Pak. J. Biochem. Mol. Biol. 2010; 43(1): 37-40

Aflatoxin B_1 contamination in poultry feed available in local markets of Peshawar

Shahid Ali[3], Arbab Riaz Khan[1], Ghazal Miraj[2], Shams-ur-Rehman Afridi[1] and Ghulam Mueen-ud-din[1]*

[1]Food Biochemistry Section, PCSIR Laboratories Complex, Peshawar, Pakistan

[2]Biochemistry Section, Agricultural Research Institute, Tarnab, Peshawar, Pakistan

[3]Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar, Pakistan

Abstract: Poultry feed samples (starter and finisher) of seven companies available at local markets of Peshawar were collected and analyzed for their Afla B_1 content using thin layer chromatography (TLC). The proximate composition of these feeds was also determined to correlate Afla B_1 with some of these compounds in the feed. The result regarding Afla B_1 of poultry feed (finisher) indicated maximum (27.07%) content as compared to starter feeds. Among the various samples Company # G had highest Afla B_1 (26.60% and 27.07%) in both starter and finisher feed respectively. The moisture content revealed that there was positive correlation ($R \geq 0.97$) between moisture and Afla B_1. Other parameters including protein, fiber, fat, NFE and energy had negative correlation ($R \leq 0.6$) with Afla B_1. From the present data it could be concluded that the samples contained a substantial amount of toxin, which might become a risk for poultry industry. As this was mostly affected by the moisture level of the feed, so the moisture must be kept reduced to minimize such dangerous risk of toxin contamination.

Keywords: Aflatoxin B_1, poultry, feed, moisture, chromatography.
Received: December 20, 2009 **Accepted:** February 02, 2010
***Author for Correspondence:** arkhan144@yahoo.com

INTRODUCTION

Feed plays an important role in the economics of poultry production. These constitute about 60 to 70 percent in cost of production of eggs and poultry meat[1]. A balanced poultry ration is based on cereals for energy, vegetable and animal protein sources for amino acids and additives containing vitamins and minerals[2]. Poultry feed consist of cereals (coarse grains), plant protein source, animal protein source, agro-based industrial by-products, vitamins, minerals, antibiotics, maize, sorghum, broken rice, fish meal, meat meal, blood meal, decorticated cotton seed meal, til cake, toria cake, corn gluten meal, guar meal, ground-nut cake, sun flower cake and soybean meal[3] which contributes carbohydrates, protein, fat, minerals and vitamins to the feed. The dehydrated lucern improve the quality of meat and egg in case of poultry[4].

Poultry feed may be contaminated with several toxins that cause adverse effects to poultry health. Among these toxins, mycotoxins (Alfatoxins) are most fatal and produced by a group of common fungal molds including *Aspergillus parasiticus* and *Aspergillus flavus*. These molds are ubiquitous in areas of the world with hot, humid climates. They are found in animal feed and contaminate human dietary staples in these climates[5]. The secondary metabolites of fungi are produced in cereals and other crops in the field during growing stage, harvesting stage and in storage stage. As cereal grains progress through harvesting, storage, feed manufacturing and delivery to the farm, the level of mycotoxins contamination generally increase[6]. Among several mycotoxins that occur frequently in naturally contaminated food and feed, aflatoxins have gained considerable attention because they are most toxic and potent carcinogenic even in small quantities[7&8]. The maximum limit for aflatoxins in food in 1981 was fixed to be 50 parts per billion (ppb) or 50micro/kg[9,10].

The well-known aflatoxins are aflatoxin B_1 (AFB_1), aflatoxin B_2, (AFB_2), aflatoxin G_1 ($AFLG_1$) and aflotoxin G_2 ($AFLG_2$) in which aflatoxin B1 is a human hepatocarcinogen and liver carcinogen when fed to certain rodent species[11-13]. It also cause economic losses by decreasing growth performance in poultry and make the poultry susceptible to carcase bruising leading to downgrading[14]. They are widespread in food and always present to varying degrees in poultry feed. Poultry are probably the most sensitive to its toxic effects, and even small amount of AFB_1 cause reduction in growth rate, feed efficiency, hatchability and increased susceptibility to diseases[15]. The signs of aflatoxicosis in poultry are not specific. Impaired growth rates and feed conversion, increased mortality from infectious diseases and signs resembling nutritional disorders are typical. Loss of pigmentation and passage of undigested feed particles have been noted. Young poultry are much more susceptible to aflatoxicosis than are mature birds.

Therefore, the study was designed to observe the contamination of Aflatoxins B_1 in the poultry feeds collected from the local markets of Peshawar. This study equipped us with information about prevalence and severity of Aflatoxin B_1 in poultry feed. The information is helpful for higher authorities to establish regulation and safe limits for this toxin in feed, and would be utilized to improve poultry industry in Pakistan.

MATERIALS AND METHODS

Sample collection

Total 14 samples (7 starter and 7 finisher) of poultry feed from different companies encoded A, B, C, D, E, F, G were collected from the local markets of Peshawar. All the information related to feed i.e. type; manufacturing and expiry date was noted separately for each sample. Information like storage temperature, humidity of stores, and length of time for storage of feed was also recorded.

Storage

To facilitate handling and for further storage, each sample was reduced to 1 kg. All the samples were grinded with a laboratory grinding mills and kept under ambient room conditions (temp, 25-30 ^{o}C and RH 50-68%) in ploythene bags for analysis. All the biochemical analysis was carried out in the Nutrition Lab. of Veterinary Research Institute (VRI) Peshawar.

Aflatoxin B_1 determination

AFB_1 was determined by the method of Jones[16] using thin layer chromatographic technique. Aflatoxin from feed was extracted using aqueous acetone (19 ml distilled water and 81ml acetone) procedure. After completion of drying in oven the residue was dissolved in 5 ml chloroform. It was spotted on silica gel plate of about 0.5 mm thickness by TLC spotter of Romer Lab. After development the plates were air dried and observed under UV light. The fluorescence intensities of aflatoxin spot of sample were compared with those of standard spot. The sample spot which would match one of the standard spot was selected. The amount of aflatoxin was estimated using the tabulated value (Table 1).

Table 1: amount of aflatoxin in standard and samples.

AFB_1/G_1 = 1µg/ml		AFB_2/G_2 =.025µg /ml	
Standard	**Sample (0.225g)**	**Standard**	**Sample (0.225g)**
10 µl (10ng)	44.4 ppb	10 µl (2.5ng)	11 ppb
20 µl (20 ng)	88.8 ppb	20 µl (5ng)	22 ppb
30 µl (30 ng)	133.3 ppb	20 µl (7.5 ng)	33 ppb
40 µl (40ng)	177.7 ppb	40 µl (10ng)	44 ppb
60 µl (6ong)	266.6 ppb	60 µl (15 ng)	66.6 ppb

Proximate composition

Moisture, ash, crude fat, crude protein and crude fiber were determined by standard method of AOAC[17]. Nitrogen free extract (NFE) value was obtained by subtracting the sum of the percentages of moisture, crude fiber, crude protein, crude fat and Ash from 100. Energy value in calories was computed by multiplying the protein and carbohydrate percentages by 4.1 and that of Crude fat by 9.1[18].

Statistical analyses

The analysis of variance of data was computed by Randomized Complete Blocked Design (RCBD). The means were compared by least significant difference test (*LSD*). The aflatoxin B_1 was correlated with proximate composition of poultry feed by correlation analysis. All the statistical analysis was carried out by using computer software Mstatc.

RESULTS AND DISCUSSION

Aflatoxin B_1

Statistical analysis of data (Table 3) revealed that Aflatoxin B1 content of starter feed was significantly ($P \leq 0.05$) different from one another. Mean values of starter data indicates that maximum (26.6%) AFB_1 was noted in sample G while minimum (20.57%) was recorded in sample A. Finisher feed followed the same pattern having maximum (27.07%) AFB_1 in sample G and minimum (22.57%) in sample A. This was above the safe limit (maximum 20 µg / kg as recommended by FDA) for poultry. Similar results were obtained by Bahri[19] who analyzed 86 sample of poultry feed and found that most of the samples contained aflatoxins with the highest prevalence of AFB_1 (96.5%), followed by AFB_2 (76.6%), AFG_1 (45.3%) and AFG_2 (25.6%). Proximate composition for starter and finisher feed of various brands is presented in Table 2.

Table 2: Composition of starter and finisher feed.

Sample	Starter	Finisher
A	20.57 b	22.57d
B	20.80 f	22.80 d
C	22.57 e	24.60 c
D	22.80 d	24.80 c
E	24.60 c	26.57 b
F	24.80 b	26.80 ab
G	26.60 a	27.07 a

Moisture content

Mean values of moisture indicate that Maximum (8.60%) moisture was noted in starter sample G while minimum (6.20%) was recorded in sample A. Also finisher feed followed the same pattern having maximum (9.80%) moisture of sample G and minimum (6.33 %) in sample A. The optimum moisture content for the growth of *A.flavus* and production of aflatoxin is about 8% and 85-90%

respectively[20]. This suggests that the risk of aflatoxin contamination can be greatly minimized if the moisture content is kept below these critical levels during storage.

Protein content

Statistical analysis of data of feed revealed that protein content of starter feed was significantly (P≤ 0.05) different from one another. Maximum (22.40%) protein was noted in sample A while minimum (20.60%) was recorded in sample G. In Finisher feed maximum (20.80%) protein was observed in sample A and minimum (19.20%) in sample G. The protein contents of poultry feed (starter and finisher) in this study was in agreement with that of Bhatti[21], who reported 15.86 -18.809% protein in starter and 18.63-19.21% in finisher poultry feed.

Crude fat

Fat concentrations in both starter and finisher feed of various brands are also significantly (p≤ 0.05) different from each other. For starter feed maximum (4.80%) value fat was recorded in sample A and minimum (2.5%) in sample G. Also finisher feed followed the sample pattern having maximum (4.50%) fat in sample A and minimum (2.20%) in sample G. The value found Crude fats in the present study are less in comparison with that of Roy[4] who reported that fat content in different brands of commercial poultry feed in the range of 5.3-7.17% for starter and 6.00-8.00 in finisher feed. The low content of fat may be due to contamination of aflatoxin in poultry feed[21].

Crude fiber

Fiber concentrations of both starter and finisher feed of various brand was also significantly (P≤ 0.05) different from each other. Mean value of data presented in Table 2 indicated that fiber content of starter feed is maximum (5.50%) in sample B and minimum (3.20%) in sample G. Finisher feed also showed the same trend with the highest value (45.33%) in sample A and minimum 3.00% in sample G. These observations fairly corresponds to those of Bhatti[3] who reported crude fiber in the range of 3.5-9.5% of starter and 3.0-9.1% for finisher poultry feed.

Ash content

Mean values of starter data showed that maximum ash (4.93%) was present in sample G, and minimum in sample A (3.20%). Exactly the same pattern was followed by finisher feed. Results of the present analysis was in good line with that of Roy[4] who reported in the range of 5.5-11.7% and 5.3-8.6% ash in starter and finisher poultry feed respectively.

Nitrogen free estimation (NFE)

NFE content for starter and finisher feed presented in Table 2, which shows significantly (P≤ 0.05) difference in samples. Mean value of the starter and finisher data indicates maximum (60.17%) NFE in sample G while minimum (58.20%) in sample A. The results of the presents study are in the agreement of Roy[4] who reported NFE in the range of 53.00 - 61.4% and 58.1 - 60.61% respectively in starter and finisher feed in various brands of poultry feed.

Energy content

The means energy content of both starter and finisher feeds were significantly (P≤ 0.05) different. It could be seen from the data that sample A of starter and finisher feed had maximum energy content (390.3 Kcal/g and 389.0 Kcal/g respectively) as compared to started and finisher sample G with minimum energy value of 370.0 Kcal/g and 360.6 Kcal/g, respectively.

Table 1: Determination of Aflatoxin B1 (ppb) for different starter and finisher feed.

	Protein		Moisture (%)		Ash (%)		Fat (%)		Fiber (%)		Energy (Kcal)		NFE	
Sample	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
A	22.40 a	20.80 a	6.200 g	6.333 g	3.200 f	3.700 g	4.800 a	4.500 a	5.200 b	4.533 a	390.3 a	389.0 a	58.20 b	60.13 bc
B	22.10ab	20.50 b	6.400 f	6.700 f	3.500 e	4.200 f	4.500 b	4.200 b	5.500 a	4.500 ab	385.4 b	383.9 b	58.00 b	59.90 cd
C	21.80bc	19.87 c	7.300 e	7.633 e	3.800 d	4.500 e	4.200 c	3.500 c	4.800 c	4.200 b	381.8 c	376.6 c	58.10 b	60.30 ab
D	21.50 c	19.80 cd	7.500 d	7.800 d	4.300 c	4.800 d	3.700 d	3.300 d	4.500 d	3.800 c	377.7 d	375.3 c	58.50 b	60.50 a
E	21.87 bc	19.70 d	8.200 c	8.800 c	4.600 b	5.200 c	3.300 e	2.800 e	3.800 e	3.500 cd	374.5 e	368.2 d	58.23 b	60.00 cd
F	20.83 d	19.50 e	8.400 b	9.400 b	4.833 a	5.500 b	2.800 f	2.500 f	3.500 f	3.300 de	371.5 f	363.7 e	59.63 a	59.80 d
G	20.60 d	19.20 f	8.600 a	9.800 a	4.933 a	5.800 a	2.500 g	2.200 g	3.200 g	3.000 e	370.0 g	360.6 f	60.17 a	60.00bcd

Correlation between AFB1 and proximate composition

The moisture content revealed that there was positive correlation (R≥0.97) between moisture and AFB1 that was with the increase in moisture content the AFB1 content also increased. Other parameters including protein, fiber, fat, NFE and energy had negative correlation (R≤0.6) with AFB1.

CONCLUSIONS

From the present data it could be concluded that the samples contained a substantial amount of toxin, which might become a risk for poultry industry. As this was mostly affected by the moisture level of the feed, so the moisture must be kept reduced to minimize such dangerous risk of toxin contamination.

As the aflatoxin content of the feeds (starter and finisher) was positive correlates with moisture content and also affected by storage condition so feeds should be dried before storage to reduce the risk of fungal infection and subsequent toxin contamination. Aflatoxin contamination of different stored poultry feed should be monitored to ensure feed safety.

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