

3-17-1997

# Reaction with Fructose Detoxifies Fumonisin B1 while Stimulating Liver-Associated Natural Killer Cell Activity in Rats

Z. Lu

*Iowa State University*

W. R. Dantzer

*Iowa State University*

E. C. Hopmans

*Iowa State University*

V. Prisk

*Iowa State University*

Joan E. Cunnick

Follow this and additional works at: [http://lib.dr.iastate.edu/fshn\\_ag\\_pubs](http://lib.dr.iastate.edu/fshn_ag_pubs)

Iowa State University, [jcunnick@iastate.edu](mailto:jcunnick@iastate.edu)

 Part of the [Animal Sciences Commons](#), [Food Science Commons](#), [Human and Clinical Nutrition Commons](#), [Other Microbiology Commons](#), and the [Other Veterinary Medicine Commons](#)  
See next page for additional authors.

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/fshn\\_ag\\_pubs/88](http://lib.dr.iastate.edu/fshn_ag_pubs/88). For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

---

This Article is brought to you for free and open access by the Food Science and Human Nutrition at Iowa State University Digital Repository. It has been accepted for inclusion in Food Science and Human Nutrition Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

---

# Reaction with Fructose Detoxifies Fumonisin B1 while Stimulating Liver-Associated Natural Killer Cell Activity in Rats

## Abstract

Fumonisin B1 (FB1) was reacted with fructose in an attempt to detoxify this mycotoxin. Fischer 344/N rats were initiated with diethylnitrosamine (15 mg/kg body weight) and then fed 69.3  $\mu\text{mol}$  FB1/kg diet or 69.3  $\mu\text{mol}$  FB1 reacted with fructose (FB1–fructose)/kg diet for 4 weeks. In comparison with the rats fed basal diet or FB1–fructose, the FB1-fed rats had significantly increased plasma cholesterol ( $P < 0.01$ ), plasma alanine aminotransferase activity ( $P < 0.05$ ), and endogenous hepatic prostaglandin production ( $P < 0.05$ ). Placental glutathione S-transferase-positive and  $\gamma$ -glutamyl transferase-positive altered hepatic foci occurred only in the FB1-fed rats. Liver-associated natural killer (NK) cell activity was significantly decreased in the FB1-fed rats and increased in the group fed FB1–fructose, as compared with the basal group ( $P < 0.03$ ). Therefore, modifying FB1 with fructose seems to prevent FB1-induced hepatotoxicity and promotion of hepatocarcinogenesis while stimulating liver-associated NK cell activity in rats.

## Keywords

Department of Microbiology, Immunology and Preventive Medicine, Fumonisin B1, Fructose, Prostaglandins, NK cell, Placental glutathione S-transferase

## Disciplines

Animal Sciences | Food Science | Human and Clinical Nutrition | Other Microbiology | Other Veterinary Medicine

## Comments

Reprinted with permission from Journal of Agricultural and Food Chemistry, 45(3):803-809. doi: [10.1021/jf9607775](https://doi.org/10.1021/jf9607775). Copyright 1997 American Chemical Society.

## Rights

One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions).

## Authors

Z. Lu, W. R. Dantzer, E. C. Hopmans, V. Prisk, Joan E. Cunnick, Patricia A. Murphy, and Suzanne Hendrich

# Reaction with Fructose Detoxifies Fumonisin B<sub>1</sub> while Stimulating Liver-Associated Natural Killer Cell Activity in Rats

Z. Lu,<sup>†</sup> W. R. Dantzer,<sup>†</sup> E. C. Hopmans,<sup>†</sup> V. Prisk,<sup>†</sup> J. E. Cunnick,<sup>‡</sup> P. A. Murphy,<sup>†</sup> and S. Hendrich<sup>\*,†</sup>

Department of Food Science and Human Nutrition and Department of Microbiology, Immunology and Preventive Medicine, Iowa State University, Ames, Iowa 50011

Fumonisin B<sub>1</sub> (FB<sub>1</sub>) was reacted with fructose in an attempt to detoxify this mycotoxin. Fischer 344/N rats were initiated with diethylnitrosamine (15 mg/kg body weight) and then fed 69.3  $\mu$ mol FB<sub>1</sub>/kg diet or 69.3  $\mu$ mol FB<sub>1</sub> reacted with fructose (FB<sub>1</sub>-fructose)/kg diet for 4 weeks. In comparison with the rats fed basal diet or FB<sub>1</sub>-fructose, the FB<sub>1</sub>-fed rats had significantly increased plasma cholesterol ( $P < 0.01$ ), plasma alanine aminotransferase activity ( $P < 0.05$ ), and endogenous hepatic prostaglandin production ( $P < 0.05$ ). Placental glutathione S-transferase-positive and  $\gamma$ -glutamyl transferase-positive altered hepatic foci occurred only in the FB<sub>1</sub>-fed rats. Liver-associated natural killer (NK) cell activity was significantly decreased in the FB<sub>1</sub>-fed rats and increased in the group fed FB<sub>1</sub>-fructose, as compared with the basal group ( $P < 0.03$ ). Therefore, modifying FB<sub>1</sub> with fructose seems to prevent FB<sub>1</sub>-induced hepatotoxicity and promotion of hepatocarcinogenesis while stimulating liver-associated NK cell activity in rats.

**Keywords:** Fumonisin B<sub>1</sub>; fructose; prostaglandins; NK cell; placental glutathione S-transferase

## INTRODUCTION

Fumonisin B<sub>1</sub> (FB<sub>1</sub>), a mycotoxin produced by the commonly occurring corn fungi, *Fusarium moniliforme* and *Fusarium proliferatum*, was hepatocarcinogenic in rats when fed in amounts of 69.3  $\mu$ mol/kg diet (50 ppm) for approximately 2 years (Gelderblom et al., 1991). Human esophageal cancer rate was high in areas where FB<sub>1</sub> concentration in corn reached approximately 11.1  $\mu$ mol/kg (Sydenham et al., 1990a). Corn products for human and animal consumption were determined to contain 0.3–4.2  $\mu$ mol FB<sub>1</sub>/kg (Hopmans and Murphy, 1993; Murphy et al., 1993; Sydenham et al., 1990b).

The hepatocarcinogenic effect of FB<sub>1</sub> can be evaluated *in vivo* by measuring changes in placental glutathione S-transferase (PGST) (Lebepe-Mazur et al., 1995) and  $\gamma$ -glutamyl transferase (GGT) activity (Gelderblom et al., 1988), which are markers of altered hepatic foci (AHF) (Sato et al., 1984; Goldsworthy et al., 1985). Also, plasma alanine aminotransferase (ALT) activity was related to fumonisin hepatotoxicity (Voss et al., 1992) and hepatocarcinogenesis in rats (Hendrich et al., 1993). Increased plasma total cholesterol was also an indicator of FB<sub>1</sub> toxicity in vervet monkeys (Fincham et al., 1992) and in rats (Hendrich et al., 1993). Production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and other eicosanoids from the cyclooxygenase pathway was stimulated in Yoshida hepatoma cells in rats and in human hepatocellular carcinomas (Trevisani et al., 1980; Hanai et al., 1993). Effects of FB<sub>1</sub> on PG production in rat liver have not been investigated yet.

Fumonisin B<sub>1</sub> can alter immunological functions mediating antitumor mechanisms. Macrophage structure and phagocytic function were down-regulated *in*

*vitro* by FB<sub>1</sub> (Chatterjee and Mukherjee, 1994; Chatterjee et al., 1995) as was lymphocyte proliferation in response to lipopolysaccharides (LPS) (Dombrink-Kurtzman et al., 1994). The effects of *in vivo* administration of FB<sub>1</sub> produced both increases and decreases in plaque-forming cell response in BALB/c mice depending upon the timing of the fumonisin injections and the number of injections (Martinova and Merrill, 1995).

Efforts have been devoted to detoxifying FB<sub>1</sub> in several ways. Thermostability of FB<sub>1</sub> proved to be great, because nearly 85% of FB<sub>1</sub> was recovered after different heat treatments: 75 °C for 135 min, 100 °C for 45 min, and 125 °C for 5 min (Dupuy et al., 1993). Treatment of fumonisin-contaminated corn with 2% ammonia for 4 days, a process that detoxified aflatoxin B<sub>1</sub>, led to slight reduction in the concentration of FB<sub>1</sub> without decreasing the toxicity in rats (Norred et al., 1991). Hydrolyzed FB<sub>1</sub>, which was produced by boiling *F. proliferatum*-contaminated corn in 1.2% calcium hydroxide solution for 1 h, was similar in toxicity to FB<sub>1</sub> when the nutritional status of rats was adequate (Hendrich et al., 1993). *In vitro* toxicity studies on several analogs of FB<sub>1</sub> showed that the analogs containing FB<sub>1</sub>'s amine group and the tricarballic side chains were more toxic than FB<sub>1</sub>, whereas the analog containing only the tricarballic side chains was not toxic (Kraus et al., 1992). The *N*-acetyl derivative of FB<sub>1</sub> at 1 mM was less toxic in primary rat hepatocytes than FB<sub>1</sub> at the same concentration. In addition, rats fed *N*-acetyl-FB<sub>1</sub> at about 1.3 mmol/kg diet did not exhibit hepatic neoplastic nodules or increased hepatic GGT activity (Gelderblom et al., 1993). Therefore, FB<sub>1</sub>'s amine group is likely to be critical for its toxicity. The *N*-acetyl-FB<sub>1</sub> may be produced during the isolation and purification of FB<sub>1</sub> from corn cultures of *F. moniliforme*. This paper describes a dedicated and more practical method to block FB<sub>1</sub>'s amine group by reacting the amine group with reducing sugars such as fructose in a Maillard reaction. It was hypothesized that modifying FB<sub>1</sub> with fructose would reduce or prevent promotion of hepatocarcinogenesis and hepatotoxicity in rats.

\* Author to whom correspondence should be addressed [telephone (515) 294-4272; fax (515) 294-6193; e-mail shendric@iastate.edu].

<sup>†</sup> Department of Food Science and Human Nutrition.

<sup>‡</sup> Department of Microbiology, Immunology and Preventive Medicine.

## MATERIALS AND METHODS

**Preparation of Crude Fumonisin B<sub>1</sub>.** Fumonisin B<sub>1</sub> is a class 2B carcinogen (International Agency for Research on Cancer, 1993), and standard safety precautions were taken during its handling.

*F. proliferatum* strain M5991 (from Dr. Paul Nelson, Pennsylvania State University, College Station, PA) predominantly produces FB<sub>1</sub>. Sterile, aflatoxin-free corn was inoculated with lyophilized cultures of *F. proliferatum* strain M5991 which had been reconstituted in pH 7.4 phosphate-buffered saline. This corn culture was incubated at 22 °C in the dark for 3 weeks. After incubation, freeze-dried corn culture was ground and extracted with 1:1 acetonitrile:water. The extract was partitioned with ethyl acetate, after which the water phase was loaded onto a 1 kg XAD-16 column (7.5 × 100 cm, Sigma Chemical Co., St. Louis, MO). The column was washed with water, and FB<sub>1</sub> was eluted with 4 L of methanol. The eluate was dried under vacuum, reconstituted in water, and loaded onto a Lobar LiChroprep RP-8 column (25 × 310 mm; EM Separations, Gibbstown, NJ). The column was washed with 20% acetonitrile:80% water containing 0.1% trifluoroacetic acid (TFA), and FB<sub>1</sub> was eluted with 50% acetonitrile:50% water containing 0.1% TFA. The purity of the isolated FB<sub>1</sub> was determined by analytical HPLC of the *o*-phthalaldehyde (OPA) derivative (Hopmans and Murphy, 1993). The FB<sub>1</sub> standard curve was prepared with FB<sub>1</sub> generously donated by Dr. P. G. Thiel (Research Institute for Nutritional Diseases, South African Medical Research Council, South Africa). The isolated FB<sub>1</sub> was approximately 40% pure, containing 1.5 g of FB<sub>1</sub>. The preparation also contained trace amounts of fumonisins B<sub>2</sub> and B<sub>3</sub>.

**Preparation of Highly Purified Fumonisin B<sub>1</sub>.** Liquid cultures were prepared as in Dantzer et al. (1996a) by inoculating capped baffled Erlenmeyer flasks containing 500 mL of modified Myro medium with a 4 day shake flask culture of *F. proliferatum* strain M5991. Fumonisin B<sub>1</sub> was isolated and purified by the procedure as described in Dantzer et al. (1996b). Briefly, harvested liquid cultures were filtered and run over a series of chromatographic steps, including Amberlite XAD-16, reverse phase Lobar C<sub>8</sub>, ion exchange DEAE Sepharose, and reverse phase YMC C<sub>18</sub>, until FB<sub>1</sub> purity >95% was obtained. Purified FB<sub>1</sub> solutions were freeze-dried, and a dry weight was determined. The purified FB<sub>1</sub> was dissolved volumetrically and assayed for FB<sub>1</sub> as described by Hopmans and Murphy (1993). The mass of FB<sub>1</sub> was calculated from the standard curve and compared with the weighed mass of freeze-dried FB<sub>1</sub>. This ratio was taken as the percent purity of FB<sub>1</sub> (Dantzer et al., 1996b). The purity of the FB<sub>1</sub> was confirmed to be >95% by Dr. Ronald Plattner (USDA-ARS, National Center for Utilization Research, Peoria, IL).

**Preparation of Fumonisin B<sub>1</sub>-Fructose Adduct.** Both crude and highly purified FB<sub>1</sub> were conjugated with fructose as described by Murphy et al. (1995). Briefly, 725 μM FB<sub>1</sub> in 50 mM potassium phosphate buffer, pH 7.0, containing 1 M fructose, was heated for 48 h at 80 °C. Less than 5% of the FB<sub>1</sub> was left unreacted with fructose. Fumonisin B<sub>1</sub>-fructose was hydrolyzed by refluxing in 2 N KOH for 2 h at 100 °C, after which the pH was adjusted to 2.8 with 2 N HCl. All of the FB<sub>1</sub> could be recovered as hydrolyzed FB<sub>1</sub>. In addition, FB<sub>1</sub> was heated under the same conditions without fructose and retained 100% reactivity with OPA.

**Diets.** Three experimental diets were fed to rats in each of the two studies. Basal diet supplying 40% of energy as fat was modified from AIN-76 (American Institute of Nutrition, 1977) in the study feeding crude FB<sub>1</sub>, or from AIN-93G (American Institute of Nutrition, 1993) in the study feeding highly purified FB<sub>1</sub> (Table 1). Crude or highly purified FB<sub>1</sub>-containing diets were prepared by incorporating 69.3 μmol FB<sub>1</sub>/kg diet into the basal diets. Crude or highly purified FB<sub>1</sub> reacted with fructose were incorporated into the basal diets at a level equivalent to 69.3 μmol FB<sub>1</sub>/kg diet. On the basis of the proposed reaction between FB<sub>1</sub> and a reducing sugar such as fructose (Murphy et al., 1996), it was estimated that

**Table 1. Basal Diet Composition<sup>a</sup>**

ingredient	crude FB <sub>1</sub> expt (g/kg)	purified FB <sub>1</sub> expt (g/kg)
beef tallow	139.6	139.6
corn oil	66.5	
soybean oil		66.5
casein	224.1	224.1
corn starch	228.5	228.5
dextrose	224.1	224.1
cellulose	56.0	56.0
vitamin mix (AIN-76)	11.2	
vitamin mix (AIN-93G-VX)		10.0
mineral mix (AIN-76)	39.2	
mineral mix (AIN-93G-MX)		39.2
CaCO <sub>3</sub>	5.0	
choline chloride	2.2	
L-methionine	3.4	
ascorbate	0.1	
L-cystine		3.0
choline bitartrate		2.5
TBHQ		0.014

<sup>a</sup> Basal diet modified from AIN-76 was used in crude FB<sub>1</sub> experiment; basal diet modified from AIN-93G was used in highly purified FB<sub>1</sub> experiment.

**Table 2. Differences in Body Weight but Not Feed Intake among Female Rats Fed Fumonisin B<sub>1</sub> (FB<sub>1</sub>), FB<sub>1</sub> Reacted with Fructose (FB<sub>1</sub>-Fructose), or a Basal Diet**

	group		
	basal	FB <sub>1</sub> -fructose	FB <sub>1</sub>
total feed intake (g)	366 ± 37	354 ± 43	320 ± 25
average daily feed intake (g/d)	13 ± 1	13 ± 2	11 ± 1
final body weight (g)	191 ± 19	191 ± 10	171 ± 14 <sup>a</sup>
body weight increase (g)	128 ± 15	128 ± 8	108 ± 15 <sup>a</sup>

<sup>a</sup> Significantly different from the basal group, *P* < 0.05.

approximately 17 g of unreacted fructose was added per kilogram of FB<sub>1</sub>-fructose diet. All the diets were stored at 4 °C.

**Animals.** The use of animals and the experimental procedures were approved by the Iowa State University Animal Care Committee. In the experiment with crude FB<sub>1</sub>, 20 10-day-old male F344/N rats obtained from Harlan Sprague-Dawley (Madison, WI) were injected intraperitoneally with diethylnitrosamine (DEN, 15 mg/kg body weight) in 0.1 mL of corn oil. At 3 weeks of age, the weaned rats were randomly assigned into one of the three treatment groups with six or seven rats each. In the experiment with highly purified FB<sub>1</sub>, 39 10-day-old female F344/N rats obtained from Harlan Sprague-Dawley were initiated as described above, randomly assigned into the treatments with 12–15 rats each. In both experiments, rats were given free access to the experimental diets and water for 4 weeks in an animal facility with a 12-h light/dark cycle maintained at 22–25 °C and 50% humidity. Body weight and feed intake of the female rats were recorded weekly.

**Plasma and Liver Sample Preparations.** Part of the plasma obtained from heparinized blood was analyzed within 24 h for ALT activity. The remaining plasma was stored at –80 °C and later analyzed for plasma total cholesterol.

The liver was perfused with 40 mL of Hank's balanced salt solution containing 1% EDTA and 25 mM HEPES. The perfusate (10–12 mL) containing red blood cells, Pitt cells, and other leukocytes was used as the source of effectors in the NK cell activity assay.

Each of the three largest lobes of the liver was sliced into 1 cm slices. Three slices, one from each lobe, were immediately frozen as a block on dry ice and stored at –80 °C. From each of the frozen liver blocks, two 10-μm serial sections were cut with a Histostat Microtome (Model 855, Leica Inc., Deerfield, IL) for later stainings for GGT activity and PGST.

From each rat, 0.5 g of minced liver portions was immediately homogenized in an ice bath with 10 passes of a

**Table 3. Increased Plasma Total Cholesterol Concentration, Plasma Alanine Aminotransferase (ALT) Activity, and Endogenous Hepatic Prostaglandin (PG) Production in Rats Fed Crude or Highly Purified Fumonisin B<sub>1</sub> (FB<sub>1</sub>), Compared with Feeding FB<sub>1</sub> Reacted with Fructose (FB<sub>1</sub>-Fructose) or a Basal Diet**

	plasma cholesterol (mg/dL)	plasma ALT activity (units/L)	hepatic PGF <sub>2α</sub> (ng/g)	hepatic PGE <sub>2</sub> (ng/g)
Expt 1. Male Rats Fed Crude FB <sub>1</sub>				
basal ( <i>n</i> = 6)	143 ± 24	58 ± 13	49 ± 8	4 ± 1
FB <sub>1</sub> ( <i>n</i> = 7)	261 ± 45 <sup>b</sup>	105 ± 58 <sup>a</sup>	77 ± 22 <sup>a</sup>	7 ± 2
FB <sub>1</sub> -fructose ( <i>n</i> = 7)	145 ± 33	51 ± 8	53 ± 13	5 ± 3
Expt 2. Female Rats Fed Highly Purified FB <sub>1</sub>				
basal ( <i>n</i> = 15)	113 ± 28	45 ± 11	5 ± 2	2 ± 1
FB <sub>1</sub> ( <i>n</i> = 12)	261 ± 68 <sup>b</sup>	75 ± 22 <sup>a</sup>	10 ± 4 <sup>a</sup>	4 ± 2 <sup>a</sup>
FB <sub>1</sub> -fructose ( <i>n</i> = 12)	121 ± 27	50 ± 11	5 ± 3	2 ± 1

<sup>a</sup> Significantly different from the basal group in respective study, *P* < 0.05. <sup>b</sup> Significantly different from the basal group in respective study, *P* < 0.01.

Potter-Elvehjem homogenizer in 5 mL, pH 7.4, 50 mM potassium phosphate buffer containing 4.2 mM acetylsalicylic acid. The liver homogenates were frozen on dry ice and stored at -80 °C for later analyses of endogenous hepatic PGF<sub>2α</sub> and PGE<sub>2</sub> levels.

**Plasma Total Cholesterol Concentration and Alanine Aminotransferase Activity.** Plasma total cholesterol concentration was determined by using Sigma diagnostic kit, procedure 352-3 (Sigma). Plasma ALT activity was measured by using Sigma diagnostic kit for glutamate/pyruvate transaminase optimized ALT assay (Sigma).

**Histochemical Staining and Computerized Stereology of AHF.** One of the frozen serial sections was stained for the presence of PGST-positive altered hepatic foci (AHF). Placental glutathione S-transferase was detected by the peroxidase-anti-peroxidase (PAP) method using a Vectastain ABC avidin-biotin universal rabbit PAP kit (Vector Laboratories, Burlingame, CA). Placental glutathione S-transferase was purified by the method of Sato et al. (1984), and rabbit anti-rat PGST antiserum was prepared as described by Hendrich and Pitot (1987).

The second frozen serial section was stained for GGT activity as described by Rutenburg et al. (1968). The substrate for GGT was  $\gamma$ -glutamyl-4-naphthylamide (GMNA) (United States Biochemical Corp., Cleveland, OH).

Altered hepatic foci were quantified via computerized stereology by slight modification of a program which was developed by Dr. Harold Campbell at McArdle Laboratories, University of Wisconsin, Madison (Campbell et al., 1986). Two magnified images of the serial sections stained for GGT activity and PGST were projected onto a Summagraphics Microgrid II digitizer screen and automatically plotted on an HP 9872C plotter controlled by an HP9845B computer (Hewlett-Packard, Palo Alto, CA) connected to an LA120 printer (Digital Equipment Corp., Maynard, MA) to quantify AHF.

**Radioimmunoassay for Endogenous Liver Prostaglandin F<sub>2α</sub> and E<sub>2</sub> Levels.** Endogenous liver PGF<sub>2α</sub> and PGE<sub>2</sub> levels were determined by radioimmunoassay according to the method of McCosh et al. (1976). Anti-rat PGF<sub>2α</sub> rabbit antiserum was purchased from Sigma. Anti-rat PGE<sub>2</sub> rabbit antiserum was a gift from Dr. Jaqueline Dupont, USDA-ARS, Beltsville, MD. Goat anti-rabbit gamma globulin (ARGG) was obtained from Western Chemical Research Corp., Fort Collins, CO. Concentrated [<sup>3</sup>H]PGF<sub>2α</sub> and [<sup>3</sup>H]PGE<sub>2</sub> with specific activities of 168.0 and 154.0 Ci/mmol, respectively, were purchased from Dupont New England Nuclear, Boston, MA. Standards of PGF<sub>2α</sub> and PGE<sub>2</sub> were obtained from Sigma. Scintiverse BD (Fisher Chemical, Fair Lawn, NJ) was added to each tube before counting. Sample-containing tubes were run in duplicate. Total count tubes and background tubes were counted in triplicate. Six replicates were made to determine total binding. Along with the samples, a standard curve was run in duplicate, one set at the beginning of the sample run and the other set at the end. Radioactivity was determined as cpm using a Packard liquid scintillation analyzer model 1900 TR (Packard Instrument Co., Downers Grove, IL).

**Natural Tumor Cytotoxicity Assay.** In the experiment with purified FB<sub>1</sub>, tumor cytotoxicity of liver NK cells was assessed on the first seven animals sacrificed from each group

due to time constraints in performing the assay. The liver perfusate containing Pitt cells was layered over 3 mL of Accupaque (Accurate Chemical Co., Westbury, NY). The cells were centrifuged for 15 min at 15 000 rpm in a swinging bucket centrifuge (Model CR312, Jouan, Winchester, VA). The cells at the interface of the density gradient media were collected, washed twice with complete media (RPMI-1640 supplemented with 50  $\mu$ g/mL gentamicin, 25 mM HEPES, 2 mM L-glutamine (all from Life Technologies, Gaithersburg, MD) and 10% fetal bovine serum (FBS) (JRH Scientific, Lenexa, KS)), and enumerated on a Celltrack II (NOVA Biomedical, Waltham, MA). The cells from each sample were diluted to 5 × 10<sup>6</sup> and plated in 96-well plates (Model 3595, Costar, Cambridge, MA) to obtain 20, 10, 5, and 2.5 × 10<sup>5</sup> cells (effectors)/well in triplicate. The targets for the assay were YAC-1 cells, which had been labeled with 200  $\mu$ Ci of <sup>51</sup>Cr (401 mCi/mg, Dupont New England Nuclear) for 70 min, and maintained in complete media. The targets were washed three times prior to dilution, and 10<sup>4</sup> targets were plated in each well of the NK assay including control wells to determine spontaneous and maximum release. The plates were incubated for 4.5 h in a humidified CO<sub>2</sub> incubator (5% CO<sub>2</sub>, 95% air) (Fisher Scientific). At the end of the incubation the plate was centrifuged at 500 rpm for 5 min, and 100  $\mu$ L of supernatant was collected to determine the amount of <sup>51</sup>Cr released by dying cells using a Gamma Trac 1191 (TM Analytic, Inc., Elk Grove Village, IL). The cpm detected were used to calculate the lytic units (LU) of activity at 20% lysis in 10<sup>7</sup> effectors using a computer program.

**Statistical Analysis.** The liver PGF<sub>2α</sub> and PGE<sub>2</sub> concentrations were determined by a computer program based on a logit transformation of the standard curve (Duddleson et al., 1972). One-way ANOVA was performed to analyze feed consumption, body weight, plasma total cholesterol level and ALT activity, endogenous hepatic PGF<sub>2α</sub> and PGE<sub>2</sub> concentrations, and natural tumor cytotoxicity, using the Statistical Analysis System (Cary, NC). Student's *t*-test was performed to compare group differences after ANOVA. A *P* value of <0.05 was considered to be statistically significant. For the analysis of computerized stereology of AHF, only the means and standard deviations were given because the means equalled zero in basal and FB<sub>1</sub>-fructose groups.

## RESULTS

**Experiment 1. Male Rats Fed Crude Fumonisin B<sub>1</sub>.** In comparison with the rats fed basal diet and the rats fed FB<sub>1</sub>-fructose, the FB<sub>1</sub>-fed rats had significantly increased plasma total cholesterol concentration and ALT activity by 85% (*P* < 0.01) and 100% (*P* < 0.05), respectively (Table 3).

The PGF<sub>2α</sub> concentration was significantly increased by approximately 51% in the rats fed FB<sub>1</sub> as compared with those in the other groups (*P* < 0.05) (Table 3). There were no significant differences in PGE<sub>2</sub> levels among the groups (Table 3).

All FB<sub>1</sub>-fed rats had both PGST- and GGT-positive AHF. The average PGST-positive AHF area percentage

**Table 4. Placental Glutathione S-Transferase (PGST)- and  $\gamma$ -Glutamyl Transferase (GGT)-Positive Altered Hepatic Foci (AHF) Occurred Only in Rats Fed either Crude Fumonisin B<sub>1</sub> (FB<sub>1</sub>) or Highly Purified FB<sub>1</sub>**

	no. of PGST lesions	no. of GGT lesions	% of PGST lesion area	% of GGT lesion area
Expt 1. Male Rats Fed Crude FB <sub>1</sub>				
basal ( <i>n</i> = 6)			0	0
FB <sub>1</sub> ( <i>n</i> = 7)			5 ± 6	1 ± 1
FB <sub>1</sub> -fructose ( <i>n</i> = 7)			0	0
Expt 2. Female Rats Fed Highly Purified FB <sub>1</sub>				
basal ( <i>n</i> = 15)	0	0	0	0
pure FB <sub>1</sub> ( <i>n</i> = 12)	50 ± 17	45 ± 13	68 ± 18	61 ± 13
FB <sub>1</sub> -fructose ( <i>n</i> = 12)	0	0	0	0

was  $5.0 \pm 6.3$ , and the average GGT-positive AHF area percentage was  $1 \pm 1$  in the rats fed FB<sub>1</sub> at  $69.3 \mu\text{mol/kg}$  diet (Table 4). There were no detectable PGST- or GGT-positive AHF in the group fed  $69.3 \mu\text{mol/kg}$  FB<sub>1</sub> reacted with fructose or in the control group (Table 4).

**Experiment 2. Female Rats Fed Highly Purified Fumonisin B<sub>1</sub>.** The average daily feed intake in FB<sub>1</sub> group, FB<sub>1</sub>-fructose group and basal group was  $11 \pm 1$ ,  $13 \pm 2$ , and  $13 \pm 1$  g, respectively. There were no significant differences in the total feed intake or the average daily feed intake among the three groups (Table 2).

The rats in the FB<sub>1</sub>-treated group had significant reductions in both body weight (by 12%,  $P < 0.05$ ) and body weight gain (by 16%,  $P < 0.05$ ) as compared with the rats in the groups fed FB<sub>1</sub>-fructose and basal diet (Table 2).

In comparison with the basal group and the group fed FB<sub>1</sub>-fructose, rats fed FB<sub>1</sub> at  $69.3 \mu\text{mol/kg}$  diet had significantly increased plasma total cholesterol concentration and ALT activity by about 2.3-fold ( $P < 0.01$ ) and 1.7-fold ( $P < 0.05$ ), respectively (Table 3).

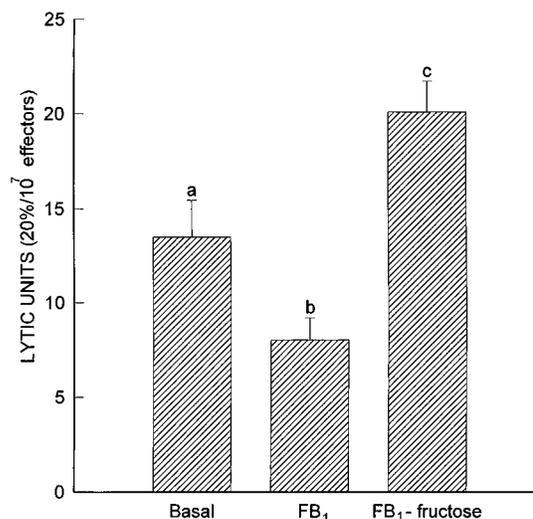
The PGF<sub>2 $\alpha$</sub>  and PGE<sub>2</sub> concentrations were both significantly increased by 2-fold in rats fed  $69.3 \mu\text{mol/kg}$  FB<sub>1</sub>-containing diet as compared with those in the other groups ( $P < 0.05$ ) (Table 3).

All of the rats fed FB<sub>1</sub> developed PGST- and GGT-positive AHF. The average number of PGST-positive AHF and the average PGST-positive AHF area percentage were  $50 \pm 17$  and  $68 \pm 18$ , respectively, and the average number of GGT-positive AHF and the average GGT-positive AHF area percentage were  $45 \pm 13$  and  $61 \pm 13$ , respectively, in the rats fed FB<sub>1</sub> (Table 4). There were no detectable PGST- or GGT-positive AHF in either the group fed FB<sub>1</sub>-fructose or basal diet (Table 4).

Compared with the control rats, tumor cytotoxicity was significantly suppressed in animals fed FB<sub>1</sub> ( $P < 0.03$ ) and significantly elevated in animals fed FB<sub>1</sub>-fructose diet ( $P < 0.01$ ) (Figure 1). Thus, feeding highly purified FB<sub>1</sub> or FB<sub>1</sub>-fructose induced significant and opposing changes in liver tumor cytotoxicity.

## DISCUSSION

These studies demonstrated that subjecting FB<sub>1</sub> to nonenzymatic browning conditions with fructose eliminated FB<sub>1</sub> toxicity as reflected in body weight, plasma total cholesterol concentration, and ALT activity, development of GGT- and PGST-positive AHF, and concentrations of endogenous hepatic PGF<sub>2 $\alpha$</sub>  and PGE<sub>2</sub>. Loss of amine group reactivity toward OPA was taken as an indication that the amine had condensed with fructose (Murphy et al., 1995). Results with either crude or highly purified FB<sub>1</sub> were generally similar, showing detoxification of FB<sub>1</sub>, because in both cases FB<sub>1</sub> reacted with fructose did not promote hepatocarcinogenesis in rats and did not increase plasma cholesterol



**Figure 1.** Tumor cytotoxicity of liver-associated NK cells against YAC-1 target cells, expressed as mean lytic units (LU)  $\pm$  SE ( $n = 7/\text{group}$ ), in female rats fed fumonisin B<sub>1</sub> (FB<sub>1</sub>), FB<sub>1</sub> reacted with fructose (FB<sub>1</sub>-fructose), or a basal diet. Treatments marked by different letters were significantly different from basal group: b,  $P < 0.03$ ; c,  $P < 0.01$ .

concentration, plasma ALT activity, or hepatic PGF<sub>2 $\alpha$</sub> , whereas feeding crude or highly purified FB<sub>1</sub> significantly increased all of these parameters compared with rats fed a basal diet (Tables 3 and 4). The greater development of PGST- and GGT-positive AHF, by 5- and 13-fold, respectively, caused by highly purified FB<sub>1</sub> compared with crude FB<sub>1</sub> (Table 4), may be due simply to inherent variability of the two cohorts of rats in their responsiveness to the carcinogenesis protocol used. Rats are highly variable in individual response to such protocols. Other signs of FB<sub>1</sub> toxicity were quite similar whether crude or purified FB<sub>1</sub> was fed. This result may suggest that females are more susceptible to promotion of cancer by FB<sub>1</sub> than the males, but no other studies have examined this.

The chemical modification of FB<sub>1</sub> by fructose by a Maillard reaction probably caused the formation of a Schiff's base, FB<sub>1</sub>-fructose adduct, in which the FB<sub>1</sub> amine group was combined with the fructose ketone. The addition of the bulky fructose did not cause FB<sub>1</sub> to be less absorbed in the intestine even though this may increase both water solubility and molecular size (Hopmans et al., submitted). At the molecular level, FB<sub>1</sub> toxicity is most likely due to its inhibition of ceramide synthase, a key enzyme in *de novo* sphingolipid biosynthesis (Wang et al., 1991). Perhaps the presence of fructose blocked the inhibitory binding of FB<sub>1</sub> to ceramide synthase.

The putative effectiveness of the detoxification of FB<sub>1</sub> by reaction with fructose suggested that the amine group of FB<sub>1</sub> was critical to FB<sub>1</sub> toxicity. This was in accordance with another study in which the *N*-acetyl

derivative of FB<sub>1</sub> showed no hepatotoxicity and no hepatocarcinogenicity (Gelderblom et al., 1993).

The significant reductions in body weight and body weight gain of female rats fed FB<sub>1</sub> compared with the other groups were accompanied by normal feed consumption (Table 2). This did not agree with another study in which both body weight and feed intake in male Sprague–Dawley rats were significantly decreased after intraperitoneal injection of FB<sub>1</sub> (Bondy et al., 1995). Neither body weight nor feed intake was significantly decreased after FB<sub>1</sub> consumption by male and female Fischer 344 rats (Voss et al., 1995). The difference between our results and the others cannot be readily explained.

Fumonisin B<sub>1</sub>-induced hypercholesterolemia as observed in the present studies (Table 3) was reported in vervet monkeys (Fincham et al., 1992), as well as in rats fed FB<sub>1</sub> (Hendrich et al., 1993). Hypercholesterolemia is an observed effect of other tumor-promoting agents and toxicants, such as phenobarbital (Katayama et al., 1991). The hypercholesterolemic mechanism of FB<sub>1</sub> is unknown.

In agreement with the findings of Hendrich et al. (1993), FB<sub>1</sub> fed to rats at 69.3 μmol/kg diet for 4 weeks increased not only plasma total cholesterol but plasma ALT activity as well (Table 3). Elevated plasma ALT activity indicated hepatocyte membrane damage which led to the leakage of ALT into the blood. Such damage was associated with the development of AHF caused by FB<sub>1</sub> (Hendrich et al., 1993).

Placental glutathione *S*-transferase-positive AHF and GGT-positive AHF were found only in FB<sub>1</sub>-fed rats in both studies (Table 4). Placental glutathione *S*-transferase and GGT are useful markers of FB<sub>1</sub> hepatocarcinogenesis. The induction of PGST could be demonstrated in single putatively initiated hepatocytes within 48 h after DEN treatment (Moore et al., 1987). Both PGST and GGT persisted during hepatocarcinogenesis (Hendrich and Pitot, 1987). In the present study, placental glutathione *S*-transferase- and GGT-positive AHF were detected after 4 weeks of FB<sub>1</sub> treatment. This indicated that both PGST and GGT were induced at an early stage of hepatocarcinogenesis and could serve as sensitive markers of FB<sub>1</sub>-induced hepatocarcinogenesis. Because PGST- and GGT-positive AHF were virtually undetectable in DEN-initiated rats fed FB<sub>1</sub> reacted with fructose, FB<sub>1</sub> promotion of carcinogenesis may be blocked by modifying FB<sub>1</sub> with fructose.

Only FB<sub>1</sub>-treated rats showed significantly greater amounts of endogenous PGF<sub>2α</sub> and PGE<sub>2</sub> compared with the control group, and PGST- and GGT-positive AHF were only present in FB<sub>1</sub>-fed rats. The elevation of hepatic PGF<sub>2α</sub> and PGE<sub>2</sub> concentrations paralleled the induction of AHF in the liver. Therefore, increased PG production is related to promotion of rat hepatocarcinogenesis caused by FB<sub>1</sub>. Both PGF<sub>2α</sub> and PGE<sub>2</sub> may contribute to cell proliferation in the liver during FB<sub>1</sub> treatment. Prostaglandins of the F series stimulated the proliferation of neonatal rat hepatocytes (Armato and Andreis, 1983). Prostaglandins including PGF<sub>2α</sub> and PGE<sub>2</sub> also played a role in proliferation of hepatoma cells (Trevisani et al., 1980). Direct evidence of stimulatory effect of PGs on the proliferation of hepatoma cells was shown by the reversal of decreased hepatoma cell numbers by PGE<sub>2</sub> in indomethacin-treated rats (Trevisani et al., 1980).

Prostaglandins may also exert indirect effects on proliferation of tumor cells by suppressing the local

immune response. Prostaglandins were able to make macrophages and/or lymphocytes less sensitive to various stimuli (Pelus and Strausser, 1977; Schultz et al., 1978). Moreover, prostaglandin E<sub>2</sub> suppressed NK cells and lymphokine-activated killer (LAK) cells (Ohnishi et al., 1991; Roth and Golub, 1993; Baxevanis et al., 1993). The present studies showed that hepatic PGF<sub>2α</sub> and PGE<sub>2</sub> concentrations were significantly increased in FB<sub>1</sub>-induced promotion of rat hepatocarcinogenesis (Tables 3 and 4), and in the female rats fed purified FB<sub>1</sub>, liver-associated antitumor response was decreased (Figure 1). However, the mechanism for enhancement of tumor cytotoxicity in the FB<sub>1</sub>–fructose-fed group is not clear as this group did not differ from the basal diet group in any of the other measures. The tumor cytotoxic cells of the liver are Pitt cells, which are similar to blood NK cells, but with higher lytic activity. The possible immune-enhancing effect of FB<sub>1</sub>–fructose deserves further study.

The mechanism of FB<sub>1</sub>-induced carcinogenesis is still not fully understood. Fumonisin B<sub>1</sub> is an inhibitor of sphingolipid synthesis (Wang et al., 1991). Sphingolipids may be anticarcinogenic because they are down-regulators of protein kinase C (Merrill, 1991), which could affect many enzyme activities after it is activated by carcinogens (Weinstein, 1987). It is not known whether FB<sub>1</sub> can stimulate phospholipase A<sub>2</sub> which hydrolyzes phospholipids to release the PG precursor, arachidonate, or activate cyclooxygenase to produce more PGs in the liver. Prostaglandin F<sub>2α</sub> was shown to stimulate the formation of intracellular diacylglycerol in Swiss 3T3 cells (Macphee et al., 1984), which can in turn activate protein kinase C. Perhaps FB<sub>1</sub>-induced alteration of sphingolipid metabolism stimulates PG production, by an unknown mechanism.

Possibly decreased inhibitory binding of FB<sub>1</sub> to ceramide synthase as a result of FB<sub>1</sub> reaction with fructose may be the mechanism of FB<sub>1</sub> detoxification in this experiment. Further study is needed to identify the structure of the putative FB<sub>1</sub>–fructose conjugate and investigate its stability, possible processing occurrence, bioavailability and toxicity.

#### ABBREVIATIONS

Used FB<sub>1</sub>, fumonisin B<sub>1</sub>; DEN, diethylnitrosamine; ALT, alanine aminotransferase; PG, prostaglandin; PGST, placental glutathione *S*-transferase; GGT, γ-glutamyl transferase; AHF, altered hepatic foci; NK cell, natural killer cell; and OPA, *o*-phthaldialdehyde.

#### ACKNOWLEDGMENT

We thank Dr. Paul Nelson of Pennsylvania State University for supplying *Fusarium proliferatum* strain M5991, Dr. Jaqueline Dupont of USDA–ARS for donating anti-rat PGE<sub>2</sub> rabbit antiserum, Dr. Pieter Thiel of South African Medical Research Council for donating pure FB<sub>1</sub>, and Dr. Ronald Plattner for confirming the purity of our purified FB<sub>1</sub>.

#### LITERATURE CITED

- American Institute of Nutrition: Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **1977**, *107*, 1340–1348.
- American Institute of Nutrition: AIN-purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

- Armato, U.; Andreis, P. G. Prostaglandins of the F series are extremely powerful growth factors for primary neonatal rat hepatocytes. *Life Sci.* **1983**, *33*, 1745–1755.
- Baxeavanis, C. N.; Reclus, G. J.; Gritzapis, A. D.; Dedousis, G. V. Z.; Missitizis, I.; Papamichail, M. Elevated prostaglandin E<sub>2</sub> production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. *Cancer* **1993**, *72*, 491–501.
- Bondy, G.; Suzuki, C.; Barker, M.; Armstrong, C.; Fernie, S.; Hierlihy, L.; Rowsell, P.; Mueller, R. Toxicity of fumonisin B<sub>1</sub> administered intraperitoneally to male Sprague-Dawley rats. *Food Chem. Toxicol.* **1995**, *33*, 653–665.
- Campbell, H. A.; Xu, Y.; Hanigan, M. H.; Pitot, H. C. Application of quantitative stereology to the evaluation of phenotypically heterogeneous enzyme-altered foci in rat liver. *J. Natl. Cancer Inst.* **1986**, *76*, 751–765.
- Chatterjee, D.; Mukherjee, S. K. Contamination of Indian maize with fumonisin B<sub>1</sub> and its effects on chicken macrophages. *Lett. Appl. Microbiol.* **1994**, *18*, 251–253.
- Chatterjee, D.; Mukherjee, S. K.; Dey, A. Nuclear disintegration in chicken peritoneal macrophages exposed to fumonisin B<sub>1</sub> from Indian maize. *Lett. Appl. Microbiol.* **1995**, *20*, 184–185.
- Dantzer, W. R.; Pometto, A. L.; Murphy, P. A. Fumonisin B<sub>1</sub> production by *Fusarium proliferatum* strain M5991 in a modified Myro liquid medium. *Natural Toxins* **1996a**, *4*, 168–173.
- Dantzer, W. R.; Hopmans, E. C.; Clark, A.; Hauck, C. C.; Murphy, P. A. Purification of fumonisin B<sub>1</sub> from liquid cultures of *Fusarium proliferatum* in a modified Myro medium. *J. Agric. Food Chem.* **1996**, *44*, 3730–3732.
- Dombrink-Kurtzman, M. A.; Bennett, G. A.; Richard, J. L. An optimized MTT bioassay for determination of cytotoxicity of fumonisins in turkey lymphocytes. *J. AOAC Int.* **1994**, *77*, 512–516.
- Duddleson, W. G.; Midgley, A. R. Jr.; Niswender, G. D. Computer program sequence for analysis and summary of radioimmunoassay data. *Comput. Biomed. Res.* **1972**, *5*, 205–217.
- Dupuy, P.; Le Bars, P.; Boudra, H.; Le Bars, J. Thermostability of Fumonisin B<sub>1</sub>, a mycotoxin from *Fusarium moniliforme*, in corn. *Appl. Environ. Microbiol.* **1993**, *10*, 2864–2867.
- Fincham, J. E.; Marasas, W. F. O.; Taljaard, J. J. F.; Kriek, N. P. J.; Badenhorst, C. J.; Gelderblom, W. C. A.; Seier, J. V.; Smuts, C. M.; Faber, M.; Weight, M. J.; Slazus, W.; Woodroof, C. W.; van Wyk, M. J.; Kruger, M.; Thiel, P. G. Atherogenic effects in a non-human primate of *Fusarium moniliforme* cultures added to a carbohydrate diet. *Atherosclerosis* **1992**, *94*, 13–25.
- Gelderblom, W. C. A.; Marasas, W. F. O.; Jaskiewicz, K.; Combrinck, S.; van Schalkwyk, D. J. Cancer promoting potential of different strains of *Fusarium moniliforme* in a short-term cancer initiation-promotion assay. *Carcinogenesis* **1988**, *9*, 1405–1409.
- Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B<sub>1</sub>, in rats. *Carcinogenesis* **1991**, *12*, 1247–1251.
- Gelderblom, W. C. A.; Cawood, M. E.; Snyman, S. D.; Vleggaar, R.; Marasas, W. F. O. Structure-activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* **1993**, *31*, 407–414.
- Goldsworthy, T. L.; Pitot, H. C. The quantitative analysis and stability of histochemical markers of altered hepatic foci in the rat liver following initiation by diethylnitrosamine administration and promotion with phenobarbital. *Carcinogenesis* **1985**, *6*, 1261–1269.
- Hanai, T.; Hashimoto, T.; Hishiwaki, K.; Ono, M.; Akamo, Y.; Tanaka, M.; Mizuno, I.; Yura, J. Comparison of prostanoids and their precursor fatty acids in human hepatocellular carcinoma and noncancerous reference tissues. *J. Surg. Res.* **1993**, *54*, 57–60.
- Hendrich, S.; Pitot, H. C. Enzymes of glutathione metabolism as biochemical markers during hepatocarcinogenesis. *Cancer Metastasis Rev.* **1987**, *6*, 155–178.
- Hendrich, S.; Miller, K. A.; Wilson, T. M.; Murphy, P. A. Toxicity of *Fusarium proliferatum*-fermented nixtamalized corn-based diets fed to rats: effects of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 1649–1654.
- Hopmans, E. C.; Hauck, C. C.; Hendrich, S.; Murphy, P. A. Bioavailability of fumonisin B<sub>1</sub>, hydrolyzed fumonisin B<sub>1</sub>, and the fumonisin B<sub>1</sub>-fructose adduct in rats. *J. Agric. Food Chem.*, submitted.
- Hopmans, E. C.; Murphy, P. A. Detection of fumonisin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and hydrolyzed fumonisin B<sub>1</sub> in corn-containing foods. *J. Agric. Food Chem.* **1993**, *41*, 1655–1658.
- International Agency for Research on Cancer. *Toxins derived from Fusarium moniliforme: Fumonisin B<sub>1</sub>, B<sub>2</sub> and Fusarin C*; Monograph on the evaluation of carcinogenic risk to humans. **1993**, No. 56, 445–466.
- Katayama, T.; Momoto, Y.; Yasutomo, W.; Kato, N. Elevated concentrations of  $\alpha$ -tocopherol, ascorbic acid, and serum lipids in rats fed polychlorinated biphenyls, chlorobutanol, or phenobarbital. *J. Nutr. Biochem.* **1991**, *2*, 92–96.
- Kraus, G. A.; Applegate, J. M.; Reynolds, D. Synthesis of analogs of fumonisin B<sub>1</sub>. *J. Agric. Food Chem.* **1992**, *40*, 2331–2332.
- Lebepe-Mazur, S.; Wilson, T.; Hendrich, S. *Fusarium proliferatum*-fermented corn stimulates development of placental glutathione S-transferase-positive altered hepatic foci in female rats. *Vet. Human Toxicol.* **1995**, *37*, 55–59.
- Macphée, C. H.; Drummond, A. H.; Otto, A. M.; Jimenez de Asua, L. Prostaglandin F<sub>2 $\alpha$</sub>  stimulates phosphatidylinositol turnover and increases the cellular content of 1,2-diacylglycerol in confluent resting Swiss 3T3 cells. *J. Cell. Physiol.* **1984**, *119*, 35–40.
- McCosh, E. J.; Meyer, D. L.; Dupont, J. Radioimmunoassay of prostaglandins E<sub>1</sub>, E<sub>2</sub> and F<sub>2 $\alpha$</sub>  in unextracted plasma, serum, and myocardium. *Prostaglandins* **1976**, *12*, 471–486.
- Martinova, E. A.; Merrill, A. H. Fumonisin B<sub>1</sub> alters sphingolipid metabolism and immune function in BALB/C mice: Immunological responses to fumonisin B<sub>1</sub>. *Mycopathologia* **1995**, *130*, 163–170.
- Moore, M. A.; Nakagawa, K.; Satoh, K.; Ishikawa, T.; Sato, K. Short communications: single GST-P positive liver cell-putative initiated hepatocytes. *Carcinogenesis* **1987**, *8*, 483–486.
- Murphy, P. A.; Hendrich, S.; Hopmans, E. C.; Hauck, C. C.; Lu, Z.; Buseman, G.; Munkvold, G. Effect of processing on fumonisin content of corn. In *Fumonisin in Food*; Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 323–334.
- Murphy, P. A.; Hopmans, E. C.; Miller, K.; Hendrich, S. Can fumonisins in foods be detoxified? In *Natural Protectants and Natural Toxicants in Food, Vol. 1*; Bidlack, W. R., Omaye, S. T., Eds.; Technomic Publishing Co.: Lancaster, PA, 1995; pp 105–117.
- Murphy, P. A.; Rice, L. D.; Ross, P. F. Fumonisin B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> content of Iowa, Wisconsin and Illinois corn and corn screenings. *J. Agric. Food Chem.* **1993**, *41*, 263–266.
- Norred, W. P.; Voss, K. A.; Bacon, C. W.; Riley, R. T. Effectiveness of ammonia treatment in detoxification of fumonisin-contaminated corn. *Food Chem. Toxicol.* **1991**, *29*, 815–819.
- Ohnishi, H.; Lin, T. H.; Nakajima, I.; Chu, T. M. Prostaglandin E<sub>2</sub> from macrophages of murine splenocyte cultures inhibits the generation of lymphokine-activated killer cell activity. *Tumor Biol.* **1991**, *12*, 99–110.
- Pelus, L. M.; Strausser, H. R. Prostaglandins and the immune response. *Life Sci.* **1977**, *20*, 903–913.
- Ross, P. F.; Rice, L. G.; Plattner, R. D.; Osweiler, G. D.; Wilson, T. M.; Owens, D. L.; Nelson, H. A.; Richard, J. L. Concentrations of fumonisin B<sub>1</sub> in feeds associated with animal health problems. *Mycopathologia* **1991**, *114*, 129–135.
- Roth, M. D.; Golub, S. H. Human pulmonary macrophages utilize prostaglandins and transforming growth factor  $\beta$ 1 to suppress lymphocyte activation. *J. Leukocyte Biol.* **1993**, *53*, 366–371.
- Rutenburg, A. M.; Kim, H.; Fischbein, J. W.; Hanker, J. S.; Wasserhrug, H. L.; Seligman, A. M. Histochemical and

- ultrastructural demonstration of gamma-glutamyl transpeptidase activity. *J. Histochem. Cytochem.* **1968**, *17*, 519–526.
- Sato, K.; Kitahara, A.; Satoh, K.; Ishikawa, T.; Tatematsu, M.; Ito, N. The placental form of glutathione S-transferase as a new marker protein for preneoplasia in rat chemical hepatocarcinogenesis. *Gann* **1984**, *75*, 199–202.
- Schultz, R. M.; Pavlidis, N. A.; Stylos, W. A.; Chirigos, M. A. Regulation of macrophage tumoricidal function: A role for prostaglandins of the E series. *Science* **1978**, *202*, 320–321.
- Sydenham, E. W.; Thiel, P. G.; Marasas, W. F. O.; Shephard, G. S.; van Schalkwyk, D. J. K.; Koch, K. R. Natural occurrence of some *Fusarium moniliforme* mycotoxins in corn from low and high esophageal cancer prevalence area of the Transkei, Southern Africa. *J. Agric. Food Chem.* **1990**, *38*, 1900–1903.
- Sydenham, E. W.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O.; Stockenstrom, S. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* **1991**, *39*, 2014–2018.
- Trevisani, A.; Ferretti, E.; Cappuzo, A.; Tomasi, V. Elevated levels of prostaglandin E<sub>2</sub> in Yoshida hepatoma and the inhibition of tumor growth by non-steroidal anti-inflammatory drugs. *Br. J. Cancer* **1980**, *41*, 341–347.
- Voss, K. A.; Norred, W. P.; Bacon, C. W. Subchronic toxicological investigations of *Fusarium moniliforme*-contaminated corn, culture material, and ammoniated culture material. *Mycopathologia* **1992**, *117*, 97–104.
- Voss, K. A.; Chamberlain, W. J.; Bacon, C. W.; Herbert, R. A.; Walters, D. B.; Norred, W. P. Subchronic feeding study of the mycotoxin fumonisin B<sub>1</sub> in B6C3F1 mice and Fisher 344 rats. *Fundam. Appl. Toxicol.* **1995**, *24* (1), 102–110.
- Wang, E.; Norred, W. P.; Bacon, C. W.; Riley, R. T.; Merrill, A. H., Jr. Inhibition of sphingolipid biosynthesis by fumonisins. *J. Biol. Chem.* **1991**, *266*, 14486–14490.

Received for review October 19, 1996. Accepted December 10, 1996.® This research was supported by Center for Advanced Technology Development, Iowa State University, and Pioneer Hybrid International, Inc. This paper is published as Journal Paper J-16170, Iowa Agriculture and Home Economics Experiment Station, Project 2995, a contributing project to regional NC-129.

JF9607775

---

® Abstract published in *Advance ACS Abstracts*, February 1, 1997.