Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis

A. M. Shareef

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

(Received June 19, 2008; Accepted July 13, 2009)

Abstract

Forty five finished poultry feed samples, collected from different broilers, broiler breeders and layers farms were divided into two parts, for mycological and mycotoxins examination**.** In counting of molds**,** dilute plate technique was used, whereas feed parts were used for mycotoxin estimation, they were subjected to four standard kits of Aflatoxin, Ochratoxin, T-2 toxin and Fumonisins. Mold counts were around 10^5 cfu.g^{-1} sample. Fourteen mold genera were recovered. From the systematic point of view, 2 genera belonged to *Zygomycetes* (i.e. *Mucor, Rhizopus*,), 1 genus belong to *Ascomycetes* (i.e. *Eurotium*); the majority, within so-called mitotic fungi (formerly *Deuteromycetes*), encompassed 11 genera (i.e. *Acremonium, Alternaria, Aspergillus, Fusarium, Paecilomyces, Penicillium, Scopulariopsis,, Trichothecium, Ulocladium and Aerobasidium*). The most frequent fungi were those from the genus *Aspergillus*. The concentrations of the four analyzed mycotoxins in the poultry finished feeds, and the percentages of the recovered mycotoxins, revealed that aflatoxins was recovered in 91.1% of the examined samples, with a mean value of 179.1µg/kg. The same percentage was found with Ochratoxins, but with lower mean concentration of 159.4µg/kg. In the third order were Fumonisins mycotoxins were in the third order, and they were recovered in 51.1% of the tested samples with a mean value of 127µg/kg. In the fourth order was T-2 toxin, with a percentage of 2.2% and a value of 50.0µg/kg.

Key words: Molds, Mycotoxins, Mycotoxicosis, Poultry feed.
Available online at http://www.vetmedmosul.org/ijvs

الاعفان وسمومها في الاعلاف الناهية لحقول الدواجن ذات التسمم الفطري المحتمل

عقيل محمد شريف

فرع الصحة العامة البيطرية كلية الطب البيطري جامعة الموصل الموصل العراق

الخلاصة

تم تحليل خمس وأربعون نموذج عليقه جمعت من حقول دواجن فروج اللحم وحقول أمهات فروج اللحم وحقول الدجاج البياض**.** قسّمت النماذج إلى جزأين، احدهما لفحص الاعفان، والآخر للكشفِ عن سموم الاعفان. في عد الاعفان استخدمت طريقة تخفيف الإطباق، بينما عومل الجزء الثاني من العينات مع أربعة عُدَدِ قياسيةِ لسموم الافلا والاوكرا وسم ت-٢ وسم الفومونيسين. تَراوح العد الكلي للاعفان مِنْ ٠,١ × ١٠^{١} إلى ٦,٥ x ١٠^{٦} لكل غم عليقة، بمعدلِ ٧,٢ x ١٠^{٥} لكل غم عليقة. أربعة عشرَ جنس للاعفان سجل في هذه الدراسةِ. يَعُودُ نوعان منها إلى Zygomycetes (Mucor, Rhizopus) و جنسُ واحد إلى Ascomycetes (Eurotium) الأغلبية، ضمن ما تسمّى بفطر mitotic سابقا ً(Deuteromycetes)، والذي شمل ١١ نوع (Acremonium)، Alternaria، Aspergillus، Fusarium Paecilomyces، Penicillium Ulocladium, Trichoderm و Scopulariopsis, Aerobasidium. العفن الأكثر تكراراً كَان مِنْ جنسAspergillus. اظهرت تراكيز السموم التي تم الكشف عليها في ٤٥ عينة من اعلاف الدواجن ونسب تواجدها في هذه الاعلاف ان سموم الافلا والاوكرا وجدتا بنسبة ٩١،١ % وبتركيز ١٧٩ مايكروغرام/ كغم

لسم الافلا و١٥٩،٤ مايكروغرام /كغم لسم الاوكرا على الترتيب .سموم الفومونيسين وجدت في ٥١,١% العيناتِ المفحوصة وبمعدل ١٢٧ مايكروغرام كغم. في الترتيب الرابع كَانَ لسم ت-٢ بنسبة مئوية ٢,٢% وبمعدل ٥٠ مايكروغرام/ كغم.

Introduction

Mold occurrence and growth on poultry feeds is one of the major threats to poultry economic and health. Besides their negative impacts on nutritional and organoleptic properties, moulds can also synthesize different mycotoxins. More than 100.000 fungal species are considered as natural contaminants of agricultural and food products. However, due to genetical and ecological factors, relatively few can actually generate mycotoxins (1). According to Leibetseder (2), 30 to 40 % of existing moulds can elaborate toxic substances under favorable conditions. The majority of the toxic species belong to the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* (3). The effects of mycotoxins on higher animals include hepatotoxicity, nephrotoxicity, immunotoxicity, oncogenesis and genotoxicity (4-6). Despite the great attention that has been paid to the study of toxigenic moulds and their mycotoxins in various foods and feeds, little is known about fungal and mycotoxin contamination of poultry feed in Mosul governorate yet. During the last two or three decades, the production of mixed poultry feeds were significantly increased in parallel with the evolution of poultry industry in the country. Furthermore, it is well established that contamination of poultry feeds with mycotoxins may induce sanitary disturbances and mortality among the birds and secondary contamination of the human consumer via eggs, poultry meat and giblets (7,8). Variety of complex and divers clinical signs of potential mycotoxicosis were always observed in different broiler, broiler breeder and layer farms with potential mycotoxicosis. Affected flocks showed one or more of the following symptoms; decreased weight gain; anorexia; reduced feed conversion efficiency; decreased egg production; poor egg shell quality ;increased egg blood spots; spiking mortalities; immunosuppression and failure of vaccination programs; increased susceptibility to diseases especially E-coli infection; reduced fertility and hatchability; visceral hemorrhages; leg weakness and high percentages of leg deformities; pale bird syndrome; fatty liver with pale, muddy to yellowish discoloration; increased bruising; enlarged pale kidneys; wet litter; urate deposition in the body cavities; increased incidence of viral diseases like Newcastle disease, infectious bursal disease and inclusion body hepatitis; oral lesions; tibial dyschondroplasia; gizzard erosions; paralysis; extension of leg and neck.

The purpose in the present work was to initiate a study on the toxigenic mycoflora of poultry feed in Ninevah governorate/ Iraq that It includes: enumeration and identification of moulds genera naturally contaminating different kinds of poultry feeds and detection and quantification of the 4 major mycotoxins in the feed samples, aflatoxin, ochratoxin A, fumonisins and T-2 toxin.

Materials and methods

Samples

The samples of finished poultry feed were delivered to the College of Veterinary Medicine, University of Mosul from different broilers, broiler breeders and layers farms. These farms were located in Nineveh governorate and were claimed from potential mycotoxicosis, feed samples were collected during a 2-years-long period from Apr 2005 to Sep 2007. Random 10 to 30 representative samples of I kg were collected from several locations within a batch of feed and combined thoroughly to provide a composite sample of 1 Kg for submission. They were then divided to two parts, one for mycological examination, and the other for mycotoxins detection. Feed samples intended for mycological examination were usually analyzed immediately upon arrival or, if necessary, they were stored for 2-3 days in paper bags at room temperature (22-25ºC). The other parts of feed samples intended for mycotoxins analysis were stored at -20C^{o}.

Isolation of poultry feed fungi

Dilute plate technique was used for isolation of fungi from the samples (9). General molds count was carried out by weighing 20 g of the poultry mixed feed samples and their mixing with 180 ml of saline solution (0.85% sodium chloride) with 0.05% Tween 80 on a horizontal shaker for ca. 30 minutes. Then, 0.1 ml of appropriate dilutions made up to 10^{-5} was applied on Dichloran Rose Bengal Chloramphenicol agar (DRBC (10, 11). Plates were incubated at 25^{o}C for 5-7 days. The mold genera were identified according to (11-15).

Mycotoxins analyses

Feed parts intended for mycotoxin estimation were subjected to four standard kits for aflatoxin, ochratoxin, T-2 toxin and fumonisins using Neogen mycotoxin extraction kits (Neogen corporation) as follows:

Samples preparation and extraction: Twenty five gram-samples were collected for analysis. These samples were finely ground,so that at least 75% of them pass through a 20 mesh. Five gram samples were blended with 25 ml of 70%

v/v methanol/ water solution for 3 minutes. Extracts were filtered through a Whattman No.1 filter paper.

Test procedure: All Neogen extraction reagents were allowed to warm at room temperature (18-30 ºC) before use. Red marked mixing wells were prepared, one for each sample plus four red wells for controls. All red- marked wells prepared were placed in the well holder. An equal number of antibody coated (AB) white wells to those red-marked wells were also prepared. Hundred µl of conjugate were transferred to each red-marked mixing well. To those red wells containing the conjugate another (with new pipette tips) 100 µl of controls and samples were added by using a 12-cannel pipettor liquid in wells were mixed and pipetting it up and down for 3 minutes. After mixing 100 µl of the (conjugate + samples, or conjugate + controls) were transferred to AB – coated wells. These wells were moved back and froth for well mixing the contents in each well for 10-20 seconds without splashing reagents from the wells. Antibody-coated wells were then incubated at room temperature (18-30 ºC). The contents in AB- coated wells were shacked out, by filling the wells with deionized water and dumping them out. This step was repeated 5 times. Turning the wells upside down and tamping them out on a paper was carried out until the remaining water has been removed. Substrate was then added to AB- coated wells, by using the 12-channel pipettor through pipetting 100 µl of substrate to these wells. Mixing was done by sliding the well holder back and froth for 10-20 seconds, followed by incubation for 3 minutes. Stop solution was poured to these wells (100 µl) to each, mixing was done by sliding well holder back and froth on a flat surface. Within 20 minutes after the addition of stop solution. Results were read, using a micro well reader (ELx00) with a 650 nm filter. More blue color means less toxins. Results of the yield optical densities of the controls and samples were obtained by using computerized Neogen Verotex Softwere Program version 2.9.16 (Neogen Corporation**).**

Results

The total number and percentages of enumerated mold genera in Nineveh finished poultry feed are presented in Table 1, and the ability to produce appropriate mycotoxins is shown in Table 2. Total fungal counts were ranged from 0.1×10^1 to 6.5×10^6 cfu.g-1 of feed sample, with an average 7.2×10^5 cfu.g-1 sample (Table 1. Fourteen mold genera were recovered during this study.

From the systematic point of view, 2 genera belong to *Zygomycetes* (i.e. *Mucor, Rhizopus*,), 1 genus belong to *Ascomycetes* (i.e. *Eurotium*); the majority, within so-called mitotic fungi (formerly *Deuteromycetes*), encompassed 11 genera (i.e. *Acremonium, Alternaria, Aspergillus, Fusarium,, Paecilomyces, Penicillium, Scopulariopsis,, Trichothecium, Ulocladium and Aerobasidium*). The most frequent fungi were those from the genus *Aspergillus*, recovered from 40 samples (88%) with a range of 0.1×10^5 - 5.3×10^6 and a mean value of 2.6×10^6. In the second order were the molds from the genus *Penicillium and Mucor,* both recovered from 28 samples (64%) with a range of 0.2×10^5 - 4.4×10^6 and 3×10^4 - 2.6×10^5 and a mean of 2.2×10^6 and 1.4×10^5 respectively. To the third and fourth orders were the molds from the genera *Rhizopus*, and *Scopulariopsis*, recovered from 23 and 22 samples (50% and 48%) respectively with a range of 1.5×10^4- 2.1×10^5 and 0.2×10^4- 1.8×10^5, and a mean of 1.1×10^5 and 9.1×10^5 respectively. The following recovered genera were the molds of *Alternaria* and *Eurotium*, from 20 and 19 samples with percentages of 45% and 42% and a range of 8×10^3 - $1.8 \times 10^5$3 and $\times 10^3$ - 1.7×10^5, with a mean of 9.4×10^4 and 8.5×10^4 respectively. In the descending pattern occurred the molds of the genera *Cladosporium* and *Fusarium*, recovered from 17 and 14 samples (37% and 31%), representing a range between0.2×10^3- 9.8×10^4 and 0.4×10^3 - 9.2×10^4, and a mean of 4.9×10^4 and 4.6×10^4 respectively. From less than 10 samples recovered the genera of the mitotic molds from *Acremonium,, Paecilomyces, Ulocladium, Aureobasidium*, and *Trichothecium,* with 11%,7%,7%,2% and 2% respectively, with an average of 4300,110,70,50 and 25 CFU g^{-} 1respectively.

Table 2 illustrate the frequency of recovered mold genera out of 45 finished poultry feeds tested through the study period, of these 41 samples were positives for aflatoxins and ochratoxins (91.1%).To a lower extent were the positive samples of Fumonisins (51.1%). The lowest positive sample was that of T-2 Toxin (2.2%). The mycotoxins concentrations were in general positive proportion to the number of respective molds producing them, being highest in aflatoxins and ochratoxins. These mycotoxins are produced mainly by aspergillus and penicillium mold genera, the most prominanat mold enumerated in this study. In a descenging manner were also the average levels of aflatoxins, ochratoxin A, fumonisins and T-2 toxin. The highest levels of these mycotoxins were as follows; aflatoxins: < 475 µg/kg ; ochratoxin A: < 460 µg/kg ; fumonisins: < 350 µg/kg and T-2 toxin: 50 µg/kg.

Table 3 shows the distribution concentration of different mycotoxins in the tested finished poultry feeds. Only aflatoxins and ochratoxin A were ranged from 0-500 µg/kg. The highest number of samples contaminated with aflatoxin were within levels of 200-300 µg/kg. Although ochratoxin level was also ranged from 0-500 µg/kg, but the highest levels were occurred in concentrations of 50 and 250 µg/kg. Fumonisins highest levels were occurred in concentrations between 50-150 µg/kg, and the highest levels did not exceeds 350 µg/kg. The only one sample contaminated with T-2 toxin had level of 50 µg/kg.

Table 1: Frequency and average count of recovered mold genera from 45 Nineveh poultry finished feed samples.

Molds	Frequency of Positive samples	Percentage of mold genera frequencies	Range	Average counts cfu g^{-1}
Aspergillus spp.	40	88.8	0.1×10^5 - 5.3×10^6	2.6×10^6
Penicillium spp.	28	62.2	0.2×10^5 - 4.4×10^6	2.2×10^6
Mucor spp.	29	62.2	3×10^4 - 2.6×10^5	1.4×10^5
Rhizopus spp.	23	51.1	1.5×10^4 - 2.1×10^5	1.1×10^5
Scopulariopsis spp.	22	48.8	0.2×10^4 - 1.8×10^5	9.1×10^5
Alternaria spp.	20	44.4	8×10^3 - 1.8×10^5	9.4×10^4
Eurotium spp.	19	42.2	3×10^3 - 1.7×10^5	8.5×10^4
Cladosporium spp.	17	37.7	0.2×10^3 - 9.8×10^4	4.9×10^4
Fusarium spp.	14	33.3	0.4×10^3 - 9.2×10^4	4.6×10^4
Acremonium spp.	5	11.1	0.6×10^2 - 8.7×10^3	4.3×10^3
Paecilomyces spp.	3	6.6	1×10^1 - 2.1×10^2	1.1×10^2
Ulocladium spp.	3	6.6	2×10^1 - 1.2×10^2	7×10^1
Trichothecium spp.	1	2.2	0.2×10^1 - 0.8×10^1	5×10^1
Aureobasidium spp.	1	2.2	0.2×10^1 - 0.8×10^1	2.5×10^1

Table 2: Number of tested, positive, percentage and levels of detected mycotoxins in PMFS tested.

Parameters	Mycotoxins			
	Aflatoxins	Ochratoxin A	Fumonisins	T-2 Toxin
No. of samples tested	45	45	45	45
No. of positive samples	41	41	23	1
Percentage positive (%)	91.1	91.1	51.1	2.2
Average level (µg/kg)	179.1	159.4	127	50
Highest level (µg/kg)	475	460	350	50

Figure 4 shows the different AF concentrations (ppb) in positive AF-PFF samples. It is evident that 24% of the samples had AF concentration between 201-250 ppb, followed by 16.6% between 151-200ppb and 11.1% between 351-400 ppb. The remaining concentrations were recovered in less than 10% of the samples.

Figure 5 shows the different Ochratoxin concentrations (ppb) in positive Ochratoxin -PFF samples. From figure it is clear that 20% of the samples had Ochratoxin up to 50 ppb, followed by 17.7% between 201-250 ppb, 13.3% between 51-199 and 351-400, 11.1% between 101-150, while the remaining concentration were occurred in percentages less than 10%.

Figure 6 shows the different fumonisins concentrations (ppb) in positive fumonisins -PFF samples. It is evident that 48.8% were negative samples. The positive samples showed that 22% of them had fumonisins concentrations up to 50 ppb, and 13.3% between 101-150 ppb, while the concentrations up to 350 ppb had percentages of less than 10%. No more than 350 ppb of fumonisins mycotoxin was detected.

Figure 7 shows that 97.75 of the tested feed samples were negative for T-2 toxin, and only one sample with 50 ppb was detected.

Discussion

Both field and storage fungi were recovered in this study through the examination of poultry finished feed samples collected from different poultry farms with potential mycotoxicosis. These molds include members of the genera *Aspergillus, Penicillum, Fusarium, Alternaria and Cladosporium* which could contaminate many agricultural commodities used in the formulation of poultry finished feed samples like corn, wheat, soybean, barley and others commodities. These molds are of great importance because of potential mycotoxin production (16). The total fungal loads in the analyzed finished feed samples in our study were around 10^5 $cfug^{-1}$ which is higher than that reported in Slovakia of 10^3 $cfu.g^{-}1$ (17), and 10^4 $cfu.g^{-1}$ (18). Similar results were found to those reported in Turky (19), Spain (20) and from Argentina (21,22). The predominance of mold genera were to large extent resembles those found in Saudi Arabia (23), which could be due to some resemblance in the ecological and climatologically conditions. The main contaminating molds appeared to be from the genus Aspergillus with a percentage recovery of 91.11%.

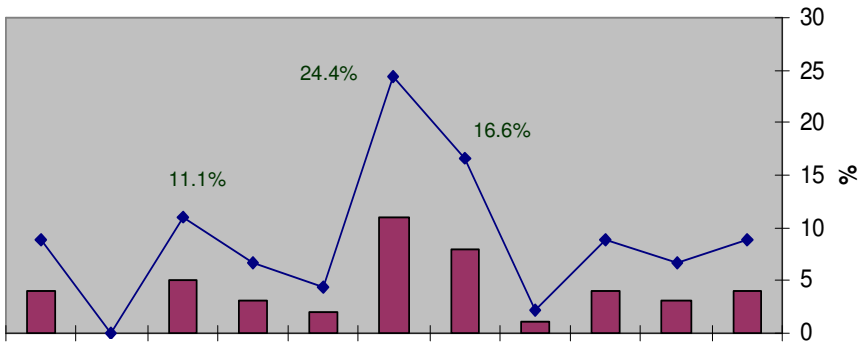
Table 3**:** Mycotoxins recovered from Nineveh poultry finished feed.

Sample No.	Aflatoxins ppb	Ochratoxins ppb	Fumonisins ppm	T-2 toxin ppb
PFF 1*	57	30	ND	ND**
PFF 2	189	290	100	ND
PFF 3	254	230	ND	ND
PFF 4	370	61	ND	ND
PFF 5	69	291	ND	ND
PFF 6	165	242	200	ND
PFF 7	ND.	159	250	ND
PF F8	250	ND.	ND	ND
PF F9	350	42	ND	ND
PF F10	452	460	200	ND
PFF 11	54	91	ND	ND
PFF 12	152	420	ND	ND
PF F13	250	223	150	ND
PFF 14	340	133	150	ND
PF F15	ND.	173	30	ND
PF F16	458	ND.	ND	ND
PFF 17	146	228	ND	ND
PFF 18	ND	191	150	ND
PF F19	240	ND.	ND	ND
PF F20	36	133	200	ND
PFF 12	362	23	350	ND
PFF 22	475	214	ND	ND
PFF 23	76	38	ND	ND
PF F24	158	28	150	ND
PF F25	370	212	30	ND
PF F26	356	273	ND	ND
PF F27	235	ND.	20	ND
PFF 28	230	19	50	ND
PF F29	235	12	130	ND
PFF 30	453	73	20	ND
PF F31	ND.	260	ND	ND
PFF 32	293	142	20	ND
PF F33	250	212	ND	ND
PF F34	323	57	ND	ND
PF F35	230	72	ND	ND
PF F36	196	12	250	ND
PF F37	226	68	ND	ND
PF F38	180	50	20	ND
PFF 39	400	252	250	ND
PFF 40	196	222	10	ND
PF F41	188	142	ND	ND
PFF 42	250	123	20	ND
PF F43	219	183	ND	ND
PF F44	90	280	150	ND
PF F45	22	320	150	50

*PFF=Poultry finished feed from farm 1 ND**=Not detect.

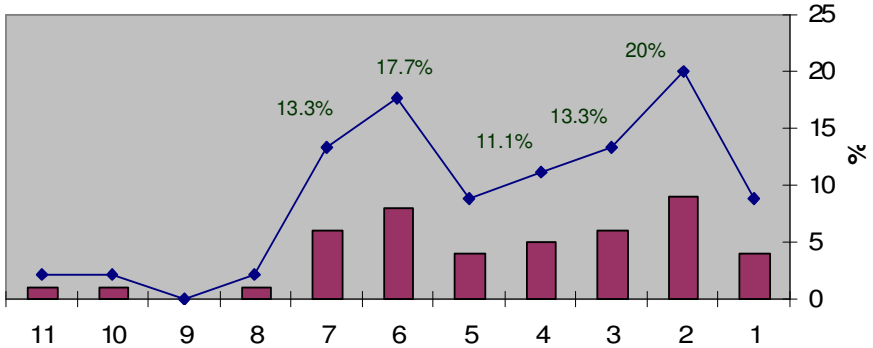
This finding in consistent with (22) and (24) and (25) who found that the most dominant species isolated of poultry feed samples belonged to the genus *Aspergillus*, but not in consistent with (17), (22) and (25), who found that the most frequent fungi were those from the genus *Penicillium*. Although molds of Aspergillus species are more often soil fungi or saprophytes but several are important because they produce mycotoxins. *Penecilli* in our results were in the second order in counting and percentages among recovered molds, as they are one of the main contaminants of stored cereals as well as feeds with worldwide occurrence (26-28) being producers of toxicologically significant mycotoxins (29-31). In order, our results were in consistent with (17) that the third mostly encountered fungi were representatives of the genus *Mucor*, who found this genus with frequency of 44%, followed by the genus *Rhizopus*. Our results also revealed that molds from the genus *Eurotium* were recovered in 19%, and *Fusarium* in 14% of the samples. These results were less than that reported by (17), who found that *Eurotium* and *Fusarium* genera were widespread through the samples they examined and were occurred with the same frequency of 42%. In this study, the mean of aflatoxins were recovered at a rate of 91.1%, with an average of 179.1µg/kg of finished poultry feed samples, confirming our previous results of aflatoxin contamination to poultry feed samples (32). Comparing with other Asian countries, our results were higher than that reported in north Asia (China, Taiwan and Korea) of 3%, and higher than south East Asia (Malaysia, Philippines, Thailand and Vietnam) of 36%, and higher than south Asia (India, Pakistan and Bangladesh) of 53% (33). The second mycotoxin recovered was Ochratoxin (91.1%), in an average of 159.4 µg/kg, confirming our previous results of ochratoxin contamination of poultry finished feeds (34). Our results were also more than those reported in north Asia (China, Taiwan and Korea) of 15%, and higher than south East Asia (Malaysia, Philippines, Thailand and Vietnam) of 9%, and higher than south Asia (India, Pakistan and Bangladesh) of 50% (33). Again our high results here were due to sampling feeds from mycotoxicosis suffering poultry farms. Ochratoxin has been implicated in significant field outbreaks of mycotoxicosis in poultry (35). Fumonisins mycotoxins were the third type of mycotoxins recovered in this study, in a percentage of 53.3% and an average of 127µg/kg of finished poultry feed samples. These mycotoxins recovered here in Mosul governorate in corn samples intended for use in finished poultry feeds (36). Our results agreed with the percentages reported in north Asia (China, Taiwan and Korea) of 52%, and with those of south East Asia (Malaysia, Philippines, Thailand and Vietnam) of 52%, but higher than hose reported in south Asia (India, Pakistan and Bangladesh) of 32% (33). The fourth mycotoxin recovered in this study was T-2 toxin in a percentage of 2% at a rate of 50 µg/kg. This toxin was

Figure 4:Distribution of different AF concentrations(ppb) in positive AF- PFF samples



1=ND 2=0-50ppb 3=51-100ppb 4=101-150ppb 5=151-200ppb 6=201-250ppb
7=351-300ppb 8=301-350ppb 9=351-400ppb 10=401-450 ppb 11=451-500ppb

Figure5: Distribution of different Ochratoxin concentrations (ppb) in positive Ochratoxin-PFF samples



1=ND 2=0-50ppb 3=51-100ppb 4=101-150ppb 5=151=200ppb 6=201-250ppb
7=351-300ppb 8=301-350ppb 9=351-400ppb 10=401-450 ppb 11=451-500ppb

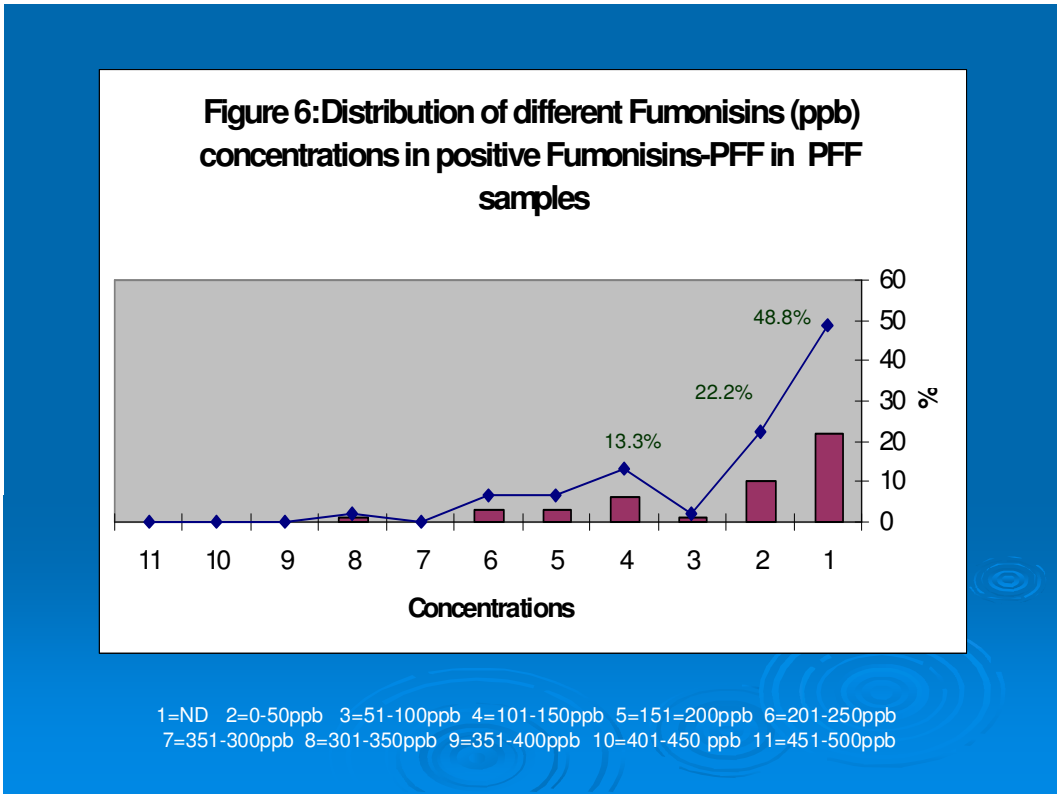


Figure 6:Distribution of different Fumonisins (ppb) concentrations in positive Fumonisins-PFF in PFF samples
60
50
40
30
20
10
0
%
48.8%
22.2%
13.3%
11 10 9 8 7 6 5 4 3 2 1
Concentrations
1=ND 2=0-50ppb 3=51-100ppb 4=101-150ppb 5=151=200ppb 6=201-250ppb
7=351-300ppb 8=301-350ppb 9=351-400ppb 10=401-450 ppb 11=451-500ppb

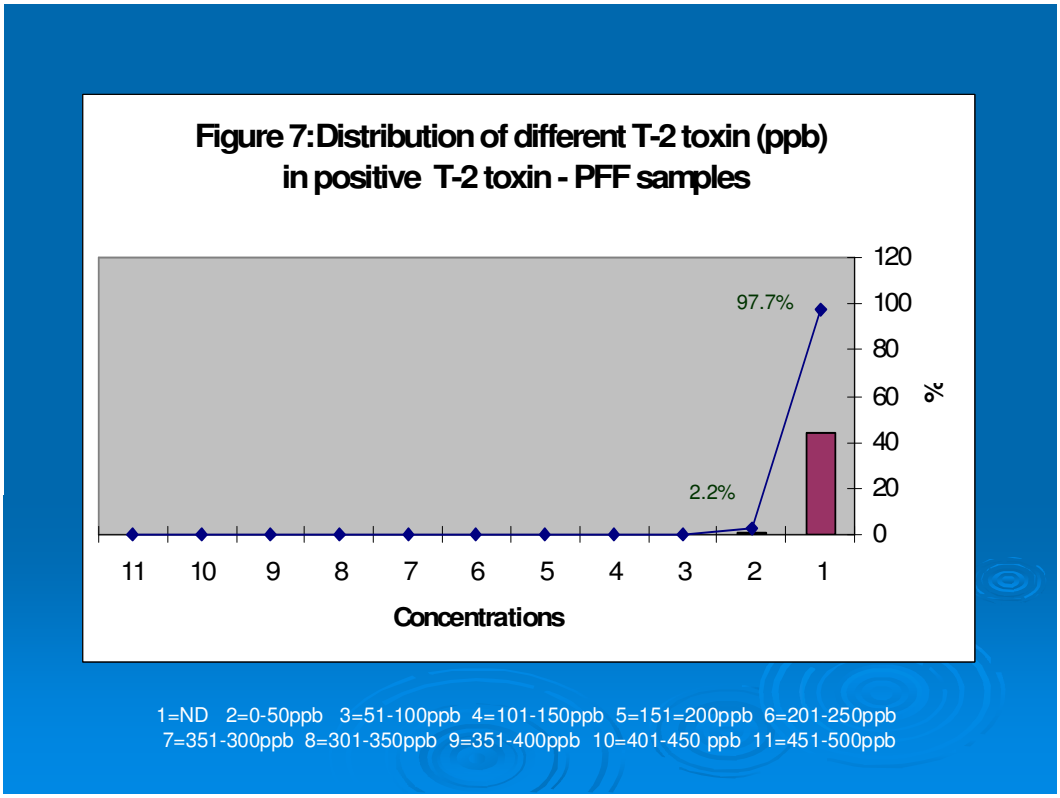


Figure 7:Distribution of different T-2 toxin (ppb) in positive T-2 toxin - PFF samples
120
100
80
60
40
20
0
%
97.7%
2.2%
11 10 9 8 7 6 5 4 3 2 1
Concentrations
1=ND 2=0-50ppb 3=51-100ppb 4=101-150ppb 5=151=200ppb 6=201-250ppb
7=351-300ppb 8=301-350ppb 9=351-400ppb 10=401-450 ppb 11=451-500ppb

also recovered by us through the study performed during 2004-2005 in Mosul governorate (37). Our results were close to those reported in north Asia (China, Taiwan and Korea) of 0%, and those found in south East Asia (Malaysia, Philippines, Thailand and Vietnam) of 1%. In general our results were agreed with those reported by Pacin et al., (38) who found that the most recovered mycotoxins from feed associated fungi were ochratoxin A, aflatoxin and fumonisin. It may be stated that *Aspergillus* (including *Eurotium*), *Penicillium* and *Fusarium* are the typical fungal genera inhabiting poultry feed mixtures. In fact, they are very important contaminants being renowned for their ability to form a huge number of various types of toxic extrolites-mycotoxins (39,40), and that the outcomes of this study clearly show that finished poultry feeds in Nineveh governorate represent a rich source of significant mycotoxin producers, especially those from the *Penicillium*, *Fusarium* and *Aspergillus* genera. It is possible that these co-contaminant of estimated mycotoxins in our study, of two carcinogenic mycotoxins, aflatoxin and fumonisins, and two cancer promoting mycotoxins, ochratoxin and T-2 toxin, could exert great negative effects on farms health and productivity than do each of them singly and of public health concern to the health of consumer through the food chain by ingestion residual levels of these toxins in poultry meat and poultry products (meat and eggs) (41,42). These toxic substances are known to be either carcinogenic (e.g. aflatoxin B1, fumonisin B1, Ochratoxin A), neurotoxic (fumonisin B1), nephrotoxic (ochratoxin A), dermatotoxic (trichothecenes), or immunosuppressive (aflatoxin B1, ochratoxinA and T-2 toxin) (33). the senerginestic effects of these mycotoxins on poultry productivity and health were well documented.(43-45).

Acknowledgements

The author thanks the College of Veterinary Medicine, University of Mosul for providing all supports for this experiment.

References

1. Jemmali M. Les moisissures et leurs toxines. La Recherche, 1979;10: 124-131.
2. Leibetseder J. Die bedeutung der Mykotoxine für Mensch und Tier. Ernähr Nut.1989;13:739.
3. Kaushal KS, Sinha S.P. Mycotoxins. Asean Food J. 1993;8:87-93.
4. Dierheimer G. Recent advances in the genotoxicity of mycotoxins. Rev Méd Vét. 1998;149:585-590.
5. Gabal MA, Azzam AH. Interaction of aflatoxin in the feed and immunization against selected infectious diseases in poultry. II. Effect on one day old layer chicks simultaneously vaccinated against Newcastle diseases infectious bronchitis and infectious bursal disease. Av Pathol. 1998;27:290-295
6. Kurata A, Ueno Y. Toxigenic fungi: Their toxins and health hazard. Odansha-Elseviers Tokyo-Amsterdam-Oxford-NewYork; 1984. 247 p.
7. Pennington LJ. Mycotoxin: thin layer chromatography and densitometric determination of aflatoxins in mixed feeds containing citrus pulp. JAOAC. 1986;69:690-696.
8. Naoom RAF. Estimation of aflatoxin residues for some ruminant and poultry (Local and imported) livers in Mosul. (masters thesis) Mosul; 2007. 94 p.
9. Edmund M., Roger L. and Yoshito M.: Microbiology of processed spices. J Milk Food Technol. 1975;38:683-687.
10. Pitt JI, Hocking AD. Fungi and food spoilage. Sydney: Academic press; 1985. 185p.
11. Pitt JI. *Penicillium viridicatum, Penicillium verrucosum* and production of ochratoxin A. Appl Environm Microbiol. 1987;53:266-269.
12. Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces.* Academic Press. London; 1979. 206p.
13. Pitt JI. Laboratory guide to common *Penicillium species.* Academic Press London, 1988. 87p.
14. Raper K, Fennel DI. The genus *Aspergillus*. The Williams and Wilkins co, Baltimore, U.S.A. 1965. 306p.
15. Domsch KH, Gams W. Compendium of soil fungi. Academic Press. London, New York, Toronto, Sydney, San Francisco; 1980. 566p.
16. David MW, Wellington M, Zeljko J. Biology and ecology of mycotoxigenic Aspergillus species as related to economic and health concerns, In: Mycotoxins and food safety, DeVries W, Truksess W, Jackson LS. Advances in experimental medicine and biology. Kluwer Academic/Plenum Publishers.USA.2002;504:3-17.
17. Labuda R, Tančinová D. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. Ann Agric Environ Med. 2006;13: 193–200
18. Magnoli CE, Saenz MA, Chiacchiera SM, Dalcero AM. Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains isolated from poultry feeds in Argentina. 1999;145: 35-41
19. Heperkan D, Alperden I. Mycological survey of chicken feed and some feed ingredients in Turkey. J Food Protect. 1988;51:807-810.
20. Bragulat MR, Abarca ML, Castella G, Cabanes FJ. A mycological survey on mixed poultry feeds and mixed rabbit feeds. J Sci Food Agric. 1995;67:215-220.
21. Dalcero A, Magnoli C, Chiacchiera S, Palacios G, Reynoso M. Mycoflora and incidence of aflatoxin B1, zearlaenone and deoxynivalenol in poultry feeds in Argentina. Mycopathologia. 1997; 137:179-184.
22. Dalcero A, Magnoli C, Luna M, Ancasi G, Reynoso MM, Chiachiera S, Miazzo R, Palacio G. Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia. 1998;141: 37-43.
23. Abdul Wahab R. Hashem. Seed-borne fungi in domestic bird feed in Saudi Arabia. 1996;37:223-226.
24. Pacin AM, González HHL, Etcheverry M, Resnik SL, Vivas L, Espin S. Fungi associated with food and feed commodities from Ecuador. 2003;156:87-92.
25. Glenda RO, Jessika MR, Marcelo EF, Lilia RC, Gloria MD, Kelly MK, Ana MD, Carlos AR. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. 2006;162:355-362.
26. Filtenborg O, Frisvad JC, Samson RA. Specific association of fungi to foods and influence of physical environmental factors. In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (Eds): Introduction to Food- and Airborne Fungi,. Centraalbureau voor Schimmecultures, Utrecht 2002. 306-320p.
27. Leistner L. Toxinogenic penicillia occurring in feeds and foods. A review. Food Technology in Australia. 1984;36:404-413.
28. Pitt JI. Biology and ecology of toxigenic *Penicillium* species. In: Trucksess *et al.* (Eds): Mycotoxins and Food Safety,. Kluwer Academic Plenum Publishers, London 2002. 29-41p.

29. Frisvad JC, Filtenborg O: Terverticillate *Penicillia*. Chemotaxonomy and Mycotoxin Production. Mycologia. 1989;81:837-861.
30. Frisvad JC, Smedsgaard J, Larsen TO, Samson RA. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud Mycol. 2004;49:201-242.
31. Frisvad JC, Thrane U. Mycotoxin production by common filamentous fungi. In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (Eds). Introduction to Food- and Airborne Fungi,. Centraalbureau voor Schimmecultures, Utrecht 2002. 321-330p.
32. C.A.S.T. Mycotoxins:Economic and health risk.Task force report No. 116. Concil for Agricultural Science and Technology. Ames. IA. USA. 1989. 1-91p.
33. Lee-Jiuan C, Li-Mien T. High occurrence of mycotoxins in Asian feedstuffs. World poultry. 2006;10,4:13-16.
34. Shareef AM. Natural contamination of some broilers feed commodities with ochratoxin, Iraqi J Vet Sci. 2006;20:9-17.
35. Leeson S, Diaz G, Summers JD. Poultry metabolic disorders and mycotoxins. University books. P.O. Box 1326. Canada.1995. 277-248p.
36. Shareef AM. Fumonisns mycotoxin contamination level of corn meal used for poultry feeding. Accepted in Raf J Sci. 2008: Under publication
37. Shareef AM. Natural occurrence of T-2 toxin in broiler's feed commodities determined with Elisa. Iraqi J Vet Sci. 2007;21:167-173.
38. Pacin AM, González HHL, Etcheverry M, Resnik SL, Vivas L, Espin S. Fungi associated with food and feed commodities from Ecuador. 2003;156:87-92.
39. Samson RA. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. Stud Mycol. 2004;49:1-173.
40. Frisvad JC, Thrane U. Mycotoxin production by common filamentous fungi. In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (Eds): Introduction to Food- and Airborne Fungi,. Central bureau voor Schimme cultures, Utrecht. 2002. 321-330p.
41. Hiba SA. Estimation of aflatoxin levels for some raw milk types and cheeses (Local & imported) in Mosul city. (Masters thesis) Mosul. Iraq. 2007.115 p.
42. Whitlow L. Mycotoxins are a bombshell in distiller's grains. World Poultry. 2007;23:13
43. Huff WE, Harvey RB, Kubena LF, Rottinghaus GE. Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. Poult Sci. 1988; 67:1418–1423.
44. Kubena LF, Harvey RB, Buckley SA, Edrington TS, Rottinghaus GE. Individual and combined effects of moniliformin present in Fusarium fujikuroi culture materialand aflatoxin in broiler chicks. Poult Sci. 1997;76:265–270.
45. Harvey RB, Kubena LF, Rottinghaus GE, Turk JR, Harvey HH, Kubena LF. Fujikuroi culture material and deoxynivalenol from naturally contaminated wheat incorporated into diets of broiler chicks. Poult Sci. 2004;41:957–963.