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Sterigmatocystin in Dairy Cattle Feed Contaminated with *Aspergillus versicolor*

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Sterigmatocystin (7.75 μg/g of feed) and a high-propagule-density of *Aspergillus versicolor* were detected in feed associated with acute clinical symptoms of bloody diarrhea and death in dairy cattle. Nine isolates of *A. versicolor* from the feed produced 13 to 89 μg of sterigmatocystin per g on cracked corn and lower amounts in liquid culture. This is the first report of sterigmatocystin in dairy cattle feed in the United States.

Aspergilli are ubiquitous fungi, and many species occur regularly in grains stored at 13.5 to 18% moisture (1, 8, 14). Included among these aspergilli are members of the *Aspergillus glaucus* and *A. restrictus* groups, *A. candidus*, *A. ochraceus*, and *A. versicolor*. Of particular interest is *A. versicolor* because of its frequent occurrence in stored grains and food commodites (3, 5, 6, 9, 10, 15, 22, 23) and its ability to elaborate the carcinogen sterigmatocystin (12, 16). Some members of the *A. flavus*, *A. nidulans*, *A. ustus*, and *A. glaucus* groups, as well as *Chaetomium* species (21) and the field fungus *Bipolaris* (12), are also known to produce sterigmatocystin (13, 16).

Reports documenting the occurrence of sterigmatocystin in foodstuffs, feedstuffs, and cereal grains reveal a low level of incidence. Sterigmatocystin has been found in wheat (0.3 μg/g, Canada [17]), corn (0.15 μg/g, India [4]), rice (0.8 to 16.3 μg/g, Japan [9]), cattle feed (0.1 μg/g, Poland [7]; 0.1 μg/g, Britain [19]) and green coffee beans (12 μg/g, Italy [3]; 1.1 μg/g, South Africa [11]). The Food and Drug Administration did not detect sterigmatocystin in an analysis of more than 500 samples of U.S. cereal grains conducted in 1974 to 1975 (20). In this study sterigmatocystin was detected in dairy cattle feed; the feed was mycologically examined for molds, and the toxin-producing potential of *A. versicolor* isolates was evaluated.

A dairy cattle feed sample (1,100 g) consisting of corn, cottonseed, and protein mix was received in 1983 from a farmer in Tennessee. The farmer reported that cattle eating this feed exhibited bloody diarrhea, with subsequent loss of milk production and death in some cases. When the dairy herd was fed a different ration, these symptoms disappeared.

The mycoflora of the feed sample were determined by separately blending three 20-g subsamples with 180 ml of sterile water (precooled to 5°C) for 2 min at high speed. A 10^{-5} dilution was made from the resultant slurry, and 0.2 ml was spread on each of 12 plates of potato glucose agar containing streptomycin (25 mg/liter) and tetracycline (1.25 mg/liter). Plates were incubated for 5 days at 25°C. The total number of fungal colonies was determined, and representative colonies were subcultured for identification.

The feed sample contained approximately 8×10^6 fungal propagules per g of feed, as determined by dilution plating. *A. versicolor* and *A. candidus*, the two dominant molds, accounted for 28.6 to 60.8% and 31.4 to 61.0% of the total propagule density, respectively. Fungi of lower densities included *Trichosporon* sp. (2.0 to 5.1%), *Penicillium restrictum* (2.0 to 4.0%), *A. conicus* (0 to 3.9%), Mycelia Sterilia (0 to 1.3%), *Penicillium implicatum* (0 to 1.3%), *A. amstelodami* (0 to 0.2%), *A. ruber* (0 to 0.2%), *Candida* sp. (0 to 0.2%), and *Microascus trigonosporus* (0 to 0.2%). Three colony types of *A. versicolor* were readily distinguished on potato glucose agar. Type A (represented by NRRL 13144, NRRL 13145, and NRRL 13146) was floccose and white to orange-brown with sparse sporulation; type B (represented by NRRL 13147, NRRL 13148, and NRRL 13149) was tightly textured, blue-green, and heavily sporulating; type C (represented by NRRL 13150, NRRL 13151, and NRRL 13152) was also tightly textured and heavily sporulating, but colonies were gray-green. Colony types retained their characteristics upon subculture.

Nine isolates of *A. versicolor* were tested for sterigmatocystin production on autoclaved cracked corn and in 2% yeast extract–4% sucrose liquid medium. Triplicate solid-state fermentations were carried out in 500-ml Erlenmeyer flasks containing 50 g of sterigmatocystin-free cracked corn. The moisture level of the corn was adjusted to 35% with distilled water before autoclaving. Each flask was inoculated with a 1.0-ml spore suspension prepared by adding 5 ml of sterile distilled water to a 7-day-old malt extract agar slant of each *A. versicolor* isolate and agitating. Flasks were incubated at 25°C as static cultures for 11 days. Isolates of *A. versicolor* were also cultured in duplicate in 300-ml Erlenmeyer flasks containing 50 ml of 2% yeast extract–4% sucrose liquid medium. Cultures were inoculated and incubated as described above.

Sterigmatocystin in each corn and liquid culture sample, as well as in the original feed sample (six analyses; 50 g each), was determined by thin-layer chromatography (18). The procedure involved the extraction of fermented cracked corn or feed (50 g) or liquid culture mycelia in a Waring Blendor for 3 min with methanol–4% KCl (180 and 20 ml, respectively), followed by partial purification on a Florisil column. The column was eluted with hexane followed by acetone-methylene chloride (5:95, vol/vol). The eluates were examined by the thin-layer chromatography developed in benzene-ethanol-acetic acid (90:5:5, vol/vol/vol). The plates were air-dried in a hood, sprayed with 20% ethanolic aluminum chloride, and heated 10 min in an oven at 90°C. Sterigmatocystin was visualized as a yellow fluorescent spot under short-wavelength UV light (254 nm) in each of the acetone-methylene chloride eluates. Confirmation of sterigmatocystin was by treatment of the positive eluates with trifluoroacetic acid-benzene developed on thin-layer chromatographic plates with the solvent system used above.

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TABLE 1. Sterigmatocystin production by *A. versicolor* isolates from dairy cattle feed

Colony type[a]	*A. versicolor* strain	Sterigmatocystin production on following substrate[b]:	
		Cracked corn (μg/g)[c]	YES medium (μg/ml)[d]
A	NRRL 13144	67.5	0.69
	NRRL 13145	89.3	0.42
	NRRL 13146	14.4	1.35
B	NRRL 13147	13.1	0.14
	NRRL 13148	37.6	0.13
	NRRL 13149	42.8	0.27
C	NRRL 13150	61.2	0.77
	NRRL 13151	67.3	0.28
	NRRL 13152	71.5	0.35

[a] Duncan's analysis ($P = 0.05$) of the average mean of each of the three colony types for sterigmatocystin production on cracked corn and 2% yeast extract–4% sucrose medium shows that colony types B and C are significantly different.
[b] Incubated for 11 days at 25°C.
[c] Mean of three flasks.
[d] Mean of two flasks. YES, 2% yeast extract–4% sucrose.

Isolates of *A. versicolor* produced 13.1 to 89.3 μg of sterigmatocystin per g on cracked corn and 0.13 to 1.35 μg/ml on 2% yeast extract–4% sucrose liquid medium (Table 1). All isolates produced less sterigmatocystin on 2% yeast extract–4% sucrose liquid medium than on cracked corn. In the 1,100-g dairy feed sample, 7.75 μg of sterigmatocystin was detected per g of feed.

The detection of substantial levels of sterigmatocystin in feed associated with diarrhea and death in dairy cattle suggests need for further studies on mycotoxins produced by aspergilli. Although sterigmatocystin was detected in dairy feed, its role in the observed intoxication is unknown. Toxic effects of sterigmatocystin laboratory animals have been associated with kidney and liver damage and diarrhea (2). This report illustrates a further example of the potential economic significance of mycotoxins.

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