NATURAL AND SYNTHETIC BINDERS TO REDUCE FUMONISIN TOXICITY IN CORN

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Abstract:
Fumonisins are toxins (mycotoxins) produced by fungi (*Fusarium*) that are frequently identified in corn (maize). Mycotoxins are toxic to animals and humans and contaminated crops are discounted or destroyed. Clay feed additives effectively bind and reduce the toxicity of aflatoxins and might be used to reduce the toxicity of other mycotoxins. Adsorption of mycotoxins to feed additive surfaces can limit the bioavailability and toxicity of contaminated feed that has been ingested. An enzyme-linked immunosorbent assay (ELISA) method was used to measure fumonisin concentrations in adsorption isotherms from water and from aqueous corn meal. Unlike the aflatoxins, fumonisins are acidic, anionic compounds at neutral pHs that would not likely bind to the negatively-charged surfaces of natural clay minerals. Hence, modified clays, cationic synthetic clays, or other materials that might bind fumonisins and reduce the toxicity were examined. Various potential fumonisin sorbents were tested and compared to cholestyramine resin, which has reduced fumonisin toxicity in animal feeding studies. Activated carbon and bentonites have also been used in animal feeding studies, but do not effectively reduce fumonisin toxicity. Cholestyramine is an anion exchange resin and pharmaceutical product, which is used to treat human medical conditions. 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 structural polysaccharide found in the exoskeletons of crustaceans and insects. 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.

Research Findings

Fumonisin B1 adsorption from water

Batch adsorption isotherms are commonly used to measure the sorption of contaminants to 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. Contaminants initially sorbed to clays in the stomach might be partially desorbed in the intestines. The acidic pH in the stomach should protonate the NH_2 group (Figure 1) in fumonisins to NH_3^+ to yield a cation that might adsorb to bentonites. However, the neutral pH in the intestines might deprotonate the $NH_3\,^+$ group, 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. A critical biochemical needed for the ELISA measurements (fumonisin B1-horse radish peroxidase) was not commercially available and was necessarily 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.

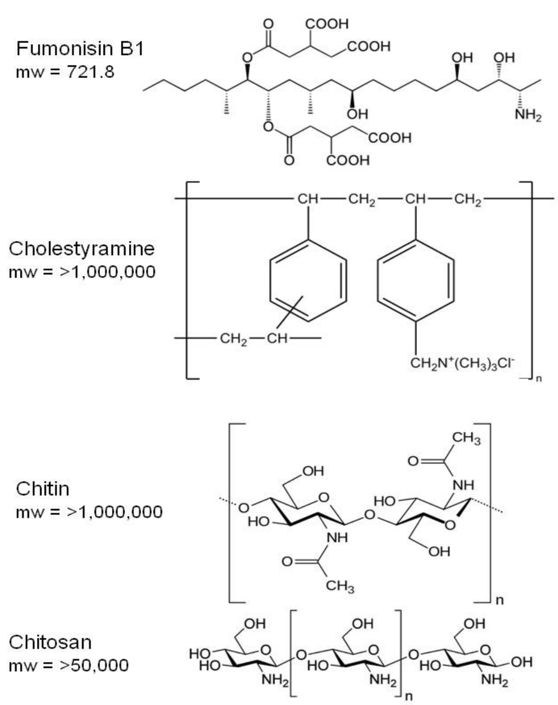


Figure 1. Chemical structures of fumonisin B1, cholestyramine, chitin, and chitosan.

Cholestyramine and chitosan are both positively-charged polymers that can adsorb anions at acidic pHs. However, unlike cholestyramine, chitosan is uncharged at neutral or alkaline pHs. The amine groups in cholestyramine have been quaternized with methyl groups to yield a permanent positive charge. Chitosan is thought to act much like cholestyramine when ingested. Chitosan is not toxic and it is sold as a human food supplement, but the health benefits are not proven.

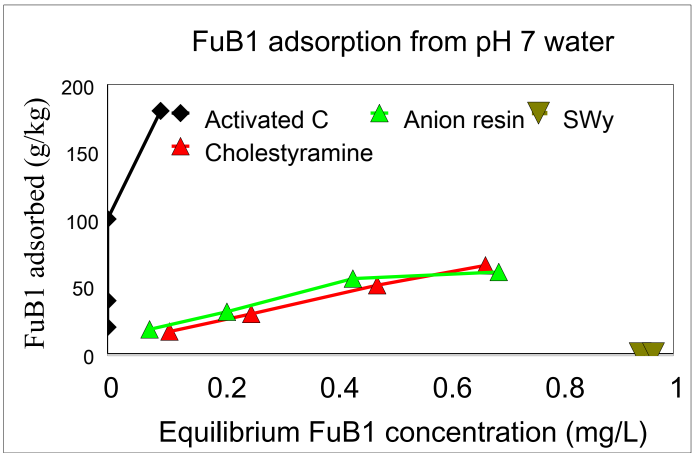
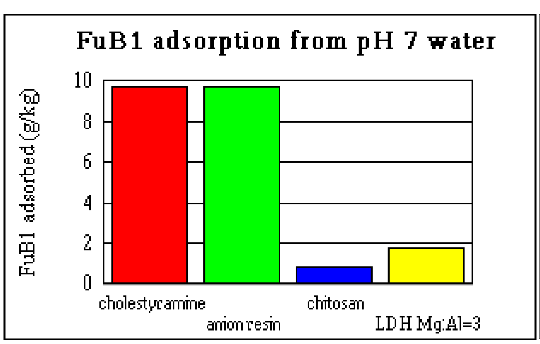
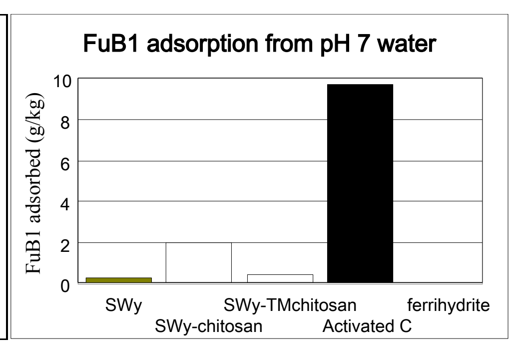


Figure 2. Fumonisin B1 (FuB1) adsorption from pH7 water.

FuB1 more strongly adsorbs to activated carbon than cholestyramine and bentonite (SWy) from pH7 water (Figure 2). Sofrizzo et al. (2000) used very large (260 mg/L) initial FuB1 concentrations in adsorption isotherms and achieved a very high adsorption of ~190 g FuB1/kg of cholestyramine and 124 g FuB1/kg of activated carbon. In contrast, Sofrizzo et al. (2000) measured FuB1 adsorption of only 3.3 g FuB1/kg of bentonite. Similarly, other feeding studies have not shown bentonites to be effective in reducing the toxicity of fumonisins. In this study, the much lower initial FuB1 concentrations of 1mg/L used in isotherms resulted in only ~60 g FuB1/kg of cholestyramine. The very high FuB1 concentrations used by Sofrizzo et al. (2000) are much larger than would likely occur in contaminated corn. Sofrizzo et al. (2000) also added FuB1 to rat diets and determined that cholestyramine consistently reduced the toxic effects of FuB1 to rats. Activated carbon adsorbed much more FuB1 from pH7 water (Figure 2) than cholestyramine, but Piva et al. (2005) showed that activated carbon does not effectively reduce FuB1 toxicity to piglets.





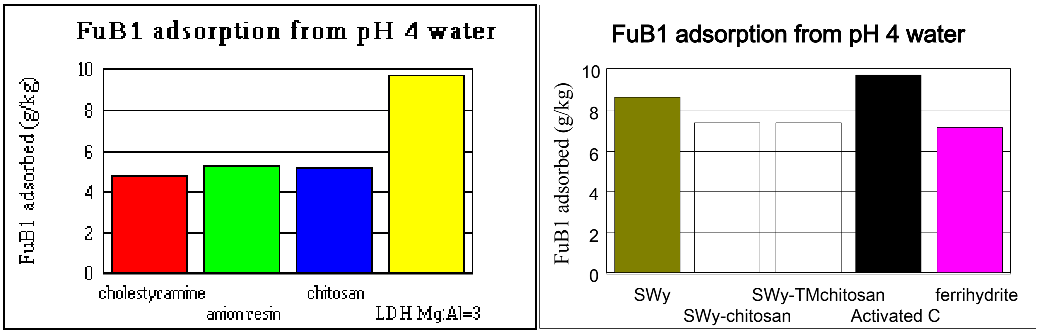


Figure 3. Fumonisin B1 (FuB1) adsorption from water at pHs 7 and 4.

At pH 7, cholestyramine, anion resin, and activated carbon adsorbed comparable amounts of FuB1 from water (Figure 3). The LDH mineral was not effective at pH 7 probably because of adsorbed CO_3^{2-} anions that block the exchange sites. At pH 4, SWy (bentonite), LDH, and activated carbon were more effective FuB1 adsorbents than cholestyramine or the anion resin. Chitosan FuB1 adsorption was comparable to cholestyramine at pH 4. At pH 4, even ferrihydrite, a weakly crystalline mineral found in iron rust and soils, adsorbed more FuB1 than cholestyramine. Ferrihydrite is positively charged at acidic pHs and effectively adsorbs anions, such as phosphate and arsenate. FuB1 should be a cation at pH 4 and how it adsorbs to ferrrihydrite is not clear. FuB1 adsorption to ferrihydrite at pH 4 but not at pH 7 demonstrates the importance of pH on FuB1 adsorption, but it probably will not be an effective feed additive to bind ingested fumonisins. Fumonisin adsorption is clearly pH dependent for the charged sorbents. Activated carbon is not charged and FuB1 adsorption is not pH dependent. FuB1 adsorbs to bentonites at acidic pHs, but not at neutral or alkaline pHs. The chitosan-modified bentonites (SWy-Chitosan, SWy-TMChitosan) effectively adsorbed FuB1 at pH 4. The TMChitosan is chitosan that was reacted with methyl iodide to quaternize the amine groups (Donard et al., 1986) and make it more similar to cholestyramine.

Fumonisin adsorption from corn meal

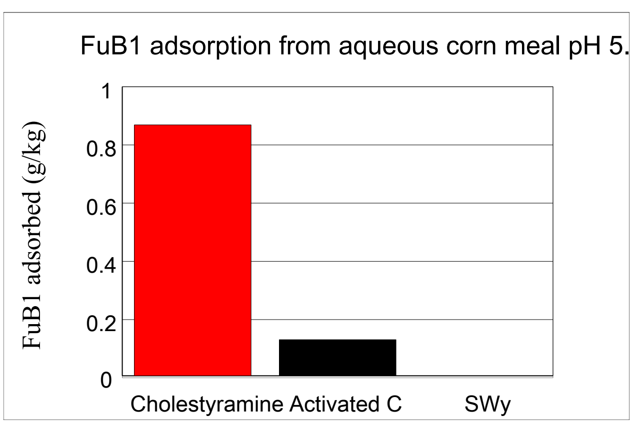


Figure 4. FuB1 adsorption from aqueous corn meal for materials that have been used in feeding studies.

FuB1 adsorption from aqueous corn meal (Figure 4) is consistent with animal feeding studies. Cholestyramine, which effectively prevented FuB1 toxicity in a feeding study, adsorbed much more FuB1 than activated carbon and bentonite, which feeding studies indicate do not prevent FuB1 toxicity. In comparison to FuB1 adsorption from water (Figure 2), much less FuB1 was adsorbed from aqueous corn meal. The dissolved compounds (e.g. proteins) present in aqueous corn meal apparently inhibit FuB1 adsorption to cholestyramine and other feed additives. Orthophosphate and other anions might compete with FuB1 for feed additive adsorption sites and reduce FuB1 adsorption.

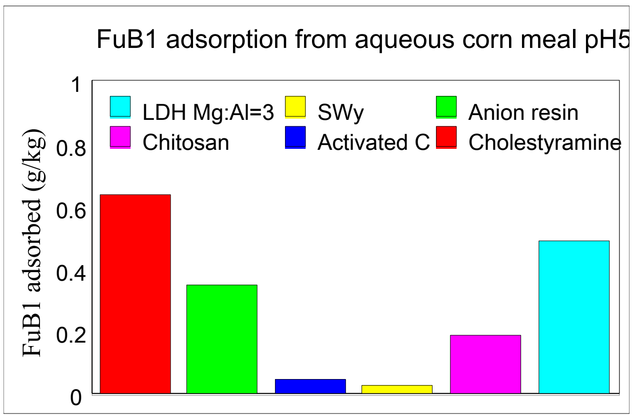


Figure 5. FuB1 adsorption from aqueous corn meal to various sorbents.

In Figure 5, cholestyramine, anion resin, chitosan, and the layered double hydroxide (LDH) mineral were effective FuB1 adsorbents from aqueous corn meal, but the bentonite (SWy) and activated carbon were not effective adsorbents. Other anion resins, chitosan, and layered double hydroxides might also reduce fumonisin toxicity if mixed with animal feed based on the relative adsorption of FuB1 to cholestyramine. Animal feeding studies are needed to establish that chitosan and layered double hydroxide minerals can effectively prevent fumonisin toxicity. Anion resins other than cholestyramine appear to be comparable materials in FuB1 adsorption and might be substituted for cholestyramine, which is probably more expensive.

CONCLUSIONS

Cholestyramine and a comparable anion exchange resin (Spectra/Gel ion exhange 2x8 strong base anion, chloride form) adsorbed fumonisin B1 from water and aqueous corn meal similarly. Chitosan and a layered double hydroxide mineral also effectively adsorbed fumonisin B1 from water and aqueous corn meal. All of these materials show promise as feed additives to bind fumonisins in feed and prevent toxicity.

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