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RESEARCH ARTICLE

Extrusion cooking with glucose supplementation of fumonisin-contaminated corn grits protects against nephrotoxicity and disrupted sphingolipid metabolism in rats

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Scope: Fumonisin B1 (FB1) is a mycotoxin found in maize and maize-based foods. It causes animal diseases and is a suspected risk factor for cancer and birth defects in humans. Extrusion cooking reduces FB1 concentrations in maize however toxicity caused by unknown degradation or FB1-matrix reaction products might persist.
Methods and results: To test the efficacy of extrusion to reduce FB1 toxicity, Fusarium verticillioides fermented corn (= maize) grits (Batch-1= 9.7 ppm FB1; Batch-2= 50 ppm FB1) were extruded without (Batch-1E; Batch-2E) or with 10% glucose supplementation (Batch-1EG; Batch-2EG). FB1 concentrations were reduced 64% (Batch-2E) to 94% (Batch-1EG) after cooking. When the uncooked and processed grits were fed (50% w/w in rodent chow) to rats for up to 8 weeks, FB1 intakes averaged 354, 103, and 25.1 çg/kg body weight/day for Batch-1, Batch-1E and Batch-1EG and 1804, 698, and 222 çg/kg body weight/day for the Batch-2, Batch-2E and Batch-2EG, respectively. Nephrotoxicity including apoptotic lesions and elevated sphingoid base concentrations decreased in a dose-dependent manner in groups fed Batch-1, Batch-1E, Batch-2, Batch-2E, or Batch-2EG and was absent in the Batch-1EG group.
Conclusion: Extrusion cooking, especially with glucose supplementation, is potentially useful to reduce FB1 concentrations and toxicity of FB1-contaminated maize.

Keywords:
Extrusion cooking / Fumonisin / In vivo toxicity / Sphinganine / Sphinganine 1-phosphate

1 Introduction

Fumonisins are mycotoxins produced by *Fusarium verticillioides*, *F. proliferatum*, other *Fusarium* and some species of the *Aspergillus niger* complex [1–3]. They are common contaminants of maize and maize-based products but have also been found in a variety of other foodstuffs [3]. Fumonisin B_1 (FB_1) is the most predominant and thoroughly studied congener [1, 2]. Its toxic and carcinogenic effects in animals include leukoencephalomalacia in *Equidae*, pulmonary edema in swine, and hepato- and nephrotoxicities in a variety of piscine, avian and mammalian species [2, 4, 5]. FB_1 is a liver and kidney carcinogen in rodents [6, 7]. The impact of FB_1 or other fumonisins on human health is unknown although evidence suggests that FB_1 is a risk factor for birth (neural tube) defects or cancer in populations consuming large amounts of contaminated maize [1, 2, 8, 9].

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Abbreviations: FB_1, Fumonisin B_1; **deoxy Sa,** 1-deoxysphinganine; **NDF-FB_1,** *N*-(1-deoxy-D-fructos-1-yl)-FB_1; **Sa,** sphinganine; **Sa 1-P,** sphinganine 1-phosphate; **So,** sphingosine

FB_1 is generally stable during cooking and therefore efforts to reduce its concentrations in foods have yielded mixed results [10, 11]. Extrusion cooking is a process combining high temperature and pressure that has been shown to significantly reduce fumonisin concentrations in maize. Greater reductions are achieved when a reducing sugar is added to the mixture prior to processing [11–13]. However, analytical results alone are insufficient to determine whether cooking reduces toxicity as unknown thermal decomposition or other reaction products, including difficult to detect matrix-associated or bound fumonisins [14, 15] might contribute to toxicity.

In earlier studies, FB_1 concentrations in spiked (30 ppm) or *F. verticillioides*-fermented (33–48 ppm) grits were reduced 10–28% by cooking alone and 75–85% by cooking with 10% glucose supplementation using a single-screw extruder [13]. Extrusion with glucose partially reversed toxicity of the less contaminated fermented grits when they were fed to rats, suggesting that the process was potentially useful for reducing fumonisin exposure [16].

The purpose of this investigation was to determine the effectiveness of cooking *F. verticillioides*-fermented corn (= maize) grits by twin-screw extrusion to reduce FB_1 bioavailability and toxicity. The bioassay was more rigorous than an earlier one [16] in that it (i) tested the process on grits having FB_1 concentrations both below (9.7 ppm, Batch-1) and above (50 ppm, Batch-2) that for which partial protection against toxicity was demonstrated, (ii) evaluated toxicity over a longer, 8 wk exposure period, (iii) included hematology and clinical chemistry indicators of kidney and liver dysfunction as experimental endpoints and (iv) utilized changes in target organ sphingoid base concentrations as a biomarker of exposure.

2 Materials and methods

2.1 Grits

Contaminated corn grits were prepared as previously reported [13]. Briefly, two batches were fermented with *F. verticillioides* M-2552 until contamination levels of about 10 (Batch-1) or 50 (Batch-2) ppm FB_1 were achieved.

2.2 Extrusion

Extrusion conditions are described in detail by Jackson et al. [17]. Briefly, Batch-1 and Batch-2 grits (20% moisture content, dry weight basis) were extruded without (Batch-1E, Batch-2E) or with 10% food-grade glucose (ADM Corn Processing, Decatur, IL, USA) supplementation (Batch-1EG, Batch-2EG) using a model CTSE-V twin-screw apparatus (C.W. Brabender, South Hackensack, NJ, USA) with conical mixing configuration at 160°C, 40 rpm and 60 g/min feed rate. A portion of the uncontaminated grits (20% moisture content, dry weight basis) was similarly processed without adding glucose. The extruded grit preparations were ground and they and the uncooked grits were stored frozen. FB_1 and hydrolyzed FB_1 (HFB_1) concentrations in the grits before and after processing were quantified by HPLC with fluorescence detection following derivatization with *o*-phthaldialdehyde (Sigma, St. Louis, MO, USA). The FB_1-glucose reaction product *N*-(1-deoxy-D-fructos-1-yl)-FB_1 (NDF-FB_1) [18] was quantified by LC-MS analysis [13].

2.3 Animals

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN, USA), 3 wk of age were acclimated for 2 wk and then randomly assigned to eight groups ($n = 10$/group) having mean body weights of 73 ± 3.1 g (SD) to 74 ± 3.7 g. The animals were individually housed in stainless-steel, wire-mesh cages in an environmentally controlled room having a 12-h light/dark cycle. Fresh tap water and the appropriate control or test diet were provided ad libitum.

2.4 Diets

Animals were fed standard rodent diet (TD2019, Teklad, Madison, WI, USA) during acclimation. The basal feed (Certified Teklad TD.09.119, < 0.1 ppm FB_1) used to mix the control and test diets was formulated to ensure adequate amounts of protein, vitamins, lipotropes and other nutrients. Basal feed and the grits preparations were mixed (50% w/w) using a Patterson Kelley V blender with intensifier bar. FB_1 concentrations of each diet (Table 1) were calculated on the basis of the FB_1 concentrations in the raw or processed grits, which in turn were determined by chemical analysis [17]. The diets were stored frozen and dispensed fresh each wk.

2.5 Bioassay

Procedures were approved by the USDA-ARS Russell Research Center Institutional Animal Care and Use Committee. The groups were fed uncooked uncontaminated grits (uncooked control group), extruded uncontaminated grits (extrusion control group) or one of the test diets (Table 1). The animals were observed at least once per day. Body weights and food consumptions were measured weekly. After 3 and 8 wk of feeding, five animals/group were fasted overnight in metabolism cages for collection of urine samples. Thereafter, they were lightly anaesthetized (Isoflurane USP, Isoflo®, Abbott Laboratories, Chicago, IL, USA) and blood collected (periorbital plexus) for hematology and serum chemistry determinations. Following blood collection, the animals were euthanized (CO_2 inhalation) and examined by necropsy. The kidneys, liver, brain, heart,

lungs with trachea, testes, adrenals and spleen were weighed. Representative specimens of these and other major organs were fixed in 10% neutral buffered formalin. Additional samples of liver and kidney were collected and frozen (−80°C) for sphingolipid analyses.

2.5.1 Clinical laboratory studies

Hematology, serum chemistry and urinalysis were conducted on all animals. Hematology profiles were obtained using a Bayer Advia 120 Hematology Analyzer and commercial reagents (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Profiles included erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, total and differential leukocyte counts, platelet count and mean platelet volume. Clinical chemistry procedures were automated (Hitachi Model 912) and done using commercial reagents (Roche Diagnostics Corporation, Indianapolis, IN, USA). Serum enzyme determinations were aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and γ-glutamyl transpeptidase. Other parameters were total protein, albumin, triglycerides, cholesterol, glucose, total bilirubin, blood urea nitrogen, creatinine and electrolytes (sodium, potassium, calcium, bicarbonate, chloride phosphorus and anion gap). Urine samples were screened for color, turbidity, pH, protein, glucose, ketones, bilirubin and blood using Siemens Multistix® (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) as well as for specific gravity (TS refractometer) and by microscopic examination.

2.5.2 Histopathology

Hematoxylin and eosin (H & E)-stained kidney specimens from all animals were examined in a random order and without knowledge of the animals' group assignments. Lesions consistent with fumonisin exposure [4] were scored on a scale of 0 = no effect; 1 = few apoptotic cells in an unremarkable parenchyma indicating a minimal change; 2 = mild, generalized effect of FB_1 exposure; 3 = more obvious, moderate effect and 4 = severe effect with overt necrosis and fibrosis [16]. Hematoxylin and eosin-stained liver (all groups, both time periods) and heart, testes, spleen, adrenals and lung (all groups, 8 wk only) specimens also were examined.

2.5.3 Tissue sphingoid base profiles

The concentrations of free sphinganine (Sa), sphingosine (So), 1-deoxysphinganine (deoxy Sa), sphinganine 1-phosphate (Sa 1-P) and sphingosine 1-P (So 1-P) in the kidneys of all animals and in the livers of the control, extruded control, Batch-2E and Batch-2EG groups were determined using a previously reported LC-MS method [19] as modified for deoxy Sa [20].

2.5.4 Statistical analysis

Statistical procedures were done using SigmaStat software (Jandel Scientific, San Rafael, CA, USA). Each test group was compared with the control groups and the two other groups fed grits preparations made from the same batch. Continuous, homogenous data were analyzed by ANOVA and differences among groups identified post hoc using Student–Newman–Keul's method. Nonparametric data including kidney scores were analyzed using the Kruskal–-Wallis Test and Fisher's exact test was used to evaluate pathology incidence data. All tests were two-tailed and significance was determined at $p < 0.05$.

3 Results

3.1 Extrusion and diets

Analytical procedures and results for the effects of twin-screw extrusion cooking on FB_1 in maize grits are reported in detail elsewhere [17]. Briefly, extrusion reduced FB_1 concentrations by 72 and 64% for Batch-1E and Batch-2E, respectively (Table 1). Glucose supplementation enhanced reductions so that FB_1 concentrations were reduced by 94% for Batch-1EG and 89% for Batch-2EG. As a result of dilution (50% w/w) with basal feed, the FB_1 concentrations ranged from 0.3 to 4.9 ppm for the lower level Batch-1 series (Batch-1, Batch-1E and Batch-1EG) and 2.9 to 25 ppm for the higher level Batch-2 series (Batch-2, Batch-2E and Batch-2-EG) of experimental diets. Uncooked and extruded control diets contained < 0.1 ppm FB_1. Processing did not affect HFB_1 concentrations, which were low (< 0.5 ppm) in all diets. Minor amounts of the glucose reaction product, NDF-FB_1, were present in the uncooked control diet. Extrusion lowered its dietary concentration from 0.4 ppm (Batch-1) to < 0.1 ppm (Batch-1E) and from 2.1 ppm (Batch-2) to 0.5 ppm (Batch-2E). Extrusion with glucose supplementation did not affect NDF-FB_1 in Batch-1E but increased its amount in Batch-2E: concentrations were 0.5 ppm in the Batch-1EG and 3.9 ppm in Batch-2EG diets.

3.2 Body weight and food consumption

No differences in body weight were found between the uncooked control and other groups. Body weights of the extruded control group were significantly higher than in the Batch-2 group at wk 2 and the Batch-2, Batch-2E and Batch-2EG groups at wk 3. No other differences were found. Group mean weights (g) at wk 8 were as follows: uncooked

control = 380 ± 23 (SD), extruded control = 383 ± 23, Batch-1 = 364 ± 31, Batch-1E = 366 ± 26, Batch-1EG = 377 ± 38.0, Batch-2 = 364 ± 23, Batch-2E = 373 ± 25 and Batch-2EG = 359 ± 27.

Weekly food consumption of the Batch-1 and Batch-2 groups was significantly lower than in the two control groups through wk 3 (data not shown). Food consumption of rats fed Batch-1 also was significantly less when compared with the Batch-1E and Batch-1EG groups and reduced during weeks 1 and 2 in rats fed Batch-2 compared with those fed Batch-2E or batch-2EG. No significant differences were found after wk 3.

3.3 FB_1 intake

Daily FB_1 intakes (μg FB_1/kg body weight/day) were calculated using the formula: ((weekly food consumption (g) × FB_1 concentration (μg/g))/average body weight (kg) during the week)/7. For Batch-1 (Table 1), extrusion reduced daily FB_1 intake about 70% and extrusion with glucose reduced FB_1 daily intake >90%. Reductions of 60–88% were achieved by extrusion and extrusion with glucose, respectively, of Batch-2. Exposures (μg FB_1/kg body weight/day) declined as the study progressed. Daily intakes of all groups were reduced significantly (39–44% lower) during weeks 4–8 compared with weeks 1–3 and, with one exception (Batch-1EG), to the entire 8-wk feeding period.

Table 1. FB_1 concentrations (ppm) of the diets prepared from two batches of *F. verticillioides* fermented grits that were uncooked, extruded (-E), or extruded with 10% glucose supplementation (-EG) for 3 or 8 wks and estimated daily FB_1 intake of rats fed the diets for 3 or 8 wk

Diet	FB_1[a)] (ppm)	Estimated daily FB_1 intake during weeks[b)]		
		1–3 (n = 10)	4–8 (n = 5)	1–8 (n = 5)
Fermented Grits Low FB_1				
Batch-1	4.9	$468^{c\ C} \pm 27.0$	$288^{d\ C} \pm 34.2$	$354^{d\ C} \pm 150$
Batch-1E	1.4	$143^{c\ D} \pm 28.7$	$80^{d\ D} \pm 6.9$	$103^{d\ D} \pm 34.4$
Batch-1EG	0.3	$33.6^{c\ E} \pm 7.7$	$19.3^{d\ E} \pm 1.7$	$25.1^{cd\ D} \pm 10.5$
Fermented Grits High FB_1				
Batch-2	25	$2419^{c\ C} \pm 391$	$1439^{d\ C} \pm 212$	$1804^{d\ C} \pm 560$
Batch-2E	9.0	$923^{c\ D} \pm 38.2$	$563^{d\ D} \pm 57.3$	$698^{d\ D} \pm 201$
Batch-2EG	2.9	$298^{c\ E} \pm 50.2$	$179^{d\ E} \pm 22.5$	$222^{d\ D} \pm 63.7$

a) Dietary concentration (wet weight basis) as determined by chemical analysis of the grits [17]; control and extruded control diets, <0.1 ppm.

b) Calculated from body weight, food consumption and FB_1 concentration data, see text for formula; 1–8 wk results do not include animals necropsied after week 3. cdValues in ROWS not sharing lower-case superscript are significantly different, $p < 0.05$. CDEBatch-1 and Batch-2 series groups not sharing upper-case superscripts (in COLUMNS) are significantly different, $p < 0.05$.

3.4 Clinical laboratory studies

There were no exposure-related hematology or serum chemistry findings. Urinalysis revealed signs of kidney dysfunction in the animals fed Batch-2 or Batch-2E, which included traces of blood and increased numbers of (presumably sloughed kidney tubule) cells at wk 3 (data not shown). These findings were persistent but markedly less severe at wk 8.

3.5 Necropsy and organ weights

There were no treatment-related gross lesions. Absolute kidney weights (g) of animals fed Batch-1, Batch-1E or Batch-1EG were not different from those fed the uncooked control diet (Table 2). Absolute kidney weights of the Batch-1 group were however significantly lower than those of the extruded controls at wk 3. Kidney weights of the Batch-2 group were significantly reduced relative to both control groups and the Batch-2EG group at wks 3 and 8.

Relative kidney weights (% body weight) (Table 2) of the Batch-1 group were significantly lower than those of the two control, Batch-1E and Batch-1EG groups at week 3. Relative kidney weights of rats fed Batch-2 were decreased significantly at both wks 3 and 8 when compared with the controls. At wk 3, relative kidney weights of the Batch-2E and Batch-2EG groups were not different from those of the Batch-2 or the uncooked control groups but were less than those of the extruded control group. At wk 8, relative kidney weights of animals fed Batch-2 or Batch-2E were significantly lower than those fed the Batch-2EG or the two control diets.

3.6 Histopathology

Treatment-related effects were limited to the kidneys. Extrusion and, to a greater extent, extrusion with glucose reduced nephrotoxicity although protection was complete only in rats fed Batch-1EG. Specifically, the number of animals (n = 5/group) in the Batch-1 series exhibiting apoptotic proximal tubule cells or apoptosis together with other features consistent with FB_1 nephropathy at wk 3 were as follows: Batch-1 = 5; Batch-1E = 4; Batch-1EG = 1 and the two control groups ≤ 1. The group incidences of rats with lesions at wk 8 were as follows: Batch-1 = 4; Batch-1E = 2; Batch-1EG and the control groups = 0. Mean histopathology severity scores (Fig. 1) for the Batch-1 series decreased at

Table 2. Absolute and relative (% body weight) kidney weights of rats fed diets prepared from uncontaminated grits or two batches of *F. verticillioides* fermented grits that were uncooked, extruded (-E), or extruded with 10% glucose supplementation (-EG) for 3 or 8 wk

Diet	Week 3		Week 8	
	Absolute (g)	% Body weight	Absolute (g)	% Body weight
Controls				
Uncooked	$2.07^{ab\ /AB}$	$0.81^{a\ AB}$	$2.40^{a\ AB}$	0.66^{A}
	± 0.08	±0.04	±0.19	±0.03
Extruded	$2.25^{a\ A}$	$0.87^{a\ A}$	$2.51^{a\ B}$	0.69^{A}
	± 0.23	±0.05 (4)	±0.19	±0.02
Fermented Grits Low FB_1				
Batch-1	1.79^{b}	0.72^{b}	2.26^{a}	0.65
	± 0.09	±0.02	±0.08	±0.05
Batch-1E	2.06^{ab}	0.82^{a}	2.49^{a}	0.71
	±0.17	±0.05 (4)	±0.31	±0.04
Batch-1EG	2.11^{ab}	0.87^{a}	2.54^{a}	0.71
	±0.27	±0.08 (4)	±0.37	±0.09
Fermented Grits High FB_1				
Batch-2	1.73^{C}	0.70^{C}	2.03^{C}	0.58^{B}
	±0.08	±0.03 (4)	±0.11	±0.04
Batch-2E	1.83^{BC}	0.76^{BC}	2.17^{BC}	0.61^{B}
	±0.18	±0.06	±0.17	±0.04
Batch-2EG	1.86^{B}	0.75^{BC}	2.37^{AB}	0.69^{A}
	±0.18	±0.07	±0.16	±0.04

Note: values are the mean ± SD; $n = 5$ animals/group unless indicated otherwise in parentheses. abc Control and Batch-1 series groups not sharing lower-case superscripts are significantly different, $p < 0.05$; ABC Control and Batch-2 series groups not sharing upper-case superscripts are significantly different, $p < 0.05$. Fumonisin B_1 concentrations of the diets are given in Table 1.

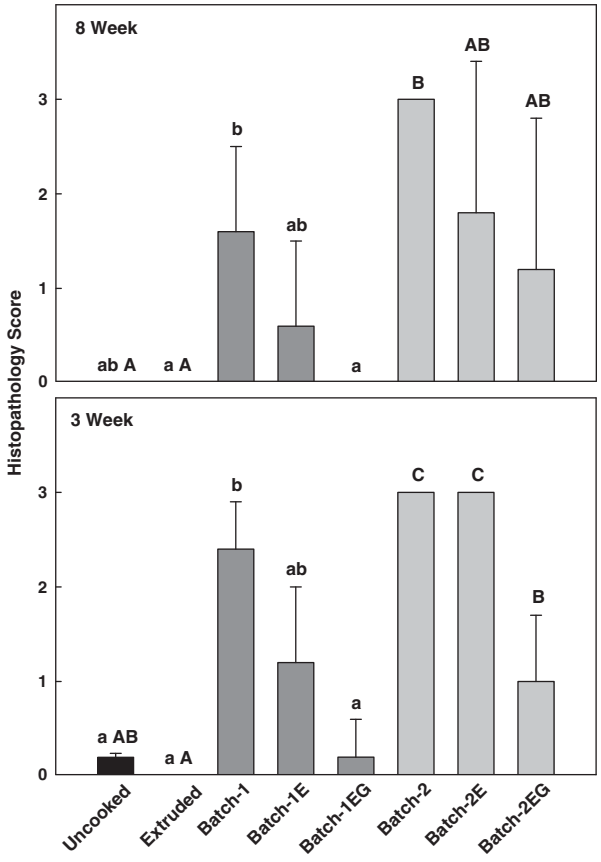

Figure 1. Histopathology scores for severity of renal lesions consistent with fumonisin exposure in rats fed diets containing (50% w/w) uncooked (uncooked) or extruded (extruded) uncontaminated maize grits, or two batches of uncooked (Batch-1, Batch-2) FB_1-contaminated grits, contaminated grits after extrusion (Batch-1E, Batch-2E) or contaminated grits after extrusion with glucose supplementation (Batch-1EG, Batch-2EG) for 3 or 8 wk. Values indicate the group mean score, $n = 5$; errors bars denote standard deviation. See text for explanation of scores. Control and Batch-1 series groups not sharing lower-case superscripts are significantly different and Control and Batch-2 series groups not sharing upper-case superscripts are significantly different, $p < 0.05$.

week 3 in the order Batch-1 > Batch-1E > Batch-1EG ≥ two control groups and at week 8 in the order Batch-1 > Batch-1E > Batch-1EG = two control groups. The number of animals ($n = 5$/group) in the Batch-2 series having nephropathy were as follows: Batch-2 and Batch-2E = 5; and Batch-2EG = 4 at wk 3 and Batch-2 = 5; Batch-2E = 3; and Batch-2EG = 2 at week 6. The severity of lesions in the Batch-2 series groups was greater than in their Batch-1 counterparts and the controls. Scores for the Batch-2EG group were none the less lower than those of the Batch-2 and Batch-2E groups at wk 3 and wk 8 ($p < 0.05$). Scores for the latter groups were equal at wk 3 while at wk 8, the mean severity score for the Batch-2E group was between those of the Batch-2 and Batch-2EG groups.

When average daily FB_1 intake during weeks 1–3 or 4–8 and corresponding pathology scores of the groups were considered (Fig. 2), it was evident that the scores were dose dependent and, for all groups, both daily intakes and histopathology scores were lower at wk 8.

3.7 Sphingoid bases

Sphingolipid metabolism was disrupted in the kidneys of all animals fed uncooked Batch-1 and Batch-2 grits as indicated by significant increases in the concentrations of Sa, So, their 1-P metabolites (Table 3) and total Sa (Sa plus Sa 1-P) (Fig. 3). Lesser, but statistically significant increases also were found in the Batch-1E and Batch-2E groups. Concen-

trations of individual sphingoid bases and total Sa of the Batch-1EG group were not elevated. Sphingoid base concentrations of the Batch-2EG group tended to be lower than in the Batch-2E and Batch-2 groups although, due to relatively large standard deviations, statistical differences were not always demonstrated. Sphingoid base concentrations of the Batch-2EG group were in contrast up to 88-fold higher (Sa, wk 8) than in the control groups and 28-(Sa, week 3) to 69-fold (total Sa, wk 8) higher than in the Batch-1EG group.

Total Sa concentrations in kidneys increased in a (FB_1 daily intake) dose-dependent manner (Fig. 4) but the magnitude of the changes was diminished at week 8. A high degree of correlation (Fig. 5) was found between total Sa and mean histopathology scores at both times. Furthermore, higher pathology scores were associated with lower total Sa concentrations at week 8 than at wk 3.

Sa, Sa 1-P, So and So 1-P in the livers of rats fed Batch-2 and Batch-2E were not elevated and deoxy Sa was not found.

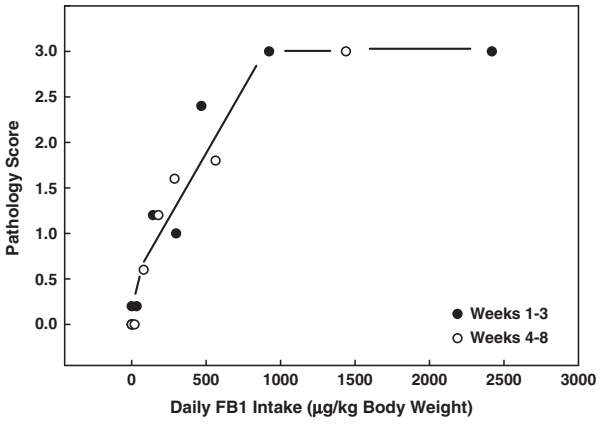

Figure 2. Group mean histopathology scores (from Fig. 1) plotted against group mean daily FB_1 intakes as determined from weekly body weight, food consumption and diet FB_1 concentration data.

4 Discussion

The efficacy of extrusion cooking, with and without glucose supplementation, using a twin-screw extrusion configuration to reduce FB_1 concentrations and in vivo toxicity of contaminated grits was tested. The experiment complemented an earlier but less rigorous 3-wk rat feeding bioassay of fermented grits containing ≥ 33 ppm FB_1 after single-screw extrusion with or without glucose supplementation [13, 16]. As before, diet formulations were based solely on FB_1 concentrations. The lower amounts of FB_2, FB_3, HFB_1 and NDF-FB_1 present in the grits [17] were not considered because these fumonisin species were not toxic when evaluated by various rodent bioassays [21–24]. Furthermore, some evidence suggests FB_2 and FB_3 do not accumulate in

Table 3. Kidney sphinganine (Sa), Sa 1-phosphate (Sa 1-P), sphingosine (So) and So 1-phosphate (So 1-P) concentrations (nmol/g) of rats fed diets prepared from uncontaminated grits or two batches of *F. verticillioides* fermented grits that were uncooked, extruded (-E), or extruded with 10% glucose supplementation (-EG) for 3 or 8 wk

Diet	Week 3[a)]				Week 8			
	Sa	Sa 1-P	So	So 1-P	Sa	Sa 1-P	So	So 1-P
Controls								
Uncooked	$4.49^{bc\ B} \pm 3.51$	$0.96^{b\ B} \pm 0.81$	$8.00^{b\ B} \pm 2.77$	$0.28^{b\ B} \pm 0.16$	$1.60^{b\ B} \pm 0.81$	$<0.30^{b\ B} \pm 0$	$4.12^{b\ B} \pm 1.96$	$0.65^{b\ B} \pm 0.20$
Extruded	$2.56^{b\ B} \pm 0.73$	$0.74^{b\ B} \pm 0.97$	$7.93^{b\ B} \pm 1.89$	$0.25^{b\ B} \pm 0.20$	$1.50^{b\ B} \pm 0.22$	$<0.30^{b\ B} \pm 0$	$4.14^{b\ B} \pm 0.78$	$0.58^{b\ B} \pm 0.16$
Fermented Grits Low FB_1								
Batch-1	$316^{d} \pm 99.1$	$306^{d} \pm 143$	$33.9^{c} \pm 7.84$	$18.7^{d} \pm 7.72$	$120^{d} \pm 38.2$	$40.7^{d} \pm 24.0$	$17.4^{c} \pm 4.09$	$9.35^{d} \pm 4.84$
Batch-1E	$181^{d} \pm 96.6$	$89.3^{c} \pm 71.1$	$29.5^{c} \pm 6.01$	$6.98^{c} \pm 4.73$	$67.4^{c} \pm 22.3$	$6.30^{c} \pm 6.57$	$20.0^{c} \pm 7.86$	$2.71^{c} \pm 1.56$
Batch-1EG	$6.32^{c} \pm 4.14$	$1.03^{b} \pm 1.28$	$8.81^{b} \pm 0.55$	$0.37^{b} \pm 0.34$	$1.92^{b} \pm 0.66$	$<0.30^{b} \pm 0$	$4.60^{b} \pm 0.76$	$0.40^{b} \pm 0.09$
Fermented Grits High FB_1								
Batch-2	$443^{D} \pm 74.7$	$581^{C} \pm 141$	$43.3^{D} \pm 5.76$	$23.7^{D} \pm 5.65$	$265^{C} \pm 144$	$115^{D} \pm 23.1$	$45.6^{D} \pm 20.6$	$17.7^{D} \pm 5.28$
Batch-2E	$275^{C} \pm 29.5$	$402^{C} \pm 95.0$	$28.7^{C} \pm 4.51$	$19.2^{D} \pm 4.52$	$175^{C} \pm 52.6$	$96.5^{D} \pm 35.1$	$26.3^{C} \pm 6.79$	$18.1^{D} \pm 8.13$
Batch-2EG	$177^{C} \pm 85.0$	$49.0^{B} \pm 76.4$	$24.4^{C} \pm 7.51$	$4.71^{C} \pm 5.23$	$132^{C} \pm 43.0$	$17.2^{C} \pm 19.6$	$23.2^{C} \pm 8.67$	$4.38^{C} \pm 2.03$

a) Values are the mean ($\pm$SD) for $n = 5$ animals. bcd Control and Batch-1 series groups not sharing superscript are significantly different, $p < 0.05$. BCD Control and Batch-2 series groups not sharing superscript are significantly different, $p < 0.05$.

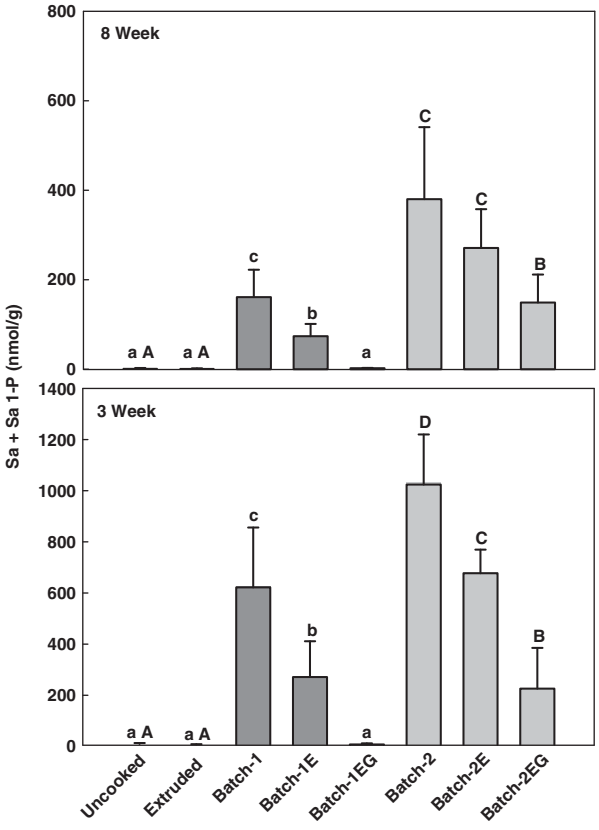

Figure 3. Total concentrations of sphinganine (Sa) plus sphinganine 1-phosphate (Sa 1-P) in the kidneys of rats fed diets containing (50% w/w) uncooked (uncooked) or extruded (extruded) uncontaminated maize grits, or two batches of uncooked (Batch-1, Batch-2) FB_1-contaminated grits, contaminated grits after extrusion (Batch-1E, Batch-2E) or contaminated grits after extrusion with glucose supplementation (Batch-1EG, Batch-2EG) for 3 or 8 wk. Values indicate the group mean score, $n = 5$; errors bars denote standard deviation. See text for explanation of scores. Control and Batch-1 series groups not sharing lower-case superscripts are significantly different and Control and Batch-2 series groups not sharing upper-case superscripts are significantly different, $p < 0.05$.

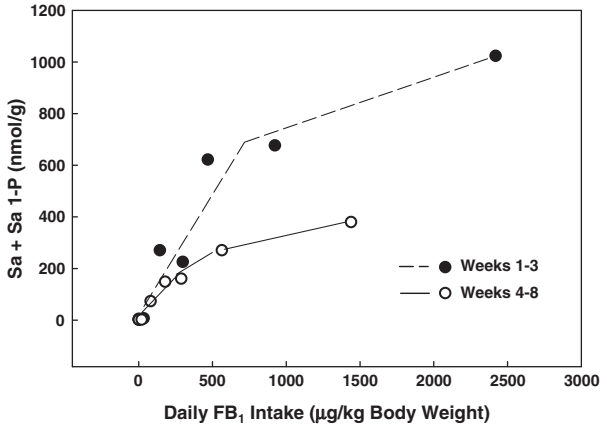

Figure 4. Group mean total kidney sphinganine concentrations (Sa + Sa 1-P; from Fig. 3) plotted against group mean daily FB_1 intakes as determined from weekly body weight, food consumption and diet FB_1 concentration data.

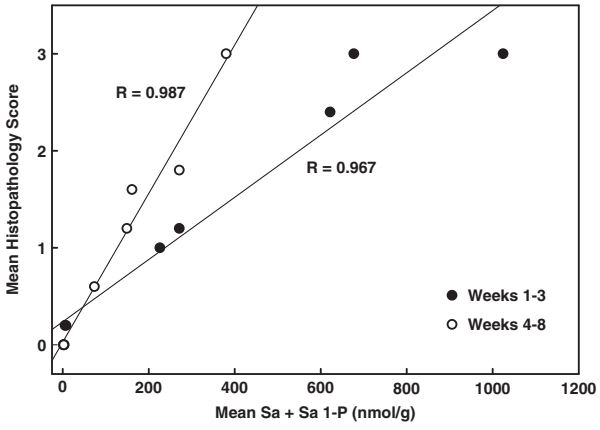

Figure 5. Group mean histopathology scores for kidney lesions consistent with fumonisin exposure (from Fig. 1) plotted against group mean total kidney sphinganine concentrations (Sa + Sa 1-P; from Fig. 3). R = linear correlation coefficient.

the tissues of rats exposed to these mycotoxins via the diet [25]. The bioavailability and toxic potential of matrix bound FB_1 or unknown FB_1 reaction or breakdown products in the cooked grits are unknown. However, because the in vivo effects were tightly correlated with the diets' measurable (not bound to matrix) FB_1 concentrations, it is unlikely that unknown fumonisin reaction products or matrix bound forms remaining in the extruded products made a significant contribution to toxicity. This issue requires further study.

FB_1 concentrations of the diets made with uncooked Batch-1 or Batch-2 grits were not high enough to cause liver lesions, influence clinical chemical variables or elevate liver sphingoid base concentrations. The Batch-1 and Batch-2 diets were however clearly nephrotoxic and effects on relative organ weight, histopathology and sphingoid base concentrations were consistent with well-defined apoptotic and biochemical outcomes following exposure to FB_1 [2, 4–7] or FB_1-contaminated culture materials [16, 25, 26]. That kidney weights and histopathology scores of the Batch-1EG and control groups did not differ constitutes clear evidence that extrusion with glucose prevented toxicity of the less contaminated Batch-1 grits. Extruding Batch-1 grits without glucose was only partially protective, as was extrusion or extrusion with glucose supplementation of the more highly contaminated Batch-2 grits.

Sa increases in target tissues as a consequence of ceramide synthase inhibition by FB_1, the mechanistic trigger causing sphingolipid metabolism disruption [25, 27]. The increases are dose-dependent and occur at lower doses or

earlier (time-course) than the appearance of microscopic lesions [2, 4, 26]. Consequently, elevated sphingoid base concentrations are a more sensitive indicator of low-dose FB_1 exposure than tissue pathology [24–26]. Sphingolipid metabolism in the kidneys of rats fed the uncooked Batch-1 and Batch-2 grits was clearly disrupted. Extrusion and extrusion with glucose reduced the sphingoid base elevations in the Batch-1 series of groups. Concentration decreased in the order: Batch-1 > Batch-1E > Batch-1EG = controls. Therefore, extrusion with glucose reduced the FB_1 concentration of Batch-1 grits to levels that, when mixed 1:1 with the basal diet, were not only insufficient to induce nephropathy but also too low to disrupt sphingolipid metabolism. Kidney sphingoid base concentrations in the Batch-2 series of groups exhibited a similar pattern of reduction by extrusion and greater reduction by extrusion with glucose. However, sphingoid base concentrations of the Batch-2 series of groups were higher than their Batch-1 counterparts and, with the exception of Sa 1-P of the Batch-2E group at wk 3, were consistently elevated ($p < 0.05$) compared with the control groups.

Altogether, histopathology and sphingoid base findings established that extrusion with glucose supplementation using a twin-screw apparatus was an effective processing method for reducing FB_1 in contaminated grits and consequently for lowering daily intakes and reducing toxicity. The consequences of chronic exposure to maize-based foods processed by extrusion with glucose is unknown but should be investigated and consider the potential consequences arising from the high sugar content of the cooked product. In this regard, clinical laboratory results and microscopic examination of liver and other tissues in this study did not reveal evidence of any ''non-fumonisin'' effects.

The findings have also provided additional information on the relationship between sphingolipid metabolism disruption and toxicity. Both microscopic pathology scores and total Sa were tightly correlated with daily FB_1 intakes. Therefore, the lower scores and lesser sphingoid base elevations in the animals at 8 wk were likely a consequence of the lower doses (i.e. lower daily intakes) incurred after week 3 because of the animals' growth, rather than a consequence of physiological adaptation. Second, while the severity of kidney pathology was correlated with total Sa concentrations at both time periods, it is interesting that the scores indicative of the most extensive tissue injury were associated with lower total Sa concentrations at wk 8 than at wk 3. Determining the reason for this was beyond the scope of this experiment but should be considered in the future. Progressive depletion of complex sphingolipids [8, 24, 27], compromised vitamin or nutrient utilization [8, 28], further disruptions of overall lipid metabolism and signaling, compositional changes in cell membranes [29], induction of nitric oxide [30, 31], or up- or down-regulation of cytokines [29, 32, 33] are among the factors that could be involved. It is noteworthy that deoxy Sa was not found in the livers or kidneys. Deoxy Sa, which is produced when alanine is substituted for serine during de novo sphingolipid biosynthesis [20], accumulated in the livers of mice following exposure to FB_1 in other studies [20, 24]. Its absence in rat liver suggests that sphingolipid metabolism in rats and mice differ in some respects and that these differences might influence their target organ-specific responses to fumonisin exposure.

In conclusion, extrusion with 10% w/w glucose supplementation using a twin-screw configuration reduced FB_1 concentrations in maize grits more effectively than extrusion alone. Extrusion with glucose of grits containing ca. 10 ppm FB_1 significantly reduced daily FB_1 intakes and prevented the development of kidney lesions and disruption of sphingolipid metabolism when the processed grits were fed, 50% w/w in the formulated diet, to male rats. No evidence suggesting the formation of unknown toxic FB_1 reaction products was obtained. Therefore, extrusion with glucose supplementation is a potentially useful process to reduce the toxicity of fumonisin-contaminated maize.

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