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Note

Quantitation of deoxynivalenol and its metabolite in bovine urine and feces by gas chromatography with electron-capture detection

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Deoxynivalenol (DON, vomitoxin) is a naturally occurring trichothecene mycotoxin produced by several species of the fungus *Fusarium* [1]. DON-contaminated feed has been associated with numerous cases of sublethal toxicosis in animals resulting in feed refusal, reduced weight gain, emesis and diarrhea [2–4]. Knowledge of residue transmission to humans through animal tissue remains incomplete.

DOM-1 is a metabolite of DON which is formed by the reduction of the epoxide group of DON leading to the loss of oxygen and formation of a carbon–carbon double bond (Fig. 1). This metabolite was originally described in rats [5], it has been found in cattle [6], and it can be produced in vitro [7, 8] by rumen microflora. The toxicity of DOM-1 remains unknown.

Diagnostic procedures for DON exposure in cattle have to date relied upon

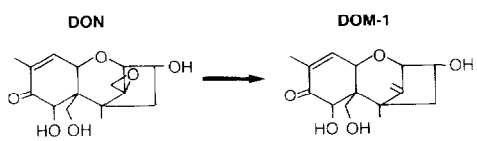

Fig. 1. Reduction of deoxynivalenol (DON) to DOM-1.

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the analysis of feedstuffs, and several methods have been reported [9–11]. This report presents procedures for rapid quantitative analysis of DON and its metabolite DOM-1 directly from bovine urine and feces. Gas chromatography (GC) with electron-capture detection is used for the quantitative determination of these two mycotoxin compounds as trimethylsilyl ethers in both urine and feces.

EXPERIMENTAL

Chemicals

Deoxynivalenol (3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) was purchased from Myco Labs (Chesterfield, MO, U.S.A.) for standard preparation. DOM-1, the de-epoxy metabolite of DON, was supplied by T. Yoshizawa.

Trimethylsilyl (TMS) derivatizing reagent was prepared by mixing 5 parts trimethylsilylimidazole with 1 part trimethylchlorosilane purchased from Sigma (St. Louis, MO, U.S.A.). Clin-Elut columns were purchased from Analytichem International (Harbor City, CA, U.S.A.). Prep-Sep prepacked silica cartridges containing 300 mg silica were purchased from Fisher Scientific (Chicago, IL, U.S.A.), and C_{18} cartridges used in the initial feces extraction were Baker, 500 mg (J.T. Baker, Phillipsburg, NJ, U.S.A.). All solvents were distilled in glass.

Instrumentation

GC was performed using a Hewlett-Packard Model 5840A equipped with a ^{63}Ni electron-capture detector, a 1.8 m × 2 mm I.D. glass column packed with 3% OV-17 on 100–120 mesh Supelcoport, and a Hewlett-Packard Model 7671A autosampler. Other conditions were as follows: carrier gas of argon–methane (95:5) at a flow-rate of 35 ml/min, column oven temperature 220°C, injector temperature 275°C and detector temperature 325°C.

Standard solutions

DON and DOM-1 were dissolved in absolute ethanol to give stock standard concentrations of 25 ng/μl. Spike recoveries were calculated by comparing peaks heights of DON from urine and feces extracts with a calibration curve prepared from 50, 100, 200, 400 and 800 pg of DON standards. Control samples of urine and feces were collected from experimental animals prior to addition of DON-contaminated feed to their ration.

Urine

Saturated sodium chloride solution (1 ml) was introduced into a 10-ml Clin-Elut column 3 min prior to addition of 9 ml urine. After 5 min, the mycotoxins were eluted with 100 ml of ethyl acetate into a boiling flask. The ethyl acetate was evaporated with a rotary evaporator, and the residue was transferred with two (2 ml) rinses of chloroform to a silica cartridge (preconditioned with chloroform). The flask was rinsed a third time with 200 μl of acetone followed by 1.8 ml of chloroform, then transferred to the silica cartridge and allowed to drain. The cartridge was then rinsed with an additional

3 ml of chloroform—acetone (9:1). The toxins were eluted from the silica cartridge with 9 ml of chloroform—acetone (3:1) collected in a disposable test tube, and the eluate was concentrated to dryness under an air stream.

Feces

Whole feces (12 g) or lyophilized feces (4 g) were extracted with 80 ml of 10% methanol in water containing 1% sodium chloride. Samples were agitated for 1 h on a wrist action shaker and filtered through Whatman No. 4 filter paper lined with glass wool. Aliquots (20 ml) of sample extracts were partitioned twice with 20 ml of hexane, and the hexane layers were discarded. The aqueous sample was added to a 20-ml Clin-Elut column and left for 5 min to allow adsorption onto the diatomaceous earth packing. The toxins were eluted with 160 ml ethyl acetate into a boiling flask. The ethyl acetate was evaporated with a rotary evaporator, and the remaining residue was transferred to a silica cartridge with chloroform and eluted by the same method described for urine.

Derivatization

The extracts of urine or feces were redissolved for derivatization in 25 μl of TMS derivatizing reagent and 100 μl ethyl acetate, vortexed and heated at 60°C for 5 min. The sample was then diluted with 9.9 ml of hexane and placed at room temperature overnight (18 h). Diluted extract (200 μl) was then added to 800 μl of hexane in autosampler vials, and 2 μl were injected into the gas chromatograph.

An alternative method for feces extraction was utilized prior to discovery of silica column effectiveness. Following evaporation of ethyl acetate from Clin-Elut columns, the extract was transferred with 3 × 2 ml chloroform to a 1 cm I.D. glass column containing 2.5 g of 60—100 mesh Florisil packed in chloroform. The Florisil columns were rinsed with 30 ml dichloromethane—acetone (95:5) and eluted with 50 ml dichloromethane—methanol (9:1). The extract was evaporated, and the remaining residue was dissolved in 100 μl of methanol followed by addition of 1 ml of water. The sample in methanol—water solution was then loaded onto Baker C_{18} columns, rinsed with 2.0 ml water and eluted with 2.0 ml of methanol—water (1:1). The C_{18} extracts were evaporated under air stream at 50°C and derivatized as previously described for silica column extracts.

RESULTS AND DISCUSSION

Deoxynivalenol and its metabolite DOM-1 were quantitated in urine and feces by GC with a ^{63}Ni electron-capture detector as the corresponding TMS derivatives. The detection limits in both urine and feces for both trichothecenes were 50 ppb. The overall recoveries from urine and feces spikes (Table I) were 86.7 and 87.4% for DON and 93.6 and 81.3% for DOM-1, respectively. The relatively low number of DOM-1 samples with respect to DON samples was due to a limited availability of standard. The overall coefficient of variation (C.V.) for DON was 8.5% in urine and 10.2% in feces.

TABLE I

RECOVERY OF DON AND DOM-1 FROM SPIKED URINE AND FECES

Sample	Compound	Concentration (ng/ml)	*n*	Recovery (%)	S.E.
Urine	DON	200	15	97.8	3.9
		1000	15	85.0	1.5
		5000	15	77.3	1.3
Overall			45	86.7	1.9
	DOM-1	200	3	93.6	2.9
Feces	DON	500	10	78.9	3.8
		1000	10	88.4	3.8
		5000	10	94.9	2.7
Overall			30	87.4	2.3
	DOM-1	500	2	76.4	
		1000	2	86.1	

Urine analysis and quantitation

Control cow urine was collected and frozen prior to analysis. Aliquots of 100 ml of urine were spiked with appropriate volumes of toxin 1 h prior to analysis. Centrifugation of urine at 100 *g* prior to addition to Clin-Elut columns aided recovery of the toxins probably due to a better adsorption of materials onto packing materials after removal of solid precipitate. Addition of saturated sodium chloride solution directly to the Clin-Elut columns rather than to the sample prior to loading on-column reduced the amount of salt residue after evaporation of the elution solvent and increased transfer efficiency. Disposable silica cartridges were an inexpensive and efficient method for purification of DON and DOM-1 in urine, and use of chloroform and acetone mixtures as solvents allowed for quick evaporation. TMS derivatization of extracts allowed short GC runs of approx. 6 min at 220°C (Fig. 2). No late eluting or interfering peaks were observed.

Feces analysis and quantitation

Cow feces were collected and determined to be trichothecene-free by GC analysis prior to addition of DON and DOM-1 for recovery studies (Fig. 3). Individual samples were spiked prior to extraction with appropriate volumes of mycotoxin standard solution. Partitioning of hexane-soluble compounds from the aqueous extract aided the removal of extraneous GC peaks upon injection.

The use of a silica cartridge reduced the volume of solvent required and provided more rapid clean-up over the Florisil and C_{18} method originally utilized. Recoveries of DON and DOM-1 were consistent between both methods, so, owing to the relative ease of use of silica cartridges, the silica cartridges were used.

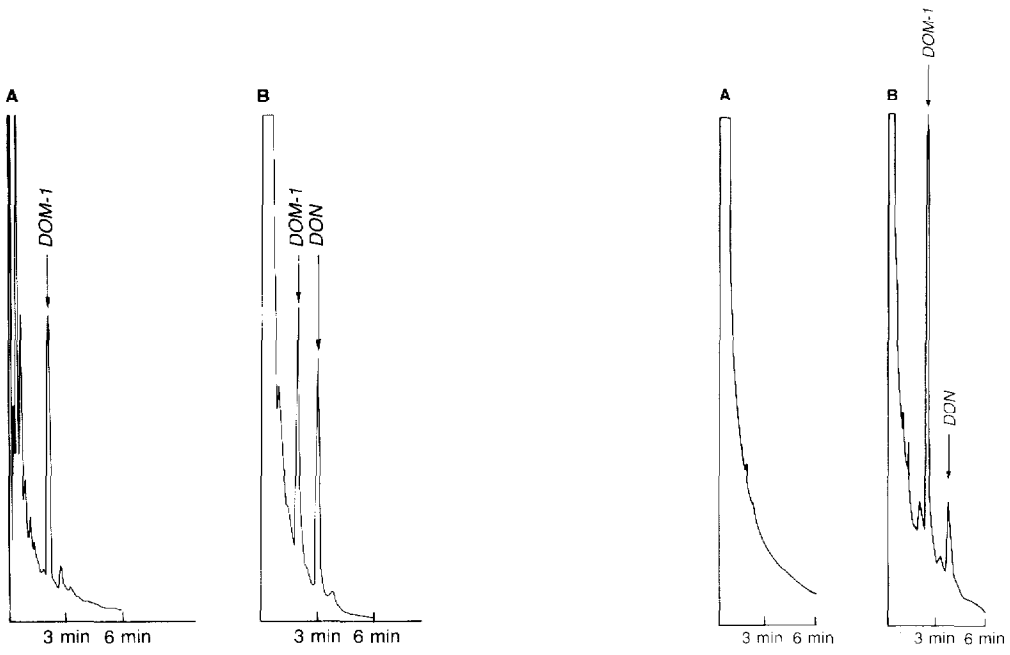

Fig. 2. Gas chromatograms of TMS-derivatized urine extracts. (A) Urine containing 2.70 ppm DOM-1 obtained from a cow fed DON-contaminated ration; (B) DON/DOM-1 cow urine spike (200 ng/ml).

Fig. 3. Gas chromatograms of TMS-derivatized feces extracts. (A) Control feces; (B) feces containing 0.28 ppm DON and 1.36 ppm DOM-1 obtained from a cow fed a DON-contaminated ration.

Analysis of urine and feces from orally dosed cow

We have utilized this method for analysis of urine and feces obtained from cows fed DON-contaminated feed for five days. DOM-1 was the major metabolite detected in both urine and feces and was present in most samples at six to ten times the concentrations of DON. Maximal concentrations of DOM-1 in urine and feces were 18 ppm and 13 ppm, respectively. Urine was the specimen of choice for detection of DON exposure owing to the relative ease of analysis and the high levels contained within, but feces provided a readily collectable and available specimen for analysis also.

Information on the metabolism and excretion of DON in dairy cattle fed DON-contaminated feed will be reported elsewhere [6].

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