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Mycotoxins Contamination Levels in Broiler Feeds and Aflatoxin Residues in Broiler Tissues

Anwaar M. El-Nabarawy[1*], Elshaimaa Ismael[2], Khaled A. Shaaban[1], Sawsan S. El Basuni[3,] Mohamed M. Batikh[4] and Mohamed Shakal[1]

[1]*Poultry Disease Department, Faculty of Veterinary Medicine, Cairo University, Egypt.*
[2]*Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt.*
[3]*Avian and Rabbit Diseases Department- Faculty of Veterinary Medicine, Benha University, Egypt.*
[4]*Poultry Disease Department, Animal Health Research Institute, Kafr El-sheikh Provisional Lab, Egypt.*

*Corresponding author's E-mail: anwaar.elnabarawy@gmail.com; ORCID: 0000-0003-1618-6842

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ABSTRACT

The need for regulations to limit the concentration of mycotoxins in feed and food requires the availability of data on levels of contamination in different feedstuffs and estimation of the mycotoxin residues in animal meat. Therefore, this study was conducted to determine contamination levels with different mycotoxins in broiler feed and aflatoxin residues in broilers' muscle and liver. A total of 194 feed samples, including 148 compound feeds and 46 feed ingredients, were collected from feed manufacturing companies and broiler farms. Feed samples were analyzed for detecting aflatoxins, ochratoxins, zearalenone, and fumonisins using an official analytical method. Moreover, aflatoxin residues were estimated in 64 broiler's muscle and liver tissues. Obtained results revealed that 100% of compound broiler feed sampled from manufacturing companies were contaminated with aflatoxin and ochratoxin. Also, 96.4% and 92.8% of compound broiler feed sampled from broiler farms were contaminated with aflatoxin and ochratoxin, respectively. Furthermore, 30.6% and 91% of the feed samples were above the permissible levels of aflatoxin and ochratoxin. Aflatoxin residues were detected in all meat and liver samples with levels above the permissible limits. Large scale surveys for determination of different mycotoxins in poultry feed and mycotoxins residues in poultry products are of national and international importance.

Key words: Aflatoxin, Broiler feed, Fumonisin, Mycotoxin residue, Ochratoxins, Zearalenone.

INTRODUCTION

Mycotoxins are secondary metabolites produced by mycotoxigenic fungi infecting feed ingredients under field and storage conditions and they remain long after the death of the mold (Aravind et al., 2003). Moreover, the co-occurrence of mycotoxins in poultry feed is more prevalent than a single mycotoxin (Atalla et al., 2003; Kana et al., 2013; Kovalsky et al., 2016). Concomitant contamination by several mycotoxins may augment their toxic effects (Huff and Doerr, 1981; Chandrasekaran 1996; Pappas et al., 2014). Aflatoxin, ochratoxin A, zearalenone, T-2 toxin, vomitoxin, and fumonisin are the most significant mycotoxins affecting poultry species through naturally contaminated feeds and have serious toxic effects and probable synergistic properties (Njobeh et al., 2012). The combined effect of ochratoxin and aflatoxin at a dose of 23 and 16 ppb; respectively, resulted in depressed T and B lymphocytes activity, suppressed immunoglobulin and antibody production (El Nabarawy et al., 2016). Naturally contaminated broiler diet by aflatoxin, ochratoxin, and zearalenone at permissible levels resulted in a significant reduction in feed conversion rate, body weight and antibody titers to infectious bursal disease virus (El Nabarawy et al., 2020). Permissible limits of mycotoxins in poultry feed and feed ingredients are 20 ppb for aflatoxins (FDA, 2000; van Egmond and Jonker, 2004a), 25 ng/g for ochratoxins (EC, 2006), 10 ppm for zearalenone (FDA, 2010) and 100 ppm for fumonisin (FDA, 2001). Besides, zearalenone is receiving serious attention for control, since it is considered a mycotoxin indicator in addition to its synergistic action with other mycotoxins, but its regulation needs further attention (Park and Troxell, 2002).

The contaminated animal feed is the major cause of exposure to mycotoxins in animals and therefore ultimately in humans (Bryden, 2012). In the last few decades, the increase in the incidence of various types of cancers between various categories of people may be contributed to dietary factors, aflatoxins and agrochemical

contaminated foods (Maiyoh and Tuei, 2019). In the same respect, van Egmont and Jonker (2004b) reported that dietary contamination of aflatoxins represents a major risk to public health and aflatoxins are known to have a strong hepatotoxic and carcinogenic effect. In general, the consumption of contaminated food induces neurotoxic, immunosuppressive, teratogenic, mutagenic and carcinogenic effects in humans (Fernandez et al., 2000). Mohd-Redzwan et al. (2013) reported on cumulative evidence from humans revealed a strong linkage occurs between aflatoxin and hepatic chronic carcinoma (HCC). Also, acute aflatoxicosis induced abdominal pain, vomiting, edema, and death. Moreover, aflatoxicosis outbreak was recorded four times in Kenya from 2004 to 2014, with mean 600 individuals were affected, and 211 deaths were estimated from this outbreak (Awuor et al., 2017). Hence, the European community and many other countries have determined 2 ng/g aflatoxin B1 (AFB1) and 4 ng/g total aflatoxin as maximum tolerance levels in human food products (Van Egmond and Jonker, 2004b; Wild and Gong, 2009). The accumulation of AFB1 residues in broiler meat and liver leads to the toxin carryover through the food chain. AFB1 residues may persist unchanged in the liver even when exposure levels are relatively low (Magnoli et al., 2002). The occurrence and incidence of aflatoxins, ochratoxins, and zearalenone in chicken meat are alarming and urged the need for continuous monitoring for these toxins in chicken meat and eggs (Iqbal et al., 2014).

There are very limited data on the epidemiological status of mycotoxins in broilers feed and meat in Egypt. Therefore, the objective of this work was to study the situation of the contamination levels of aflatoxin, ochratoxin, zearalenone, and fumonisins in broilers feed and the level of aflatoxin residues in broiler tissues.

MATERIALS AND METHODS

Sampling, extraction and determination of mycotoxins in broilers feed and feed ingredients

Sampling of broiler feed

A surveillance study was carried out on different mycotoxins contamination levels in broilers feed in 2014 and 2018. A total of 194 broiler feed samples including 148 compound broilers feeds and 46 feed ingredients were collected from feed manufacturing companies (n=37) and broilers farms (n= 111) for mycotoxins detection and determination. The samples were collected by a representative method according to the recommendation of the FAO for detection and determination of aflatoxins, ochratoxin A, zearalenone, and fumonisin mycotoxins.

Requirements and consumable materials

A) Mycotoxins columns: Aflatest, ochratest, zearalatest, and fumonitest, each type of toxin has its specific column which is consumed for one sample.

B) Chemicals and reagents: Methanol, HPLC grade (4X4L), Distilled deionized water, and Nonionized sodium chloride (salt, NaCl), Afla test developer, Phosphate buffered saline (PBS) Lot: 17021PBS, Ochratest eluting solution Lot: 17061E, 0.1 tween PBS Lot: 17011G2, Zearalatest developer Lot: 102594-4, and fumonisin A and B developer, were utilized in the analysis.

C) Mycotoxins calibration standards: One vial; each of 3 levels, for aflatest, ochratest, zearalatest, and fumonitest calibration.

D) Fluorometer series 4: Fluorometer series 4 provides an accurate and sensitive measurement for aflatoxin, ochratoxin, zearalenone, and fumonisins mycotoxins.

Extraction of mycotoxins

Mycotoxins were extracted from representative samples of broilers feed where 50g of each ground sample was mixed with 5g sodium chloride analar and 100 ml methanol: water (80: 20 by volume) solution. The mixture was blended at high speed for 1 minute and then the extract was filtered through fluted filter paper. For aflatoxin; 10ml filtered extract was mixed with 40ml distilled water, then filtered through a glass microfiber filter (VICAM, 1999). For ochratoxin; 10ml filtered extract was mixed with 40ml phosphate-buffered saline (PBS), then filtered through a 1.5 µm glass microfiber filter (VICAM, 2008). For zearalenone, 1ml filtered extract was mixed with 49ml distilled water and then filtered through microfiber filter (VICAM, 2013). For fumonisin, 10ml filtered extract was mixed with 40ml of 0.1% Tween-20/2.5%PEG/PBS wash buffer, then filtered through a 1.5 µm microfiber filter (VICAM, 2015).

For aflatoxins, 10ml of the filtered diluted extract was passed through the affinity column at a rate of about 1 drop/second (10ml = 1.0g sample equivalent). Then, the column was washed with 10ml distilled water at a rate of 1-2 drops/second. The affinity column was then eluted with 1.0ml HPLC grade methanol at a rate of 1 drop/second, and the elute was collected in a glass cuvette, to where 1ml of freshly made test developer solution was added (VICAM, 1999). For ochratoxin, 10ml of the filtered diluted extract was passed through the affinity column, then the column was washed with 10ml 0.1%

Tween 20/PBS followed by 5ml purified water, and 1.5ml OchraTest™ Elution Solution was used to elute the column (VICAM, 2008). For zearalenone, 1ml of the filtered diluted extract was passed through the affinity column, then the column was washed with 10ml distilled water, and eluted with 1ml HPLC grade methanol, on which a 1ml ZearalaTest ™ Developer was added (VICAM, 2013). For fumonisin, 5ml of the filtered diluted extract was passed through the affinity column, then the column was super-washed with 5ml of 0.1% Tween 20/2.5% PEG/PBS followed by 5ml of PBS, and 1ml HPLC grade methanol was used to elute the column, and a 1ml mixture of Developers A and B was added (VICAM, 2015).

Standardization of Fluorometer series 4

Mycotoxin calibration standards (1 vial each of 3 levels). For aflaTest, calibration settings are adjusted to -1, 27, and 13±2, with detection range 0 – 100 ppb, and limit of detection 1ppb (VICAM, 1999). For Ochratest, calibration settings are adjusted to -1.3, 30, and 14±2, with detection range 0 – 100 ppb, and limit of detection 2ppb (VICAM, 2008). For ZearalaTest, calibration settings are adjusted to 16, -2, and 8±2, with detection range 2 – 100 ppm, and limit of detection 2ppm (VICAM, 2013). For FumoniTest, calibration settings are adjusted to -0.50, 12, and 5.8±0.3, with detection range 0 – 10 ppm, and limit of detection 0.25ppm for corn (VICAM, 2015).

Determination of mycotoxins in broilers feed

Fluorometer series 4 provides accurate and sensitive measurement of mycotoxins. AflaTest® WB SR, OchraTest™, ZearalaTest ™, and FumoniTest ™ have been used for quantitative measurement of aflatoxins, ochratoxin A, zearalenone, and fumonisins in broilers feed and feed ingredients. These test kits are based on immunoaffinity chromatography. The fluorescence of the mycotoxin in the elution solution can then be measured in a fluorometer series 4. Quality assurance and validation of Series-4 Fluorometer procedures were validated by the AOAC Research Institute under the Performance Tested Program to detect and determine mycotoxins, and were licensed under certification mark no. 940801.

Sampling, extraction and ELISA screening of total aflatoxin residues in the muscles and liver of broiler chickens

Sampling of broilers liver and meat

Upon obtaining the approval of the Institutional Animal Care and Use Committee (IACUC) on the Animal Use Protocol (AUP) (VetCU10102019093); 64 broilers' muscles and liver were collected from markets located in different governorates (1, 2 and 3), to determine aflatoxin residues.

Extraction of aflatoxin residues

The aflatoxin residues were extracted from Liver and muscles where 20g of each ground sample was added to 100ml of the extraction solvent (70% methanol), in which the ratio of sample to extraction solvent is 1:5 (w/v). After blending for 2min, 5-10ml of the extract was filtered through a Whatman filter paper (Kensler et al., 2003; Williams et al., 2004; Klich, 2007).

ELISA screening of total aflatoxin residues in the muscles and liver of broiler chickens

The concentration of total aflatoxin residues in the tissue of muscles and liver of broiler chickens was determined by a solid-phase competitive inhibition enzyme-linked immune-assay (ELISA), using HELICA® Low Matrix Total Aflatoxin Assay Kits (HELICA Biosystems, Inc. Santa Ana, CA). The extracted filtrate and the aflatoxin- horse-radish peroxidase (HRP) enzyme conjugate were mixed and added to the antibody-coated microwell. After a step of 5 washes, an enzyme-substrate was added, and the blue color was developed. This was followed by the addition of a stop solution. Absorbances were read at 450 nm by a computerized microplate reader and the optical densities (OD) of the samples were compared to the ODs of the kit standards and a result was determined by interpolation from the standard curve and the total concentration expressed in ng/g (Kensler et al., 2003; Williams et al., 2004; Klich, 2007).

Statistical analysis

Descriptive analysis of mycotoxin levels was performed using PASW Statistics software, version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Feedstuffs contamination by mycotoxins represents a great threat to broilers industry and public health. As shown in Table 1 and Figures 1 and 5, rates of mycotoxins in compound broiler feed in 2014 and 2018 revealed that all 37 analyzed samples were positive to aflatoxin and ochratoxin and their levels ranged from 1 to 55 ppb (mean = 14.33 ppb in 2014 and 20.36 ppb in 2018) and 1.8 to 71 ppb (mean = 27.85 ppb in 2014 and 3.12 ppb in 2018), respectively. In addition, zearalenone and fumonisins were detected in 21 (56.8%) and 6 (16.2%) of the examined samples, respectively, with levels range of 0.48 to 10 ppm (mean = 1.06 ppm in 2014; 3.80 ppm in 2018) and 1.2 to 12 ppm (mean = 7.17 ppm), respectively.

Table 1. Levels of mycotoxins contamination in broiler feed sampled from manufacturing companies in 2014 and 2018

Year	No. of sample	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm	Year	No. of sample	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm
2014	1	13	19	-	8.3	2018	1	19	2.9	3.9	-
	2	32	66	1.2	12		2	52	2.3	5.1	-
	3	27	71	1.2	-		3	41	1.8	5.1	-
	4	9	8.9	-	-		4	51	1.9	7.2	-
	5	9	18	-	-		5	55	2.8	10	-
	6	6	29	-	1.2		6	16	2.3	8.6	-
	7	12	3.3	0.79	-		7	27	2.7	8.2	-
	8	8	43	-	-		8	1	2.2	0.62	-
	9	12	12	-	-		9	3	22	0.52	-
	10	13	7.5	-	-		10	2	2.5	0.48	-
	11	17	17	-	-		11	4	3	1	-
	12	15	20	-	-		12	2	6	1	-
	13	13	19	-	-		13	2	4	1	-
	14	32	66	1.2	8.3		14	4	4	0.59	-
	15	27	71	1.2	12		15	11	3.9	4.2	-
	16	9	8.9	-	-		16	23	17	-	-
	17	9	18	-	-						
	18	6	29	-	-						
	19	12	3.3	0.79	1.2						
	20	8	43	-	-						
	21	12	12	-	-						

- Not analyzed.

Table 2. Levels of mycotoxins contamination in broiler feed sampled from broiler farms in 2014.

Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm	Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm
1	14	71	1.3	8.5	**31**	12	56	-	-
2	85	28	8.9	-	**32**	11	54	-	-
3	22	16	9.7	-	**33**	13	7.5	3.4	3.5
4	18	15	1.6	14	**34**	17	17	-	-
5	25	14	1.6	5.7	**35**	15	20	-	-
6	9	8.6	-	-	**36**	6	-	-	-
7	9	18	-	-	**37**	12	12	-	-
8	12	0	1.1	6.1	**38**	3	11	-	-
9	12	26	1.4	3.6	**39**	4	27	-	-
10	13	44	0.98	5.2	**40**	13	13	-	-
11	30	28	2.4	-	**41**	20	62	-	-
12	2	12	2.3	3.8	**42**	10	57	-	-
13	8	13	-	-	**43**	17	56	-	-
14	14	17	1	4.1	**44**	12	56	-	-
15	14	12	1.1	-	**45**	11	54	3.4	1.3
16	1	16	1.1	5	**46**	15	20	-	-
17	29	15	0.96	-	**47**	13	19	-	-
18	0	21	1.3	5.6	**48**	13	7.5	-	-
19	12	-	-	-	**49**	17	17	-	-
20	17	22	1.4	19	**50**	9	8.9	-	-
21	93	3	-	-	**51**	9	18	-	-
22	8	24	-	-	**52**	32	66	-	-
23	6	-	-	-	**53**	27	71	-	-
24	12	12	-	-	**54**	10	57	-	-
25	3	11	-	-	**55**	17	56	-	-
26	4	27	-	-	**56**	12	56	-	-
27	13	13	-	-	**57**	26	62	-	-
28	20	62	-	-	**58**	3	11	-	-
29	10	57	-	-	**59**	4	27	-	-
30	17	56	-	-	**60**	12	3.3	0.79	1.2

Table 3. Levels of mycotoxins contamination in broiler feed sampled from broiler farms in 2018.

Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm	Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm
1	14	71	1.2	8.5	**27**	20	12	2.3	3.8
2	9	8.6	0	0	**28**	0	0	13	0
3	9	18	0	0	**29**	14	17	1	4.1
4	0	21	1.2	5.6	**30**	14	12	1.1	-
5	17	12	1.4	1.9	**31**	1	16	1.1	-
6	25	14	0	0	**32**	29	15	0.96	5
7	5	22	0	0	**33**	36	46	8.1	0
8	17	12	1.4	1.9	**34**	34		6.2	0
9	33	18	0	0	**35**	19	1.7	7.8	0
10	8	24	0	0	**36**	35	3.8	8.4	0
11	18	15	1.6	14	**37**	43	2.1	7.9	0
12	15	15	70	0	**38**	2	6	2	0
13	10	10	40	0	**39**	25	30	1.5	0
14	13	10	30	0	**40**	30	55	30	0
15	25	15	20	0	**41**	50	40	0	0
16	0	11	20	0	**42**	60	15	0	0
17	0	10	0	0	**43**	100	30	0	0
18	12	26	1.4	3.6	**44**	40	90	0	0
19	22	16	3.7	0	**45**	60	50	0	0
20	10	51	2.4	4.9	**46**	140	30	0	0
21	10	57	0	-	**47**	55	30	0	0
22	17	24	0	0	**48**	560	40	0	0
23	35	22	8.9	0	**49**	40	20	0	0
24	12	0	1.1	6.1	**50**	30	0	0	0
25	13	44	0.98	5.2	**51**	60	0	0	0
26	30	28	2.4	-					

Table 4. Levels of mycotoxins contamination in feed ingredients

Type of feed ingredient	Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm	Type of feed ingredient	Sample No.	Aflatoxin ppb	Ochratoxin ppb	Zearalenone ppm	Fumonisin ppm
Yellow corn	1	7	8.5	13	-	**Soya bean**	1	11	8	-	-
	2	8	43	14	-		2	11	5	-	-
	3	0	3.3	15	-		3	-	5.2	-	-
	4	0	29	19	-		4	-	1.3	-	-
	5	7	8.5	-	-		5	-	20	-	-
	6	7	8.5	-	-		6	14	48	5.5	-
	7	8	43	-	-		7	11	5	-	-
	8	0	26	-	-		8	11	8	-	-
	9	-	3.3	-	-		9	11	8	-	-
	10	7	8.5	-	-		10	11	5	-	-
	11	29	-	-	-		11	-	5.2	-	-
	12	-	3.3	-	-		12	-	1.3	-	-
	13	29	-	-	-		13	-	20	-	-
	14	-	3.3	-	-		14	14	48	5.5	3.5
Wheat germ	1	28	20	-	-		15	11	5	-	-
	2	28	20	-	-		16	11	8	-	-
	3	28	20	-	-		17	-	20	-	-
	4	28	20	-	-		18	14	48	-	-
Nutritive concentrates	1	-	24	-	-		19	-	1.3	-	-
	2	-	24	-	-		20	-	5.2	-	-
	3	-	24	-	-		21	-	1.3	-	-
Lysine	1	44	19	15	-		22	-	5.2	-	-
	2	44	19	19	15	**Feed additive**	1	44	19	15	-

- Not analyzed.

Table 5. Mean level of total aflatoxin residues in liver and muscle samples collected from commercial broiler chickens from different provinces of Egypt

Province	Total aflatoxin residues (ng/g) in tissue samples (n)					
	Breast (15)		**Thigh (28)**		**Liver (21)**	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
1	20.30±2.80	13.15-30.00	12.90±4.90	0.20-27.10	22.30±4.40	11.30-34.90
2	17.00±0.90	16.05-17.85	37.18±4.22	13.15-69.40	33.66±3.42	6.80-4.20
3	20.30±1.10	17.20-23.60	19.20±1.40	13.35-24.80	17.70±2.70	7.10-28.70
Total	19.2±1.6	13.15-30.0	23.09±3.51	0.20-69.40	24.55±3.51	7.10-54.20

SE: Standard error. n: number

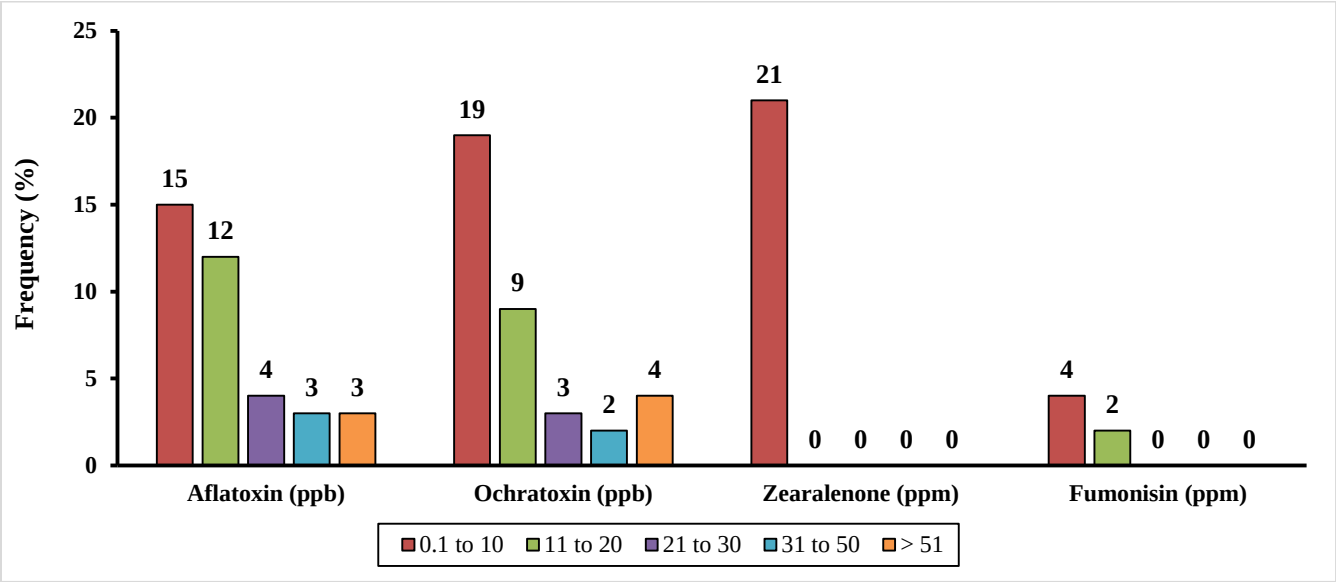

Figure 1. Mycotoxins contamination levels in compound broilers feed sampled from feed manufacturing companies, Egypt

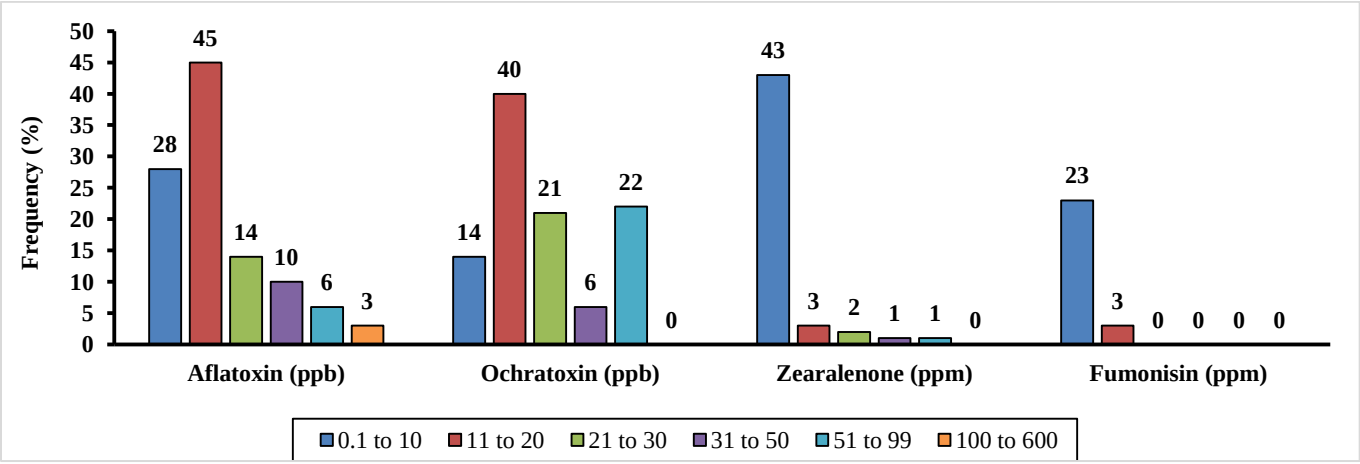

Figure 2. Mycotoxins contamination levels in compound broilers feed sampled from broiler farms, Egypt

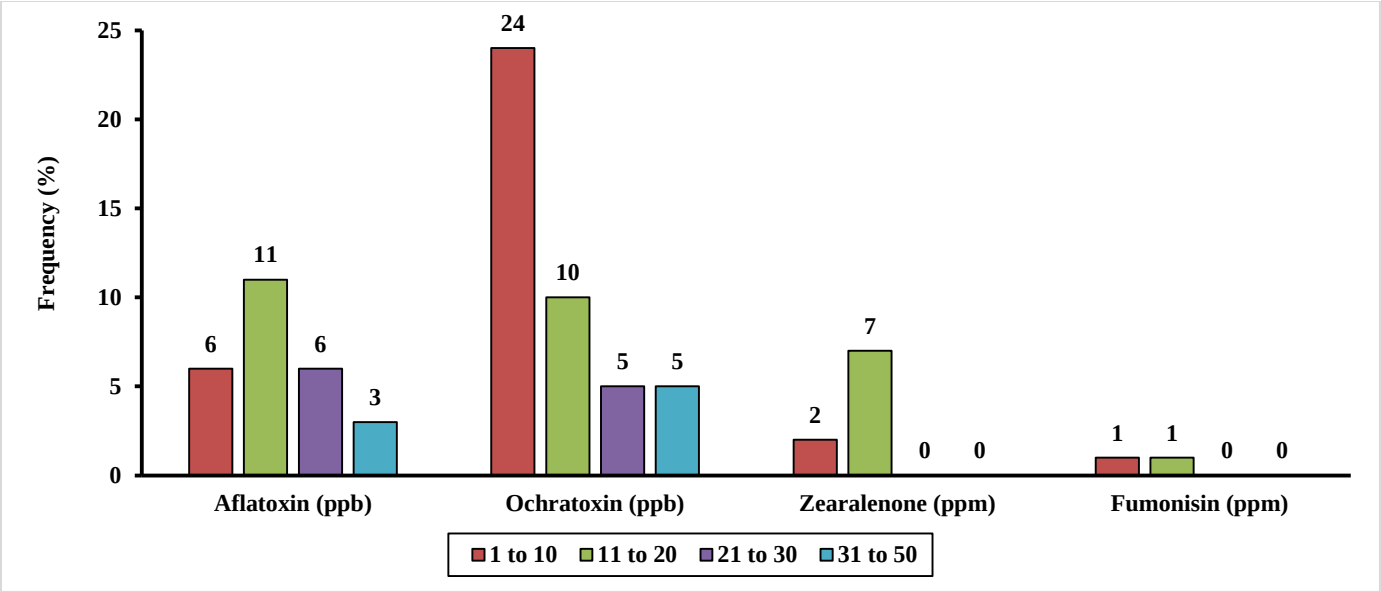

Figure 3. Mycotoxins contamination levels in feed ingredients

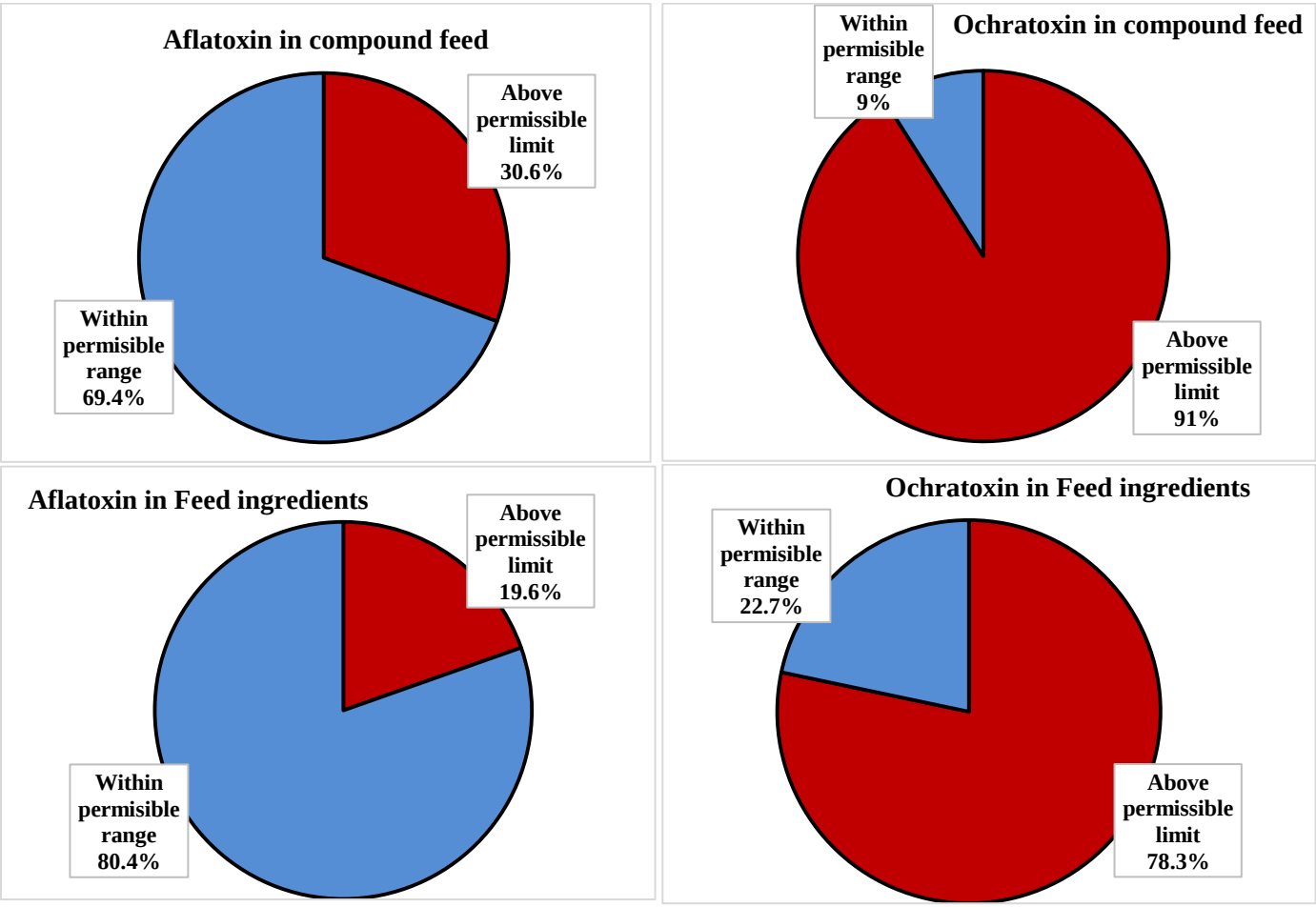

Figure 4. Percentage of aflatoxin and ochratoxin contamination exceeding the permissible limits (20 and 5 ppb; respectively according to FDA, 2000 and EC, 2006) in compound broilers feed and feed ingredients samples, Egypt.

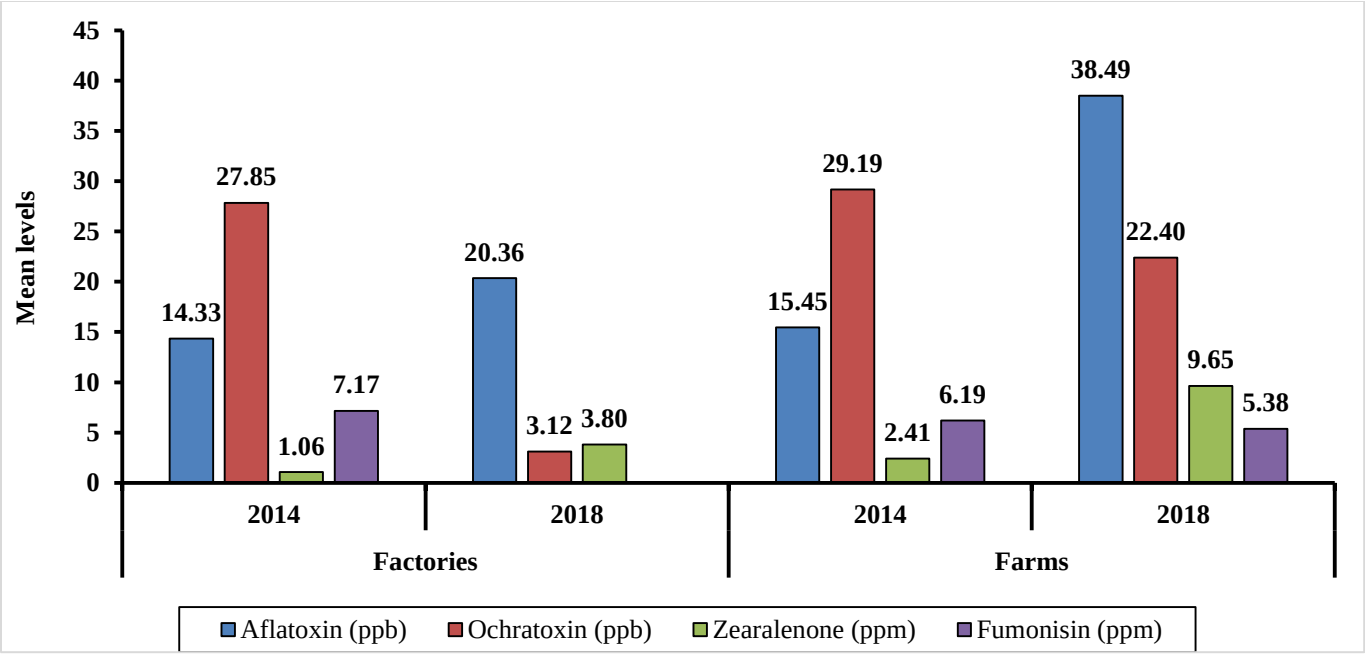

Figure 5. Mean levels of mycotoxins contamination in compound broilers feed in 2014 and 2018, Egypt

As illustrated in Tables 2 and 3 and Figures 2 and 5, rates of mycotoxins in compound broiler feed in 2014 and 2018 revealed that 96.4% and 92.8% of totally analyzed samples were positive to aflatoxin and ochratoxin with levels ranged from 1 to 560 ppb (mean = 15.45 ppb in 2014; 38.49 ppb in 2018) and 1.7 to 90 ppb (mean = 29.19 ppb in 2014; 22.40 ppb in 2018), respectively. Also, zearalenone and fumonisins were detected at a rate of 45.5% and 23.4%, respectively, with levels range of 0.79 to 70 ppm (mean= 2.41 ppm in 2014; 9.65 ppm in 2018) and 1.2 to 19 ppm (mean= 6.19 ppm in 2014; 5.38 ppm in 2018), respectively. Table 4 and Figures 3 and 5 revealed that aflatoxin and ochratoxin in different types of feed ingredients were detected with rates 54.5% and 95.5%, respectively and their levels ranged from 7 to 44 and 1.3 to 48 ppb, respectively. In addition, the prevalence of zearalenone and fumonisin was 20.5% and 4.5% in the analyzed samples with a range of 5.5 to 19 and 3.5 to 15 ppm, respectively. The obtained results revealed that the contamination levels of aflatoxins and ochratoxins were above the permissible values (20 and 5 ppb, respectively) in compound broilers feed at 30.6% and 91% and in feed ingredients at 19.6% and 78.3%, respectively (Tables 1-4, and figure 4).

The FDA restricts levels of aflatoxin in food

and animal feeds to 20 ppb and the EU limits levels of aflatoxin to 15 ppb (Yang et al., 2020). The reported levels of aflatoxins in poultry feed and their ingredients are parallel to those previously reported in Egypt and other countries. In a previous study, from 87-broiler feed samples collected from a poultry feed production unit in Kuwait, aflatoxin was detected in broiler starter at 0.48 ppb level (range 0 to 3.26 ppb), and in broiler finisher at 0.39 ppb level (range 0 to 1.05 ppb) (Beg et al., 2006). Moreover, in Kuwait, aflatoxins were detected in 63.9% of poultry feed; with range 6 to 201 ppb for AFB1, and 8 to 335 ppb for aflatoxin B2 (Natour et al., 1983). In Egypt, 80% of the sampled maize contained aflatoxins at 480 ppb level (Mahmoud, 1993). In Turkey, 71% of layer feed samples showed an aflatoxin level of less than 5 ppb, and only 2 samples exceeded acceptable levels (20-ppb) (Nizamlýolu and Oguz, 2003). In Bangladesh, poultry feed showed aflatoxin levels ranged from 7 to 160 ppb (Dawlatana et al., 2002), while 216- feed ingredients from a poultry feed factory in India, showed contamination with aflatoxin in 60% of the mixed feed samples, with range 10 to 1500 ppb (Thirumala-Devi et al., 2002). In Nigeria, analysis of 102 samples of poultry feed and feed ingredients from poultry farms showed AFB1 in 83% of feed samples (range, 0.5–760 ppb; mean, 74 ppb) (Akinmusire et al., 2019). In South Africa, aflatoxins reported the lowest prevalence (30% of samples) with levels ranged between 0.2 to 71.8 ppb (mean: 9.0 ppb) (Njobeh et al., 2012). In Cameroon, Abia et al. (2013) analyzed 20 feed samples pools collected from different

poultry farms and reported the rate of contamination with aflatoxins of 75-95%. In Argentina, Greco et al. (2014) detected aflatoxins in 90% of poultry feed samples (median 2.685 ppb).

Since ochratoxin A was discovered in 1965, it has been ubiquitous as a natural contaminant of moldy food and feed. The multiple toxic effects of ochratoxin A are a real threat to human beings and animal health. Humans exposed to ochratoxin A can develop a range of chronic disorders and plays the main role in the pathogenesis of some renal diseases including Balkan endemic nephropathy, kidney tumors occurring in certain endemic regions of the Balkan Peninsula, and chronic interstitial nephropathy occurring in Northern African countries and likely in other parts of the world (Malir et al., 2016). Worthwhile, the EU has set a maximum limit of 5 ppb for cereal products (Yang et al., 2020). In our study, the vast contamination of the poultry feed and feed ingredients with ochratoxin-A agrees with several previous reports. In Kuwait, broiler feed showed ochratoxin levels ranged from 4.6 to 9.6 ppb (Beg et al., 2006). In Argentina, ochratoxin was found in 38% of the poultry feed samples with levels ranged from 25 to 30 ppb (mean 27 ppb) (Dalcero et al., 2002). In South Africa, ochratoxin reported the lowest prevalence (4% of samples) with levels ranged between 6.4 and 17.1 ppb (mean: 9.9 ppb) (Njobeh et al., 2012). In Cameroon, Abia et al. (2013) analyzed 20 feed samples pools collected from different poultry farms and reported the ochratoxins rate of 80-90%. In Argentina, Greco et al. (2014) detected ochratoxin in 90% of the poultry feed samples (median 5 ppb) and aflatoxins (median 2.685 ppb).

The EU has set a concentration limit for zearalenone in raw maize to 100 ppb and in cereal products to 20 ppb (Yang et al., 2020). The incidence of detectable Zearalenone is similar to that found by previous studies conducted in several regions. In Cameroon, Abia et al. (2013) analyzed 20 feed samples pools collected from different poultry farms and reported feeds contamination with zearalenone in 100% of samples, with mean concentrations 155 (range 0.7-600) ppb. In Kuwait and Egypt, zearalenone ranged from 46.4 to 67.6 ppb in broiler feed samples collected from a poultry feed production unit (Beg et al., 2006), and 40 ppb in 80% of the maize samples integrated in poultry feeds (Mahmoud, 1993); respectively. In Swedish, zearalenone was detected in 2 of 68 mixed feed samples, with one showed a very high level (1200 ppb) and the other was 100 ppb (Pettersson and Kiessling, 1992). In Argentina, Greco et al. (2014) detected Zearalenone in 86% of the poultry feed samples (median 50 ppb).

The EU has set a maximum limit of fumonisin in raw corn 4000 ppb (Yang et al., 2020). The high incidence of detectable fumonisin is similar to that found by previous studies conducted in several countries. In Kuwait, fumonisin ranged from 1.4 to 3.2 ppm in broiler feed samples collected from a feed factory (Beg et al., 2006). In Nigeria, fumonisin B1 was detected in most of the samples (97%) (Range, 37–3760 ppb; mean, 1014 ppb) (Akinmusire et al., 2019), as well as Ezekiel et al. (2012) detected fumonisins in 83% of 58 commercial poultry feed samples in Nigeria, with concentrations range, 31– 2733 ppb; and mean, 964 ppb. In South Africa, Njobeh et al. (2012) detected fumonisins in 87% of 92 compound feeds samples with concentrations range, 104–2999 ppb; and mean 903 ppb. In Cameroon, Abia et al. (2013) analyzed 20 feed samples pools collected from poultry farms and reported fumonisins in 100% of samples, with concentrations range, 16– 1930 ppb; and mean, 468 ppb. In Taiwan, Tseng and Liu (2001) detected fumonisin in few samples of imported maize at level exceeded 0.3 ppm. In Iran, Shephard, et al. (2002) detected fumonisin B1 in maize at average levels of 3.18 ppm (range 0.68 to 7) and 0.22 ppm (range <0.01 to 0.88) in two areas. Likewise, In the United Kingdom, maize feedstuffs were reported to frequently contain fumonisin B1 and B2 at levels up to 5 ppm (Scudamore et al., 1997). In Argentina, Greco et al. (2014) detected Fumonisins in all the samples in a range of 222–6,000 ppb (median 1,750 ppb).

The combined toxic effects of aflatoxin, ochratoxin, zearalenone, and fumonisins in feed and food might pose a veterinary and public health risk. Overall, results indicated that 86.60% of compound feed and feed ingredient samples contained two or more mycotoxins. Combined contamination with aflatoxin and ochratoxin was detected in 76.8% of the samples. In Argentina, Greco et al. (2014) found that 90% of poultry feed samples were contaminated with ochratoxin and aflatoxins. Beg et al. (2006) detected the coexistence of ochratoxin A, fumonisin, and zearalenone in poultry feed from Kuwait, although in lower concentrations than the permissible limits defined for the poultry feed. In Bangladesh, Dawlatana et al. (2002) confirmed the possibility of multiple mycotoxins contamination in poultry feed and detected five mycotoxins in one sample of maize. While in Nigeria, Akinmusire et al. (2019) reported contamination with at least four mycotoxins in 102 samples of feed and feed ingredients collected from poultry farms, as they detected fumonisin B1 in most of the samples (97%) and

AFB1 in 83% of feed samples. Also, Scudamore et al. (1997) detected multi-mycotoxin contamination with both aflatoxin and fumonisin in some samples of maize. Magnoli et al. (2002) reported that fumonisins had the highest incidence (97%) followed by AFB1 (46%) and zearalenone (18%). Moreover, Aravind et al. (2003) analyzed a commercial broilers diet naturally contaminated with mycotoxins in India, and reported contamination with aflatoxins, ochratoxin, and zearalenone toxins, and suggested the possible synergistic toxic effect from the combination of multiple mycotoxins offered in the contaminated feed.

The presence of mycotoxins in animal products is the most critical aspect and a serious factor affecting meat quality that has a special public health concern. As presented in Table 5, aflatoxins residues were detected in all examined tissue samples that collected from the commercial broiler chickens in three provinces, Egypt with detectable levels above the recommended permissible limit for human consumption (4 ng/g) according to FDA regulations. These results came in agreement with Herzallah (2009) who documented the levels of AFB1, AFB2, aflatoxin G1 and G2 ranged from 1.10 to 8.32 mg/kg and 0.15 to 6.36 mg/kg in imported and fresh meat samples. Moreover, Iqbal et al. (2014) and Markov et al. (2013) reported that AFB1 was detected in 10% and 35% of the collected chicken samples from Croatia and Pakistan, with maximum levels 3.0 mg/kg and 8.01 mg/kg, respectively.

Collectively, mean total aflatoxin residues in the liver of all examined samples were higher than their levels in the breast and thigh muscle tissues (Table 5). Similarly, Magnoli et al. (2011) and Herzallah et al. (2014) reported aflatoxin and its metabolites residues in the different tissues of broiler chicks fed aflatoxin-contaminated diets with different concentrations and various treatments. These recorded results related to the ability of poultry to metabolize and eliminate aflatoxin from their tissues.

CONCLUSION

In this study, all broiler compound feed samples collected from feed production units were contaminated with aflatoxins and ochratoxins, while zearalenone and fumonisin were detected at 56.8% and 16.2% of samples, respectively. Moreover, 30.6% of aflatoxin-positive samples and 91% of ochratoxin-positive samples exceeded the permissible limit in compound feed. Co-contamination with two or more types of different mycotoxins was recorded in 86.6% of tested compound feed and feed ingredients and the combined contamination with aflatoxin and ochratoxin was found in 76.8% of positive samples. Aflatoxin residues that exceeded the recommended permissible limit for human consumption were detected in 100% of broiler meat and liver samples.

DECLARATIONS

Competing interests

The authors have no competing interests.

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Authors' contribution

Anwaar Mettwally El-Nabarawy designed the experiment, provided the facilities and the material needed, performed mycotoxins detection and determination, wrote and revised the manuscript. Elshaimaa Ismael contributed to mycotoxins detection and determination, designed the figures, wrote and revised the manuscript. Sawsan El Basuni collected broiler tissue samples, performed the extraction of aflatoxin residues and wrote the manuscript. Khaled Shaaban contributed to mycotoxin detection and determination. Mohamed Mohamed Ismail Batikh collected feed and broiler tissue samples

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