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Prevalence of Mycotoxins in Poultry Rations

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Abstract. Samples of poultry broiler and layer rations (n = 865) were received at Romer Laboratories Pakistan from different parts of the country over a period of 31 months for the purpose of their mycotoxin analysis. The samples were analyzed by thin layer chromatography and by HPLC. The mycotoxins analysed included aflatoxin-B_1 (AfB_1), zearalenone (Zon), deoxynivalenol (Don), 3-acetyldeoxynivelenol (3ac-Don), 15-acetyldeoxynivelenol (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). The mycotoxin AfB_1 was noted to be the major contaminant in the feed samples analyzed (84.70% in 182 feeds), followed by OtA (51% in 41 feeds), Zon (49.33% in 150 feeds), Don (38% in 150 feeds), T-2 (34.65% in 101 feeds), 3ac-Don (19.41% in 67 feeds), and 15ac-Don (11.94% in 67 feeds). Mean values with standard deviation for AfB_1, 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 observed to be negative for HT-2 toxin, Das, neosolaniol, nivalenol, and fusarenon-x. This study is the first report on the occurrence of a range of mycotoxins in the Pakistani poultry rations, which shows that AfB_1, OtA, Zon, T-2 toxin, Don, 3ac-Don and 15ac-Don may be present at levels, which adversely affect poultry production.

Keywords: mycotoxins, aflatoxins, toxic metabolites, poultry feed, poultry rations

Introduction

Mycotoxins are toxic secondary metabolites of moulds that are produced on a wide range of commodities and under a diverse range of situations (Moss, 1991). Deleterious effects in poultry production are caused mainly by aflatoxins, zearalenone, trichothecenes and ochratoxins (Binder *et al*., 2000). The toxic effects of mycotoxins on poultry are dependent upon age, sex, and the physiological and nutritional status of the bird at the time of exposure. Since the mould growth at various stages during the feed production and distribution systems can magnify the mycotoxin problem, it is difficult to diagnose the contributing factors in the field situations (Cortyl and Heidler, 2002). Apart from this, some mycotoxins are formed under specific climatic conditions under which the plants are growing in the field, while others are produced under high humidity prevalent during inadequate storage conditions. Once produced, nevertheless, it is very difficult to get rid of these mycotoxins or even reduce their contamination, because these toxins have a high physical and chemical stability (Wilson and Abramson, 1992).

Mycotoxins are frequently associated with serious diseases of poultry, livestock and humans (CAST, 2003). Presence of aflatoxins in the poultry feed may cause reduced growth rates, poor feed conversion ratios, increased stress susceptibility, kidney damage, anaemia, suppressed immune system, and greater interference with normal protein and lipid metabolism (Butool *et al.,* 1990; Huff *et al.,* 1988). Ochratoxin-A (nephrotoxin) is the mycotoxin traditionally considered to be of importance in the poultry birds. The ochratoxin-A contaminated diet, when consumed by poultry birds, may cause listlessness, huddling, diarrhoea and tremors in broilers, whereas in layers it may cause decreased egg shell quality and increased percentage of egg with blood or meat spots (Cortyl and Heidler, 2002). Trichothecene mycotoxins, on the other hand, are the *Fusarium*-produced toxins that occur naturally at levels potentially toxic for chicken (Leeson and Summer, 2001).

Feed is the major financial input in poultry production, amounting to 60-70% of the total cost (Butool *et al.,*1990). Mycotoxins cause serious economic losses by damaging upto 25% of the world's poultry crop (CAST, 1989). Generally, poultry feed contains 40-60% grains, mainly maize, rice and wheat. The poultry feed in Pakistan is almost entirely dependent upon agricultural by-products (Anjum and Naseem, 2000). Usually, and particularly in the developing countries, the best quality grains and cereals are exported or reserved for human consumption, whereas the poorer quality harvests are consumed for the production of animal feeds (Jones,1995). Human population growth is another factor for the non-availability of acceptable quality grains for poultry feeds (Anjum and Naseem, 2000). In addition, inadequate storage facilities, humid envi-

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ronment and elevated temperatures, particularly from May to November, are conducive for the growth of fungi, such as *Aspergillus* species, which produce mycotoxins in these conditions (Sabri *et al*., 1989). The low temperature in the winter season is favourable for the production of certain other mycotoxins, like ochratoxin-A, trichothecenes and zearalenone by *Fusarium* species (Richard, 2000).

The mycotoxin analysis in Pakistan is usually limited to aflatoxins. Pakistan's environment has been reported to favour aflatoxicosis, which is quite common in commercial broilers, breeders and layers (Bhatti, 1989; Siddique *et al*., 1987). Isolated attempts have been made to evaluate the occurrence of ochratoxin-A (Rehman *et al*., 2003). However, little information is available on the presence of other mycotoxins in poultry feeds and feed ingredients in Pakistan. It is reported that some moulds are able to produce more than one mycotoxin, whereas 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-B_1 and ochratoxin-A, aflatoxin-B_1 and T-2 toxin, ochratoxin-A and citrinin, etc., exert additive, antagonistic or synergistic effects on the health status of birds (Huff *et al*., 1988). The present study shows the general trend of occurrence of aflatoxin-B_1, trichothecenes-A, trichothecenes-B, ochratoxin-A, and zearalenone in the poultry rations in Pakistan.

Materials and Methods

The various steps involved in analytical procedures have been summarized (Fig.1).

Sample collection and preparation. Commercial samples of different types of poultry feeds were collected from various parts of Pakistan (Rawalpindi, Islamabad, Abbotabad, Mansehra, Lahore, Gujrawala, Faisalabad, Sumundri, Kamalia, Multan, Rahim Yaar Khan, Thatha) over a period of 31 months from December 2001 to July 2004. The feed samples (1-2 kg) were collected from feed mills and domestic/cottage industry mixers. The samples were ground and sub-sampled by Romer Series-II Sub-sampling Mill (Richard, 2000) for the purpose of obtaining a homogeneous and representative sample. The sieve size of the mill was 16 mesh. The surplus materials were stored as file samples.

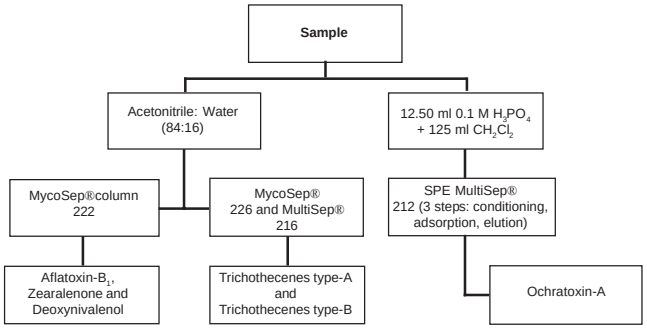

Fig. 1. The various steps involved in analytical procedures for the detection and quantification of mycotoxins in poultry rations.

Sample extraction and clean-up. Aflatoxin-B_1, ochratoxin-A, zearalenone, trichothecenes type-A (neosolaniol, diacetoscirpenol, Ht-2 toxin,T-2 toxin) and trichothecenes type-B (nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon-x) were analysed by the procedures developed by Romer Laboratories Inc., 1301-Stylemaster Drive, Union, MO, USA (Richard, 2000).

Sample analysis. For the analysis of aflatoxin-B_1 (AfB_1, deoxynivalenol (Don) and zearalenone (Zon), the analysis protocol of 3-toxin test (method code: tox-tl-01-02.1, Romer Laboratories Inc., USA) was followed (Richard, 2000). A 25 g portion of finely ground sample in acetonitrile : water (84 : 16) was blended at high speed in a blender (Osterizer; Sunbeam-Oster Household Products, New Maxico, USA). For clean-up, Romer MycoSep® column 226 (Romer Laboratories Inc., USA) was used and the residue was evaporated by Romer Evap® System (Romer Laboratories Inc., USA). The redissolved sample was spotted against the standard solution (3-toxin standard: 0.4 µg/ml AfB_1, and 20 µg/ml Zon and Don, Romer Laboratories Inc., USA). For this, Romer® Autospotter was used, which was followed by the visual observation of blue fluorescence of AfB_1(R_f = 0.5), Zon (R_f = 0.7) and Don (R_f = 0.3) under longwave UV light (365 nm), with reference to the standard spots.

For the analysis of trichothecenes types-A and B, the protocol of types-A and B trichothecenes dual column quantitative test (method code : tri-tl-01.00.2, Romer Laboratories Inc., USA) was followed. The initial extraction step was exactly similar to the 3-toxin test (for AfB_1, Zon, Don). For clean-up, MycoSep® column 227 in combination with MultiSep ® column 216 were used. For toxin estimation, standard spots [B-trich: 10 µg/ml of deoxynivalenol (Don), nivalenol (Niv) and fusarenon-x (Fus-x)/A-trich, trichothecenes type-A (neosolaniol : Neos, diacetoscirpenol : Das, Ht-2 toxin : Ht-2, T-2 toxin : T-2 : 10 µg/ml of T-2, HT-2, Das and Neos] were spotted along with the samples and observed under longwave UV light.

For the analysis of ochratoxin-A, the analysis protocol of ochratoxin-A quantitative TLC test (method code: och-tl-01-00.3, Romer Laboratories Inc., USA) was followed. For clean-up,

MultiSep® column 212 was used. The toxin in the samples was quantified with reference to standard solution (1µg/ml ochratoxin-A, Romer Laboratories Inc., USA) under longwave UV light.

Results and Discussion

A total of 862 samples were analyzed in poultry rations of broilers, broiler breeders, layers, layer breeders, and grand-parent flocks for aflatoxin-B_1 (AfB_1), zearalenone (Zone), ochratoxin-A (OtA), trichothecene type-A toxins (T-2 and HT-2), diacetoscirpenol (Das), deoxynivalenol (Don), 3-acetyldeoxynivalenol (3ac-Don), 15-acetyldeoxynivalenol (15ac-Don), nivalenol (Niv) and neosolaniol (Neos). The analytical data with regard to contents of different mycotoxins so obtained have been given in Table 1, which indicate the presence of different mycotoxin levels estimated in different commercial poultry rations.

Aflatoxin-B_1 (AfB_1). For the detection of AfB_1, 182 feed samples were analyzed. Results of these analyses are presented in Table 2, which show that 84% of the samples tested

Table 1. Spectrum of mycotoxins in poultry rations collected from different areas in Pakistan

Type of mycotoxins	Samples (nos.)	Positive samples (nos.)	Contamination detected (%)	Mycotoxin level* (µg/kg)	Range of mycotoxin levels (µg/kg)	Detection limits (µg/kg)
Aflatoxin-B_1	182	155	84.70	13.11	1-120	1
Zearalenone	150	74	49.33	213.58	125-3600	125
Ochratoxin-A	41	21	51.21	10.02	2-75	2
T-2 toxin	101	35	34.65	442.56	100-7500	100
HT-2 toxin	23	nd	nd	nd	nd	100
Diacetoxyscirpenol	26	nd	nd	nd	nd	250
Neosolaniol	10	nd	nd	nd	nd	500
Deoxynivalenol	150	57	38.00	456	100-8100	100
3-acetyldeoxynivalenol	67	13	19.41	41	100-499	100
15-acetyldeoxynivalenol	67	8	11.94	38.92	100-988	100
Nivalenol	20	nd	nd	nd	nd	500
Fusarenon-x	28	nd	nd	nd	nd	500

* = mean value of the feed samples analyzed; nd = not detected

Table 2. General guidelines for mycotoxin contamination in poultry feeds in respect of their low, medium and high contamination

Type of mycotoxin*	Low contamination (µg/kg)	Medium contamination (µg/kg)	High contamination (µg/kg)
Aflatoxins (B_1, B_2, G_1, G_2)[a]	<20	20-50	>50
Ochratoxin-A[b]	<10	10-60	>60
Zeralenone(Zon)[b,c]	<50	50-250	>250
T-2 Toxin[b,c]	<150	150-400	>400
HT-2 Toxin[b,c]	<150	150-400	>400
Neosolaniol (Neos)[b,c]	<150	150-400	>400
Diacetoxyscirpenol (Das)[b,c]	<150	150-400	>400
Fusarenon-x (Fus-x)[b,c]	<250	250-1000	>1000
Deoxynivalenol (Don)[b,c]	<250	250-1000	>1000
3-acetyldeoxynivalenol[b,c]	<250	250-1000	>1000
15-acetyldeoxynivalenol[b,c]	<250	250-1000	>1000
Nivalenol (Niv)[b,c]	<250	250-1000	>1000

* = compiled from: a = FDA (1994); b = Anjum and Naseem (2000); c = Cortyl and Heidler (2002)

were found positive for AfB_1,with the minimum of 1 ppb and the maximum of 120 ppb. Mean levels of AfB_1 were noted to be below the safe limit of 20 ppb recommended by FDA (Richard, 2000). It was observed that variations in the levels of mycotoxins in poultry feeds were due to marked fluctuations in the environmental temperature and humidity conditions during the course of the year. Presumably, the constituted feeds stored under appropriate conditions were subject to lesser direct influence of temperature and humidity. However, increased production of AfB_1 in feedstuffs may be expected if the storage was for a longer period under unsatisfactory ventilation and storage conditions (Richard, 2000).

Zearalenone (Zon). The detection of zearalenone appeared to be quite common, which occurred at levels from low to high with reference to the recommended levels mentioned in Table 2. Results reported in Table 1 show that 49% (74 out of 150) of the feed samples analyzed, contained detectable levels of zearalenone. A wide range of 125-3600 ppb of zearalenone was detected, with an average of 213 ± 440 ppb. The mean value falls within the medium contamination range, i.e., 50-250 ppb (Table 2). Major cause for zearalenone contamination in the poultry feed is corn (Richard, 2000). However, broiler chicks and laying hens are not significantly affected by zearalenone, even when this toxin is consumed in large levels (Christensen *et al*., 1977).

Ochratoxin-A (OtA). The analytical data obtained indicated that 51% samples were found to be positive for OtA, with the mean value of 10 ppb, having the range of 2-75 ppb. The mean value was at the safe recommended level of 10 ppb (Anjum and Naseem, 2000).

Trichothecenes type-A. For the trichothecenes type-A mycotoxins, 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 noted to be positive (34.65% in 101 feeds) with the mean value of 442 µg/kg . The mean value for T-2 toxin was at the high-contamination level (Table 2). The T-2 toxin is one of the most toxic compounds in the trichothecenes type-A group (Bamburg and Strong, 1971), which is responsible for drastic and sudden decreases in egg production, eggs with thin shells, abnormal feathering and slow growth in chicken (Cortyl and Heidler, 2002).

Trichothecenes type-B. For the detection of trichothecenes type-B mycotoxins, deoxynivalenol (150 samples), 3-acetyldeoxynivalenol (67 samples), 15-acetyldeoxynivalenol (67 samples), nivalenol (20 samples) and fusarenon-x (28 samples) were analyzed. In trichothecenes type-B group, deoxynivalenol (38%), 3-acetyldeoxynivalenol (13%), 15-acetyldeoxynivalenol (8%) were detected. Deoxynivalenol appeared with medium level contamination (Table 2), which was 456 ppb. While 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol occurred at below the low contamination levels (Table 2), the other two toxins of the group, namely, nivalenol and fusarenon-x, were not detected.

The present study reveals that more than one mycotoxins were detected in poultry feeds. Among these toxins, aflatoxin-B_1 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%). These observations are in conformity with Beg *et al*. (2006), Dawlatana *et al.* (2002) and Anjum and Naseem (2000), who have reported the presence of these toxins in combinations, in poultry feed samples. This study has indicated that though aflatoxin-A is commonly prevalent (84.70%), yet there is a wider range of other important mycotoxins which may be threatening the health of poultry in Pakistan. These observations indicate that mycotoxins may adversely damage 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 (Cortyl and Heidler, 2002). No level is a safe level. Even low levels of mycotoxins can affect the immune system of the bird. The mycotoxins in combination (either synergistic or additive) appear to exert greater negative impact on the health and productivity of poultry/animals in comparison with their individual effects (Smith and Seden, 1998).

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