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Detoxification of Aflatoxin B1 in Poultry and Fish Feed by Various Chemicals

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Abstract. In this study various poultry and fish feed samples were initially analyzed for presence of aflatoxin. All the samples were found contaminated with aflatoxin B1 only. Contaminated samples were treated with different organic and inorganic chemicals to detoxify aflatoxin B1 in poultry and fish feed samples. The maximum reduction in the aflatoxin B1 concentration was observed with 0.5% HCl as 14.20 ppb to 2.09 ppb (86.50%) in the poultry and 69.26 ppb to 10.46 ppb (84.89%) in fish feed samples.

Keywords: aflatoxin, detoxification, chemicals, thin layer chromatography

Introduction

Aflatoxins are harmful substances that can cause severe health hazards to human and animals due to which many economical problems originate. The contamination of animal feed with mycotoxins represents a worldwide problem for farmers. These toxins originate from molds whose growth on living and stored plants is almost unavoidable particularly under moist conditions (Alexander *et al.*, 2001). The fungal species *Aspergillus flavus* and *Aspergillus parasiticus* are very harmful for food and feed stuff, therefore the removal of these aflatoxins is extremely necessary. A close relationship exists between the quality of feed and the quality of animal products offered for human consumption. However, feeds can also be contaminated with a wide variety of compounds (Frank, 2011). Various chemicals have been used to kill pathogens in feed and feed ingredients. The detoxification effect of citric acid was investigated in rice samples and the results revealed the effectiveness of 1N citric acid in reducing aflatoxins levels in rice samples (Safara *et al.*, 2010). Some countries adjust aflatoxin levels in their foods e.g. USA and EU (Europe Union) permit level lower than 20 ppb and Korea and Japan 10 ppb (Chiavaro *et al.*, 2001). The alkali treatment using inorganic or organic bases is an effective and economically feasible method of degrading aflatoxins**.** Treatment of corn with less than 0.5% calcium hydroxide decreased aflatoxin levels by 43% and boiling 1600-ppb naturally contaminated corn with 3% sodium hydroxide at 100 °C for 4 min decreased total aflatoxins levels by 93% (Hamed, 2005). Detoxification of aflatoxins in the poultry mixed feed naturally contaminated at the level of 775.25 ppb was done using chemicals, *viz.* sodium bisulphite and sodium hydroxide (Singh *et al.*, 2003). Aflatoxins can be destroyed with calcium hydroxide (Bauer, 1994). Reduction to less than 10% of the original AFB1 content within 2 h was recorded when the medium contained 1.5% potassium permanganate, 2.5 and 5% chloramin B (lachema) or soda, and when there was 5% ammonia heated to 60 °C, 5% sodium hydroxide, potassium hydroxide or calcium hydroxide, or 50% chromo-sulphuric acid (Dvorak, 1990). Several compounds such as activated charcoal, aluminosilicates and processed cell wall of *Saccharomyces* sp. (esterified glucomannan) have shown to be effective as mycotoxin adsorbents in poultry feeds (Vieira, 2003). Chemical detoxification methods such as use of hydrogen peroxide (Sreenivasa *et al.*, 1967) and calcium hydroxide (Coker *et al.*, 1984), sodium hydroxide and ammonification have been investigated. Ammonia treatment was found to be the most effective and practical method for use in large scale feed processing plants with 95% of successful detoxification. Aflatoxin-contaminated commodities can be detoxified by a variety of methods based to some extent on economics and the physical and chemical

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characteristics of the substance being treated. In this study, use of different chemicals is evaluated for the detoxification of aflatoxin B1 in the contaminated samples of poultry and fish feed.

Materials and Methods

This study was conducted in Food and Biotechnology Research Centre of Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore. The poultry and fish feed samples were prepared for aflatoxin analysis by method of Begum *et al*. (1985). Aflatoxins were detected by Romer's method (Romer *et al.*, 1976). Estimation of aflatoxins in toxic extracts was made by comparison with standard technique (AOAC, 2005). Analytical laboratories use one of several procedures such as thin-layer chromatography, mini columns, gas chromato-graphy, or mass spectroscopy to determine aflatoxin levels. These procedures are highly accurate and quantitative. In this study thin layer chromatographic (TLC) technique was used for the determination of aflatoxin in all samples.

Materials. In the present study, all the chemicals used were of analytical grade procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (St. Louis, USA).

Preparation of samples for aflatoxins determination. ***Extract.*** The half laboratory sample was ground through Romer grinding mill. The other half sample was kept for reference. The ground sample was mixed properly and test portion was taken from this mixture. 50 g of ground sample was taken into 500 mL conical flask and 25 mL of water, 25 g diatomaceous earth and 150 mL chloro-form was added. After shaking for 30 min filtered through filter paper. Collected 2nd 50 mL portion $CHCl_3$ and evaporated on a steam bath.

Qualitative determination. Immediately spotted 5, 10 and 15 µL on TLC plate (Approximately 1.5 cm from the base). Spotted 5 µL standard on one spot in a duplicate as internal standard. The plate was developed with anhydrous ether in developing tank uptill half. After development in ether removed the plate from tank and let it dry. Redeveloped in same direction in TLC tank with acetone-chloroform (1:9) (v/v). Adjusted the acetone-chloroform ratio as needed to modify R_f of aflatoxins. Finally presence or absence of aflatoxins in test solution spot was observed.

Quantitative determination. For Quantitative analysis 1, 2, 3, 4 and 5 µL of test solution was spotted on silica gel coated plates. Similarly on same plate 1, 2, 3, 4 and 5 µL of aflatoxin standard was spotted. The fluorescence intensities of the spots were compared and the concentration of aflatoxins was calculated by applying the following formula

$$\text{Aflatoxins } (\mu g/kg) = \frac{S \times Y \times V}{W \times Z}$$

Where:

Z = Volume in µL of sample extract required to give fluorescence intensity comparable to that of S = µL of aflatoxins standard
S = Volume in µL of aflatoxins standard of equivalent intensity to Z (µL of sample)
Y = Concentration of aflatoxins standard in µg/mL
V = Volume in µL of solvents required to dilute final extract
W = Weight in g of original sample contained in final extract

Detoxification. There are many methods for detoxification of aflatoxins. Aflatoxins can be detoxified physically (sunlight), biologically (bacteria, soil) and chemically (Basappa and Shantha, 1996). In this study aflatoxins positive samples were detoxified by the treatment of different chemical solutions.

Detoxification by various chemicals. Ground samples (50 g) were kept into different 500 mL conical flasks. Chemical solutions of different compositions of HCl, CaOH, citric acid, Iso-propanol, sodium hypochlorite, sodium bisulphate, acetone and ethanol were added into different flasks. Conical flasks were shaken on wrist action shaker for 2 h and then filtered through filter paper and dried for 2 days.

Quantification after detoxification. Quantification of detoxified sample for aflatoxins was carried out by same method such as chloroform extraction, detection by thin layer chromatography, estimation through UV light and calculation by formula.

Results and Discussion

Aflatoxins are among the most powerful carcinogens, naturally occurring fungal toxic metabolites and pose significant health risks and acute toxicological effects to human beings as well as animals. Approximately 20 aflatoxins have been isolated from various fungal species. Among these aflatoxin B1 is most toxic and potent. Aflatoxin B1 received greater attention than any other mycotoxins because of its demonstrable carcinogenic

effect in susceptible animals and its acute toxic effects in human (Bressac *et al.,* 1991). The detoxification of aflatoxin B1 in poultry feed samples by using different chemicals is shown in Table 1.

It was observed that aflatoxin B1 is greatly reduced by 0.5% hydrochloric acid (Table 1) while no reduction in aflatoxin B1 was observed when 1% Iso-propanol was used in poultry feed samples. 50% calcium hydroxide and 0.4% sodium hypochlorite may be effective as detoxifying chemicals for aflatoxin B1 as 80% and 82.98% reduction of aflatoxin B1 was noted, respectively. There was no reduction of aflatoxin B1 in fish feed samples when 1% and 2% Iso-propanol were used as detoxifying chemicals. In case of fish feed samples 0.4% sodium hypochlorite reduced aflatoxin B1 upto 80.97% while 77.84% reduction in aflatoxin was observed when 50% calcium hydroxide was used (Table 2).

Table 1. Detoxification of aflatoxin B1 in poultry feed samples by using different chemicals

Poultry feed samples	Aflatoxin B1 (ppb)	Solvent used for detoxification (%)	Aflatoxin B1 (ppb) after treatment	Reduction in aflatoxin (%)
1.	B_1= 0.73	0.1 Hydrochloric acid	B_1= 0.49	32.80
2.	B_1= 15.20	0.3 Hydrochloric acid	B_2= 7.70	49.30
3.	B_1= 14.20	0.5 Hydrochloric acid	B_1= 2.09	86.50
4.	B_1= 4.30	5 Calcium hydroxide	B_1= 1.23	71.39
5.	B_1= 25.80	50 Calcium hydroxide	B_1= 5.16	80.00
6.	B_1= 3.14	10 Citric acid	B_1= 2.08	33.00
7.	B_1= 7.70	30 Citric acid	B_1= 2.80	63.00
8.	B_1= 4.38	1 Iso-propanol	B_1= 4.38	0.00
9.	B_1= 11.70	5 Iso-propanol	B_1= 10.89	6.90
10.	B_1= 74.78	0.3 Sodium hypochlorite	B_1= 22.59	69.70
11.	B_1= 63.90	0.4 Sodium hypochlorite	B_1= 10.87	82.98
12.	B_1= 30.73	2 Sodium bisulphate	B_1= 22.12	28.01
13.	B_1= 49.20	3 Sodium bisulphate	B_1= 31.0	36.90
14.	B_1= 10.20	99 Acetone	B_1= 3.18	68.82
15.	B_1= 25.15	96 Ethanol	B_1= 11.34	54.90

Table 2. Detoxification of aflatoxin B1 in fish feed samples by using different chemicals

Fish feed samples	Aflatoxin B1 (ppb)	Solvent used for detoxification (%)	Aflatoxin B1 (ppb) after treatment	Reduction in aflatoxin (%)
1.	B_1= 4.30	0.1 Hydrochloric acid	B_1= 2.89	32.79
2.	B_1= 12.56	0.3 Hydrochloric acid	B_1= 6.44	48.72
3.	B_1= 69.26	0.5 Hydrochloric acid	B_1= 10.46	84.89
4.	B_1= 28.65	5 Calcium hydroxide	B_1= 7.68	73.19
5.	B1= 3.52	50 Calcium hydroxide	B_1= 0.78	77.84
6.	B_1= 46.82	10 Citric acid	B_1= 31.36	33.02
7.	B_1= 10.63	30 Citric acid	B_1= 4.15	60.95
8.	B_1= 2.07	1 Iso-propanol	B_1= 2.07	0.00
9.	B_1= 1.23	2 Iso-propanol	B_1= 1.23	0.00
10.	B_1= 7.28	5 Iso-propanol	B_1= 6.78	6.86
11.	B_1= 6.55	0.3 Sodium hypochlorite	B_1= 2.01	69.31
12.	B_1= 25.12	0.4 Sodium hypochlorite	B_1= 4.78	80.97
13.	B_1= 23.62	3 Sodium bisulphate	B_1= 14.89	36.96
14.	B_1= 2.40	99 Acetone	B_1= 0.74	69.16
15.	B_1= 13.06	96 Ethanol	B_1= 6.02	53.90

The sodium hypochlorite concentration and pH were the most important factors involved in reducing high-toxin levels to non detectable amounts; e.g., at pH 8, 0.4% sodium hypochlorite reduced aflatoxin Bl from 725 ppb to trace amounts in ground raw peanuts; at pH 9, only 0.3% NaOCl was required (Natarajan *et al.,* 1975). Sodium hypochlorite is chemical substance used with commercial bleaches for detoxification of aflatoxins (Yang, 1972). The results showed a complete destruction of aflatoxin in a very short time when high concentrations of 5-6% or 0.67 M to 0.81 M of sodium hypochlorite were used.

The maximum detoxification of aflatoxin B1 in poultry feed and fish feed samples was observed when 0.5 % HCl was used. Acid treatment was the most effective, significantly increasing the ability of tested isolates to remove more aflatoxin B1. These results agree with that reported by El-Nezami *et al*. (1998), who found that HCl treatment of *L. rhamnosus* GG and *L. rhamnosus* pellets significantly enhanced the binding ability of it toward aflatoxin B1.

It was concluded that the detoxification of aflatoxin B1 may be affected by alkali solutions according to their concentration but it is degraded greatly by acid addition (WenLi *et al.,* 2008). Strong acids convert aflatoxin to its hemiacetal form through hydration that is much less toxic.

Conclusion

Aflatoxin contamination is unavoidable and unpredictable which make it unique challenge to feed safety as it directly or indirectly suffers animals and human beings. Although there are many chemicals but it is found that 0.5% hydrochloric acid is the pre-eminent chemical for decontamination of aflatoxin B1 in poultry and fish feed samples. The study revealed a high incidence of aflatoxin contaminated feed and feed ingredients and that low concentrations of acid removal of AFB1 from feed may be used on large scale to minimize economic loss due aflatoxin contamination and to improve animal health condition.

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