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Mycological examination of poultry feed with special reference to Aflatoxin and Ochratoxin interaction in poultry farms in El- Behaira Province

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ABSTRACT

Moulds are capable of reducing the nutritional value of feed stuff as well as elaborating several mycotoxins. Mycotoxin-contaminated feed has adverse effects on animal health and productivity. Also, mycotoxins may be carried over into meat and eggs when poultry are fed with contaminated feed. In a point prevalence study of feed stuff used for poultry nutrition in El Behaira were analyzed for fungal flora, natural incidence of Aflatoxin and Ochratoxin. the mycological examination of 100 poultry feed samples revealed *that Penicillium, Fusarium, Alternaria, Aspergillus Flavus, Aspergillus Ochraceus, Aspergillus Candidus, Aspergillus Niger*, and *Mucor* were the dominant mould groups with a percentages 40, 23, 36, 30, 33, 8, 40, 30 % respectively.

Samples were examined for the detection of total aflatoxin (TA) and ochratoxin A (OTA). Overall incidence of TA was recorded as 80% (n=80/100). The OTA was detected in 83% (n=83/1oo). However the co-occurance of TA and OTA was 63% (n=63/100).

2/80 samples (2.5%) contain TA above the permissible limit (20ppb) while OTA was (34/83) samples (41%) at a rate of (6-10) ppb and (3) samples (3.6%) above (10ppb) above the acceptable limit. all samples collected in -summer were contained aflatoxin and ochratoxin (100%), and aflatoxin was at low level below the permissible limit while ochratoxin at high level 30 samples above 5 ppb.

On the other hand, in winter season comparatively lower incidence and levels of aflatoxins than that detected in summer season in which 30/50 samples (60%) were contained aflatoxin at low level while 33/50 samples (66%) contained ochratoxin with 5 samples at high level above the permissible limit.

This study indicates the need for continuous assessment of the mycological and mycotoxins status of poultry feed, in order to feed poultry for optimal performance ensuring food safety and to ensure safe poultry for human consumption.

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INTRODUCTION

Mycotoxins are often found as natural contaminants in raw ingredients of poultry feed **(Khan et al. 2011).** Poultry are highly susceptible to mycotoxicoses caused by aflatoxins (AF) and ochratoxins (OTA) **(Anjum et al. 2011).** The AF and OT are the major components of secondary metabolites group produced by several toxigenic fungi.

Aflatoxicosis results in severe economic loss in the poultry industry that affecting ducklings, broilers, layers, turkeys and quails **(CAST 2003).** In poultry, AF impairs most of the important production parameters including weight gain, feed intake, feed conversion efficiency, processing yield, egg production, and male and female reproductive performance **(Hussain et al. 2010).** As a common rule, poultry should not get more than 20 µg/kg TA in the feed.

Aflatoxin contamination in feed induces immunsupression in poultry, thus the birds become more susceptible to several diseases **(Dhanasekaran et al. 2009).**

Toxigenic *Aspergillus flavus* isolates generally produces aflatoxins B1 and B2, whereas *A. parasiticus* produces aflatoxins B1, B2, G1 and G2 **(Davis and Diener 1983).** The major hosts of *A. flavus* among food and feed commodities are cereal grains, peanut, cotton seed and protein sources such as rapeseed meal, cotton seed meal, soyabeen meal, sunflower meal, corn gluten meal, copra meal, and palm kernel meal **(Anjum et al. 2012).**

Ochratoxins are another group of mycotoxins that are produced by several species of *Aspergillus and Penicillium*; these include certain members of *Aspergillus ochraceus* group and *Penicillium verrucosum* type I and II **(Reverberi et al. 2010).** The OTA is constituted of three compounds; these are Ochratoxin A, B or C. Among these three, ochratoxin A (OTA) is the most harmful one.

Ochratoxins are considered as powerful nephrotoxins, carcinogens, teratogens, and immune-toxins in rats, humans and likely in poultry **(Frisvad 1995 Romani et al. 2000 Bozzo et al. 2008).**

Ochratoxin is highly stable during feed storage and feed preparation procedures, thus it may endanger humans as the final consumers of contaminated food of plant or animal origin. Among the feed of animal origin, poultry is frequently consumed **(Schiavone et al. 2008).** Poultry diets are based on cereals and cereal by -products upto 50-60% on a dry matter basis, and these raw materials are the preferred substrates for *Penicillium* and *Aspergillus* growth **(Petzinger and Weindenbach 2002).**

Different feed ingredients that are used in poultry feeds are likely to be contaminated with AF and OT producing fungi due to suitable environments for fungal growth like improper harvesting and storage, unhygienic method of processing and production, poor methodology of consumption and utilization. Therefore, regular monitoring of AFand OT in poultry feeds is an important precondition to check toxins build up in poultry feeds.

Most toxogenic species belong to the genera *Aspergillus, Penicillium, Fusarium,* and *Alternaria* **(Pitt and Hocking 2009).** According to several authors mycotoxins such as aflatoxins, zearalenone, T2-toxin, deoxynivalenol, ochratoxin A, fumonisins, and patulin can be considered the most common mycotoxins found in feed and food **(Hussein and Brasel 2001 Firdous 2003).**

Most mycotoxicosis cases in poultry are caused by an intake of low concentration of contaminants over a long time that lead to typical chronic symptoms of poor growth, poor feed efficiency, and suboptimal production. however ingestion of higher concentration leads to acute clinical symptoms associated with specific vital organs, the immune system, and other aspects of avian physiology as well as mortality **(Mabbett 2004).**

For quality control, the identification of the contaminating fungi is essential because it provides data on the potential production of its mycotoxins and is a helpful indicator to determine feed hygienic quality **(Khosravi et al. 2008).**

The aim of the present study was to determine the AF and OT contents in poultry feed in El-Behaira Province. Samples were collected from poultry farms and samples were received in Animal Health Research Institute Damanhour branch. The study includes isolation and identification of mould genera and species, natural levels of aflatoxin, ochratoxin and their interaction.

2. MATERIALS AND METHODS

2.1. Samples

A total of 100 representative samples (1-2 kg per sample) of poultry ration (starter and grewer) were collected from poultry farms in El Behaira, then homogenized and divided to obtain a 1 kg of working sample for analysis. Each sample was ground in a laboratory mill and divided into two parts, one part for mycological examination and the other for mycotoxin determination. The investigation period was carried out during June 2020 - May 2021.

For mycological examination feed samples were immediately analyzed upon arrival or they were stored for 2-3 days in paper bags at room temperature (about 25°C). Feed samples intended for mycotoxin analysis were examined immediately. Sampling procedure followed the principles of the Romer® Guide **(Richard 2000).**

Information including date of sample collection, type of feed or ingredients, place, name of feed mill or farm, date of manufacture or purchase of feed, batch number, brand name, number of feed bags out of which sample collected, sample weight, storage conditions, number of birds at poultry farm, breed, age and mortality of flock were recorded.

Isolation and identification of mould from feed samples according to **(Cruickshank et al. 1975** and **Koburger 1970)**

Ten grams of each milled feed sample was mixed with 90 mL of 0.1% peptone water and shaken on a horizontal shaker for 20 minute each sample was cultured on the surface of Sabourad Dextrose Agar (SDA). The culture grown media were incubated at 25 °C for 3- 5 days. Colonies were subculture on freshly prepared SDA media to obtain pure cultures. Pure cultures were also maintained in SDA slants at 5±1°C. Mould and yeast were identified by their colonial morphology and microscopic characteristics. The fungal isolates were mounted in lacto phenol cotton blue stain solution on slides with cover slips and microscopically examined for spores and vegetative bodies according to the method described by **Barnet and Hunter (1972).**

The identification was based on colonial features, pigment production and the micro morphology of the spores produced. Cultures were examined at 4 or 5 days intervals from the onset. Some characteristics were also noted on the texture, colour, shape and the production of pigment on the underside. Fungi were identified at genus level according to **Samson et al. (2004), Pitt and Hocking (2009), Nelson et al. (1983), Simmons (2009).**

2.3. Detection of mycotoxins residues.

Total aflatoxin and ochratoxin were detected in poultry feed, using immuno-affinity method **(Scot and Trucksess 1997).** Series-4 Fluorometer (VICAM) was used in this procedure. This method depend on Sample extraction by using methanol (80%) and Column chromatography by passing the filtered extract through AflaTest-p affinity column. Reading of total aflatoxin was obtained after 60 second as part per billion (ppb).

2.4. Detection of aflatoxin and ochratoxin producing ability of *Aspergillus flavus* and *Aspergillus ochraceus* isolates.

Aflatoxin and ochratoxin were produced by growing *A.flavus* and *A.ochraceus* on sterile polished maize by the method of **Shotwell et al. (1966)** in which 5 isolated strains of *A.Flavus* and A. O*chraceaus* were growing on potato dextrose agar for 7-10 days at 28°C ,the cultures had a heavy crop of green conidia was removed and a uniform suspension of the spores were inoculated in each flask containing (50 gm of ground maize and 25 ml of sterile distilled water) which previously autoclaved and placed on shaker and incubated at 28 c for 7- 10 days, it is important that the maize does not remain as a compact mass. then the mouldy maize was autoclaved to kill the fungus, dried and ground to fine powder and mycotoxin was detected by Series-4 Fluorometer (VICAM).

Table (1). Incidence of mould species isolated from examined samples .

Mould species	No.	%
A.flavus	30	30%
A.ochraceus	33	33%
A.candidus	8	8%
A.niger	40	40%
Pencillium	40	40%
Fusarium	23	23%
Alternaria	36	36%
Mucor	30	30%

3. RESULTS

Table (2). Incidence of aflatoxin and ochratoxin in examined poultry feed.

Number of examined samples	Positive samples for aflatoxin		Positive samples for ochratoxin		Positive samples for aflatoxin and ochratoxin		Positive samples for aflatoxin and negativefor ochratoxin		Positive samples for ochratoxin and negativefor aflatoxin	
	No.	%	No.	%	No.	%	No.	%	No.	%
100	80	80%	83	83%	63	63%	17	17%	20	20%

Table (3). Level of aflatoxin and ochratoxin in examined poultry feed.

No. of samples (100)	No.	%	Residues expressed in ppb level of mycotoxin					
Aflatoxin contaminated samples			1-10 ppb.		10-20 ppb		>20 ppb	
			No.	%	No.	%	No.	%
	80	80%	76	95	2	2.5	2	2.5
Ochratoxin contaminated samples			1-5 ppb		6-10 ppb		>10ppb	
			No.	%	No.	%	No.	%
	83	83%	46	55.4	34	41%	3	3.6%

Table (4). Incidence of samples positive for Aflatoxin and/or Ochratoxin but negative for Aspergillus spp. Isolation.

No. of samples	Samples negative for isolation.		Residues level of mycotoxin					
Aflatoxin producing samples (80)			1-10 ppb.		10-20 ppb		>20 ppb	
	No.	%	No.	%	No.	%	No.	%
	50	62.5	50	100%	0	0	0	0
Ochratoxin producing 5amples (83)			1-5 ppb		6-10 ppb		>10ppb	
	No.	%	No.	%	No.	%	No.	%
	50	60.2%	40	80%	10	20%	0	0

Table (5). Toxigenicity of *Aspergillus flavus* isolates.

Isolate serial No.	1	2	3	4	5
Amount of AFS (ppb)	25	14	120	40	12

Table (6). Toxigenicity of *Aspergillus Ochraceus* isolates.

Isolate serial No.	1	2	3	4	5
Amount of OT (ppb)	20	15	4	17	38

Table (7). Incidence of aflatoxin and ochratoxin in examined samples in summer and winter season.

No. of examined samples	Positive aflatoxin producing samples			Positive ochratoxin producing samples		
(50) in summer	No.	%	>20 pp	No.	%	>5ppb
	50	100%	2	50	100%	30
(50) in winter	30	60%	0	33	66%	7

DISCUSSION

The mycotoxins are natural contaminants of poultry feed ingredients as 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.

On the other hand, *A.flavus* and *A.ochraceus* were found to constitute a public health hazard due to production of aflatoxins and ochratoxins that cause some degree of acute toxicity when consumed in high mounts and are potential carcinogen. (**FDA 2000, (Bahtnager and Ehrlich 2002).** The 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 (**Saleemi et al. 2010).**

Fungal contamination of animal feed, with the consequent mycotoxin production, is one of the major threats to human and animal health **(Castillo et al. 2004**), For quality control the identification of the contaminating fungi is essential because it provides data on the potential production of its mycotoxins and is a helpful indicator to determine feed hygienic quality **(Khosravi et al. 2008).**

In this work, most tested samples were contaminated with fungi and mycotoxins. table (1) show the incidence of *Aspergillus spp* in which *A.Flavus, A. ochraceus, A candidus,* and *A. niger* were isolated and their frequencies of occurrence were 30%, 33%, 8% and 40% respectively. This was in agreement with (**Petzinger and Weindenbach 2002)** who reported that Poultry diets are based on cereals and cereal by-products up to 50-60% on a dry matter basis, and these raw materials are the preferred substrates for *Penicillium* and *Aspergillus* growth. The toxicogenic fungal contamination of the raw materials occurs during the pre-harvest and/or the post-harvest periods, and the finished feeds are exposed during production, processing, transportation, and storage .It has been observed that moulds and mycotoxins presence varies depending on the geographical location and the year. Temperature and humidity play important roles not only in the development of fungi but also in mycotoxin production **(Saleemi et al. 2010)**.

Other four mould genera were isolated *Penicillium, Fusarium Mucor,* and *Alternaria* and their frequencies of occurrence were 40%, 23%, 30% and 36% respectively. **(Hassan and Omran 1996)** 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 **(Nooh et al. 2014)** recovered many types of fungi such as *Aspergillus* and *Penicillium* from Egyptian maize at field and earlier storage.

Table (2)show the incidence of total aflatoxin (TA) and ochratoxin (OTA) in poultry feed in which out of (100) samples were examined (80) samples (80%) were positive for (TA), (83) samples (83%) were positive for (OTA), (17) samples (17%) were positive for (TA) only and (20) samples (20%) positive for (OTA) only and (63) samples (63%) were positive for both aflatoxin and ochratoxin. Co-occurrence of mycotoxins was determined in about 63% of tested samples This agree with **(Binder et al. 2007)** who reported that concurrent exposure to multiple mycotoxins is more likely in feed stuff. The combined action of mycotoxins can generate an interactive effect such as additivity, synergism, or antagonism. The synergistic interaction causes the most toxic effects in the case of aflatoxins and OTA. Synergistic effects in poultry were observed in the case of aflatoxins and OTA. This interaction lead to reduction of body weight and increased mortality **(Constanzo and Murphy 2012**) .

Table (3) show the incidence of aflatoxin in examined feed in which 76 samples (95%) at rate of (1-10) ppb., 2 samples (2.5%) at a rate of (10-20) ppb and 2 samples (2.5%) above the permissible limit (20 ppb). The high incidence and low values of TA were observed in examined feed samples. This low incidence of aflatoxin could be due to selection of good quality graded samples **(Beg et al. 2006).**

Table (3) show the incidence of ochratoxin in examined feed in which 46 samples (55.4%) at rate of (1-5)ppb, 34 samples (41%) at a rate of (6-10) ppb and 3 samples (3.6%) above (10ppb), in this study the examined samples have high incidence and levels of ochratoxin (OTA) that reach up to the permissible limit**. (Akinmusire et al. 2019)**.

In Table (4) 50 samples (62.5%) out of 80 samples produced TA at a rate of (1-10)ppb but we could not isolate *A. flavus* from the samples also 50 samples (62.2%) out of 83 samples contained OTA but *A. ochraceus* could not be isolated from examined samples, 40 samples (80%) produced ochratoxin at a rate of (1-5) ppb and 10 samples (20%) produced ochratoxin at a rate of (6-10) ppb. these results may be due to that mycotoxins were synthesized by organisms when metabolically active in moist stored feed in sealed and unsealed silos and the species which synthesized these toxins may have been killed by heat treatment during the preparation of the feed or may be feed ingredients treated with antifungal additives.

In table (5) the results of screening of isolated strains of *A. flavus* for aflatoxins production revealed that 3 isolates out of 5 tested isolates were found to be aflatoxin producers at a rate of above the permissible limit and 2 isolates produced aflatoxin at a rate below the permissible limit but all are toxins producers. The permissible limits are ranged between 15 ppb. **(WHO 1975)** and 20 ppb **(FAO 1995 & FDA 2000)**.

Also the results of screening of isolated strains of *A. ochrceaus* for ochratoxin production revealed that 4 isolates out of 5 tested isolates were found to be ochratoxin producers at a rate of above the permissible limit and 1 isolates produced ochratoxin at a rate below the permissible limit but all are toxins producers In table (6) The same findings were detected by **Hassan et al. (2012) El-Hamaky et al. (2016).**

In this study, about 50% of the samples were collected in winter season and the other 50 samples were collected in summer. the results revealed that all samples collected in summer were contained aflatoxin and ochratoxin (100%), and aflatoxin was at low level below the permissible limit while ochratoxin at high level 30 samples above 5 ppb. On the other hand, in winter season comparatively lower incidence and levels of aflatoxins than that detected in summer season in which 30 samples (60%) were contained aflatoxin at low level while 33 samples (66%) contained ochratoxin with 5 samples at high level above the permissible limit (table 7). **Abidin et al. (2013), (Saleemullah et al. (2006**) and **Anjum et al. (2012)** studied the effect of storage conditions on TA production and found a positive rela-

tionship between TA level and moisture content. Interactions between available water and temperature are the most important two factors in which fungi be able to germinate, grow and produced toxins these results also agree with **Marín et al. (2012) and Nooh et al. (2014**). detected incidence rates of OTA in feeds at winter season were lower than that detected in summer season, Where, the OTA 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 OTA, respectively.

In this study, high humid environment and elevated temperature during June to August were conducive for the growth of fungi producing mycotoxins **(Sabri et al. 1989**). The high incidence of significant levels of aflatoxins and ochratoxin A in feed at summer season rather than winter season warrants that there is urgent need to under take mycotoxins awareness creation programs among different foods and feedstuffs in Egypt to secure the bad environmental condition that enhanced mycotoxins production in feed and food.

In this work, although the levels of the Aflatoxin detected on poultry feed was lower than the regulation limits established, while the ochratoxin detected with high incidence and level and the co-occurrence of them was demonstrated. The scientific literature offers a broad spectrum of information on the effects of individual mycotoxins in various animal species but concurrent exposure to multiple mycotoxins is more likely in feed stuff. while mycotoxins have attracted worldwide attention due to their direct impact on human health, due to food contamination, it is also relevant to consider to what extent mycotoxins can be carried over into edible tissues like meat and eggs when poultry are fed with contaminated feed.

CONCLUSION

Higher incidence and low level of TA are detected in poultry feeds On the other hand, OTA contamination is present at high incidence and high levels in poultry feed. However, this situation demands for immediate necessary control measures. Adequate post-harvest drying should be done, and proper storage condition should be maintained. Finally, strict regulations and surveillance programs for testing food and feed for aflatoxin and ochratoxin contamination are highly recommended to improve the health status of the consumers.

We suggest that periodic monitoring and the application of the Hazard Analysis and Critical Control Points (HACCP) on the prevention and control of mycotoxins in the animal feed industry are needed in order to guarantee the hygienic quality of feed to ensure health and productivity of poultry as well as prevent human food borne diseases.

REFERENCE

Abidin ZU, Khatoon A, Qureshi MA, Butt TM. 2013. Determination of aflatoxin B1 in finished poultry feed samples collected from different poultry farms and markets of Lahore, Pakistan. International Journal of Veterinary Science, (2): 28-31.

Akinmusire OO, ElYuguda AD, Musa JA, Oyedele OA, Sulyok M, Somorin YM, Krska R. 2019. Mycotoxins in poultry feed and feed ingredients in Nigeria Mycotoxin Res, 35 (2): 149-155.

Anjum MA, Khan SH, Sahota AW, Sardar R. 2012. Assessment of aflatoxin B1 in commercial poultry feed and feed ingredients. The Journal of Animal and Plant Sciences, (22) : 268-272.

Anjum MA, Sahota AW, Akram M, Ali I. 2011. Prevalence of mycotoxins in poultry feeds and feed ingriedents in Punjab (Pakistan). The Journal of Animal and Plant Sciences, (2) : 117-120.

Barnett HL, Hunter BB, 1972. Illustrated genera of imperfect fungi. 2nd., Burgess Put. Co.

Beg MU, Al-Mutairi M, Beg KR, Al-Mazeedi HM, Ali LN, Saeed T 2006. Mycotoxin in poultry feed in Kuwait. Archives of Environmental Contamination and Toxicology, (50) : 595-602.

Binder EM, Tan LM, Chin LJ, Handl J, Richard J. 2007. "Worldwide occurrence of mycotoxins in commodities, feeds and feed in-

gredients," Animal Feed Science and Technology, (137(3-4): 265–282). View at: Publisher Site | Google Scholar

Bozzo G, Ceci E, Bonerba E, Desantis S, Tantillo G. 2008. Ochratoxin A in laying hens: HighPerformance Liquid Chromatography detection and cytological and histological analysis of target tissues. Journal of Applied Poultry Research, (17): 151-156.

CAST. 2003. Mycotoxins: Risk in Plants, Animals and Humans. Task Force Report No. 139, Council for Agricultural Science and Technology (CAST), Ames, Iowa, USA.

Castillo MD, González HHL, Martínez EJ, Pacin AM, Resnik SL. 2004. "Mycoflora and potential for mycotoxin production of freshly harvested black bean from the Argentinean main production area," *Mycopathologia*, (158 (1): 107–112. View at: Publisher Site | Google Scholar

Constanzo AD, Murphy M. 2012. "St rategies for Feeding Mycotoxin and Mold Contaminated Grains to Cattle.

Cruickshank KR, Duguid JP, Marmion BD, Swain RHA. 1975. Medical Microbiology. 12th Ed., Vol.2, churchill Livingston Limited, Edinburgh, London and New York.

Davis ND, Diener VL. 1983. Some characteristics of toxigenic and non toxigenic isolates of *A. flavus and A. parasiticus*. In: Diener VL, Asquith RL, Dickens JW Edn. Aflatoxin and A. flavus is corn. Southern Coop. Series, Bull. 279, Opelika, Ala; pp 112.

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

El-Hamaky AM, Atef A, Hassan Heidy Abo El Yazeed MK, Refai 2016. Prevalence and Detection of Toxigenic *A. flavus, A .niger and A. ochraceus* by traditional and molecular biology methods in feeds. .International Journal of Current Research, (8 Issue, 1, pp.. 25621-25633).

FAO (Food and Agriculture Organization of the United Nations) 1995. "Food and Nutrition paper."Worldwide Regulation of Mycotoxins Advanced Copy.

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

Frisvad JC. 1995. Mycotoxins and mycotoxigenic fungi in storage: Stored grain ecosystems. Jayas, DS; White, ND and Muir WE, Ed. Marcel Dekker, NY; (pp 251-288).

Food and Drug Administration FDA 2000. Conference on mycotoxins in animal feeds, grains and food related to human and animal health. Rockville, Maryland.

Hassan AA, RMA Omran. 1996. Seasonal variation in mycoflora and mycotoxins in feeds and pathological changes due to ochratoxins. J. Egypt. Vet. Med. Ass., (56 (1): 73-96).

Hassan Atef A, Mogeda K, Mansour Howayda M, El Shafei Nouha HA, Oraby Rasha MH, Sayed El Ahl 2012. Studies on Mycosis and Mycotoxicosis in Cattle. Bulletin of Environment, Pharmacology and Life Sciences (Volume 1, Issue 3, 12-22).

Hussein HS, Brasel JM. 2001. "Toxicity, metabolism, and impact of mycotoxins on humans and animals," *Toxicology*, (vol. 167, no. 2, pp. 101–134.) View at: Publisher Site | Google Scholar

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

Khan SH, Shamsul H, Rozina S, Muhammad AA. 2011. Occurrence of Aflatoxin B1 in Poultry Feed and Feed Ingredients in Pakistan. International Journal of Agro Veterinary and Medical Sciences, (5: 30-42).

Khosravi AR, Dakhili M, Shokri H. 2008. "A mycological survey on feed ingredients and mixed animal feeds in Ghom province, Iran," *Pakistan Journal of Nutrition*, (vol. 7, no. 1, pp. 31–34). View at: Publisher Site | Google Scholar

Koburger JA. 1970. Fungi in foods. 1-Effect of inhibitor and incubation temperature on enumeration. J. Milk and food Technol., 33(10): 433-434).

Mabbett T. 2004. "Keep feeds free from fungi," *African Farming*, (pp. 15–16.) View at: Google Scholar

Marín S, Ramos AJ, Sanchis V. 2012. Modeling *Aspergillus flavus* growth and aflatoxins production in pistachio nuts. Food Microbiol. (32): 378–388.

Nelson P, Toussoun T, Marasas W. 1983. *Fusarium Species: An Illustrated Manual for Identification*, Pennsylvania State University Press, University Park, Pa, USA.

Nooh A, Amra H, Youssef MM, El-Banna AA. 2014. Mycotoxin and toxigenic fungi occurrence in Egyptian maize. International Journal of Advanced Research, (Volume 2, Issue (2): 521-532).

Petzinger E, Weindenbach A. 2002. Mycotoxins in the food chain: the role of ochratoxins. Livestock Production Science, (76): 245-350.

Pitt JI, Hocking AD. 2009. *Fungi Food Spoilage*. 3rd Ed. published by Springer, Dordrecht Heidelberg, London, New York.

Reverberi M, Punelli F, Scarpari M, Camera E, Zjalic S, Ricelli A. 2010. Lipoperoxidation affects ochratoxin A biosynthesis i*n Aspergillus ochraceus* and its interaction with wheat seeds. Applied Microbiology and Biotechnology, (85): 1935-1946.

Richard J. 2000. Sampling and sample preparation for mycotoxin analysis. Romer Labs Guide to Mycotoxins, 2. Romer Labs Inc., 1301 Stylemaster Drive, Union, MO 63084-1156, USA (76-85).

Romani S, Sacchetti G, Chaves Lo´pez C, Pinnavaia G, Dalla Rosa M. 2000. Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. Journal of Agriculture and Food Chemistry, (48) : 3616-3619.

Sabri MA, Siddique M, Khan MZ, Samad HA. 1989. Prevalence and pathology of mycotoxicosis in young broiler chicks in and around Faisalabad. Pakistan Veterinary Journal, (9:106-108).

Saleemullah IA, Khalil IA, Shah HU. 2006. Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chemistry, (98): 699-703.

Saleemi MK, Khan MZ, Khan A, Javed I. 2010. "Mycoflora of poultry feeds and mycotoxins producing potential of Aspergillus species," *Pakistan Journal of Botany*, (vol. 42, no. 1, pp. 427–434) View at: Google Scholar.

Samson RA, Hoekstra ES, Frisvad JC. 2004. *Introduction to Food and Airborne Fungi*, Centraalbureau voor Schimmelcultures, Utrech, The Netherlands, 7th edition.

Schiavone A, Cristina C, Luigi G, Luisa P, Sara A , Laura C 2008. A survey on the occurrence of ochratoxin A in feeds and sera collected in conventional and organic poultry farms in Northern Italy. Italian Journal of Animal Sciences, (7) : 495-503.

Scott PM, Trucksess MW. 1997. "Application of immunoaffinity columns to mycotoxin analysis," *Journal of AOAC International*, (vol. 80, no. 5, pp. 941–949).

Simmons E 2009. *Alternaria: An Identification Manual*, American Society of Microbiology, Washington, DC, USA, 1st edition.

Shotwell OL, Hesseltine CW, Stubblefield RD, Sarsenoson WG. 1966. Production of aflatoxin on rice. Appl. Microbiol., (14) :425-428.

World Health Organization WHO 1975. "Technical Rep6rt series. "Recommended health based limits in occupational exposure to aflatoxins."Report of a WHO study Group. Technical Report Series No. 643. World Health Organization Geneva.