Journal of Food Protection, Vol. 71, No. 10, 2008, Pages 2036–2041

Reduced Toxicity of Fumonisin B_1 in Corn Grits by Single-Screw Extrusion

KENNETH A. VOSS,[1]† LLOYD B. BULLERMAN,[2] ANDREIA BIANCHINI,[2] MILFORD A. HANNA,[2] AND DOJIN RYU[3]*†

[1]*Russell Research Center, U.S. Department of Agriculture, Agricultural Research Station, 950 College Station Road, Athens, Georgia 30605;* [2]*Department of Food Science and Technology, University of Nebraska, 143 Filley Hall, Lincoln, Nebraska 68583; and* [3]*Department of Nutrition and Food Sciences, Texas Woman's University, P.O. Box 425888, Denton, Texas 76204, USA*

MS 08-034: Received 17 January 2008/Accepted 2 May 2008

ABSTRACT

Corn grits spiked with 30 μg/g fumonisin B_1 and two batches of grits fermented with *Fusarium verticillioides* (batch 1 contained 33 μg/g, and batch 2 contained 48 μg/g fumonisin B_1), which were extruded by a single-screw extruder with and without glucose (10%, dry weight basis) supplementation were fed to rats. Control groups were fed uncontaminated grits. Extrusion with glucose more effectively reduced fumonisin B_1 concentrations of the grits (75 to 85%) than did extrusion alone (10 to 28%). With one exception, the fumonisin B_1–spiked and fermented extrusion products caused moderately severe kidney lesions and reduced kidney weights, effects typically found in fumonisin-exposed rats. Lesions in rats fed the least contaminated grits (batch 1) after extrusion with 10% glucose were, however, significantly less severe and not accompanied by kidney weight changes. Therefore, extrusion with glucose supplementation is potentially useful for safely reducing the toxicity of fumonisins in corn-based products and studies to determine the optimal conditions for its use are warranted.

In all corn (maize)–growing regions worldwide, *Fusarium verticillioides* and *Fusarium proliferatum* frequently contaminate the crop. These fungi produce fumonisins, a group of toxic secondary metabolites, with fumonisin B_1 (FB_1) being the most abundant and the most potent analog found in corn and corn-based food products *(16).* FB_1 is also the most thoroughly studied fumonisin from a toxicological standpoint, and its toxicities in domestic and laboratory animals are well documented. These include equine leukoencephalomalacia *(17),* porcine pulmonary edema *(10),* and hepato- and nephrotoxicity in a number of species including laboratory rodents *(1, 28).* FB_1 is a liver and kidney carcinogen in male rats *(7)* and induces liver cancer in female mice *(12).* Its human health effects are unclear; however, evidence suggests that FB_1 is a risk factor for neural tube defects and esophageal cancer in populations consuming contaminated corn as a diet staple *(1, 4, 8, 11, 18, 21).*

Attempts to eliminate or reduce this mycotoxin in foods have had limited success, largely due to its heat stability *(14)* and, consequently, FB_1 and its analogs are found in finished corn-based foods destined for human consumption *(9, 19, 24).* Results of previous studies suggest that extrusion processing, a cooking method combining high heat and high pressure, may significantly reduce fumonisin concentrations in corn *(3, 15).* These studies considered only the concentrations of fumonisins and known reaction products such as *N*-(deoxy-D-fructos-1-yl)-FB_1 *(20),* as determined by chemical (high-performance liquid chromatography; HPLC) and immunological (enzyme-linked immunosorbent assay) methods. Thus, the effect of extrusion on in vivo fumonisin toxicity has not been determined. This information is crucial to evaluate if extrusion processing can be a useful strategy for reducing the toxic potential of fumonisins in corn-based feeds and foods, because the possibility remains that FB_1 and other fumonisins might be converted by the high heat and pressure conditions of extrusion to reaction products that remain biologically active. It has been demonstrated that cooking by single-screw extrusion with 10% glucose supplementation significantly reduces the concentration of FB_1 in batches of spiked and fermented corn grits *(2).* The specific objective of this study was to determine the extent to which the in vivo toxicity of the batches extruded with 10% glucose was reduced.

MATERIALS AND METHODS

Preparation of contaminated grits. Contaminated corn grits were prepared as described previously *(2)* and used for both the analytical study to determine the fate of fumonisins in the materials *(2)* and this bioassay. In short, one batch of grits (''spiked grits'' or SG) was contaminated by direct addition of FB_1 at 30 μg/g, and two other batches (''fermented grits'' or FG) were cultured with *F. verticillioides* M-2552 until contamination levels of about 33 (FG1) to 48 (FG2) ppm of FB_1 in the grits were achieved. Moisture content of all batches of grits were adjusted to 20% (dry weight basis) prior to extrusion.

Extrusion of corn grits. The spiked and fermented grits were extruded in the presence and absence of 10% food-grade glucose (ADM Corn Processing, Decatur, Ill.), in a model 2003 GR-8 single-screw extruder (C.W. Brabender Instruments, South Hackensack, N.J.) at 160°C and 60 rpm as described previously *(2).* The extruded and unextruded samples were ground using an S-500 disc mill (Glen Mills, Inc., Clifton, N.J.). A batch of un-

* Author for correspondence. Tel: 940-898-2468, Fax: 940-898-2634; E-mail: dryu@twu.edu.

† K. A. Voss and D. Ryu contributed equally to this study.

TABLE 1. *Fumonisin concentrations in the control and test diets*[a]

Basal diet mixed with:	Control/test diet code	Total fumonisins in diet (ppm)[b]	FB_1 in diet (ppm)	Estimated daily FB_1 intake (mg/kg/body wt)[c]
Clean grits (negative control)	—	—	None detected	—
Clean grits, extruded (extrusion control)	—	—	None detected	—
Spiked grits	SG	13.0	12.7	1.69 D[d] e[e] (0.20)
Spiked grits, extruded	SG-E	12.0	11.5	1.45 E e (0.20)
Spiked grits plus glucose, extruded	SG-EG	10.4	3.3	0.43 F e (0.05)
Fermented grits (batch 1)	FG1	21.1	13.3	1.76 D e (0.2)
Fermented grits (batch 1), extruded	FG1-E	14.6	9.7	1.23 E e (0.20)
Fermented grits (batch 1) plus glucose, extruded	FG1-EG	6.5	1.9	0.23 F f (0.03)
Fermented grits (batch 2)	FG2	30.1	19.4	2.59 D d (0.28)
Fermented grits (batch 2), extruded	FG2-E	24.3	16.5	2.05 E d (0.25)
Fermented grits (batch 2) plus glucose, extruded	FG2-EG	11.1	3.8	0.49 F d (0.06)

[a] Nominal FB_1 concentrations calculated from HPLC analysis data of Bullerman et al. *(2)*.

[b] Sum of FB_1, FB_2, FB_3, hydrolyzed FB_1, and the glucose reaction product *N*-(deoxy-D-fructos-1-yl)-FB_1.

[c] Values indicate mean of 3 weeks and are estimated from body weight, food consumption, and nominal dietary fumonisin concentration data; standard deviation is indicated in parentheses.

[d] Where indicated, groups not sharing the same capital letter are significantly different, $P < 0.05$. Three analyses were done. For each, the three X, X-E, and X-EG (X = SG, FG1, or FG2) groups were compared to test the effect of extrusion method on each grits preparation.

[e] Where indicated, groups not sharing the same lowercase letter are significantly different, $P < 0.05$. Three sets of analyses were done. For each, the groups fed diets containing the grits preparations that were processed in the same manner (for example, the SG-EG, FG1-EG, and FG2-EG groups) were compared.

contaminated corn grits was processed (without glucose supplementation) in the same manner and served as an extrusion control. All materials were stored frozen until the diets were prepared (see below).

Bioassay: diets. The control and test diets were prepared by blending the extruded materials with a basal feed (Rodent Diet 2019, Teklad, Madison, Wis.), using a Patterson Kelley V blender with intensifier bar. The FB_1 concentration of the basal feed was <0.1 ppm, as determined by HPLC *(26)*. The test diets were formulated to contain equivalent amounts (dry weight equivalents equal to 50% [wt/wt] uncooked SG, FG1, or FG2) of their respective materials. The formulated diets were stored frozen and dispensed fresh each week.

Bioassay: animals. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, Ind.), 4 weeks of age at initiation of the study, were acclimated for 1 week and then randomly assigned to 11 groups ($n = 5$ per group). Group mean body weights averaged from 73.4 ± 3.05 (standard deviation) to 74.4 ± 3.65 g at the beginning of the study. The animals were individually housed in stainless steel, wire-mesh cages in an environmentally controlled room (23 ± 1°C, relative humidity of 56% ± 9.5%, 12-h light–dark cycle). Basal feed (during acclimation), control or test diet, and fresh tap water were available ad libitum.

Bioassay: feeding study. Procedures conformed to federal guidelines for the care and use of laboratory animals and were approved by the U.S. Department of Agriculture, Agricultural Research Station, Russell Research Center Institutional Animal Care and Use Committee. Each group was fed the negative control, extrusion control, or one of the nine test diets prepared from fumonisin-contaminated unprocessed or extruded grits (Table 1). The animals were observed daily and their body weights and food consumptions determined weekly. After 3 weeks, the animals were fasted overnight, euthanized with Isoflurane, USP (Isoflo, Abbott Laboratories, Chicago, Ill.) and examined by necropsy. The liver and kidneys were excised, weighed, and specimens thereof fixed in 10% neutral buffered formalin.

Bioassay: histopathology. Hematoxylin and eosin–stained sections of liver and kidney from each animal were microscopically examined, without knowledge of the animal's number or group assignment. Kidney lesions were scored on a scale of 0 to 4, based on the following subjective criteria *(23)*: 0 = no lesions consistent with fumonisin exposure; 1 = minimal effect, essentially normal with a few scattered apoptotic epithelial cells in the tubules of the outer medulla; 2 = mild effect, obvious fumonisin-like lesions with apoptotic epithelial cells in the outer medulla, sloughing of epithelial cells into tubule lumina, basophilia, variation of epithelial cells size and shape, variable nucleus size, or some focal tubule regeneration in outer medulla; 3 = moderate effect, same features as criterion 2, but more extensive with focal extension of lesions into tubules of the cortex; 4 = severe lesions, extensive tissue injury with overt necrosis, tubular atrophy, interstitial inflammation, and sclerosis or deposition of amorphous eosinophilic material around tubules (no lesions of this severity were observed).

Bioassay: statistical analysis. Statistical procedures were done using SigmaStat software (Jandel Scientific, San Rafael, Calif.). Each group was compared with the negative control, the extrusion control, and the two other groups fed the same materials (i.e., SG, SG-E, and SG-EG; FG1, FG1-E, and FG1-EG; or FG2, FG2-E, and FG2-EG were compared with each other and with the negative and extrusion control groups) or otherwise as indicated in Table 1. Continuous data were analyzed by analysis of variance and differences among groups identified using Duncan's multiple range test. Pathology score and other nonparametric data were analyzed with the Kruskal-Wallis test and differences between groups established using the Student-Newman-Keuls test. All tests were two tailed, and significance was judged at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical fate of the fumonisins, including the concentrations of FB_1, FB_2, FB_3, hydrolyzed FB_1, and the glucose reaction product *N*-(deoxy-D-fructos-1-yl)-FB_1 of the uncooked SG, FG1, and FG2 and their extrusion products used in this study are reported elsewhere *(2).* In short, extrusion reduced FB_1 concentrations (in parts per million) in the spiked grits, batch 1 of fermented grits, and batch 2 of fermented grits by 10, 28, and 14%, respectively, a finding that is consistent with results of earlier reports *(3, 15).* Maximum reductions of fumonisins were observed when extrusion was combined with glucose supplementation *(17, 20)* and, accordingly, the addition of 10% glucose to the grits just prior to extrusion increased the amount of FB_1 reduction achieved. As a result, the FB_1 concentrations in the diets prepared from the extruded, glucose supplemented grits in this study were reduced by 75% (spiked grits) to 85% (batch 1 fermented grits). These reductions are reflected in the FB_1 concentrations of the formulated test diets used in this bioassay (Table 1). Hydrolyzed FB_1 concentrations were low (<10% of total fumonisins) in all nonextruded and extruded materials. Significant amounts of *N*-(deoxy-D-fructos-1-yl)-FB_1 were formed only when extrusion was combined with 10% glucose supplementation: in this case, *N*-(deoxy-D-fructos-1-yl)-FB_1 made up 68, 55, and 52% of the total fumonisins. FB_2 and FB_3 were not detected in the spiked grits. They were present in the nonextruded fermented grits, constituting 34 to 35% of the total fumonisins (sum of FB_1, FB_2, FB_3, hydrolyzed FB_1, and *N*-(deoxy-D-fructos-1-yl)-FB_1). FB_2 and FB_3 were also found at similar levels (29 to 31% of the total fumonisins) in the extruded fermented grits preparations and in lesser amounts (14% of the total fumonisins) in the fermented grits after extrusion with 10% glucose supplementation *(2).*

Fumonisin concentrations of the test diets were nominal and based on the FB_1 content of the unprocessed and extruded materials (Table 1), as reported previously *(2).* FB_2, FB_3, and hydrolyzed FB_1 (also known as aminopentol) were not considered when formulating the test diets because they, in contrast to equivalent μmolar dietary concentration of FB_1, were not toxic when fed to mice *(12)* or when hydrolyzed FB_1 was given to rats by gavage *(5).* *N*-(deoxy-D-fructos-1-yl)-FB_1 was also not considered because of its relatively low concentration (<6% of the total fumonisins) in the nonextruded materials and because fumonisin-glucose reaction products were significantly less toxic than was FB_1 when fed to swine *(6).*

Appearance, body weight, food consumption, and daily FB_1 intake. No outward signs of toxicity were observed in any group. With one exception, no significant differences in body weight were found. Body weights of the FG2 (98.8 ± 3.7 g) group were slightly but significantly less than those of the other groups (106.8 ± 4.55 to 112.2 ± 6.65 g) after week 1 and were also slightly less (135 ± 5.8 g) than those of the two control groups (152 ± 11.8 and 154.2 ± 10.5 g) after week 2. Body weights of the FG2 rats remained 8% (FG2-E) to 12% (extrusion control) lower than those of the two control groups as well as the FG2-E and FG2-EG groups after week 3, although the differences were not statistically significant.

Feed consumption of the FG2 (132 ± 5.9 g) and FG2-E (129 ± 13.7 g) groups was decreased 12 to 14% compared with the negative controls (149 ± 5.5 g) during week 2. No other significant differences were found, although feed consumptions of the test groups tended to be about 10% less than those consumptions of the control groups (data not shown).

Daily intake of FB_1 was calculated from the weekly body weight and food consumption data (Table 1). Animals fed the extruded spiked or fermented grits preparations ingested (milligram per kilogram of body weight per day) significantly less FB_1 than did their counterparts fed the unextruded grits. In turn, FB_1 intake of the three groups fed grits extruded with 10% glucose supplementation was significantly less than those of the animals fed diets prepared from unextruded or extruded (without glucose) grits. Daily intakes paralleled the nominal dietary concentrations of FB_1 and ranged from 70 to 85% (SG-E, FG1-E, and FG2-E) and 13 to 25% (SG-EG, FG1-EG, and FG2-EG) of the daily intakes of their respective SG, FG1, or FG2 groups. Daily FB_1 intake of the FG1-EG group which, as discussed below, was the only group of the six given an extruded grits preparation to show reduced toxicity, were significantly lower than those of the SG-EG and FG2-EG groups.

Organ weights. The kidney of the male Sprague-Dawley or F344 rat is a sensitive indicator of fumonisin toxicity *(22, 25, 27),* and the FB_1 concentrations in the SG, FG1, and FG2 diets were selected to cause moderate kidney lesions and mild to moderately decreased (<15% compared with controls) relative kidney weight *(23).* In this way, the kidney served as the bioassay target organ for showing if extrusion alone or with glucose supplementation exacerbates or ameliorates toxicity of the spiked or fermented grits.

No differences in absolute (grams) or relative (percent final body weight) liver weights were found. Absolute kidney weights of the FG2 group were less than those weights of the two control groups (Table 2); otherwise, no differences in absolute weight were found. Relative kidney weight of the SG group was less than that of the negative control, while relative kidney weights of the SG-E and SG-EG groups were less than those relative kidney weights of both the negative and extrusion control groups. Relative kidney weights of the FG1 and FG1-E groups were significantly decreased compared with the negative controls and the FG1-EG (which did not differ from the controls) groups, suggesting that the addition of glucose to the FG1 grits ameliorated toxicity. This was not the case for FG2, however, as the relative kidney weights of the FG2, FG2-E, and FG2-EG groups were similar to each other and significantly less than those weights of the negative control group. Relative kidney weights of the extrusion control group did not differ from the negative control group. However, they averaged about 4% less and, as a result, signifi-

TABLE 2. *Absolute and relative kidney weights of rats fed control diets or diets formulated with the unextruded or extruded grits for 3 weeks*

Diet[a]	Kidney wt[b]	
	Absolute (g)	Relative (% body wt)
Clean corn grits		
Negative control	1.42 A[c] (0.22)	0.82 A (0.03)
Extrusion control	1.42 A (0.15)	0.79 AB (0.04)
Spiked corn grits		
SG	1.24 A (0.11)	0.74 BC (0.08)
SG-E	1.29 A (0.11)	0.72 C (0.04)
SG-EG	1.29 A (0.11)	0.72 C (0.03)
Fermented corn grits (batch 1)		
FG1	1.22 A (0.07)	0.76 B (0.02)
FG1-E	1.39 A (0.09)	0.75 B (0.05)
FG1-EG	1.38 A (0.10)	0.83 A (0.04)
Fermented corn grits (batch 2)		
FG2	1.13 B (0.08)	0.72 C (0.03)
FG2-E	1.25 AB (0.06)	0.74 BC (0.04)
FG2-EG	1.29 AB (0.14)	0.76 BC (0.06)

[a] SG, spiked grits; SG-E, spiked grits extruded; SG-EG, spiked grits extruded with glucose; FG1, fermented grits batch 1 unextruded; FG1-E, fermented grits batch 1 extruded; FG1-EG, fermented grits batch 1 extruded with glucose; FG2, fermented grits batch 2 unextruded; FG2-E, fermented grits batch 2 extruded; FG2-EG, fermented grits batch 2 extruded with glucose.

[b] Values are group means (SD), $n = 5$.

[c] Where indicated, groups not sharing the same capital letter are significantly different, $P < 0.05$. Three sets of analyses were done. For each, the X, X-E, and X-EG (X = SG, FG1, or FG2) groups were compared with each other and with the two common (negative and extrusion) control groups. Statistics were run separately for each material (groups fed unextruded, extruded, extruded plus glucose, negative control, and extrusion control diets were compared).

cant differences were found between the extrusion control group and the SG-E, SG-EG, and FG2 groups (Table 2).

Pathology. Gross lesions were not found in any group, and microscopic examinations revealed no evidence of a test-related liver effect. Incidental findings included cytoplasmic vacuolation of hepatocytes, scattered small foci of chronic inflammation or necrosis, and mitotic figures. Apoptotic hepatocytes were rare: one to two were noted in two to four animals per group, including the controls.

No noteworthy microscopic lesions were found in the kidneys of the negative or extrusion control groups. In contrast, kidney lesions typically found in Sprague-Dawley rats fed diets containing fumonisins *(22, 23, 25, 27)* were noted, with one exception, in all rats from the remaining groups (Table 3). Lesions in the FG1-EG group were significantly ($P < 0.05$) less severe (mean score was 1.2) than lesions found in the FG1 and FG1-E groups and were lowest among the nine test groups (mean scores ranged from 2.6 to 3.0). The kidney of one FG1-EG animal appeared unaffected (score of 0), two others had only minimal (score of 1) lesions, and the remaining kidneys from the FG1-EG group were considered mildly affected (score of 2). Thirty-six of the remaining 40 animals, 3 to 5 per group, fed the diets prepared from FB1-spiked corn or the fermented corn preparations had more extensive (score of 3) kidney lesions (Fig. 1).

TABLE 3. *Summary of histopathological kidney findings in rats*

Diet[a]	Incidence/mean score[b]	Score distribution[c]			
		0	1	2	3
Controls					
Negative control	0/0 A[d]	5	0	0	0
Extrusion control	0/0 A	5	0	0	0
Spiked corn grits					
SG	5/3 B	0	0	0	5
SG-E	5/2.8 (0.44) B	0	0	1	4
SG-EG	5/2.6 (0.55) B	0	0	2	3
Fermented corn grits (batch 1)					
FG1	5/3 B	0	0	0	5
FG1-E	5/3 B	0	0	0	5
FG1-EG	4/1.2 (0.84) A	1	2	2	0
Fermented corn grits (batch 2)					
FG2	5/3 B	0	0	0	5
FG2-E	5/3 B	0	0	0	5
FG2-EG	5/2.6 (0.89) B	0	1	0	4

[a] SG, unextruded FB_1-spiked corn; SG-E, extruded SG; SG-EG, extruded SG plus glucose; FG1, unextruded fermented corn (batch 1); FG1-E, extruded FG1; FG1-EG, extruded FG1 plus glucose; FG2, unextruded fermented corn (batch 2); FG1-E, extruded FG2; FG2-EG, extruded FG2 plus glucose for 3 weeks.

[b] Entry indicates the number of animals having fumonisin-like lesions/mean score (SD) for severity of the lesions, $n = 5$ rats per group.

[c] Values indicate the number of rats in each group having kidneys having minimal (1), mild (2), or moderate (3) kidney lesions consistent with fumonisin nephropathy. No animals had severe (4) lesions. 0 = no lesions. See text for detailed explanation of the scoring criteria.

[d] Distribution of scores for groups not sharing the same capital letter are significantly different, $P < 0.05$.

Kidney lesions were consistent with the well-documented apoptotic and other effects of fumonisins *(22, 23, 25, 27),* and, taken together, histopathology and organ weight findings indicated that extrusion and glucose reduced the toxicity of the FG1 grits. Extrusion alone was not effective for any of the three materials and extrusion and glucose did not significantly affect toxicity of the SG or FG2. The difference in the degree to which extrusion plus glucose supplementation reduced the toxicity of the two batches of extruded grits is attributable to their different FB_1 concentrations before (FG1 = 32.7 ppm, FG2 = 47.9 ppm, dry weight) and after (FG1-EG = 4.8 ppm, FG2-EG = 9.5 ppm) processing *(2).* Consequently, both FB_1 concentration of the FG1-EG diet and estimated daily FB_1 intake of rats fed the FG1-EG diet were less than half of their respective FG2-EG values (Table 1). The FG1-EG diet was also less toxic, contained less FB_1, and resulted in a

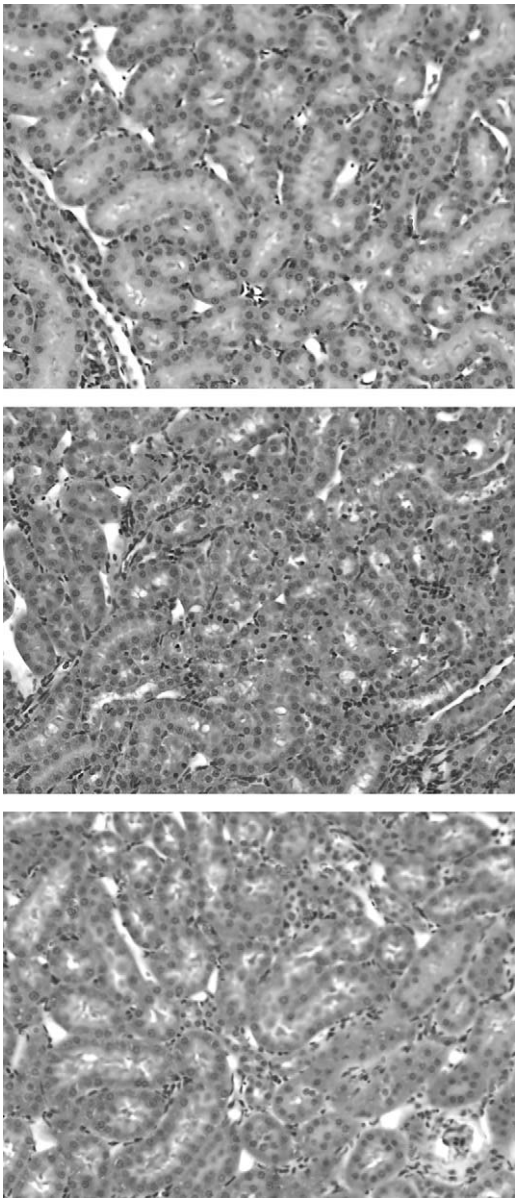

FIGURE 1. *Photomicrographs illustrating the appearance of the kidneys (outer strip of outer medulla) from rats fed control group (top), batch 1 of fermented grits showing the lesions (middle), and the same batch of FB_1-contaminated grits after extrusion with 10% glucose supplementation, showing the less severe lesions (hematoxylin and eosin stained, magnification of ×220). Note the basophilia, variation in cell and nucleus size, widespread pyknotic nuclei and apoptosis and sloughing of epithelial cells into the tubule lumen in the middle frame.*

significantly lower (about 50%) daily intake of FB_1 than did the SG-EG diet, even though the initial FB_1 concentrations of the two materials and, consequently, the SG (12.7 ppm) and FG1 (13.3 ppm) diets were similar. Interestingly, the concentration of *N*-(deoxy-D-fructos-1-yl)-FB_1 in FG1 grits extruded with glucose (9.0 μg/g) was about half of that found in SG grits extruded with glucose and, furthermore, recovery of total fumonisins from the former (30%) was considerably less than from the latter (78%) *(2)*. This suggests the possibility that fumonisin binding or other mycotoxin–matrix interactions during extrusion are influenced by physical and chemical differences in the matrices of the unfermented, FB_1-spiked, and the fermented grits. It is also a likely reason for the lower dietary FB_1 concentration found in the FG1-EG diet and significantly reduced daily FB_1 intakes of the FG1-EG group compared with the SG-EG group.

In any event, histopathology and kidney weight findings indicate that extrusion with glucose supplementation, but not extrusion alone, reduced the in vivo toxicity of one batch of *Fusarium*-fermented corn (FG1). Extrusion, with or without glucose, was ineffective for detoxifying FB_1-spiked corn having a similar FB_1 concentration or a second, more highly contaminated batch of fermented corn (FG2). The results obtained with the FG1 grits suggests that extrusion plus glucose not only just safely reduce fumonisin concentrations and toxicity in corn-based products, but also show that toxicity will not be reduced if fumonisin concentrations of materials prior to cooking exceed some as yet undefined limit. Therefore, additional bioassays incorporating dose and time-course parameters, tissue sphingoid base measurements to assess fumonisin bioavailability at doses not including overt toxicity, as well as more extensive chemical analyses of fumonisin–food matrix reaction products are needed to fully characterize how extrusion affects fumonisins in food matrices and to determine the practicability and limitations of extrusion for reducing fumonisins in corn-based products.

It must be noted that FB_1 is a liver and kidney carcinogen in rodents and is classified as possibly carcinogenic to humans (International Agency for Research on Cancer group 2B) *(13)*; therefore, FB_1 should be handled using proper precautionary measures.

ACKNOWLEDGMENTS

This study was supported in part by the National Research Initiative of the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service, grant no. 2005-35201-16329, and the Anderson Research Grant Program of North Central Regional Project NC-213. The technical assistance of J. Showker, P. Stancel, N. Stewart, and E. Wray are gratefully acknowledged.

REFERENCES

1. Bolger, M., R. D. Coker, M. DiNovi, D. Gaylor, W. C. A. Gelderblom, M. Olsen, N. Paster, R. T. Riley, G. Shephard, and G. J. A. Speijers. 2001. Fumonisins, p. 103–279. *In* Safety evaluation of certain mycotoxins in foods. Food and Agriculture Organization of the United Nations Food and Nutrition paper 74. World Health Organization, Geneva.
2. Bullerman, L. B., A. Bianchini, L. S. Jackson, J. Jablonski, M. A. Hanna, and D. Ryu. 2008. Reduction of fumonisin B_1 in corn grits by single-screw extrusion. *J. Agric. Food Chem.* 56:2400–2405.
3. Castelo, M. M., L. S. Jackson, M. A. Hanna, B. H. Reynolds, and L. B. Bullerman. 2001. Loss of fumonisin B1 in extruded and baked corn-based foods with sugars. *J. Food Sci.* 66:416–421.
4. Chu, F. S., and G. Li. 1994. Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidence of esophageal cancer. *Appl. Environ. Microbiol.* 60:847–852.
5. Collins, T. F., R. L. Sprando, T. N. Black, N. Olejnik, R. M. Eppley, M. E. Shackelford, P. C. Howard, J. I. Rorie, M. Bryant, and D. I. Ruggles. 2006. Effects of aminopentol on in utero development in rats. *Food Chem. Toxicol.* 44:161–169.
6. Fernandez-Surumay, G., G. D. Osweiler, M. J. Yaeger, G. E. Rotting-

haus, S. Hendrich, L. K. Buckley, and P. A. Murphy. 2005. Fumonisin B–glucose reaction products are less toxic when fed to swine. *J. Agric. Food Chem.* 18:4264–4271.
7. Gelderblom, W. C. A., N. P. J. Kriek, W. F. O. Marasas, and P. G. Thiel. 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1 in rats. *Carcinogenesis* 12:1247–1251.
8. Gelineau-van Waes, J. B., L. Starr, J. R. Maddox, F. Aleman, K. A. Voss, J. Wilberding, and R. T. Riley. 2005. Maternal fumonisin exposure and risk for neural tube defects: mechanisms in an in vivo mouse model. *Birth Defects Res. Part A Clin. Mol. Teratol.* 73:487–497.
9. Gutema, T., C. Munimbazi, and L. B. Bullerman. 2000. Occurrence of fumonisins and moniliformin in corn and corn-based food products of U.S. origin. *J. Food Prot.* 63:1732–1737.
10. Harrison, L. R., B. M. Colvin, J. T. Greene, L. E. Newman, and J. R. Cole, Jr. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin FB_1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* 2:217–221.
11. Hendricks, K. 1999. Fumonisins and neural tube defects in south Texas. *Epidemiology* 10:198–200.
12. Howard, P. C., L. H. Couch, R. E. Patton, R. M. Eppley, D. R. Doerge, M. I. Churchwell, M. M. Marques, and C. V. Okerberg. 2002. Comparison of the toxicity of several fumonisin derivatives in a 28-day feeding study with female B6C3F1 mice. *Toxicol. Appl. Pharmacol.* 185:153–165.
13. International Agency for Research on Cancer (IARC). 2002. Monographs on the evaluation of carcinogenic risks to humans, p. 301–366. IARC Press, Lyon, France.
14. Jackson, L. S., J. J. Hlywka, K. R. Senthil, L. B. Bullerman, and S. M. Musser. 1996. Effects of time, temperature and pH on the stability of fumonisin B_1 in an aqueous model system. *J. Agric. Food Chem.* 44:906–912.
15. Katta, S. K., L. S. Jackson, S. S. Sumner, M. A. Hanna, and L. B. Bullerman. 1999. Effect of temperature and screw speed on stability of fumonisin B_1 on extrusion-cooked corn grits. *Cereal Chem.* 76: 16–20.
16. Marasas, W. F. O. 2001. Discovery and occurrence of the fumonisins: a historical perspective. *Environ. Health Perspect.* 109:239–243.
17. Marasas, W. F. O., T. S. Kellerman, W. C. A. Gelderblom, J. A. W. Coetzer, P. G. Thiel, and J. J. van Lugt. 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* 55:197–203.
18. Missmer, S. A., L. Suarez, M. Felkner, E. Wang, A. E. Merrill, Jr., K. J. Rothman, and K. A. Hendricks. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. *Envrion. Health Perspect.* 114:237–241.
19. Pittet, A., V. Parisod, and M. Schellengerg. 1992. Occurrence of fumonisins B_1 and B_2 in corn-based products from the Swiss market. *J. Agric. Food Chem.* 40:1352–1354.
20. Poling, S. M., R. D. Plattner, and D. Weisleder. 2002. *N*-(Deoxy-D-fructos-1-yl) fumonisin B_1, the initial reaction product of fumonisin B_1 and D-glucose. *J. Agric. Food Chem.* 50:1318–1324.
21. Rheeder, J. P., W. F. O. Marasas, P. G. Thiel, E. W. Sydenham, G. S. Shepard, and D. J. Van Schalkwyk. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* 82:353–357.
22. Riley, R. T., D. M. Hinton, W. J. Chamberlain, C. W. Bacon, E. Wang, A. H. Merrill, and K. A. Voss. 1994. Dietary fumonisin B_1 induces disruption of sphingolipid metabolism in Sprague Dawley rats: a new mechanism of nephro-toxicity. *J. Nutr.* 124:594–603.
23. Riley, R. T., and K. A. Voss. 2006. Differential sensitivity of rat kidney and liver to fumonisin toxicity: organ-specific differences in toxin accumulation and sphingoid base metabolism. *Toxicol. Sci.* 92: 335–345.
24. Sydenham, E. W., G. S. Shephard, P. G. Thiel, W. F. O. Marasas, and S. Stockenstorm. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* 25:767–771.
25. Voss, K. A., W. J. Chamberlain, C. W. Bacon, and W. P. Norred. 1993. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B_1. *Nat. Toxins* 1:222–228.
26. Voss, K. A., F. I. Meredith, and C. W. Bacon. 2003. Effect of baking and frying on the in vivo toxicity to rats of cornmeal containing fumonisins. *J. Agric. Food. Chem.* 51:5546–5551.
27. Voss, K. A., R. D. Plattner, R. T. Riley, F. I. Meredith, and W. P. Norred. 1998. In vivo effects of fumonisin B_1–producing and fumonisin B_1–nonproducing *Fusarium moniliforme* isolates are similar: fumonisins B_2 and B_3 cause hepato- and nephrotoxicity in rats. *Mycopathologia* 141:45–58.
28. Voss, K. A., R. T. Riley, W. P. Norred, C. W. Bacon, F. I. Meredith, P. C. Howard, R. D. Plattner, T. F. X. Collins, D. Hansen, and J. K. Porter. 2001. An overview of rodent toxicities: liver and kidney effects of *Fusarium moniliforme* and fumonisins. *Environ. Health Perspect.* 109(Suppl.):259–266.