Zag. J. Pharm. Sci., Vol. 3, No. 1, April 1994

A COMPREHENSIVE SCREENING FOR AFLATOXINS IN ANIMAL FEEDS IN EGYPT

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ABSTRACT

A total of 1503 of commercially mixed feeds, cereal grains, milk replacers, protein concentrates and processed animal feeds were collected during the years 1991-1994 from commercial mills and animal feeding stores located throughout Egypt. Aflatoxins were detected in 619 (41%) samples. The presence of aflatoxins in the range of 1-2000 ppb was evidented in the positively tested samples. The commercially mixed feeds were found to be more contaminated with aflatoxins than were in the cereal grains. Poultry rations showed the highest % of aflatoxins (55%) among the total tested (362) samples. On the other hand milk replacer proved to have the lowest aflatoxins contamination (7%). These results may explain the recent increase of some diseases as hepatocellular carcinoma and nephropathy among animals and humans in Egypt.

INTRODUCTION

Mycotoxins screening can be used to assist in the maintenance of low levels of contamination in commodities or manufactured animal feeds, thereby protecting the consumer of milk, meat, eggs, etc., from residues of mycotoxins and their metabolities. It is now well recognized that almost any type of food or feed can harbor toxigenic strains of Aspergillus which can produce aflatoxins under suitable conditions of growth. There is also experimental evidence that some of the mycotoxins can pass from the alimentary tract into animal tissues, milk and eggs[1-6]. This means that people may expose their health to high risk either by consuming food primarily contaminated with mycotoxins or via an animal being fed a dietary ration containing fungal toxins.

Therefore, the potential problem of naturally occurring mycotoxins in various animal feeds, their consequent animal toxicity, and their passage from animal to man is a matter of great concern.

EXPERIMENTAL

Samples :

A total of 1503 animal feed samples were collected during the years 1991 to 1994 from licensed feed stores and commercial feed mills located throughout Egypt. In addition 240 samples of the commercially mixed feeds and milk replacer were selected at random for the analysis. The single collection were made according to the same sampling procedure in the same period of time for the entire country.

Aflatoxin standards were kindly supplied by Prof. E. Creepy, Bourdeaux, France.

Chromatography :

Thin layer chromatography (TLC) was performed on silica gel GF_{254} (Merck). Solvent system used for developing TLC was chloroform : methanol : water (85 - 15 - 0.5), visualysed under UV light at 366 nm.

High performance liquid chromatography (HPLC) of aflatoxin B_1 (AfB_1)

was performed on LKB, Model 2140, Sweden, Ultrapac Column; flow rate : 0.8 ml/min., column lichosorb RP-18, 10µm (4 x 250 mm); Mobile phase : H_2O : CH_3CN : CH_3OH : glacial acetic acid, (54.4 : 24 : 9.6 : 0.5) UV detection : 366 nm, injection volume : 10 µl, Retention time (T_R) was calculated at 12.81 min (for AfB_1).

Analysis of Aflatoxins :

Twenty grams of each sample were defatted by extraction with pet. ether (50 ml x 2). The residue was extracted with chloroform (50 ml x 2). The chloroformic extract was dried over anhydrous sodium sulphate and then evaporated under vaccum at 45°C to dryness. The residue were reconstituted with 1 ml of $CHCl_3$.

Qualitative analysis of the chloroformic extracts were carried out by TLC and the R_f values were reported as 0.84 for aflatoxin B_1. Quantitative analysis of the purified samples were performed using HPLC. An aliquot of 10 µl of the cleaned extract was injected on reversed phase C-18 column.

RESULTS

Analytical results of the tested samples are presented in table 1. The result of TLC analysis of the different samples under investigation showed that 41% of the samples were contaminated with aflatoxin B_1. The concentrations of aflatoxin B_1 were ranged between 1-2000 ppb, as measured quantitatively by HPLC. The commercially mixed feeds proved to be more contaminated with aflatoxins than were grains (Table 1). The lowest percentage of samples contaminated with aflatoxins was found in milk replacer. The highest contaminated samples occurred among the samples of poultry rations (55%) followed by cereal grains and processed animal feeds (49% and 48%, respectively).

DISCUSSION AND CONCLUSION

This survey was conducted to disclose the main source of animal feed contamination. On the basis of the collected data, it seems that the principal source of aflatoxins in animal feeds and in human food of animal origin, under Egyptian conditions at least, is aflatoxin.

These results showed a higher percentage of contamination of feed stuffs compared with the results of the analysis of freshly imported ingredients for processing mixed feeds.

This is most probably due to storage under uncontrolled conditions of temperature and humidity, which may allow fungi that invade stored feeds to produce various mycotoxins.

In the preliminary multimycotoxin examination of grains and mixed feeds, over 41% of the samples showed blue fluoresent spots on TLC plates, which in appearance and R_f value corresponded to AFB_1. However, the great number of the primarily positive samples, should not be taken as a rapid decision about acceptance or rejection of the sample testedunless the specific and sensetive method of analysis has to be chosen to avoid misclassification.

Therefore, it is important to perform specific chemical tests for every single case when the presence of aflatoxins in feed on food stuffs is revealed by simplified preliminary procedure[8] .

The survey showed that the contamination rate of grain and feed stuffs is dangerous. The necessity of permanent monitoring for mycotoxins in feed stuffs and foods of animal origin seems to be obvious in order to reduce the hazards of mycotoxicoses in farm animals and prevention of human exposure to fungal toxins.

One should be aware that high levels of mycotoxins are often accompanied by visible moulding of food or feed stuffs, which are normally rejected. However, high toxin level can be found without visible signs of moulding, as mycotoxins will persist after moulds have disappeared.

Table (1) : Detection of Aflatoxins in Egyptian animal feeds.

Type of Feed	Total number of samples tested	% +ve samples	Estimation of AFB_1 in +ve samples (ppb)		
			1 - 50	51 - 200	201-2000
1- Poultry ration	362	55	32	14	9
2- Processed animal feeds	225	48	34	10	4
3- Cereal grains	176	49	32	9	8
4- Concentrates and fish meals	432	30	6	8	16
5- Milk replacer	57	7	0	5	2
6- Rice straw and hay	50	48	18	16	24
7- Wheat bran and flour	203	33	23	7	3

Total samples 1503, +ve sample 619 and % of contamination 41%.

N.B. : Food laws in most countries prohibit the sale of any food or feed that contains 5-20 ppb of aflatoxin B_1 [7].

It is of great concern for both human and animal health the effect of repeated subacute exposure at $\mu g\ kg^{-1}$, parts per billion (ppb) levels, to mycotoxins because toxicological experiments have now been shown that AFB_1 is regarded as the most potent naturally occurring carcinogen[9].

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مسح مكثف للأفلاتوكسينات (السموم الفطرية) الموجودة فى الاعلاف بمصر

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تم فى هذه الدراسة عمل مسح وتقدير كمى لوجود الافلاتوكسين فى ١٥٠٣ عينة من الاعلاف مختلفة المصادر والانواع الموجودة فى مصر. وقد وجد الافلاتوكسين ب١ فى ٦١٩ عينة أى تمثل ٤١٪ من مجموع العينات التى فحصت.

وقد تبين أن أعلاف الدواجن هى اكثر الاعلاف تلوثا بالافلاتوكسين (٥٥٪) واقلها اعلاف بدائل الألبان (٧٪).

والمعروف أن الحيوانات التى تتغذى على اعلاف ملوثة بالافلاتوكسين تنتقل هذه الى الالبان واللحوم والبيض وهذه النتائج تفسر ظهور بعض الامراض فى الوقت الحديث مثل امراض سرطان الكبد والفشل الكلوى التى تصيب الحيوان والانسان.