Natural Co-occurrence of Fumonisins and Zearalenone in Cereals and Cereal-Based Foods from Eastern and Southern Africa

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The natural co-occurrence of fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), and zearalenone was investigated in 40 randomly selected cereals and cereal-based commodities collected in 1994 from Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe. FB1 was detected in 37 of the samples (92.5%) at concentrations ranging from 20 to 1910 ng/g, while total fumonisin (FB1 + FB2 + FB3) concentrations in the same samples ranged from 20 to 2735 ng/g. The highest total fumonisin levels were detected in maize kernels from Zimbabwe (2735 ng/g). In contrast to the high incidence of fumonisins (92.5%), zearalenone was detected in only five samples (12.5%) at concentrations ranging from 40 to 400 ng/g. Linear regression analysis showed no correlation between the occurrence of fumonisins and zearalenone in the samples tested. Although limited in sample numbers, this survey ranks the fumonisins as major contaminants of cereals and cereal-based foods in Eastern and Southern Africa.

Keywords: *Fumonisins; zearalenone; cereals; Eastern Africa; Southern Africa*

INTRODUCTION

The mycotoxins frequently encountered in cereals include those produced by *Fusarium* (fumonisins, trichothecenes, and zearalenone), *Aspergillus* (aflatoxins), and *Penicillium* (ochratoxin A). *Fusarium* species are now recognized as a major agricultural problem. They occur worldwide on a variety of plant hosts, primarily on cereal grains. The three most important species of *Fusarium* that have been associated with human and/or animal health problems are *Fusarium sporotrichioides* (Sherb), *Fusarium moniliforme* (Sheldon), and *Fusarium graminearum* (Schwabe). *Fusarium* species produce a variety of mycotoxins with widely divergent biological and toxicological effects in humans and animals consuming the infected grain (Marasas, 1994).

In surveys carried out in Western European countries such as Italy, France, and Spain, *F. moniliforme* is the most frequently isolated fungal species from maize in the field, harvested maize, and maize-based commodities (Logrieco and Bottalico, 1988; Bottalico et al., 1989; Rapior et al., 1993; Cabanes et al., 1993). *F. moniliforme* is also common on maize in African countries such as South Africa (Transkei) and Zambia, where maize is the dietary staple of humans (Marasas et al., 1978). The presence of toxigenic *Fusaria*, with a prevalence of *F. moniliforme*, has also been reported on millet and sorghum from Nigeria, Lesotho, and Zimbabwe (Onyike et al., 1991; Onyike and Nelson, 1993). *F. moniliforme* produces a variety of secondary metabolites that include mycotoxins. Fumonisins are a group of recently discovered mycotoxins that are produced primarily by *F. moniliforme* (Gelderblom et al., 1988).

Six structurally related fumonisins, fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), fumonisin B4 (FB4), fumonisin A1 (FA1), and fumonisin A2 (FA2), have been described. Of the six fumonisins, FB1, FB2, and FB3 are the major compounds usually isolated from maize fungal cultures and from naturally contaminated maize (Hopmans and Murphy, 1993; Visconti and Doko, 1994). These toxins have been associated with toxicoses of animals and are the causal agents of equine leukoencephalomalacia (ELEM) and porcine pulmonary edema (PPE). FB1 has also been linked to several field outbreaks of mycotoxicoses among animals (Harrison et al., 1990, Kellerman et al., 1990; Gelderblom et al., 1991; Ross et al., 1992; Thiel et al., 1991).

The fumonisins exhibit cancer-promoting activity in rats and may play an important role as human carcinogens (Gelderblom et al., 1991). In the Transkei region of South Africa where maize is the human staple food, consumption of fumonisin-contaminated maize has been linked to esophageal cancer (Sydenham et al., 1990, Thiel et al., 1992). In this region, fumonisin levels of up to 117.5 μg/g were detected in moldy maize used for brewing beer. Similar association between consumption of fumonisin-contaminated maize and esophageal cancer has been reported in China and Northeastern Italy (Chu and Li, 1994; Franceschi et al., 1990). Furthermore, fumonisins have been detected in maize and maize-based foods marketed in several countries worldwide (Sydenham et al., 1991; Pittet et al., 1992; Stack and Eppley, 1992; Hopmans and Murphy, 1993; Visconti and Doko, 1994).

Zearalenone (ZEA) is a mycotoxin produced primarily by *F. graminearum*. Zearalenone has been shown to have estrogenic and anabolic activity on various species. It causes hyperestrogenism and infertility in swine and has been associated with infertility and abortions in other farm animals (Mirocha and Christensen, 1974; Prelusky et al., 1994). Zearalenone has been implicated in several incidents of precocious pubertal changes in children (Kuiper-Goodman et al., 1987).

Many toxigenic species of *Fusarium* produce more than one toxin (Marasas et al., 1984). The natural co-

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S0021-8561(96)00257-9 CCC: $12.00

occurrence of several *Fusarium* mycotoxins, such as trichothecenes, moniliformin, zearalenone, and the fumonisins, on samples of moldy or healthy maize has been reported in several countries worldwide (Mirocha et al., 1977; Jemmali et al., 1978; Siame and Lovelace, 1989; Sydenham et al., 1990; Chu and Li, 1994). The co-occurrence of fumonisins and/or zearalenone with other mycotoxins can lead to exposure to a combination of rather than individual toxins. The synergistic action of different mycotoxins has been reported in laboratory animals (Bhavanishankar et al., 1988).

Cereals and cereal-based products (especially maize) form the main dietary staple food of most people in Eastern and Southern Africa. The extent to which consumers are exposed to fumonisins and zearalenone was determined in a limited survey of cereals and cereal-based products originating from Eastern and Southern Africa. In this study, we report the natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based products from Eastern and Southern Africa.

MATERIALS AND METHODS

Sample Source and Preparation. A total of 40 randomly selected cereals and cereal-based products were analyzed for fumonisins and zearalenone. Samples, meant for human consumption, were either purchased from retail outlets (Botswana and South Africa) or drawn from sample lots (Kenya, Malawi, Mozambique, Tanzania, Uganda, Zambia, and Zimbabwe). One sample consisting of cereal-based mixed feed intended for rabbits (Zambia) was also analyzed. Sample size varied from 1 to 5 kg (except for samples from Tanzania which were less than 1 kg). Samples were finely ground in a Bueheler laboratory mill and thoroughly mixed before taking aliquots for fumonisin and zearalenone analysis.

Fumonisin Analysis. The fumonisins were analyzed by the method given by Sydenham et al. (1992), with the modifications described by Doko et al. (1995). Fumonisins were extracted from 50 g subsamples into methanol−water. The methanol−water extract was passed through a strong anion-exchange cartridge, and the fumonisins were eluted from the cartridge with acetic acid−methanol. The acetic acid−methanol solvents were evaporated under a gentle stream of nitrogen. Fumonisins were then derivatized with 200 μL of *o*-phthaldialdehyde (OPA). The OPA derivatives (10 μL) were analyzed by reverse-phase HPLC (M-45 pump and Model 754 data system from Waters Corp., Milford, MA) connected to a Perkin-Elmer 650S fluorescence detector (Perkin-Elmer, Norwalk, CT). The fluorescence of the OPA derivatives was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. Quantitation was by comparison with reference standards. The detection limit was 20 ng/g for each toxin.

Zearalenone Analysis. The method by Bennett et al. (1985), with modifications described by Siame and Lovelace (1989), was used for zearalenone. Zearalenone was extracted from the samples with chloroform and partitioned with sodium hydroxide. The pH of the aqueous layer was lowered with citric acid, and zearalenone was taken up in dichloromethane. The dichloromethane was evaporated to dryness, and the residue was taken up in 200 μL of chloroform for thin layer chromatography (TLC). TLC was carried out on 0.2 mm silica gel plates (E. Merck, Germany). Quantitation was by visual comparison of the fluorescence intensity with that of standards. The detection limit was 40 ng/g.

Statistics. Linear regression analysis was performed for correlation between the occurrence of fumonisins and zearalenone in samples, using the GraphPAD software Instat V2.03 (Sigma, St. Louis, MO). The result was obtained following ANOVA test.

RESULTS AND DISCUSSION

Forty cereal and cereal-based samples were screened for the simultaneous presence of fumonisins (B1, B2, and B3) and zearalenone. The levels of FB1, FB2, FB3, total fumonisins (FB1 + FB2 + FB3), and zearalenone are given in Table 1.

Table 1. Fumonisins (FB1, FB2, and FB3) and Zearalenone (ZEA) Levels in Cereals and Cereal-Based Commodities from Eastern and Southern Africa Countries[a]

cereal and cereal-based products		mycotoxin concentration (ng/g)				
origin	identification	FB1	FB2	FB3	FB1 + FB2 + FB3	ZEA
Botswana						
1	coarse maize	165	50	40	255	nd
2	maize meal	35	nd	nd	35	nd
3	maize meal	255	85	30	370	nd
4	maize meal	220	nd	nd	220	nd
5	maize meal	230	75	nd	305	100
6	maize kernels	350	105	70	525	nd
7	sorghum meal	20	nd	nd	20	nd
8	sorghum meal	20	nd	nd	20	nd
Mozambique						
9	maize kernels	245	75	25	345	nd
10	maize kernels	240	110	45	395	nd
11	maize kernels	295	85	50	340	nd
South Africa						
12	maize meal	70	nd	nd	70	nd
13	maize meal	60	nd	nd	60	nd
Malawi						
14	maize kernels	20	nd	nd	20	nd
15	maize kernels	30	nd	nd	30	nd
16	maize kernels	115	nd	nd	115	nd
17	maize kernels	95	nd	nd	95	nd
18	maize kernels	75	nd	nd	75	nd
19	maize kernels	105	30	nd	135	nd
20	maize kernels	30	nd	nd	30	nd
21	maize kernels	nd	nd	nd	nd	400
Zambia						
22	maize meal	740	380	85	1205	nd
23	rabbit pellets	70	nd	nd	70	nd
Zimbabwe						
24	maize meal	55	nd	nd	55	nd
25	maize meal	1910	620	205	2735	nd
26	maize meal	115	nd	nd	115	nd
27	maize meal	420	150	55	625	nd
28	maize kernels	nd	nd	nd	nd	nd
29	maize kernels	125	40	nd	165	nd
Kenya						
30	maize kernels	780	275	130	1185	40
Tanzania						
31	maize kernels	50	nd	nd	50	nd
32	maize kernels	40	nd	nd	40	nd
33	maize kernels	nd	nd	nd	nd	40
34	maize kernels	160	nd	nd	160	nd
35	maize kernels	50	nd	nd	50	80
36	maize kernels	25	nd	nd	25	nd
37	maize kernels	55	nd	nd	55	nd
38	maize kernels	165	60	nd	225	nd
39	maize kernels	90	nd	nd	90	nd
Uganda						
40	maize kernels	605	155	85	845	nd

[a] nd = not detected, <20 ng/g for fumonisins and <40 ng/g for zearalenone.

Fumonisins were detected in 37 of the 40 samples (92.5%). All samples (100%) from Botswana, South Africa, Mozambique, and Zambia were contaminated with fumonisins. The single samples, from Kenya and Uganda, were also contaminated with fumonisins. The extent of contamination of the samples from the other countries, although less than 100%, was still very high: Malawi, 87.5%; Tanzania, 88.7%; and Zimbabwe, 83.3%.

The levels of fumonisins varied from sample to sample. When the samples were considered individually, sorghum meal from Botswana and maize kernels from Malawi had the lowest total fumonisin levels (20 ng/g). Very high levels of total fumonisins were detected in samples from Zambia (70−1205 ng/g) and Zimbabwe (55−2735 ng/g). The samples from Kenya and Uganda

Table 2. Occurrence and Distribution of Total Fumonisins (FBs) and Zearalenone (ZEA) in Cereals and Cereal-Based Commodities from Eastern and Southern African Countries

		toxins (ng/g)		number of positive samples		
	occurrence[a]	range	mean[b]	<100	100−1000	>1000 (ng/g)
			Botswana			
FBs	8/8 (100)	20−525	220	3	5	0
ZEA	1/8 (12.5)	100	100	0	1	0
			Malawi			
FBs	7/8 (87.5)	20−135	70	5	2	0
ZEA	1/8 (12.5)	400	400	0	1	0
			Mozambique			
FBs	3/3 (100)	340−395	360	0	3	0
ZEA	0/2					
			South Africa			
FBs	2/2 (100)	60−70	65	2	0	0
ZEA	0/2					
			Zambia			
FBs	2/2 (100)	70−1205	638	1	0	1
ZEA	0/2					
			Zimbabwe			
FBs	5/6 (83.3)	55−2735	740	1	3	1
ZEA	0/6					
			Kenya			
FBs	1/1 (100)	1185	1185	0	0	1
ZEA	1/1 (100)	40	40	1	0	0
			Tanzania			
FBs	8/9 (88.9)	25−225	90	6	2	0
ZEA	2/9 (22.2)	40−80	60	2	0	0
			Uganda			
FBs	1/1 (100)	845	845	0	1	0
ZEA	0/1					
			Total			
FBs	37/40 (92.5)	20−2735	288	18	16	3
ZEA	5/40 (12.5)	40−400	132	4	1	0

[a] Percentage incidence is reported in parentheses. [b] Mean of positive samples.

were also highly contaminated, 1185 and 845 ng/g, respectively. When the mean of the positive samples was taken, the lowest levels were detected in samples from South Africa, Malawi, and Tanzania with 65, 70, and 90 ng/g, respectively (Table 2). Among the positive samples, 18 (48.8%) had fumonisin levels less than 100 ng/g, 16 (43.2%) had fumonisin levels ranging between 100 and 1000 ng/g, and only three samples (5.4%) contained fumonisin levels over 1000 ng/g.

Fumonisins were detected in 92.5% of the samples analyzed, suggesting widespread occurrence in Eastern and Southern Africa. Although the data are from a limited survey, the high incidence of fumonisin contamination is consistent with what has been reported in maize from the United States, South Africa, and some European countries (Sydenham et al., 1991; Stack and Eppley, 1992; Doko et al., 1995). In a recent study of fumonisin content of different maize genotypes with varied characteristics and geographical origins, Doko et al. (1995) reported a similar high incidence of toxin contamination across genotype and geographical origin. The percentage of contaminated samples from different regions was also high: Benin, 82%; Zambia, 100%; Italy, 100%; and Portugal, 100%.

The data reported here and that reported from surveys elsewhere indicate widespread occurrence of fumonisins in maize products (Pittet et al., 1992; Scudamore and Chan, 1993). The potential for human and animal exposure to fumonisins is a worldwide problem. Maize consumed as such or in its different processed forms remains a staple food for large numbers of people in the developing world, providing not only nutrients but a significant amount of toxins as well. Data from FAO (1992) showed daily maize intakes of more than 200 g/person/day in Eastern and Southern African countries. The highest daily maize intakes were reported in Malawi (468.8), Zambia (418.6), and Zimbabwe (330.9). This means that a person from Zimbabwe may be consuming an average of 245 μg of fumonisins/day. This level of contamination may have serious health implications on the consumers if one considers that 10 and 100 μg/g have been proposed to be dangerous to horses and pigs, respectively (Marasas, 1995). However, a wider survey of cereals in Eastern and Southern Africa needs to be undertaken in order to determine precisely the extent to which consumers are exposed to mycotoxins such as fumonisins.

In contrast to the high incidence and levels of fumonisins in the samples analyzed, zearalenone was only detected in 5 of the 40 samples (12.5%) with levels ranging from 40 to 400 ng/g. Only one sample had zearalenone levels above 100 ng/g, the sample from Malawi which had levels of 400 ng/g. No zearalenone was detected in samples from Mozambique, South Africa, Uganda, Zambia, and Zimbabwe. However, previous studies have revealed a higher incidence of naturally occurring zearalenone in cereals and cereal-based products. Concentrations of up to 6 and 10 μg/g have been reported in moldy maize from Zambia and South Africa, respectively (Siame and Lovelace, 1989; Beardall and Miller, 1994).

Linear regression analysis performed for correlation between the presence of fumonisins and zearalenone in contaminated samples showed a slope close to zero: -0.01071 ($P = 0.6214$), with a correlation coefficient of -0.08052 ($r^2 = 0.006483$). Thus, there is no apparent correlation between the simultaneous occurrence of fumonisins and zearalenone in the samples tested. The significant negative correlation between the simultaneous occurrence of fumonisins and zearalenone in the samples tested is consistent with other reports that there is antagonism between *F. moniliforme* and *F. graminearum* (Rheeder et al., 1990; Sydenham et al., 1990).

The high incidence of fumonisins (at high levels) in cereals and cereal-based foods may have serious implications on the health of the intended consumers in Eastern and Southern Africa. This is especially so since these foods constitute the staple food diet of most people in these regions.

ACKNOWLEDGMENT

We thank all participants of the FAO−UB Workshop for providing the samples used in this study. We also thank W. F. O. Marasas, P. G. Thiel, and S. Stockenstrom for useful suggestions.

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Received for review April 15, 1996. Revised manuscript received July 24, 1996. Accepted July 24, 1996.[⊗] This work was done as part of the Joint FAO-University of Botswana (UB) Workshop on mycotoxins other than aflatoxins, held at the University of Botswana, Gaborone, Botswana, Dec 12−16, 1994, and supported in part by FAO/UN, Rome, Italy. Some of the analyses were done at PROMEC-MRC, Tygerberg, South Africa.

JF960257+

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.