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Seasonal Variation in Prevalence of Mycotoxins in Feed and Feedstuffs at Beni-Suef Governorate in Egypt

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Abstract

The prevalence of mycotoxins in animal and poultry feeds under different environmental variations in summer and winter seasons were investigated. One hundred and six samples of feeds were collected (58 samples during summer and 48 during winter seasons)for detection of aflatoxins(Afs) and ochratoxin (OA)contamination. The samples were collected from farms at Beni-Suef governorate in which the animal and poultry suffered from signs of toxicities as vomiting, diarrhea, diminished production of meat and milk and stunted growth. The results revealed that the incidence and levels of mycotoxins in feed during summer season were comparatively higher than in winter season. Where, the Afs residues were detected in all examined samples of (white corn, wheat bran, barseem hay, poultry feed, concentrated feeds and silage)(100%), during summer season . Whereas, in winter season comparatively lower incidence and levels of aflatoxins were detected in samples(yellow corn, white corn, soya bean, wheat bran, barseem hay, poultry feed, concentrated feeds and silage, cotton seed cake , dairy feed and broiler concentrates) at the rates of incidence of 16.6%, 50%, 33.3%, 70%, 80%, 60%, 75%,62.5%, 50%0% and 66,6%), respectively. Similarly, the detected incidence rates of OA in winter season were also comparatively lower than that detected in summer season, Where, the OA residues were detected in all examined samples of (white corn, concentrated feeds and silage)(100%), during summer season. Whereas, 83.3% , 50%, 33,3% and 72% of examined yellow corn, wheat bran, barseem hay and poultry feed samples were contaminated with OA, respectively. But, in winter season, the OA residues were not detected in all samples of (yellow corn, white corn, wheat bran, poultry feed and broiler concentrates), While, the rates of incidence of OA in samples of (soya bean, barseem hay, concentrated feeds silage, cotton seed cake and dairy feed) in winter season were (33.3%, 30%, 60%, 25%, 80% and 50%), respectively. Therefore, frequent testing program of the animal feeds and other environmental factors for mycotoxigenic fungi and mycotoxins contamination are a critical demand today to safe the animal and human health.

Key words: Aflatoxins ,Ochratoxin A, Feeds, TLC , Detection, Seasons , Limits , Mycotoxins.

1-Introduction

Recently, the Food and Agriculture Organization [1] reported that in areas such as Asia and Africa, 8–18% of cereals commodities, seeds, fruits and vegetables are lost during postharvest handling and storage and about 13.5% of the total value of grain production was lost. The majority of these losses can be attributed to fungal growth and contamination with mycotoxins. Nearly every food or feed commodity can be contaminated by fungal organisms and many of these fungi are capable of producing one or more mycotoxins, which are toxic metabolites of concern to human and animal health. It is estimated that 25 to 50% of the crops harvested worldwide are contaminated with mycotoxins. The percentage is highest in tropical regions, where, up to 80% of the crops are reported to contain significant amounts of mycotoxins [2,3, 4,5]. Mycotoxins are secondary metabolites produced by filamentous fungi

that have deleterious effects on human and animal consumers. They are structurally diverse, deriving from a number of biosynthetic pathways and their effect upon consumers is equally diverse ranging from acutely toxic to immunosuppressive or carcinogenic to human, animal, invertebrates, plants, and microorganisms [6]. The production of a particular mycotoxin is restricted to a limited number of fungal species and in some instances may be limited to particular strains within a species. Although over 400 mycotoxins have been described, relatively few are of major concern with respect to human and animal health, as they are responsible for the production of the great majority of the mycotoxicosis. The majority of these fungi infect plants and can be

regarded as phyto-pathogens and mycotoxin contamination is an economic problem for livestock and feed industries. The presence of mycotoxins in feedstuffs reduces the feed quality in terms of both energy and protein value. The high moisture content (>12%) and grain damage favor mold growth. Mycotoxins may get concentrated from 30-500 times in broken grain as compared to whole grain [5, 7].

In addition, human food can be contaminated with mycotoxins at various stages in the food chain [5,8] and the most important genera of mycotoxigenic fungi are belonged to some members of *Aspergillus sp.*, *Alternaria sp*, *Claviceps sp*, *Fusarium sp.*, *Penicillium sp.* and *Stachybotrys sp.*. The principal classes of mycotoxins include a metabolite of *A. flavus* and *A. parasiticus* (aflatoxinB_1)(AFB_1), the most potent hepato-carcinogenic substance which has been recently proven to be genotoxic. Similarly,[9] reported that the naturally occurring Afs were classified as carcinogenic to humans (Group 1) while OTA and Fumonosins were classified as possible carcinogens (Group 2). However, Trichothecenes and ZEN, were not classified as human carcinogens (Group 3). The health hazards of mycotoxins to humans or animals have been reviewed extensively in recent years [10, 11].However, ochratoxin A (OTA) is produced by fungi of the genera *Aspergillus and Penicillium* and the major species implicated in OTA production includes *A.ochraceus,A.carbonarius,A.melleus,A.sclerotiorum,A.sulphureus,Pichiaverrucossum*[7,12].

Whereas, aflatoxins and ochratoxins were detected in animal feeds and feedstuffs of cereals[11]. Other products of concern are human food of animal products as poultry meat and kidneys, sausages[13, 14]and Milk and their products chees [15].

Because of the toxic and carcinogenic potential of mycotoxins, there is an urgent need to develop detection methods that are rapid and highly specific. These mycotoxins have diverse chemical structures and it is not possible to develop one method to detect all relevant mycotoxins, even if some progress was achieved in the simultaneous detection of several mycotoxins[16].

On the other hand, the development of accurate and sensitive techniques for qualitative and quantitative analysis of mycotoxins and various factors can affect fungal colonization and/or production of the mycotoxins was investigated in various studies [5, 10, 11]. These factors can be categorized as the environmental conditions which conducive to fungal colonization and mycotoxin production such as temperature, relative humidity and insect infestation [5, 17]. Also, the use of fungicides and/or fertilizers, stresses such as drought, an increase in temperature, and an increase in relative humidity may selectively alter colonization and metabolism of mycotoxigenic fungi and thus alter mycotoxin production [5, 18]. Therefore, the present work was undertaken to screen feeds and rations in farms at Beni-Suef governorate at different two main seasons to illustrate the role of environmental climatic conditions in aflatoxins and ochratoxins contaminations of animal feed.

2. Material and Methods

2.1. Material

2.1.1. Feed samples:

The samples were collected during summer and winter seasons from farms at Beni-Suef governorate, in which the animal and poultry suffered from signs of toxicities as vomiting, diarrhea, diminished production of meat and milk and stunted growth. One hundred and six samples of feed and feedstuffs were collected(58 samples during summer and 48 during winter seasons), included yellow corn, white corn, wheat, Soya bean, barseem hay, wheat bran, concentrated feed, poultry feed, broiler concentrates ,silage, cotton seed cake and dairy feed. The samples were collected in polyethylene pages and kept fridge at 5-8 ^{o}C till subjected to laboratory examination. They were brought to laboratory of mycology and mycotoxins, Animal Health Research Institute, Cairo, for detection of aflatoxins and ochratoxin contamination.

2.1.2. Standards of aflatoxins and ochratoxin A: Standards of aflatoxins B_1, B_2, G_1 and G_2 and ochratoxin A were purchased from Sigma Chemical Company (USA).

2.2. Methods:

2.2.3. Detection of aflatoxins contaminations in feed by TLC [19]:

2.2.3.1.. Preparation of the samples :

One hundred grams of feed sample was finely ground and thoroughly mixed in an electric mill to pass sieve No. 10.25 g of each sample were transferred to a 500 ml glass stopper Erlynmeyar flask for the extraction of toxin.

2.2.3..2. Extraction: The samples were extracted with 50 ml of methanol: water solution (55:45 V/V) after being well shaken for 30 min., the suspention was filtered with Whatman No.1 filter paper. The filterate was collected in a separating funnel and the toxin extracted twice with 50 ml

portion of chloroform and the lower layer of chloroform was then drained and passed over a thin layer of 10 gm of anhydrous sodium sulphate (Na_2SO_4). The filterate was then evaporated in a boiling water bath till dryness.

2.2.3.3. Purification and clean up of extracted filtrate :

Each sample residue was dissolved in 2-3 ml of chloroform and purified (clean up) using the column chromatography. After purification, the extract was evaporated till dryness. The residue was cooled and stored at 0 ºC for chromatography study on thin layer plates for detection of the aflatoxin.

2.2.3.4.Thin layer Chromatographic analysis of choloroform extract :

2.2.3.4.1. Preparation of thin layer plates:

A- Preparation of silica gel suspension:

The silica gel suspension for coating glass plates was prepared by adding 80 ml distilled water to 40 gm silica gel in a conical flask shaked vigorously for 1 minute.

B- Coating of galss plates:

The silica gel suspension was pourd into the spreader without let slit in closed position. The spreader was then placed on starting glass plates (20 x 20 cm) on the tray and the level was rotated 180 degrees and immediately the glass plates were coated with 0.25 mm thickness of silica gel suspension. The plates were lifted for 10 minutes until gelled for chromatography, a line was scraped at 16 cm from the bottom edge of TLC plate as a solvent stop and 0.5 cm from silica gel was removed from each side of TLC plate to prevent edge effects.

2.2.3.4.3. Detection of aflatoxins by TLC :

Resolutions of reference aflatoxin B_1, B_2, G_1 and G_2 solution were prepared to give a final dilution with Benzene–acetonitrile (9:1) the same as those prepared individually (0.5 µg aflatoxin B_1, G_1 or M_1 and 1/5 concentration for B_2 or G_2).A vial of sample extract residue was uncapped and 0.1µl Benzene – acetonitrile (9-1) was added and mixed. Activation of thin layer plates for 1 hr in hot air oven at 110 ºC and removed immediately to the dessicator to cool.

A known volume of the sample solution spots of (5, 10, 20 and 40 µl) was stopped on an imaginary line from the bottom edge of the plate . Standard solution was spotted on the plate with known concentration using 10-20 µl capillary pipette.The plate was developed with (toluene:ethyl acetate:90 % formic acid)(5:4:1,V/V/V) in an equilibrate jar or developing tank for 30 minutes.When the solvent travels about 12 cm front, the plates were removed from the jar, air dried and inspected under long wave ultraviolet light lamp (360 nm) for examining the tested and standard spotes matches.

2.2.4. Detection of Ochratoxin (OA) contaminations in feed by TLC [19, 20]:

2.2.4.1.. Preparation of the samples :

One hundred grams of feed sample was finely ground and thoroughly mixed :in an electric mill to pass sieve No. 10.25 g of each sample were transferred to a 500 ml glass stopper Erlynmeyar flask for the extraction of toxin.

2.2.4..2. Extraction:

The samples were extracted with 50 ml of methanol: water solution (55:45 V/V) after being well shaken for 30 min., the suspention was filtered with Whatman No.1 filter paper. The filtrates were acidified with 0.1 N hydrochloric acid, and then ochratoxins were extracted from the acidified filtrates by using chloroform. Each 50 ml filtrate was extracted twice with 50 ml chloroform using a separator funnel. The chloroform extracts were refrigerated over night to allow the separation of insoluble diffuse white material that surfaced at 5°C.

2.2.4.3. Purification of extract [21].

A-An equal amount of 0.1 N sodium bicarbonate solution was added to the acidified chloroform extract and Shaken in separator funnel.

B-The sodium bicarbonate (Na_2HCO_3) extract was acidified with 0.1 N hydrochloric acid and once more re-extracted by chloroform.

C-The purified chloroform extract was evaporated till dryness using rotatory evaporator and weight of the dry samples were determined.

2.2.4.4. Estimation of the prepared ochratoxin A:

2.2.4.4.1. Qualitative estimation [19].

A-Thin layer chromatographic plates of silica gel (0.25 mm layer) were prepared by the usual procedure, air dried for at least 10 minutes and activated at 110°C for 1 hour.

B-The dried samples were dissolved in 250 µl chloroform using a microsyringe, 10 µl were spotted on TLC plate along with 10 µl chloroform solution of the standard ochratoxin A.

C-The spots were air dried and the plates were put in the developing tank containing the solvent system [Toluene - ethyl acetate - formic acid (6: 3: 1 v/v/v)]. When the solvent 41traveled 13 – 15 cm above the origin, the plates were removed from the tank.

D-The plates after spotting were air dried and inspected under UV light (256 nm and 365 nm) and the intensities of the green fluorescence of ochratoxin A spot of the standard solution was compared with the green fluorescence sample spots at the same R_f.

2.2.4.4.2. Quantitative estimation [21].

Different known concentration of standard ochratoxin A and different unknown concentration of produced ochratoxin A were spotted on chromatographic plates, then the plates were developed in developing tank with the developing solvent system. After the solvent traveled 13 cm above the starting line, the plates were removed, air dried and inspected under UV (256 nm and 365 nm). From the color intensities, the nearest known concentration of the standard to the unknown samples was determined. The densitometric analysis of ochratoxin A was done according to **[22]**, where the developed thin layer chromatographic plates were placed on the stage of a fluoro-densitometer at such a position that the spots of ochratoxin A would be in the light path. The plates were scanned at a wave length of 333 nm for excitation to read the intensity of fluorescent of each spot as recorded on a chart by the integrator attached to the densitometer. The amount of toxin calculated against the used standard from the following equation:

$$\mu g/kg = \frac{BYSV}{ZXW}$$

B: Reading of sample - Y: Concentration of standard in µg/ml- S: UL of standard spotted. V: Final dilution of sample extract- Z: Reading of standard- X: µl of sample extract spotted. W: gm sample represent by final extract

2.2.5. Statistical analysis:

The obtained data were computerized and analyzed for significance, calculation of standard error and variance according to **[23].**

Results and Discussion

The mycotoxins are natural contaminants of foods and feeds even when the most efficient condition of culture, harvest, storage and handling are used. The prevalence of these toxins in feed samples varies depending on geographical location and seasons of the year and the specific qualities of climate, vegetation and land are the important factors in connection with certain geographical location the air with the wind or in combination of wind and rain**[24, 25].**On the other hand, *A.flavus* and *A.ochraceus* were recorded to constitute a public health hazard due to production of aflatoxins and ochratoxins that cause some degree of acute toxicity when consumed in high amounts and are potential carcinogen. In developing countries, it appears that there is a direct correlation between dietary aflatoxins intake and the incidence of liver cancer**[26, 27].**

However, aflatoxicosis is a toxic hepatitis leading to jaundice and death in severe cases. Various studies reported the incidence of these symptoms in Kenya (during 1981, 2001, 2004 and 2005), India, and Malaysia**[28, 29]**. Aflatoxin B_1 has been extensively linked to human liver cancer in which it acts synergistically with HBV infection and was classified as a human carcinogen (Group 1 carcinogen) **[9].** This combination represents a heavy cancer burden in developing countries. A recent comparison of the estimated population risk between Kenya and France highlighted the greater burden that can be placed on developing countries **[28].**

The dangerous largest risk of Afs to humans is usually the result of chronic dietary exposure and associated with human hepatocellular carcinomas, which may be accompanied with hepatitis B virus infection **[30]**. In addition, Afs had been found in tissues of children suffering from Kwashiorkor and Reye's syndrome and were thought to be a contributing factor to these diseases and Reye's syndrome, which are characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral edema and considered a form of protein energy malnutrition in animals **[31]**. However, aflatoxins exposure was associated with reduced levels of secretory immunoglobulin A (IgA) in Gambian children **[32]**and cause functional alterations of specific lymphocyte subsets in Ghanaian adults and indicate that aflatoxins could cause impairment of human cellular immunity that could decrease resistance to infections **[33].**

On the other hand, significant losses to the poultry industry occur due to effects of ochratoxin A on performance and health. It causes a reduction in growth rate and feed consumption, poorer feed conversion and increased mortality **[34].** Ochratoxicosis has been implicated in a diverse range of toxicological effects, including renal toxicity, mutagenicity, teratogenicity, neurotoxicity and immunotoxicity in both animals and human **[35].** In human, OTA is implicated in Balkan endemic nephropathy (BEN) a disease characterized by tubulonephritis**[36].**

The most important factors that influence growth and mycotoxin production are environmental temperature, substrate water activity (aW), relative humidity, gas composition, substrate composition, inoculum concentrations, microbial interactions and mechanical or insect damage **[37, 38, 39].** In particular, it is the interaction between some or all of these factors that determines whether contamination increases and mycotoxins are produced. Interactions between available water and temperature are fundamental because they represent the two-dimensional niche in which fungi may be able to germinate, grow and actively compete for the allocation of the available resources **[40, 41, 42].**

The genus *Aspergillus* represents a large group of fungi that occupies very diverse ecological niches. Where, **[43, 44]** reported that the isolated *A. flavus* and *A. ochraceus* from yellow corn, white corn, soya bean meal, wheat and beans produced significant levels of Afs and OA and these samples were collected from store houses under bad condition of storage at high temperatures and moisture. Mycotoxins associated with

Aspergillus species include AFs, ochratoxins, versicolorins, sterigmatocystin, gliotoxin, citrinin, CPA, patulin, citreoviridin, cyclopiazonic acid, penicillic acid and tremorgenicmycotoxins**[5, 45, 46, 47].**

Currently, **[24]** isolated various molds including *A.flavus* and *A. ochraceus* from yellow corn and mixed feed during summer season, where high moisture content and high temperature existed and they detected significant levels of OA in samples. While, **[48]** recovered many types of fungi such as *Aspergillus and Penicillium* from Egyptian maize at field and earlier storage. Ten governorates located between latitudes 22 southwards to 32 northwards, led to a variety climate of temperature, wind and rainfall during the same season, this diversity impact on type and number of toxigenic fungi that grown on maize that followed by mycotoxins type and amount influences. In the present work, One hundred and six samples of feeds were collected (58 samples during summer and 48 during winter seasons), included yellow corn, white corn, wheat, Soya bean, barseem hay, wheat bran, concentrated feed, poultry feed, broiler concentrates ,silage, cotton seed cake and dairy feed. The samples were examined for detection of aflatoxins and ochratoxin A residues during summer and winter seasons (Tables 1-4 & figures 1, 2).

The current results revealed that the incidence and levels of aflatoxins (Afs) residues in animal feeds during summer season were comparatively higher than in winter season.

Where, the Afs residues were detected in all examined samples of (white corn, wheat bran, barseem hay, poultry feed, concentrated feeds and silage)(100%) during summer season with the mean levels of(56±4.0 ppm , 80±6.0 ppm, 16±00 ppm, 8.3±1.7 ppm, 11.73±1.2 ppm and 32±00 ppm), respectively. Whereas, 72.2% of examined yellow corn samples were contaminated with aflatoxins at the mean level of (16.26±3.2 ppm)(Tab.,1 and fig. 1).

Table (1) Prevalence of aflatoxins in single and compound feeds during summer season at Beni-Suef governorate

Types of feed		Prevalence of aflatoxins				
		% of +ve	Levels of aflatoxins in samples (mg/kg – ppm)			Types of AFs
			Maximum	Minimum	Mean ±SE	
Single feed	Yellow corn	72.2	32	3.2	16.26±3.2	B_1,B_2,G_1, G_2
	White corn	100	96	16	56±4.0	B_1, B_2,G_1, G_2
	Wheat bran	100	96	64	80±6.0	B_1, B_2,G_1, G_2
	Barsem Hay	100	16	16	16±00	B_1, B_2,G_1, G_2
Compound feed	Poultry feeds	100	32	1.6	8.3±1.7	B_1, B_2,G_1, G_2
	Concentrated feed	100	32	3.2	11.73±1.2	B_1, B_2,G_1, G_2
	Silage	100	32	32	32±00	B_1, B_2,G_1, G_2

The permissible limits of WHO**[49]** (15 ppb) and FAO**[50**]&**[26]** (20 ppb).

On the other hand, in winter season comparatively lower incidence and levels of aflatoxins were detected in samples(Table, 2 and figure, 1). Where, the Afs residues were detected in samples of (yellow corn, white corn, soya bean, wheat bran, barseem hay, poultry feed, concentrated feeds and silage, cotton seed cake , dairy feed and broiler concentrates) at the rates of incidence of (16.6%, 50%, 33.3%, 70%, 80%, 60%, 75%,62.5%, 50%0% and 66,6%), respectively. The detected levels of Afs in winter season were also comparatively lower than that detected in summer season, where, the mean levels of detected Afs were (0.8±00 ppm, 1.6±00 ppm, 0.8±00 ppm, 4.4±3.6 ppm, 2.66±1.16 ppm, 0.8±0.22 ppm, 1.8±0.16 ppm, 2.2±0.6 ppm, 1.6±00 ppm, 00 ppm and 0.8±0.2 ppm), respectively.

Table (2) Prevalence of aflatoxins in single and compound feeds during winter season at Beni-Suef governorate

Types of feed		Prevalence of aflatoxins				
		%of +ve	Levels of aflatoxins in samples (mg/kg – ppm)			Types of AFs
			Maximum	Minimum	Mean ±SE	
Single feed	Yellow corn	16.6	0.8	0.8	0.8±00	B_1, B_2
	White corn	50	1.6	1.6	1.6±00	B_1, B_2,G_1, G_2
	Soya bean	33.3	0.8	0.8	0.8±00	B_1, B_2
	Wheat bran	70	8	0.8	4.4±3.6	B_1, B_2
	Barsem Hay	80	6.2	0.4	2.66±1.16	B_1, B_2,G_1, G_2
Compound feed	Poultry feeds	60	1.6	0.4	0.8±0.22	B_1, B_2,G_1, G_2
	Concentrated feed	75	3.2	1.6	1.8±0.16	B_1, B_2,G_1, G_2
	Silage	62.5	3.2	0.8	2.2±0.6	B_1, B_2
	Cotton seed cake	50	1.6	1.6	1.6±00	B_1
	Dairy feed	0	0	0	00	0
	Broiler concentrates	66.6	1.2	0.4	0.8±0.2	B_1

The permissible limits of WHO**[49]** (15 ppb) and FAO**[50**]&**[26]** (20 ppb).

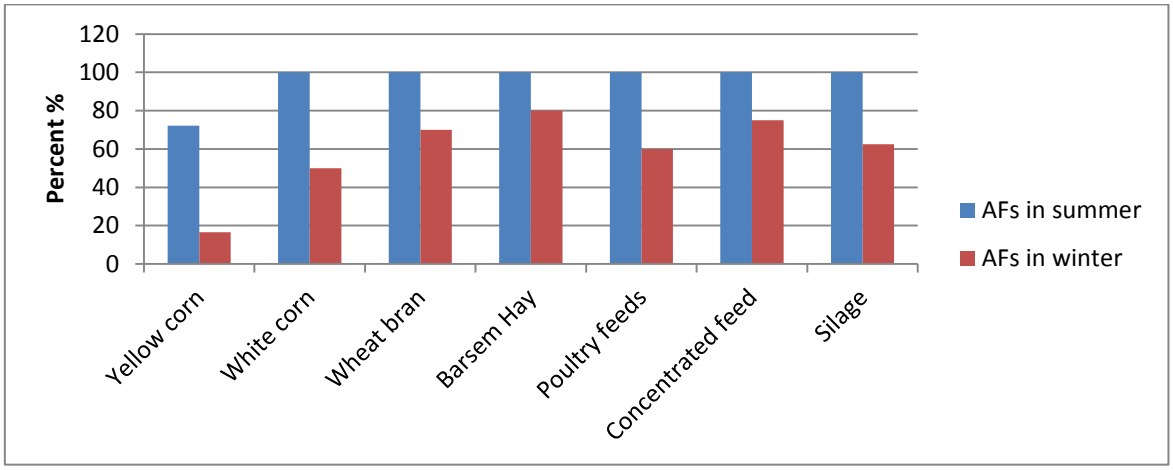

Fig. (1)Prevalence of aflatoxins in feeds during summer and winter seasons at Beni-Suef governorate

It is interesting to report her that all types of aflatoxins (B_1, B_2,G_1, G_2) were detected in all positive feed samples for aflatoxins contamination during summer season. Whereas, in winter season the positive samples of yellow corn soya bean wheat bran and silage showed the detection of only Afs $B_{1\&}$ B_2 and samples of cottonseed cake and broiler concentrates yielded the detection of AfB_1 alone.

In addition, the detected levels of aflatoxins in positive samples during different seasons were over the permissible limits that recommended by WHO**[49]** (15 ppb) and FAO**[50]**&**[26]** (20 ppb).Thus, the consumption of these feeds can be resulted a health hazard for human and animal.

Table (3) Prevalence of ochratoxin A in single and compound feeds during summer season at Beni-Suef governorate

Types of feed		Prevalence of ochratoxin A			
		% of +ve	Levels of ochratoxin A in samples (mg/kg –ppm)		
			Maximum	Minimum	Mean ±SE
Single feed	Yellow corn	83.3	160	4	71.26±11.6
	White corn	100	16	4	10±6.0
	Wheat bran	50	40	40	40±0.0
	Barsem Hay	33.3	4	4	4±00
Compound feed	Poultry feeds	72	80	2.0	31.7±7.86
	Concentrated feed	100	80	8	49.3±14.5
	Silage	100	16	16	16±00

Legal limits for ochratoxin A in different Cereal grains and theire products set by the European Commission (**[51]:** not ,more than 0.25 mg/kg (ppm)and 0.05 ppm in other feedstuffs.

Table (4) Prevalence of ochratoxin A in single and compound feeds during winter season at Beni-Suef governorate

Types of feed		Prevalence of ochratoxin A			
		%of +ve	Levels of ochratoxin A in samples (mg/kg – ppm)		
			Maximum	Minimum	Mean ±SE
Single feed	Yellow corn	0	0	0	0±00
	White corn	0	0	0	00
	Soya bean	33.3	8	8	8±00
	Wheat bran	0	0	0	00
	Barsem Hay	30	8	2	3.66±0.8
Compound feed	Poultry feeds	0	0	0	00
	Concentrated feed	60	8	4	5±1.0
	Silage	25	4	2	3±1.0
	Cotton seed cake	80	4	4	4±00
	Dairy feed	50	40	40	40±00
	Broiler concentrates	0	0	0	00

Legal limits for ochratoxin A in different Cereal grains and theire products set by the European Commission**[51]**: not ,more than 0.25 mg/kg (ppm)and 0.05 ppm in other feedstuffs.

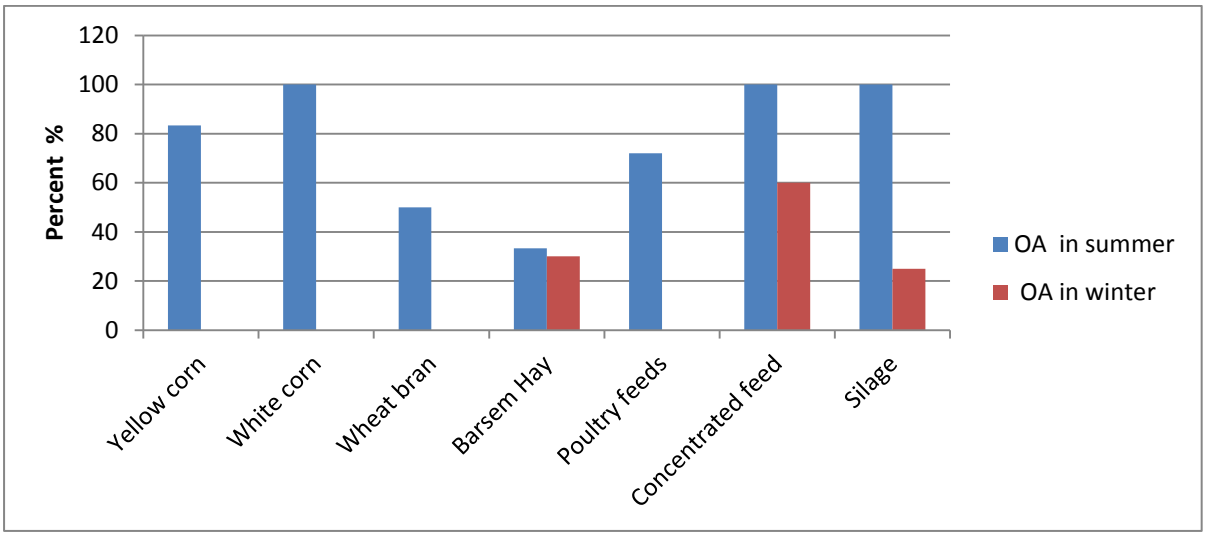

Fig. (2) Prevalence of ochratoxin A in feeds during summer and winter seasons at Beni-Suef governorate

Regarding the incidence and levels of ochratoxin A (OA) residues in feeds during summer season were comparatively higher than in winter season. Where, the OA residues were detected in all examined samples of (white corn, concentrated feeds and silage)(100%) during summer season with the mean levels of(10±6.0 ppm , 49.3±14.5 ppm and 16±00 ppm), respectively. Whereas, 83.3% , 50%, 33,3% and 72% of examined yellow corn, wheat bran, barseem hay and poultry feed samples were contaminated with OA at the mean levels of (71.26±11.6 ppm, 40.0±0.0 ppm, 4.0±0.0 ppm and 31.7±7.86 ppm), respectively (Table,3 and figure 2).

Currently, in winter season comparatively lower incidence and levels of OA were detected in samples(Table, 4 and figure, 2). Where, the OA residues were not detected in all samples of (yellow corn, white corn, wheat bran, poultry feed and broiler concentrates)(100%). While, the rates of incidence of OA in samples of (soya bean, barseem hay, concentrated feeds, silage, cotton seed cake and dairy feed) were (33.3%, 30%, 60%, 25%, 80% and 50%), respectively with the mean level of (8±00 ppm, 3.66±0.8 ppm, 5±1.0 ppm, 3±1.0 ppm, 4±0.0 ppm and 40±0.0 ppm), respectively.

The detected levels of OA in positive samples of feeds were significantly higher than the international limits that recommended by the European Commission **[51]** who reported that the legal limits for ochratoxin A in different Cereal grains and their products must be not more than 0.25 mg/kg (ppm)and 0.05 ppm in other feedstuffs. Thus its consumption can be resulted a health hazard for human and animal

Nearly every food or feed commodity can be contaminated by fungal organisms and many of these fungi are capable of producing one or more mycotoxins, which are toxic metabolites of concern to human and animal health. It is estimated that 25 to 50% of the crops harvested worldwide are contaminated with mycotoxins. The percentage is highest in tropical regions, where, up to 80% of the crops are reported to contain significant amounts of mycotoxins**[5, 7].**

In other study, **[52]**detected aflatoxins in 30% of feed samples with the mean value of (3.4 ± 0.1 ppm). While**,[3]**reported that 60% of cattle blood had the mean levels of aflatoxins (15.20±0.01 ppb) and the used feed samples in breeding of these animals had the amounts of AFB_1 detected in (60%) of feed samples, with the mean levels of (55.00±1.50 ppb).Whereas, **[7]** screened one hundred feed samples for mycotoxigenic fungi and recovered 106 fungal isolates comprising, *Aspergillus flavus*, *A. ochraceus* and *A. niger*. Thirty three isolates of 47 *A. flavus* produced aflatoxin B_1 and B_2 at average levels of (170-750 ppb), while, 22 of 44 tested isolates of *A. niger* produced OTA with average levels of (100-550 ppb), whereas, 12 of 15 *A. ochraceus* isolates produced OTA at average levels of (300-700 ppb).

The effects of mycotoxins in human and animals varied from carcinogenic; nephrotoxic and immunosuppressive health effects [**10, 11, 53]**. In developing countries, it appears that there is a direct correlation between dietary aflatoxins intake and the incidence of liver cancer**[26, 27]**.The exposure to AFB_1 can result in suppressed immune response, malnutrition, proliferation of the bile duct, centrilobular necrosis, fatty infiltration of the liver, hepatic lesions and even hepatomas. It is one of the most commonly found metabolites and has a highest toxigenic effect [**10, 11, 54]**.

The mycotoxins are formed by certain fungal species, whenever environmental factors are conductive during the growth of these frequently occurring mycomycetes on foodstuffs and animal feeds; the process takes place as a secondary metabolism. The mycotoxin inhibits cell division, RNA/ DNA synthesis and causes apoptosis **[10, 11, 55].**The effects of mycotoxins in human and animals varied from carcinogenic; nephrotoxic and immunosuppressive health effects **[53, 56].**Although the main route of human exposure to mycotoxins has been identified as the direct ingestion of contaminated cereals, grains and food of animal origin **[56].** There are many studies about whether or not the ingestion of meat, milk, and eggs originating from mycotoxin exposed food production animals is a significant pathway for mycotoxins among humans **[14, 57]** However,**[58]**reported that no strains of *A. foetidus* produced OTA and the consistent with this analysis, the strain of *A. foetidus* that was described as an OTA producer was later shown to be *A. niger* and not *A. foetidus*. In other study, **[52]** detected aflatoxins in 30% of feed samples with the mean value of 3.4 ± 0.1 ppm and ochratoxins in 20% with the mean values of 2.2 ± 0.02 ppm . Whereas, T-2 toxins and zearalenone were gained from 20% and 16% of samples with the mean levels of 36.0 ± 1.0 and 22 ± 0.3 ppm, respectively, but fuminosin B_1 (FB_1) toxin was found in 2% of samples at mean levels of 70 ± 0.01 ppm.

The Food and drug administration has established recommended maximum levels for aflatoxins in animal feed as 20 µg/kg of feed **[59],** while, the permissible limits of aflatoxin for large ruminants varied between 700-1000 ppm of feed. This limit will cause loss of weight gain, high food consumption and low feed efficiency **[51]**whereas, the OA produced significant pathological changes in animal at the levels of 2.5 ppm of animal body weight for 5 days when given orally **[21].**

The detected levels of mycotoxins in the present study were significantly over the permissible limits in feeds as a result of continuous feeding of toxic feed. The same findings were detected by **[3, 4,7, 10, 11, 15, 52, 53],** in association with significant high mycotoxin levels in feed and sera of diseased animals. The mycotoxins pollution of animal feeds results in significant losses in animal health and causes important burdens to the country's economy with regard to meat, milk, wool and leather industries. Therefore, frequent testing program of the animal feeds and their environment for fungi and mycotoxins contamination is a critical demand particularly during summer season.

Conclusion

The high incidence of the significant levels of aflatoxins and ochratoxin A in feed at summer season rather than winter season as recorded in present study warrants that there is urgent need to undertake mycotoxins awareness creation programs among different foods and feedstuffs in Egypt to secure the bade environmental condition that enhanced mycotoxins production in feed and food. Application of Thin layer chromatography technique for screening of feeds for mycotoxins was found to be still rapid, highly specific, easy to perform and cost effective method to assist creation of such programs and reduction of the risk of harmful effects of toxigenic fungi and their toxins to human and farm animals health.

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