers keep indigenous chicken as an enterprise because they are hardy, adapt well to rural environments, survive on low inputs, require small space, and scavenge on scattered grains, leafy vegetables and grass and adapt well to fluctuations in feed resources (Kingori *et al.,* 2010). However, Shuaib *et al.* (2010) reported that poultry are more susceptible to aflatoxins B1 even at small doses compared to other farm animals. Consuming mycotoxin in feed can result into toxic and carcinogenic effects in animals and humans (Bennett and Klich, 2003; Iheshiulor *et al.,* 2011). Depending on duration of exposure and dosage, mycotoxins can cause immune suppression, anorexia, poor growth rate, increased morbidity and mortality and decreased productivity (Akende *et al.,* 2006; Ezekiel *et al.,* 2011; Githanga, 2019). Mycotoxin intake compromises important production parameters like weight gain, feed intake, feed conversion efficiency and reproductive performance (Nemati *et al.,* 2014; Monson *et al.,* 2015).Exposure to mycotoxins was positively associated with low birth weight and concentration of antibodies toasexual malaria parasites and hepatitis B surface antigen in children (Githanga *et al.,* 2019). The study aimed at identifying common fungal genera that produce major mycotoxins and level of contamination in indigenous chicken feeds and chicken products, sampled from three counties of Western Kenya.

Materials and Methods

Study area and design. A cross-sectional household survey covering 180 smallholder farmers, 60 from each county, was conducted in Siaya, Busia and Kakamega Counties in Western Kenya between February and March 2016. In each county, three sub-counties were selected, viz: Siaya (Gem, Alego and Ugenya), Busia (Teso South, Matayos and Nambale) and Kakamega (Lurambi, Lugari and Navakholo). A three-stage purposive cluster sampling design (Chromy, 1979) was used; first, selecting the counties because they were within agro-ecological zones experiencing warm and humid weather conditions that promote mould growth and subsequent mycotoxins production, and indigenous chicken production is a major enterprise. Secondly, three sub-counties were selected based on number of active poultry farmer groups. Third, random sampling was used to select four farmer groups per sub-county and five farmers were chosen in each group, distinguished as either youth or women group, with guidance from county front-line extension officers.

Sample collection. Three out of the five farm-households per group were systematically selected at an interval of 2 for feed sampling, which was done by taking grabs from top, middle and bottom of storage container then mixing to obtain homogenous sample, then 0.5 kg sample drawn from mixture. To control moisture loss, samples were placed in paper bags, carefully sealed and stored at -20 °C, awaiting mycotoxins analysis. From each county, 20 eggs were sampled from 1st and 5th farmer for a total of 60. These samples were stored in egg trays kept at 4 ºC in a cold chamber until analysis for aflatoxin. A total of 60 chicken, 2 from 1st and 5th farmer (20 per county) were slaughtered and samples of thigh muscle, breast muscle, liver and kidney (total of 240) obtained. Each sample was placed in labelled zip-lock bag, packed in a cooler-box filled with ice and transported to laboratory for storage at -15 ºC until determination of aflatoxin levels was done. Analysis of fungal genera and mycotoxins in feeds, eggs and chicken tissues. From the 235 feed samples, fungi were isolated using dilution plating technique (Pitt and Hocking, 2009). A sub-set of indigenous chicken feeds that were collected during the baseline survey intended for mycotoxigenic fungi analysis was cultured for fungal growth at BeCA- ILRI Hub Laboratory in Nairobi, Kenya. Whole-grain feed samples intended for mycological and mycotoxins (aflatoxins, fumonisins and ochratoxins) were ground using a Romer mill. Out of 235 chicken feeds analysed for fungal contamination, 217 isolates representing each feed and region, were selected from a pool of 568 fungal isolates for further molecular identification. Selection was based on morphology of

fungi, feed type and region. Only morphologically different fungal species from the same feed type from different regions were selected for molecular identification. Among the 217 isolates, 195 fungal isolates were identified successfully up to species level. Extraction of aflatoxins was done using commercially available competitive Enzyme-Linked Immuno-sorbent Assay (ELISA) kits (Helica Biosystems Inc., CAT. No. 981AFL01LM-96), according to manufacturer's instructions. Extraction of fumonisin was done using commercially available ELISA kits (Helica Biosystems Inc., CAT. No. 951FUM01C-96) following manufacturer's instructions. Ochratoxin A was also extracted using commercially available ELISA kits (Helica Biosystems Inc., CAT. No. 941OCH01M-96). Egg samples were analyzed for aflatoxin levels using commercially available ELISA kit (Helica Biosystems Inc., Total Aflatoxin Assay, Cat. No. 941AFL01M-96, USA) following the manufacturer's instructions. The same was used in chicken tissue sample analysis.
Data analysis. Data were analyzed using Statistical Package for Social Sciences (SPSS), Version 20 and MS Excel 2016 to generate values for fungus, aflatoxin, fumonisin and ochratoxin A contamination and the means wereseparated by Scheffe' procedure for significance at $p< 0.05$). GENSTAT, 14th Edition – ANOVA, was used to analyze egg and tissues data.

Results and Discussion

Fungal incidence and mycotoxin contamination of indigenous chicken feeds from Western Kenya. The frequencies of 16 fungi genera identified in chicken feed samples differed significantly (Table 1). *Aspergillus* spp. was the most frequently (32%) isolated fungi genera, followed by *Penicillium* spp (29%) and *Fusarium* spp (23%), while others such as Sarocladium spp, Rhizomucor spp and Monascus spp were among the least, at 1%. Fungi are the most frequently found microorganism in feeds and their primary source in feeds is usually plant material (Cegielska-Radziejewska *et al.,* 2013). In this study, the most commonly isolated fungal species were *Aspergillus, Penicillium* and *Fusarium* genera in descending order.These are fungal isolates of great concern since they produce mycotoxins including, aflatoxins, fumonisins and ochratoxins A, respectively,; which can have carry-over effects to animal products, with negative consequences on human health if consumed (Adeyeye, 2016). Results here are similar to other studies which reported that *Aspergillus*, *Penicillium* and *Fusarium* genera are the most frequently isolated fungi in chicken feeds (Greco *et al.,* 2014; Krnjaja *et al.,* 2014; Josephat *et al.,* 2015). In the present study, A. flavus was most prevalent in homemade rations and commercial feeds as opposed to maize and sorghum. This may be due to poor handling along the value chain, exposing them to high risk of mycotoxin contamination. These findings are alsocomparable to those of Ariyo *et al.* (2013), who reported that both locally and commercially processed feeds had high levels of *Aspergillus* flavus. Several *Penicillium* spp and their associated toxins contaminate agricultural products, causing losses in various part of the world (Ismaiel and Papenbrock, 2015).Another dominant potential mycotoxin-producing genera present in samples were *Fusarium* species that are best known to produce an array of toxins like trichothecenes, zearalenone and deoxynivalenol (DON) beside fumonisins (Ismaiel and Papenbrock, 2015; Adeyeye, 2016). Results here concur with those of Greco *et al.* (2015) and Marijani *et al.* (2017), who reported low or no incidence of *Fusarium* spp in commercial feeds.

Contamination of feeds by mycotoxins. All feed samples were contaminated with aflatoxin at different levels (Table 2). Among the feeds analysed, commercial feeds were significantly ($p<0.05$) contaminated (44.25 ppb), followed by maize bran (14.03 ppb).Low levels of aflatoxin were recorded in sorghum, millet, cassava and maize (0.17, 0.21, 0.25 and 0.33 ppb, respectively); however, homemade ration, posho mill waste and brewers' waste had slightly high levels (7.70,

Table 1. Incidence of fungal genera isolated in chicken feeds sampled from Western Kenya

Fungi Genus	Frequency (N=195)	Incidence (%)
Aspergillus spp.	62	32
Penicillium spp.	56	29
Fusarium spp.	23	12
Talaromyces spp.	11	6
Epicoccum spp.	10	5
*Phom*a spp.	6	3
Sarocladium spp.	6	3
Cladosporium spp.	4	2
Coniothyrium spp.	2	1
Eurotium spp.	2	1
Chaetomium spp.	1	1
Davidiella spp.	1	1
Microsphaeropsis spp.	1	1
Monascus spp.	1	1
Rhizomucor spp.	1	1
Tricholoma spp.	1	1

6.77 and 6.63 ppb, respectively). Among all feed types analysed, commercial feeds were the most contaminated, having levels higher than regulatory limits (>10 ppb), with means significantly different ($p < 0.05$) from other feed types. Findings in this study are similar to that of Mutiga *et al.* (2015), who observed greater fumonisin than aflatoxin contamination in maize from Western Kenya; with only 2% and 8% of maize samples from Bungoma and Kisii, respectively, recording over 10 ppb levels of aflatoxins. These findings are also similar to that of Kana *et al.* (2013) who recorded an average of 1.0 ppb with a range of 2 to 42 ppb in maize used in the formulation of chicken feeds in Cameroon. Typically, maize used in commercial chicken feed production are neither clean nor sorted since they are cheap (Okiki *et al.,* 2010). It is possible that higher contamination levels observed in commercial feeds is due to prolonged duration taken from production, processing, and storage to marketing (Kajuna *et al.,* 2013). During processing, feeds risk being contaminated with mould spores, especially when cereals grains are ground, exposing large surface area to fungal attack and proliferation, and subsequent toxin production.

Feeds with significantly ($p < 0.05$) high mean levels of fumonisin included posho-mill waste, sorghum, maize and sorghum mixture, maize bran and maize; being 5,101.14; 4,161.01; 3,099.71; 2,490.65 and 1,126.83 ppb, respectively (Table 2). Like aflatoxins, fumonisins are a significant concern in food and feed safety. They are carcinogenic in human, while in animals they damage tissues, including liver, brain, and lungs (Patriarca and Pinto, 2017; Pleadin, 2015). In this study, some feeds were contaminated with fumonisin levels above (>1,000ppb) the legal limit in Kenya. Fumonisin was the most prevalent mycotoxin compared to ochratoxin A and aflatoxin, having a mean of 1,378.18 ppb. Among the common feeds tested, sorghum and maize had higher levels of fumonisin, with means of 4,161.01 and 1,126.83ppb, respectively, compared to other feed types. A study by Ayalew *et al.* (2006) also reported higher (range: 1370–2117 ppb) levels of fumonisin in sorghum samples. Mutiga *et al.* (2015) reported higher (> 50%) number of maize samples with

fumonisins levels above 1,000 ppb from Western Kenya. In Western Kenya, maize harvesting period coincided with second rainsand given that fungus prevalence is generally increased by wet weather conditions during late stages of season, this increases chances ofmould infestation, particularly with those in *Fusarium genera* (Alakonya *et al.,* 2008; Josephat *et al.,* 2015). Moreover, maize being the staple food in the region, continuous planting in same or neighbouring fields favour field fungi (*Fusarium* spp) proliferation and infection, hence accumulation of fumonisin in maize grains.

Table 2 indicates that maize bran and sorghum samples had significantly ($p< 0.05$) high ochratoxin A levels (22.43 and 18.38 ppb, respectively) compared to maize, cassava and millet at (3.35, 3.12 and 2.04 ppb, respectively). No feed had levels above regulatory limit of > 100ppb prescribed for poultry. In feeds, OTA can be carried over in chicken products, such as eggs and meat and contaminated products can have harmful health effects in human (Iqbal *et al.,* 2014; Lee *et al.,* 2016). All feedsanalysed had ochratoxin A levels below regulatory limits of <100 ppb. Elbashir and Ali (2014) also reported low levels (3.3%) of ochratoxins incidence in sorghum and its derived products. However, Ayalew *et al.* (2006) reported much higher levels of ochratoxin A, with a mean of 174.8 ppb in sorghum; this was attributed to a steady increase in moisture content due to underground storage pits. There is growing public health concern over potential consumption of animal-derived food products, like meat, eggs and milk containing metabolites or residues of mycotoxins. Therefore, prevention of chronic exposure is imperative, especially in developing countries where chronic exposure is of critical concern.

Table 2. Mycotoxin levels detected in chicken feed sampled from Western Kenya

Feed sample (n = 235)	Mean mycotoxin levels, ppb		
	Aflatoxin	Fumonisin	Ochratoxin A
Maize (95)	0.33^{a}	1126.83^{abc}	3.35^{a}
Sorghum (28)	0.17^{a}	4161.01^{abc}	18.38^{bc}
Cassava (24)	0.25^{a}	43.87^{a}	3.12^{a}
Millet (20)	0.21^{a}	60.73^{a}	2.04^{a}
Peanut (8)	0.53^{a}	10.47^{a}	3.74^{a}
Commercial (20)	44.25^{b}	$544.73a^{b}$	7.55^{ab}
Brewers' waste (3)	6.63^{a}	$382.04a^{b}$	8.45^{ab}
Posho-mill waste (4)	6.77^{a}	5101.14^{c}	4.50^{a}
Soya bean (7)	0.26^{a}	18.88^{a}	2.76^{a}
Beans (4)	0.48^{a}	183.25^{ab}	5.38^{ab}
Homemade ration (17)	7.70^{a}	693.02^{ab}	5.54^{ab}
Maize and sorghum (2)	0.51^{a}	3099.71^{abc}	3.35^{a}
Maize bran (3)	14.03^{ab}	2490.65^{abc}	22.43^{c}
Overall mean	6.316	1378.18	6.97

$^{a, b, c}$ Means within a column with different superscripts are significantly different ($p< 0.05$). Kenyan regulatory limits in poultry feed for aflatoxin, fumonisin and ochratoxin A are 10, 1000 and 100 ppb, respectively.

Aflatoxin residues in chicken eggs and tissues. Egg samples in all the three counties were found to contain only traces of aflatoxin as shown in Figure 1. Among all the tissues, liver had the high mean level of aflatoxin (Table 3). In Busia, Kakamega and Siaya, liver had 5.24±0.66, 2.68±0.85 and 4.67±0.73 ppb, respectively with a mean of 4.19 ppb. Breast muscle showed a mean of 3.57 ppb. Kidney had the least (2.02±0.56) level of aflatoxin. Results revealed that there is no big difference of aflatoxin levels in chicken tissues under the two production systems even though free-range had slightly higher levels (Table 4). Aflatoxins in feed consumed by chicken have been shown to pass to eggs and tissues causing contamination of products (Gareis *et al.,* 2000; Tarus, 2018). Eggs and tissues of indigenous chicken contained aflatoxin residues, with the former showing only trace levels. This could be attributed to the fact that eggs take only 24 hours to be laid while tissues act as deposition (muscles especially the breast muscle) and detoxification sites (liver and kidney) of these toxins. Among the tissues sampled, liver had the highest mean aflatoxin level across the Counties. This could be due to exposure of chicken to aflatoxins while in the field (free ranging). Chicken consume contaminated waste grains, vegetation, soils, wild seeds, among others, in the field since it is hard to control. These results are in agreement with Darwish *et al.* (2016) who found that liver had higher total aflatoxin levels. The presence of aflatoxins in chicken tissues is an indication contamination of feeds. Although there is no safe level, WHO/FAO have set permitted amounts of 20 ppb and KEBS of 10 ppb so as to control exposure because the levels are significant to public health.

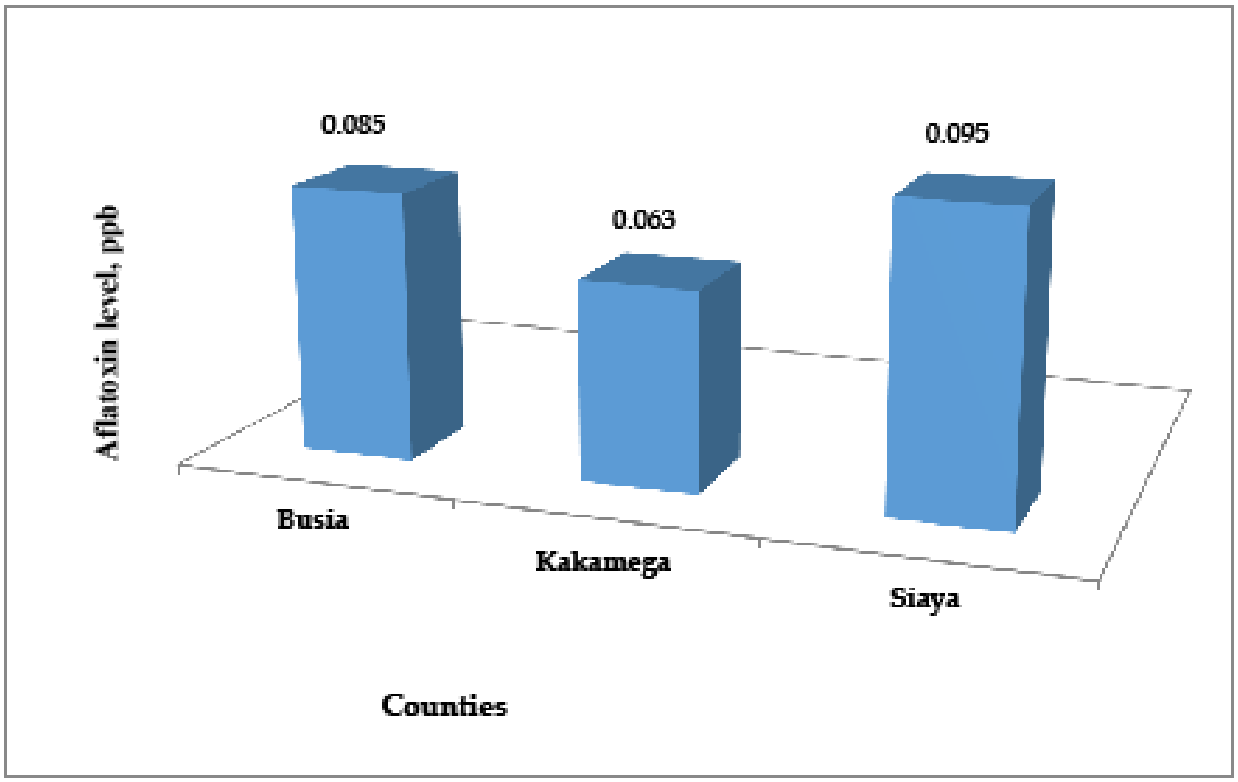

Figure 1. Mean aflatoxin levels in indigenous chicken eggs sampled from three counties of Western Kenya

Table 3. Mean aflatoxin levels in chicken tissues sampled from three counties of Western Kenya

County	Aflatoxin level in chicken tissue, ppb (n = 60)			
	Breast muscle	Kidney	Liver	Thigh muscle
Busia	4.96±0.51	0.83±0.40	5.24±0.66	1.76±0.60
Kakamega	2.74±0.84	1.91±0.75	2.68±0.85	3.85±1.17
Siaya	3.01±0.41	3.34±0.54	4.67±0.73	2.38±0.76
Mean	3.57±0.59	2.02±0.56	4.19±0.75	2.66±0.84

Mean ± SEM (standard error of mean)

Table 4. Mean aflatoxin levels in tissues of chicken kept under different production systems in Western Kenya

Production system	Tissue	Mean	Standard deviation	Coefficient of variation
	BM	3.753±0.44	2.71	72.10
	K	1.926±0.39	2.39	123.9
Semi-free range	L	3.953±0.52	3.21	81.09
	TM	2.276± 0.51	3.15	138.3
Free range	BM	3.143±0.68	3.10	98.68
	K	2.157± 0.77	3.53	163.8
	L	4.619± 0.86	3.93	85.07
	TM	3.371± 1.11	5.07	150.4

Mean ± SEM (Standard error of mean)

Conclusions and Recommendation

Common fungal genera isolated from the feeds were *Aspergillus, Penicillium* and *Fusarium* which are known to have the potential of producing aflatoxins, ochratoxin A and fumonisins, respectively. Commercial feeds were found to be more contaminated with aflatoxins; however, fumonisin and ochratoxin A levels in all feeds did not exceed the regulatory limits. Eggs had traces of aflatoxin while liver and breast muscle had higher levels; however, these levels also did not exceed regulatory limits. A multi-actor, multi-pronged approach is necessary, from farm to fork, pre-production to postharvest, marketing and distribution, supported by an enabling policy, regulatory and institutional framework, to mitigate mycotoxin contamination challenge.

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