NATURAL AND SYNTHETIC BINDERS TO REDUCE FUMONISIN TOXICITY IN CORN: ACQUISITION AND TESTING OF COMMERCIAL PRODUCTS

<u>Annual Report to Texas Corn Producers Board</u>.

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<u>Abstract:</u>
Fumonisins are toxins (mycotoxins) produced by fungi (*Fusarium*) that are frequently identified in corn (maize). An enzyme-linked immunosorbent assay (ELISA) method was used to measure fumonisin concentrations in adsorption isotherms from water and from aqueous corn meal. Various potential fumonisin sorbents were tested and compared to cholestyramine resin. Activated carbon and bentonites have been used in animal feeding studies, but do not effectively reduce fumonisin toxicity. Cholestyramine is a strong base anion exchange resin and pharmaceutical product, which is used to treat high cholesterol in humans. Cholestyramine reduced fumonisin toxicity in animal feeding studies. Other anion exchange resins might be as effective as cholestryramine in reducing fumonisin toxicity. Chitosan is a non-toxic biopolymer derived from chitin, which is the ubiquitous structural polysaccharide found in crustaceans, insects, and fungi. Chitosan is a modified form of chitin with amine groups that protonate in acidic solutions to yield a positive charge and might bind fumonisins like cholestryamine. Layered double hydroxides (LDH) are synthetic minerals with anion exchange properties that are chemically and structurally related to magnesium hydroxide. Fumonisin molecules can exist as anions or cations depending on pH and adsorption to feed additives is consequently more complex than aflatoxin adsorption. Consistent with animal feeding studies, cholestyramine and another anion exchange resin effectively adsorbed fumonisin B1 (FuB1) from aqueous corn meal, whereas, bentonite (SWy) and activated carbon were not effective. Chitosan and a layered double hydroxide mineral also effectively adsorbed FuB1 from aqueous corn meal and might also reduce fumonisin toxicity in ingested feed. Commercial samples of anion exchange resins and chitosan were acquired and tested. Fumonisin adsorption by other anion exchange resins was comparable to cholestyramine. A great variety of anion exchange resins are produced worldwide and might be used instead of the more expensive cholestyramine. Chitosan and chitosan salts are produced worldwide and are comparable to the Sigma-Aldrich material. Chitosan is the only anion-exchanger potential feed additive for fumonisn adsorption that has received US FDA approval as a generally recognized as safe material. A gastrointestinal incubation procedure was developed to more effectively model fumonisin adsorption by additives in ingested corn. Soluble compounds in corn interfere with fumonisin adsorption to feed additives and phytates appear to be the most active interferents. Use of phytase enzyme to decompose phytates or additives to precipitate phytates might used to increase fumonisin adsorption to feed additives and more effectively decrease the toxicity of contaminated corn.

Research Findings

Fumonisin B1 adsorption from water

Batch adsorption isotherms are commonly used to measure contaminant sorption by clays and other materials. Sorption from water is relevant to contaminants dispersed in the environment. It should also be relevant to ingested contaminants because the digestive systems of animals are aqueous systems. However, the aqueous environment in digestion is in a constant state of change. Soluble compounds in feed can adsorb to feed additives and limit mycotoxin adsorption. Contaminants initially adsorbed to feed additives in the stomach might be partially desorbed in the intestines. The acidic pH in the stomach should protonate the NH_2 groups (Figure 1) of fumonisins to NH_3^+ to yield a cationic form can sorb to bentonites. However, the neutral to alkaline pHs in the intestines can deprotonate the $NH_3^{\ +}$ groups, dissociate the COOH groups to COO^-, and convert fumonisins into anions. Bouhet and Oswald (2007) concluded that fumonisin toxicity most likely occurs in the intestines. Enzyme-linked-immunoassay (ELISA) methods were used to quantify fumonisin B1 concentration and measure adsorption to feed additives. A critical, but commercially-unavailable biochemical needed for the ELISA measurements (fumonisin B1-horse radish peroxidase) was by necessity synthesized in the laboratory (Yu and Chu, 1999; Barno-Vetro et al., 2000; Bird et al., 2002; Shiu et al., 2010.). For adsorption from water and aqueous corn meal, fumonisin B1 concentrations were measured in water after equilibration, centrifugation, filtration, and pH adjustment.

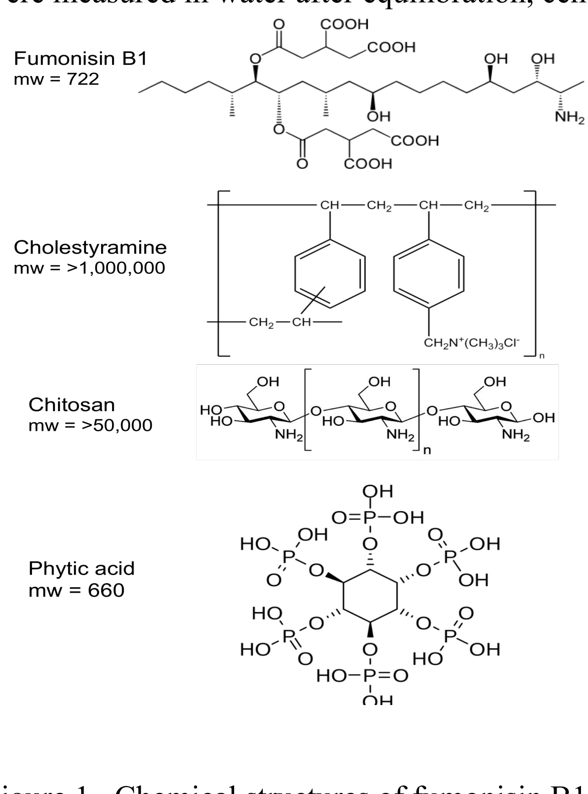

Figure 1. Chemical structures of fumonisin B1, cholestyramine, chitosan, and phytate.

The chemical structures of fumonisin B1, cholestyramine, chitosan, and phytic acid indicate properties relevant to the adsorption of fumonisins in ingested feed to feed additives. Depending on pH, fumonisins can be either anions or cations. Cholestyramine is a strong base anion exchange resin with permanent positively-charged sites. Questran is one of the pharmaceutical products based on cholestyramine used for cholesterol control. Cholestyramine is the only potential feed additive shown to reduce fumonisin toxicity in animal feeding studies. Sofrizzo et al. (2000) added FuB1 to rat diets and determined that cholestyramine consistently reduced the toxic effects of FuB1 to rats. In contrast, Piva et al. (2005) showed that activated carbon used as a feed additive does not effectively reduce FuB1 toxicity to piglets. Chitosan is a chitin-derived biopolymer, soluble fiber, antibacterial hemostatic wound dressing, low-mw oligomer seed coating, foliar antifungal, and OTC food supplement. Chitosan has a pH-dependent charge and is positively-charged at neutral to acidic pHs. Chitosan is insoluble at neutral to alkaline pHs. However, chitosan acetate, lactate, and other salts are soluble forms of chitosan that might be more effective fumonisin sorbents. The European Food Safety Authority (EFSA) concluded that regular chitosan ingestion by humans lowers total and low-density lipoprotein (LDL) cholesterol. Hence, ingested chitosan acts much like cholestyramine in lowering cholesterol and might similarly adsorb fumonisins in ingested feed. In 2012, the US FDA approved chitosan as a generally recognized as safe (GRAS) food additive. Layered double hydroxides (LDH) are a group of synthetic minerals (e.g. $Mg_{1-x}Al_x(OH)_2Cl_x$, $Mg_{1-x}Fe_x(OH)_2Cl_x$) that have large anion exchange capacities and might adsorb fumonisins in feed like cholestyramine. LDHs have been used in human antacid products, such as Rolaids. Cholestyramine is a strong-base anion exchange resin and many similar products are produced worldwide. Three other anion exchange resins were acquired and tested. Chitosan is also produced worldwide and samples from China and Washington State were acquired and tested. Several layered double hydroxides were prepared in the laboratory and tested. Layered double hydroxides are industrial minerals used and produced worldwide. Many such products would be available for animal feeding studies.

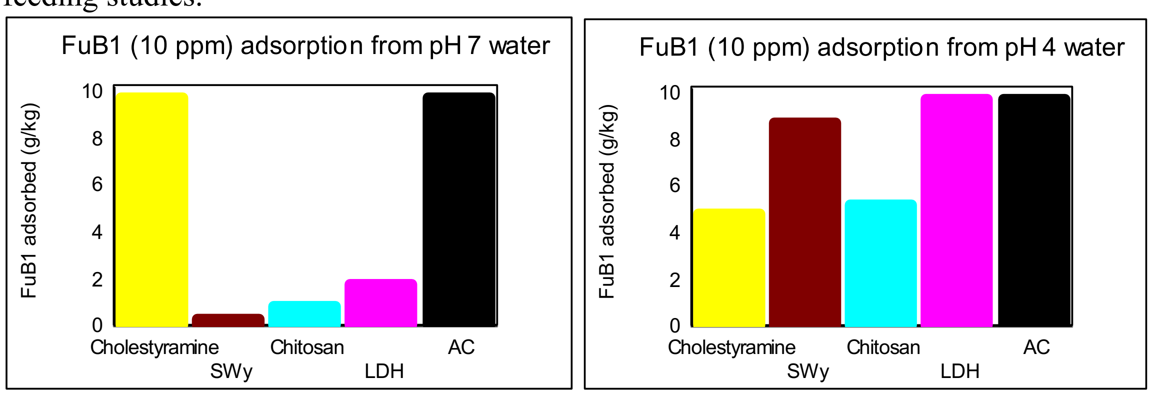

Figure 2. Fumonisin B1 (FuB1) adsorption from pH 7 and pH 4 water.

In water at pH 7, fumonisin B1 (FuB1) more strongly adsorbs to cholestyramine and activated carbon (AC) than to bentonite (SWy), chitosan, or a layered double hydroxide

(LDH) mineral (Figure 2). However at pH4, FuB1 was effectively adsorbed by all five materials. Activated carbon effectively adsorbed FuB1 from pH7 water (Figure 2, but

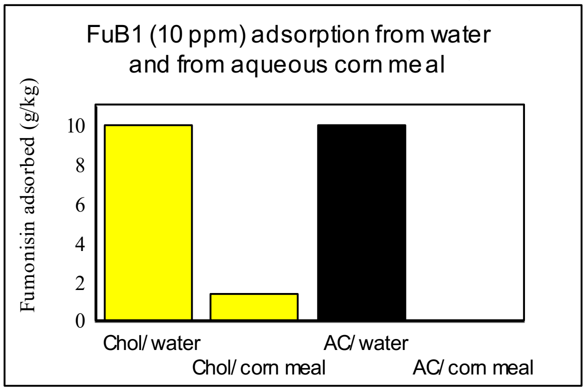

Figure 3. Fumonisin B1 (FuB1) adsorption to cholestyramine and activated carbon from water and aqueous corn meal.

Sofrizzo et al. (2000) used very large (260 mg/L) initial FuB1 concentrations in water adsorption isotherms and achieved a very high adsorption of ~190 g FuB1/kg of cholestyramine, 124 g FuB1/kg of activated carbon, but only 3.3 g FuB1/kg of bentonite. If cholestyramine could adsorb 190 g fumonisin/kg cholestyramine in animal feed, very little cholestyramine would be needed to control fumonisin toxicity. However, Sofrizzo et al. (2000) found that only 2.4 g FuB1/kg cholestyramine were adsorbed from aqueous corn flour. As indicated in Figure 3, compounds in corn meal clearly decreased FuB1 adsorption to cholestyramine and activated carbon. The fact that cholestyramine more effectively adsorbs fumonisins from corn meal might explain why cholestyramine decreases fumonisin toxicity in animal feeding studies, but activated carbon is not effective. Proteins and carbohydrates are soluble feed compounds that might interfere with fumonisin adsorption to feed additives. Proteins and carbohydrates, however, are essential nutrients in feed and little might be done to prevent interference. Phytates are another group of soluble compounds in feed (~1% phytates in corn) and a potential fumonisin adsorption blocker that might be controlled or eliminated. Fumonisin adsorption with and without added sodium phytate was measured to examine the effect of phytates on fumonisin adsorption to feed additives. Phytates and phytic acid consist of a cyclohexane central ring bonded to six orthophosphate groups. Each of the six orthophosphate groups contains two acidic hydroxyls which can dissociate to produce a super anion with up to 12 negatively-charged sites. Cholestyramine, chitosan, and LDHs are anion exchangers and adsorption of phytate anions might block fumonisin adsorption.
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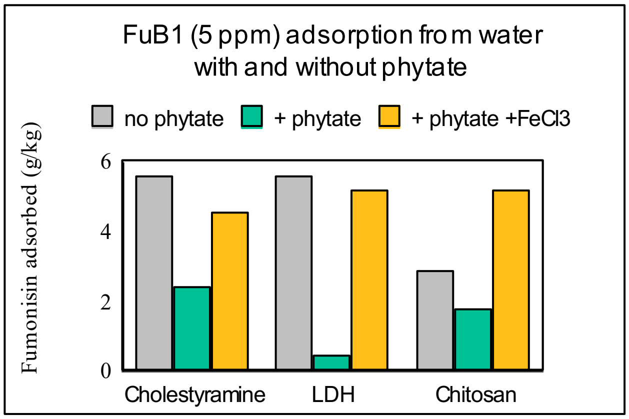

Figure 4. FuB1 adsorption from water with and without added phytate.

Fumonisin B1 adsorption from aqueous corn meal (Figure 3) was comparable to FuB1 adsorption from water with added sodium phytate (Figure 4). Phytates in corn meal appear to account for much of the reduction in FuB1 adsorption from aqueous corn relative to water. Iron chloride ($FeCl_3$) can precipitate phytate and prevent interference. Phytates in feed might be either precipitated (e.g. $FeCl_3$) or decomposed with phytase enzyme (Phytase-EZ) to prevent interference and increase fumonisin adsorption to feed additives. Samples of corn meal with 10 mg/kg fumonisin B1 were incubated with and without phytase enzyme. Phytase is added to swine and poultry feed to reduce soluble phosphate concentrations in the manure. Non-ruminant animals, such as poultry, swine, and humans cannot digest phytates. Soluble phosphate in runoff waters is an environmental problem caused by large animal feeding operations. Phytates in manure are rapidly decomposed to orthophosphates by microorganisms. Hill et al. (2008) showed that phytase addition to distillers dried grain reduced the need for phosphate supplementation in the feed and reduced soluble phosphate concentrations in sow manure.

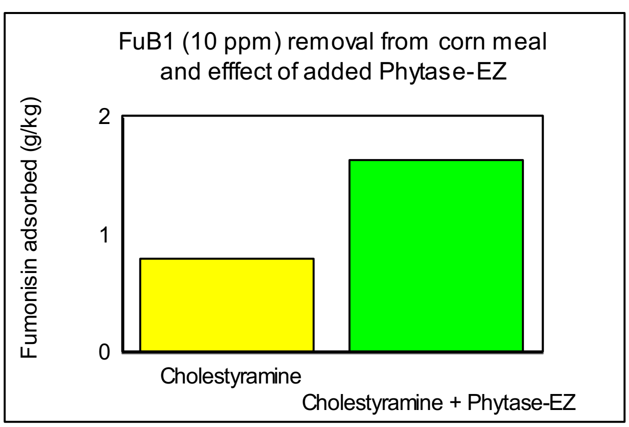

Figure 5. Effect of phytase enzyme on FuB1 adsorption from aqueous corn meal.

The addition of phytase enzyme to corn meal clearly increased fumonisin adsorption from aqueous corn meal to cholestyramine (Figure 5). This suggests that phytase addition might increase fumonisin adsorption to feed additives as well as reduce soluble phosphate concentrations in animal manure.

A gastrointestinal incubation method for fumonisins was adapted from procedures developed to model contaminant absorption from ingested food and soils by animals and humans (Crews et al. (1983), Malagelada et al. (1976), and Rodriquez et al. (1999). The procedure was developed to more effectively model fumonisin adsorption to feed additives during digestion. The procedure uses aqueous fumonisin-contaminated corn meal, simulated gastric fluid with 1% pepsin, a two-hour incubation at pH 1.8 (stomach model), and a two-hour incubation with simulated intestinal fluid at pH 7.0- 7.5 (intestine model). Preliminary results suggest that a Dow Chemical Company anion exchange resin (Dowex 1x4-400) might adsorb fumonisins in ingested corn meal more effectively than cholestyramine. The Dowex material has a smaller grain size than cholestyramine. A test that combines phytase enzyme incubation with gastrointestinal incubation will be used to further examine the potential effects of phytate removal on fumonisin adsorption.

CONCLUSIONS

Fumonisins have a pH-dependent charge and the adsorption behavior changes with pH. Fumonisins are anions at neutral to alkaline pHs and cations at acidic pHs. Cholestyramine, other anion exchange resins, chitosan, and layered double hydroxides are potential additives to bind fumonisins in feed. Only cholestyramine, activated carbon, and bentonites have been tested in animal feeding experiments. The anion exchange resin, cholestyramine, is the only material that has been tested in animal feeding experiments and shown to effectively reduce fumonisin toxicity. Many potentially less expensive anion exchange resins similar to cholestyramine are produced worldwide. Chitosan and layered double hydroxides are anion exchange materials produced worldwide that might also bind fumonisins in feed. A gastrointestinal incubation procedure was developed to model fumonisin adsorption to feed additives during digestion. Phytates in corn meal bind to feed additives, such as cholestyramine, and limit fumonisin adsorption to feed additives. The addition of phytase enzyme to feed, which is done to control soluble phosphates in manure, might also enhance fumonisin adsorption to feed additives. The data collected in this study has identified promising fumonisin adsorbents that might be further tested in animal feeding studies and other feed treatments that might be used to enhance fumonisin adsorption to feed additives and more effectively decrease fumonisin toxicity to animals.

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