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Acceptance by Swine and Rats of Corn Amended with Trichothecenes

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Swine and rats demonstrated the same response factor (i.e., the average amount of corn amended with trichothecenes consumed by animals per the average amount of uncontaminated corn consumed by animals) for consumption of corn amended with 40 ppm of either T-2 toxin or diacetoxyscirpenol. Rat response factor for corn containing 40 ppm of vomitoxin was 1.8 times more than corn containing either T-2 toxin or diacetoxyscirpenol at 40 ppm. For the corn containing 40 ppm of vomitoxin, swine response factor was 1.8 times greater than rat response factor.

Frequently, trichothecenes are associated with mycotoxicoses in animals that ingest grains naturally molded with *Fusarium* sp. (A. Ciegler, H. R. Burmeister, R. F. Vesonder, and C. W. Hesseltine, *Mycotoxins: Occurrence in the Environment*, in press). Disorders in animals observed upon the ingestion of corn in which trichothecenes were detected are the hemorrhagic syndrome in cattle and the phenomenon of refusal and emesis exhibited by swine. Hsu et al. (3) found 2 ppm of T-2 toxin in corn-fed dairy cows in which 7 of 35 died. T-2 toxin was detected in brewers grain, used as one constituent of a diet fed to 115 dairy cattle, which resulted in nine deaths (6). One of the major clinical disease features in these dairy cattle was multiple petechial hemorrhages of the mucus membrane. Experimentally, this hemorrhagic syndrome could not be produced in cattle fed rations containing T-2 toxin (7) or in swine (15). However, Kosuri et al. (4), upon parenteral administration of T-2 toxin to cattle, observed hemorrhaging of the mucosal surfaces. Vomitoxin (3,7,15-trihydroxy-12,13-epoxytrichothec-9en-8-on) (VOM) was encountered at a level of 40 ppm in corn naturally contaminated with *F. graminearum* that caused refusal and emesis in swine (12, 13). Results of experiments by Forsyth et al. (2) further document the emetic and refusal activity of VOM in swine.

Trichothecene synthesis by molds has not been studied in the field, but under laboratory conditions toxin production is dependent on culture, temperature, and media (10). It was shown recently that grains fermented with *Fusarium* strains produced refusal factors which made it unacceptable to swine (11).

To follow the isolation of refusal factors from grains contaminated with *Fusarium* sp., it became desirable to use small animals. Chemical methods for trichothecene analysis are laboriously long (8) and usually require a gas chromatograph and mass spectrometer in tandem for detection. Currently the animal of choice is the pig. However, their relatively large size, their cost, the low number available to researchers, and the quantities of grain required for feeding trials dictated a search for a more convenient laboratory animal. Rats have been reported to discriminate between noncontaminated grains and grains contaminated with *Fusarium* sp. Roine et al. (8) reported that rats refused to eat a diet containing oats, barley, and wheat fermented with *F. roseum graminearum* and *F. culmorum*. Forsyth (1) observed that rats dislike a diet composed of more than 5% *Gibberella*-damaged corn. Kotsonis et al. (5) also showed that corn fermented with *F. graminearum* and *F. roseum equiseti* was refused by rats and that corn amended with T-2 toxin causes dramatic refusal activity by rats.

Our study was undertaken to determine whether there was a mutual relationship between rats and pigs expressed by a refusal response when they were fed corn containing trichothecenes. The differences expressed by rats and pigs for corn not containing trichothecenes versus corn amended with trichothecenes (Tables 1 and 2, respectively) are so apparent as to preclude statistical analyses. In Table 2 there are seven values, each the mean of two rats; in every instance for two of the treatments, and except for one instance with VOM, the control values are higher than the test values and statis-

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TABLE 1. *Consumption by swine of uncontaminated corn spiked with VOM T-2 toxin and DAS*

Sample (500 g)	Toxin (ppm)	Corn consumed (g)				RF
		Feeding trial I	Feeding trial II	Δ (I − II)[a]	Avg.	
VOM	40	178	68	110	123	0.25
T-2 toxin	40	125	100	25	112	0.22
T-2 toxin	20	230	125	105	177	0.35
T-2 toxin	10	200	225	25	212	0.42
T-2 toxin	2	500	425	75	462	0.92
DAS	40	110	140	30	125	0.25
Control[b]		500	500	0	500	1.00

[a] Sufficiency of the size of the experiment is related to the magnitude of the differences observed: $\Delta^2/2 = si^2$; $\sqrt{\Sigma\Delta_2^2}/7 = S = 46.98$; LSD = 2.365($\sqrt{2}$ = constant)/(2 = mean number of observations); LSD = 2.365(46.98) = 111. (See Snedecor and Cochran [9].)

[b] Uncontaminated corn.

TABLE 2. *Feed consumption by rats of corn containing T-2 toxin, DAS, or VOM*[a]

Sample	Corn consumed (g) during the following feeding trial:					RF
	I	II	III	IV	Avg.	
Control[b]	13.1	14.1	9.08	7.13	9.6	1.00
			8.00	7.55		
			8.56			
DAS	3.75	0.25	3.7	2.9	2.5	0.26
			3.2	3.4		
				1.3		
T-2 toxin	0.25	1.25	4.17	0.63	2.4	0.25
			4.33	3.50		
				4.00		
VOM	2.35	6.5	5.25	1.37	4.4	0.46
	8.50		4.17	3.00		
			4.17	4.25		

[a] Values indicate average results for two rats per trial for 2 days; toxins were at 40 ppm. LSD at the 5% level was 3.30 and is based on the variation between rats fed the same test material.

[b] Uncontaminated corn.

tical analyses showed significant differences. A least significant difference (LSD) (9) was calculated for the pig-feeding study and the rat study and appears at the bottom of each table.

In previous work (11–13), refusal factors were evaluated by a pig assay based on the degree of acceptance of corn spiked with extracts obtained from moldy corn. Uncontaminated corn (500 g) amended with the test extract in question was fed to 40- to 60-pound (ca. 18.1- to 27.2-kg) swine (two per trial; duplicate) that had been given no feed for 24 h, and the amount they consumed was compared with swine fed moldy corn and uncontaminated corn. A positive response was indicated if the swine refused the corn containing an extract from the moldy corn in the same proportion as the molded grain. The test was confined only to amounts of corn consumed by swine over a 48-h period, with no other factors such as weight gains or disorders considered.

Uncontaminated corn (1 kg) used in these feeding trials was analyzed for VOM, diacetoxyscirpenol (DAS), and T-2 toxin by extracting with 40% aqueous methanol (2 liters). The residue obtained from evaporation of the aqueous methanol to dryness was chromatographed on silica-gel column chromatography. The silica-gel column was eluted with 5% methanol-chloroform (500 ml), and this eluate was taken to dryness. This residue was analyzed for the aforementioned trichothecenes by converting it to its trimethylsilyl ethers (TMS) with TBT (Pierce Chemical Co.) at 120°C for 15 min. The TMS solution was analyzed for VOM, DAS, and T-2 toxin by gas-liquid chromatography–mass spectrometry (14). These trichothecenes could not be detected. The control corn was spiked with 1 ppm of these toxins and analyzed in the same manner as the unspiked corn. A recovery of 60% VOM and 90% for the T-2 toxin and DAS was obtained. Hence, it was concluded that DAS, VOM, and T-2 toxin were not present in the control corn at ≥1 ppm. This uncontaminated corn spiked with VOM, DAS, and T-2 toxin, respectively, was offered to swine as described above; at a level of 40 ppm, the same response factor (RF) (0.25) resulted. A progressively higher RF was exhibited by swine as less T-2 toxin was added to uncontaminated corn (Table 1). The swine accepted corn spiked with 2 ppm of T-2 toxin.

Rats (two per trial; duplicate; male and female of the Sprague-Dawley strain) with an average body weight of 280 g and housed in individual cages were fed uncontaminated corn in the form of extruded kurls for 2 days. We observed no differences between male and female rats in terms of the intake of control corn or test corn amended with the trichothecenes DAS, VOM, or T-2 toxin. Corn fed to rats as kurls was preferred, since wastage was not observed as it was with corn fed in the coarsely cracked form. Then the rats were fed uncontaminated corn amended with the trichothecenes VOM, T-2 toxin, and DAS, respectively, for 2 days. Their preference for grains containing these toxins is shown in Table 2. Rats consumed corn containing VOM at a level of 40 ppm about 1.8 times better than corn spiked with 40 ppm of T-2 toxin and DAS, respectively. This greater degree of refusal exhibited by rats for the trichothecenes T-2 toxin and DAS versus VOM may be due to the strong vesicant properties of these toxins (R. F. Vesonder and C. W. Hesseltine, *in* P. E.

Nelson, ed., *Metabolites of Fusarium*, in press).

These feeding trials show swine to be twice as sensitive as rats to corn containing 40 ppm of VOM. The dosage levels tested for each of these trichothecenes show rats to be suitable animals for their detection as exhibited by a refusal response.

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