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Prevalance of Mycotoxins in Poultry Finished Feed

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Abstract : Samples of poultry finished feed (n = 865) received from different parts of country over a period of two years and seven months at Romer Labs Pakistan. The samples were analyzed by TLC at Romer labs, Pakistan and by HPLC at Romer Labs Singapore and Austria. Toxin analyses included aflatoxin B1 (AfB1), zearalenone (ZON), deoxynivalenol (DON), 3 acetyl-deoxynivelenol (3ac-DON), 15 acetyl-deoxynivelenol (15ac-DON), nivalenol (NIV), fusarenon-x (Fus-x), T-2 toxin (T-2), HT-2 toxin (HT-2) diacetoscirpenol (DAS), neosolaniol (NEOS) and ochratoxin A (OTA). Aflatoxin B1 was the major contaminant in feed (84 %) followed by OTA (51 %), zearalenone (49.33 %), DON (38 %), T-2 (34.65 %), 3ac-DON (19.41 %) and 15ac-DON (11.94 %). Mean values with standard deviation for AfB1, OTA, ZON, DON, T-2 toxin, 3ac-DON and 15ac-DON were 13 ± 16.80 μg/kg, 10 ± 19.63 μg/kg, 213.58 ± 440 μg/kg 456 ± 1122 μg/kg 442.56 ± 1191 μg/kg, 41 ± 102 μg/kg and 38.92 ± 149.58 μg/kg respectively. All samples were negative for HT-2 toxin, DAS, Neosolaniol, Nivalenol, and Fusarenon-x. This first reported natural occurrence of a range of mycotoxins in Pakistan poultry finished feedstuff shows that AfB1, OTA, ZON, T-2 toxin, DON, 3ac-DON and 15ac-DON may be present at levels which may affect poultry production.

Key words : Mycotoxins, Natural occurrence, Poultry feed

Introduction

Mycotoxins are secondary metabolites of 6900 fungal species, 5% of world's total fungal moulds formed under certain environmental conditions (Hawkworth, 1991). Negative effects in poultry production are caused mainly by aflatoxins, zearalenone, trichothecenes and ochratoxins. These effects of mycotoxins on poultry are dependent upon the age, sex, physiological state and nutritional status of the animals at the time of exposure. Since the mold growth at various stages within the feed production and distribution system can magnify mycotoxin problem, hence it is difficult to diagnose in the field situation (M. Cortyl and D. Heidler, 2002). Table 1. Apart from this, mycotoxins are formal under special climatic conditions on the growing field plant, while others are produced in cases of high humidity due to inadequate storage conditions. Mycotoxins are being frequently associated with serious diseases of poultry, livestock and man. Once produced, it is very difficult to get rid of or even to reduce the contamination because toxins have a high physical and chemical stability.

Feed is the major expanse in poultry production amounting to about 60-70% of the total cost. Mycotoxins cause great economic loss by damaging 25 % of world's crop (Task Force Report, 2003). Generally, poultry feed contains 40-60% grains mainly maize, rice and wheat. While in Pakistan, poultry feed is almost dependent upon agricultural by-products. Usually, and especially in developing countries, (like Pakistan) the best quality grains/cereals are exported or reserved for human consumption and the crops with poorer quality are consumed for the animal feed. Human population explosion is another factor for non-availability of acceptable quality grains for poultry feed. (Anjum and Naseem, 2000). In addition, inadequate storage facilities, humid environment and elevated temperature particularly from May to November are conducive for fungal growth, which produces mycotoxins like of *Aspergillus* species in local conditions. The low temperature in winter season is favorable for certain mycotoxins like Ochratoxin A, trichothecenes and Zearalenone/*Fusarium* species.

In Pakistan the mycotoxin analysis was just limited to aflatoxins and is documented that Pakistan's environment favors aflatoxicosis, which is quite common in commercial broiler, breeder and layer (Siddique *et al*., 1987; Bhatti, 1989). Isolated attempts have been made for Ochratoxin A. However, little information is available for the presence of rest of mycotoxins in poultry feeds and feed ingredients. It is reported that some moulds are able to produce more than one mycotoxins and some mycotoxins are produced by more than one mould species, and thus several mycotoxins are often simultaneously found in a single commodity (CAST, 2003). Co-occurrence of certain mycotoxins (like aflatoxin B1 and Ochratoxin A, aflatoxin B1 and T-2 toxin, Ochratoxin A and Citrinin etc.) exerts additive, antagonistic or synergistic effects on bird's health status (Huff *et al*., 1988). This study is presented to see the general occurrence trend of aflatoxin B1, Trichothecenes A and B, Ochratoxin A and Zearalenone in poultry finished feed in Pakistan.

Analytical methods : The flow chart summarizing the analytical procedure is given in Fig. 1.

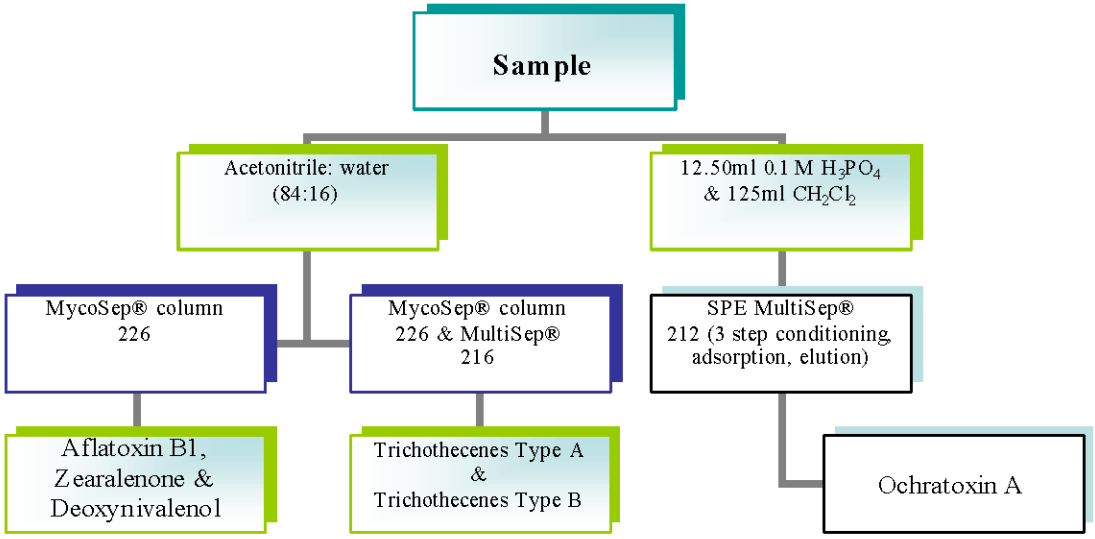

Fig. 1 : Flow chart for analytical procedure

Sample Collection: This study was conducted at Romer Labs, Pakistan. The commercial samples of poultry feed (various categories) were collected from all areas of Pakistan, over a period of December 2001 to July 2004 (two years and seven months).

Sample Preparation: The samples were ground to produce free-flowing representative sub sample material using Romer Series II Subsampling mill (Romer Labs Inc., 1301 Stylemaster Union, MO, USA). The surplus materials were stored as file samples.

Sample Extraction and Clean-up: Aflatoxin B1, Ochratoxin A, Zearalenone, Trichothecene Type A (Neosolaniol; Neos, Diacetoscirpenol; DAS, HT-2 toxin; HT-2, T-2 Toxin; T-2) and Trichothecene Type B (Nivalenol; NIV, Deoxynivalenol; DON, 3-acetyl Deoxynivalenol; 3-acetyle Deoxynivalenol, 15-acetyl Deoxynivalenol; 15ac-DON, Fusarenon-x; Fus-x) were considered for analysis by the procedures developed by Romer Labs, Inc., USA.

3-Toxin (AfB1, ZON, DON): For analysis protocol of 3-toxin test (method code: tox-tl-01-02.1, Romer Labs Inc., USA) was followed. A 25 g portion of finely ground sample in Acetonitrile: water (84:16) was blended at high speed with Osterizer blender (Osterizer Company Europe). For clean-up Romer MycoSep column #226 (Romer Labs Inc., USA) was used and the residue was evaporated by Romer Evap® System (Romer Labs Inc., USA). The re-dissolved sample was spotted against the standard solutions (3-toxin standard, 0.4μg/mlAfB1, and 20μg/ml ZON and DON concentration, Romer Labs Inc., USA). For this, Romer® Autospotter was used. Then the AfB1 of blue fluorescent (Rf value 0.5), ZON (Rf value 0.7) and DON (Rf value 0.3) were visually estimated under long wave UV light (365nm) with reference to standard spots.

Trichothecenes A and B: For analysis protocol of Type A and B Trichothecenes Dual Column Quantitative test (method code: tri-tl-01.00.2, Romer Labs Inc., USA) was followed. The initial extraction step was exactly similar to 3-toxin test. For clean-up, MycoSep® 227 columns in combinations with MultiSep® 216 column were used. For toxin estimation, standard spots (B-trich: 10μg/ml of DON, NIV and Fus-x/A-trich: 10μg/ml of T-2, Ht-2, DAS and NEOS) were spotted along with the samples and estimated under long wave UV light.

Ochratoxin A: For analysis protocol of Ochratoxin A Quantitative TLC test (Method code: och-tl-01-00.3, Romer Labs Inc., USA) were followed. For clean-up MultiSep® 212 columns were used. The toxin in the samples was quantified with reference to standard solution (1μg/ml Ochratoxin A, Romer Labs Inc., USA) under long wave UV light.

Results and Discussion

A total of 862 samples were analyzed in poultry feed for Aflatoxin B1, Zearalenone, Ochratoxin A, T-2 toxin, HT-2

toxin, Diacetoxyscirpennol, Neosolaniol, Deoxynivalenol, 3ac-DON, 15ac-DON, Nivalenol and Neosolaniol. The analyses with regards to contents of different mycotoxins have been given in Table 2 which illustrates different mycotoxin levels estimated in commercial poultry feeds.

Aflatoxin B1 (A fB1) : For AfB1 182 feed samples were analyzed. Results presented in the Table 2 reveals that 84% samples were found positive for AfB1 with minimum value of 1 ppb and maximum value of 120 ppb. Mean levels of AfB1 were below than safe levels of 20 ppb recommended by FDA. It was seen that varied levels in poultry feed was due to marked fluctuation in environmental temperature and humidity during the course of year. Presumably, the compound feed stored under appropriate conditions is subject to lesser direct influence of temperature and humidity. However, increased production of aflatoxins in feedstuffs could be expected if there is storage for a longer period under unsatisfactory ventilation and storage conditions.

Zearalenone (ZON): Zearalenone appeared to be quite common occurring at levels from low to high with reference to levels mentioned in Table 3. Results mentioned in Table 2 show that 49 % (74 out of 150) feed samples contained detectable levels of zearalenone. A wide range of 125-3600 ppb of zearalenone was found with an average of 213 $\pm$ 440 ppb. The mean value fall in the medium contamination range i.e. 50-250 ppb (FDA recommendations). Major cause for Zearalenone contamination in poultry feed is corn. However, broiler chicks and laying hens are not greatly affected by Zearalenone, even when they consume large amount of compound (Christensen *et al*. 1988).

Ochratoxin A (OTA): The analyses made in the Romer laboratory showed that 51 % samples were found positive with a mean value of 10 $\pm$ 19.63 having range from 2-75 ppb. The mean value was as safe level recommended by FDA as 10 ppb.

Trichothecenes Type A: For Trichothecenes Type A, T-2 toxin (101 samples); HT-2 toxin (23 samples); Diacetoscirpenol (26 samples) and Neosolaniol (10 samples) were analyzed. Among these toxins, only T-2 toxin was found positive (34.65 %) with a mean value of 442S $\pm$ 1191. The mean value for T-2 toxin was at high-contamination level (Table 3). However, T-2 toxin is one of the most toxic compounds in the group responsible for drastic and sudden decreases in egg production, eggs with thin shells, abnormal feathering and slow growth in chicken.

Trichothecenes Type B: Similarly, for Trichothecenes Type B, Deoxynivalenol (150 samples); 3-acetyl-Deoxynivalenol (67 samples); 15-acetyl- Deoxynivalenol (67 samples); Nivalenol (20 samples) and Fusarenon-x (28 samples) were analyzed. In Trichothecenes Type B group, Deoxynivalenol (38 %), 3-acetyl- Deoxynivalenol (13 %); 15-acetyl- Deoxynivalenol (8 %) were detected. Deoxynivalenol was appeared with medium contamination (Table 3) 456 $\pm$ 1121. While 3-acetyl- Deoxynivalenol and 15-acetyl- Deoxynivalenol occurred with below low contamination levels (Table 3). On the other hand the other two toxins of the group Nivalenol and Fusarenon-x were not detected.

Table 1: Clinical signs of mycotoxins on poultry (layers / broilers)

Mycotoxins	Effects
Aflatoxins	Hepatotoxic (fatty liver), ataxia (nervous syndrome), a cause for vaccine failure, Immunosu ppression (susceptibility for bacterial infection e.g. Salmonella, various viruses and other infectious agents commonly found around the farm yard), kidney disorder, outbreaks of coccidiosis, feedlot or poultry house that normal healthy animals ward off, decreased blood clotting results in a greater downloading and condemnation of the birds because of massive bleeding and bruises, less carcass pigmentation is exhibited and egg yolks are paler, decreased hatchability, reduced egg production and mortality.
Ochratoxin A	Increased mortality, poor feed conversion, poor growth rates and feed refusal, in broiler (OTA) poultry production are listlessness, huddling, diarrhea, tremors and other neural abnormalities, decrease egg shell quality and increased percentage of eggs with blood and meat spots.
Deoxynivalenol	Reduced feed consumption in layers and broilers breeders, acute signs included (DON) widespread haemorrhage and deposition of urates, neural toxicity and irritation of upper gastrointestinal tract.
T-2 Toxin	Lesions at the edges of beaks, intestinal lesions, decreased feed consumption, weight loss, abnormal feathering in chicks, a drastic and sudden drop in egg production with thinner shells, reduced weight and mortality.

Source: Asian Poultry Magazine, 2002

Table 2: Spectrum of Mycotoxins in poultry Finished Feed

Types of Mycotoxins	No. of collected samples	No. of Positive samples	Percent contamination	Average Mycotoxins (μg/kg)	Mycotoxin Levels (μg/kg)
Aflatoxin B1	182	155	84.70	13.11 ± 16.80	1-120
Zearalenone	150	74	49.33	213.58 ± 440.30	125-3600
Ochratoxin A	41	21	51.21	10.02 ± 19.63	2-75
Trichothecenes Type A					
T-2 Toxin	101	35	34.65	442.56 ± 1191.02	100-7500
HT-2 Toxin	23	ND	ND	ND	ND
Diacetoxyscirpenol	26	ND	ND	ND	ND
Neosolaniol	10	ND	ND	ND	ND
Trichothecenes Type B					
Deoxynivalenol	150	57	38.00	456 ± 1122	100-8100
3 acetyl-Deoxynivalenol	67	13	19.41	41 ± 102	100-499
15 acetyl-Deoxynivalenol	67	8	11.94	38.92 ± 149.58	100-988
Nivalenol	20	ND	ND	ND	ND
Fusarenon-x	28	ND	ND	ND	ND

- ND (Not Detected)

Table 3: General Guideline for Mycotoxin Contamination In Poultry Feed

Type of Mycotoxin	Low Contamination	Medium Contamination	High Contamination
Aflatoxin(B1,B2,G1,G2)	< 20ppb	20-50ppb	> 50ppb
Ochratoxin A	< 10ppb	10-60 ppb	> 60 ppb
Zeralenone(ZON)	< 50ppb	50-250 ppb	> 250 ppb
T-2 Toxin	< 150ppb	150-400 ppb	> 400 ppb
HT-2 Toxin	< 150ppb	150-400 ppb	> 400 ppb
Neosolaniol(Neos)	< 150ppb	150-400 ppb	> 400 ppb
Diacetoxyscirpenol (DAS)	< 150ppb	150-400 ppb	> 400 ppb
Fusarenon-x(Fus-x)	< 250ppb	250-1000 ppb	> 1000 ppb
Deoxynivalenol (DON)	< 250ppb	250-1000 ppb	> 1000 ppb
3 acetyl- Deoxynivalenol	< 250ppb	250-1000 ppb	> 1000 ppb
15 acetyl- Deoxynivalenol	< 250ppb	250-1000 ppb	> 1000 ppb
Nivalenol(NIV)	< 250ppb	250-1000 ppb	> 1000 ppb

Source: FDA 2000

The present study reveals that more than one mycotoxin occur in poultry feeds. Among these toxins, Aflatoxin B1 was the main mycotoxin found in 155 samples (84 %) in combination with Ochratoxin A (51.21 %), T-2 toxin (34.65 %), Deoxynivalenol (38 %), 3ac-DON (19.41 %) and 15ac-DON (11.94 %). The observations were in confirmation with Harbans *et al*. 1991 and Anjum and Naseem 2000 who have reported the presence of these toxins in combination in poultry feed samples. This study has explained that aflatoxin is not commonly prevalent but there is wider range of mycotoxins threatening the health of poultry in Pakistan.

The observations are suggestive to the fact that mycotoxins are damaging the poultry flock/industry. The effects of mycotoxicosis depend upon the nature of the mycotoxin, time of exposure, general health conditions of the poultry birds and immunity status. Even low levels of mycotoxins can affect the immune system of the bird. The simultaneous presence of mycotoxins with low levels can effect synergistically with severe losses to poultry.

The overall pattern of mycotoxins in poultry feed suggest that the environmental conditions during field condition, harvest, storage, processing and manufacturing of feed and then subsequent storage under farm conditions play vital role in mycotoxicosis.

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