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AFLATOXINS AND AFLATOXIGENIC FUNGI OCCURRENCE IN ANIMAL AND POULTRY FEED OF ASSIUT GOVERNORATE FARMS, EGYPT

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ABSTRACT

Sixty representative pelleted animal and poultry feed samples were collected from Assiut governorate farms for aflatoxins (B₁, B₂, G₁, and G₂) analysis by thin layer chromatography (TLC) and ultra-performance liquid chromatography (UPLC) in the EGAC-accredited central lab. of the Faculty of Veterinary Medicine, Assiut University, as well as for their aflatoxigenic fungi prevalence assessment. TLC results showed that 35% of the analyzed feed samples were positive for AFs. UPLC results showed that means \pm SE of AFB₁, AFB₂, AFG₁, AFG₂ and total AFs were 23.36 \pm 13.12, 2.88 \pm 1.07, 1.33 \pm 0.64, 0.52 \pm 0.25 and 28.08 \pm 13.98 µg kg⁻¹ in animal feed and 34.88 \pm 25.18, 2.55 \pm 0.78, 1.80 \pm 1.51, 1.30 \pm 0.52 and 40.83 \pm 25.59 µg kg⁻¹ in poultry feed, respectively. The prevalence of aflatoxins was 70% and 100% in animal feed and poultry feed samples, respectively. *Aspergillus flavus* isolates from the analyzed feed samples showed different degrees of aflatoxigenic ability on coconut agar medium, and the poultry feed samples were more contaminated with AFs and aflatoxigenic fungi than animal feed samples.

Key words: Aflatoxins; animal feed; poultry feed; TLC; UPLC

INTRODUCTION

Fungi not only cause the deterioration of food and feed but also can produce mycotoxins as secondary toxic metabolites. According to the Food and Agriculture Organization (FAO), mycotoxins contaminate 25% of the world's crops (Pankaj *et al.*, 2018). Mycotoxins have threatened the poultry industry all over the world (Diaz and Murcia, 2011), especially aflatoxins (AFs), which act as the most toxic

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E-mail address: hebafawzy907@aun.edu.eg *Present address:* Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Egypt. ORCID: <u>https://orcid.org/0000-0002-7578-9822</u>. category among various types of mycotoxins and are produced mainly by *Aspergillus falvus* and *Aspergillus parasiticus* (Nakai *et al.*, 2008). In food, aflatoxins B₁, B₂, G₁, G₂, M₁, and M₂ are ubiquitous (Sumit *et al.*, 2010), but in animal and poultry feed stuffs, only AFB₁, B₂, G₁, and G₂ are the major naturally occurring aflatoxins. Aflatoxin B₁ is the most potent and toxic one, followed by G₁, B₂, and G₂ (Bryden, 2007).

Aflatoxin is the most potent mycotoxin due to its acute toxicological impacts and chronic hepatocellular carcinoma. The International Agency for Research on Cancer (IARC) classified aflatoxins as human carcinogens in "group 1," except AFM₁, which was classified as possibly carcinogenic to humans in "group 2B" (IARC, 2010). In addition to their carcinogenic effects, AFs have cytotoxic, genotoxic, mutagenic, and teratogenic effects (Deabes et al., 2012). Poultry are the most vulnerable to aflatoxicosis, which can impair their production and reproduction parameters and result in significant economic loss (Hussain et al., 2010).

More than 100 countries have established regulations for AFs on food and feed imports due to their serious impacts on humans, animals, and the worldwide economy. Contamination of food and feed by aflatoxins threatens food security for humans and animals (Udomkun *et al.*, 2017). According to the European Food Safety Authority (EFSA), no tolerable daily intake (TDI) for aflatoxins was established due to the highly toxic effects of this group of mycotoxins (EFSA, 2020).

The current Egyptian regulatory limits for total AFs and AFB1 in animal feed are 20 μ g kg⁻¹ and 10 μ g kg⁻¹, respectively (Egyption Standards, 2010). According to the European Union, the maximum tolerable levels for total AF and AFB₁ in cereals for human consumption are 4 and 2 μ g kg⁻¹, respectively, and 20 μ g kg⁻¹ for total AF in poultry feeds (EFSA 2010).

These regulatory limits obstruct crops' export and import from different parts of the world; thus, our study aimed to evaluate the AFs level in animal and poultry feed in Upper Egypt with TLC and UPLC and detect its validity according to national and international permitted limits. Animal and poultry feed was intended to be chosen in the current study due to the further residues of AFs in animal and poultry products, especially milk, meat, and eggs.

MATERIALS AND METHODS

1. Chemicals

• Chemicals used for aflatoxins analysis were of analytical reagent

grade and included dichloromethane, chloroform, acetone, methanol, acetonitrile sodium sulphate

acetonitrile, sodium sulphate anhydrous, acetic acid, and deionized water. Thin-layer chromatography aluminate silica gel plates were obtained from Merck (Germany) and aflatoxins standards (B_1 , B_2 , G_1 , and G_2) from Wesel (Germany).

• Aspergillus flavus and parasiticus agar media were prepared in the lab. according to Pitt and Hocking (2009) used as a selective media for Aspergillus spp. and coconut agar media also was prepared in the lab. according to Yazdani *et al.* (2010) and they were used as a screening method for the aflatoxigenic ability of Aspergillus spp.

2. Sampling and sample preparation

Sixty representative pelleted feed samples (30 animal feed and 30 poultry feed samples) were collected in proper paper bags from twenty Assiut governorate farms through 2022. During the analysis, each of the three samples per farm was mixed and represented with one sample. Each sample was ground in a laboratory mill and used for aflatoxins analysis and mycological examination.

3. Detection of aflatoxins in animal and poultry feeds

3.1. Using Thin Layer Chromatography

Finely ground feed samples were prepared for immediate aflatoxins analysis on TLC, according to Braicu *et al.* (2008). Then the plates were viewed under long-wavelength UV light (365 nm) for AF fluorescence detection. Aflatoxins were identified by comparing them to aflatoxin standards on a TLC plate. The retention factor (RF) on TLC was estimated according to Abd-Elaah and Samya (2005) as the following equation:

Retention factor = Distance moved by substance (spot)

Distance moved by the solvent (solvent front)

3.2. Using Ultra-Performance Liquid Chromatography

Thin layer chromatography was followed with an ACQUITY® UPLC H-class system equipped with an ACQUITY BEH C18 column (2.1 x 100 mm, particle size 1.7 μ m) with a fluorescence detector (excitation 365 nm and emission 429 nm), 64 water, 18 methanol, and 18 acetonitrile (HPLC grade) mobile phases, and a 0.4 ml/min flow rate, 1 μ l injection volume, and a 4-minute running time according to the method described by Benvenuti and Burgess (2010) in the central laboratory of the Faculty of Veterinary Medicine at Assiut University.

The LOQ was 0.02, 0.03, 0.16, and 0.20 ng ml⁻¹; the LOD was 0.0061, 0.0100, 0,0539, and 0.0659 ng ml⁻¹; recovery % was 90.39, 94.47, 87.82, and 87.14; and repeatability% was 85.35 ± 3.83 , 86.84 ± 6.87 , 93.77 ± 7.96 , and 89.77 ± 8.2 for AFB₁, AFB₂, AFG₁, and AFG₂, respectively.

3.3. Standard preparation

According to Benvenuti & Burgess (2010), standard working solutions were immediately prepared by diluting an appropriate amount of Mycotoxin Mix 1 (Aflatoxin) standard with 100% methanol, where it is light sensitive and storage occurs at -8 to -22°C or below. Mycotoxin Mix 1 (Aflatoxins) standard included AFB₁, AFG₁, AFB₂, and AFG₂ with 2.00 μ g/ml, 2.01 μ g/ml, 0.500 μ g/ml, and 0.503 μ g/ml concentrations, respectively.

4. Isolation of *Aspergillus* species from feed samples on *Aspergillus flavus parasiticus* agar (AFPA) medium

Ten grams of each milled feed sample were mixed with 90 ml of distilled water and shaken for 20 minutes, followed by the culturing of 1 ml on AFPA medium as a selective medium for *Aspergillus flavus* and *Aspergillus parasiticus* (dissolve dichloran 2 mg, ferric ammonium citrate 0.5 g, peptone 10 g, yeast extract 20 g, agar 15 g, and 100 mg chloramphenicol in one liter of distilled water by heating till boiling on a hot plate, then autoclave for 15 minutes at 121°C), followed by cooling, pouring in Petri dishes, culturing the samples, and incubating for 7 days in the dark at 25–28°C.

5. Determination of the aflatoxigenic ability of *Aspergillus* spp. on coconut agar medium (CAM):

Fungi were identified by their colonial morphology and microscopic characteristics. After that, their aflatoxigenic ability in CAM was determined (100 g of shredded coconut was homogenized for 5 minutes with 300 ml hot distilled water, then the homogenate was filtrated through four layers of cheesecloth, the pH of the clear filtrate was adjusted to 7, then completed to 1000 ml distilled water and add 20 g agar, the medium was autoclaved for 15 minutes at 121°C, cooled to about 40–45°C, poured into Petri dishes, and the isolates were cultured).

Aspergillus spp. isolates were inoculated centrally in a petri dish containing 10–15 ml of CAM and incubated for 7 days in the dark at 25° C. Cultures were observed for fluorescence under long-wave UV light (365 nm) after 3, 5, and 7 days. The positive results were shown as a blue fluorescence zone around colonies of *A. flavus* with different intensities (Yazdani *et al.*, 2010).

6. Statistical analysis:

Results were expressed as a mean \pm SE or SD. Aflatoxins level results were analyzed statistically using the computer program SPSS for Windows, version 16.0, through analyses of variance (Green & Salkind, 2010).

RESULTS

1. Detection of aflatoxins in animal and poultry feeds with TLC and UPLC

Animal and poultry feed samples were analyzed for aflatoxins with TLC, followed by UPLC. Poultry feed samples were more contaminated with AFs than animal feed samples (**Figure 1**). TLC results showed that 35% of the analyzed feed samples were positive for AFs, compared to the standard by UV lamp (**Tables 1, 2, and Figure 2**). UPLC results showed that aflatoxin B₁, B₂, G₁, G₂, and total AFs prevalence were 70%, 70%, 40%, 40%, and 70% in animal feed and 100%, 100%, 20%, 60%, and 100% in poultry feed samples, respectively. Mean \pm SE of AFB₁, AFB₂, AFG₁, AFG₂, and total AFs were 23.36 \pm 13.12, 2.88 \pm 1.07, 1.33 \pm 0.64, 0.52 \pm 0.25, and 28.08 \pm 13.98 µg kg⁻¹ in animal feed samples and 34.88 \pm 25.18, 2.55

 \pm 0.78, 1.80 \pm 1.51, 1.30 \pm 0.52, and 40.83 \pm 25.59 µg kg⁻¹ in poultry feed samples, respectively. A comparison of the previous results with AF's regulatory permissible limits showed that about 35% and 53% of positive samples exceeded EU and Egyptian regulatory permissible limits, respectively **Table (1 and 2).**



Figure 1: Comparison between AFs level (µg kg⁻¹) in animal and poultry feed samples.



Figure 2: Thin layer chromatography results of feed samples were either negative (-), suspect (±), or positive (+) for AFs compared with standard (St.).

Animal feed samples	UPLC Aflatoxins level (µg kg ⁻¹)							European	Egyptian Regulation
	AFB ₁	AFB ₂	AFG1	AFG ₂	Total AFs	TLC	CAM	Union Acceptance	Acceptance
1	4.43	1.7	3.5	1.8	11.43	<u>±</u>	+		
2	6.3	3.2	0.82	ND	10.32	+	+		
3	ND	ND	ND	ND	ND	-	+	V	
4	28.5	5.4	ND	ND	33.9	+	+	, ×	×
5	46.7	8.31	3.82	1.96	60.79	т 1	Т	~	~
6	ND	ND	ND	ND	ND	+	+	×	×
7	133.3	8.62	ND	ND	141.7	-	+	N	γ
8	ND	ND	ND	ND	ND	++	++	×	×
9	4.3	0.74	ND	0.8	5.84	-	-	V	
10	10.1	0.88	5.2	0.66	16.8	-	-		
						. ±	+		×
Mean	23.36	2.88	1.33	0.52	28.08				
SE	13.12	1.07	0.64	0.25	13.98				
Prevalence	70%	70%	40%	40%	70%				
Min.	ND	ND	ND	ND	ND				
Max.	133.3	8.62	5.2	1.96	141.7	•			

Table 1: Aflatoxin level (μg kg⁻¹), TLC, CAM results, and acceptance in animal feed samples according to permissible regulations

Ultra Performance Liquid Chromatography aflatoxin levels in animal feed samples (μ g kg⁻¹) were represented as means \pm SE, (ND): Non-Detected. Thin layer chromatography results in feed samples were represented with negative (-), suspect (\pm), positive (+), or highly positive (++) for AFs. According to European Union and Egyptian regulations, animal feed samples were either accepted ($\sqrt{}$) or rejected (\times). On CAM, *A. flavus* isolates were highly toxigenic (++), moderately toxigenic (+), or atoxigenic (-)

Table 2: Aflatoxin level (μg kg⁻¹), TLC, CAM results, and acceptance in poultry feed samples according to permissible regulations

Poultry	UPLC Aflatoxins level (µg kg ⁻¹)						САМ	European Union	Egyptian Regulation
samples	AFB ₁	AFB ₂	AFG1	AFG ₂	Total AFs	ILC	CAM	Acceptance	Acceptance
1	13.32	1.74	ND	ND	15.06	+	+		×
2	6.3	0.8	ND	1.8	8.9	±	+		
3	1.185	0.735	ND	2.185	4.105	-	+		V
4	260.669	6.914	ND	ND	267.58	++	++	×	×
5	20.4	3	ND	ND	24.717			~	~
6	3.5586	4.317	ND	ND	4.335	Ŧ	+		à
7	6.6	0.776	ND	2.03	10.43	-	+	N	N
8	4.9	1.8	ND	0.2	5.5	±	-	N	N
9	21.778	0.4	15.171	5.101	51.574	-	+		
10	10.13	6.524	2.81	1.66	16.13	+	++	×	×
		1.53				±	+		×
Mean	34.88	2.55	1.80	1.30	40.83	-			
SE	25.18	0.78	1.51	0.52	25.59				
Prevalence	100%	100%	20%	60%	100%	-			
Min.	1.185	0.4	ND	ND	4.105	_			
Max.	260.669	6.914	15.171	5.101	267.583	-			

Ultra Performance Liquid Chromatography aflatoxin levels in poultry feed samples (μ g kg⁻¹) were represented as means \pm SE, (ND): Non-Detected. Thin layer chromatography results in feed samples were represented with negative (-), suspect (\pm), positive (+), or highly positive (++) for AFs. European Union and Egyptian regulations showed that animal feed samples were either accepted ($\sqrt{}$), or rejected (×). On CAM, *A. flavus* isolates were highly toxigenic (++), moderately toxigenic (+), or atoxigenic (-)

2. Isolation of *Aspergillus* species on AFPA and determination of their aflatoxigenic ability on CAM

On AFPA medium, Aspergillus niger, Aspergillus flavus, and Aspergillus terreus were the most frequent Aspergillus spp., which were 56%, 33%, and 11%, respectively, in animal feed samples, but in poultry feed samples only *Aspergillus niger* (60%) and *Aspergillus flavus* (40%) were observed (**Figure 3**). *Aspergillus parasiticus* was not isolated in poultry and animal feed samples during the current study.



Figure 3: Prevalence % of *Aspergillus* species (*A. niger*, *A. flavus*, and *A. terreus*) in animal and poultry feeds.

Thin-layer chromatography and coconut agar medium are used for aflatoxins and aflatoxigenic fungi presumptive analysis, respectively. On CAM, *Aspergillus flavus* isolates were tested for AFs-producing potential, and results indicated that 85% of A. *flavus* isolates had suitable conditions to produce aflatoxins in feed with different blue fluorescent haloes around the *Aspergillus flavus* colony according to (**Table 1, 2 and Figure 4**).



Figure 4: *A. flavus* isolates on coconut agar medium showing (a) moderately toxigenic and (b) highly toxigenic isolates based on the degree of blue fluorescent halo around the *Aspergillus flavus* colony (arrow).

DISCUSSION

A contaminated feed with aflatoxigenic fungi and aflatoxins is considered a health risk for humans and animals, where aflatoxins are the most toxic and potent secondary fungal metabolites.

In the current study, TLC analysis results for animal and poultry feed samples confirmed the findings of Kotinagu *et al.* (2015) that 30% of livestock feed and feed ingredients were contaminated with AFB₁. However, Ramesh *et al.* (2013) results were higher than the results of our study, where 79.66% of feed samples were positive for aflatoxin B₁ on TLC.

UPLC results showed that aflatoxin B_1 , B_2 , G_1 , G_2 , and total AFs prevalence were 70%, 70%, 40%, 40%, and 70% in animal feed and 100%, 100%, 20%, 60%, and 100% in poultry feed samples, respectively. Mean ± SE of AFB₁, AFB₂, AFG₁, AFG₂, and total AFs were 23.36 \pm 13.12, 2.88 \pm 1.07, 1.33 \pm 0.64, 0.52 ± 0.25 , and $28.08 \pm 13.98 \ \mu g \ kg^{-1}$ in animal feed samples and 34.88 ± 25.18 , 2.55 \pm 0.78, 1.80 \pm 1.51, 1.30 \pm 0.52, and 40.83 \pm 25.59 μ g kg⁻¹ in poultry feed samples, respectively. These results agreed with previous studies of poultry feeds where aflatoxins' occurrence was between 64% and 100% (Aboagye-Nuamah et al., 2021; Kana et al., 2013; Mokubedi et al., 2019; Taylor & Ezekiel, 2012).

Taylor and Ezekiel (2012) showed that among the regulatory detected toxins in poultry feed in Nigeria's districts and states, aflatoxins had a prevalence of 62% with levels above 20 μ g kg⁻¹. In Cameroon, the prevalence of aflatoxins in mixed poultry feeds was 93.3% and 83% at levels 2 to 52 μ g/kg and 2 to 23 μ g/kg in broiler and layer feed samples, respectively (Kana *et al.*, 2013).

Mokubedi *et al.* (2019) analyzed different mycotoxins in poultry feed samples from South Africa and observed that the AF incidence was 92%, with generally low concentration levels ranging from 0.1 to 3.7 μ g/kg.

In sub-Saharan Africa, Kemboi *et al.* (2020) showed that aflatoxins' occurrence in feed and feed ingredients was 70%, with a range of 0.2-318.5 μ g/kg. According to Nakavuma *et al.* (2020), aflatoxins levels in poultry feed produced by feed processors and farmers were 7.5 ± 0.71 to 393.5 ± 19.09 and 19.0 ± 1.41 to 188.5 ± 2.12 μ g kg⁻¹, respectively.

Our study results also compared with Abdelhamid (1990) who observed that peanuts have the highest contamination mean of AFB₁ (400 μ g kg⁻¹), but the lowest AFB₁ mean was in soybean samples (5 μ g kg⁻¹). Salem (2002) showed that total AF levels in feedstuff samples from Assiut province were between 2 and 60 µg kg⁻¹. Njobeh et al. (2012) revealed that the incidence of AFs was 30% with a 0.2–71.8 μ g kg⁻¹ range and a 9.0 µg kg⁻¹ mean in South African compound feeds, and Abdallah et al. (2017) showed that only AFB₁ was present in commercial feed samples in upper Egypt with a 4% incidence and 11 μ g kg⁻¹ as the maximum level, and only one of the positive samples was above the maximum Egyptian regulatory limits, which were lower than our total AFs mean.

The maximum permissible level of aflatoxins in food and feed was set by various national and international organizations to provide food security and safety for consumers (Udomkun et al., 2017). A comparison of our results with AF's regulatory permissible limits showed that about 35% and 53% of positive samples exceeded EU and Egyptian regulatory permissible limits, respectively. Kang'ethe and Lang'a (2009) reported that 67% and 58% of the AF-positive samples from farmers and grain millers exceeded the FAO recommended limits, respectively. According to the European regulatory limit for aflatoxins, Gruber-Dorninger et al. (2018) showed that 54.4% of different African countries' finished feed samples were unfit for consumption and exceeded permissible limits and Nakavuma et al. (2020) revealed that all the analyzed feed samples from feed processing plants were contaminated with aflatoxins, but only 18.8% were within the limit recommended and safe for consumption.

The prevalence of *Aspergillus* spp. and the ability of *Aspergillus flavus* isolates for AFs-production were tested, our results disagree with previous studies, as Aliyu *et al.* (2016) showed that the most prevalent fungal species isolated from poultry feeds in Sokoto

metropolis were Aspergillus fumigatus (58.8%), followed by Aspergillus flavus (41.2%), but Hassan *et al.* (2021) showed that *A. flavus* (62.5%) was the most predominant *Aspergillus* spp., followed by *A. niger* (25.9%), and *A. fumigatus* (15.8%). Only 57.3% of *A. flavus* isolates could produce AFs.

The production of aflatoxin is dependent on the growth of fungi strains and the availability of suitable environmental conditions. Moisture is the first factor where A. flavus can produce aflatoxins at a moisture content of 16%–28% (Dorner, 2008). Temperature is the second factor, where; A. flavus can grow at 12–47°C, but the optimum temperature for aflatoxin production is 25-32°C (Smalley, 1986). Aflatoxins and aflatoxins-producing fungi have a high occurrence in tropical and subtropical regions where humidity and temperature conditions are optimal for toxin production (Jacobsen et al., 2007) and contaminate different agricultural commodities that will be used in processing rations in the future (Rushing & Selim, 2019; Tajkarimi et al., 2011).

The variation in aflatoxigenic and aflatoxin level incidence in the current study is not only due to differences in atmospheric conditions in Egypt and feed storage conditions, but also due to the ingredients of animal and poultry feed, where raw materials include corn, rice, wheat, soybeans, and other additives. This is based on animal or poultry needs and eating habits. These cereals are highly susceptible to aflatoxigenic and AF contamination, and most industrial processes do not detoxify aflatoxins.

CONCLUSION

This study revealed that pelleted poultry feed is more contaminated with AFs and aflatoxigenic fungi than pelleted animal feed. Thus, the continuous monitoring of AFs in food and feed by TLC and UPLC and strict application of permissible limits are necessary for human security and safety. Predisposing factors for AF production such as temperature, relative humidity, and moisture must be controlled, as in suitable conditions, aflatoxigenic fungi can produce different levels of AFs.

Declarations

Author Contribution

(1) Heba Fawzy Kamaly has contributed substantially to the practical section, the composition of the paper, data analysis, and statistical analysis. (2) Zakaria Mukhtar Zaky has contributed to the practical section and the revision of the manuscript.

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REFERENCES

- Abdallah, M.F.; Girgin, G.; Baydar, T.; Sulyok, М. Krska, *R*. and (2017):Occurrence of multiple mycotoxins fungal and other metabolites in animal feed and maize samples from Egypt using LC-MS/MS. Journal of the Science of Food and Agriculture, 97(13), 4419-4428 https://doi.org/10.1002/jsfa.8293.
- Abd-EIaah, G.A. and Samya, S.A. (2005): Occurrence of fungal species and mycotoxins from decayed sugarcane (Saccharrum officinarum) in Egypt. *Mycobiology*, 33(2), 77–83 doi: 10.4489/MYCO.2005.33.2.077
- Abdelhamid, A.M. (1990): Occurrence of some mycotoxins (aflatoxin, ochratoxin A, citrinin, zearalenone and vomitoxin) in various Egyptian feeds.
 Archives of Animal Nutrition, 40(7), 647–664 DOI: 10.1080/1745039900 9428413

- Aboagye-Nuamah, F.; Kwoseh, C.K. and Maier, D.E. (2021): Toxigenic mycoflora, aflatoxin and fumonisin contamination of poultry feeds in Ghana. *Toxicon*, 198, 164–170 DOI: 10.1016/j.toxicon.2021.05.006
- Aliyu, R.M.; Abubakar, M.B.; Yakubu, Y.; Kasarawa, A.B.; Lawal, N. and Bello, M.B. (2016): Prevalence of potential toxigenic Aspergillus species isolated from poultry feeds in Sokoto metropolis. 14(1), 39–44 http://dx.doi.org/10.4314/sokjvs.v14i1.
 7
- Benvenuti, M.E. and Burgess, J.A. (2010): Rapid analysis of aflatoxins in corn, cereals, and almonds using ACQUITY UPLC H-Class System with fluorescence detection. Corporation W, Editor. Milford, MA2010.
- Braicu, C.; Puia, C.; Bodoki, E. and Socaciu, C. (2008): Screening and quantification of aflatoxins and ochratoxin a in different cereals cultivated in Romania using thin-layer chromatographydensitometry. Journal of Food Quality, 31(1), 108–120. https://doi.org/10.1111/j.1745-4557.2007.00187.x
- *Bryden, W.L. (2007):* Mycotoxins in the food chain: human health implications. Asia Pacif. J. Clin. Nutr, 16(1), 95–101.
- Deabes, M.M.; Darwish, H.R.; Abdel-Aziz, K.B.; Farag, I.M.; Nada, S.A. and Tawfek, N.S. (2012): Protective effects of Lactobacillus rhamnosus GG on Aflatoxins-induced toxicities in male Albino Mice. J Environ Anal Toxicol, 2(3), 2–9 http://dx.doi.org/10.4172/ 2161-0525.1000132
- Diaz, G.J. and Murcia, H.W. (2011): Biotransformation of aflatoxin B1 and its relationship with the differential toxicological response to aflatoxin in commercial poultry species. Aflatoxins-Biochemistry and Molecular Biology, 1, 3–20 DOI: 10.5772/22109
- Dorner, J.W. (2008): Management and prevention of mycotoxins in peanuts. Food Additives and Contaminants, 25(2), 203–208

https://doi.org/10.1080/026520307016 58357

- *EFSA. (2010):* report for 2010 on the results from the monitoring of vetrinary medicinal product residues and other substances in live animals and animal products. *The European Food Safety Authority Journal, 212, 1–64.*
- *EFSA. (2020):* Risk assessment of aflatoxins in food. European Food Safety Authority Journa, 18.
- *Egyption Standards (2010):* egyptian standards regulation, 7136/2010, Maximum Levels for Certain Contaminants in Foodstuffs. Egyptian Organization for Standarization and Quality Control.
- Green, S.B. and Salkind, N.J. (2010): Using SPSS for Windows and Macintosh: Analyzing and understanding data. Prentice Hall Press.
- Gruber-Dorninger, C., Jenkins, T., *and* Schatzmayr, G. (2018). Multimycotoxin screening of feed and feed raw materials from Africa. World Mycotoxin Journal, 11(3), 369–383. https://doi.org/10.3920/WMJ2017.229 2
- Hassan, W.H.; Mostafa, S.R.; Khalil, H.A. and Abed, A.H. (2021): Detection of Aflatoxigenic Fungi in Poultry Feed. 6(2), 92–97 DOI: 10.21608/JAVS. 2021.68213.1074
- Hussain, Z.; Khan, M.Z.; Khan, A.; Javed, I.; Saleemi, M.K.; Mahmood, S. and Asi, M.R. (2010): Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin B1 levels. Food and Chemical Toxicology, 48(12), 3304– 3307 DOI: 10.1016/j.fct.2010.08.016
- IARC. (2010): cancer classified agents. International IARC France Lyon, IARC, 1 Vol. Monographs.
- Jacobsen, B.J.; Coppock, R.W. and Mostrom, M.S. (2007): Mycotoxins and Mycotoxicoses. Bozeman, MT: Montana State University, Extension publication EBO174 https://doi.org/10. 1177/0748233709348263
- Kana, J.R.; Gbemenou, B.; Gnonlonfin, J.; Harvey, J.; Wainaina, J.; Wanjuki, I.;

Skilton, R.A. and Teguia, A. (2013): Assessment of Aflatoxin Contamination of Maize, Peanut Meal and Poultry Feed Mixtures from Different Agroecological Zones in Cameroon. 884–894. https://doi.org/10.3390/toxins5050884

- Kang'ethe, E.K. and Lang'a, K.A. (2009): Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. African Health Sciences, 9(4), 218–226. http://ajol.info/index.php/ahs/article/vi ewFile/52140/40768\nhttp://ovidsp.ovi d.com/ovidweb.cgi?T=JS&PAGE=ref erence&D=emed9&NEWS=N&AN=2 010174817
- Kemboi, D.C., Ochieng, P.E.; Antonissen, G.; Croubels, S.; Scippo, M.; Okoth, S.; Kangethe, E.K.; Faas, J.; Doupovec, B.; Lindahl, J.F. and Gathumbi, J.K. (2020): Multi-Mycotoxin Occurrence in Dairy Cattle and Poultry Feeds and Feed Ingredients from Machakos Toxins 12(12): 762. https://doi.org/10.3390/ toxins12120762
- Kotinagu, K.; Mohanamba, T. and Kumari, R.L. (2015): Assessment of aflatoxin B1 in livestock feed and feed ingredients by high-performance thin layer chromatography. Veterinary World, 8(12), 1396–1399 doi: 10.14202/vetworld.2015.1396-1399
- Mokubedi, S.M.; Phoku, J.Z.; Changwa, R.N.; Gbashi, S. and Njobeh, P.B. Analysis of mycotoxins (2019): contamination in poultry feeds manufactured in selected provinces of South Africa using UHPLC-MS/MS. Toxins. 11(8), 1 - 17.https://doi.org/10.3390/toxins1108045 2
- Nakai, V.K.; De Oliveira Rocha, L.; Gonçalez, E.; Fonseca, H.; Ortega, E.M.M. and Corrêa, B. (2008): Distribution of fungi and aflatoxins in a stored peanut variety. Food Chemistry, 106(1), 285–290 https://doi.org/10.1016/j.foodchem.200 7.05.087

- Nakavuma, J.L.; Kirabo, A.; Bogere, P.; Nabulime, M.M.; Kaaya, A.N. and Gnonlonfin, B. (2020): Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. 2, 1–10 https://doi.org/10. 1186/s40550-020-00079-2
- Njobeh, P.B.; Dutton, M.F.; Åberg, A.T. and Haggblom, P. (2012): Estimation of Multi-Mycotoxin Contamination in South African Compound Feeds. 836– 848. https://doi.org/10.3390/toxins 4100836
- Pankaj, S.K.; Shi, H. and Keener, K.M. (2018): A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends in Food Science and Technology, 71, 73–83 https://doi.org/

10.1016/j.tifs.2017.11.007

- Pitt, J.I. and Hocking, A.D. (2009): Fungi and food spoilage (Vol. 519). Springer.
- Ramesh, J.; Sarathchandra, G. and Sureshkumar, V. (2013): Original Research Article Analysis of feed samples for aflatoxin B 1 contamination by HPTLC - a validated method. 2(5), 373–377 http://www.ijcmas.com
- Rushing, B.R. and Selim, M.I. (2019): Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and Chemical Toxicology*, 124, 81–100 DOI: 10.1016/j.fct.2018.11.047
- Salem, D.A. (2002): Natural occurrence of aflatoxins in feedstuffs and milk of dairy farms in Assiut province, Egypt. Wiener Tierarztliche Monatsschrift, 89(4), 86–91.
- Smalley, E. B. (1986). *Identification of mycotoxin producing fungi and conditions leading to aflatoxin contamination of stored food grains.*
- Sumit, R.; Kim, J.E. and Coulombe Jr, R. (2010): Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Research in Veterinary

Science, *89*(3), 325–331 DOI: 10.1016/j.rvsc.2010.04.011

- *Tajkarimi, M.; Shojaee, M.H.; Yazdanpanah, H. and Ibrahim, S.A. (2011):* Aflatoxin in Agricultural Commodities and Herbal Medicine, Aflatoxins-Biochemistry and Molecular Biology, Dr. *Ramon G. Guevara-Gonzalez (Ed.)* DOI: 10.5772/23729
- *Taylor, P. and Ezekiel, C.N. (2012):* Food Additives and Contaminants : Part A Fungal and bacterial metabolites in commercial poultry feed from Nigeria. August, 37–41 DOI: 10.1080/19440049.2012.688878
- Udomkun, P.; Wiredu, A.N.; Nagle, M.,;Müller, J.; Vanlauwe, B. and Bandyopadhyay, R. (2017): Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application–A review. Food Control, 76, 127–138 doi: 10.1016/j. foodcont.2017.01.008.
- Yazdani, D.; Zainal Abidin, M.A.; Tan, Y. H. and Kamaruzaman, S. (2010): Evaluation of the detection techniques of toxigenic aspergillus isolates. African Journal of Biotechnology, 9(45), 7654–7659. https://doi.org/ 10.5897/AJB10.1128

مدي تواجد الأفلاتوكسينات والفطريات المفرزة للأفلاتوكسينات في أعلاف الحيوانات والدواجن بمزارع محافظة أسيوط، مصر

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تعد الأفلاتوكسينات من أخطر السموم الفطرية التي تُهدد صحة الإنسان والحيوان علي مستوي العالم. أوضحت الدراسة الحالية مدي تواجد الأفلاتوكسينات والفطريات المفرزة لها في الأعلاف حيث تم تجميع ٢٠ عينة من أعلاف الحيوانات و الدواجن من مزارع محافظة أسيوط وتم تحليلها باستخدام تقنية كروماتوجر افيا الضغط العالي السائل بالمعمل المركزي بكلية الطب البيطري جامعة أسيوط وكشفت نتائج هذه الدراسة أن أعلاف الدواجن أكثر تلوثا بالأفلاتوكسينات والفطريات المفرزة لها من أعلاف الحيوانات حيث أوضحت نتائج هذه الدراسة أن أعلاف الدواجن أكثر تلوثا بالأفلاتوكسينات والفطريات المفرزة لها من أعلاف الحيوانات حيث أوضحت نتائج كروماتوجر افيا الضغط العالي السائل أن متوسط مستوي الافلاتوكسينات بي ١، بي ٢، جي ١ وجي ٢ كانت ٢٣,٣٦ - ١٣,١٢ - ١، ١٠, ١٠ + ١, ١٠ و ٢٠, ± ٢٠,٠ ميكروجرام / كيلو جرام في أعلاف الحيوانات و ٢٥,١٨ - ٢٥,١٨ - ١٠, ١٠ + ١٠,٠ و ٢٠, ± ٢٠,٠ ميكروجرام / كيلو جرام في أعلاف الدواجن علي الترتيب. كما أوضحت النتائج أيضا أن عزلات الأسبر جلس فلافس التي تم عزلها من هذه الأعلاف أعلاف الدواجن علي الترتيب. كما أوضحت النتائج أيضا أن عزلات الأسبر جلس فلافس التي تم عزلها من هذه الأعلاف والأعلاف الدواجن علي الترتيب. كما أوضحت النتائج أيضا أن عزلات الأسبر جلس فلافس التي تم عزلها من هذه الأعلاف والأعلاف باستخدام تقنية كروماتوجر افيا الضغط العالي السائل وتطبيق الحدود المسموح بها بصر امة من أجل أمن وسلامة والأعلاف باستخدام والرحوبة التحكم في العوامل المساعدة علي انتاج الإفلاتوكسينات في الأغذية الحرارة والرطوبة والرطوبة النسبية.