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Determination of Aflatoxin and Ochratoxin in poultry feed ingredients and finished feed in humid semi-tropical environment

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ABSTRACT

A total of 186 samples comprising of poultry feed ingredients (n=114) and finished poultry feeds (n=72) were analyzed for the detection of total aflatoxin (TA) and ochratoxin A (OTA). The concentrations of TA and OTA in the samples were determined using direct competitive Enzyme-Linked Immunosorbent Assay (ELISA). Overall incidence of TA was recorded as 80.64% (n=150/186); whereas, in the feed ingredients, it was 86.84% (n=99/114), and in the finished feeds, the incidence of TA was 70.83% (n=51/72). Corn, cotton seed meal, sunflower meal, and cotton gluten meal were found to be highly (100%) contaminated with TA. The OTA was determined in 63.15% (n=72/114) and 29.17% (n=21/72) feed ingredients, and finished feed samples, respectively, with an overall incidence of 50% (n=93/186). Maximum level of OTA contamination (100%) was recorded in corn gluten meal. However, no feed contained OTA above the acceptable level as set by the European Union on OTA contamination in poultry finished feed. On the other hand, a number of samples contained TA above the acceptable limit. Thus, immediate control measures should be taken to ensure safe poultry for human consumption.

Keywords

Aflatoxin, Moisture, Ochratoxin, Poultry feed, Poultry feed ingredients

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INTRODUCTION

Although mycotoxins are found world-wide, their contamination generally occurs in the tropical and sub-tropical regions in the world. 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 (TA) and ochratoxins (OTA) (Anjum et al., 2011). The TA and OTA are the major components of a group of secondary metabolites produced by several fungi.

Aflatoxicosis causes severe economic loss in the poultry industry affecting ducklings, broilers, layers, turkeys and quails (CAST, 2003). In poultry, TA impairs most of the important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, 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 may cause reduction of immune response in poultry, thus the birds become vulnerable to several diseases (Dhanasekaran et al., 2009). Toxigenic *Aspergilus flavus* isolates generally produces aflatoxins B_1 and B_2, whereas *A. parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2 (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). Aflatoxin producing fungi utilize the nutrients present in the ingredients for their metabolism and propagation, and thereby reduce the nutritional quality of the feed ingredients (Akande et al., 2006).

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). In 2006, during a program of Hazard Analysis Critical Control Point (HACCP) in the Apulian region of Southern Italy, ochratoxin was detected in commercial feed for laying hens. 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 foods 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).

In Pakistan, different feed ingredients that are used in poultry feeds are likely to be contaminated with TA and OTA producing fungi. Because, most commercial feed mills in Pakistan provide suitable environments for fungal growth provoked by improper harvesting and storage, unhygienic method of processing and production, poor methodology of consumption and utilization. Therefore, regular monitoring of TA and OTA in poultry feeds is an important precondition to check toxins buildup in poultry feeds. The prevalence study on mycotoxins in feeds is regularly and frequently practiced in many countries like Brazil (Rosa et al., 2006), Kuwait (Beg et al., 2006), Nigeria (Osho et al., 2007), India (Vijayasamundeeswari et al., 2009), Iran (Beheshti and Asadi, 2014), and Malaysia (Reddy and Salleh, 2011). Few studies have also been conducted upon presence of mycotoxins in poultry feeds and agricultural products in Pakistan (Saleemullah et al., 2006; Khan et al., 2011; Anjum et al., 2011, 2012). However, regular monitoring is crucial for ensuring the safety of animal or poultry feeds. The present study was designed to quantify total TA and OTA contents in poultry feed raw material (cereals and plant protein sources) and finished feed kept in the humid semi-tropical environment of Punjab Province in Pakistan during June to August 2012.

MATERIALS AND METHODS

Samples and sampling procedure: A total of 186 samples comprising of different types of raw feed materials (n=114), and finished feed (n=72) were collected directly from poultry farms or poultry feed production sites in a humid semi-tropical environment. The samples were analyzed at the Feed Testing Laboratory, Poultry Research Institute, Rawalpindi, Pakistan. Sampling procedure followed the principles of the Romer® Guide (Richard, 2000). First, the sample was collected as a lot (500 g), which was composed of several small samples (100 g each) taken randomly from the whole lot. After grinding the sample, a sub-sample was taken for analytical process. A questionnaire was also designed for collecting 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, mortality of flock and so on. The experimental period was carried out during June-July, 2012 with an average temperature of 38.47°C, and a relative humidity of 65.97%.

Determination of moisture content: Moisture content in the sample was determined using the hot air oven method (AOAC International, 2011). The samples were dried at 105°C to constant weight, and the average content was calculated as percentage on wet basis.

Toal Aflatoxin and Ochratoxin assays in feed: The concentration of total TA in the feed samples was determined by a direct competitive Enzyme-Linked Immunosorbent Assay (ELISA), using Agra Quant® Total Aflatoxin Assay 4/40 Kits (Romer Laboratory Inc. Singapore). Twenty grams of grinded feed sample was added to 100 mL of 70% methanol for extraction of TA and filtered. The filtrate and enzyme conjugated TA were mixed, and added to antibody coated microwell. Aflatoxin in the samples and standards were allowed to compete separately with enzyme conjugated TA for antibody binding sites. After a step of 5 washes, an enzyme substrate was added, and blue color was developed. This was followed by addition of stop solution. Absorbances were read at 460 nm by a computerized microplate reader and TA expressed in μg/kg.

The concentration of OTA in each feed sample was determined using Agra Quant® Ochratoxin Assay Kits (Romer Laboratory Inc., Singapore). The technique is also a direct competitive ELISA. The procedure was same as described for TA assay. All data were determined for average and median by using the SPSS version 9.5 (SPSS, Cary, NC, USA) statistical analysis program.

RESULTS AND DISCUSSION

The occurance of TA in different poultry feed ingredients and finished feed are presented in **Table 1**. Overall incidence of TA in feed ingredients and finished feed samples was 80.64%. Out of total 114 different poultry feed ingredients analyzed, overall incidence of 86.84% of TA was observed, with average and maximum contamination levels as 74.4 and 165 µg/kg, respectively. In our previous 3 years survey study (2006 to 2009) in the Punjab province, we analyzed 1,021 samples, of which 646 were found positive for the presence of TA, among which 47, 51, 60 and 66% were cereals, cereal byproducts, oilseed meals and poultry feeds, respectively (Khan et al., 2011). However, in the present study, relatively high incidence of TA was determined in cereals (86.66%), oilseed meals (86.95%) and poultry feeds (70.8%). In another study, 1,200 raw feed ingredients and finished feed samples collected from Pakistan, Bangladesh, China, Korea, Malaysia, Philippines, Singapore, Sri Lanka, Thailand and Vietnam between 1998 and 2001 were analyzed for the presence of TA. The average contamination and maximum level were found as 109 and 585 µg/kg, respectively (Wei, 2004). This level of TA was much higher than that of the present study.

Corn is the most commonly used feed ingredient in poultry diets, in which 100% TA incidence was recorded. The average contamination level and maximum level of TA were 80 and 110 µg/kg, respectively, with high moisture contents (10.5 to 15%). Corn is more susceptible for TA production throughout the world as compared to canola, soybean and rapeseed (Firdous, 2003).

Aflatoxin production may occur in the field, during post-harvest, processing, storage or feeding under suitable environmental conditions. Although prevention of aflatoxin formation in feed prior harvesting is difficult due to high moisture, it is possible to attain favorable results by ensuring proper storage (Richard, 2007). Iram et al. (2014) reported that positive corn samples contained 77.3% TA B1 and 28% TA B2; the levels were above the limits as mentioned by the European Union (EU). Reddy and Saleha (2011) found that 22.5% samples of corn had TA B1 ranging from 20.6 to 135 µg/kg exceeding the international regulatory limits for poultry feed (*i.e.*, >20 µg/kg). In this study, TA content in corn was higher than the report of Shah et al. (2010), who found 77.8% and 88.9%

Table 1: Aflatoxin contamination in poultry feed ingredients (n=114) and finished feed (n=72).

Ingredients/Feed	Samples analyzed (n)	Positive Samples (n; %)	Moisture Range (%)	Mean (µg/kg)	Max Level (µg/kg)
Corn	15	15 (100)	10.5-15	80	110
Rice broken	15	10 (66.66)	8.7-11.5	10.70	26
Rice polish	15	14 (93.33)	6.9-12	25.10	59.32
Cotton seed meal	15	15 (100)	11-15.8	90	165
Canola meal	12	6 (50)	8.7-9.0	5.0	10.5
Soybean meal	12	9 (75)	11-15	25	56.9
Sunflower meal	10	10 (100)	10-12	25	95
Corn gluten meal (30%)	10	10 (100)	10.6-12.5	57.8	81.51
Corn gluten meal (60%)	10	10 (100)	11-14	89.9	96.56
Broiler Starter (Mash)	10	8 (80)	8.7-14.0	26.9	40.50
Broiler Starter (crumb)	10	6 (60)	6.5-7.9	10.0	22.00
Broiler Finisher (Mash)	10	9 (90)	7.5-15.0	25.0	44.70
Layer Chick Starter (Mash)	10	9 (90)	8.9-14.7	33.0	55.90
Layer Chick Starter (crumb)	10	5 (50)	6.8-9.0	9.0	17.50
Layer Grower (Mash)	10	9 (90)	9.76-15.0	29.9	56.0
Layer (Mash)	12	5 (41.66)	7.6-18.5	25.09	11.90
Total	114* + 72** = 186	99# (86.84) + 51## (70.83) = 150 (80.64)	-	-	-

* = total number of feed ingredients, ** = total number of finished feeds, # = total positive feed ingredients, ## = total positive finished feeds.

TA contamination in corn with mean values of 14.9 µg/kg and 16.2 µg/kg in the upper and lower Swat regions in Pakistan, respectively. However, in the present study, TA was present in corn with average value of 80 µg/kg and maximum value of 110 µg/kg. For all 15 rice broken samples tested, TA was found in 66.66% (maximum of 26 µg/kg) samples. However, concentration of TA was relatively higher in rice polish samples (93.33%) as compared to rice broken samples (maximum of 59.32 µg/kg).

Among vegetable protein sources, cotton seed meal, canola meal, soybean meal, sunflower meal and corn gluten (30% & 60%) were tested. All the 15 cotton seed meal were contaminated with TA at an average level of 90 µg/kg, and maximum level of 165 µg/kg with high moisture contents (11 to 15.8%). About half of the canola meal samples were found positive for TA. The average and maximum contamination level in canola meal was observed as 5.0 and 10.50 µg/kg, respectively. Beheshti and Asadi (2014) reported that canola meal samples could contain TA.

Soybean meal is the most frequently used feed ingredient as vegetable protein source in poultry diet. For all 12 soybean meal samples tested, concentration of TA toxin was relatively high (mean 25 and maximum level 56.9 µg/kg). Similarly, average concentration and maximum level in 12 sunflower meal was observed as 25 and 95 µg/kg, respectively. In the current study, mean values of TA in all feed ingredients (except rice broken and canola meal) and finished feeds (except broiler starter and layer chick starter in crumb forms) were found higher than safe limit of 20 µg/kg, as recommended by FDA (Richrad, 2000).

The results revealed that TA incidence in finished poultry feed was 70.83%. The average contamination level and maximum level were 26.28 and 56 µg/kg, respectively. The results showed that incidence and average concentration of TA were less in the finished feed samples as compared to feed ingredients.

The less incidence and mean values of TA were observed in broiler starter and layer chick starter feed in the form of crumb as compared to other mash feeds. This might be due to low moisture content, as recorded in crumb feed. Maximum TA value (56 µg/kg) was observed in layer grower (mash) with high level of moisture range (9.76-15%). A survey was conducted in Kuwait for determination of TA in the samples of broiler starter feed, broiler finisher feed, and layer mash feed, and was revealed low average TA concentration for poultry feed. This low incidence of aflatoxin could be due to selection of good quality graded samples (Beg et al., 2006). Furthermore, in this study, mean moisture content of all feed ingredients and poultry feed was not higher than the safe storage level of 15% (Alam et al., 2007), whereas it has been reported that high moisture content, long storage and heavy rains during storage may increase the level of TA (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 relationship between TA level and moisture content. 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 occurrence of OTA in different poultry feed ingredients and finished feed are presented in **Table 2**. Overall incidence of OTA was 50%. The average and maximum OTA contamination level were recorded as 21.95 and 97.5 µg/kg, respectively. Within 114 poultry feed ingredients, 63.15% samples were positive for OTA, with an average contamination level of 36.49 µg/kg.

About 86.6% corn samples were contaminated with OTA. The average contamination level and maximum level of OTA in corn were 60 and 97.5 µg/kg, respectively. OTA was found in 46.66 and 33.33% rice broken and rice polish samples, respectively, with maximum levels of 20 and 18 µg/kg, respectively.

Among vegetable protein sources, cotton seed meal samples were found contaminated with OTA at an average level of 34 µg/kg, and maximum level of 48 µg/kg. About 41.66% of the canola meal samples were found positive for OTA. For soybean meal samples, concentration of OTA was found as mean 22 µg/kg and maximum level of 45 µg/kg. Average concentration and maximum level of OTA in sunflower meal was relatively high as 51 and 72 µg/kg, respectively. Like TA, OTA was also extensively contaminated with corn gluten meal (30 or 60%) samples; particularly 100% contamination was found in corn gluten 60%.

The incidence of OTA in finished feed was 29.17%. The average and maximum contamination levels of OTA were 7.11 and 23 µg/kg, respectively; however, these levels were less than that of feed ingredients.

No feed sample was found above the limits set by the EC recommendation 2006/576/CE on OTA contamination in poultry feed (100 µg OTA/kg in 12%

Table 2: Ochratoxin contamination in poultry feed ingredients (n=114) and finished feed (n=72).

Ingredients/Feed	Samples Analyzed (n)	Positive Samples (n; %)	Mean (µg/kg)	Max Level (µg/kg)
Corn	15	13 (86.66)	60	97.5
Rice broken	15	7 (46.66)	8.8	20
Rice polish	15	5 (66.66)	5.9	18
Cotton seed meal	15	9 (60)	34	48
Canola meal	12	5 (41.66)	17	26
Soybean meal	12	8 (66.66)	22	45
Sunflower meal	10	8 (80)	51	72
Corn gluten meal (30%)	10	7 (70)	32	44
Corn gluten meal (60%)	10	10 (100)	70.70	95.71
Broiler Starter (Mash)	10	3 (30)	9.0	18.0
Broiler Starter (crumb)	10	2 (30)	5.0	11.0
Broiler Finisher (Mash)	10	3 (30)	5.3	12.0
Layer Chick Starter (Mash)	10	4 (40)	11.0	23.0
Layer Chick Starter crumb)	10	3 (30)	4.4	10.78
Layer Grower (Mash)	10	2 (20)	5.5	12.7
Layer (Mash)	12	4 (33.33)	9.6	22.65
Total	114* + 72** = 186	72# (63.15) + 21## (29.16) = 93 (50)	-	-

* = total number of feed ingredients, ** = total number of finished feeds, # = total positive feed ingredients, ## = total positive finished feeds.

moisture). Similar to our findings, Jaimez et al. (2004) detected OTA in 33% feed and raw materials in Spain. Domijan et al. (2005) in Croatia found OTA in 39% corn. In a survey conducted in the UK on ingredients for animal feeding stuffs, only 5% of the analyzed corn gluten samples were found positive for OTA (Scudamore et al., 1998). Schiavone et al. (2008) found in Italy that all the tested poultry feed samples were contaminated with OTA ranging from 0.04 to 6.50 µg/kg. However, in the current study, values of OTA in all samples were found higher as compared to previous reports; this might be due to high humidity and elevated temperature present in Pakistan.

CONCLUSION

Higher incidence and contamination level of TA are detected in local poultry feeds and feed ingredients. On the other hand, OTA contamination is present at low levels in both poultry feed and feed ingredients. 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 TA contamination are highly recommended to improve the health status of the consumers.

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