

AFLATOXIN CONTAMINATION OF POULTRY FEED AND ITS INGREDIENTS

Part. I. Cotton Seed, Cotton Seed Cake and Cotton Seed Meal

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Five hundred and twenty-six samples of cotton seed, cotton seed cake, and cotton seed meal were collected during the period of study and had 44.6%, 49.3% and 70.6% positive samples for aflatoxin B₁ (AFB₁) respectively. Cotton seed meal had maximum average content of 160.6 µg/kg AFB₁, while cotton seed and cotton seed cake had 115 µg/kg and 114 µg/kg AFB₁ respectively. Eight samples of cotton seed meal and aflatoxin B₁ in excess of 1000 µg/kg. The highest amount of AFB₁ (1629 µg/kg) was found in a sample of cotton seed meal collected in 1984. The samples were analysed by the methods laid down in Association of Official Analytical Chemists.

Key words: Aflatoxins, Cotton seeds, *Aspergillus flavus*.

INTRODUCTION

Samples of cotton seed, meal and cake have been collected over the last eight years to determine the magnitude of aflatoxin contamination. Cotton seed is the major edible oil crop of Pakistan and its meal and cake are an important vegetable source of protein for poultry and cattle. Due to the frequent outbreak of mild to severe aflatoxicosis among the poultry birds around Karachi, a survey of poultry feed and its ingredients was started in 1978 to screen out the ingredients more susceptible to aflatoxin contamination. Cotton seed, meal and cake were found to be highly and frequently contaminated by this toxin. Samples of these ingredients were subjected to routine analysis to find out the levels of aflatoxin. Among the fungi previously isolated from various oil seeds *A. flavus* was found to be the most prevalent fungus with a frequency of 25% [1]. Bollenbacher and Marsh were the first to report the infection of cotton *Gossypium hirsutum* L. by *A. flavus* in 1954 [2]. Insects like pink boll worm *Pectinophora gossypiella* (Saunders) predisposed cotton seed to invasion by *A. flavus* in the field [3] and subsequent aflatoxin production. Occasionally in high-risk areas in certain parts of the United States a 100 percent incidence of aflatoxin contamination of incoming cottonseed lots were observed while going into storage with a mean total aflatoxin level of 2600 µg/kg (4).

MATERIAL AND METHOD

Samples of approximately 2 kg of cottonseed, cotton seed cake and cotton seed meal were collected randomly from godowns of the local market and various poultry

feed mills. The sampling of cotton seed cake is extremely difficult; firstly, due to the highly heterogeneous distribution of aflatoxins and, secondly, due to its marketing in pieces of various sizes; and thirdly, due to the presence of lint on the seeds.

To get the most representative sample of a lot a rigorous sampling plan was followed. The samples were collected by sample probe directly in the plastic bags which were pierced diagonally from two or more places. The samples were collected from several bags of the lot, mixed thoroughly and ground in a mill and passed through a sample divider to reduce it approximately to 100g for the purpose of analysis. The major error in the final results lies in an improper sampling plan, because the amount of aflatoxin in an individual cotton seed may go up to 5.75 million µg/kg [5], enough to contaminate a large lot. A few highly contaminated individual seeds from a particular lot may result in extremely high levels of contamination of the lot [6]. Thus the simple presence or absence of few contaminated seeds in the sample may result in high consumer or producer risk.

The samples were analysed according to the American Association of Analytical Chemist, official methods of analysis 26.048 – 26.055 [7] procedure on cotton seed products. Estimation of aflatoxin was made through thin layer chromatography by comparison with standards. Confirmation was achieved by forming derivatives by trifluoroacetic acid, H₂SO₄ spray of TLC plates and also by co-chromatography. Two-dimensional chromatography was performed whenever the final extract was not clean enough for simple chromatography. The fungi were isolated from the cotton seed and its products on Czapek's Dox Agar

medium to determine the extent of infection of *A. flavus*. The cotton seeds were autoclaved and then inoculated by the spores of the isolates of *A. flavus* to screen out the potential aflatoxigenic isolates and the amount of aflatoxin produced by them. The seeds were incubated for seven days at relative humidity of around 85 %, temperature of $28^{\circ} \pm 1^{\circ}$ and moisture content of about 19 %.

RESULTS AND DISCUSSION

Cotton seed: Hundred and one samples of cottonseed have been analysed for aflatoxin. Forty five samples

(44.6%) were found to contain aflatoxin with an average level of 115.1 $\mu\text{g}/\text{kg}$ of AFB₁ (Table 1). The maximum number of positive samples were 69.2%. The highest average content of 183.5 $\mu\text{g}/\text{kg}$ of AFB₁ was found in 1984 and the least contaminated in 1978 (26.9%) but the average content was quite high at 113 $\mu\text{g}/\text{kg}$ AFB₁ in that year and the lowest average contamination was found in 1980 at 57.3 $\mu\text{g}/\text{kg}$ AFB₁. No sample was collected in 1982. Throughout the period of study 14 samples had an aflatoxin B₁ content less than 20 $\mu\text{g}/\text{kg}$, whilst 12 samples had a level between 21 $\mu\text{g}/\text{kg}$ and 100 $\mu\text{g}/\text{kg}$ AFB₁. 19 samples had this toxin above 100 $\mu\text{g}/\text{kg}$ AFB₁ but less than 1000 $\mu\text{g}/\text{kg}$.

Table 1. Presence of Aflatoxin B₁ $\mu\text{g}/\text{kg}$ in cottonseed, cottonseed cake and cottonseed meal, over the last eight years (1978-1985).

Year/Commodity	No. of samples collected	No. of positive samples	Percentage contaminated samples	Average aflatoxin	Aflatoxin range
1978					
Cotton seed.	26	7	26.9	113.0	11 - 302
Cotton seed cake	16	6	37.5	116.3	9 - 237
Cotton seed meal	19	12	63.2	135.0	4 - 441
1979					
Cotton seed.	21	9	42.9	97.7	18 - 367
Cotton seed cake	20	8	40.0	117.5	5 - 401
Cotton seed meal	56	29	51.8	151.7	5 - 562
1980					
Cotton seed.	08	03	37.5	98.3	16 - 172
Cotton seed cake	13	07	53.8	83.0	12 - 215
Cotton seed meal	42	27	64.3	73.6	7 - 473
1981					
Cotton seed	07	03	42.9	57.3	18 - 117
Cotton seed cake	07	04	57.1	77.0	18 - 193
Cotton seed meal	30	23	76.6	125.8	7 - 617
1982					
Cotton seed	-	-	-	-	-
Cotton seed cake	01	-	-	-	-
Cotton seed meal	08	05	62.5	107.6	18 - 383
1983					
Cotton seed	07	04	57.1	83.3	8 - 126
Cotton seed cake	05	03	60.0	90.0	22 - 195
Cotton seed meal	53	39	73.4	125.7	3 - 971
1984					
Cotton seed	13	09	69.2	183.5	4 - 625
Cotton seed cake	09	07	77.8	216.0	33 - 532
Cotton seed meal	85	69	81.2	228.8	11 - 1629
1985					
Cotton seed.	19	10	52.6	74.7	6 - 262
Cotton seed cake	04	02	50.0	59.5	37 - 82
Cotton seed meal	57	43	75.4	176.4	8 - 1338

The maximum amount 625 $\mu\text{g}/\text{kg}$ AFB₁ was found in a sample collected in 1984.

Cotton seed cake: During the survey 75 samples of cotton seed cake were collected. Among these, 37 samples (49.3%) were found to be contaminated having, an average content of 114.2 $\mu\text{g}/\text{kg}$ AFB₁, approximately the same average as that in cotton seed (115.1 $\mu\text{g}/\text{kg}$ AFB₁). The highest average amount of AFB₁ 216 $\mu\text{g}/\text{kg}$ was found in the year 1984 and the lowest average content 59.5 $\mu\text{g}/\text{kg}$ AFB₁ in 1985. The highest number of positive samples (77.8%) were collected in 1984 and the lowest number (37.5%) were found in 1978. The highest amount of this toxin (532 $\mu\text{g}/\text{kg}$ AFB₁) was found in a sample collected in 1984. Aflatoxin could not be detected from the sample collected in 1982. Eight samples had aflatoxin less than 20 $\mu\text{g}/\text{kg}$ AFB₁ while 13 samples were found contaminated between 21 100 $\mu\text{g}/\text{kg}$ of AFB₁, while 16 samples (the maximum number of positive samples) contained levels between 100 and 532 $\mu\text{g}/\text{kg}$ AFB₁.

Cotton seed meal. The maximum number of samples collected (350) were that of cotton seed meal, an important and rich source of vegetable protein for poultry feed. Of these 247 samples (70.6%) were found positive for aflatoxin B₁ at levels of 3 to 1629 $\mu\text{g}/\text{kg}$, with an average contamination of 160 $\mu\text{g}/\text{kg}$ AFB₁. As in the cases of cotton seed and cotton seed cake, the highest average (228.8 $\mu\text{g}/\text{kg}$ AFB₁) and the highest number of positive sample (81.4%) were found in 1984. The lowest number of positive number (51.8%) were from 1979 whilst the lowest average content 73.6% $\mu\text{g}/\text{kg}$ AFB₁ was in the succeeding year. The maximum number of samples (104) were found to contain aflatoxin B₁ between 21 and 100 $\mu\text{g}/\text{kg}$ and only 8 sample had aflatoxin in excess of 1000 $\mu\text{g}/\text{kg}$. 56 samples were found to be contaminated below 20 $\mu\text{g}/\text{kg}$ of aflatoxin B₁. 79 samples (31.9% of positive samples) were found to be contaminated with high amounts of aflatoxin in the range of 101 to 1000 $\mu\text{g}/\text{kg}$.

Aflatoxin B₁ was found in all the positive samples collected throughout the period of study. 13 samples (12.8%) of cotton seed were found positive for aflatoxin B₂ (AFB₂) with an average content of 11.7 $\mu\text{g}/\text{kg}$ whilst aflatoxin G₁ (AFG₁) was detected in 7 samples (6.9%) with an average content of 9.6 $\mu\text{g}/\text{kg}$ and aflatoxin G₂ (AFG₂) was found in one sample in traces only. In the case of cotton seed cake, 8 samples (10.7%) were positive for AFB₂ with an average of 8.3 $\mu\text{g}/\text{kg}$. 5 samples (6.7%) were found to contain AFG₁ with an average amount of 5.9 $\mu\text{g}/\text{kg}$ while AFG₂ detected in traces in two samples. Aflatoxin B₂ was detected in 35 samples (10%) in cotton

seed meal with an average content of 17.7 $\mu\text{g}/\text{kg}$ while AFG₁ was detected in 17 samples 2.9%), the average content being 11.2 $\mu\text{g}/\text{kg}$ and in case of AFG₁, it was found in two samples at an average of 7.7 $\mu\text{g}/\text{kg}$. 245 samples (78.9%) were found contaminated in excess of 20 $\mu\text{g}/\text{kg}$ of aflatoxin B₁. 114 samples (25.3%) were found containing this toxin in excess of 100 $\mu\text{g}/\text{kg}$ AFB₁ which is quite a high contamination. Eight samples had this toxin in excess of 1000 $\mu\text{g}/\text{kg}$ which if incorporated in feed can produce serious problems in birds.

Aspergillus flavus was isolated from all the samples of cotton seed meal and cake while 42% cotton seeds were found contaminated by this fungus. About 31% isolates were of *A. flavus* in relation to the other fungi isolated making it the most prevalent fungus. Fifty four percent of isolates produced aflatoxin in the range of 113 $\mu\text{g}/\text{kg}$ to 5.9 mg/kg. Once the toxigenic fungi become established within the seed coat, it grows and produces toxins during storage and the seed coat protects it from the chemicals used for their control [8].

To reduce the aflatoxin contamination of cotton seed in the field, it is essential to adopt good agronomic methods like skip row planting and defoliation of the lower parts of plants which play an important role in reducing aflatoxin accumulation in cottonseed [8], screening of fluorescent seeds, which contain an average concentration of aflatoxin 400 to 2,300 times greater than non fluorescent seeds [9], and its products. It has been shown that even though fluorescent seeds are 2-9% (mean 6%) of the total seed weight lot, 80-100% (mean 96%) of aflatoxin was concentrated in them [10]. It is also important to select varieties of crops which are less prone to infection by fungi responsible for the production of aflatoxins [8].

The occurrence of aflatoxin is highly undesirable in cotton seed cake/meal for animal feed as it may produce nutritional problems. Moreover, the biologically active metabolite can be carried over in the meat [11] and eggs [12]. Large-scale detoxification of aflatoxin in cotton seed meal can be made by ammoniation process [13] for use in poultry and animal feed. Effective methods for pink boll worm control will be necessary if the aflatoxin problem is to be minimized in cotton seed.

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