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Determination of multiple mycotoxins levels in poultry feeds from Cameroon

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Abstract

For the first time in Cameroon, this paper reports on multiple mycotoxins occurrences in poultry feeds. Twenty feed samples collected from different poultry farms were analyzed for 320 fungal metabolites by liquid chromatography electrospray ionization tandem mass spectrometry. Results showed feeds contamination by 68 metabolites including 18 mycotoxins/metabolites currently regulated in the European Union such as fumonisins B_1 (FB_1), B_2, and B_3; deoxynevalenol (DON); and beta-zearalenol recovered in all samples. FB_1 reported highest FB mean level of 468 (range 16–1930) μg kg^{-1}. Levels of DON and ZEN were mostly concentrated in feeds from western-highlands conversely for FBs and aflatoxins concentrations in Yaounde. Aflatoxin B_1 mean level of 40 μg kg^{-1} exceeded the worldwide permitted limit for aflatoxins in feed and generally inversely proportional to weight gain in chicken.

Key Words: Cameroon, LC-ESI-MS/MS, poultry feeds, multi-mycotoxins

Mycotoxins are toxic secondary metabolites produced by toxigenic fungi species. Several of these metabolites exist in the literature with only a few of them that have been extensively studied partly due to the lack of suitable methods of analysis of multiple mycotoxins. European Union regulated mycotoxins include aflatoxins (AFs), fumonisins (FBs), ochratoxins (OTs), deoxynivalenol (DON), and zearalenone (ZEN). Animals are mainly exposed via ingestion of contaminated feeds. This may result in effects such as reduction in amount of nutrients available for use by the animal (Kao and Robinson, 1972) with negative health implications, leading to increase incidence of disease, reduce production efficiency with associated huge economic loss to farmers. Dead may occur in cases of high acute intoxication of mycotoxins (Whitlow *et al.,* 2000). In Cameroon the sub-sector of agriculture known as poultry farming is notable for its production

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of meat, eggs and employments for many Cameroonians. Preliminary findings from Cameroon reported presence of aflatoxins in chicken feed and eggs of hens (Tchouanguep *et al.,* 1994), and contamination of maize (major raw material of poultry feed) by a few mycotoxins (Ngoko *et al.,* 2008; Njobeh *et al.,* 2010) and recently on multiple mycotoxins contaminations (Abia *et al.,* 2012). The aim of this study was to determine the levels to which poultry feeds from Cameroon are contaminated by multiple mycotoxins and to associate levels with effects on growth of chickens.

Sixty three (31 layers and 32 broilers) feed samples were collected from 15 and 20 farms from the western-highlands (Bamenda, Bafoussam and Bangante) and southern-tropical forest (Yaounde) regions of Cameroon, respectively from June to December 2010 and kept at −20°C. Each sample was collected after thorough mixing of the whole, and from several spots. Weights of broilers were taken at intervals of 7 days for six weeks. Enzyme-linked immunosorbent assay (ELISA; Kit from RENEKABIO Laboratory, Tustin, California, US) was applied as described by the manufacturer to assay total aflatoxins level in broiler feeds.

From the pool of 31 layer feed samples, 20 samples (50 g each) from the western-highlands (N = 9) and Yaounde (N = 11) were sub-sampled and transferred in a cooler containing dry ice to Austria for analysis with no further processing. 5 g of each sample was extracted with 20 ml extraction solvent (ES) for 90 mins at 180 rpm using a GFL 3017 rotary shaker (GFL 3017, Burgwedel, Germany). 500 µl of each extract was transferred into a 1.5 ml glass vial containing an equal volume of the dilution solvent (DS) and vortexed for 30 secs. After proper mixing, 5 µl of the diluted extract was injected into the LC-ESI-MS/MS system.

Spiking experiments were done to determine apparent recovery of the target metabolites. Three least contaminated samples (each 0.25 g) considered as blanks of feed were each spiked with 100 µl of multi-metabolite standard, thoroughly mixed and kept at ambient temperature in the dark in a fume cupboard overnight to establish equilibration between the metabolites and the matrix. Thereafter, 1 ml of ES was added and placed on a rotary shaker at 180 rpm for 90 mins. Subsequently, 300 µl of extract was diluted with 300 µl of DS and vortexed for 30 secs and 5 µl injected into the LC-ESI-MS/MS system.

A multi-mycotoxin technique described in our earlier study Abia *et al.* (2012) was employed to screen for 320 fungal metabolites. Briefly, detection and quantification was performed using a QTRAP 5500 LC-ESI-MS/MS System (AB SCIEX, Foster City, CA) equipped with a Turbo Ion Spray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent Technologies 1290 Infinity). Chromatographic separation was performed at 25°C on a Gemini® C18 column (Phenomenex, Torrance, CA, US). ESI-MS/MS was performed in the scheduled multiple reaction monitoring (sMRM) mode both in positive (54 secs) and negative (96 secs) polarities (cycle time = 1 sec) in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte.

With optimum method performance characteristics (data not shown), mycotoxins were quantified using external calibration (1/x weighted) and levels were later adjusted based on recovery, while semi-quantification was performed using the response factor of a structurally related compound for a few other cases where standards were absent, e.g., fumonisin B_6 (FB_6) using the response factor of FB_2. Each mycotoxin present in the samples was quantified by comparing its peak area to those of related standard. Limits of detection (LOD) and quantification (LOQ) were estimated at the lowest concentration in spiked samples corresponding to a signal-to-noise ratio (S/N) of 3 : 1 and 10 : 1, respectively.

Table 1 show that the studied layer feeds from Cameroon were contaminated by a total of 68 fungal metabolites amongst which were 18

mycotoxins/metabolites currently regulated in the European Union. The most frequently detected (in all samples) legislated metabolites were fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3); deoxynevalenol (DON); and beta-zearalenol (β-ZEL).

As concerns FBs, FB_1 showed highest levels of contamination (mean 468, range 16–1930) μg kg^{-1} relative to FB_2 and FB_3. The hydrolysed form of FB_1 (HFB_1) had the lowest occurrence frequency (40%) and with very high mean concentration of 12085 (range 5687–23957) μg kg^{-1}. The mean level of DON was 164 (range 19–495) μg kg^{-1}, while its derivatives were detected with lowest levels in deoxynevalenone-3-glucoside (DON-G) at mean level of 12 (range 0.2–60) μg kg^{-1} and lowest frequency of 15% in deepoxy-deoxynivalenol (DOM). Similarly, zearalenone (ZEN) had the highest mean level of 155 (range 0.7–600) μg kg^{-1} whilst zearalenone-4-sulphate (ZEN-S) had the lowest mean level of 4 (range 0.1–19) μg kg^{-1}. Alpha-zearalenol (α-ZEL) had lowest frequency of occurrence (55%).

Also detected were the aflatoxins (AFs) and ochratoxins (OTs) which occurred at frequencies of 75–95% and 80–90% respectively. As far as AFs were concerned, aflatoxin B_1 (AFB_1) was the most frequent and had the highest concentration (mean 40.4, range 1.2–200) μg kg^{-1} while aflatoxin G_2 (AFG_2) had the lowest amounts (mean 5.4, range $<$ LOQ-14) μg kg^{-1}. The maximum total AFs levels in broiler feeds from ELISA analysis were 30 and 22 μg kg^{-1} in the western-highlands and Yaounde respectively. Furthermore, it was observed that weight gain of broilers were inversely correlated with aflatoxins load ($R^2 =$ 0.517). Of the OTs, ochratoxin A (OTA) was the most frequent (90%) and concentrated form of OTs (mean 1.6 μg kg^{-1}, range $<$ LOQ-4.6 μg kg^{-1}) detected in the studied feed samples.

In addition to the legislated mycotoxins, 20/42 additional mycotoxins/metabolites (Table 1) were present in all studied samples. These included nivalenol (NIV), sterigmatocystin (STER), aurofusarin (ARF), beauvericin (BEA), brevianamide F (BREVI F), chlamydosporol, enniatin B (ENN-B) and B1 ($ENN-B_1$), kojic acid (KA), monocerin (MONO), tryptophol, chanoclavin (CNV), curvularin, emodin (EMOD), equisetin (EQUS), macrosporin, physcion, skyrin, averufin (AVEF), and norsolorinic acid (NORC-AC). Some of these metabolites occurred at high levels, e.g., KA (mean 6622 μg kg^{-1}, range 35–61353 μg kg^{-1}) whilst MONO and CNV had low mean levels of 2.4 (range 0.2–15) μg kg^{-1} and 0.5 (range $<$ LOQ-1.5) μg kg^{-1} respectively. Notwithstanding, of all the 42 additional metabolites, verrucofortin (VERF) was the least abundant (10%) with lowest mean level of 0.01 (rang 0.001–0.01) μg kg^{-1}. Both VERF and CJ21058 were not detected in samples from Yaounde.

To determine the levels to which chicken feeds from Cameroon are contaminated with multiple mycotoxins, chicken feed samples collected from different poultry farms were analysed for presence of 320 fungal metabolites by a direct dilute and shot LC-ESI-MS/MS method. Based on spiked samples, the method performance was satisfactory, with over 62/68 recoveries above 50%. The LOD of the different metabolites were between 0.0003 μg kg^{-1} for ZEN-S and ENN-B, and 5.0 μg kg^{-1} for KA. Whilst some African nations have data on feed contamination limited for a few regulated mycotoxins (FAO, 2004), to the best of our knowledge no mycotoxins regulations in feeds exist in Cameroon partly due to non-existence of data on mycotoxin contamination in feed. Therefore, the mycotoxin data reported herein is unique in chicken feed from Cameroon in terms of the variety of fungal metabolites studied.

Maize constitute over 60% of poultry feed in Cameroon and thus its quality with respected to mycotoxin contamination may grossly affect feed quality. Levels of FBs, DON, ZEN, and OTA in this study were similar to concentrations detected in maize from Cameroon while the recovered HFB_1 and ZEN derivatives were absent in the said maize samples. Conversely, AFs levels recorded in studied samples were higher than

Table 1. Natural Occurrences of multi-mycotoxin in poultry feed from Cameroon and Variation across study locations

	Mycotoxin levels in poultry feed from Cameroon					
			Variation across study locations in Cameroon			
	Cameroon		Western highlands		Yaounde	
Metabolite	F (%)	Mean (range) μgkg^{-1}	F (%)	Mean (range) μgkg^{-1}	F (%)	Mean (range) μgkg^{-1}
Aflatoxin B_1 (AFB_1)	19 (95)	40.4 (1.2–200)	8 (89)	24.2 (1.7–63)	11 (100)	52 (1.2–200)
Aflatoxin B_2 (AFB_2)	15 (75)	5.8 (0.4–20)	6 (67)	3.5 (0.4–7)	9 (82)	7.3 (2–20)
Aflatoxin G_1 (AFG_1)	18 (90)	19.9 (0.4–123)	7 (78)	13.1 (1.1–57)	11 (100)	24.3 (0.4–123)
Aflatoxin G_2 (AFG_2)	16 (80)	5.4 (< LOQ–14)	6 (67)	2.6 (< LOQ–2.7)	10 (91)	6.5 (< LOQ–14.4)
Fumonisin B_1 (FB_1)	20 (100)	468 (16–1930)	9 (100)	291 (16–1137)	11 (100)	612 (87–1930)
Fumonisin B_2 (FB_2)	20 (100)	113 (7.9–520)	9 (100)	62 (8–263)	11 (100)	154 (26–520)
Fumonisin B_3 (FB_3)	20 (100)	33.4 (< LOQ-120)	9 (100)	22.5 (< LOQ–93)	11 (100)	41.3 (11–120)
Hydrolysed Fumonisin B_1 (HFB_1)	8 (40)	12085 (5687–23957)	3 (33)	12710 (5687–23957)	5 (45)	11711 (8928–15602)
Fumonisin B_6 (FB_6)	18 (90)	5.4 (< LOQ–16)	7 (78)	4.7 (< LOQ–11)	11 (100)	5.8 (0.7–16)
Ochratoxin A (OTA)	18 (90)	1.6 (< LOQ–4.6)	8 (89)	2.1 (< LOQ–5)	10 (91)	1.2 (< LOQ–2)
Ochratoxin B (OTB)	16 (80)	1.1 (< LOQ–2.2)	8 (89)	1.3 (< LOQ–2.2)	8 (73)	0.9 (< LOQ–1.02)
Deoxynivalenol (DON)	20 (100)	164 (19–495)	9 (100)	248 (130–495)	11 (100)	95 (19–227)
Deoxynivalenol-3-Glucoside (DON-G)	18 (90)	12 (0.2–60)	9 (100)	19 (4–60)	9 (82)	4 (0.2–15)
Deepoxy-deoxynivalenol (DOM)	3 (15)	18.5 (17–20)	nd	nd	3 (27)	18 (17–20)
Zearalenone (ZEN)	19 (95)	155 (0.7–600)	9 (100)	275 (12–600)	10 (91)	47 (0.7–297)
Beta-zearalenol (β-ZEL)	20 (100)	4.7 (0.3–28)	9 (100)	8 (1–28)	11 (100)	1.9 (0.3–12)
Alpha-zearalenol (α-ZEL)	11 (55)	6.1 (0.6–20)	8 (89)	7.8 (0.6–20)	3 (27)	1.6 (0.8–2.7)
Zearalenone-4-Sulphate (ZEN-S)	17 (85)	4 (0.1–19)	9 (100)	6.7 (0.2–19)	8 (73)	1 (0.1–5.5)
Nivalenol (NIV)	20 (100)	463 (18–1429)	9 (100)	806 (126–1429)	11 (100)	182 (18–739)
Citrinin (CIT)	19 (95)	177 (4.4–759)	8 (89)	59 (4.4–283)	11 (100)	264 (25–759)
Sterigmatocystin (STER)	20 (100)	2.3 (< LOQ–12)	9 (100)	3.3 (< LOQ–12)	11 (100)	1.1 (< LOQ–2.4)
O-Sterigmatocystin (O-STER)	19 (95)	1 (< LOQ–6.7)	8 (89)	0.7 (< LOQ–3.5)	11 (100)	1.3 (0.04–6.7)
Aurofusarin (ARF)	20 (100)	8.3 (0.3–37)	9 (100)	15.9 (0.8–37)	11 (100)	2 (0.34–13)
Beauvericin (BEA)	20 (100)	16 (2–78)	9 (100)	21 (2.3–78)	11 (100)	11.4 (3.2–45)
Brevianamide F (BREVI F)	20 (100)	142 (31–249)	9 (100)	136 (31–247)	11 (100)	147 (39–249)
Chlamydosporol (CHLA)	20 (100)	62 (32–105)	9 (100)	55.8 (32–88)	11 (100)	67 (40–105)
Culmorin (CUL)	17 (85)	72 (< LOQ -167)	9 (100)	97 (39–167)	8 (73)	35 (< LOQ–58)
Cytochalasin J (CYT-J)	17 (85)	110 (< LOQ-396)	7 (78)	180 (< LOQ–396)	10 (91)	63 (< LOQ–253)
Cytochalatin H (CYT-H)	10 (50)	102 (< LOQ-273)	5 (56)	131 (< LOQ–273)	5 (45)	73 (< LOQ–187)
Enniatin A (ENN-A)	17 (85)	0.5 (< LOQ–1.8)	8 (89)	0.5 (0.04–1.6)	9 (82)	0.5 (< LOQ–1.8)
Enniatin A1 ($ENN\text{-}A_1$)	19 (95)	4 (< LOQ–17)	9 (100)	4.5 (0.02–13)	10 (91)	3.7 (< LOQ–17)
Enniatin B (ENN-B)	20 (100)	6.6 (0.02–27)	9 (100)	9 (0.02–27)	11 (100)	4.7 (0.02–17)
Enniatin B1 ($ENN\text{-}B_1$)	20 (100)	11 (0.03–41)	9 (100)	14.5 (0.09–41)	11 (100)	8.5 (0.03–41)
Kojic acid (KA)	20 (100)	6622 (35–61353)	9 (100)	11307 (35–61353)	11 (100)	2789 (35–18445)
Monocerin (MONO)	20 (100)	2.4 (0.2- 15)	9 (100)	2.7 (0.4–14)	11 (100)	2 (0.2–15)
Tryptophol (TRYP)	20 (100)	581 (59–8311)	9 (100)	1138 (79–8311)	11 (100)	126 (59–199)
3-Methylviridicatin (3-MV)	19 (95)	1 (< LOQ–3)	8 (89)	1.4 (< LOQ–3)	11 (100)	0.08 (< LOQ–0.08)
Cycloaspeptite A (CA-A)	15 (75)	3 (< LOQ–11)	6 (67)	2.8 (< LOQ–6)	9 (82)	3.2 (< LOQ–11)
Chanoclavin (CNV)	20 (100)	0.5 (< LOQ–1.5)	9 (100)	0.4 (< LOQ–0.7)	11 (100)	0.6 (< LOQ–1.5)
Ergocristine (ERT)	12 (60)	11 (0.9–46)	6 (67)	15 (5–46)	6 (55)	6.8 (0.9–15)
Ergocominine (ERCN)	6 (30)	3 (1–8)	4 (44)	3.9 (1–8.4)	2 (18)	2.2 (1.3–3.2)

Metabolite	Mycotoxin levels in poultry feed from Cameroon					
			Variation across study locations in Cameroon			
	Cameroon		Western highlands		Yaounde	
	F (%)	Mean (range) μgkg^{-1}	F (%)	Mean (range) μgkg^{-1}	F (%)	Mean (range) μgkg^{-1}
Ergocryptinin (ERCT)	9 (45)	0.5 (< LOQ–1.4)	4 (44)	0.7 (0.2–1.4)	5 (45)	0.4 (< LOQ–0.7)
Pestalotin (PEST)	19 (95)	3.4 (< LOQ–16)	9 (100)	1.9 (0.7–4.9)	10 (91)	4.9 (< LOQ–16)
Secalonic Acid D (S-A-D)	8 (40)	23 (0.5–69)	4 (44)	13 (1.3–32.4)	4 (36)	33 (0.5–69)
Nonactin (NONC)	4 (20)	0.1 (0.03–0.2)	1 (11)	0.1	3 (27)	0.1 (0.03–0.2)
Verrucofortin (VERF)	2 (10)	0.01 (0.001–0.01)	2 (22)	0.01 (0.001–0.01)	nd	nd
Alternariolmethylether (AME)	15 (75)	1.1 (0.01–7)	7 (78)	1.6 (0.1–7)	8 (73)	0.6 (0.01–1.5)
Andrastin A (ANDR-A)	5 (25)	3479 (367–15298)	1 (11)	684	4 (36)	4178 (367–15298)
CJ21058 (CJ)	8 (40)	0.4 (0.1–1.6)	8 (89)	0.4 (0.1–1.6)	nd	nd
Curvularin (CURV)	20 (100)	5 (0.2–19)	9 (100)	3.9 (0.2–12)	11 (100)	5.9 (0.6–19)
Emodin (EMOD)	20 (100)	15 (1.6–38)	9 (100)	17.2 (3.3–38.4)	11 (100)	13 (1.6–27)
Equisetin (EQUS)	20 (100)	11 (2–25)	9 (100)	7.8 (2.1–13)	11 (100)	14 (2–25)
Macrosporin (MAC)	20 (100)	7 (0.1–31)	9 (100)	6.5 (2.1–14.6)	11 (100)	7.6 (0.1–31)
Physcion (PHYS)	20 (100)	125 (13–1215)	9 (100)	199 (25–1215)	11 (100)	64 (13–158)
Radicicol (RAD)	8 (40)	5 (0.8–18)	5 (56)	5.3 (0.8–18)	3 (27)	3.9 (2.4–6)
Skyrin (SKY)	20 (100)	26 (1–153)	9 (100)	23.3 (2.5–75)	11 (100)	29 (1.3–153)
Averantin (AVET)	19 (95)	0.7 (0.03–5)	9 (100)	0.4 (0.2–0.9)	10 (91)	0.9 (0.03–5)
Averufin (AVEF)	20 (100)	681 (22–4156)	9 (100)	389 (22–962)	11 (100)	921 (28–4156)
Versicolorin A (VER-A)	17 (85)	282 (12–1842)	8 (89)	169 (12–372)	9 (82)	383 (88–1842)
Versicolorin C (VER-C)	18 (90)	257 (0.5–1375)	8 (89)	180 (13–518)	10 (91)	318 (0.5–1375)
Averufanin (AVFN)	18 (90)	891 (280–3670)	8 (89)	616 (361–1230)	10 (91)	111 (280–3670)
Norsolorinic acid (NORC-AC)	20 (100)	104 (23–369)	9 (100)	70 (28–111)	11 (100)	131 (23–369)
Tentoxin (TNTX)	16 (80)	2.2 (0.3–6.3)	8 (89)	2.2 (0.5–6)	8 (73)	2.2 (0.3–4)
Apicidin (APIC)	18 (90)	5 (0.2–21)	9 (100)	5.6 (2–12)	9 (82)	5.3 (0.2–21)
Chrysophanol (CHRY)	19 (95)	10 (1.1–37)	9 (100)	9.7 (1.9–34)	10 (91)	10.8 (1–37)
Rugulosin (RUG)	14 (70)	24 (5–76)	8 (89)	27 (5.7–76)	6 (55)	20.7 (5–52)
Methylsulochrin (MS)	7 (35)	0.7 (0.04–3)	5 (56)	1 (0.1–3)	2 (18)	0.2 (0.04–0.4)
Alternariol (AOH)	12 (60)	1.5 (0.1–10)	5 (56)	0.6 (0.09–1.8)	7 (64)	2.2 (0.1–10)

nd: not detected

those in maize from Cameroon which did not had AFG_2 contamination employing the same method (Abia *et al.*, 2012). This was not surprising considering the inclusion of other ingredients in feed preparations. The mean total AFs levels exceeded the maximum permitted level of 20 μg kg^{-1} for AFs for feed (FAO 2004) and were generally inversely proportional to increase in weight of chickens. In addition, the high mean level of AFB_1 shows the high prevalence of unsafe levels of AFs in chicken feed from Cameroon. Although mycotoxins such as FBs, OTA and DON did not exceed their respective MPLs, in view of their frequent occurrences and continuous exposure of chickens, attention is needed. Generally, layer feed samples from Yaounde were commonly tainted with the AFs and FBs with the exception of HFB_1, while samples from western-highlands were generally contaminated by OTs; ZEN/ZEN derivatives; and DON/DON metabolites, with the exception of DOM which was not detected in samples from the western highlands.

The frequent occurrence of the non-regulated metabolites at high levels occasionally, is a concern, though the health effects of many

remains unknown. For example, ARF may be transferred from contaminated feed when eaten by chickens into egg yolk where it damages the quality of the egg resulting in a dark brown coloration of egg yolk in lieu of orange (Kotyk *et al.*, 1995). The *Fusaria* mycotoxin ENN B2 at low levels (mean 6.6, range 0.02–27) μg kg^{-1} may induce cytotoxic effects even at very low quantities (Ivanova *et al.*, 2006). BEA has the potential to induce apoptosis and DNA fragmentation in human cell lines (Logrieco *et al.,* 2001). EMOD is known to be genotoxic and cytotoxic (Yan and Ma, 2007). Similarly, AVET, AVEF, NORC-AC, and STER are potent mutagenic and genotoxic intermediates of AFB_1 biosynthetic pathway (Wong *et al.,* 1977). As a result, chronic exposures of chickens to multiple fungal metabolites may require urgent attention especially as the demands for poultry products keep rising (Haruna and Hamidu, 2004) with increase in its socio-economic value.

Eleven non-legislated mycotoxins including HFB_1, 3-methylviridicatin, cycloaspeptite A, chanoclavin, ergocristine, ergocominine, ergocryptinin, secalonic acid D, verrucofortin, rugulosin and methylsulochrin were detected in feed samples but which were not detected in food commodities in a recent studies (Abia *et al.*, 2012). On the contrary, absent in feed samples but which were recovered in food samples were 12 metabolites including fusarenon X and fusaric acid (Abia *et al.*, 2012) frequently reported in feed samples (Ezekiel *et al.*, 2012; Warth *et al.*, 2012).

The present study reveals the usefulness of the LC-ESI-MS/MS analytical technique to the feed industry, showing several fungal metabolites and their concentrations in some cases in the same samples. This corroborates with the recent finding of Ezekiel *et al.* (2012) and Warth *et al.,* (2012) elsewhere in Africa. Based on the wide range of naturally occurring toxic metabolites detected, this study has highlighted the high risk of mycotoxin exposure of chickens and subsequently human consumer populations. Of specific interest was the high mean AFs level that exceeded the regulatory level. Therefore, further research, surveillance, and educational intervention is needed to reduce mycotoxin contamination levels in maize and other raw materials included in poultry feed, thereby improving on poultry health, increasing productivity of the chickens.

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