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AFLATOXINS IN VARIOUS FOODS AND FEED INGREDIENTS

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Cereals, pulses, nuts and other food and feed ingredients were collected from different regions of Pakistan were screened for aflatoxin contamination. The presence of mycoflora on these commodities were also examined. Samples of wheat, rice, pulses and beans showed negative results although toxigenic strains of *Aspergillus flavus* were present on some of the samples. Maize and maize products showed the presence of aflatoxin B_1 and B_2 with a concentration range of upto 800 µg/kg. It ranged from 400-800 µg/kg in pea-nuts, pistachio nuts and wal-nuts. Poultry feed and its ingredients contained 8-1140 µg/kg of aflatoxin. Non- detectable levels of aflatoxin were noticed when the contaminated products were stored at 30° for 7 days or 40° for 3 days after treatment with 1.5% ammonia at 18% moisture. Chicks given detoxified ration showed improvement in weight gain and feed efficiency.

Key words: Aflatoxin, Mold, Food

Introduction

Molds produce a host of chemicals such as antibiotics, which are very useful to human beings and others like mycotoxins which cause serious disease in man and animals.

It was in early sixties that a mysterious disease called Turkey X disease decimated one lakh Turkeys and thousands of other farm birds in south-east of England. The culprit was located to be the pea-nut meal [1-3] that had been imported from Brazil. It was given the name "Rosetti meal" from the name of the ship that brought thousands of tonnes of it to England. The meal was found to be contaminated with aflatoxins, a group of highly fluorescent compounds designated as B_1, B_2, G_1 and G_2: These toxins have the ability to cause cancer of liver. Of the many chronic effects, primary liver cancer is the major worry for man and poultry production. A 10% incidence of tumours has been recorded in Fisher in-bread rats when 1 ppb of aflatoxin B_1 was included in their diet [4] and rainbow trout produced a similar tumour at an aflatoxin diet level of 0.1 ppb [5].

Mycotoxins can enter the human food chain directly, if people eat contaminated flour, pea-nuts, pistachio-nuts and wal- nuts as dry fruit and bakery and confectionary products or indirectly through meat or milk from animals feed on contaminated feedstuffs. Tropical countries like Pakistan are ideally suited for the growth of *Aspergillus flavus* and the production of aflatoxins. The present studies were undertaken to investigate the frequency of aflatoxin contamination in common food and feedstuffs of Pakistan.

Materials and Methods

The food/feed samples were either purchased from the local market or were supplied by various Government and Private agencies. Shina, pistachio-nuts and almond samples were received from Baluchistan. Raw ground-nuts from Rawalpindi area and dried skim milk from LPRI Bahadarnagar. PASSCO supplied wheat samples. Feed and feed ingredients were supplied by poultry feed manufacturers of the country.

Romer's minicolumn methods [6] was followed for the detection of aflatoxins. The sample after extraction with acetone water mixture (85 : 15) was purified with $FeCl_3$ slurry and $CuCo_3$. It was further extracted with chloroform. The chloroform extract was allowed to drain through minicolumn by gravity (packed with drierite, florisil, silica gel, neutral alumina and drierite). A blue fluorescent band at the top of florisil was judged to contain aflatoxins. Confirmation of aflatoxin was done by the derivative formation [7].

Estimation was carried out by T.P.I. standard procedure [8,9] TLC plates coated with silica gel G and silica gel GHR (Merck) were used alongwith extra pure solvents. Detoxification was carried out with ammonia at ambient temperature and pressure [10].

Biological experiments were conducted on day old (Hubbard strain) broiler chicks for eight weeks [11].

Results and Discussion

The moisture content of the samples (Table 1) varied from 1.64% to 25.94%. Similarly moisture content of maize samples (6.11 - 14.49%) was slightly lower than wheat (6.47 - 15-13%). Three hundred and twelve samples of food were screened for the presence of aflatoxins and only 36 samples were found contaminated. Among the dry fruits, wal-nuts showed 18.75% contamination followed by pistachio-nuts 11.0% and ground-nuts 6.0% (Table 2).

In poultry feed out of 194 samples, twenty eight were contaminated with aflatoxins (Table 3). Aflatoxin content of these samples varied from 8-1140 µg/kg. Forty percent of the maize gluten samples were found to be contaminated with

TABLE 1: MOISTURE CONTENTS OF SAMPLES ANALYZED

No.	Material	Moisture Content (%)
1.	Wheat and wheat flour	6.47 - 15.13
2.	Rice	6.93 - 14.95
3.	Maize and maize flour	6.11 - 14.49
4.	Oat	8.81 - 13.02
5.	Ground nut	3.68 - 9.47
6.	Pistachio nuts	3.20 - 8.50
7.	Walnuts	1.64 - 6.63
8.	Almonds	3.05 - 4.81
9.	Dry Dates	14.50- 14.61
10.	Shina	5.77 - 8.26
11.	Fig	12.42- 15.30
12.	Coconuts	1.85 - 5.36
13.	Chalghoza	1.85 - 5.95
14.	Raisins	11.34- 16.62
15.	Pulses and beans	2.91 - 25.94
16.	Poultry feed	7.08 - 15.40
17.	Poultry feed ingredients	5.80 - 18.21

TABLE 2. AFLATOXIN CONTAMINATION IN FOOD

Food Material	No. of samples	No. of contaminated samples	Aflatoxins ($B_1 + B_2$) identification	Total aflatoxin concentration ($B_1 + B_2$) µg/kg
Maize and maize flour *Zea mays*	130	16	+	133 - 800
Groundnuts *Ahachis hypagae*	100	6	+	24 - 800
Pistachio nuts *pistachio vera*	18	2	+	00 - 200
Walnuts *Juglans regia*	64	12	+	80 - 400

Samples of Wheat and Wheat flour *Triticum vulgars* (1000), Rice *Oryza sativa* (100), Almonds *Prunus amygdalus* (14), Figs *Fecus- carica* (30), Coconut *Cocos-nucifera* (40), Chalghoza *Pinus-gardina* (24), Raisins *Vitis vinifera* (35), Dal chana *Cicer arietinum* (55), Dalmash *Phaseolus radiatus* (63), Dal Masoor *Lens esculenta* (33), Dal mong Phaseolus - mungo (25), Green beans (50), Chick pea (6), Refined sugar (8), Dried Milk (43), Supari *Betel nut* (26), Spices (12), Fresh liver (12) and Eggs (24) were also investigated but did not show any contamination.

TABLE 3. AFLATOXIN CONTAMINATION IN FEED

Feed Material	No. of samples	No. of contaminated samples	Aflatoxins (B_1+B_2) identification	Total aflatoxin concentration ($B_1 + B_2$) µg/kg
Poultry feed	194	28	+	13 -1000
Cotton seed meal	2	1	+	8
Cotton seed cake	128	6	+	24 - 160
Sunflower cake	3	2	+	24 - 200
Corn cake	2	1	+	00 - 266
Rice polish	9	1	+	00 - 240
Maize glaten	32	13	+	40-1140
Dried bread	3	1	+	00 - 640

Samples of Mustard cake (24), Rape seed meal (24), Sesame Cake (12), Wheat midlings (2), Wheat bran (3), Fish meal (13), Rice bran (7), Rice bran (3) Meat meal (10), Sorghum (6), Bone meal (9), Til cake (3), Guar meal (2), Blood meal (10), Maize bran (2), Matri (2) and Vitamin premix (1) were also investigated but did not show any contamination.

aflatoxins which ranged from 40-1140 µg/kg. Sunflower and cotton seed cake contained 24-200 µg/kg and 24-160 µg/kg respectively of aflatoxin.

A look at the fungal load of food and feed samples showed that *Aspergillus flavus* was present on most of the samples.

Detoxification experiments. Corn detoxification experiments are presented in Table 4. With 1.5% ammonia (w/w), moisture 18% and the incubation temperature 20 + 2° 14 days were required to reduce the aflatoxin B_1 content from 500 µg/kg to 6 ug/kg. Increase in incubation temperature to 30 ± 2 reduced the period to 7 days for non-detectable levels. Other parameters being the same; it was further reduced to 3 days at 40 ± 2°C.

TABLE 4: EFFECT OF AMMONIA AND MOISTURE LEVELS IN TREATED CORN ON RESIDUAL AFLATOXIN B_1 CONTENT FOR REACTION AT 20,30 AND 40°C

NH^3 added percent of dry matter	Corn moisture, percentage 12.0	15.0	18.0	21.0
	Residual aflatoxin B_1, µg/kg			
	4 days at 20 °			
0.5	160	90	50	55
1.0	75	50	33	25
1.5				
2.0	26	15	12	10
	7 days at 30 °			
0.5	90	50	33	30
1.0	45	30	20	25
1.5	20	12	ND	5
2.0	12	10	8	8
	3 days at 40 °			
0.5	50	35	20	28
1.0	33	29	15	15
1.5	16	9	ND	8
2.0	10	6	6	7

Initial aflatoxin B_1 content was 500 ug/kg
All readings are average of two sets of values
ND: Non-detected [10].
Hamid & Shah, Pak.J.Sci.Ind.Res., 26; 384(1983)

Biological Experiments. The detoxified feed [10] was fed to day old chicks and the results are detailed in Table 5. Average weight gained by the chicks fed on ration A (Control), B (Control ammoniated), C (Contaminated) and D (Detoxified) was 1641, 1916, 1345 and 1734 g respectively. Average feed consumed by the chicks fed on ration A,B,C and D was 4713, 4855, 4840 and 4944 g respectively and average cumulative feed efficiency values were 2.87, 2.53, 2.59 and 2.85 respectively.

Agricultural practices combined with tropical condition in Pakistan, especially during monsoon season, create conditions conducive to fungal invasion and the consequent production of mycotoxins. High moisture contents encourage

TABLE 5: AVERAGE WEIGHT GAIN, FEED CONSUMPTION, FEED EFFICIENCY, MORTALITY, DRESSING PERCENTAGE AND WEIGHT OF INTERNAL ORGANS

Particulars	Rations			
	A Control	B Control ammonia-ted	C Contami-nated	D Contami-nated ammoniated
No. of chicks	30	30	30	30
Days on experiment	56	56	56	56
Average initial weight/chicken	45	45	45	45
Average final weight/chicken (gm)	1686	1961	1390	1779
Average total weight gained/chick (gm)*	1641	1916	1345	1734
Average total feed consumption/chick (gm)*	1713	4855	4840	4944
Average total feed efficiency**	2.87	2.53	3.59	2.85
Average mortality(%)	10.00	6.66	30.00	13.33
Average dressing percentage*	53.03	57.21	53.55	55.02
Average heart weight (gm)	13.00	14.66	11.66	13.33
Average liver weight (gm)**	51.3	057.16	58.00	51.33
Average gizzard weight (gm)	38.60	34.25	41.00	42.66

* Significant at 5% level ** Significant at 1% level

mold propagation and production of toxins. But this is not the only factors. Although moisture content of raisins were higher than those of wal-nuts yet raisins were free of aflatoxins whereas wal-nuts were contaminated. The moisture was not available to fungi due to high sugar content in raisins but in case of wal-nuts the hairline cracks in the outer coat of the fruit, before drying, seems to be responsible for fungal invasion and production of aflatoxins. Similarly maize is harvested during wet season and is stored with high moisture contents which is conducive to fungal growth and hence toxin production. Wheat on the otherhand is harvested during the least humid and dry months which helps in further reducing the possibility of fungal attack. Presence of zinc and phytic acid in wheat bran is responsible for checking production of aflatoxin. Maize, a staple food of the people living in northern parts of Pakistan was found highly contaminated. This high concentration of toxin can cause health hazards. In fact high incidence of liver carcinoma is reported in the people whose staple diet is maize. Consumption wise contaminated ground-nuts present more health hazards than other because these are cheap and easily available.

EEC has set a tolerance limit of 20 µg/kg for feed and feed ingredients [12]. Except cotton seed meal all the contaminated samples had aflatoxin far exceeding the permissible tolerance limits. Aflatoxin contamination in poultry feeds can also be traced back to the contamination of ingredients. Left over pieces of bread are generally stocked and sold to hawkers who use it for feeding cattle. These were found to be heavily loaded with toxins (Table 3). It is a well known fact that aflatoxin M_1 and M_2 are excreted in the milk when the feed is contaminated with aflatoxins.

It can be concluded from the present investigation that the incidence of contamination is quite high and can enter human food chain via poultry meat, eggs, milk and milk products apart from causing severe economic losses to the poultry farmer.

As *Aspergillus flavus* is present on most of the samples, provision of proper environment including nutrients results in the production of toxin which will cause morbidity/mortality of poultry and animals fed on the feed and results in liver damage or cancerous growth.

Detoxification. Aflatoxin contamination of corn seems to be the result of cultural practices and uncontrolable environmental conditions. Studies were undertaken to establish optimum conditions of temperature and pressure. Corn detoxification results are in Table 4. Bagley [13] reported pronounced effect of temperature on the changes of aflatoxin level with time. It was reported that 600 µg/kg of aflatoxin contamination at 15% moisture level in the corn was reduced to below the FDA guide line of 20 µg/kg in about 3 weeks at 25°, but in only 3 days at 38°. It has been observed that low temperature reduced volatility of ammonia thus potential material handling losses in large scale application were reduced. Temperature of the ammoniated corn should be adjusted to 30 ± 2° or higher for adequate detoxification within a normally acceptable time period.

General performance. Aflatoxin contaminated feed after ammoniation gave better results in respect of weight gain than other rations. Lower weight gain was observed when contaminated ration was fed. Ammoniation of the feeds (control as well as contaminated) seems to improve the nutritive value of the feed as is evident by the weight gain results. Maximum feed was consumed by the chicks receiving ration D and minimum by ration A. Feed efficiency values were high in ration B which was ammoniated control and lower in feed C (contaminated). Feed efficiency values of ration D was comparable with the control. Results indicated that ammoniation of feeds improved the feed efficiency. It is evident from the data that ammoniation helped in lowering the mortality rate thus indicating the destruction of aflatoxin. Livers with similar weight were observed in ration A and D suggesting thereby that the detoxified feed was equal in comparison with the control regarding aflatoxin inactivation. Colour of the liver were normal in chicks given ration A and D. Spots were evident in liver of the chicks fed on contaminated feed were consistent with a report by Tung *et al* [14, 15] who stated that dietary aflatoxin increased the susceptibility of broiler chicken to bruising. Impaired blood coagulation during aflatoxicosis may also be involved in the occurrence of haemorrhages.

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