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Occurrence of Fumonisin in Forage Grass in New Zealand†

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Fumonisin B_1 (FB_1) was isolated from samples of forage grass originating in paddocks associated with an idiopathic disease of Canadian wapiti and wapiti-red deer hybrids characterized by "ill thrift" and liver dysfunction. Four of 40 samples contained 1, 3, 6, and 9 ppm (micrograms per gram) of FB_1 and 4, 0.5, 2, and 0.5 ppm, respectively, of the methyl ester of FB_1. Analyses were done by ion spray mass spectrometry and confirmed by both fast atom bombardment (solids probe) and mass spectral analysis by electron impact ionization of the trifluoroacetate derivative of the base hydrolyzed product (pentolamine) of FB_1. This article contains the first report of the presence of fumonisin B_1 in grass.

Fumonisin is a mycotoxin produced primarily by *Fusarium moniliforme* when it colonizes corn in the field and is usually in the corn screenings used as animal feed. Such feed can contain rather large amounts of this toxin that vary from 10 to more than 300 ppm (7). When compared with levels of contamination by aflatoxin, zearalenone, or T-2 toxin, this is a large amount and is certainly of concern. Fumonisin was first described by a South African research team headed by W. F. O. Marasas (1) after some 20 years of research looking for the elusive toxin produced by *F. moniliforme* that could explain equine leukoencephalomalacia. This disease has been reported in the literature for more than 50 years, and although *F. moniliforme* was known to be associated with it, no one could successfully isolate the toxin responsible for it. Marasas et al. (4) were successful in reproducing equine leukoencephalomalacia, and since that time, it has been reported to cause lung edema in swine (5), liver dysfunction in experimental rats, nondescribed poultry (ill thrift) disease, cancer in rats (2), and, it is suspected, cancer of the esophagus in humans. Although definitive proof is not available for all of the diseases described above, with the exception of equine leukoencephalomalacia, the correlative evidence is persuasive.

This study was prompted by the occurrence of an idiopathic disease among Canadian wapiti (*Cervus elaphus*) and wapiti-red deer hybrids raised on grassland in New Zealand (Waiora Farms, Invermay). The signs noted were scouring and severe hepatopathy characterized by raised levels of aspartate aminotransferase and gamma glutamyl transpeptidase in the blood as well as low levels of total protein and albumin.

In an effort to find a probable cause of the malady, we decided to investigate the possibility that *Fusarium* mycotoxins are the etiologic agents. Grass samples were collected from the paddocks in which the animals were grazing during the time that signs of the disease were noted. The samples were collected at various random sites and described as "high," i.e., from the top 2 to 6 in. (ca. 6 to 15 cm) of pasture, and "low," i.e., from the bottom 2 in. (ca. 6 cm). These were freeze-dried and kept in a freezer until the time of analysis.

The grass samples were analyzed for the presence of fumonisin B_1 (FB_1), as the deer showed signs of severe liver dysfunction. Although the mycotoxin fumonisin causes equine leukoencephalomalacia, it has been shown to also cause liver damage, i.e., one of the diagnostic signs of chronic toxicity, in rats (2).

The extraction method used was based on a previously described method (6) for corn, with modifications to eliminate chlorophyll and pigments from the grass substrate. Essentially, the extraction was made with acetonitrile-water (1:1) and then partitioned with chloroform; the aqueous phase was adjusted to pH 4 with 0.5 N HCl. The latter was loaded onto a Sep Pak C-18 column, rinsed with 6 ml of water and 2 ml of acetonitrile-water (15:85), and then eluted with acetonitrile-water (70:30). An attempt was made to analyze the *o*-pthaldehyde derivative by high-performance liquid chromatography, but this method was not successful because of interfering pigments. As an alternative, the underivatized extract was analyzed by ion spray mass spectrometry (ISMS). Aliquots of the extract were injected directly into a C-18 column interfaced to an ISMS (Taga 6000; Sciex, Thornhill, Ontario, Canada) with acetonitrile-water-10 mM ammonium acetate (50:49:1). Quantitation was based on a standard curve constructed from the recovery of known amounts of standard FB_1 added to grass samples. Additional analyses were made by subjecting the sample to base hydrolysis and making trifluoroacetate (TFA) derivatives of the products. These were then analyzed for purposes of verification by electron impact ionization on a VG7070EQ tandem mass spectrometer after resolution by gas chromatography. The gas chromatographic column used was a 250-μm (i.d.) fused silica (DB5; J & W Scientific, Folsom, Calif.) programmed at 80 to 280°C at 20°/min and an injection chamber temperature of 280°C. The presence of FB_1 was also confirmed by analysis (3) with fast atom bombardment (FAB) by direct probe insertion with glycerol-water-acetic acid (49:50:1).

The results of the analyses (ISMS) of the grass samples are shown in Table 1. Both FB_1 and its methyl ester were found in all of the positive samples and in significant amounts. The ISMS and FAB spectra are shown in Fig. 1. Both show a protonated molecular ion at *m/z* 722 and its sodium adduct at *m/z* 744. The direct-probe FAB spectrum was run to confirm the analysis found in ISMS. Further confirmation was obtained by hydrolysis of the sample to the level of its pentolamine and resolution of its TFA ester by gas chroma-

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TABLE 1. Analysis of FB_1 and its methyl ester[a]

Sample[b]	Amount (ppm)[c] of:	
	FB_1	Methyl ester
L6	6.3	2
L8	1.0	3.7
H2	9.0	0.5
H7	3.0	0.5

[a] Samples were from pasture grass collected near Invermay in the South Island of New Zealand. All analyses were made by ISMS with a Taga 6000 mass spectrometer.

[b] Four of 40 samples were positive for fumonisin. Samples L6, L8, H2, and H7 were hydrolyzed, made into TFA derivatives, and confirmed as FB_1 by electron impact mass spectrometry.

[c] Measured as micrograms per milligram.

tography and analysis by electron impact mass spectrometry. The molecular ion (*m/z* 981) obtained in negative chemical ionization was not found by electron impact mass spectrometry. However, the diagnostic ion fragments used to confirm the presence of the TFA derivative of FB_1 were *m/z* 140, 180, 428, 542, and 640. The fragment ion at *m/z* 140 represents the *N*-TFA with carbons 1 and 2.

The presence of the methyl ester of FB_1 in ISMS (Fig. 2) may be due to the method of preparation of the samples or to ion spray analysis of the toxins; however, this idea is still speculative, and it is possible that FB_1 can exist in plants as the methyl ester. This possibility is especially true in sample L8 (Fig. 2), in which the majority of the toxin was found in methyl ester form. The abundance of free carboxyl groups of

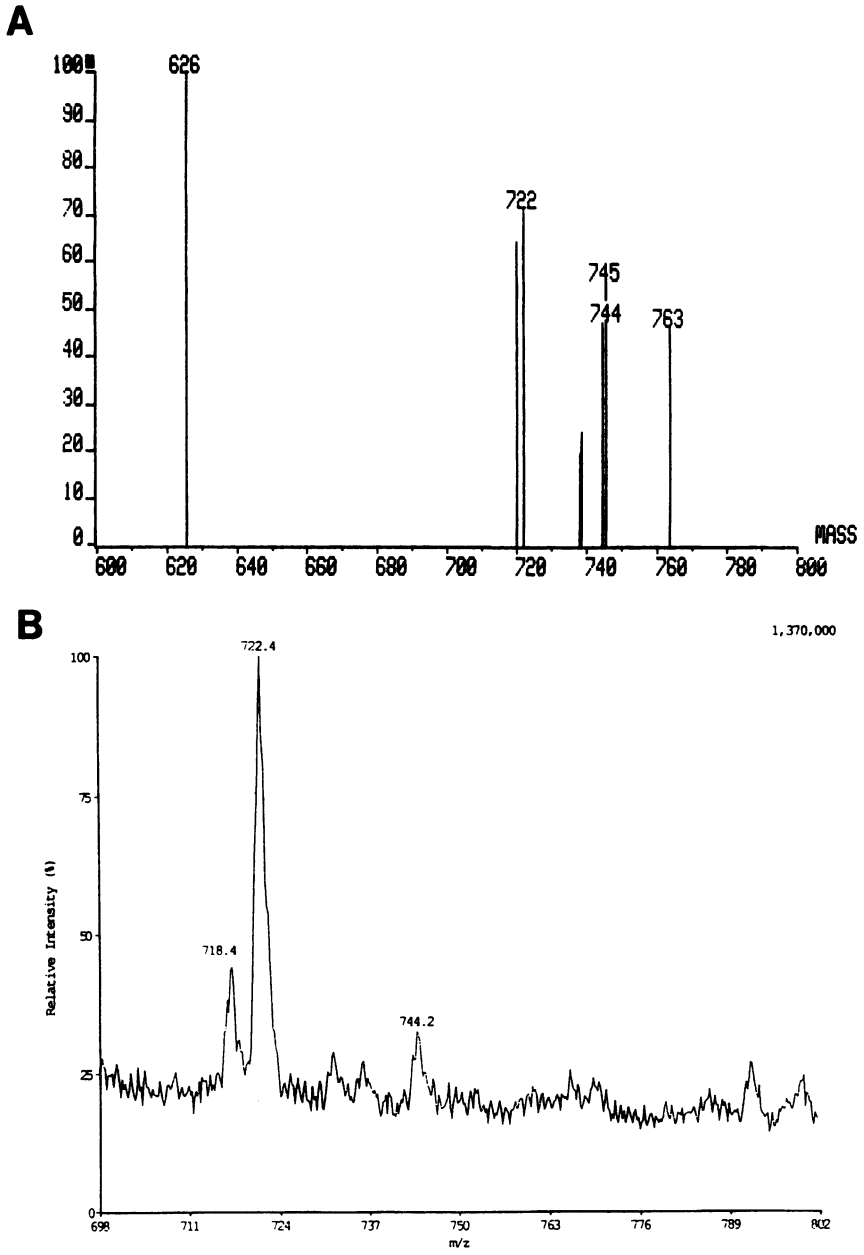

FIG. 1. Analysis of grass sample L-6 by FAB on a solids probe (A) and ISMS (B). The protonated molecular ion (*m/z* 722) and the sodium adduct (*m/z* 744) were found by both FAB and ISMS.

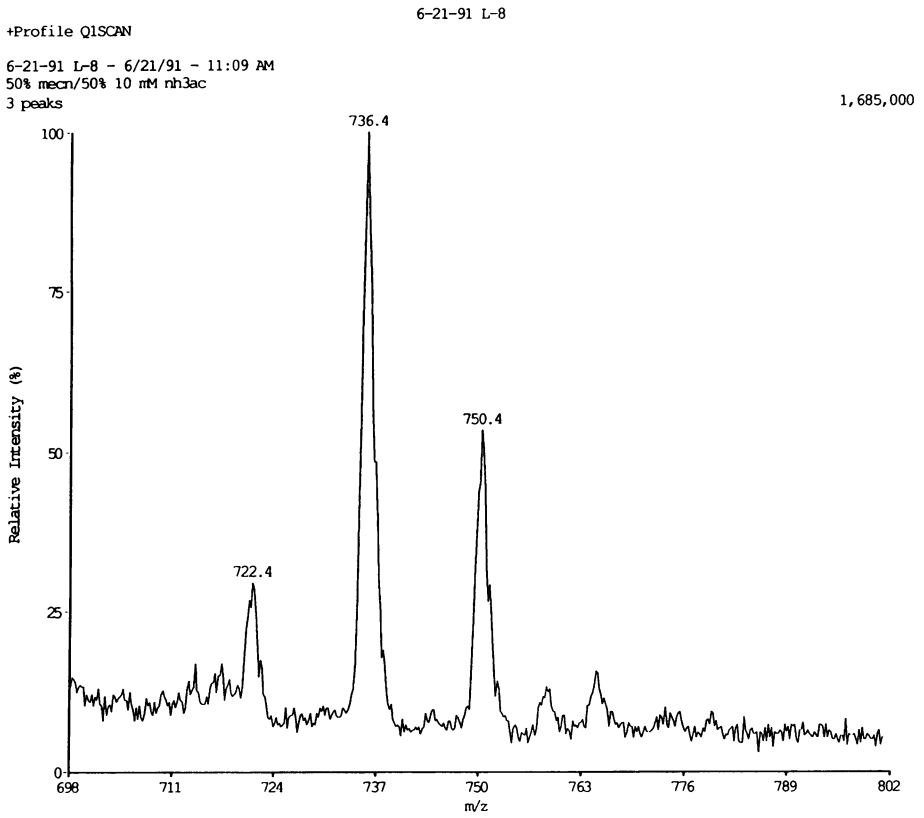

FIG. 2. Analysis of grass sample L-8 by ISMS. The molecular ion is found at *m/z* 722, the methyl ester or methoxy is found at *m/z* 736 or 750.

tricarballylic acid provides sufficient opportunity for esterification as well as glycoside formation. Nevertheless, it is recommended that, in analyses by either FAB or ion spray, both the parent compound (M^+ = 722) and its methyl ester (M^+ = 736) be included in the analysis. Fragment *m/z* 750 is either a methyl ester or a methoxy.

This article contains the first report of the presence of FB_1 in pasture grass, as other natural-occurrence reports (7) indicate that corn is the predominant substrate, with the greatest frequency in corn screenings. We consider 5 to 10 ppm of FB_1 in corn to be high risk for horses. We are not certain that the fumonisin found in the grass samples is associated with the ill thrift found in the wapiti-red deer hybrids and Canadian wapiti. However, FB_1 does cause liver dysfunction in experimental animals and can be considered a candidate along with other etiological agents.

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