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ASSESSMENT OF AFLATOXIN B1 IN COMMERCIAL POULTRY FEED AND FEED INGREDIENTS

M. A. Anjum, S. H. Khan, A. W. Sahota[*] and R. Sardar

Poultry Research Institute, Murree Road, Shamsabad, Rawalpindi, Pakistan
[*]Deptt. of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan
Corresponding author e-mail: sohailhassan64@gmail.com

ABSTRACT

A total of 487samples (77 and 410 of poultry feed ingredients and poultry feed, respectively) received from various parts of the country were analysed for detection of Aflatoxin B1 (AFB1). Overall incidence of AFB1 in feed ingredients was 60 percent. The average contamination and maximum levels of AFB1 were 37.62 and 56 µg/kg, respectively, whereas, the incidence in poultry feed samples was 44.39 percent with average contamination and maximum levels of 23.75 and 78 µg/kg, respectively. However, maximum level of AFB1 was higher (78µg/kg) in poultry feed samples as compared to feed ingredients (56µg/kg). Furthermore, higher incidence and average contamination level of AFB1 were recorded in mash feeds (49.68 percent and 25.12µg/kg, respectively) than in crumb feeds (41.32 percent and 22.37µg/kg, respectively). A higher incidence of AFB1 was observed during the rainy months i.e. July & August in 2009 (53.85 & 60.86 percent, with contamination levels of 56 & 56 µg/kg, respectively) and in 2010 (68.18 & 69.44 percent with contamination levels of 78 & 56 µg/kg, respectively) than in other months of these years.

Key words: Aflatoxin B1, incidence, poultry feed ingredients, poultry feed.

INTRODUCTION

Cereals and plant protein sources used in poultry feeds have been associated with contaminants produced by moulds during crop production and storage. Among the contaminants, aflatoxins, produced by moulds not only can deteriorate food and feeds, but also can adversely affect human and animal health since they may produce toxic metabolites "mycotoxins" (Cole and Cox, 1981).

Aspergillus flavus, *Aspergillus parasiticus* and *Aspergillus nomius* are the widespread fungi isolated from a wide range of animal and human foods, which produce highly hepatocarcinogenic aflatoxins (Klich and Pitt, 1988). The major hosts of *A. flavus* among food and feed commodities are cereal grains, peanut, cotton seed and protein sources such as rapeseed meal, cottonseed meal, soya been meal, sunflower meal, corn gluten meal, copra meal and palm kernel meal (Pitt and Hocking, 1997). Aflatoxins utilize the nutrients present in the ingredients for their metabolism and propagation and thereby reduce the nutritional quality of ingredients (Akande *et al*., 2006).

Aflatoxin B1 (AFB1) is the most prevalent toxin in cereals used in feeds and presents the greatest toxigenic threat. The AFB1 is an active hepatocarcinogen, which can inhibit nucleic acid synthesis by either direct interaction with enzymes involved or by a toxin-DNA template (Windholz *et al*, 1983). Aflatoxin B1 is rapidly absorbed from the small intestine into the mesenteric venous blood in poultry (Hsieh and Wong, 1994). In chicken, B1 is metabolized into M1 and B2a in the liver and NADP linked enzyme system reduces B1 and B2 to cyclopentanol and aflatoxinol. However, in laying chickens, both AFB1and aflatoxinol accumulates in eggs and aflatoxinol is the major metabolite in muscles and blood (Bentvihok *et al*. 2002). Aflatoxin B1 impairs all important production parameters in poultry including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance (Bentvihok *et al*. 2002).

Regular monitoring of AFB1 in poultry feeds is an essential pre-requisite to prevent aflatoxin buildup in poultry feeds. The study of prevalence of aflatoxins in animal/poultry feeds is regularly and frequently reported from many countries including Brazil (Rosa *et al*., 2006), Nigeria (Osho *et al*., 2007), India (Vijayasamundeeswari *et al*., 2009) and Malaysia (Reddy and Salleh, 2011). In Pakistan limited studies have been reported upon presence of mycotoxins in poultry feeds and agricultural products (Hanif *et al*., 2006; Saleemullah *et al.,* 2006; Anjum *et al*., 2011; Khan *et al*., 2011).

Keeping above in view, the present project has therefore been planned to examine AFB1contamination in major poultry feed ingredients and poultry feeds from different agro-ecological zones of Pakistan.

MATERIALS AND METHODS

Samples collection: For the estimation of AFB1, a total of 487 samples of different types of raw materials and finished poultry feed were received at Feed Testing Laboratory, Poultry Research Institute (PRI), Rawalpindi from all over the country over a period of 18 months from July, 2009 to December, 2010. The samples were mostly sent by the commercial poultry farmers themselves or in some cases these samples were also collected by the Feed samplers employed at PRI. The feed samples thus received were properly packed in separate polythene bags and the particulars of the commercial poultry farm and the feed stuff were recorded separately in the Performa.

A total of 77 samples of different poultry feed ingredients and 410 samples of different poultry feeds were analysed in the laboratory for AFB1 (detail of samples received is given in Tables 1 and 2).

Estimation of Aflatoxin B1:

Sample preparation and clean-up procedures: A total of 25 g of each sample was milled (for grinding and preparing sub-sampling using a Lab mill-1 QC-114, Hungary) The samples were then well-mixed and extracted with 100 ml of aceto-nitrite: water (84:16) through blending at high speed for 3 minutes. The extract was filtered through a folded filter (Whatman®, No. 1001185, England) and then 4 ml of the filtrate was slowly pressed through a Mycosep® column number 226 and the residue was evaporated. Aflatoxin B_1 standard was procured from Biopure® Referenzsubstanzen GmbH, Austria and other chemicals were purchased from Merck AG, Germany.

Thin layer chromatography (TLC): The residue after evaporation was dissolved in toluene:acetonitrile 97:3 and then sample was spotted against the standard solution (0.4 *ug*/ml AFB1) with the use of Autospotter on TLC plate at 60°C, along with a standard series corresponding to 25, 50, 100, and 200 *ng* AFB1. The plate was developed in chloroform: acetone (9:1) to about 1 cm from the top of the plate dried and dipped into methanol/sulphuric acid 90:10. After heating at 150°C the spots were visible under long wave UV light (365nm) with reference to the standard spots.

The levels of AFB1 thus determined in poultry feed ingredients and poultry feed were subjected to statistical analysis to find out mean value in each feed sample by using the SPSS version 9.5 (SPSS, Cary, NC, USA) computer software.

RESULTS

The occurrence of aflatoxin B1 in different poultry feed ingredients is presented in Table 1. Out of total 77 different poultry feed ingredients analyzed an overall incidence of 60 percent of AFB1 was observed with average and maximum contamination levels of 37.62 and 56 μg/kg, respectively.

Corn samples tested had 61.54 percent occurrence level of AFB1 (8 samples positive out of 13 samples) with average and maximum levels of contamination of 25 and 56 μg/kg, respectively. In each 5 samples of rice broken and wheat AFB1 was detected in 60 percent samples with mean and maximum concentration of 21 to 39 μg/kg and 19 to 19μg/kg, respectively). However, occurrence and concentration of aflatoxin B1 was observed to be relatively low (50 percent) in rice polishing in comparison to rice broken.

Table 1: Aflatoxin B_1 contamination levels (μg/ kg) in poultry feed ingredients

Ingredients	Total No. of samples analyzed	No. of positive samples	MeanAFB1 (μg/ kg)	Max. AFB1 levels (μg/ kg)
Corn	13	8	25	56
Rice broken	5	3	21	39
Wheat	5	3	19	19
Rice polish	6	3	30	39
Cotton seed meal	5	5	32	56
Canola meal	7	2	23	26
Guar meal	5	2	23	26
Soybean meal	6	4	19	19
Sunflower meal	5	4	33	56
Corn gluten meal (30%)	5	3	26	39
Corn gluten meal (60%)	5	3	34	56
Rape seed meal	5	3	19	19
Fish meal	5	3	24	39

Among the plant protein sources, all the cotton seed meal samples tested were found contaminated with AFB1 with average and maximum levels of 32 and 56 μg/kg, respectively. The average and maximum contamination levels in canola and guar meal were 23 and 26μg/kg, respectively. In soybean meal and rape seed meal concentration of aflatoxin B1 was found to be relatively low (both mean and maximum level of 19 μg/kg). A higher incidence (80%) of AFB1 was found in sunflower meal with mean and maximum levels of 33 and 56μg/kg, respectively. The incidence of AFB1 was observed to be 60 percent in other vegetable protein sources tested with mean and maximum values of AFB1 (34 and 56 μg/kg, respectively) in corn gluten meal (60%), and 26 to 39 μg/kg, respectively in corn gluten meal (30%).

Out of 410 different poultry feeds tested incidence of aflatoxin B1 was found 44.39 percent (Table 2) with average and maximum contamination levels of 23.75 and 78.00 μg/kg, respectively. The incidence of AFB1 was found to be low (44.39 percent) in feed samples than in poultry feed ingredients (60 percent), whereas, the maximum level of AFB1 was found in poultry feed samples (78μg/kg) as compared to feed ingredients (56μg/kg). The higher incidence and average contamination levels of AFB1 were recorded in mash feeds (49.68% and 25.12μg/kg, respectively) as compared in crumb feeds (41.32% and 22.37μg/kg, respectively).

Table 2: Aflatoxin B_1 contamination levels (μg/ kg) in poultry feeds

Type of Feed	Total No. of samples analyzed	No. of positive samples	MeanAFB1 (μg/ kg)	Max level AFB1(μg/ kg)
Broiler starter (crumbs)	11	6	20	26
Broiler starter (mash)	14	8	21	26
Broiler finisher (crumbs)	5	1	19	19
Broiler finisher (mash)	5	3	24	39
Broiler breeder starter (crumbs)	63	25	30	39
Broiler breeder starter (mash)	16	7	25	56
Broiler breeder grower (crumbs)	11	4	21	26
Broiler breeder grower (mash)	5	3	26	56
Broiler breeder (crumbs)	11	5	24	26
Broiler breeder (mash)	43	20	23	56
Layer starter (crumbs)	30	16	21	39
Layer starter (mash)	66	31	26	56
Layer grower (crumbs)	7	3	23	26
Layer grower (mash)	5	2	32.5	39
Layer (crumbs)	60	23	21	39
Layer (mash)	58	25	23.5	78

Table 3: Aflatoxin B_1 occurrence (%) and contamination levels (μg/ kg) during 2009 and 2010.

Months	Samples analyzed (No.)	Positive samples (No.)	Contamination detected (%)	Max Level (μg/ kg)
July, 2009	39	21	53.85	56
August, 2009	23	14	60.86	56
September, 2009	14	5	35.71	26
October, 2009	19	7	36.84	19
November, 2009	18	7	38.89	26
December, 2009	28	12	42.86	19
January, 2010	12	4	33.33	26
February, 2010	32	12	37.50	26
March, 2010	30	12	40.00	56
April, 2010	29	13	44.83	39
May, 2010	35	15	42.86	39
June, 2010	21	9	42.85	56
July, 2010	44	30	68.18	78
August, 2010	36	25	69.44	56
September, 2010	32	13	40.62	39
October, 2010	30	12	40.00	26
November, 2010	18	7	38.88	19
December, 2010	27	10	37.04	26

Layer starter (mash), broiler breeder (both mash and crumbs), broiler breeder starter (mash), layer (mash), layer grower (both crumbs and mash) had occurrence level of AFB1 ranging from 40 to 46.51 percent. Broiler breeder grower, broiler breeder and layer (crumbs), had similar range of occurrence of (36.36 to39.68 percent).

The highest (78 µg/kg) contamination level of aflatoxin B1 was detected in layer mash. The maximum level of AFB1 contamination in broiler breeder starter and grower, broiler breeder and layer starter mash was observed to be 56 µg/kg. Maximum level of AFB1 (39 µg/kg) was recorded in broiler finisher & layer grower mash, broiler breeder starter, layer chick starter & layer. crumbs. However, maximum AFB1 contamination level in broiler starter crumbs & mash, broiler breeder grower and broiler breeder crumbs was (26µg/kg).

In the present study, a higher incidence of AFB1 in poultry feed ingredients was found during the rainy months in July & August, 2009 (53.85 & 60.86 percent, with contamination level of 56 & 56 µg/kg, respectively) and in 2010 it was 68.18 & 69.44 percent, with contamination level 78 & 56 µg/kg, respectively than in other months of the same years (Table 3).

DISCUSSION

The poultry industry suffers greater economic loss than any of the livestock industries because of their greater susceptibility to aflatoxin (Robens and Richard, 1992). In the current study, mean levels of AFB1 in poultry feed ingredients (except wheat, soyabean meal, rape seed meal) and poultry feeds (except broiler finisher crumbs) were observed to be higher than safe limit of 20 µg/kg recommended by FDA (Richrad, 2000). All poultry feeds in crumb forms showed less contamination than in poultry mash. The pellets are made up of compressed mash and crumbles of broken up pellets, therefore usually it takes more time for toxins to build up in the pellets to a fatal level. Corn is more liable to the most critical AFB1 contaminations throughout the world. Toxin production may occur in the field during post harvest, storage, processing or feeding under appropriate environmental conditions. Although it is difficult to prevent aflatoxin formation in feed prior harvesting due to high heat and moisture, it is possible to attain favorable results by correct storage (Richard, 2007). Reddy and Saleha (2011) reported that 22.5percent samples of corn had AFB1 contamination ranging from 20.6 to 135 µg/kg exceeding above the international regulatory limits for poultry feed (> 20µg/kg). Zinedine *et al*. (2007) reported AFB1contamination ranging from 0.23 to 11.2 and 0.05 to 5.38 mg/g in corn and poultry feeds respectively. In the present study, corn had AFB1 incidence of 61 percent, which was higher than the permissible limits.

In our previous 3 years survey study conducted from 2006 to 2009) in the Punjab province out of 1021 samples analyzed, 646 were found positive for presence of AFB1 out of which 47, 51, 60 and 66 percent cereals, cereal by-products, oilseed meals and poultry feeds, respectively, were found positive for AFB1 (Khan *et al*., 2011). However, in the present study, relatively high incidence of AFB1 (61 percent) in cereals and low incidence (44 percent) was observed in poultry feeds. This difference might be due to less number of poultry feed samples tested during the present study as compared to the previous. Similar results from a study conducted by Vijayasamundeeswari *et al*. (2009) in Tamil Nadu, India, dictated that 61.3% samples of maize kernels were contaminated with AFB1 and the levels of AFB1 in 26% of the pre- and post-harvest maize kernels exceeded 20µg/kg. The highest level of AFB1 (245 µg/kg) was recorded in post-harvest maize kernel samples; in poultry feeds, AFB1 was detected in 30 out of 53 samples and the levels ranged from 0.7 to 31.6 µg/kg. During another study 1200 raw ingredients and feed samples collected from Pakistan, Bengal, China, Korea, Malaysia Philippines, Singapore, Sri Lanka, Thailand and Vietnam between 1998 and 2001 were analyzed for AFB1. The average contamination and maximum level were found 109 and 585 µg/kg, respectively (Wei, 2004). This concentration of AFB1 contamination was much higher than recorded during the present study. The quantum of samples (29 from Pakistan) was too small and highly contaminated samples appeared to have been used in the above-mentioned study. In contrast of the above studies, another survey was conducted at a poultry feed production unit in Kuwait for aflatoxin contamination in the samples of yellow maize, soybean meal, wheat bran used as raw material and the poultry feed prepared for broiler starter, broiler finisher, and layer mash. The results revealed low average aflatoxin concentration than the permissible levels, for poultry feed. This low incidence of aflatoxin could be due to selection of good quality graded samples. (Beg *et al*., 2006).

The results of the present study showed higher incidence and contamination level of AFB1 in poultry feed ingredients during rainy months of July and August. The higher occurrence of aflatoxin during rainy season of the year appears to be due to lack of proper storage facilities of poultry feed ingredients and poultry feed. It was observed that variations in the levels of AFB1 in poultry feeds and ingredients were due to marked fluctuations in the environmental temperature and humidity during different seasons of the year. Similarly, Okoli *et al*. (2007) reported higher occurrence of aflatoxin in the month of July in local fish meal, palm kernel cake, and brewers dried grain with the prevalence ranging from 13.64 to 18.18 percent. Tangendjaja, *et al*. (2008) reported that corn harvested during the wet season had higher (66.4µg/kg) level of aflatoxin than those harvested in the dry season (36.5µg/kg). A comparatively higher contamination level in maize was observed during warm and humid months (Ram *et al*., 2010).

The findings of the present study indicating higher incidence and contamination level of AFB1 in local poultry feeds and feed ingredients call for immediate necessary control measures including adequate post harvest drying and storage and also of constant monitoring of poultry feed and feed ingredients.

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