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ASSESSMENT OF AFLATOXINS IN FEEDS AND FEED INGREDIENTS OF BOTH LIVESTOCK AND POULTRY

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ABSTRACT

Aflatoxin (AFs) are secondary metabolites produced primarily by *aspergillus flavus* and *aspergillus parasiticus* in agricultural foodstuff such as peanuts, maize grains, cereals, and animal feeds. Moreover, AFs are highly toxic, mutagenic, teratogenic and carcinogenic. A total of 141 samples comprising of feed ingredients (n=58) and complete feeds (n=83) used for cattle and poultry nutrition were analyzed for detection of aflatoxin in both seasons winter and summer. The incidence and level of aflatoxin B1 in feed ingredients was 26.923% (n=7/26) by mean of 78.285 ppb in summer but in winter was 28.125% (n=9/32) by mean of 47.333 ppb. For aflatoxin B2, the incidence and level in feed ingredients was 7.692% (n=2/26) and mean (54 ppb) in summer while in winter was 9.375% (n=3/32) by mean of 52.666 ppb. The incidence and level of total aflatoxins in feed ingredients was 11.538% (n=3/26) by mean of 98.333 ppb in summer but in winter was 15.625% (n=5/32) by mean of 112 ppb. Out of 7 cotton seed cake samples, one was contaminated with total aflatoxins (TAF) (14.285%) and had 300 ppb. Among 13 maize samples, only one had TAF (7.692%) and contains 14 ppb. From 4 sorghum grain samples, 2 were contaminated with AFB1 (50%) and had 12.5±5.303 ppb. Among 8 soybean processed cake, one of them (12.5%) was had AFB1 in summer and contained 10 ppb, and 2 samples in winter (25%) with range of 3-5 ppb (4±0.707). One soybean sample was contaminated with AFB2 (12.5%) and had 3 ppb. From sunflower feed samples (n=26), 11 were contaminated with AFB1, 6 in summer (23.076%) with mean of 89.666±22.188ppb (range 80-150), and 5 in winter (19.230%) with mean level 78.6±17.226ppb (range 40-150ppb). Four samples were contaminated with AFB2, 2 in summer (7.692%) with mean level of 54±32.526ppb (range 8-10) and 2 samples in winter (7.692%) had average level of 77.5±1.767ppb (range 75-80). Six samples had TAF, 2 samples in summer (7.692%) with average level of 140.5±77.428 ppb (range 31-250) and 4 in winters (15.384%) with mean of 65±5.303 ppb. The occurrence of B1, B2 and TAF in complete feed samples was high in winter than in summer. B1 in winter samples was 42±11.798 ppb while in summer was 16.562±3.027 ppb. B2 in winter was 65±4.082 ppb while in summer was 36.833±11.996 ppb. TAF in winter samples was 140±56.319 ppb while was 22±4.242 ppb in summer. Overall incidence of detected aflatoxin in total samples as 35.82% (n=24/67) in summer, 33.783% in winter but in total samples was 34.751% (n=49/141). From these obtained results, it was clarified that about 1/3 of analyzed samples were contaminated with aflatoxins which represent great hazard for their consumers in both animals and humans. So More efforts must be done to minimize this contamination by application of suitable conditions to prevent fungal growth and thereby prevent more production of aflatoxins.

Key words: Aflatoxins - feed ingredients - corn- livestock feed - soybean cake.

INTRODUCTION

Aflatoxins (AFs) are one of mycotoxins produced by many different types of fungi such as asperigillus (*Aspergillus parasiticus*, *Aspergillus flavus*, and *Aspergillus nominus*). Aflatoxins are secondary metabolites of these fungi which contaminate a large number of feedstuffs (especially cottonseed, corn, maize, grains, cereals and peanuts) and food. Aflatoxins are difuranocoumarin chemical compounds. According to the chemical structure, there are many types of aflatoxins like B1, B2, G1, G2, M1 and M2. The main aflatoxins types found in milk are M1 and M2. AFM1 as a metabolite of AFB1 and AFB2 can found in eggs. Aflatoxins cause many toxic effects for plants, animals, human as well as microorganisms (Talebi *et al.*, 2011, Chen *et al.*, 2013, Wacco *et al.*, 2014). The production of AFs occurs in field due to the effect high temperature, stress, and drought and (Villers, 2014).

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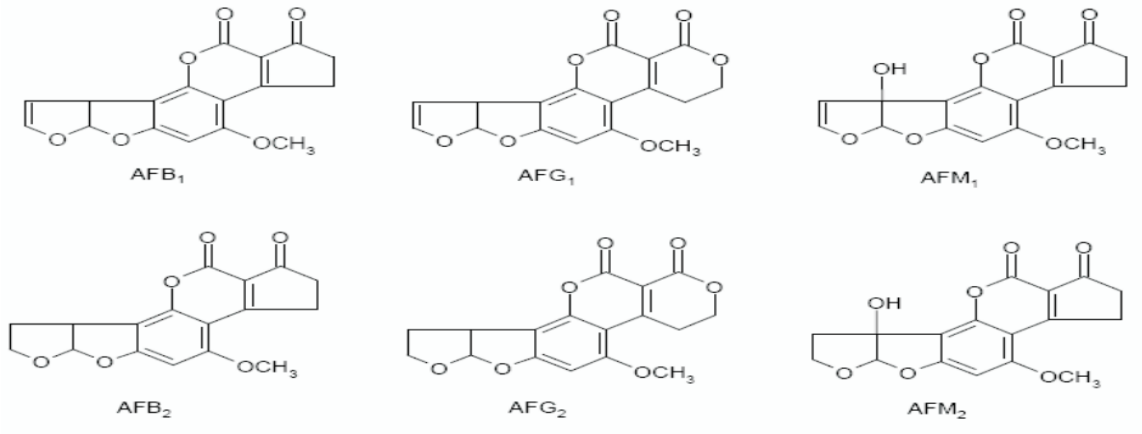

Chemical structures of aflatoxin B1, B2, G1, G2, M1 and M2 (Talebi *et al.*, 2011).

Many of world organizations as FAO found that myctoxins contaminate more than 25% of the agricultural commodities and as a result for this contamination many economic losses were occur (Kabak *et al.*, 2006). Mycotoxins are responsible for many different toxic effects for both animals and human and considered as the most dangerous problem for feeds and food (Sforza *et al.*, 2006). Over 400 well known mycotoxins, aflatoxins are the most famous one as they toxic to human beings (they have high toxicity like hepatotoxicity, carcinogenicity, teratogenicity as well as mutagenicity effects). Patients who already infected with hepatitis B and Hepatitis C are in a high risk for induction of hepatic cancer when consuming foods contaminated with aflatoxins. International Agency for Research on Cancer classified AFB1 as a human carcinogen group 1 (IARC, 2000, Costanzo *et al.*, 2015).

The fungi which responsible for production of aflatoxins utilize the nutrient materials necessary for their growth from the feed and food leading to minimizing the nutritional quality of these types of foods (Akande *et al.*, 2006). In general, birds exposed to feeds contaminated with aflatoxins will suffer from exhausted immune system which reflected in high affections with large number of diseases (Dhanasekaran *et al.*, 2009). Cereal grains, cotton seed, peanut and protein rich meals such as cotton seed meal, corn gluten meal, copra meal, palm kernel meal, rapeseed meal, soybean meal, and sunflower meal considered as the major suitable media for growth and propagation of *Aspergillus flavus* (Anjum *et al.*, 2012).

Nowadays poultry industry (broilers, duckling, layers, quails and turkeys) is suffering from severe economic loss due to consumption of feed ingredients highly contaminated with aflatoxins (CAST, 2003). Aflatoxins in poultry reduce feed intake and feed conversion rate which leading to decrease body weight gain, decrease egg production and reproduction performance of both males and females (Hussain *et al.*, 2010). As a common rule, poultry should not get more than 20 µg/kg TA in the feed.

Consumption of food or feed stuffs highly contaminated with aflatoxins can induce severe acute hepatic necrosis which ends by hepatic cirrhosis or hepatocarcinoma. This can result in acute hepatic failure which manifested by disorders in absorption, metabolism of nutrient materials, edema, bleeding and mental abnormalities and coma. Chronic exposure to highly contaminated ingredients with aflatoxins can increase the possibility for hepatocicity and cancer in the gallbladder and by that can result in induction of mutation as the metabolite of aflatoxins intercalate into DNA and alkylate the bases via epoxide moiety (Nogueira *et al.*, 2015).

Aflatoxins are natural toxins that contaminate various types of food and feed stuffs leading to health risk in both human and animals. The aim of this study was to determine the prevalence of aflatoxins contamination and then levels in animal (cattle and poultry) feed samples at Assiut Governorate.

MATERIALS AND METHODS

[1] Samples and sampling: One hundred and forty one feed ingredient (n=58, 26 samples in summer and 32 samples in winter) and complete feeds (n=83, 41 samples in summer and 42 samples in winter) used for cattle and poultry nutrition were collected from Assuit Governorate during winter and summer 2017. The feed ingredient samples were cotton seed cake (n=7, all in winter), maize (n=13, 10 samples in summer and 3 samples in winter), sorghum (n=4, one sample in summer and 3 samples in winter), soybean cake (n=8, 3 samples in summer and 5 samples in winter) and sunflower cake (n=26, 12 samples in summer and 14 samples in winter).

[2] Methods for estimation of aflatoxins levels in examined samples:
Aflatoxins were detected in qualitativly by TLC according to Park *et al.* (1994) and quantitatively for positive samples by UPLC according to Benvenuti and Burgess (2010).

[3] Chemicals: Aflatoxin standards (B_1, B_2 and total) used in this study were of analytical grade and obtained from Merck Company (Germany).

[4] Apparatus:

- Blender (15000 rpm) with a 1L glass jar and cover.
- Rotary evaporator system Cole-Parmer; Diagonal, 115 VAC
- Shaker, Model EL680, Eberbach Co.
- UV light Chamber with UV lamb at 365 excitation wave length.
- AVICAM Afla Test® Affinity column was used for Cleanup of samples.
- An ACQUITY® UPLC H-class system from Waters Company, USA.

RESULTS

The results of the analyzed samples in this survey were summarized in tables 1-6.

In table 1, out of 7 cotton seed cake samples, one contains TAF (14.285%) and had 300 ppb. Among 13 maize samples, only one had TAF (7.692%) and contains 14 ppb. From 4 sorghum grain samples, 2 were contaminated with AFB1 (50%) and had 12.5±5.303 ppb. Among 8 soybean cake, one (12.5%) contains AFB1 in summer (10 ppb) and 2 in winter (25%) with mean of 4±0.707ppb (3-5). One soybean sample was contaminated with AFB2 (12.5%) and had 3 ppb. From sunflower feed samples (n=26), 11 were contaminated with AFB1, 6 in summer (23.076%) with mean of 89.666±22.188ppb (80-150), and 5 in winter (19.230%) with mean level 78.6±17.226ppb (40-150ppb). 4 samples were contaminated with AFB2, 2 in summer (7.692%) with 54±32.526ppb (8-10) and 2 samples in winter (7.692%) with 77.5±1.767ppb (75-80). 6 samples had TAF, 2 in summer (7.692%) had140.5±77.428 ppb (31-250) and 4 in winter (15.384%) with 65±5.303 ppb.

Table 1: Estimation of aflatoxins levels (ppb) in feed ingredients used for cattle and poultry nutrition.

Feed ingredient		Summer season				Winter season			
		+ve samples	Incidence (%)	Range	Mean ± SE	+ve samples	Incidence (%)	Range	Mean ± SE
Cotton seed cake (n=7)	B1	----	----	------	-------	------	------	------	-------
	B2	----	----	------	-------	------	------	------	-------
	T. afl	----	----	------	-------	1	14.285	300	300
Maize (n=13)	B1	----	----	------	-------	------	------	------	-------
	B2	----	----	------	-------	------	------	------	-------
	T. afl	1	7.692	14	14	------	------	------	-------
Sorghum (n=4)	B1	----	----	------	-------	2	50	5 - 20	12.5 ± 5.303
	B2	----	----	------	-------	------	------	------	-------
	T. afl	----	----	------	-------	------	------	------	-------
Soybean cake (n=8)	B1	1	12.5	10	10	2	25	3 - 5	4± 0.707
	B2	----	----	------	-------	1	12.5	3	3
	T. afl	----	----	------	-------	------	------	------	-------
Sunflower cake (n=26)	B1	6	23.076	8 - 150	89.666± 22.188	5	19.230	40-150	78.6 ± 17.226
	B2	2	7.692	8 - 100	54 ± 32.526	2	7.692	75-80	77.5 ± 1.767
	T. afl	2	7.692	31 - 250	140.5 ± 77.428	4	15.384	50-80	65.00±5.303

In table 2, among 30 analyzed cattle feeds, 9 were contaminated with AFB1, 4 with AFB2 and 3 with TAF. In samples contaminated with AFB1, 5 samples were in summer (16.666%) with 15-53 ppb (33.6±6.896) and 4 in winter (13.333%) with 10-75ppb (38.75±13.074). For samples contaminated with AFB2, 2 in summer (6.666%) with 50-53 ppb (51.5±1.060) and 2 in winter (6.666%) with 60-75 ppb (67.5±1.303). 3 samples (11.538%) contaminated with TAF in winter had 70-300 ppb (185±54.211).

In table 2, out of 53 poultry feed samples, 7 had AFB1, 5 in summer (9.433%) with 7.5-30 ppb (13.5±3.714), 2 in winter (3.773%) with 60-70 ppb (65±3.535). 3 samples were contaminated with AFB2, 2 in summer (3.773%) with 7.5-10 ppb (8.75±0.883), 1 in winter (1.886%) had 60 ppb. 3 samples contaminated with TAF. 2 in summer (3.773%) with 16-28 ppb (22±4.424) and 1 in winter had 5 ppb. Among these 53 poultry feeds, 23 samples were for layers, 5 contaminated with AFB1, 3 in summer (13.043%) with 10-30 ppb (16.666±5.443) and 1 in winter (4.347%) with 70 ppb. One sample contaminated with AFB2 in summer (4.347%) which contains 10 ppb. 2 samples contaminated with TAF, 1 in summer (4.347%) which had 16 ppb and 1 in winter (4.347%) which had 15 ppb. For broiler feeds, 3 samples contaminated with AFB1, 2 in summer (7.692%) with 7.5-10 ppb (8.75±0.883) and 1 in winter (3.846%) had 60 ppb. 2 samples contaminated with AFB2, 1 in summer (3.846%) had 7.5 ppb and 1 in winter (3.846%) had 60 ppb. One sample contains 28 ppb TAF in summer.

Table 2: Estimation of aflatoxins levels (ppb) in complete feeds used for cattle and poultry nutrition.

Feed ingredient		Summer season				Winter season			
		+ve samples	Incidence (%)	Range	Mean ± SE	+ve samples	Incidence (%)	Range	Mean ± SE
(1) Cattle feeds (n=30)	B1	5	16.666	15-53	33.6 ± 6.896	4	13.333	1 0-75	38.75 ± 13.074
	B2	2	6.666	50-53	51.5±1.060	2	6.666	60-75	67.5 ± 5.303
	T. afl	--	--------	--------	--------	3	10	70-300	185 ± 54.211
(2) Poultry feeds (n=53)	B1	5	9.433	7.5-30	13.5 ±3.714	2	3.773	60-70	65± 3.535
	B2	2	3.773	7.5-10	8.75 ±0.883	1	1.886	60	60
	T. afl	2	3.773	16-28	22±4.424	1	1.886	5	5
(**2a**) Layers (n=23)	B1	3	13.043	10-30	16.666 ±5.443	1	4.347	70	70
	B2	1	4.347	10	10	----	--------	--------	--------
	T. afl	1	4.347	16	16	1	4.347	5	5
(**2b**) Broiler starter (n=26)	B1	2	7.692	7.5-10	8.75±0.883	1	3.846	60	60
	B2	1	3.846	7.5	7.5	1	3.846	60	60
	T. afl	1	3.846	28	28	----	--------	--------	--------
(**2c**) Broiler finisher (n=4)		All these samples were found free from any aflatoxins							

In table 3, for feed ingredient samples, AFB1 and AFB2 were high in summer than in winter. AFB1 in summer samples was 8-150 ppb (78.285±21.742) while in winter was 3-150 ppb (47.333±15.155). AFB2 in summer samples was 8-100 ppb (54±32.526) but in winter were 3-300 ppb (52.666±20.310). For samples contaminated with TAF, winter samples contained high TAF levels ranged from 50-300 ppb (112±42.251) while in summer contained 98.333±62.047 ppb (14-250).

Table 3: Estimation of aflatoxins levels (ppb) in feed ingredients used for cattle and poultry nutrition.

	Summer season (No. of samples = 26)				Winter season (No. of samples = 32)			
Aflatoxins	**+ve samples**	**Incidence for summer samples (%)**	**Range**	**Mean ± SE**	**+ve samples**	**Incidence for winter samples (%)**	**Range**	**Mean ± SE**
B1	7	26.923	8-150	78.285±21.742	9	28.125	3-150	47.333±15.155
B2	2	7.692	8-100	54 ± 32.526	3	9.375	3-300	52.666±20.310
T.afl	3	11.538	14-250	98.333 ±62.047	5	15.625	50-300	112±42.251

In table 4, for complete feed samples, levels of B1, B2 and TAF was high in winter. B1 in winter samples was 42±11.798 ppb while in summer was 16.562±3.027 ppb. B2 in winter was 65±4.082 ppb while in summer was 36.833±11.996 ppb. TAF in winter samples was 140±56.319 ppb while was 22±4.242 ppb in summer.

Table 4: Estimation of aflatoxins levels (ppb) in complete feeds used for cattle and poultry nutrition.

	Summer season (No. of samples = 41)				Winter season (No. of samples = 42)			
Aflatoxins	**+ve samples**	**Incidence (%)**	**Range**	**Mean ± SE**	**+ve samples**	**Incidence (%)**	**Range**	**Mean ± SE**
B1	10	24.390	7.5-30	16.562±3.027	6	14.285	10-70	42±11.798
B2	4	9.756	7.5-50	36.833±11.996	3	7.142	60-75	65±4.082
T. afl	2	4.878	16-28	22±4.242	4	9.523	5-300	140±56.319

Table 5: Incidence of the total analyzed samples from feed ingredients and complete feeds used for cattle and poultry nutrition.

	Feed Ingredient samples (No. of samples = 58)				Complete feed samples (No. of samples = 83)				Total analyzed samples
	Total samples	**-ve samples**	**+ve samples**	**Incidence of +ve samples**	**Total samples**	**-ve samples**	**+ve samples**	**Incidence of +ve samples**	
Summer	26	14	12	46.153	41	28	13	31.707	67
Winter	32	15	17	53.125	42	30	12	28.571	74
Total	58	39	29	50.000	83	58	25	30.120	141

Table 6: Estimation of aflatoxins levels (ppb) in all analyzed samples used for cattle and poultry nutrition.

Collection season	Total samples	+ve samples	Incidence of + ve samples	Range	Mean ± SE
Summer	67	24	35.820	7.5-150	49.520±11.843
Winter	74	25	33.783	3-300	72.724±13.752
Total	141	49	34.751	3-300	61.981±09.332

DISCUSSION

Many of world organizations as FAO found that myctoxins contaminate more than 25% of the agricultural commodities and as a result for this contamination many economic losses were occur (Kabak *et al.,* 2006). Mycotoxins are responsible for many different toxic effects for both animals and human and considered as the most dangerous problem for feeds and food (Sforza *et al.,* 2006).

The results of the present study revealed that, out of 7 cotton seed cake samples, one contains TAF (14.285%) and had 300 ppb. Among 13 maize samples, only one had TAF (7.692%) and contains 14 ppb. From 4 sorghum grain samples, 2 were contaminated with AFB1 (50%) and had 12.5±5.303 ppb. Among 8 soybean cake, one (12.5%) contains AFB1 in summer (10 ppb) and 2 in winter (25%) with mean of 4±0.707ppb (3-5). One soybean sample was contaminated with AFB2 (12.5%) and had 3 ppb. From sunflower feed samples (n=26), 11 were contaminated with AFB1, 6 in summer (23.076%) with mean of 89.666±22.188ppb (80-150), and 5 in winter (19.230%) with mean level 78.6±17.226ppb (40-150ppb). 4 samples were contaminated with AFB2, 2 in summer (7.692%) with 54±32.526ppb (8-10) and 2 samples in winter (7.692%) with 77.5±1.767ppb (75-80). 6 samples had TAF, 2 in summer (7.692%) had140.5±77.428 ppb (31-250) and 4 in winter (15.384%) with 65±5.303 ppb (table 1).

Among 11 crushed sorghum samples, 2 are contaminated with aflatoxin B1 (206.7ppb), 1 with B2 (5.46ppb), 2 with G1 (136.91 ppb) (Elzupir *et al.,* 2009). Bibin Becha & Devi (2013) found that the levels of aflatoxin in examined feeds samples varied from 1 to 400 ppb, in feed ingredients were 1-680 ppb, in ground nut cake (139.75±31.1 ppb) while was high in maize (122±53.36 ppb). Kotinagu and Co-workers (2015) analyzed 49 feed ingredient samples for their presence of aflatoxin B1. An incidence of 42.85% (3 of 7) of cotton seed cake was contaminated with aflatoxin B1 (23.3 ppb). In soybean cake, 1 of 3 (33.33%) was contaminated with B1 (50 ppb). One samples from three ones from sunflower de oiled cake found positive for aflatoxin B1 (10 ppb). Thirteen maize samples analyzed, 5 (38.46%) contained aflatoxin B1 (62 ppb).

Our findings are in agreement with these previous studies where aflatoxins (especially B1) were found in most analyzed samples. This variation of percentage of contamination may be due to difference in the types of substrates and handling processes from time of harvest to the time of consumption.

Poultry diets are composed from feed ingredient consists mainly from corn. Corn is the more feed ingredient in poultry diets susceptible as a media for production of aflatoxins. Firdus (2003) found that total aflatoxins level in analysed corn samples was ranged from 80 and 110 µg/kg while the moisture content of these examined corn was 10.5 to 15%.

Wei (2004) in 1200 raw feed ingredients and finished feed samples collected from 1998 to 2001 found that the total aflatoxin levels in these examined samples were ranged from 109 and 585 µg/kg. Total aflatoxin level was estimated by Khan *et al.* (2011) in 1021 different feed samples in Punjab province. From these analyzed samples, 646 were found contaminated by aflatoxins ((47% for cereals, 51% for cereal byproducts, 60% for oilseed meals and 66% for poultry feeds).

The level of contamination with aflatoxin was higher than the MPL by the EU (20 ppb). Our results for aflatoxin were in agreement with Fareed *et al.* (2014) who found that 100% of corn samples were contaminated with aflatoxin with an average contamination level of 80 ppb and a maximum level of 110 ppb. Compared to other ingredients, corn is more susceptible for aflatoxin production throughout the world (Firdous 2003). In poultry feed in Egypt, the most commonly ingredient is corn.

Among 30 analyzed cattle feeds, 9 were contaminated with AFB1, 4 with AFB2 and 3 with TAF. In samples contaminated with AFB1, 5 samples were in summer (16.666%) with 15-53 ppb (33.6±6.896) and 4 in winter (13.333%) with 10-75ppb (38.75±13.074). For samples contaminated with AFB2, 2 in summer (6.666%) with 50-53 ppb (51.5±1.060) and 2 in winter (6.666%) with 60-75

ppb (67.5±1.303). 3 samples (11.538%) contaminated with TAF in winter had 70-300 ppb (185±54.211) (table 2).

Dhand and Co-workers (1998) analyzed 28 samples of feeds for dairy cattle. Twenty one (75%) were found positive for aflatoxin B1**.** Among 59 feed samples, 47 samples (79.66) were positively contaminated with aflatoxin B1and had a level 25.53 ppb (Ramesh *et al.,* 2013). Examined 48 livestock feed samples revealed that 16(33%) were positive for aflatoxin B1. From 48 samples, 23 samples were cattle feed 6 of them (23%) were contaminated with aflatoxin B1and contained contain 32 ppb. From 48 samples, 17 were for poultry feeds 6 of them (35.2%) contain 13.4 ppb aflatoxin B1 (Kotinagu *et al.,* 2015).

The resistance of animals to mycotoxins show great variability as the monogastric animals had low resistance if compared with ruminants which had high resistant. In ruminants, the microbial content of the rumen plays an important role in detoxification. In spite of this, the rumen degrades aflatoxin ≤10% if the concentration of aflatoxin was ranged from 1-10 ppm (Westlake *et al.,* 1989). Aflatoxin concentration below 10 ppm induce inhibition for the bacterial growth as well as metabolic activity of microorganisms of the rumen (Yiannikouris and Jouany, 2002).

The susceptibility of animals to aflatoxins is affected by age and sex of exposed animals where females are less susceptible than males. On the other hand, the mature animals show high susceptibility then immature one (Arias *et al.,* 2011; Cassel *et al.,* 2012). The most characteristic signs of chronic aflatoxin intoxication are feed refusal, weight loss, reduced growth rate, rough hair coat, subcutaneous hemorrhage, anemia, listlessness and mild diarrhea may occur. Disorders in the reproductive function (as a result of abnormality in the estrous cycles and induction of abortion), rectal prolapsed and disturbance in the immune system which results in increased susceptibility to infectious diseases (Cassel *et al.,* 2012).

The Food and Drug Administration (FDA) of USA stated that 20-3000 ppb is the action levels for aflatoxin present in food or feed (Guidance for Industry, 2000). The USFDA established that the maximum for total aflatoxin residue in corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals in the USA is 20 ppb, whereas the European Commission established maximum acceptable levels ranging from 5-20 ppb for AFB1 for a variety of animal feeds (European Commission Regulation, 2002). The maximum residual level (MRL) or acceptable level for aflatoxins in complete feedstuffs for dairy animals is 5 ppb and for calves and lambs 10 ppb while is 20 ppb in most complete feedstuffs, all feed materials and complementary feedstuffs for cattle, goats, sheep, poultry and pigs (Commission European Communities, 2003).

From 53 poultry feed samples, 7 had AFB1, 5 in summer (9.433%) ranged between 7.5-30 ppb (13.5±3.714), 2 samples in winter (3.773%) with 60-70 ppb (65±3.535). 3 samples contaminated with AFB2, 2 in summer (3.773%) ranged between 7.5-10 ppb (8.75±0.883), 1 sample in winter (1.886%) had 60 ppb. 3 samples contaminated with TAF. 2 in summer (3.773%) with 16-28 ppb (22±4.424) and 1 in winter had 5 ppb. Among these 53 poultry feeds, 23 samples were for layers, 5 contaminated with AFB1, 3 in summer (13.043%) ranged between 10-30 ppb (16.666±5.443) and 1 in winter (4.347%) with 70 ppb. One sample contaminated with AFB2 in summer (4.347%) which contains 10 ppb. 2 samples contaminated with TAF, 1 in summer (4.347%) which had 16 ppb and 1 in winter (4.347%) which had 15 ppb. For broiler feeds, 3 samples contaminated with AFB1, 2 in summer (7.692%) with 7.5-10 ppb (8.75±0.883) and 1 sample in winter (3.846%) had 60 ppb. 2 samples contaminated with AFB2, 1 sample in summer (3.846%) had 7.5 ppb and 1 sample in winter (3.846%) had 60 ppb. One sample contains 28 ppb TAF in summer (table 2).

Nowadays, most of feedstuffs for large animals as well as poultry feeds are mainly dependant on the imported feed ingredients which may be contaminated with mycotoxins especially aflatoxins during production, transportation from the imported country as these feeds exposed to different environmental changes and storage (Hassan *et al.,* 2012). Azab *et al.* (2005) examined 500 samples of animal feed and feed ingredients for their aflatoxin B1. They found that AFB1 content was ranged from 25-2000 ppb.

Alam *et al.* (2012) determined aflatoxin residue in a 216 samples of poultry feed ingredients in Pakistan. The maximum aflatoxin residues which detected in summer season was 191.65 ppb for AFB1, 86.85 ppb for AFB2, 167.82 ppb for AFG1 and 89.80 ppb for AFG2 while the minimum levels was observed in the winter season. Fareed *et al.* (2014) analyzed 114 different poultry feed ingredients. The incidence of positive contaminated samples with total aflatoxins (86.84%) with a maximum level of 165 ppb and average of 74.4 ppb. Famy *et al.* (2015) examined many poultry feed samples for their aflatoxon content in Gharbia Governorate, Egypt. They found that feed samples had aflatoxin level as 73.25 ppb and all these samples were higher than the maximum permissible limit (20 ppb).

For feed ingredient samples, AFB1 and AFB2 were high in summer than in winter. AFB1 in summer

samples was 8-150 ppb (78.285±21.742) while in winter was 3-150 ppb (47.333±15.155). AFB2 in summer samples was 8-100 ppb (54±32.526) but in winter were 3-300 ppb (52.666±20.310). For samples contaminated with TAF, winter samples contained high TAF levels ranged from 50-300 ppb (112±42.251) while in summer contained 98.333±62.047 ppb (14-250) (table 3). Ramesh *et al.* (2013) tested 59 feed samples for their aflatoxin B1 content. They found that 47 samples (79.66%) were positive and their aflatoxin B1 level was 25.53 ppb.

Abbas (2005) mentioned that aflatoxins contaminated foods consumed by human beings affect children both and adults. In children, aflatoxins result retarded growth, delayed development and hepatic disorders (hepatic damage, and hepatic cancer). Adult human have high resistance and can tolerate amounts that can induce great effects in children in spite of adults are also at risk. Nearly, all of animal species is not immune against aflatoxin effects. Aflatoxins induce cancer in both animals and humans (Hudler, 1998). After exposure to aflatoxins through ingestion of contaminated food, aflatoxins may be metabolized by the liver to become the less harmful aflatoxin M1 (via hydroxylated or a reactive epoxide intermediate). The most route of exposure to aflatoxins is through the digestive tract but aflatoxin B1 can ingested and inter the body through skin (Boonen *et al.*, 2012).

Livestock in intensive production systems are at higher risk of dietary exposure than other ones. In countries where regulation for aflatoxins in animal feeds exists, the total permissible aflatoxin levels in animal feeds range from 0 to 50 ppb with an average of 20 ppb (FAO 2004) (Standards for individual feed components may be higher). In developing countries 25–50% of samples have levels >20 ppb (Binder *et al.*, 2007, Rodrigues and Naehrer 2012).

The positive correlation between the consumption of aflatoxin-contaminated foods and the increase of the occurrence of hepatic cancer in peoples in South East Asia and Africa suggests the threat posed to human health by aflatoxin (Peers and Linsell, 1973). The absolute safety is never achieved; many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed (Talebi *et al.*, 2011).

Conclusion and recommendations: Higher incidence and contamination level of aflatoxins were detected in examined samples for ruminants and poultry feeds and feed ingredients. However, this situation demands for immediate necessary control measures. Adequate pre- and post-harvest interventions should be done, and proper storage condition should be maintained. Finally, strict regulations, surveillance programs for testing food and feed for aflatoxin contamination are highly recommended to improve the health status of the consumers.

REFERENCES

Abbas, H.K. (2005): Aflatoxin and Food Safety. CRC Press. ISBN 0-8247-2303-1.

Alam, S.; Shah, H.; Khan, H. and Magan, N. (2012): The Effect of Substrate, Season, and Agroecological Zone on Mycoflora and Aflatoxin Contamination of Poultry Feed from Khyber Pakhtunkhwa, Pakistan. Mycopathologia, 174(4):341-349.

Akande, K.E.; Abubakar, M.M.; Adegbola, T.A. and Bogoro, S.E. (2006): Nutritional and health implications of mycotoxins in animal feeds: a review. Pakistan J of Nutrition, 5:398-403.

Anjum, M.A.; Khan, S.H.; Sahota, A.W. and Sardar, R. (2012): Assessment of aflatoxin B1 in commercial poultry feed and feed ingredients. The J of Animal and Plant Sci., 22:268-272.

Arias, S.L.; Mary, V.S.; Theumer, M.G. and Rubinstein, H.R. (2011): Micotoxicosis en animales de laboratorio. In: Ramos Girona AJ (ed.). Micotoxinas y micotoxicosis, Madrid, AMV Ediciones: 299-328.

Azab, R.M.; Tawakkol, W.M.; Hamad, A.; Abou-Elmagd, M.; El-Agrab, H. and Refai, M. (2005): Detection and estimation of aflatoxin B1 in feeds and its biodegradation by bacteria and fungi. Egyptian Journal of Natural Toxins, 2:39-56.

Benvenuti, M. and J. Burgess (2010): Rapid analysis of aflatoxins in corn, cereals, and almonds using ACQUITY UPLC H-Class System with fluorescence detection. Waters Corporation Application Note APNT10172781.

Bibin Becha, B. and Devi, S.S. (2013): Aflatoxin levels in feeds and feed ingredient of livestock and poultry in Kerala. J. Vet. Animal Sci., 44: 76-78.

Binder, E.M.; Tan, L.M.; Chin, L.J.; Handl, J. and J. Richard, J. (2007): Worldwide occurrence of mycotoxins in commodities, feed and food ingredients. Anim. Fed. Sci and Tech., 137:265-282.

Boonen, J.; Malysheva, S.V.; Taevernier, L.; Diana Di Mavungu, J.; De Saeger, S. and De Spiegeleer, B. (2012): Human skin penetration of selected model mycotoxins. Toxicology. 301 (1-3):21-32.

Cassel, E.K.; Campbell, B.; Draper, M. and Epperson, B. (2012): Aflatoxins hazards in grain/Aflatoxicosis and livestock. South Dakota State University (SDSU). Cooperative Extension Service / College of Agriculture & Biological Sciences / USDA. Text adapted with permission from Uni. of

Maryland Cooperative Extension Service Fact Sheet [444 & 445].

CAST (2003): Mycotoxins: risks in plant, animal, and human systems. In: J.L. Richard, G.A. Payne (Eds.), Council for Agricultural Science and Technology Task (CAST) Force Report No.139, Ames, Iowa, USA.

Chen, X.; Grenier, B. and Applegate, T.J. (2013): Afltoxins in Poultry. Purdue Extension. AS-615-W. Purdue Animal Science. www.ag.purdue.edu/ANSC.

Commission of European Communities (2003): Commission directive 2003/100/EC. Offic. J. Euroommunit., 285: 33-37.

Costanzo, P.; Santini, A.; Fattore, L.; Novellino, E. and Ritieni, A. (2015): Toxicity of aflatoxin B1 towards the vitamin D receptor (VDR). Food Chem. Toxicol., 76(2):77-79.

Dhand, N.K.; Joshi, D.V. and Jand, S.K. (1998): Aflatoxins in dairy feeds/ingredients. Indian J. Anim. Nutr., 15(4): 285-286.

Dhanasekaran, D.; Annamalai, P. and Noorudin, T. (2009): Evaluation of aflatoxicosis in hens fed with commercial poultry feed. Turkish J of Vet. and Animal Sciences, 33: 385-391.

Elzupir, A.O.; Younis, M.; Fadul, M.H. and Elhussein, A.M. (2009): Determination of aflatoxins in animal feed in Khartoum State, Sudan. J. of Animal and Vet. Advances, 8(5):1000-1003.

European Commission (2002): Regulation (EU) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed, Brussels, 2002.

Fahmy, N.E.; Eltholth, M.M.; Mohamed, R.A.; ELTras, W.F. and El-Midany, S.A. (2015): Management of Poultry Farms and the Likelihood of Contamination of Poultry Feed with Mycotoxins in Gharbia Governorate, Egypt. World Vet J, 5(4): 51-58.

FAO (Food and Agriculture Organization of the United Nations)(2004): Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81. Rome, Italy, pp. 9-35.

Fareed, G.; Khan, S.H.; Anjum, M.A. and Ahmed, N. (2014): Determination of aflatoxin and Ochratoxin in poultry feed ingredients and finished feed in humid semi-tropical environment. J. Adv. Vet. Anim. Res., 1(4): 201-207.

Firdous, S. (2003): Effect of storage, temperature and moisture on the total aflatoxin growth in indigenous feed ingredients by HPLC. M. Phil. Thesis. GC Uni., Lahore, Pakistan, 30-39.

Guidance for Industry (2000): Action levels for poisonous or deleterious substances in human food and animal feed. Food and Drug Administration. August 2000.

Hassan, A.M.; Youssef, A.I. and Reddy, P.G. (2012): Ochratoxin-A and Mold in Some Broiler Farms of Ismailia, Egypt and Evaluation of Common Mycotoxin Binders. International Journal of Poultry Science, 11(4):288-293.

Hussain, Z.; Muhammad, Z.K.; Ahrar, K.; Ijaz, J.; Muhammad, K.S.; Sultan, M. and Muhammad, R.A. (2010): Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin B1 levels. Food and Chemical Toxicology, 48: 3304-3307.

Hudler, G.W. (1998): Magical mushrooms, mischievous molds: The remarkable story of the fungus Kingdom and its impact on human affairs. Princeton Uni. Press. ISBN 978-0-691-07016-2.

IARC (2000): Monographs on the evaluation of carcinogenic risks to human. Some traditional herbal medicine, some mycotoxins, naphthalene and styrene. No.82. IARC, Lyon, France.

Kabak, B.; Dobson, A.D.W. and Var, I. (2006): Strategies to prevent mycotoxin contamination of food and animal feed: A review. Crit. Rev. Food Sci. Nutr., 46(8):593-519.

Khan, S.H.; Shamsul, H.; Rozina, S. and Muhammad, A.A. (2011): Occurrence of aflatoxin B1 in poultry feed and feed Ingredients in Pakistan. Inter J of Agro Vet and Med Sci, 5:30-42.

Kotinagu, K.; Mohanamba, T. and Rathna Kumari, L. (2015): Assessment of aflatoxin B1 in livestock feed and feed ingredients by high-performance thin layer chromatography. Veterinary World, EISSN: 2231-0916. pp. 1396-1399.

Nogueira, L.; Foerster, C.; Groopman, J.; Egner, P.; Koshiol, J. and Ferreccio, C. (2015): Association of aflatoxin with gallbladder cancer in Chile. JAMA, 313 (20): 2075-2077.

Park, D.L.; Trucksess, M.W.; Nesheim, S.; Stack, M. and Newell, R.F. (1994): Solvent-efficient thin-layer chromatographic method for the determination of aflatoxins B1, B2, G1, and G2 in corn and peanut products: Collaborative study. JAOAC International, 77:637-646.

Peers, F.G. and Linsell, C.A. (1973): Dietary aflatoxin and liver cancer- a population based study in Kenya. 27: 473-484.

Ramesh, J.; Sarathchandra, G. and Suresh Kumar, V. (2013): Analysis of feed samples for aflatoxin B1 contamination by HPTLC- A validation method. Int. J. Curr. Microbiol. Appl. Sci., 2(5):373-377.

Rodrigues, I. and Naehrer, K. (2012): Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010. Phytopathologia Mediterranea, 51:175-192.

Sforza, S.; Dall'Asta, C. and Marchelli, R. (2006): Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry. Mass Spectrometry Rev., 25(1):54-76.

Talebi, E.; Khademi, M. and Rastad, A. (2011): An Over Review on Effect of Aflatoxin in Animal Husbandry. Asian J. Eep. Biol. Sci., 2(3):754-757.

Villers, F. (2014): Aflatoxins and safe storage, Front. Microbiol., 5: 158-64.

Wacoo, A.P.; Wendiro, D.; Vuzi, P.C. and Hawumba, J.F. (2014): Methods for Detection of Aflatoxins in Agricultural Food Crops. J. Appl. Chem., 2014: 1 1-15.

Wei, G. (2004): Biomin mycotoxin survey in the Asia Pacific Region. Biomin Singapore Lab Reports; pp 35-80.

Westlake, K.; Mackie, R.I. and Dutton, M.F. (1989): In vitro metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. Anim. Feed Sci. Technol. 25:169-178.

Yiannikouris, A. and Jouany, J.P. (2002): Mycotoxins in feeds and their fate in animals: a review. Review article. Anim. Res. 51:81-99.

تقييم الأفلاتوكسين في العلف ومكونات العلف لكل من المواشي والدواجن

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الأفلاتوكسين (AFs) هي مستقلبات سرطانية ثانوية تنتج أساسًا من فطر اسبرجلس فلافس وأسبرجلس بارازيتكس في المواد الغذائية الزراعية مثل الفول السوداني وحبوب الذرة والحبوب والأعلاف الحيوانية. علاوة على ذلك فإن الـ AFs تكون شديدة السمية ، مطفرة ، مسخية ومسرطنة. اجريت هذه الدراسه لتحليل ١٤١ عينة من مكونات العلف (n = 58) والأعلاف الكاملة (n = 83) المستخدمة في تغذية الماشية والدواجن للكشف عن الأفلاتوكسين في فصلي الشتاء والصيف. وقد أظهرت النتائج أن معدل الإصابة بالأفلاتوكسين B1 ٢٦,٩٢٣٪ (n = ٢٦/٧) بما يعادل ٧٨,٢٨٥ جزء من البليون في الصيف وفي الشتاء كان ٢٨,١٢٥٪ (n = ٣٢/٩) بمتوسط ٤٧,٣٣٣ ppb. بالنسبة للأفلاتوكسين B2 ، كان معدل حدوثه فى مكونات العلف ٧,٦٩٢٪ (عدد = ٢/٢٦) وبمتوسط (٥٤ جزء في البليون) في الصيف وفي فصل الشتاء ٩,٣٧٥٪ (عدد = ٣٢/٣٢) وبمتوسط (٥٢,٦٦٦ ppb). كانت نسبة الإصابة بالأفلاتوكسين في مكونات العلف ١١,٥٣٨٪ (عدد = ٢/٢٦) وتعني (٩٨,٣٣٣ جزء من البليون) في الصيف وفي فصل الشتاء كانت ١٥,٦٢٥٪ (n = ٣٢/٥) بمتوسط (١١٢ جزء في البليون). من أصل ٧ عينات من بذور القطن كان أحدها ملوثًا TAF 14.285٪ وتحتوى ٣٠٠ جزء في البليون. من بين ١٣ عينة ذرة ، كانت واحده TAF 7.692٪ وتحتوي على ١٤ جزء في البليون. من ٤ عينات من حبوب الذرة الرفيعة ، كانت50٪ (ملوثة بـ AFB1 وبها ١٢,٥ ± ٥,٣٠٣ جزء من البليون. من بين ٨ قوالب فول الصويا المصنعة ، كانت واحده منهم (١٢,٥ ٪) تحتوي على AFB1 في الصيف وبها ١٠ جزء في البليون ، وعينتان فى الشتاء (٢٥ ٪) تحتوى على ٣-٥ جزء من البليون (٤ ± ٠,٧٠٧). تلوثت عينة فول صويا (AFB2 12.5%) وتحتوى على ٣ جزء في البليون. من عينات عباد الشمس (n = ٢٦) ١١ ملوثة بـ AFB1، 6 في الصيف (٢٣,٠٧٦ ٪) بمتوسط ٨٩,٦٦٦ ± ٢٢,١٨٨ppb (المدى ٨٠-١٥٠) و٥ في الشتاء (١٩,٢٣٠٪) بمتوسط ٧٨,٦ ± ١٧,٢٢٦ppb (المدى ٤٠-١٥٠ppb). كانت أربعة عينات ملوثة بـ AFB2 و٢ في فصل الصيف (٧,٦٩٢٪) بمتوسط ٥٤ ± ٣٢,٥٢٦ppb (المدى ٨-١٠) وعينتان في فصل الشتاء (٧,٦٩٢٪) بمتوسط ٧٧,٥ ± ١,٧٦٧ppb (المدى ٧٥-٨٠). كانت ست عينات تحتوي على TAF، 2 في الصيف (٧,٦٩٢٪) بمتوسط ١٤٠,٥ ± ٧٧,٤٢٨ جزء من البليون (المدى ٣١-٢٥٠) و ٤ في فصول الشتاء (١٥,٣٨٤٪) بمتوسط ٦٥ ± ٥,٣٠٣ ppb. كان حدوث B1 و B2 و TAF في عينات التغذية الكاملة عالية في فصل الشتاء عن فصل الصيف. كان B1 في عينات الشتاء ٤٢ ± ١١,٧٩٨ جزء في البليون بينما في الصيف ١٦,٥٦٢ ± ٣,٠٢٧ جزء في البليون. كان B2 في فصل الشتاء ٦٥ ± ٤,٠٨٢ جزء من البليون بينما في الصيف ٣٦,٨٣٣ ± ١١,٩٩٦ جزء من البليون. كانت الافلاتوكسنات الكليه في عينات الشتاء ١٤٠ ± ٥٦,٣١٩ جزء في البليون بينما كان ٢٢ ± ٤,٢٤٢ جزء في البليون في الصيف. نسبة حدوث الأفلاتوكسين في إجمالي العينات كان ٣٥,٨٢٪ (n = ٦٧/٢٤) في الصيف ، ٣٣,٧٨٣٪ في الشتاء ولكن في العينات الكلية كانت ٣٤,٧٥١٪ (n = ١٤١/٤٩). من هذه النتائج تبين أن حوالي ثلث العينات التي تم تحليلها ملوثة بالأفلاتوكسينات التي تمثل خطورة كبيرة على المستهلكين من الحيوانات والأنسان. لذا يجب بذل المزيد من الجهود لتقليل هذا التلوث عن طريق تطبيق شروط مناسبة لمنع نمو الفطريات وبالتالي منع المزيد من إنتاج الأفلاتوكسين.