Survey of Vomitoxin Contamination of the 1980 White Winter Wheat Crop in Ontario, Canada

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ABSTRACT

During the summer of 1980, there was a great deal of rainfall and high humidity in southwestern Ontario. Sprouting of the kernels on plants in the field and pink discoloration down-graded the white winter wheat crop. Samples were submitted to Agriculture Canada from elevators and boats loading wheat for export. Chemical analyses indicated low levels of vomitoxin, with some analytical results as high as 8.5 ppm. A series of feeding trials was initiated with contaminated wheat (1.0 ppm vomitoxin) to determine effects on swine and poultry. Swine diets containing 0.3 and 0.7 ppm vomitoxin resulted in decreased feed consumption and weight gains. Poultry were fed similar levels of vomitoxin without any serious effects. Gross examination of internal organs at the termination of the subacute studies revealed no apparent toxic effects. Several farmers reported feed refusal, vomiting and death in their livestock. Chemical analyses, in general, revealed relatively low levels of vomitoxin. In one case, the level of vomitoxin (0.95 ppm) in the feed was high enough to be a possible contributing factor in the observed ill effects.

INTRODUCTION

White winter wheat is an important crop in Ontario. It is sown in the autumn and harvested during the early summer before other cereal crops. Much of the 250,000 tonnes of winter wheat produced each year is exported as feed grade wheat. During the wheat harvest in July 1980, Ontario farmers noticed a great deal of sprouting and pink discoloration of wheat kernels. As a result, wheat delivered to elevators was down-graded. Preliminary chemical analyses indicated the presence of 2-deoxynivalenol (vomitoxin). Figure 1 shows a map of southern Ontario where winter wheat was most affected by vomitoxin contamination, especially in the 3 counties of Elgin, Ken and Lampton, located between Lake Huron and Lake Erie. In these areas, vomitoxin levels were as high as 8.5 ppm.

Vomitoxin is a mold metabolite (1-4) found in corn and cereal grains contaminated in the field by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schw.] Petch) (5-10). Mold infestation usually begins at the ear tip, often referred to as "pink ear rot." In the field, infection of corn by *G. zeae* is favored by low temperatures with concomitant high moisture conditions. The molecular structure of vomitoxin is shown in Figure 2.

Vomitoxin is a trichothecene mycotoxin which may produce feed refusal, vomiting and possibly other ill effects such as bloody stools. In 1973, Vesonder et al. (2) isolated, characterized and demonstrated vomitoxin to be major emetic and feed refusal factors to swine. Forsyth et al. (1) showed dose-response relationships between level of vomitoxin administered (orally and intraperitoneally) to swine and feed refusal and vomiting. There is some evidence suggesting that swine are particularly sensitive to vomitoxin; poultry and cattle are more resistant (11).

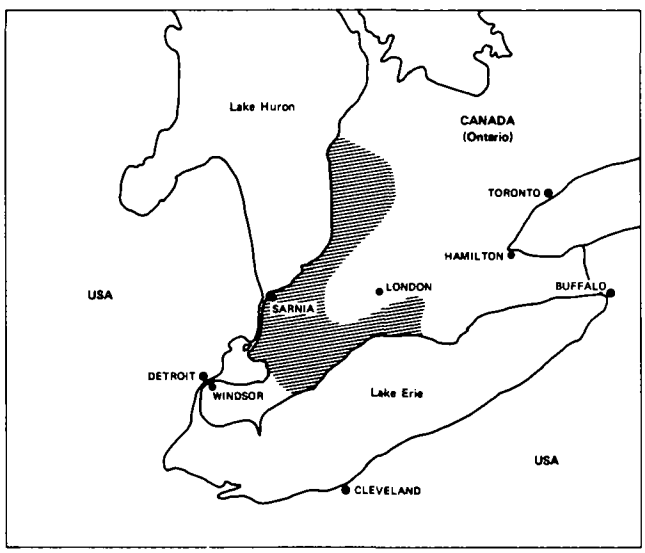

FIG. 1. Map of southwestern Ontario, Canada. Shaded areas highlight region where levels of vomitoxin contamination in white winter wheat were the highest in the 1980 crop.

In this paper, we report the results of a survey for mycotoxins of the white winter wheat crop harvested in the province of Ontario in 1980. Exploratory feeding trials were done to determine the effects of low levels of vomitoxin in swine and poultry diets. In addition, several farmers who noticed problems with their farm animals sent suspect feed samples to our laboratories for chemical analyses. The results of the field studies are also reported in this paper.

Screening Procedure

Samples (20-g) were extracted (12), dissolved in pyridine and silated at 60 C for 1 hr with TISM (N-trimethyl-silyl-imidazole) and TCMS (trimethyl-chlorosilane) from Chromatographic Specialties, Brockville, Ont. The derivatives were analyzed by combined gas chromatography-mass spectrometry (GC/MS) using a Finnigan Model 4000 GC-MS system equipped with an INCOS data system

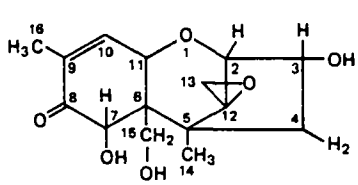

FIG. 2. Chemical structure of vomitoxin.

(Finnigan Corp., Sunnyvale, CA). The mass spectrometer was operated in the multiple ion monitoring mode to give maximal sensitivity and reasonable specificity. Five mycotoxins were screened on the GC/MS system: vomitoxin, diacetoxyscirpenol (DAS), neosolaniol (NSL), T-2 and HT-2. The lower limit of detection was 0.05 ppm.

The samples submitted from Ontario elevators were also analyzed for the mycotoxins citrinin, ochratoxin, zearalenone, sterigmatocystin, aflatoxins B_1, B_2, G_1, G_2, penicillic acid, rubratoxin, luteoskyrin and patulin using the acidified acetonitrile extraction-thin layer chromatography (TLC) method described by Stoloff et al. (13).

Analysis of Wheat Samples from Ontario Elevators

Forty-one samples of wheat representing individual producer shipments were submitted by the Canadian Grain Commission to our laboratories for chemical analyses of mycotoxins.

The results are shown in Table I. The majority of the samples were graded as "Canada Feed" grade wheat, 9 were graded as either nos. 3, 4, 5 Canada Eastern (C.E.) white winter wheat, and 4 received no official grade. The percentage of *Fusarium*-stained kernels ranged from a low of 0.01% to a high of 12%. Most samples contained less than 1% stained kernels. In general, vomitoxin levels were less than 1 ppm; the highest was 8.53 ppm. Multitoxin screening did not identify any other mycotoxins present in the wheat.

Analyses of Wheat Samples from Boats Loaded for Export

Thirty-six samples representing boatloads or partial boatloads of grain loaded from terminal or export elevators were also submitted for analyses. The results are shown in Table II. Twenty-four of the "Canada Feed" grade samples represented shipments of 207,000 tonnes of wheat. The 12 numerical grade wheat samples represented 57,000 tonnes of nos. 2-5 Canada Eastern winter wheat. In comparison to the producers' samples (Table I), the results of the wheat in boatloads (Table II) were generally much lower. *Fusarium*-stained kernels were less than 0.2%. Most samples contained vomitoxin at levels of less than 0.3 ppm. The highest vomitoxin value was 0.42 ppm.

A partial explanation may be that the blending of wheat from many producers reduced the overall vomitoxin levels in the boatload samples.

Mycology

Vomitoxin-contaminated white winter wheat samples were also analyzed for the presence of *Fusarium* species. *F. graminearum* Schwabe, *F. poae* (Peck) Wollenweber and *F. sporotrichioides* Sherbakoff sensu Seemüller were isolated (14-16). *F. graminearum* was the predominant species and, of 6 isolates tested, all produced vomitoxin in vitro.

Feeding Trials with Swine and Poultry

A series of feeding trials was done to assess the nutritional/toxicological effects of vomitoxin-contaminated wheat on swine and poultry. When 18 Yorkshire gilts were fed barley-wheat-soybean (17) grower-finisher diets containing contaminated wheat (1.0 ppm vomitoxin) feed refusal by some pigs was evident during the first 1-3 days. Average daily gains for the 21-day trial were related to vomitoxin levels

TABLE I

Vomitoxin Analysis of Producers' Samples

Fusarium-stained kernels (%)	No. of samples	Vomitoxin (ppm)
Numerical grades (nos. 2-5 C.E.)		
.01	1	ND[a]
.03	4	ND
.04	1	ND
.17	1	1.23
.20	2	.71, 1.27
	Total 9	$\bar{x}$ = 0.38
Canada feed grade		
.02	5	5-ND
.05	1	ND
.08	1	ND
.09	1	ND
.10	4	4-ND
.11	1	.32
.12	4	2-ND,.77,1.40
.15	1	.81
.20	3	ND,.50,.68
.33	1	ND
.60	1	1.51
.70	1	2.10
.80	1	1.72
1.0	1	2.00
6.0	1	5.70
12.0	1	8.53
	Total 28	$\bar{x}$ = 0.93
Ungraded		
.01	2	ND
.02	1	ND
1.0	1	3.64
	Total 4	$\bar{x}$ = 0.91

[a]ND = none detectable (0.0 ppm used in $\bar{x}$ calculations).

TABLE II

Vomitoxin Analysis—Boatload Samples

Fusarium-stained kernels (%)	No. of samples	Vomitoxin (ppm)
Numerical grades (nos. 2-5 C.E.)		
0	6	3-ND,.14,.16,.30
.03	2	.28,.41
.05	1	ND
.07	1	.32
.10	1	.16
.16	1	.14
	Total 12	$\bar{x}$ = .16
Canada feed grade		
0	2	ND
.01	2	ND
.02	3	2-ND,.25
.03	2	ND,.38
.04	2	.15,.23
.05	2	ND,.25
.06	2	ND
.08	1	ND
.10	5	ND,.06,.08, .36,.38
.12	1	.50
.16	1	.46
.17	1	.42
	Total 24	$\bar{x}$ = .15

TABLE III

Results of Feeding Trials

		Average daily gains (21 days)
Swine	Control	0.95 kg/day
	50:50	0.82 kg/day
	100 Vomitoxin-contaminated wheat	0.68 kg/day
Poultry	No major deleterious effects on feed consumption and egg production. No observed organ damage at postmortem	

TABLE IV

Suspected Mycotoxin Outbreaks, Ontario and Quebec, 1980

Location	Species	Symptoms	Vomitoxin (ppm)	Zearalenone (ppm)
Smithville, Ont.	Pig	Vomiting, feed refusal	0.95	0.13
Barrie, Ont.	Pig	Feed refusal	0.03	0.007
Buckingham, Que.	Pig	Vomiting, feed refusal 100/500 died	0.14	<0.002
	Cattle	2/7 died		
Hanover, Ont.	Pig	Vomiting	<0.05	<0.2

(Table III): 0.68 kg/day for pigs fed vomitoxin-contaminated wheat; 0.82 kg/day for pigs fed a diet containing contaminated wheat diluted 50:50 with clean wheat. Pigs fed the control diet gained 0.95 kg/day. There was no evidence of vomiting in any of the treated animals.

Experiments were done with 96 white Leghorn adult cockerels and 306 pullets. After a 24-hr fast, the cockerels were force-fed 50-g aliquots of cracked, or cracked and pelleted clean wheat, vomitoxin-contaminated wheat or a 50:50 mixture. Starting 24 hr after force-feeding, each bird was fed the same diet ad libitum for the next 7 days. Pullets were given wheat-soy diets (18) that contained either clean wheat, vomitoxin wheat or 50:50 mixtures for 10 weeks.

No marked changes in feed consumption, weight gain or egg production were noted.

Postmortem examinations of the pigs and poultry at the termination of the experiments did not reveal any gross changes to internal organs.

Field Studies

During the past year, several producers submitted feed samples for analyses when they suspected mycotoxins as the cause of feed refusal, vomiting and ill health. On-site visits to several farms were made to acquire more detailed information. Chemical analyses on representative feed samples were done to identify the mycotoxins present in the feeds. The results of 4 field cases reported in the summer-autumn of 1980 are shown in Table IV. Pigs were affected in all cases. If the feeding trials with pigs carried out in our laboratory were any indication, factors other than vomitoxin are also involved in the reactions.

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