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Natural Occurrence of *Fusarium* Toxins in Feedstuff[1]

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The mycotoxins diacetoxyscirpenol, deoxynivalenol, and zearalenone, produced by *Fusarium roseum*, were found naturally occurring in mixed feed samples. In all cases analyzed, deoxynivalenol occurred together with zearalenone. The natural occurrence of zearalenone in sesame seed is reported for the first time. Strains of *F. roseum* isolated in various parts of the world from feed implicated in animal mycotoxicosis produced monoacetoxyscirpenol, diacetoxyscirpenol, deoxynivalenol, and zearalenone.

Species of *Fusarium*, such as *Fusarium roseum*, *F. tricinctum*, and *F. oxysporum* produce mycotoxins known as the zearalenones (5) and trichothecenes (1). The former metabolites are associated with hyperestrogenism and the latter with the hemorrhagic syndrome in farm animals. Although *Fusarium* spp. are often isolated from feeds suspected of causing mycotoxicoses, trichothecenes are infrequently found; in contrast, the zearalenones are commonly encountered in nature.

The trichothecene toxins consist of about 34 derivatives; most are produced by species of *Fusarium*, *Trichoderma*, and *Trichothecium*. Hsu et al. (2) reported T-2 toxin (2 ppm) [4β,15-diacetoxy,8α-(3-methylbutyryloxy)-3α-hydroxy-12,13-epoxytrichothec-9-ene] present in moldy corn associated with a lethal toxicosis in dairy cattle. Vesonder et al. (9) found deoxynivalenol (3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) in corn refused by swine. Morooka et al. (6) found deoxynivalenol and nivalenol (3α,4β,8α,15 - tetrahydroxy - 12,13 - epoxytrichothec-9-ene-8-one) in barley infected with *F. graminearum*. We have found no other documented reports of trichothecenes in food or feedstuff. It is difficult to assign importance of mycotoxins in public and animal health if data on their incidence in nature are lacking. We wish to report the presence of diacetoxyscirpenol (4β,15-diacetoxy,3α-hydroxy-12,13-epoxytrichothec-9-ene), deoxynivalenol (vomitoxin), T-2 toxin and zearalenone [2,4-dihydroxy-6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)benzoic acid μ-lactone] in feeds implicated in mycotoxicoses of swine and cattle (Tables 1 and 2).

Additional isolates of *F. roseum* were isolated from feedstuff suspected of causing mycotoxicoses in farm animals in various parts of the world. These were grown on a solid rice medium, and analyzed for zearalenone and trichothecenes.

MATERIALS AND METHODS

Preliminary screening. Twenty-five grams of each sample was extracted with ethyl acetate; the extract was concentrated and partitioned between equal volumes of acetonitrile and petroleum ether (boiling point 60 to 70°C). The acetonitrile layer was concentrated and dissolved in 0.5 ml of acetone. A 20-μl portion of the acetone solution was converted into the trimethylsilyl (TMS) ether by evaporating the acetone under a stream of nitrogen and adding 20 μl of Tri-Sil-TBT (Pierce Chemical Co., Rockford, Ill.). The TMS ether was then analyzed for deoxynivalenol, diacetoxyscirpenol, monoacetoxyscirpenol, neosolaniol, T-2 toxin, and zearalenone by using a computerized gas chromatograph-mass spectrometer (GC-MS; LKB-9000) system. The gas chromatography was performed on a stainless steel column (90 by 8 mm) packed with 3% OV-1 on 100/120 mesh Gas Chrom Q temperature programed from 150 to 275°C at 60°C/min. The carrier gas flow was maintained at 25 ml/min. The silylated samples were analyzed by GC-MS operated in the selected ion-monitoring (SIM) mode (C. J. Mirocha et al., Abstr. Annu. Meet. Assoc. Off. Agric. Chem. 1974, p. 62). For each mycotoxin being analyzed, intensities of a set of nine characteristic ions were monitored as each component of the injected sample eluted from the column. The SIM profile, i.e., the intensities of selected ions as a function of retention time, so generated was used to identify the mycotoxin. Often the extracts of samples were too complex to yield an interpretable SIM profile; it did, however, provide an indication of the possible presence of known mycotoxins in those extracts. From the many samples screened, those shown in Tables 1 and 2 were positive for the respective mycotoxins and were subjected to further separation and purification.

Isolation, identification, and quantification of mycotoxins. The general scheme of analysis for the trichothecenes deoxynivalenol, diacetoxyscirpenol, and T-2 toxin is shown in Fig. 1. A large sample size (100 to 300 g) was used for these analyses to compensate for low recovery. Samples suspected of contain-

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TABLE 1. *Natural occurrence of Fusarium toxins in feedstuff*

Sample no.	Mycotoxins found	Concn (μg/kg)	Diagnosis	Feedstuff
FS-382	Diacetoxyscirpenol	500	Hemorrhagic bowel syndrome in swine	Mixed feed (Univ. of Minnesota)
FS-404	Diacetoxyscirpenol	380	Hemorrhagic bowel syndrome in swine	Mixed feed (Univ. of Minnesota)
FS-356	Deoxynivalenol[a] Zearalenone	1,800 250	Feed refused by swine	Maize kernels (Michigan)
FS-362	Deoxynivalenol Zearalenone	1,000 175	Feed refused by swine	Maize kernels (Indiana)
FS-398A	Deoxynivalenol Zearalenone	100 1,750	Feed refused by swine	Maize kernels (Ohio)
FS-463	Deoxynivalenol Zearalenone	40–60 3,600	Feed refused by swine and bloody stools	Commercial pelleted mixed feed
FS-417	T-2 toxin Zearalenone	76 700	Bloody stools, bovine	Mixed feed (Nebraska)
FS-483	Deoxynivalenol Zearalenone	1,000	Vomiting in dogs	Mixed feed (Iowa)
FS-489	Deoxynivalenol Zearalenone	1,000 Trace	Feed refused by swine	Mixed feed (Minnesota)

[a] Also known as vomitoxin.

TABLE 2. *Natural occurrence of zearalenone in feedstuff associated with hyperestrogenism in swine*

Sample no.	Concn (μg/kg)	Feedstuff
FS-435[a]	100–150	Corn kernels (Minnesota)
FS-449D	150	Dry sow ration (Vancouver)
FS-453A[b]	66	Farrowing ration (Vancouver)
FS-453B	150	Dry sow ration (Vancouver)
FS-443B	200	Corn kernels (Vancouver)
FS-443A[b]	250	Dry sow ration (Vancouver)
FS-447A	1,000	Lactation ration (Vancouver)
FS-447B	500	Gestation ration (Vancouver)
FS-475	2,000–5,600	Milo (Minnesota)
FS-477	1,500[c]	Sesame meal (Univ. of Minn.)
FS-468A	120	Corn kernels (Ohio)
FS-468B[b]	120	Mixed feed corn (Ohio)
FS-469	6,400	Corn kernels (Minnesota)
FS-470	6,800	Commercial pelleted, mixed feed (Minnesota)

[a] Rectal prolapse in gilts.
[b] Diethylstilbestrol was also present in these samples.
[c] Associated with hyperestrogenism in turkey poults.

ing deoxynivalenol, as indicated by the SIM profile in the preliminary screening, were extracted with 40% aqueous MeOH and those suspected of diacetoxyscirpenol and T-2 toxin contamination were extracted with ethyl acetate; samples suspected of containing both deoxynivalenol and T-2 toxin were first extracted with ethyl acetate followed by 40% aqueous methanol.

Deoxynivalenol (vomitoxin). The aqueous methanol extract was concentrated and treated with acetone with vigorous stirring to precipitate high-molecular-weight constituents such as polysaccharides and fragmented proteins. The precipitate was removed by centrifugation and the supernatant solution was chromatographed on thin-layer chromatography plates. The band corresponding to the R_f value of vomitoxin was eluted with acetone, converted into the TMS ether, and analyzed by GC-MS. The mass spectra obtained were then compared with the mass spectrum of the TMS-ether of authentic deoxynivalenol that was provided by N. Morooka.

Diacetoxyscirpenol and T-2 toxin. The ethyl acetate extract was defatted and treated with ferric gel to remove pigmented components. The filtrate was then treated with dimethoxypropane to remove water, concentrated, and resolved into its constituents on preparative thin-layer chromatography plates (silica gel GF) using chloroform-methanol (98:2) as the developing solvent. The bands corresponding to the R_f value of authentic diacetoxyscirpenol (Makor Chemicals, Jerusalem, Israel) and T-2 toxin were eluted with acetone and the eluent was concentrated. The diacetoxyscirpenol and T-2 bands were made visible by spraying an indicator strip with concentrated H_2SO_4 and heating for 3 min at 110°C. The isolated constituents were then converted into their TMS ether derivatives and analyzed by GC-MS. Mass spectra obtained were compared with those of the TMS ether of diacetoxyscirpenol and T-2 toxin. The isolated components were also converted into trifluoroacetyl derivatives by reacting them with *N*-methylbis(trifluoroacetamide) and were analyzed by GC-MS. The mass spectra of these derivatives were then compared with those of trifluoroacetate of T-2 toxin and diacetoxyscirpenol. A portion of each isolated constituent was applied to the shaved skin of a rat to determine any dermatitic reaction.

The isolates of *Fusarium* spp. isolated from toxic feeds were seeded onto previously autoclaved polished rice at a moisture content of 40% (fresh weight basis). They were grown at room temperature (24 to 27°C) for 1 week and at 14°C for 4 weeks. The cultures were harvested, dried, and extracted as described.

RESULTS AND DISCUSSION

Samples FS-382 (Table 1) and FS-404 were mixed feed obtained from the College of Veteri-

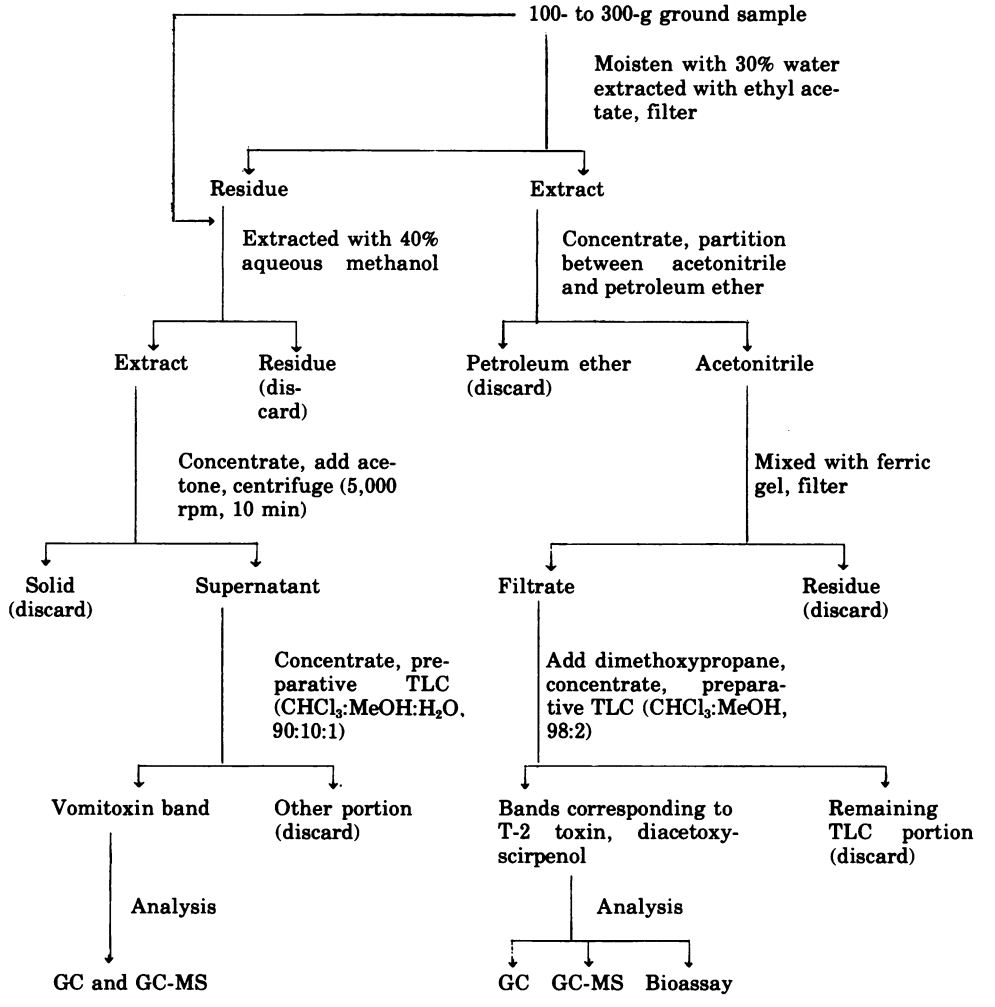

FIG. 1. *Scheme of extraction, purification and analysis of mycotoxins used in this study. TLC, Thin-layer chromatography.*

nary Medicine of the University of Minnesota and were associated with an idiopathic condition in swine called hemorrhagic bowel syndrome. The disease was first described by O'Neill (7) and is characterized by internal hemorrhages originating in the ileum. Each of our samples represented a separate incidence of this disease that occurred among experimental animals at the university. The feed samples were analyzed for T-2 toxin, aflatoxins, and ochratoxins but were negative for these toxins. However, initial screening indicated the presence of diacetoxyscirpenol. Extensive analyses resulted in isolation of the toxin that had a mass spectrum identical to that of authentic diacetoxyscirpenol. The toxin also caused a dermatitic response in the rat skin test. Gas chromatographic analysis indicated 500 to 380 μg of diacetoxyscirpenol per kg present in these samples, respectively (based on 47% estimated recovery).

Sample 417 was associated with bloody stools in cattle; analyses revealed 78 μg of T-2 toxin and 700 μg of zearalenone per kg.

Four whole kernel corn samples (FS-356, 363, 398) and one commercial mixed feed sample (FS-463), which were refused by swine, were referred to our laboratory for analysis. The corn samples originated in Michigan, Indiana, and Ohio and the mixed feed originated in Minnesota. Analysis showed the presence of deoxynivalenol (vomitoxin) at levels from 50 to 1,800 μg/kg (the percentage recovery was not estimated). Deoxynivalenol was also found in a sample of dog food (FS-483). The identity of deoxynivalenol was authenticated by mass spectroscopy. Although Vesonder et al. (8, 9) reported deoxynivalenol in corn, this is the first

TABLE 3. *Mycotoxins produced by various isolates of Fusarium associated with mycotoxicoses in farm animals*[a]

Isolate	Origin	Diagnosis	Mycotoxins[b]
Fusarium roseum	Finland	Refusal, emesis, bloody stools (swine and cattle)	DAS, DEX, F-2
F. roseum	England	Hyperestrogenism, abortion (swine)	DEX, F-2
F. roseum	Minnesota	Hyperestrogenism, abortion, death (swine)	DAS, DEX, F-2
F. roseum	Minnesota	Hyperestrogenism (swine)	F-2
F. roserum	India		MAS, DAS, DEX, F-2
F. culmorum	Washington	Hyperestrogenism (swine)	DEX, F-2

[a] The isolates were grown on a solid rice substrate and their cultures were extracted and analyzed.
[b] DAS, Diacetoxyscirpenol; DEX, deoxynivalenol; MAS, monoacetoxyscirpenol; F-2, zearalenone.

report of its occurrence in a commercial feed sample. All samples contained zearalenone, which is also produced by isolates of *F. roseum* responsible for the synthesis of deoxynivalenol.

Various corn and mixed feed samples associated with rectal prolapse, swelling of the vulva in swine, and distended, swollen teats in newborn piglets were received for analysis. The samples were analyzed for zearalenone and diethylstilbestrol by the method of Mirocha et al. (4) and by SIM. Analyses of these samples revealed the presence of zearalenone, as well as diethylstilbestrol. Sample FS-447 (sesame seed) was associated with hyperestrogenism and diarrhea in turkey poults. The signs in the turkeys were identical to those reported by Meronuck et al. (3) and Mirocha et al. (5). This is the first report of the occurrence of zearalenone in sesame seed (Table 2).

Isolates of *F. roseum*, previously isolated from feedstuff associated with suspected mycotoxicoses, were analyzed for trichothecene production after culturing on a solid rice medium (Table 3). *F. roseum* is usually easily isolated from such samples, at least more frequently than *F. tricinctum*. Results of analyses revealed that nearly all of the isolates produced a mixture of trichothecenes, as well as zearalenone. This supports our hypothesis that diacetoxyscirpenol, deoxynivalenol, and zearalenone may occur together under natural conditions.

Reports in the literature on the incidence of *Fusarium* toxins, especially the trichothecenes, in animal feeds have been lacking because of the lack of suitable methods of analysis. As these methods are developed, it will become apparent that toxins from *Fusarium* may be more prevalent in feed and foodstuff than the aflatoxins. Analyses are difficult because the analyst is often not certain for which trichothecene to analyze (there are 34 known derivatives) and each has different separation characteristics, making extraction and resolution difficult.

Most analyses were conducted in the past for the classic T-2 toxin (3,4-dihydroxy-15-acetoxy-8-methylbutyryloxy-12,13-epoxy-9-trichothecene), which is a product of *F. tricinctum*. We believe that *F. roseum* is more prevalent in problem feeds and that its products, such as monoacetoxyscirpenol, diacetoxyscirpenol, and deoxynivalenol, are more common than T-2 toxin in nature.

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