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Effect of Fermentation on Sweetpotato (*Ipomoea batatas*) Toxicity in Mice

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Unfortunate bovine fatalities occurring after ingestion of mold-damaged sweetpotatoes preclude the use of the culled tubers in livestock feed. In cattle, mold-damaged sweetpotatoes induce an acute respiratory distress syndrome resulting in asphyxiation. Because of this potential toxicity and the general abundance of culled sweetpotatoes, the detoxification efficacy of ensiling was explored since it is an easy and economically viable technique often applied to preserve livestock feed. Sweetpotato slices with or without mold damage were stored either frozen (to represent unfermented samples) or fermented for 6 weeks at room temperature. Following fermentation, organic extracts were generated for administration to mice. Thirty hours following administration of the extracts, mice were evaluated for gross and microscopic lesions affecting the lungs, liver, and kidneys. Fermentation of 6 weeks duration was observed to inadequately eliminate the lung, liver, and kidney toxicity caused by mold-damaged sweetpotatoes. In fact, fermentation exacerbated the hepatotoxicity of mold-damaged sweetpotatoes. This is also the first demonstration that sweetpotato regions lacking visible mold damage can induce lung and kidney injury, which, however, is preventable by fermentation.

KEYWORDS: 4-Ipomeanol; furanoterpenoid; *Fusarium sp.*; kidney; liver; lung; mouse; sweetpotato

INTRODUCTION

Although the sweetpotato is a rich source of carbohydrates and other nutrients, the possibility of acute respiratory distress and cattle fatalities has prevented the use of cull sweetpotatoes in the bovine diet (*1*). Toxic phytoalexins (stress metabolites) that are antimicrobial and antihelminthic are produced by sweetpotatoes in response to stresses such as mechanical trauma, cold temperature, chemicals, or infections by microbes and parasites (*2*). Two of the phytoalexins, ipomeamarone and hydroxyipomeamarone, are hepatotoxic to laboratory animals; however, liver injury is not a described feature of sweetpotato toxicity in cattle (*3*). Rather, infecting fungi (*Fusarium soloni*, *Fusarium oxysporum*, or *Ceratocystis fimbriata*) metabolize these phytoalexins to the furanoterpenoids 4-ipomeanol, 1-ipomeanol, 1,4-ipomeadiol, and ipomeanine, which are responsible for pulmonary injury (*3*, *4*).

In the United States, the major pulmonary toxin appears to be 4-ipomeanol (*3*, *4*). After ingestion, absorbed furanoterpenoids circulate to the lung where cytochrome P-450 monooxygenases metabolize the parent compound to reactive intermediates culminating in rapid pulmonary injury (*5*). An ultrastructural study has previously demonstrated 4-ipomeanol to induce in calves, within 12−96 h, necrosis and sloughing of pulmonary epithelial cells lining alveoli and terminal bronchioles (*6*). Within 1 day of ingestion, affected cattle develop pulmonary edema and interstitial pneumonia, which can result in death from asphyxiation (*7*). Historically, the disease has been referred to as "pulmonary adenomatosis" and later as "atypical interstitial pneumonia" after a 1969 Tifton, Georgia outbreak where 69 of 275 cattle died 1−3 days after ingestion of sweetpotatoes (*8*). Despite the well-described toxicity of mold-damaged tubers, significant herd losses are still occasionally reported today, particularly when sweetpotatoes are offered free choice in piles within pasture.

It is unfortunate that the risk of pulmonary injury prevents the sweetpotato from being used as cattle feed, since there is an abundance of sweetpotato waste in certain regions of the United States. Significant culling of sweetpotatoes occurs due to inferior size, weight, bruising, or mold damage. In fact, the state of North Carolina culls nearly 1.8 million bushels per year, which at $4.50 per hundred weight amounts to about a $4.2 million dollar loss (*9*). Because potential pneumotoxicity has

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excluded the sweetpotato's use as a dietary source to cattle, we have chosen to investigate fermentation (ensiling) as a potential processing method to eliminate toxicity. Ensiling offers an inexpensive processing method that is readily applied to reduce microbial contamination of feed while retaining nutritional value. Because the toxicity of the pulmonary toxin 4-ipomeanol has been well-described in the mouse, a mouse bioassay was employed to evaluate the efficacy of fermentation to ameliorate the toxicity of "black rot"-affected sweetpotatoes (*10*, *11*).

MATERIAL AND METHODS

Animals. Four week old male CD-1 mice (Charles River Laboratories, Portage, MI) were randomized and housed at five animals per polystyrene cage on Anderson Bedocobs (Granville Milling, Creedmoore, NC). Mice were allowed food and water ad libitum and were kept on a 12 h light/12 h dark cycle in a temperature-controlled animal facility. Experiments were conducted according to a protocol approved by the university Institutional Animal Care and Use Committee.

Sample Preparation. Sweetpotatoes with deteriorated (mold-damaged) and nondeteriorated surfaces were obtained from a North Carolina commercial dealer (Farm Pack, Inc., Spring Hope, NC). Deteriorated regions were defined as a dry, black surface extending into the peripheral cortex, typical of black rot, whereas nondeteriorated regions lacked such lesions (*1*, *12*). Deteriorated regions were sliced from the tubers and kept as deteriorated sweetpotato samples for subsequent processing. Following removal of the deteriorated regions, a 1 cm buffer zone was removed, and then, the next 5 cm was used to represent nondeteriorated sweetpotato.

Trimmed portions representing deteriorated and nondeteriorated sweetpotatoes were minced to 1 cm diameter pieces and stored either frozen (to represent unfermented samples) or sealed in airtight glass jars at room temperature (approximately 25 °C) for a 6 week fermentation. After 6 weeks, the sweetpotato samples were ground in a blender, and the furanoterpenoid-containing organic fraction was extracted twice with methanol. This extract was filtered, and the methanol solvent was removed in vacuo. The extract was subsequently dissolved in chloroform, filtered through glass wool, and added to autoclaved corn oil. The chloroform was removed in vacuo.

Formulation and Dosing for Mouse Bioassay. Thin-layer chromatography (TLC) with detection by Ehrlich's reagent was used to identify furan type compounds as previously described (*4*). Synthetic 4-ipomeanol (a gift from Michael Boyd at the National Cancer Institute) was used as a standard to quantify 4-ipomeanol in each of the extracts. Prior to fermentation, deteriorated sweetpotato regions were found to contain 11.1 mg of 4-ipomeanol per gram of sweetpotato. Preliminary experiments in vivo (data not shown) demonstrated intraperitoneal injection of 60 mg of synthetic 4-ipomeanol in corn oil to produce discernible lung and kidney injury at 30 h in CD-1 male mice. Because 5.4 g of sliced deteriorated sweetpotatoes contained 60 mg of 4-ipomeanol, all injections were formulated to contain extract from this amount of sweetpotato.

Mice were intraperitoneally injected with 0.3 mL of extract (equivalent to 5.4 g of sweetpotato) per 30 g of body weight. There were five mice per treatment group. Each treatment group received injections of extracts generated from sweetpotatoes that were deteriorated, deteriorated and fermented, nondeteriorated, or nondeteriorated and fermented. Control mice were injected with either 60 mg of synthetic 4-ipomeanol or vehicle (corn oil) only. The animals were periodically observed for dyspnea or lethargy during the postdosing interval. Mice were euthanized at 30 h postinjection with the exception of two animals prematurely euthanized due to lethargy and dyspnea. For euthanasia, mice were anesthetized with an intraperitoneal injection of 7.5 mg of 2,2,2-tribromoethanol (Avertin, Aldrich Chemical Co., Inc., Milwaukee, WI) and then exsanguinated via the abdominal aorta (*13*).

Histopathologic Scoring of Toxicity. A necropsy was performed on each mouse. Lungs, liver, and kidneys were weighed. Lungs were inflated and fixed with buffered formalin under 20 cm of pressure. Fixed lungs, liver, and kidneys were stored 3 days in buffed formalin, after which they were embedded into paraffin. Five micrometer thickness sections were mounted on glass slides and stained with hematoxylin and eosin.

Histologic grading of the lung parenchyma was limited to the bronchioles and scored as grade 0, 1, 2, 3, or 4. Grade 0 scores lacked bronchiolar injury. Grade 1 lesions had less than 30% of the bronchiolar epithelium affected by degeneration with rare necrosis. Grade 2 lesions had greater than 30% of the bronchiolar epithelium affected by degeneration with rare necrosis. Grade 3 lesions had greater than 30% of the bronchiolar epithelium affected by degeneration with significant necrosis. Grade 4 lesions had greater than 30% of the bronchiolar epithelium affected by necrosis and bronchioles denuded or lined by flattened epithelium.

Liver and kidney were given a histopathologic score of grade 0, 1, 2, 3, 4, or 5 adapted from criteria used in hepatoxicity and nephrotoxicity studies (*14*). Grade 0 scores lacked parenchymal injury. Grade 1 lesions had single to few degenerate or necrotic parenchymal cells. Grade 2 lesions had 5−20% of the parenchyma with degeneration or necrosis. Grade 3 lesions had 20−40% of parenchyma affected by degeneration or necrosis. Grade 4 lesions had 40−50% of the parenchyma affected by degeneration or necrosis. Grade 5 lesions had more than 50% of the parenchyma affected by degeneration or necrosis.

Statistical Analysis. For statistical analysis, all groups (vehicle, nondeteriorated, fermented nondeteriorated, and deteriorated sweetpotato treatments) had $n = 5$, except where animals died prematurely (4-ipomeanol and fermented deteriorated sweetpotato treatments had $n = 4$). Data were expressed as the mean ± standard error. Differences between groups were evaluated by analysis of variance using a Newman−Keuls post-hoc procedure to correct for multiple comparisons (GraphPad Prism, v. 3.03). Statistical significance was accepted at $p < 0.05$.

RESULTS

Detection of Furanoterpenoids in Fermented Sweetpotatoes. Using TLC analysis, the ensiled or frozen nondeteriorated sweetpotato lacked Ehrlich-positive spots (less than 25 ppm), whereas the characteristic mold-damaged blemishes of the sweetpotatoes contained high levels (250−1000 ppm) of 4-ipomeanol in the fresh material. Once the deteriorated sweetpotato material was fermented or even just frozen (not fermented) for 6 weeks, the level of 4-ipomeanol as determined by TLC was low or undetectable. However, only 4-ipomeanol was measured, as that was the only standard available. There were additional Ehrlich-positive spots that did not migrate to the position of 4-ipomeanol. These findings suggested the presence of additional potentially toxic compounds and raised the possibility that 4-ipomeanol may have been complexed or chemically modified so that it did not show up in the assay.

Gross Pathology Induced by Fermented Sweetpotatoes. One mouse receiving synthetic 4-ipomeanol and one mouse receiving fermented deteriorated sweetpotato extract died prematurely 12 h postdosing, whereas the remaining mice in these and the other groups had no observable clinical abnormalities. At necropsy, the mouse treated with synthetic 4-ipomeanol that died prematurely had clear, watery pleural effusion (approximately 0.2 mL) and pulmonary edema evidenced by froth within the trachea. Lung weight as a percent of body weight was only increased in mice treated with synthetic 4-ipomeanol (0.940 ± 0.04%, $p < 0.05$) as compared to vehicle (0.722 ± 0.026%). Significant increases in the kidney weight as a percent of body weight were observed in mice treated with synthetic 4-ipomeanol (2.03 ± 0.11%, $p < 0.05$), nondeteriorated sweetpotato (1.81 ± 0.033%, $p < 0.05$), deteriorated sweetpotato (2.22 ± 0.13%, $p < 0.05$), and fermented, deteriorated sweetpotato (2.00 ± 0.049%, $p < 0.05$) as compared to vehicle (1.48 ± 0.061%). Heavier kidneys were often pale. Mice treated with

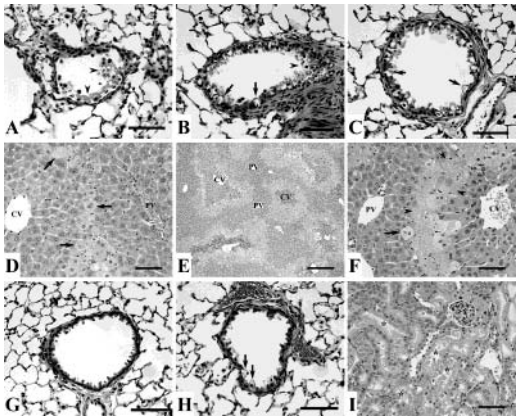

Figure 1. Representative lung, liver, and kidney histology after administration of vehicle (corn oil), 4-ipomeanol, or sweetpotato extract as described in the Materials and Methods. (**A**) Lung bronchiole epithelium necrosis (arrowheads), loss, and attenuation by 4-ipomeanol (bar = 60 μm). (**B**) Lung bronchiole epithelium necrosis (arrowhead) and degeneration (arrows) by deteriorated sweetpotato extract (bar = 60 μm). (**C**) Lung bronchiole epithelium degeneration (arrows) by fermented, deteriorated sweetpotato (bar = 60 μm). (**D**) Moderate liver midzonal hepatocellular degeneration (arrows) and rare necrosis by deteriorated sweetpotato (CV, central vein; PV, portal vein; bar, 60 μm). (**E**) Marked liver midzonal hepatocellular degeneration and necrosis by fermented, deteriorated sweetpotato (CV, central vein; PV, portal vein; bar, 240 μm). (**F**) Marked liver midzonal hepatocellular degeneration (arrow) and necrosis (arrowheads) by fermented, deteriorated sweetpotato (CV, central vein; PV, portal vein; bar, 60 μm). (**G**) Normal lung bronchiole epithelium after corn oil (bar = 60 μm). (**H**) Occasional lung bronchiole epithelium degeneration (arrows) by nondeteriorated sweetpotato (bar = 60 μm). (**I**) Kidney cortex tubular epithelial necrosis (asterisks) by nondeteriorated sweetpotato (bar = 60 μm).

vehicle or fermented, nondeteriorated sweetpotato had no discernible gross lesions affecting the liver, kidneys, or lungs.

Microscopic Pathology Induced by Fermented Sweetpotatoes. Vehicle (corn oil)-treated mice had no discernible microscopic lesions affecting the liver, kidneys, or lungs. Microscopically, the mice that received 4-ipomeanol had severe lung and kidney lesions consistent with that previously described for 4-ipomeanol in male mice (*10*, *11*). Pulmonary bronchioles contained numerous sloughed, necrotic cells with condensed, rounded, hypereosinophilic cytoplasm and pyknotic to karyorrhectic nuclei (**Figure 1A**). Much of the bronchiolar surface was denuded of cuboidal epithelium, which was replaced by flattened epithelium. The remaining nonciliated bronchiolar epithelium (Clara cells) had occasional swollen, vacuolated apical cytoplasm (degeneration). Reports of 4-ipomeanol in mice describe edema causing intra-alveolar septal thickening and fibrogranular eosinophilic alveolar material (*11*); however, such findings were not discerned in our microscopic evaluation. Synthetic 4-ipomeanol also produced severe renal tubular necrosis consisting of loss of epithelium replaced by pink, granular material and sloughed necrotic epithelial cells with karyolytic to karyorrhectic nuclei and condensed eosinophilic cytoplasm. Often, there were abundant intraluminal pink, homogeneous proteinaceous casts. The prematurely dead mouse lacked tubular necrosis. This finding is consistent with the literature since mice dead within 6−24 h from sweetpotato furanoterpenoid-induced pulmonary edema typically lack renal necrosis, which tends to only develop with prolonged survival at sublethal doses (*4*). Liver lesions were not discerned in mice receiving synthetic 4-ipomeanol, which is also consistent with the findings of others.

Extracts generated from deteriorated sweetpotatoes produced significant lung and kidney injury similar to that observed with synthetic 4-ipomeanol; however, deteriorated sweetpotatoes also induced liver injury not incurred by 4-ipomeanol exposure. Deteriorated sweetpotatoes caused pulmonary bronchiolar necrosis that was moderate in severity when compared to the synthetic 4-ipomeanol-induced lesions. In addition, deteriorated sweetpotatoes induced a greater abundance of vacuolated bronchiolar epithelial cells, with less concurrent necrosis (**Figure 1B**). Extracts of deteriorated sweetpotatoes produced renal tubular necrosis of a severity similar to that observed with synthetic 4-ipomeanol. The liver had moderate midzonal hepatocellular ballooning degeneration and necrosis (**Figure 1D**). These mice also had multifocal subcapsular hepatocellular necrosis that was interpreted to be an artifact of experimental method, since the surface hepatocytes could have been the first to encounter the intraperitoneally administered metabolic toxins. The lesions induced by fermented, deteriorated sweetpotatoes were quite similar to those induced by unfermented, deteriorated sweetpotatoes; however, there were changes in the severity of the lesions. Although pulmonary bronchioles had abundant vacuolar degeneration, there was rarely necrosis, indicating fermentation mildly attenuated pulmonary toxicity (**Figure 1C**). Whereas, on the contrary, fermentation increased the hepatotoxicity of deteriorated sweetpotato since the midzonal injury was more severe, having bridging necrosis and occasional extension into centrolobular areas (**Figure 1E**,**F**). This group had microscopic kidney lesions varying from mild to severe. The unfermented, nondeteriorated sweetpotato extracts produced lung and kidney lesions of severity less than that of deteriorated sweetpotatoes or synthetic 4-ipomeanol (**Figure 1H**,**I**). Liver lesions were not discerned in mice exposed to unfermented, nondeteriorated sweetpotato extract. No significant lung, liver, or kidney lesions were discerned in any of the mice receiving the fermented, nondeteriorated sweetpotato extract (**Figure 1G**). **Figure 2** summarizes the histopathology scores for each of the treatment groups.

DISCUSSION

This study indicated that sweetpotatoes lacking detectable 4-ipomeanol (less than 25 ppm) can induce significant pulmonary and renal injury, presumably due to additional furanoterpenoids. In addition, mice injected with nondeteriorated sweetpotato extracts developed lung and kidney lesions similar to those previously described for the furanoterpenoids 4-ipomeanol, 1-ipomeanol, 1,4-ipomeadiol, and ipomeanine (*4*). These findings suggested that related toxins can contaminate unblemished regions of sweetpotatoes, which is interesting because extracts of nondeteriorated sweetpotato lacked Ehrlich-positive spots. It is possible that other non-Ehrlich reacting toxins were present or that contaminating furanoterpenoids were below a level of detection. Ipomeamarone, 4-ipomeanol, and ipomeanine are already described to occasionally contaminate tubers lacking visible mold damage, but corroborating pathologic data have not previously been reported (*12*, *15*). Because this population of nondeteriorated sweetpotatoes was acquired by trimming the deteriorated regions away from nondeteriorated regions, these findings confirm that toxins do contaminate portions of sweetpotato distinct from black rot-affected areas. Additionally, the lack of liver injury by nondeteriorated sweetpotato suggests that the composition of toxins differed from that contained within deteriorated sweetpotatoes.

Only deteriorated sweetpotatoes produced significant hepatotoxicity localized to midzonal regions of the hepatic lobules.

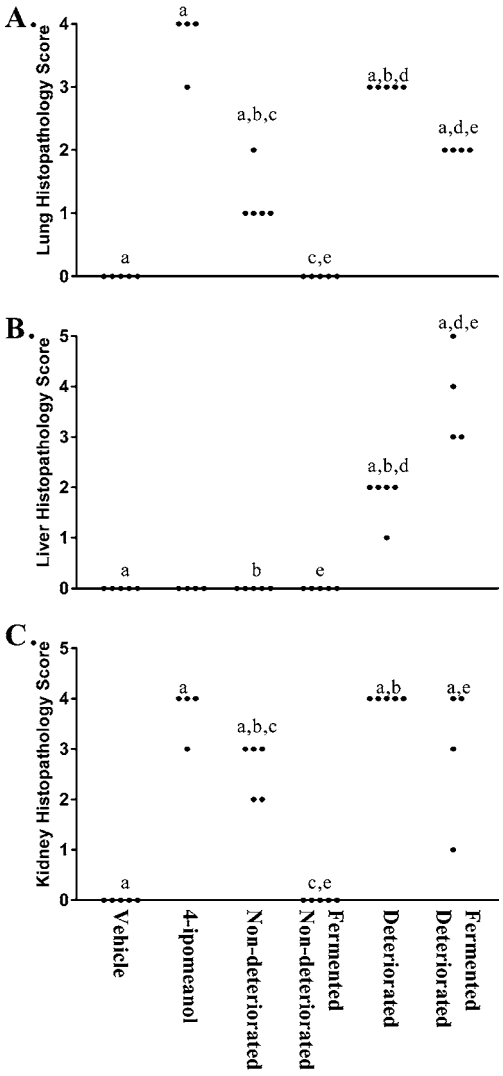

Figure 2. Histopathology scores of (**A**) lung, (**B**) liver, and (**C**) kidney injury after administration of vehicle (corn oil), 4-ipomeanol, or sweetpotato extract as described in the Materials and Methods. Data are shown as the mean histologic grade ± the standard error. The letter a denotes $p < 0.05$ for vehicle vs treatment. The letter b denotes $p < 0.05$ for nondeteriorated vs deteriorated. The letter c denotes $p < 0.05$ for nondeteriorated vs fermented, nondeteriorated. The letter d denotes $p < 0.05$ for deteriorated vs fermented, deteriorated. The letter e denotes $p < 0.05$ for fermented, nondeteriorated vs fermented, deteriorated.

Of the zonal patterns of hepatocellular injury, midzonal injury is the least common reported. Rarely, mold-damaged foodstuffs induce this pattern of hepatic injury. Aflatoxin can cause midzonal necrosis in pigs and rabbits (*16*), whereas moldy hay may cause the lesion in horses (*17*). Hepatic toxicity targeting midzonal regions is typical of furan type compounds in mice (*18*). The observed liver lesions are consistent with the presence of additional furans distinct from 4-ipomeanol, such as ipomeamarone or hydroxyipomeamarone. In fact, extracts of the Australian Ngaio tree (*Myoporum laetum*) containing the furanoterpenoid ngaione, an enantiomer of ipomeamarone, cause midzonal necrosis in mice (*19*). Although fermentation was successful in eliminating the mild pulmonary and renal toxicity of nondeteriorated sweetpotato, fermentation did not significantly ameliorate the more severe lung, liver, and kidney toxicity associated with black rot-damaged regions of sweetpotato. In fact, fermentation exacerbated the hepatoxicity of deteriorated sweetpotatoes.

In conclusion, this study demonstrated that while fermentation did slightly reduce the severity of lung toxicity, it does not eliminate the toxicity of mold-damaged sweetpotatoes. Additionally, toxins can be present in regions of sweetpotato visibly unaffected by black rot blemishes. Because this study employed only a single fermentation protocol of 1 month of duration, other biological decontamination practices cannot be totally discounted. Normal cooking (baking and microwave cooking) has already been shown to significantly reduce levels of ipomeamarone and 4-ipomeanol (*20*). More favorable detoxification conditions could perhaps be offered by a fermentation method of longer duration or cooking prior to fermentation. Feeding sweetpotatoes popped at approximately 80 °C has already demonstrated to improve pig growth rates, while ensiling for 2 months has done the same for laboratory rats because of improved carbohydrate digestibility (*21*). While fermentation did not completely detoxify the deteriorated sweetpotatoes, it does have benefits that would make it a recommendable practice for managing this potential feed resource. First, fermentation did partially detoxify the deteriorated sweetpotatoes and completely detoxified the nondeteriorated sweetpotatoes; second, this practice preserves the nutritional value and prevents additional toxins from developing as they do in outside piles; and third, storing as a silage allows the use of sweetpotatoes at a moderate level in a mixed ration diluting any toxins that are there and limiting exposure of each animal. Further research is warranted to evaluate simple detoxification methods that would allow the use of culled sweetpotatoes in livestock feed, thereby creating economic and environmental benefits for the states in which they are grown.

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NOTE ADDED AFTER ASAP

The species identification in the title was misspelled in the original ASAP posting of December 5, 2003. This was corrected December 9, 2003.

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