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TÜRKİYE'DE ÇEŞİTLİ YEMLERDEKİ AFLATOKSİN MİKTARLARI

THE AMOUNT OF AFLATOXIN IN DIFFERENT FEEDSTUFFS IN TURKEY

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SUMMARY

We have analyzed 393 differenet feedstufs for aflatoxin in the last two years between june 90 – June 92. The aim of this study was to find out the average amount and the most harmful of aflatoxin of differents feedstuff in Turkey.

KEYWORDS : Aflatoxın, Feedstuff, Turkey

ÖZET

Son iki yılda (Haziran 1990 – Haziran 1992) arasında 393 farklı yem maddesinin aflatoxin bakımından analizlerini yaptık. Amacımız Türkiye'deki yem maddelerinin ortalama aflatoxin miktarlarını saptamak ve farklı yem maddelerinin hangilerinin aflatoxin bakımından hangi aylarda daha çok zararlı olabileceğini göstermekti.

INTRODUCTION

The investigation of aflatoxin was started in 1960 by Stevens et al. Post mortem examination showed a general oedema with congested spleen and kidneys, but the most marked changes were in the liver which showed proliferation of the bile duct and degenarative changes in the parencymal cells. This condition was eventually shown to be associated with the consumtion of certain batches of feedstuffs containing groundnut meal. The production of aflatoxin is not necessarily associated with groundnut; it has been found also in bean, cottonseed and maize meals.

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Paterson et al showed that toxic groundnut are the cause of exudative hepatitis (oedema disease) in guinea–pigs, young monkeys feed with 1 mg of aflatoxin per day developed typical lesions within three weeks. Their carcinogenic effect has a bearing on any possible hazard that may be presented to humans by aflatoxins. The production of liver tumours in the rat was first noted by Lancester et al (1).

Its occurence, results from the invasion of Aspergillus flavus and is comparatively high in subtropical regions and depends on numerous factors such as weak plants after drought stress, insect or mecanical damage, delay and climatical conditions before drying and improper storage conditions.

In USA for the 1967–72 period, the percentage of lots with more than 100 µg/kg aflatoxin B1 varied from 0 to 3%. On the contrary, levels between 49 and 17 % and mean levels of 1530 and 363 µg/kg were found in Thailand and Ouganda respectively (2). Strzelecki EL, Cader–Strzelecka B, reported aflatoxin amount in 366 peanut meals from Brazil and India. Average levels of contamination 71 µg/kg for aflatoxin B1 and 16 µg/kg for B2 with total concentrations ranging from 2 to 750 µg/kg (3). R. Haliburtan et al have analyzed aflatoxin in corn from a farm, where three horses died and others became sick. They found B1, B2 and M1 at concentrations of 114,10 and 6 µg/kg respectively. Cycopiazonic acid, sterigmato cystin, and the Fusarim toxins, T–2 toxin were not detected (4).

Abdelhamid AM, has analyzed 95 samples of various Egyptian feedstuffs which were investigated for aflatoxin B1, B2, G1 and G2 (by the means of) thin layer chromotography. Out of these samples 44.2 % were positive (maize, rice crack, rice germ, rice germ cake, rice bran, wheat bran, cotton seed, cotton seed cake, peanut and mixed feed for broilers, egg production, calf fattening and milk production). High percentage 90.5 % of the positive samples were contaminated with less than 100 ppb total aflatoxins. Peanut from Ismailia showed the highest contamination–mean of 400 ppb aflatoxin B1. The comtamination relationship between kernels and shell of the same pods of the peanut was 1:7. The lowest contamination–mean was 5 ppb B1 in soye bean samples. All samples of horse bean and fish meal were negative. Aflatoxin B1 was often present alone (in 76.2 of the positive samples). The relationship between the concentrations of aflatoxins B2:G1:B1 was 1: 2.3: 22.4. (5).

We have analyezed in last two years 393 different feedstuffs in our labortory for aflatoxin. We have also tired to find the average amount of aflatoxin of feedsmaterial in Turkey.

MATERIALS AND METHODS

HPLC (WATERS), fluoresans dedektor 420–AC, Waters, pump Waters 501, C18 Column, Waters, Datamodule 745B Waters. Chemikals from Merck, Germany.

Sample prepration :

A 12.5 g sample was mixed with 1 gr NaCl in a blender and added into 62.5 ml distiled water and 62.5 ml of chloroform and blended 1 minut. It was filtered through a bed of celite and the filtrate was collected. We used Buchner funnels with Watman Nr. 4 paper and 1/2" of celite. 12.5 ml of the $CHCl_3$ was passed in silica sen–pak cartridge and then filtrate discarded.

The cartridge was washed with 10 ml of hexane followed by 10 ml of diethylether and discarded after both washes. The aflatoxin was eluted with 10 ml of 95/5; $CHCl_3$, CH_3OH and collected an a 10–15 ml screw cap vial. than it was blown and dried under nitrogen. It was dissolved in 0.2 ml of hexane and voertexed approximately for 10 seconds and added to 0.2 ml of 80 % trifluoraseticacid and vortexed approximatelly for 30 seconds.

We waited for 1 min. and then we added 2.3 ml of 10/90 ; CH_3CN / 2.5 % aceticacid and mixed thoroughly 200 µl of this solution was injected.

CHROMATOGRAPHY

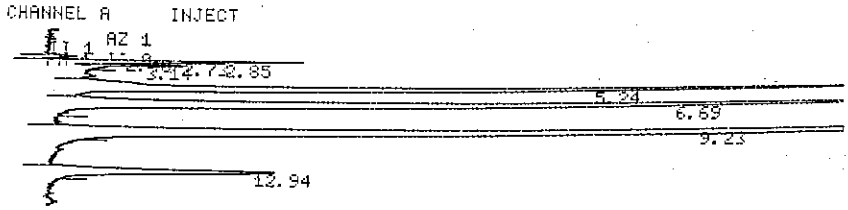
Column 5 µm resolve ve C 18 RADIAL PAK cartridge + RCSS C18 quard pak insert.

Mobile Phase : 14% CH_3OH, 14 9 CH_3CN, 72 % , (% 5 aceticacid) flow rate : 1.2 ml/min (3000 psi)

Detector : M 420 Fluorescence, 365 excitation, 425 emission with aflatoxin lamp (P/N 78409).

RESULTS

The curve for standart aflatoxin is shown in Fig. 1. The average amount of total aflatoxin and its fractions are shown in Table – 1. Positive samples which have high amount of aflatoxin are shown Table – 2.



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FILE 1. METHOD 5. RUN 1 INDEX 1 CALIB

ANALYST: ORHAN AKDOGAN

NAME	PPB	RT	AREA	BC	RF
1	0.	2.4	57511	02	
2	0.	2.73	227198	02	
3	0.	2.85	215792	02	
4	0.	3.14	229675	03	
AF G1	16.	5.24	2852546	08	178284.125
AF B1	16.	6.69	3644900	05	227806.25
AF G2	16.	9.23	9377993	01	586124.563
AF B2	16.	12.94	913039	01	57064.938
TOTALS	64.		17518654		

NEW FILE:

NAME	RF	RT
AF G1	178284.125	5.12
AF B1	227806.25	6.85
AF G2	586124.563	9.62
AF B2	57064.938	13.47

Fig. – 1 : The graphic of aflatoxin standard in HPLC injection : 200 ml, column : Resole Sum C18 Radial Pak, mobil phase : 14 % CH_3OH, 14 % CH_3CN, 72 % acetikacid solution (% 5 AcA, % 95 Water), flow rate 1.2 ml/min. (3000 psi). Dedector M 420 fluoresence, 365 excitation, 425 emission with aflatoxinlamp. The peaks are 1–G1, 2–B1, 3–G2, 4–B2.

Table – 1 : The average amount of aflatoxin and its fraction as ppb, in total samples, soonflover meal and maiz.

FRACTION	Total sample n = 393	sunflover meal n = 20	corn n = 47
B1	7.01	25.30	24.10
B2	13.00	5.90	7.20
G1	18.50	30.40	1.00
G2	0.32	1.20	0.10
TOTAL	38.83	62.80	32.40

Table – 2 : Between jun 90–jun 92, the positive samples with date and with their fraction.
APR : apricot, C: corn, F : feed, G : gluten, S.F. : sunflower meal,
TOP : tapioca. WAPR : wildapricot

Pr.Nr Dat	sample	B1	B2	G1	G2	total	mould
							gram
2359 JUL 90	F					204.0	48500
2406 JUL 90	F	3	0.1	166	–	169.1	197250
2407 JUL 90	F	2	0.2	143	–	145.2	36250
3577 JUL 91	S.F	0.1	–	132	13	145.7	14675
3575 JUL 91	S.F	6	–	84	6	96.0	9610
3911 OCT 91	C	56	14	–	–	70.0	1110000
3952 NOV 91	S.F.	29.7	–	50.5	–	80.2	1600
3953 NOV 91	C	46.7	–	0.7	–	47.4	44500
3996 NOV 91	S.F.	37.4	17.3	–	–	54.7	1000
3997 NOV 91	S.F.	41.9	17.9	–	–	59.8	3500
3998 NOV 91	S.F.	47.3	1.7	15.3	–	64.3	7000
4335 FEB 92	N	3.4	–	64	–	67.4	< 10
4367 FEB 92	G	126.2	51.8	–	–	178.0	
4385 FEB 92	C	301	160.1	9.1	–	470.2	
4386 FEB 92	C	359.1	74.2	1.7	–	435.0	
4339 APR 92	F	–	–	72.3	–	72.3	
4340 APR 92	TOP	–	–	290.0	0.6	290.6	
4547 APR 92	APR	–	–	440.5	–	440.5	
4548 APR 92	APR	–	–	439.5	–	439.5	
4573 APR 92	APR	–	–	308.0	–	308.0	
4601 APR 92	WAPR	–	–	443.0	–	443.0	
4615 APR 92	S.F.	52.8	–	111.9	–	164.7	
4624 APR 92	WAPR	–	–	354.3	–	354.3	
4719 MAI 92	S.F.	81.6	26.1	–	3.7	111.4	
4730 MAI 92	S.F.	38.8	–	83.9	–	122.	

The positive maize samples which we analyzed contained B1 with less B2 or very small amount of G1. In some sunflavermeal we found G1, G2 positive and in other samples B1 and B2. In only one sample of gluten the major component was B1. sunflowermeal contain B1, B2 and G1. B1:B2:G1 = 76:25:1 Apricot, stone of wildapricot and tapioca contain very high G1 fraction but dont contain the other fraction. The highest value of aflatoxin were measured between february-may 92 periode. We have not analyzed mould in these samples but we measured mould in positive samples between june 90 and february 92. We didn't find any correlation betwen the amount of total aflatoxin or its fraction and their mould. The result was that the stone of wildapricot, apricot, sunflower meal and maize contain more aflatoxin than the other samples. In Wintertime the samples contaned more than an average amount of aflatoxin than in summertime.

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