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Emetic and Refusal Activity of Deoxynivalenol to Swine†

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The minimum emetic dose of deoxynivalenol to swine weighing 9 to 10 kg was 0.05 mg/kg of body weight intraperitoneally and 0.1 to 0.2 mg/kg orally. There was no emesis by undosed pigs consuming vomitus from pigs orally dosed with deoxynivalenol or penned with such pigs without access to vomitus. Analysis by gas-liquid chromatography of a sample of *Gibberella zeae*-infected corn containing about 25% visually damaged kernels indicated 12 ppm of deoxynivalenol. Deoxynivalenol added to feed reduced feed consumption of 20- to 45-kg pigs, ranging from a 20% decrease with 3.6 ppm to 90% reduction with 40 ppm. Loss in weight was associated with feed refusal. Feed refusal, however, was much greater for naturally infected corn samples than for feeds with equal concentrations of the pure compound added, indicating the involvement of an additional factor(s) in the swine refusal response.

Grains damaged by *Gibberella zeae* (*Fusarium roseum, F. graminearum*) are often unsuited for swine because of feed refusal and emesis caused by one or more substances produced by the organism prior to harvest (2, 6, 15, 17). The toxin(s) can be removed by washing for 48 h (4, 5). A variety of mycotoxins are produced by *Fusarium* species, including a large number of trichothecenes (11). Many of the trichothecenes can induce vomiting (13). The trichothecene 3α, 7α, 15-trihydroxy 12, 13-epoxytrichothec-9-en-8-one (deoxynivalenol or vomitoxin) has been isolated from *G. zeae*-infected grain and culture media and implicated in the emetic (7, 10, 14–17) and the refusal responses (15). Zearalenone (a resorcylic acid lactone), an estrogen, also has been implicated in feed refusal (8).

This research quantitates the emetic and refusal activity of deoxynivalenol and its presence in a naturally infected corn sample associated with swine refusal.

MATERIALS AND METHODS

Corn sample. *G. zeae*-infected dent corn was obtained from the 1972 epidemic in Indiana and stored at about −25°C before use in both animal trials and analysis. Visual *Gibberella* damage to kernels in this sample was about 25%, as determined by Tuite et al. (12). This sample was refused by swine and rats (4, 10) and induced emesis in swine (12). It was also fed to chicks with little effect on utilization (3). Zearalenone content was about 1 ppm as determined by the method of Caldwell et al. (1).

Trichothecenes. Analytically pure deoxynivalenol and 3-acetyldeoxynivalenol were isolated from culture broth of *Fusarium roseum* 117, ATCC 28117, as described previously (16).

Refusal trials. In experiment 1, eight crossbred pigs averaging 45.5 kg were assigned at random to two dietary treatments. Sound corn was sprayed with 0 or 7.2 mg of deoxynivalenol dissolved in 100 ml of distilled water per kg of corn and fed to individually penned pigs, and corn consumption was determined each day for 4 days. Each pig received separately 0.45 kg of a protein-vitamin-mineral supplement. After 4 days, two pigs from each treatment were fed a diet containing equal parts of control corn and *G. zeae*-damaged corn (visual damage approximately 25%) for 3 days while the other four pigs received a control corn diet.

In experiment 2, the effect on consumption and weight gain of 0, 3.6, 7.2, and 40 mg of deoxynivalenol/kg of feed was investigated with pigs averaging 20 kg. A complete mixed feed composed of corn and soybean meal fortified with salt, vitamins, and minerals and containing 18% crude protein was sprayed with 25 ml of distilled water containing the appropriate amount of deoxynivalenol per kilogram of diet. Pigs were randomly assigned to treatment. There were four pigs per treatment, with each pig individually penned and fed ad libitum in a small metal creep feeder. Feed was added and consumption was determined daily. Weight gain or loss was determined after 4 days. Correlation between deoxynivalenol concentration and average daily feed consumption was determined by the least-squares method.

Experiment 3 was similar to experiment 2 except that there were three pigs per treatment for 3 days and the following treatments were used: 0, 3.6, and 16 ppm of deoxynivalenol added to control feed, a 30:70 mixture of the *G. zeae*-infected corn sample with control feed (calculated to contain naturally 3.6 ppm of deoxynivalenol), and the *G. zeae*-infected corn sam-

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ple alone. Additionally, one pig received for 1 day feed with 3.6 ppm of deoxynivalenol and 1 ppm of zearalenone added.

Emetic trials. Two experiments were conducted to determine the emetic activity of deoxynivalenol. In experiment 4, pigs averaging 9.4 kg received 0, 0.1, 0.5, or 1.0 mg of deoxynivalenol/kg of body weight (BW) intraperitoneally (i.p.), and the pigs were observed for emesis. The dosing solutions were distilled water (10.7 ml) and distilled water containing 0.5 mg of deoxynivalenol/ml.

In experiment 5, i.p. and oral (by gavage) doses were investigated in pigs averaging 9.3 kg. Three pigs per treatment received the following deoxynivalenol doses i.p.: 0.2, 0.1, 0.075, 0.05, and 0.025 mg/kg of BW, and the following oral doses: 0.4, 0.2, 0.1, 0.075, and 0 mg/kg of BW from a water solution containing 0.2 mg/ml; and six pigs received the 0.1-mg/kg oral treatment. The influences on controls of other pigs vomiting in the pen, both with and without access to the vomitus, were examined. Also the effect of i.p. water injections was studied. Three pigs per dose level received 2, 5, and 10 ml of water i.p., respectively, and were penned together. Two pens each contained four pigs that received 11 ml of water i.p. and two pigs that received 0.2 mg of deoxynivalenol per kg i.p.; in one pen pigs were allowed access to the ensuing vomitus and in the other it was washed away.

Extraction. A total of 100 g of finely ground corn was extracted three times with 400-ml portions of methanol-water (3:1, vol/vol) (Fig. 1). The aqueous methanol solutions were combined and concentrated to about 300 ml in vacuo. The aqueous concentrate was extracted three times subsequently with 100-ml portions of *n*-hexane and 150-ml portions of chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated in vacuo to give an oily residue (265 mg). The residue was dissolved in a small volume of chloroform, applied to a preparative silica gel GF_{254} thick-layer plate (20 by 20 cm), and developed in ethyl acetate-toluene (3:1, vol/vol). By using an ultraviolet lamp, a dark band corresponding to deoxynivalenol was located and eluted with ethyl acetate-methanol (9:1, vol/vol). The eluate was concentrated in vacuo and further chromatographed on the similar silica gel plate with chloroform-acetone (3:2, vol/vol). The eluate from a major band was concentrated to dryness and dissolved in 2 ml of methanol.

For recovery tests, 1-ml portions of methanol containing 1 mg of individual trichothecenes, deoxynivalenol, fusarenon, and T-2 toxin were added to 100 g of powdered sound corn and extracted.

Trichothecene determination. An Hitachi model 063 chromatograph equipped with hydrogen flame ionization detectors was used for gas-liquid chromatography analyses of the extracts from the infected corn. The column was a coil (1 m by 3 mm) of stainless-steel tubing packed with 3% silicone OV-1 on 60- to 80-mesh Chromosorb W. The following conditions were employed: column temperature, 150 to 280°C at 10°C/min; flow rate of nitrogen, 40 ml/min; hydrogen, 0.6 kg/cm^2; and air, 1.2 kg/cm^2.

A 200-μl portion of the methanol solution was evaporated to dryness in a Pre-vial (Nihon Chromato Co., Tokyo) under a nitrogen stream and reacted overnight at room temperature with 200 μl of TMS reagent (Tokyo Kasei Kogyo Co., Tokyo). A 2-μl portion of this solution was injected into the column. The standard trichothecenes were chromatographed under the same conditions as the extracts.

RESULTS

Swine feed refusal. In experiment 1, 7.2 ppm of deoxynivalenol substantially reduced feed intake (Table 1). The pigs consuming deoxynivalenol-spiked corn ate 59.1% as much as controls in 4 days. Consumption of the deoxynivalenol diet was greater on the first two days than on the third and fourth days. The observed degree of refusal, however, did not approach the almost total refusal of pigs fed *G. zeae*-damaged corn (Table 1). In fact, consumption was so little

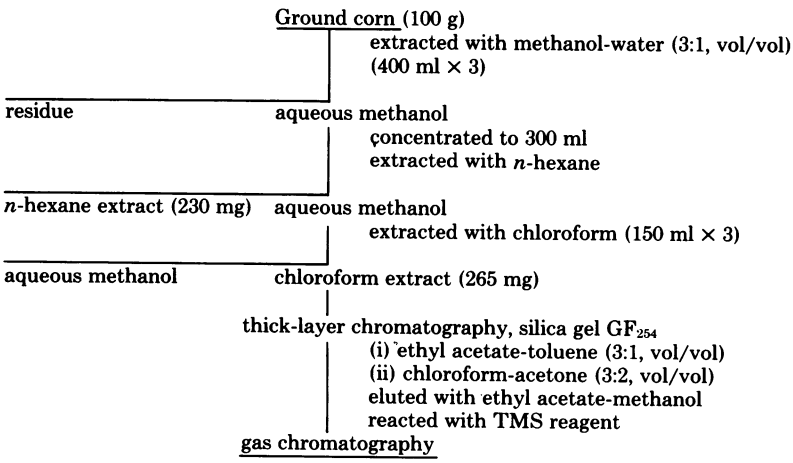

FIG. 1. *Analysis of deoxynivalenol in the Gibberella-infected corn.*

with *Gibberella* corn that it was difficult to discern from feed wastage.

In experiments 2 and 3, pigs responded to deoxynivalenol in the diet with decreased feed intake inversely proportional to the concentration added (Tables 2 and 3). A concentration of 3.6 ppm of deoxynivalenol decreased consumption in experiments 2 and 3 by 20 and 26% ($P < 0.01$), respectively. Nearly complete (90%) rejection occurred with 40 ppm of deoxynivalenol added to the diet.

TABLE 1. *Consumption of control and deoxynivalenol-treated (7.2 ppm) corn by pigs (experiment 1)*

Day	Elapsed time interval (h)	Corn consumption			
		Gibberella corn (kg)[a]	Control (kg)[a]	Deoxynivalenol (kg)[a]	% of control
1	18.3		1.411	1.148	81.4
2	28		3.143	2.159	68.7
3	26.5		2.005	0.522	26.0
4	18.5		0.799	0.522	65.3
Total	91.3		7.358	4.351	59.1
5–7[b]	71	0.122	2.26		5.4

[a] Each consumption value in kilograms is a mean representing four pigs.

[b] After 4 days, two pigs from each treatment were changed to control and the other two pigs from each treatment were changed to *G. zeae*-damaged corn diets.

The decreasing feed intake with increasing dietary deoxynivalenol additions was described by the equation $Y = Ae^{bx}$ with r value of 0.96, where $A = 1.338$, $b = -0.059$, Y = daily feed intake (kg), and x = deoxynivalenol in parts per million (Fig. 2). The consumption by pigs fed 7.2 ppm

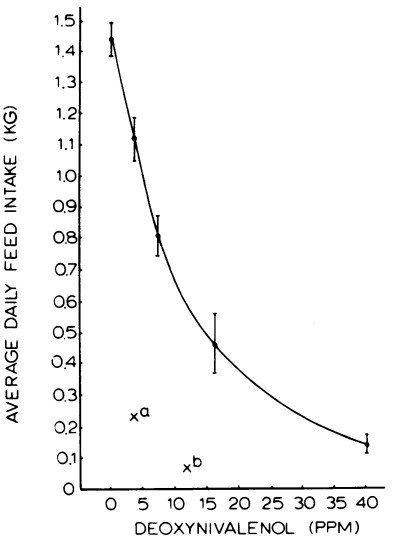

FIG. 2. *Relationship of feed consumption to dietary deoxynivalenol additions. a and b represent consumption of feed mixtures containing 3.6 and 12 ppm, respectively, of deoxynivalenol in Gibberella-infected corn.*

TABLE 2. *Effect of feeding varying levels of deoxynivalenol to pigs for 4 days (experiment 2)*

Treatment (ppm of deoxynivalenol)	Determination[a]									
	Avg initial wt	Avg final wt	Avg total gain	Avg daily gain	Avg feed intake on:				Avg daily feed intake	Total feed intake (% of control)
					Day 1	Day 2	Day 3	Day 4		
0	20.58	23.39	2.81	0.703	1.25	1.425,	1.395	1.58	1.41	100
3.6	19.50	21.43	1.93	0.483	0.91	0.945	1.213	1.463	1.13	80.2
7.2	20.01	20.69	0.68	0.170	0.755	0.783	0.843	0.835	0.81	56.9
40	20.18	17.74	−2.44	−0.610	0.278	0.123	0.090	0.08	0.14	10.1

[a] Values represent means, in kilograms, of four pigs.

TABLE 3. *Results of feeding deoxynivalenol and G. zeae-infected corn mixtures to pigs for 3 days (experiment 3)*

Treatment no.	Deoxynivalenol added (ppm)	Determination[a]								
		Avg initial wt	Avg final wt	Avg total gain	Avg daily gain	Avg feed intake (g) on:			Avg daily feed intake (g)	Total feed intake (% of control)
						Day 1	Day 2	Day 3		
1	0	19.82	21.49	1.67	0.56	1,394	1,576	1,464	1,478	100
2	3.6	19.52	20.51	0.99	0.33	1,140	1,256	879	1,092	73.9
3	16	19.82	18.46	−1.36	−0.45	486	504	404	465	31.4
4	—[b]	19.82	18.54	−1.28	−0.43	207	327	152	229	15.5
5	—[b]	20.13	18.77	−1.36	−0.45	76	107	11	65	4.4

[a] Values represent means, in kilograms, unless otherwise stated, of three pigs.

[b] Treatment 4: 30% *G. zeae*-damaged corn (25% visual damage, 12 ppm of deoxynivalenol) and 70% sound feed; treatment 5: 100% *G. zeae*-damaged corn.

of deoxynivalenol, expressed as a percentage of feed consumed by controls, corresponded well in experiment 2 (57%) with that in experiment 1 (59%) even though the pigs were of different sizes, about 45 kg and 20 kg, respectively.

In experiment 3, refusal of diets containing 30% by weight and 100% of the *G. zeae*-damaged corn sample (containing about 25% visually damaged kernels) was 84.5 and 95.6%, respectively, as compared with controls.

Body weight gain reflected feed consumption and was also negatively correlated with deoxynivalenol added. All pigs fed *G. zeae*-damaged corn (diets 4 and 5) or 16 or 40 ppm of deoxynivalenol lost weight and those fed 7.2 and 3.6 ppm gained only 24 and 69% (59% in experiment 3) as much as controls, respectively (Tables 2 and 3). No emesis was noted in the feeding trials.

Emesis. In the preliminary trial, experiment 4 (Table 4), the lowest deoxynivalenol dose i.p. (0.1 mg/kg of BW) caused vomiting, but the response was delayed compared with that of the higher doses. The lowest i.p. level to cause emesis in trial 5 (Table 5) was 0.05 mg/kg of BW, when two of three pigs vomited. The lowest active oral dose was 0.1 mg/kg of BW and only one of six pigs vomited only once. At 0.2 mg of deoxynivalenol/kg of BW, two of three pigs vomited. Thus, the oral route is about one-quarter to one-half as effective as the i.p. route in inducing vomiting, and the response was generally delayed. Vomiting induced by both routes generally ceased by 1.5 h after administration and there appeared to be no subsequent ill effects.

Distilled water injection, or consumption of vomitus, or presence of vomiting pigs did not cause control pigs to vomit.

Trichothecene quantitation. A quantitative estimation of the amount of trichothecene in the infected corn was calculated from a standard curve prepared by plotting peak height against sample size injected. The percent recovery of added trichothecenes of deoxynivalenol, fusarenon, and T-2 toxin averaged about 85, 75, and 71%, respectively. Deoxynivalenol was identified by co-chromatography with added authentic standard, which changed the height, but not the shape or position (retention time 8.3 min), of the peak and by the absence of any such peak in a control chromatogram of material similarly prepared from the sound corn.

The identification of deoxynivalenol was also confirmed by comparing it with the pure sample on silica gel GF_{254} thin-layer plates by using chloroform-acetone (3:2, vol/vol) and ethyl acetate-toluene (3:1, vol/vol). From gas-liquid chromatography tracing, 10.7 ppm of deoxynivalenol was detected in the extract from the infected corn (Fig. 3). Therefore, about 12 ppm of the toxin occurred in the Indiana sample of corn with 25% *Gibberella* damage. No other trichothecenes such as fusarenon, T-2 toxin, and 3-acetyldeoxynivalenol were detected in the corresponding extracts prepared by thin-layer chromatography.

TABLE 4. *Response to i.p. administration of deoxynivalenol (experiment 4)*

Dose (mg/kg)	Response	
	Time to first vomition (min)	No. of vomitions
0	0	0
0.1	46	10
0.5	20	15
1.0	13	13

DISCUSSION

Deoxynivalenol caused both emesis and feed refusal in pigs. Orally, 0.1 mg/kg was the minimal emetic dose, but the i.p. route was two to four times more effective. The feed refusal of naturally infected corn blended with sound corn was much greater than that of the deoxynivalenol-spiked corn believed to contain the same amount of mycotoxin. This estimation was based

TABLE 5. *Emetic response of pigs to i.p. and oral administration of deoxynivalenol (experiment 5)*

Deoxynivalenol (mg/kg of BW)	Determination							
	i.p. dose				Oral dose			
	No. tested	No. vomiting	Avg time to first vomition (min)	Avg no. of vomitions	No. tested	No. vomiting	Avg time to first vomition (min)	Avg no. of vomitions
0.4	0				3	3	59	4.3
0.2	3	3	27	8.3	3	2	68.5	5
0.1	3	3	39	9.3	6	1	82	1
0.075	3	3	42	6.7	3	0		
0.05	3	2	29	4.5	0			
0.025	3	0			0			
0	17	0			4	0		

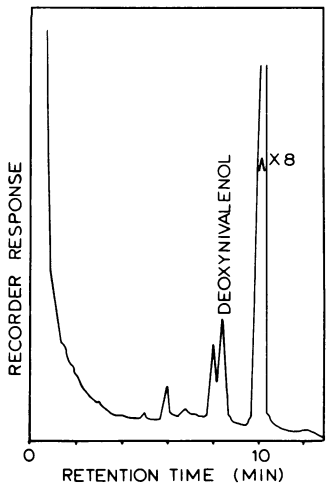

FIG. 3. *GLC analysis of TMS ether of deoxynivalenol in the Gibberella zeae-infected corn.*

on the 12-ppm amount of deoxynivalenol found in the heavily damaged corn (25% visual *G. zeae* damage) (Chester Mirocha in a separate analysis reports 13.5 ppm of deoxynivalenol in this sample). The 7.2-ppm treatment of deoxynivalenol therefore should correspond to a visual damage of approximately 15% or three times the 5% amount considered to cause rejection (12). The mixture of control and *G. zeae*-damaged corn to give 15% damage in experiment 1 was almost completely rejected (5% of consumption of controls), whereas consumption of the 7.2-ppm deoxynivalenol diets were 59 and 57% in experiments 1 and 2, respectively.

In experiment 3, diets 2 (3.6 ppm of deoxynivalenol added) and 4 (30:70 mixture of damaged to sound corn) were formulated to contain the same amounts of deoxynivalenol. Consumption was much less of the feed containing naturally infected corn (229 g/day) than of feed with deoxynivalenol added (1,092 g/day). Pigs fed straight *G. zeae*-damaged corn, with an estimated 12 ppm of deoxynivalenol, ate significantly less (65 g/day) than those fed feed with 16 ppm of deoxynivalenol added (465 g/day).

The average daily consumption for pigs fed 40 ppm of deoxynivalenol (experiment 2) was 142 g, which was 10% of the consumption of controls. Vesonder et al. (15) reported consumption of 123 g of feed containing 40 ppm of the compound (the only concentration they tested), which led them to conclude that the emetic and refusal factors are the same. The very heavily damaged corn sample we used contained about 12 ppm of deoxynivalenol, as calculated from an estimated 85% recovery, recognizing that there are limitations in the use of fortified samples to evaluate an extraction procedure. Mirocha et al. (9) reported levels in corn from Michigan, Indiana, and Ohio and mixed feeds from Minnesota (which were refused by swine) of from 0.05 to 1.8 ppm of deoxynivalenol. Ishii et al. (7) reported 7.4 ppm in a sample of corn from Ohio, whereas the report by Vesonder et al. (15) indicated a level of 40 ppm. None of these previous reports indicate the percent recovery of their method so the amounts may vary. The refusal response from *G. zeae*-infected corn is much greater, therefore, than can be accounted for by the amount of deoxynivalenol usually present.

Deoxynivalenol is often accompanied by zearalenone (9), which has been reported to cause feed refusal in rats (8). Zearalenone is less active than T-2 toxin in causing refusal but is thought to be additive or synergistic (8). At the conclusion of experiment 3, we fed one pig feed for one day with 3.6 ppm of deoxynivalenol and 1 ppm of zearalenone added. Consumption was 1,005 g, tentatively indicating no effect from zearalenone.

Vesonder et al. (15) reported the presence of small amounts of two other trichothecenes in refused corn, but their importance is unknown. Thus, the present evidence indicates that deoxynivalenol is an important emetic and refusal compound but appears, because of its reported concentration and activity, not to account for all of the refusal of *G. zeae*-infected corn.

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LITERATURE CITED

1. **Caldwell, R. W., J. Tuite, M. Stob, and R. S. Baldwin.** 1970. Zearalenone production among *Fusarium* species. Appl. Microbiol. **20**:31-34.
2. **Curtin, T. M., and J. Tuite.** 1966. Emesis and refusal of feed in swine associated with *Gibberella zeae*-infected corn. Life Sci. **5**:1937-1944.
3. **Featherston, W. R.** 1973. Utilization of Gibberella-infected corn by chicks and rats. Poult. Sci. **52**:2334-2335.
4. **Forsyth, D.** 1974. Studies on *Gibberella zeae*-infected corn in diets of rats and swine. J. Anim. Sci. **39**:1092-1098.
5. **Forsyth, D. M., L. A. DeUriarte, and J. Tuite.** 1976. Improvement for swine of *Gibberella zeae*-damaged corn by washing. J. Anim. Sci. **42**:1202-1206.
6. **Hoyman, W. G.** 1941. Concentration and characterization of the emetic principle present in barley infected with *Gibberella saubenetii*. Phytopathology **31**:871-885.
7. **Ishii, K., Y. Ando, and Y. Ueno.** 1975. Toxicological approaches to the metabolites of Fusaria. IX. Isolation of vomiting factor from moldy corn infected with Fusarium species. Chem. Pharm. Bull. (Tokyo) **23**: 2162-2164.
8. **Kotsonis, F. N., E. B. Smalley, R. A. Ellison, and C. M. Gale.** 1975. Feed refusal factors in pure cultures of *Fusarium roseum 'graminearum'*. Appl. Microbiol. **30**:362-368.

9. **Mirocha, C. J., S. V. Pathre, B. Schauerhamer, and C. M. Christensen.** 1976. Natural occurrence of *Fusarium* toxins in feedstuffs. Appl. Environ. Microbiol. **32**:553–556.
10. **Morooka, N., N. Uratsuji, T. Yoshizawa, and H. Yamamoto.** 1972. Studies on the toxic substances in barley infected with *Fusarium* spp. J. Food Hyg. Soc. Jpn. **13**:368–375.
11. **Purchase, I. F. H. (ed.).** 1974. Mycotoxins. Elsevier Scientific Publishing Company, Amsterdam.
12. **Tuite, J., G. Shaner, G. Rambo, J. Foster, and R. W. Caldwell.** 1974. The *Gibberella* ear rot epidemics of corn in Indiana in 1965 and 1972. Cereal Sci. Today **19**:238–241.
13. **Ueno, Y., K. Ishii, N. Sato and K. Ohtsubo.** 1974. Toxicological approaches to the metabolites of *Fusaria*. VI. Vomiting factor from moldy corn infected with Fusarium spp. Jpn. J. Exp. Med. **44**:123–127.
14. **Vesonder, R. F., A. Ciegler, and A. H. Jensen.** 1973. Isolation of the emetic principle from *Fusarium*-infected corn. Appl. Microbiol. **26**:1008–1010.
15. **Vesonder, R. F., A. Ciegler, A. H. Jensen, W. K. Rohwedder, and D. Weisleder.** 1976. Co-identity of the refusal and emetic principle from *Fusarium*-infected corn. Appl. Environ. Microbiol. **31**:280–285.
16. **Yoshizawa, T., and N. Morooka.** 1973. Deoxynivalenol and its monoacetate: new mycotoxins from *Fusarium roseum* and moldy barley. Agric. Biol. Chem. **37**:2933–2934.
17. **Yoshizawa, T., and N. Morooka.** 1974. Studies on the toxic substances in the infected cereals (III). Acute toxicities of new trichothecene mycotoxins: deoxynivalenol and its monoacetate. J. Food Hyg. Soc. Jpn. **15**:261–269.