the resolution of many phenolic peaks on the HPLC column, it facilitates the collection of unknown peaks individually, which makes possible their analysis and identification.

Registry No. *trans*-Caffeoyl tartrate, 67879-58-7; *cis*-*p*-coumaroyl tartrate, 67920-37-0; *trans*-*p*-coumaroyl tartrate, 27174-07-8; catechin, 154-23-4; epicatechin, 490-46-0; procyanidin B2, 29106-49-8; procyanidin B3, 23567-23-9.

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Detoxification of Deoxynivalenol with Sodium Bisulfite and Evaluation of the Effects When Pure Mycotoxin or Contaminated Corn Was Treated and Given to Pigs[1]

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Deoxynivalenol- (DON-) contaminated corn was treated with aqueous sodium bisulfite in order to (a) determine optimum conditions for reduction in free DON levels and (b) evaluate in a feeding trial the effects of the bisulfite treatment on feed intake and weight gains in pigs. The greatest reductions (up to 95% DON) were achieved when the contaminated corn was autoclaved for 1 h at 121 °C in the presence of 8.33% aqueous sodium bisulfite (600 mL/kg of corn, by weight). In the feeding trial, a diet containing 7.2 mg of DON/kg from infected corn caused reduction in feed consumption and weight gains by pigs. When the infected corn was autoclaved with sodium bisulfite, mixed with a basal diet, and fed to pigs for 7 days, feed intake and body weight gain were improved compared with pigs fed untreated inoculated corn and were similar to the cases of the controls. In an additional toxicological trial using pure compounds, no effects were seen when DON sulfonate was administered orally to swine at the same level (molar equivalent) at which nonderivatized DON caused severe emesis.

Cereals contaminated with the fungus *Fusarium graminearum* Schwabe may contain variable amounts of the mycotoxin deoxynivalenol (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one). This mycotoxin is known to affect the feed intake and weight gains of pigs (Friend et al., 1983). However, toxicity studies with DON in swine indicate that pigs may tolerate up to 2 mg of DON/kg before these effects occur (Trenholm et al., 1984).

Previous studies (Young, 1986a,b; Young et al., 1986) have shown that pure DON in aqueous solution or DON in contaminated wheat or corn reacted readily with sodium bisulfite. The product is the 10-sulfonate adduct (DON-S), which is stable in acid but hydrolyzes to DON under alkaline conditions (Young, 1986b).

The present study was done to determine optimum conditions for sodium bisulfite reduction of DON levels in contaminated corn. In addition, as an initial approach to determine whether DON-S is toxic to animals, pure DON-S was given orally and bisulfite-treated contaminated corn was fed to pigs.

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MATERIALS AND METHODS

Materials. DON was prepared biosynthetically from liquid cultures of *Fusarium culmorum* (CMI 14764) (Greenhalgh et al., 1986), and DON-S was prepared as per Young (1986b).

Corn. Two sources of corn were used. One was field corn that had been artificially inoculated with *F. graminearum* strain M69 (Miller et al., 1983) during the growing period. It was harvested, ground, and stored frozen (−18 °C) until treated with sodium bisulfite under a variety of conditions. The other source of corn, used for the feeding study, was inoculated (*F. graminearum* DAOM 180378) and after harvest was stored as the cob in a corn crib. It was ground (3-mm screen) and treated immediately before the preparation of diets fed to pigs. The latter source of corn was chosen because the response of pigs to a diet containing the same inoculated corn appeared similar to that observed with a diet containing the same amount of pure DON (Foster et al., 1986). Consequently, interference by other toxic metabolites was probably low.

Analysis of Deoxynivalenol. DON in corn was extracted by the method of Trenholm et al. (1985) and analyzed by high-performance liquid chromatography (Young, 1986b). DON in diet samples was extracted and analyzed by the method of Trenholm et al. (1985).

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Table I. Deoxynivalenol Concentrations in Contaminated Corn following Treatment with Aqueous Sodium Bisulfite

reaction[a]		
time, h	temp, °C	DON remaining,[b] %
18	22	67
1	80	47
18	80	15

[a] Corn mixed with 1.25% aqueous sodium bisulfite (80 mL/kg of corn, by weight). [b] 100% = 4.4 ppm.

Table II. Deoxynivalenol Concentrations in Contaminated Corn[a] following Treatment with Aqueous Sodium Bisulfite and Autoclaving[b]

sodium bisulfite added, g/kg of corn, by wt	water added, mL/kg of corn, by wt	DON remaining, %
0	0	88
10	100	66
	300	59
	600	51
20	100	57
	300	39
	600	21
50	100	35
	300	13
	600	5

[a] 100% = 540 ppm. [b] At 121 °C for 1 h.

Treatment of Freezer-Stored Corn. Typically, a 5-, 10-, or 25-g sample was treated with a freshly prepared aqueous solution of sodium bisulfite and allowed to stand at room or elevated temperature in a sealed container or in an autoclave at 12 °C and then immediately analyzed for the presence of DON.

Treatment of Crib-Stored Corn. Separate samples (0.5 kg) of clean and contaminated ground crib corn were thoroughly mixed with 300 mL of a freshly prepared aqueous solution of sodium bisulfite (83.3 g/L) in 2.8-L Fernbach flasks. The flasks were loosely sealed with a cheesecloth plug and autoclaved at 121 °C for 1 h. After being allowed to cool, the samples were combined and air-dried at room temperature for 3 days. The dried materials were then reground (1.5-mm screen) and incorporated into diets for swine.

Diets. These were prepared as a meal containing 90% of a corn–soybean base grower diet, with the remaining 10% of the diet being clean crib corn, sodium bisulfite treated clean crib corn, DON-contaminated (69 mg/kg) crib corn, or sodium bisulfite treated DON-contaminated crib corn.

Feeding of Diets to Pigs. Twenty-one Yorkshire pigs (11 female, 10 male) ranging in body weight from 18 to 24 kg were randomly allocated to the experimental diets in individual pens. Feed was provided ad libitum from hoppers, and water was available from automatic water bowls. For the first week, a settling-in period, all pigs received the standard corn–soybean grower diet. The experimental diets were then fed for 7 days; the pigs were weighed when allocated to the pens and 7, 10, and 14 days afterwards, with cumulative individual feed consumption also being recorded on those days.

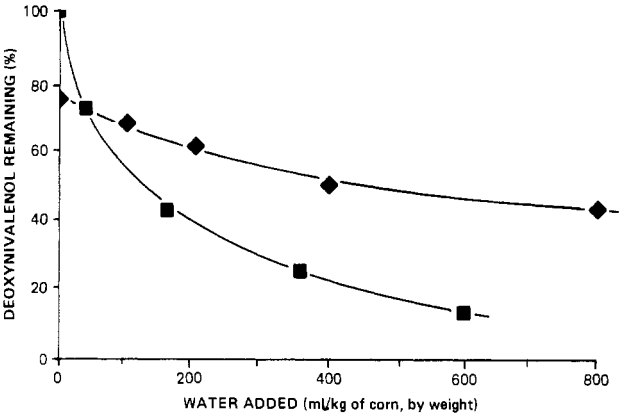

Figure 1. Deoxynivalenol content of contaminated corn following treatment with sodium bisulfite in the presence of varying amounts of water. Treatment conditions: (◆) 100% = 11 mg of DON/kg, sodium bisulfite (10 g/kg of corn, by weight) in water (mL/kg of corn, by weight) added, 2 days, room temperature; (■) 100% = 4 mg of DON/kg, sodium bisulfite (20 g/kg of corn, by weight) in water (mL/kg of corn, by weight) added, 4 days, room temperature.

DON and DON-S Administration to Pigs. In the acute toxicity study, Yorkshire pigs (ca. 30 kg) were dosed orally with an ethanolic solution (5% aqueous HCl–EtOH, 1:9; 5 mL) of either pure DON or DON-S via a soft plastic feeding tube inserted down the esophagus into the stomach. Dose levels were based on previous unpublished results that examined the dose–response relationship or emetic effect dose (ED) of DON administered orally to pigs (Prelusky, 1986). Doses used during the current study were 0.096 mg of DON/kg body weight (ED_{66}; i.e., a dose that would result in emesis in 66% of pigs tested) and/or 0.160 mg of DON/kg (ED_{98}); the molecular equivalent was used when DON-S was given. Following dosing, animals were observed for 3 h for the occurrence of emesis.

RESULTS AND DISCUSSION

Sample Treatment. The extent of reduction in DON levels was earlier found to be directly proportional to the amount of sodium bisulfite added and the reaction time (Young, 1986a). In this study, increased moisture content (Figure 1) and temperature (Table I) also aided in reducing DON concentrations. A more rapid method involving autoclaving was then investigated. A 95% reduction in DON levels was achieved after 1 h at 121 °C when 8.33% aqueous sodium bisulfite (600 mL/kg of corn, by weight) was added (Table II). Autoclaving itself contributed only a 12% reduction. On the basis of these results, 10 kg each of contaminated (69 mg of DON/kg) and clean ground crib corn was autoclaved under the same sodium bisulfite

Table III. Weight Gains and Feed Consumption by Pigs Fed Deoxynivalenol-Contaminated Corn Treated with Aqueous Sodium Bisulfite and Autoclaved[a]

		wt gains[b]		feed consumed[b]	
diet	DON concn, mg/kg	days 0–3	days 3–7	days 0–3	days 3–7
control[d]	0.11	530 ± 90 a[c]	930 ± 110 a	1490 ± 110 a	1490 ± 100 a
control + sodium bisulfite[e]	0.18	570 ± 40 a	760 ± 60 a	1530 ± 50 a	1640 ± 140 a
contaminated[e]	7.21	−420 ± 160 b	640 ± 140 a	810 ± 120 b	870 ± 150 b
contaminated + sodium bisulfite[e]	0.79	480 ± 100 a	850 ± 110 a	1460 ± 50 a	1400 ± 80 a

[a] Corn contaminated with deoxynivalenol (69 ppm) or sound corn was mixed with 8.33% aqueous sodium bisulfite (600 mL/kg of corn, by weight), autoclaved at 121 °C for 1 h, air-dried, and formulated into diet. [b] Means (g/pig per day) ± standard errors. [c] Means in the same column with different letters differ significantly at $P < 0.01$ according to the Student–Newman–Keuls multiple-range test. [d] Six pigs treated. [e] Five pigs per treatment.

treatment conditions. Analysis revealed that an 87% reduction in DON levels was achieved in the contaminated corn by this treatment. It was also observed that some lipidlike material separated out from both materials treated.

Effect of Feeding Contaminated Corn and Sodium Bisulfite Treated DON to Pigs. Reduced feed consumption and reduced weight gains in pigs, especially during the first 1–2 weeks on test, are two major symptoms associated with the consumption of low concentrations of DON-contaminated feed (Friend et al., 1986). The effect of sodium bisulfite treatments on reducing some effects of DON was investigated in two initial experiments.

The data in Table III show the performance of pigs offered the four diets used in the feeding study. Records for one of the pigs fed the clean crib corn treated with bisulfite were aberrant and were discarded. The pigs offered the untreated DON-contaminated corn diet (7.2 mg of DON/kg) gained less weight ($P < 0.01$; in fact, they suffered a net weight loss) than those on the other three diets, but only for the first 3 days of the experimental period [cf. Friend et al. (1986)]. The weight gains for the last 4 days for any diet did not show a significant difference ($P > 0.05$), although the pigs on the DON-contaminated diet had the lowest gains. The feed intake pattern was similar to that for weight gain except that the difference between pigs on the contaminated treated and contaminated untreated diets was now highly significant ($P = 0.002$) for both periods. An average weight loss of 420 g/day was recorded (Table III) for pigs fed 810 g/day of the contaminated diet during days 0–3, but on a similar feed intake between days 3–7, a weight gain of 640 g was achieved. This phenomenon might have been due, in part, to an initial metabolism response followed by a better, subsequent, utilization of the same diet. Such an explanation would be supported by the data associated with the control pigs for which corresponding gains of 530 and 930 g/day were recorded for feed intakes of 1490 g/day during each of the two periods. The data indicate that whereas the DON-contaminated diet at 7.2 mg of DON/kg caused a reduction in feed intake, treatment of the DON-contaminated corn prior to diet formulation made the resulting diet acceptable to pigs.

Sodium bisulfite treatment of the clean source of crib corn did not have any deleterious short-term effects on the pigs, who consumed the equivalent of 0.3% SO_2 in the diet. Our results are consistent with those of Til et al. (1972) who reported no decrease in feed consumption and growth of weanling pigs fed a diet containing the equivalent of 0.7% SO_2 (as sodium metabisulfite) for 48 weeks. In a review of the mammalian toxicity of ingested sulfites, Gunnison (1981) concluded that, apart from an indirect toxicity from some dietary changes and a direct irritant effect on the gastrointestinal tract at relatively high intake levels, no serious effects had resulted from chronically administered sulfite.

In the second experiment, pure DON and DON-S were administered orally to pigs. DON, when administered at the ED_{66} level, caused emesis in four of the six pigs dosed and at the ED_{98} level caused severe emesis within 20 min in all five pigs dosed. DON-S, when given at levels equivalent to DON at ED_{66} and at ED_{98}, failed to elicit any observable response in six and three animals, respectively, even up to 3 h after dosing. Thus, DON-S showed no acute toxic effects on pigs at levels equivalent to those of DON that had caused emesis.

Conclusions. These results show that autoclaving contaminated corn in the presence of sodium bisulfite effected a marked reduction in the DON concentration. Such treatment appeared to remove short-term toxic effects on pigs due to the presence of DON in the diet.

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Registry No. DON, 51481-10-8; DON-S, 103776-36-9; $NaHSO_3$, 7631-90-5.

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