

Aflatoxins in bird feed collected from various areas of Lahore city

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Abstract

The objective of this study was to estimate total aflatoxin in bird feed. Total 50 birds feed samples were collected from different markets and birds shops in Lahore. Total aflatoxin B₁, B₂, G₁, and G₂ were estimated using the Thin Layer Chromatography (TLC) method. The daily intake of aflatoxin in bird's feed is a 300ppb threshold by FDA. Total 50 samples were examined, among them 22% were contaminated with aflatoxin B₁ and B₂ and the rest of 40% samples were contaminated only with aflatoxin B₁. Aflatoxin G₁ and G₂ were not detected in any feed. The results showed that 42% of samples were contaminated within the permissible limit, 20% were unfit due to having above the allowable limit, and 38% were fit, which did not show any aflatoxin. The current study has demonstrated the easy way of providing the determination of aflatoxin in bird's feed.

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Introduction

The discovery and separation of aflatoxin are well known to be an aftereffect of examinations on the puzzling Turkey-X disease in 1960, which brought about the loss of a few thousand turkey poults in the United Kingdom. The reason for significant mortality in turkey poults and similar outbreaks in other farm animals could be connected with the utilization of rotten Brazilian shelled nut meals in the diet of affected animals (Yunus *et al.*, 2011). The name aflatoxin using the first letter from *Aspergillus* and the initial three letters from *flavus* were proposed in 1962 (Patterson, 1977). Aflatoxin was around the same time, detached in crystalline shape in the Netherlands (Vander *et al.*, 1962) and isolated into two segments, B and G, in the United Kingdom (Nesbet *et al.*, 1962).

One of the major groups of mycotoxins that cause aflatoxicosis in living creatures is aflatoxin (Aquino and Corrêa, 2011). Aflatoxin, presumably the most considered and primarily known mycotoxins, was initially noted in the early 1960s. They are among the most potent mutagenic substances known; there is extensive trial and epidemiological confirmation that they initiate liver cancer (Boutrif, 1998). The significant aflatoxins (afs) comprise

aflatoxins B₁, B₂, G₁ and G₂ created by certain toxigenic strains of *A. flavus*, *A. parasiticus*, and *A. nominus*. Aflatoxin M₁ (AFM₁), a hydroxylated metabolite, found essentially in animal tissues and liquids (milk and urine) is a metabolic result of aflatoxin B₁ (AFB₁), and these mycotoxins are not found in feed grains. The term aflatoxins allude to a class of substance mixes of related structures; among them, aflatoxin B₁ is considered the most potent cancer-causing agent (Boutrif, 1998). Aflatoxins can affect various animals, including fishes, rodents, waterfowl, poultry, swine, and dairy cattle (Kumar *et al.*, 2017). Aflatoxins can be available in the milk of dairy cows, the meat of swine, or chicken eggs if the creatures devour adequate quantity in their feed (Aquino and Corrêa, 2011).

A wide variety of nuts, cereals, and grains are available for birds in the food market. Mycotoxins probably contaminate these feed ingredients under some circumstances. Among all mycotoxins the most important aflatoxin has been detected in wild bird's food (Scudamore *et al.*, 1997).

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On the International Union for Conservation of Nature (IUCN) Red List, the red-crowned Crane has been classified as an endangered and rarest species. The current population is evaluated to be 2750 individuals. In late October, a large proportion of the people migrated from China to Yenchang. In early March, it overwinters in Yenchang Biosphere Reserve, China's largest wintering area (Ma and Tang, 1999; Wang, 2008).

Diets for Red-crowned Crane are rice, grains, wheat seedlings, shrimps, crabs, shellfishes, fishes, seepweed seeds, and leaves. However, they can eat both plants and animal origin, so they are called omnivorous (Dong *et al.*, 2005). Some foods are contaminated with mycotoxins, especially aflatoxin, due to the climatic zone in which Yenchang is located with temperature and moistness suitable for mold growth and aflatoxin production (Fan, 2008). Such conditions may have a harsh effect on the fitness and reproduction of these cranes and thus probably threatens to population (Liu *et al.*, 2016).

Aflatoxin also impacts wildlife populations, and the significance of the problem for wildlife is mainly unknown (Higgins *et al.*, 1992). In the field of nature, data concerning the impacts of aflatoxins are limited. Exposure of agricultural grains to moisture and other environmental conditions present in the fields can lead to significant production of AF. However, in 1977-1978 wintering season, extensive waterfowl died off in the two separate areas of Texas; approximately 7000 ducks and 500 geese scumbled to aflatoxicosis after consuming contaminated grain. Because of supplemental feeding, northern bobwhite may also encounter AF contaminated grain (Hernández and Guthery, 2012). Most feeders have open feeder ports that permit coordinated access of dampness to grain within the feeder. *Aspergillus* requires humidity between 70 and 90% and temperature between 6 and 46°C. If the moisture content of the substrate is between 12 and 17%, *Aspergillus* spp. can grow with this lower relative humidity. In the rolling planes of Texas and Oklahoma, these environmental conditions may occur throughout the year. Though fungal growth on grain in feeders is often readily apparent, feeders are rarely cleaned, and new feeds are often added to that already presents in feeders. This practice may promote the production of AF in feeders and cause aflatoxin in northern bobwhite (Smith and Moss, 1985).

In poultry and livestock, the cause of liver damage is mostly occurring by aflatoxin, the most frequently known mycotoxins. They lower the profitability of poultry production by reduced growth, feed conversion efficiency, egg production, and break in immunity leading to heavy financial losses. Many investigators did a survey of mycotoxins in different feedstuffs of plant origin done worldwide (Ueno, 1977; Siame and Lovelace, 1989). Poultry feed, namely starter, grower, developer layer, and branare found to be most common. During pre-harvest and post-harvest, fungal attack and aflatoxin production may occur, which are encouraged by environmental factors like temperature and humidity (Khan *et al.*, 2005).

During crop production and storage, cereals and plant proteins used in poultry feeds have been associated with contaminants produced by molds that can deteriorate food and feeds and adversely affect living creatures (Khan *et al.*, 2005).

In poultry mesenteric venous blood, the Aflatoxin B1 gets absorbed immediately by the small intestine (Hsieh and Wong, 1994). To prevent aflatoxin buildup in poultry feeds, regular monitoring of AFB1 in poultry feed is essential (Rosa *et al.*, 2006).

Materials and methods

Sample collection

Bird feed samples were collected from different bird shops of Lahore. Among them, 10 samples each were collected from Samanabad, Allama Iqbal town, Itihad colony, 10 from Shadman, and Bagh Gul Begum as shown in the map of Lahore city. Samples were prepared for Aflatoxin determination by the Begum's method (Begum *et al.*, 1985). Aflatoxins were detected by Romer's method. Estimation of Aflatoxins in extracts was made by comparison with standard technique (AOAC, 2005).

Chemical and reagents

Distilled water (25 ml), Diatomaceous earth (25g) (JJS Minerals Co., Ltd., UK), Chloroform (150 ml) (Fine Chemicals and Scientific Co., India), Diethyl Ether (Nandkrishna Chemicals Private Limited, India), Acetone (Kodak Solvent Recovery, Rochester, NY), Chloroform (Pharma Traders Pvt., Ltd., India), Benzene (Buneesha Chemicals Pvt., Ltd., India), Acetonitrile (Hudson Chemicals, Staten Island, NY).

Table I. Results of total aflatoxins B₁, B₂, G₁, G₂ in bird's feed

Sr. No	Samples	Aflatoxin				Total Aflatoxin
		B1	B2	G1	G2	B ₁ +B ₂ +G ₁ +G ₂
1	S 1	98.917	--	ND	ND	98.917
2	S 2	118.7	--	ND	ND	118.7
3	S 3	385.77	--	ND	ND	385.77
4	S 4	93.67	23.32	ND	ND	116.99
5	S 5	--	--	ND	ND	--
6	S 6	96.54	--	ND	ND	96.54
7	S 7	--	--	ND	ND	--
8	S 8	96.97	--	ND	ND	96.97
9	S 9	102.8	--	ND	ND	102.8
10	S 10	96.03	--	ND	ND	96.03
11	S 11	--	--	ND	ND	--
12	S 12	--	--	ND	ND	--
13	S 13	--	--	ND	ND	--
14	S 14	97.45	--	ND	ND	97.45
15	S 15	93.32	--	ND	ND	93.32
16	S 16	91.7	--	ND	ND	91.7
17	S 17	--	--	ND	ND	--
18	S 18	79.13	--	ND	ND	79.13
19	S 19	92.71	23.06	ND	ND	115.77
20	S 20	101.976	25.36	ND	ND	127.336
21	S 21	356.10	--	ND	ND	356.10
22	S 22	--	--	ND	ND	--
23	S 23	96.50	24	ND	ND	120.5
24	S 24	--	--	ND	ND	--
25	S 25	103.47	25.7	ND	ND	129.17
26	S 26	365.99	--	ND	ND	365.99
27	S 27	--	--	ND	ND	--
28	S 28	105.2	26.17	ND	ND	131.37
29	S 29	103.0	25.64	ND	ND	128.64
30	S 30	--	--	ND	ND	--
31	S 31	336.31	--	ND	ND	336.31
32	S 32	346.21	--	ND	ND	346.21
33	S 33	--	--	ND	ND	--
34	S 34	326.42	--	ND	ND	326.42
35	S 35	--	--	ND	ND	--
36	S 36	136.5	33.95	ND	ND	170.45
37	S 37	108.80	27.06	ND	ND	135.86
38	S 38	375.88	--	ND	ND	375.88
39	S 39	--	--	ND	ND	--
40	S 40	--	--	ND	ND	--
41	S 41	316.53	--	ND	ND	316.53
42	S 42	--	--	ND	ND	--
43	S 43	--	--	ND	ND	--
44	S 44	306.6	--	ND	ND	306.6
45	S 45	--	--	ND	ND	--
46	S 46	109.7	27.3	ND	ND	109.7
47	S 47	--	--	ND	ND	--
48	S 48	--	--	ND	ND	--
49	S 49	395.66	--	ND	ND	395.66
50	S 50	150.35	37.40	ND	ND	187.75

aflatoxin in bird's feed is 300ppb (Grace *et al.*, 2015). If the amount of aflatoxin exceeds this level, it may cause toxigenic effects on birds and be considered unfit samples. If the amount of aflatoxin in feed samples is within the permissible limit, these samples are considered contaminated but have fewer toxigenic effects. The results showed that 42% were contaminated within the allowable limit, 20% samples were unfit, which were above the permissible range, and 38% samples were fit that didn't show any aflatoxin.

Conclusion

The present study concluded that the levels of aflatoxins in the given feed samples were 62%. Among them, 22% were contaminated with aflatoxin B1 and B2 and the rest of 40% samples were contaminated only with aflatoxin B1. Aflatoxin G1 and G2 were not detected in any feed. The permissible level of U.S. Food and drug administration of aflatoxin in birds' feed is 300ppb. The present study is concluded that this level of aflatoxins mostly above the permissible limit. Thus, the consumption of average amounts of these feeds poses a health risk for the consumer.

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