Excretion of Fumonisin B1, Hydrolyzed Fumonisin B1, and the Fumonisin B1–Fructose Adduct in Rats

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Abstract
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Keywords
Fumonisin, bioavailability, fumonisin-fructose, hydrolyzed fumonisin

Disciplines
Food Science | Human and Clinical Nutrition | Other Animal Sciences

Comments

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Excretion of Fumonisin B₁, Hydrolyzed Fumonisin B₁, and the Fumonisin B₁–Fructose Adduct in Rats

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The excretion of fumonisin B₁ (FB₁), hydrolyzed FB₁ (HFB₁), and FB₁−fructose addition products (FB₁−fructose) was determined in male Fisher 344/NHsd rats. Rats were dosed by gavage with 0.69, 6.93, or 69.3 µmol/kg of body weight FB₁, HFB₁, or FB₁−fructose. Excretion of unchanged FB₁, HFB₁, and HFB₁ after hydrolysis was determined in urine and feces by analytical reverse phase HPLC and fluorometric detection of the o-phthalaldehyde derivatives. Average total FB₁ backbone excretion in feces was 101, 76, and 50% of the dose for FB₁, HFB₁, and FB₁−fructose, respectively. No effect of dose level was found on the percentage of the dose excreted as total FB₁ after hydrolysis. FB₁−fructose appears to be absorbed to the highest extent, followed by HFB₁. FB₁ appears to be excreted nearly completely in the feces. The greater absorption of HFB₁ may explain the greater toxicity of HFB₁ compared to FB₁ on a molar basis. However, the detoxification of FB₁ by formation of the fructose adduct cannot be explained by reduced absorption. Average total FB₂ backbone excretion in urine was 2.7, 5.0, and 5.3% of the dose for FB₁, HFB₁, or FB₁−fructose, respectively.

Keywords: Fumonisin; bioavailability; fumonisin-fructose; hydrolyzed fumonisin

INTRODUCTION

Fumonisins are a recently discovered family of mycotoxins, produced mainly by the corn pathogens Fusarium moniliforme and Fusarium proliferatum (Marasas, 1996). Gelderblom et al. (1988) first isolated fumonisin B₁ (FB₁), the main member of this group of toxins. Its structure was elucidated by Bezuidenhout et al. (1988). Equine leukoencephalomalacia, a disease long associated with the consumption of moldy feed, was reproduced in horses by dosing FB₁ intravenously or orally (Marasas et al., 1988; Kellerman et al., 1990). Harrison et al. (1990) showed that porcine pulmonary edema was caused by FB₁. The fumonisins have been associated with high human esophageal cancer risk in the Transkei, South Africa (Sydenham et al., 1990; Rheeder et al., 1992). FB₁ was shown to be hepatocarcinogenic and hepatotoxic in rats by several researchers (Gelderblom et al., 1991; Voss et al., 1993). Although FB₁ is a complete carcinogen (Gelderblom et al., 1988), the fumonisins are poor cancer initiators and seemingly lack genotoxicity (Gelderblom et al., 1992; Norred et al., 1993). Fumonisins strongly resemble the long-chain base backbones of sphingolipids, and several researchers have shown a disruption of sphingolipid biosynthesis by FB₁, both in vitro and in vivo (Wang et al., 1991, 1992; Yoo et al., 1992; Riley et al., 1993; Schroeder et al., 1994). The importance of this biochemical change in the diverse toxic effects of fumonisins has not yet been clearly established.

FB₁ has recently been declared a class 2B carcinogen (IARC, 1993). The presence of fumonisins in corn-containing foods is a potential risk to human health.

Levels of FB₁ ranging from 0 to 2790 µg/g and FB₂ levels ranging from 0 to 1065 µg/g have been found in US corn foods (Sydenham et al., 1991; Stack and Eppley, 1992; Hopmans and Murphy, 1993). Additionally, fumonisin has been found in corn foods in South Africa (Sydenham et al., 1991), Switzerland (Pitett et al., 1992), Spain (Sanchis et al., 1994), Italy (Doko and Visconti, 1994), and Asia (Ueno et al., 1993).

Hydrolyzed FB₁ (HFB₁), or FB₁ backbone, is defined as FB₁ without tricarballylic acid side chains. HFB₁ has been produced when corn was nixtamalized, a traditional treatment of corn with calcium hydroxide and heat. In a 4 week feeding study, diethylnitrosamine (DEN, 15 mg/kg of body weight)-initiated male Fisher 344/N rats were fed corn containing 62−67 µmol/kg of diet FB₁ from F. proliferatum corn culture material, or fed nixtamalized corn containing 17−25 µmol/kg diet HFB₁, with or without nutrient supplementation (Hendrich et al., 1993). Rats receiving nutrient-supplemented FB₁ or HFB₁ diets showed approximately equal signs of toxicity, such as elevated cholesterol and glutamate/pyruvate transaminase (GPT) levels and hepatic adenomas. Although it is possible that other carcinogens were present in these diets prepared with F. proliferatum corn culture material, it seems likely that the predominant toxicant was FB₁ (Lu et al., 1997), and after nixtamalization, HFB₁. The results suggested that HFB₁ could be more toxic than FB₁ itself (Hendrich et al., 1993), because 17 µmol/kg of diet HFB₁ caused toxic signs approximately equal to 62 µmol FB₁/kg of diet in rats with good nutritional status. Voss et al. (1996) have confirmed the Hendrich et al. (1993) findings. In a short-term liver cancer initiation/promotion model, HFB₁ was more cytotoxic than FB₁ but was unable to initiate cancer (Gelderblom et al., 1993). The authors speculated that HFB₁ is poorly absorbed from the gut and proposed an active role for the tricarballylic acid side chains in the absorption of fumonisins. HFB₁ has been detected in commercial masa, tortilla chips, and canned sweet corn (Hopmans and Murphy, 1993).
The possibility of detoxifying F₁ by derivatizing this amine with a reducing sugar in the Maillard reaction was examined because the primary amine in F₁ appears to be crucial for its toxic potential (Kraus et al., 1992) and naturally occurring N-acetyl-F₁ is not toxic (Gelderblom et al., 1993). The Maillard reaction is a common reaction in foods between primary amines and reducing sugars under the influence of heat and might provide a practical approach to FB₁ detoxification. Murphy et al. (1995) reported that heating 6.93 µM F₁ in a model system (adapted from Petriella et al. (1988)), with 100 mM glucose or fructose and 50 mM potassium phosphate, pH 7.0, resulted in an apparent first-order reaction and produced an OPA-reactive peak. When the reaction mixture was hydrolyzed, all of the F₁ was recovered as HFB₁. F₁ heated under identical conditions without a reducing sugar did not lose reactivity with OPA. The loss of reactivity was most likely due to the formation of F₁-sugar addition products. DEN-initiated (15 mg/kg body weight) male Fisher 344/NHsd rats were fed either 69.3 µM F₁ or 69.3 µM F₁ incubated with fructose (F₁-fructose) diets for 4 weeks. Rats fed F₁ had significantly increased levels of several markers of hepatocarcinogenicity, while rats receiving F₁-fructose showed no signs of hepatocarcinogenicity (Lu et al., 1997).

The removal of both tricarballylic acid side chains by hydrolysis or the formation of a fructose adduct is likely to change the bioavailability of F₁, due to changes in hydrophobicity, which may help explain the changes in toxicity observed. HFB₁ is a slightly more apolar molecule than F₁, which could facilitate its absorption by diffusion across cell membranes. Absorption of the F₁-fructose adduct was not anticipated as the Maillard reactions typically produces large, polymeric products, and the formation of a fructose adduct appears to completely detoxify F₁. The objective of this study is to determine the excretion of F₁, HFB₁, and the F₁-fructose adduct.

MATERIALS AND METHODS

Fumonisins B₁ is a class 2B carcinogen (IARC). Its hydrolysis product, hydrolyzed Fumonisin B₁, could be a carcinogen. Caution should be taken during handling of either.

Analytical Standard. F₁ was donated by P. G. Thiel from the Research Institute for Nutritional Diseases, South African Medical Research Council, South Africa. All other reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise.

F₁ Purification. F₁ was produced in F. proliferatum M 5991 liquid culture (Dantzer et al., 1996a). Mycelia were removed from the liquid culture by filtration before application of media to a 1 kg XAD-16 column (Sigma, St. Louis, MO). The column was washed with 3 L water, and F₁ was eluted with 3 L methanol. The eluant was dried under vacuum at 50 °C and redissolved in 5 mL of water:acetonitrile (1:1). All subsequent fractions were loaded onto a Lobar LiChroprep RP-8 column (25 × 310 mm; Merck, Darmstadt, Germany). After the mixture was washed with approximately 300 mL of water:trifluoroacetic acid (TFA) (1000:1), and 300 mL of water:acetonitrile:TFA (800:200:1), F₁ was eluted with water:acetonitrile:TFA (500:500:1) and collected in 15 mL fractions. Fractions were screened for F₁ content by reversed phase TLC (Rottinghaus et al., 1992). F₁-containing fractions were pooled, concentrated under vacuum at 50 °C, and applied to a semipreparative C₁₈ column (YM-C Pack ODS-AM, 10 × 250 mm, YMC Co., Ltd., Kyoto, Japan). F₁ was eluted using a linear gradient of water:acetic acid (1000:1) to acetonitrile:acetic acid (1000:1) in 100 min and a flow rate of 2 mL/min. F₁-containing fractions, selected by TLC, were pooled and lyophilized. F₁ and HFB₁ purity was evaluated by measurement of the OPA derivative as described in Dantzer et al. (1996b).

Preparation of HFB₁ and F₁-Fructose. F₁ was produced by refluxing 125 µmol of F₁ in 50 mL of 2 N KOH for 1 h (Hopmans and Murphy, 1993). The reaction mixture was acidified to pH 4.0 with 2 N HCl and applied to a XAD-16 column (2 × 35 cm). The column was washed with 100 mL of water, and HFB₁ was eluted with 100 mL of methanol. The eluant was dried under vacuum at 50 °C and redissolved in water. F₁-fructose was prepared by heating 125 µmol of F₁ in 1 L of 0.05 M potassium phosphate, 0.10 M fructose, pH 7.0, for 48 h at 80 °C (Murphy et al., 1995). The reaction mixture was dried under vacuum at 50 °C and redissolved in water.

Experimental Design. Animal use was approved by the Iowa State University Animal Care Committee. Male Fisher 344/NHsd rats, 9–10 weeks old, 160–190 g, were dosed by gavage with 0.69, 6.93, or 69.3 µmol/mL of F₁ by 50% BW F₁, HFB₁, or F₁-fructose (n = 6). The experiment was divided into three blocks with two rats of each group per block. Rats were housed individually in metabolic cages and received a purified diet, AIN 93M (Reeves et al., 1993), and distilled water ad lib during the experiment. Rats were weighed immediately before the initial dose administration and 96 h after the dose. Food consumption was determined daily. Urine and feces produced during these time points were collected at 12, 24, 48 h, and 96 h after the initial dose administration and lyophilized feces were stored at −20 °C until analysis.

Sample Preparation. For each time increment, urine was diluted to 10 mL with water. Five milliliters was acidified to pH 2.8 with 0.5 N HCl, centrifuged, and cleaned up using C₁₈ solid phase extraction according to Ross et al. (1991). The remaining 5 mL were refluxed at 100 °C with 5 mL of 2 N KOH for 2 h, after which the pH was adjusted to 2.8 with 2 N HCl. The hydrolyzed urine was centrifuged and cleaned up as above. Urine of rats dosed with HFB₁ and all hydrolyzed urine were eluted from the C₁₈ solid phase extraction cartridges with acetonitrile instead of acetonitrile:water (7:3) for additional recovery.

Extraction of feces, for each time increment, was modified from Shephard et al. (1992b). Feces were ground using mortar and pestle. Ground feces were vortexed for 1 min with 10 mL of 0.1 M EDTA, pH 5.2, centrifuged, and the supernatant collected. This procedure was repeated a total of nine times. Half of the combined supernatants was cleaned up using C₁₈ solid phase extraction as above. Before cleanup, fecal extracts of rats dosed with HFB₁ were acidified to pH 2.8 with 2 N HCl. The C₁₈ solid phase extraction cartridges for these samples were eluted with acetonitrile. The remaining half of the combined supernatants was placed in a 100 mL round-bottom flask and dried under vacuum at 50 °C. The residue was redissolved in 10 mL of 2 N KOH and refluxed at 100 °C for 2 h. After adjustment of the pH to 4.0 with 2 N HCl, the hydrolyzed fecal extracts were centrifuged and applied to a 1.5 × 5 cm XAD-16 column. The column was washed with 50 mL of water and 50 mL of water:methanol (3:1) and eluted with 50 mL of methanol. The eluant was dried under vacuum at 50 °C and redissolved in 5 mL of water:acetonitrile (1:1).

HPLC Analysis. All urine and fecal extracts were analyzed before and after hydrolysis. F₁ and HFB₁ were detected as their OPA derivatives following reversed-phase HPLC (Hopmans and Murphy, 1993). F₁ and HFB₁ were quantified by comparison of peak areas to a standard curve (0.1–4.5 nmol of F₁ or HFB₁/mL) (Hopmans and Murphy, 1993). All samples were analyzed in duplicate, and the results were averaged. Limits of determination were 0.4 nmol of F₁ or HFB₁ per urine, hydrolyzed urine, or fecal sample, and 1.1 nmol of HFB₁ per hydrolyzed fecal sample.

Recoveries. A sample of 138 nmol of F₁, HFB₁, or F₁-fructose was added to control feces or urine (n = 3). Samples were spiked 16 h prior to analysis and stored at −4 °C and analyzed as described above. All results were corrected for recovery.

LC–MS. LC–MS analysis of urine containing an unknown OPA-reactive peak was done according to Thakur and Smith (1996). A Hewlett-Packard (HP) Series II, 1090A HPLC
Hewlett-Packard, Palo Alto, CA) equipped with an HP ODS hypersil analytical column (100 × 2.1 mm) and guard column was used. OPA derivatives were prepared by mixing 25 µL of urine and 25 µL of fluoraldehyde reagent (premixed OPA reagent purchased from Pierce Chemical Co., Rockford, IL). After 1 min the reaction mixture was injected onto the column and eluted using a linear gradient from 0.1 M ammonium acetate, pH 3.5:acetonitrile (95:5) to ammonium acetate:acetonitrile (15:85) in 9 min at 0.8 mL/min. OPA derivatives were detected using an HP 1046A programmable fluorescence detector (excitation, 229 nm, emission, 440 nm). Mass analyses were performed using an HP 5989A quadrupole mass spectrometer connected to the HPLC with an HP Thermospray (TSP) interface (90:10 split), operated in the filament off, discharge on, positive ion mode. TSP tip temperature was 192 °C, source temperature was 225 °C, and the quadrupole was 100 °C. Linear scanning was performed between m/z 350 and m/z 650. Data analysis was performed using a Model 59970C processor.

Sulfatase Enzyme Assay. Urine (200 µL) was dried under nitrogen and redissolved in 0.9 mL of Tris buffer (0.4 M, pH 7.4). Type VI sulfatase from Aerobacter aerogenes (EC 3.1.6.1) (Sigma, St. Louis, MO) was diluted using 0.4 M Tris, pH 7.4, to approximately 1 unit/mL (1 unit hydrolyzes 1.0 µmol of p-nitrophenyl sulfate per minute at pH 7.1, 37 °C). One-tenth milliliter diluted enzyme was mixed with the redissolved urine and incubated at 37 °C for 24 h. The reaction mixture was acidified to pH 4.0 with 0.5 N HCl and applied to a XAD-16 column (50 × 5 mm). The column was washed with water and eluted with methanol. The eluant was dried under vacuum.

Table 1. Recovery of FB₁, HFB₁, and FB₁-fructose from Urine, Hydrolyzed Urine, Feces, and Hydrolyzed Feces

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>hydrolyzed urine</th>
<th>feces</th>
<th>hydrolyzed feces</th>
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<tbody>
<tr>
<td>FB₁</td>
<td>88 ± 9</td>
<td>69 ± 5</td>
<td>79 ± 5</td>
<td>70 ± 5</td>
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<tr>
<td>HFB₁</td>
<td>80 ± 7</td>
<td>70 ± 1</td>
<td>47 ± 1</td>
<td>65 ± 8</td>
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<tr>
<td>FB₁-fructose</td>
<td>88 ± 8</td>
<td>61 ± 8</td>
<td>79 ± 5</td>
<td>75 ± 18</td>
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</table>

a Percent of amount added.
at 50 °C, redissolved in 400 µL of water:acetonitrile (1:1), and analyzed by HPLC as described above.

**Statistical Analysis.** Statistical analysis was performed using the General Linear Models procedure and Student’s t-test with the SAS package (version 6.03, Cary, NC). Differences were considered significant if p < 0.05.

**RESULTS AND DISCUSSION**

The purification process resulted in 70% pure FB1, containing traces of FB2 and FB3 but no other primary amines. Upon alkaline hydrolysis, 99% of the FB1 was recovered as HFB1. Incubation with 100 mM fructose at pH 7.0 resulted in an 89 ± 1% loss of OPA reactivity. Hydrolysis of the reaction mixture resulted in full recovery (101%) of FB1 as HFB1. The formation of FB1—fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole fructose addition products reported by Murphy et al. 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of $[^{14}\text{C}]$FB$_1$ was excreted in bile, making this course of events highly unlikely. Our observations on FB$_1$ excretion in urine and feces were very similar to the results of two studies by Shephard et al. (1992a,b). Shephard et al. (1992b) reported that, within 24 h after dosing rats with 10.4 $\mu$mol/kg of BW $[^{14}\text{C}]$FB$_1$, a total of 101% of the activity was recovered in feces. Over 95% of the radioactivity in feces was accounted for as unchanged FB$_1$. Only 0.1% of the dose was recovered in urine as unmetabolized FB$_1$. In an earlier study by Shephard et al. (1992a), 0.4% of a similar dose of FB$_1$ was recovered unchanged in urine. Excretion in feces was not determined by Shephard et al. (1992a).

Different excretion results were obtained by Norred et al. (1993). Eighty percent of 1 mg of $[^{14}\text{C}]$FB$_1$ was recovered in feces after 96 h, while excretion of radioactivity in urine totaled 2–3% after 4 days. No analytical determination of FB$_1$ was performed to distinguish unchanged FB$_1$ from possible metabolites. A major design difference of our study compared with that of Norred et al. (1993) was that their rats were fasted prior to dosing. The presence of food in the stomach can make a considerable difference for bioavailability (Melander, 1983). It appears that fasting increases the absorption of FB$_1$, since Norred et al. (1993) recovered less of the dose in feces and more in the urine compared with our study.

After 4 days, 4.9, 0.4, and 0.1% of the 0.69, 6.93, and 69.3 $\mu$mol/kg of BW HB$_1$ dose were found in urine as unchanged HB$_1$ (Table 2). After hydrolysis, 15.1, 1.3, and 0.2% of the respective doses were recovered. Although the percent of HB$_1$ recovered in urine after hydrolysis at the 0.69 $\mu$mol/kg of BW dose was not significantly different from the 6.93 or 69.3 $\mu$mol/kg of BW dose, the trend was similar to excretion of FB$_1$.

A new OPA reactive peak, which eluted slightly before FB$_1$ and well before HB$_1$, was seen in chromatograms (Figure 3A) of urine samples of two rats dosed with 6.93 and 0.69 $\mu$mol/kg of BW HB$_1$, respectively. When these samples were hydrolyzed, this peak appeared to convert to HB$_1$ (Figure 3B). Analysis by LC–MS identified a mass of 485 units, corresponding to a sulfated HB$_1$. In this case, the sulfate could have been esterified with one of the hydroxyl groups and not the

Table 2. Percent of FB$_1$, HB$_1$, or FB$_1$–Fructose Dose Recovered in Urine, Hydrolyzed Urine, Feces, and Hydrolyzed Feces

<table>
<thead>
<tr>
<th>µmol/kg of BW</th>
<th>FB$_1$</th>
<th>HB$_1$</th>
<th>FB$_1$–fructose</th>
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<tbody>
<tr>
<td>0.69</td>
<td>176$^a$</td>
<td>179</td>
<td>177</td>
</tr>
<tr>
<td>6.93</td>
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</tr>
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<tr>
<td>69.3</td>
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<td>12821</td>
<td></td>
</tr>
<tr>
<td>urine (%)</td>
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<td>4.9</td>
<td>8.3</td>
</tr>
<tr>
<td>hydrolyzed urine (%)</td>
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<td>1.3</td>
<td>9.8$^a$</td>
</tr>
<tr>
<td>LSD</td>
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<td>15.1</td>
<td>18.2</td>
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<tr>
<td>feces (%)</td>
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<td>29$^a$</td>
<td>13$^a$</td>
</tr>
<tr>
<td>hydrolyzed feces (%)</td>
<td>60$^b$</td>
<td>72$^y$</td>
<td>39$^y$</td>
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<tr>
<td>LSD</td>
<td>28</td>
<td>71</td>
<td>16</td>
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</table>

$^a$ Amounts reported were the average of six animals. $^b$ Percentages of dose recovered for an individual compound (FB$_1$, HB$_1$, and FB$_1$–fructose, respectively) in a row with different superscript (a, b) were significantly different at $p \leq 0.05$. LSD for urine: FB$_1$ = 5.0; HB$_1$ = 16.6; FB$_1$–fructose = 7.7. LSD for feces: FB$_1$ = 39; HB$_1$ = 46; FB$_1$–fructose = 22. $^c$ Percentages of dose recovered for urine and hydrolyzed urine or for feces and hydrolyzed feces in a column with different superscripts (x, y) were significantly different at $p \leq 0.05$.

Figure 3. Chromatogram of urine of a rat dosed with 6.93 $\mu$mol/kg of BW HB$_1$ showing (A) the new OPA-reactive peak (retention time 5.5 min) and (B) its conversion to backbone after alkaline hydrolysis (retention time HB$_1$ 7.0 min). Peak at 15 min was observed in all urine samples including controls.
primary amine, since the amine was still available for reaction with OPA. Attempts to confirm the identity of this possible HFB$_1$-sulfate by enzymatic hydrolysis using Aerobacter aerogenes sulfatase could not confirm a sulfate derivative available to enzymatic hydrolysis. Other attempts at enzymatic modification of FB$_1$ have led to mixed results (Murphy et al., 1996).

In the fecal extracts, 25, 29, and 29% of the 0.69, 6.93, and 69.3 µmol of HFB$_1$/kg of BW dose were recovered as unchanged HFB$_1$ (Table 2). After hydrolysis of the fecal extract, 103, 60, and 72% were recovered. Compared across the three dose levels, a highly significant difference ($p < 0.0001$) was found in fecal extracts between unchanged HFB$_1$ and total HFB$_1$ after hydrolysis after 4 days (28 and 76%, respectively). Two possible mechanisms could result in more total HFB$_1$ excreted than unchanged HFB$_1$: modification by GI microorganisms or phase I or II metabolism after absorption and subsequent excretion in the bile. No information is available on biliary excretion of HFB$_1$ nor could this study distinguish between nonabsorbed HFB$_1$ in feces or HFB$_1$ excreted in bile. It is therefore not possible to conclude whether the modification of HFB$_1$ occurs by one or both mechanisms.

FB$_1$-fructose was measured after alkaline hydrolysis as HFB$_1$. After hydrolysis, 9.8% of the 0.69 µmol of FB$_1$-fructose/kg of BW dose and 0.8% of the 6.93 µmol of FB$_1$-fructose/kg of BW dose were recovered from urine. Surprisingly, in unhydrolyzed urine, 8.3% of the lower dose and 0.2% of the higher dose of FB$_1$-fructose were excreted in urine as intact FB$_1$ (Table 2). There was no difference ($p < 0.05$) between the amount excreted as FB$_1$ or total HFB$_1$ determined after hydrolysis at either dose. If the FB$_1$-fructose addition products were excreted intact, more HFB$_1$ should have been recovered after hydrolysis than could be accounted for by FB$_1$. A higher percentage ($p < 0.05$) of the 0.69 µmol/kg of BW FB$_1$-fructose dose was excreted in urine after 96 h compared to the 6.93 µmol/kg of BW dose.

FB$_1$-fructose in feces extracts, as FB$_1$, totaled 13 and 14% of the 0.69 and 6.93 µmol/kg of BW doses, respectively, after 4 days (Table 2). Fifty-eight and 39% of the 0.69 and 6.93 µmol/kg of BW dose, respectively, were recovered in the fecal extract as HFB$_1$ after hydrolysis. More total HFB$_1$ was recovered after hydrolysis ($p < 0.0001$) than unchanged FB$_1$. This shows that intact FB$_1$-fructose was excreted in feces. Eleven percent of the FB$_1$, in the original FB$_1$-fructose dose, was unreacted. In urine and in feces more than 11% of the total HFB$_1$ detected after hydrolysis was accounted for by FB$_1$ excreted. Apparently some of the FB$_1$-fructose was hydrolyzed prior to excretion.

In comparing the three forms of FB$_1$ administered, no difference was found in the percentage total HFB$_1$ in urine between FB$_1$, HFB$_1$, and FB$_1$-fructose at 0.69 and 6.93 µmol/kg of BW. However, at 69.3 µmol/kg of BW, more ($p < 0.04$) total HFB$_1$ was recovered after dosing with FB$_1$ compared to HFB$_1$. For all three forms of fumonisin, a higher percentage of the dose was excreted in urine at the lowest dose, compared to the two higher levels, although this was not statistically significant for HFB$_1$. The dose level had no effect on excretion of total HFB$_1$ in feces for either FB$_1$, HFB$_1$, or FB$_1$-fructose. The major portion of the dose was excreted in the feces between 24 and 48 h after dosing in all cases. More than 100% of the 0.69 µmol/kg of BW FB$_1$ and HFB$_1$ dose was recovered in urine and feces combined. The absolute amount of unchanged FB$_1$ or HFB$_1$ or total HFB$_1$ after hydrolysis in individual samples at 0.69 µmol of FB$_1$, HFB$_1$, or FB$_1$-fructose/kg of BW was very small and in many cases close to the limit of determination. This could have affected the accuracy of the analytical assay, leading to an artificially high percentage of the dose apparently recovered in urine, and a total recovery of over 100% of the dose in those cases. In contrast, at both dose levels of FB$_1$-fructose and at 6.93 and 69.3 µmol of HFB$_1$/kg of BW, far less than 100% of the dose was recovered in feces and urine combined. One possible explanation for the effect of dose levels may be binding of HFB$_1$ or FB$_1$-fructose to fecal matter, thus preventing extraction in the protocol used here. However, in experiments to evaluate efficiency of the extraction procedures, at least 16 h transpired between addition of FB$_1$-fructose or HFB$_1$ to feces and extraction. If binding to fecal material occurred, the resulting losses would have been accounted for in the recoveries shown in Table 1 and used in recovery corrections.

Our data do not support the hypothesis posed by Gelderblom et al. (1992) that HFB$_1$ is absorbed to a lesser extent than FB$_1$ or that the tricarballylic acids play an active role in the absorption of FB$_1$. The two tricarballylic acids probably do not play a role in FB$_1$ absorption, except to modify polarity. The loss of the two tricarballylic acids would render HFB$_1$ a considerably less polar molecule within the GI tract environment, possibly facilitating its absorption.

Unexpectedly, FB$_1$-fructose appeared to be absorbed to the greatest extent. Evidence of active transport of fructose has been reported by Gracey et al. (1972), Sigrist-Nelson and Hopfer (1974), and more recently by Crouzoulon and Korieh (1991). It is therefore possible that fructose could facilitate absorption of FB$_1$. Several authors reported partial absorption of fructose–amino acids produced by the Maillard reactions from the GI tract, but subsequent incorporation in the liver and utilization of these amino acids in protein synthesis did not take place or was severely reduced (Sgarbieri et al., 1973; Tanaka et al., 1975). We propose that, although absorption of the FB$_1$-fructose addition products is greater than the absorption of FB$_1$ itself, the formation of these products results from the FB$_1$-fructose detoxifies FB$_1$ by masking the primary amine group, leaving the molecule unable to exert its toxic effects.

Alteration of sphingolipid levels is a sensitive biomarker for exposure to FB$_1$s (Wang et al., 1992; Riley et al., 1993). If FB$_1$ is truly masked by the formation of fructose addition products, sphingolipid ratios should remain at control value in rats exposed to FB$_1$-fructose. Therefore, future evaluation of FB$_1$-fructose detoxification should include sphingolipid ratios to further elucidate the mechanism of detoxification. Further studies on the bioavailability of FB$_1$, HFB$_1$, and FB$_1$-fructose, using radiolabeled FB$_1$s, are needed to determine the fate of the portion of the FB$_1$, HFB$_1$, and FB$_1$-fructose doses not accounted for by excretion in urine or feces.

**ABBREVIATIONS USED**

FB, fumonisin B; HFB, hydrolyzed fumonisin B; OPA, o-phthaldialdehyde.

**LITERATURE CITED**

Bezuilenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allerman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggar, R. Structure elucidation of the fumonisins, myc-


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