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Aflatoxin Contamination in Grains and Grain Products During the Dry Season in Guatemala

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The mycotoxins, mold-produced toxic metabolites, have been widely investigated during the last 15 years. The most widely studied variety are the aflatoxins, which are produced mostly by *Aspergillus flavus.*

As food contaminants, they are in very small amounts toxic to man and animals (CAMPBELL and STOLOFF 1974). Various studies have shown them to be highly carcinogenic to animals (CAMPBELL and STOLOFF 1974, MILLER 1966), and recent evidence demonstrates this same fact to be applicable to man (VAN RENSBURG *et al* 1974).

The aflatoxins also produce acute intoxications, being ducklings, chicks, horses and calves especially vulnerable. Monogastric animals are more susceptible to these intoxications than ruminants (ZINTZEN 1975). A fact that should be kept in mind is that calves are monogastric during at least the first six weeks of life. Some of the important symptoms of aflatoxicosis are allergies, gastrointestinal inflammation and impairments of the nervous system (ZINTZEN 1975).

In various countries, aflatoxicosis has been the cause of great economic losses due to death or illness in fish, poultry and swine (BEACHMAN 1976, HAMILTON 1971, SMITH *et al* 1976). In Guatemala aflatoxicosis in chicken has been observed (ALMENGOR Personal communication).

Grains stored under hot and humid conditions are very susceptible to toxin-producing mold invasions; at temperatures above 25°C, grains with 15-20% humidity may develop appreciable quantities of aflatoxin in a week's time (BEACHMAN 1974). The contamination may even take place before harvest time (BEACHMAN 1974). Corn from blight-infested plantations is especially prone to secondary mold invasion (ZINTZEN 1975).

Aflatoxins are highly heat-resistant but are destroyed under alcaline conditions (ULLOA-SOSA and SCHROEDER 1969).

In the United States, the FDA applies a 20 ppb (parts per billion) action level for all affected commodities, except peanuts where 15 ppb has been proposed (FEDERAL REGISTER 1974). Other countries, like Sweden, Poland and Holland, use 5 ppb as a guideline. In protein supplements for undernourished children FAO/WHO proposes a 30 ppb limit (JARVIS 1975).

In the Central American area very little information is available on the subject. In cottonseed, CABRERA (1976) reported aflatoxin contamination in 4% of 99 samples analyzed. MARTINEZ *et al.* (1970) reported a high prevalence of *Penicillium, Fusarium* and *Aspergillus* in 62 Guatemalan corn samples. The fungus

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most frequently found was *A. versicolor* that was present in 57°/o of the samples. The possible mycotoxin contamination was not established (MARTINEZ *et al.* 1970).

In 1975 our laboratory started to analyze occasional samples of imported and national grains. The results indicated a high incidence of aflatoxin contamination, and for this reason a broader monitoring program was begun in 1976.

MATERIALS AND METHODS

Due to altitude variations that range from 0 to 15,000 feet, Guatemala is naturally divided into zones with very different climatic conditions. In the northern part of the country as well as on the Pacific and the Atlantic coasts, the climate is hot and humid; in the interior of the northeastern part it is hot and dry and in the highlands the climate is cold or temperate and humid. Each zone cultivates crops according to its climate.

From each of these zones samples were collected, most of which were grain samples. (Some other products were also examined for comparison purposes). Some were bought at the local market in Guatemala City and occasionally commercial samples of national and imported grains were also included. Because of this, their origin could not always be established. A total of 264 samples were collected, most of which were harvested at the end of the rainy season and stored during the dry season (November through May). They were collected from different storage facilities, silos, sacks, small shops, homes and fields, in polyethylene bags in quantities that varied from 1 to 2 pounds. Corn is the most common staple food in Guatemala and for this reason, corn and corn meal represented 50°/o of the samples. Black beans are the next important staple food, and rice is also heavily consumed.

Once received in the laboratory, the whole grain samples were finely ground, and immediate moisture determinations were made in about 50°/o of the samples. When aflatoxin analysis could not be made at once, the samples were stored at −20°C to inhibit mold growth.

All the samples were analyzed following the Romer method (ROMER 1975) with a few modifications:

We found that the use of glass wool instead of filter paper in the first filtration step speeds up the analysis. And, instead of Hyflo Super Cel as a filter aid, regular diatomaceous earth, which is used for bleaching in oil hydrogenation plants, can be used and is a cheap substitute when it can be obtained.

Instead of visual quantification of the minicolumns a "Velasco Flurotoxin Meter" (Neotec Instruments, Inc.) was utilized. The minicolumns used were a combination of those recommended by ROMER (1975) and VELASCO (1975), as follows: 2 mm glasswool, 5 to 7 mm sea sand, 7 to 10 mm Florisil, 20 mm silica gel, 10 mm neutral alumina and, finally 5 to 7 mm sea sand. The ingredients were previously dried for 1 hour at 110°C. The columns were tapped on the tabletop after each addition.

As sea sand, which is much easier to handle, was substituted for drierite, the chloroform extract was dried with a small quantity of anhydrous Na_2SO_4 before passing it through the minicolumn.

The instrument was zeroed with a blank column prepared with 2 ml of chloroform and 3 ml eluting solution (chloroform-acetone 9+ 1), and calibrated to 20 ppb with a column prepared with 50 ng aflatoxin B_1 in the amounts of chloroform and eluting solution already mentioned. A reagent blank was periodically run through the complete method and the reading, usually equivalent to 3 - 4 ppb, was deducted from the samples readings.

Uncontaminated samples fortified with aflatoxin B_1 at the 20 ppb level gave a fairly consistant recovery of 80%.

All the positive sample extracts were prepared for TLC confirmation and individual aflatoxin identification. The Romer's method (1975) was used, but instead of 4 ml, 5 ml aliquots were taken. The 10 ml extract was dried with a small amount of anhydrous Na_2SO_4, and for the final evaporation 8 ml were used, which represents 5.1 g of the sample. For the TLC assay we used the AOAC method (1975). When low levels of aflatoxin were detected by the flurometer, the residue was redissolved in a small quantity of benzene-acetonitrile (98+ 2), and the total was spotted. In this case the vial was washed with an additional quantity of solvent, and spotted.

To further remove extract impurities, the plate was left in a tank with ethyl ether until the solvent front reached the top of the plate (TROPICAL PRODUCTS INSTITUTE 1975). Further development was done according to the AOAC method. This procedure changes the R_f's to somewhat higher values.

When confirmed the TLC levels concurred fairly well with those obtained with the minicolumns. The fluorometric values are reported.

The necessity to confirm all positive results by TLC should be stressed. In our particular case, the positive samples that could not be confirmed had, with a few exceptions, given fluorometer readings close to our 4 ppb detection limit, which means that samples reported as "not detected" might contain 3 ppb or less. In a few cases, however, quite high fluorometric values could not be confirmed by TLC. This was the case for a sample of black beans with a fluorometer reading of over 50 ppb and a 15 ppb coffee pulp sample. In these cases strong blue fluorescence was observed on the plate, but with R_f values much higher than the aflatoxins.

RESULTS AND DISCUSSION

As can be seen in Table I, a high proportion of the 264 samples analyzed were contaminated with aflatoxins (17%), 8% exceeded the 20 ppb level. The highest value (240 ppb) was found in a silo-stored yellow corn sample that came from a hot and humid area. This corn showed visible mold contamination.

Aflatoxin B_1, alone or in combination with other aflatoxins, was found in all the positive samples. Aflatoxin G_1 was found in corn, peanuts, rice, beans and meat meal, while aflatoxin G_2 was detected only in rice and beans.

As expected, it became evident that when the samples are distributed in groups according to origin, the highest incidence of contamination is found in samples coming from the hot and humid region (Table II). Twenty-six percent of the samples from that same area were contaminated, 7% from the hot and dry region and 2% from the cold or temperate and humid regions. The difference is significant ($P < 0.05$).

Table I

Aflatoxin Contamination in Food and Feed during the dry Season in Guatemala, C. A. (ppb)

Commodity	Samples analyzed n	Positive samples n	<5	6-20	21-50	50	Max. value	Aflatoxins present (in 1 sample or more)
Corn, white	68	7	2	4	1		28	B_1+B_2
Corn, yellow	50	7	1	3	1	2	240	$B_1+B_2+G_1$
Corn meal	13	4	1	1	1	1	96	$B_1+B_2+G_1$
Cottonseed meal	14	2		1	1		30	B_1
Protein supplement meal	19	6	2	2	1	1	56	B_1+B_2
Rice, with bran	18	9		2	4	3	83	$B_1+B_2+G_1$
Rice, grain	6	2	1	1			8	$B_1+B_2+G_1+G_2$
Beans, black	17	1			1		22	$B_1+B_2+G_1+G_2$
Beans, others	9	0						
Peanuts, grain	10	1				1	72	$B_1+B_2+G_1$
Peanuts, shell	2	1			1		48	$B_1+B_2+G_1$
Peanut butter	2	0						
Mixed nuts	1	0						
Coffee, untoasted beans	1	1		1			8	$B_1+B_2+G_1$
Coffee, pulp	1	0						
Cocoa, beans	1	0						
Wheat, grain	3	0						
Wheat, flour	7	0						
Sorghum, red & white	7	0						
Sorghum, flour	1	0						
Sesame, seed	3	0						
Poultry feed (concentrate)	3	3		1		2	71	B_1+B_2
Meat meal	4	1		1			13	G_1
Biscuits	2	0						
Cheese Cake Mix	1	0						
Sugar	1	0						
Total	264	45	7	17	11	10		
Positive samples, percent of total:		17%	3%	6%	4%	4%		

Table II

Aflatoxin Contamination According to Origin of Samples (Positive Samples/Total Samples Analyzed)

Commodity	Hot and humid region	Hot and dry region	Cold or temperate and humid region	National products of unknown origen	Imported products, origin (when known)	
Corn, white	5/34	1/21	1/11		0/2	U.S.A. Honduras
Corn, yellow	4/13	0/5	0/15	2/10	1/7	
Corn meal				4/13		
Cottonseed meal				2/14		
Protein supplement meal				6/19		
Rice, with bran	9/16		0/2			
Rice, grain	1/2	1/4				
Beans, black	0/4	1/9	0/2		0/2	Colombia
Beans, others		0/8	0/5		0/1	U.S.A.
Peanuts, grain	1/6				0/4	U.S.A.
Peanuts, shell	1/2					
Peanut butter	0/1				0/1	U.S.A.
Mixed nuts				0/1		
Coffee, untoasted beans	1/1					
Coffee pulp			0/1			
Cocoa, beans	0/1					
Wheat, grain			0/3			
Wheat, flour			0/7			
Sorghum, red and white		0/4	0/2	0/1		
Sorghum, flour				0/1		
Sesame, seed	0/3					
Poultry feed (concentrate)				3/3		
Meat meal				1/2	0/2	El Salvador
Biscuits					0/2	U.S.A.
Cheese Cake Mix					0/1	U.S.A.
Sugar	0/1					
Total:	22/84	3/46	1/48	18/64	1/22	
Positive samples, percent of total:	26%	7%	2%	28%	5%	

The moisture content in the analyzed samples, excluding nuts, ranged from 7.0 to 17.1%; no correlation was found between moisture content and aflatoxin contamination.

An important fact to consider is that the great majority of samples were collected and stored during the dry season which should lower the incidence of contamination. A greater contamination is expected to occur during the rainy season.

It has not yet been established whether nutritional stress decreases the resistance to the toxicity of aflatoxins (MARTINEZ *et al.* 1970); it should be remembered, however, that the incidence of protein-calorie malnutrition is high in Guatemala (VITERI 1976).

The results reveal that of the 118 corn samples, 12% were contaminated. As already mentioned, corn is the most important staple food in the country, mostly consumed as "tortillas", an unleavened corn-meal bread. The tortillas are prepared from corn previously cooked with lime (CaO). Fortunately, this treatment has proved to reduce the aflatoxin content to about one-third (ULLOA-SOSA and SCHROEDER 1969). However, as far as we know, nothing has been published on the possible toxicity of the decomposition compounds.

Rice, another staple food, was also found to be heavily contaminated. The highest levels were found in grains analyzed with the bran; the maximum value being 83 ppb.

The second most important staple food, the black beans *(Phaseolus vulgaris),* did not present a contamination problem. One out of 26 samples was contaminated with 26 ppb.

CONCLUSIONS AND RECOMMENDATIONS

The results presented in this paper show that aflatoxin contamination is high in Guatemalan grains. The study was carried out in samples stored during the dry season, therefore, greater contamination is expected to occur during the rainy season.

From the findings it can be stated that further studies in this field should be carried out. Very little is known on the subject in the area, so an information campaign on aflatoxin toxicity and possible preventive measures should also be included.

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