



UPSN Clay Inclusion in Food Reduces Aflatoxin Bioavailability in a Crossover Study in Ghana



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INTRODUCTION:

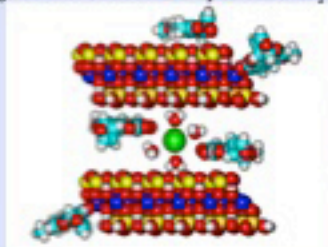
Cancer incidence is rising in Africa with approximately 650,000 people developing cancer annually and an 80% mortality rate as a result of unavailable treatment. Hepatocellular carcinoma (HCC) is one of the most common malignancies in Ghanaian men and women with mortality among Ghanaian patients accounting for 23.2% and 10.9% of all cancer related deaths respectively. Multiple factors play a role in the etiology of HCC; one major risk factor is chronic exposure to low levels of aflatoxins (AFs) from the diet (i.e. contaminated maize, groundnuts and other cereal grains). It is well established that the predominant congener aflatoxin B₁ (AFB₁) is one of the most potent naturally-occurring hepatocarcinogens in humans. The humid, yet drought-stricken, environment of Ghana promotes growth of the *Aspergillus* fungi responsible for the production of Afls while food insecurity and maize storage practices (top right) in the Ejura district of Ghana make these populations at high risk for exposure.



Enterosorption intervention strategies using montmorillonite clay (UPSN) in animals and humans have proven effective in reducing biomarkers of exposure as well as symptoms of toxicity. UPSN inclusion as a feed additive in areas with high incidence of aflatoxin exposure would be a sustainable approach to alleviating many public health issues associated with aflatoxins. Palatability of foods containing UPSN and efficacy of UPSN to bind AFB₁ in foods was assessed both *in vitro* and in a well-established human population in the Ejura District of Ghana (bottom right).

MATERIALS AND METHODS:

Figure 1. Montmorillonite Clay Structure: Al₂Si₄O₁₀(OH)₂



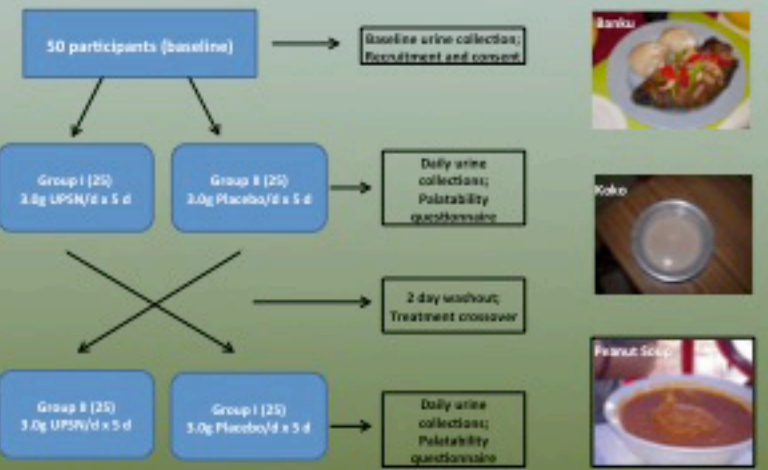
Schematic representation of 2:1 layer-lattice montmorillonite clay showing hydrated calcium in the interlayer cation with AFB₁ bound. A net negative charge is responsible for high cation exchange capacities in soils. Common substitutions: Mg²⁺ for Al³⁺; Al³⁺ for Si⁴⁺; and Fe³⁺ for Al³⁺. Key: Si⁴⁺ (yellow), silica tetrahedra and Al³⁺ (blue), aluminum octahedra, O (red), H (white), Ca²⁺ (green).

Figure 2. AFB₁ Extraction from Maize



Preliminary *in vitro* study to analyze binding capability of UPSN throughout a traditional Ghanaian cooking process. Maize meal (56g) was spiked in triplicate with AFB₁ at levels ranging from 5ppb-2ppm with and without inclusion of 1.5g UPSN. Samples were run with, and without, fermentation. Extraction of AFB₁ from maize slurry was conducted according to VICAM methods.

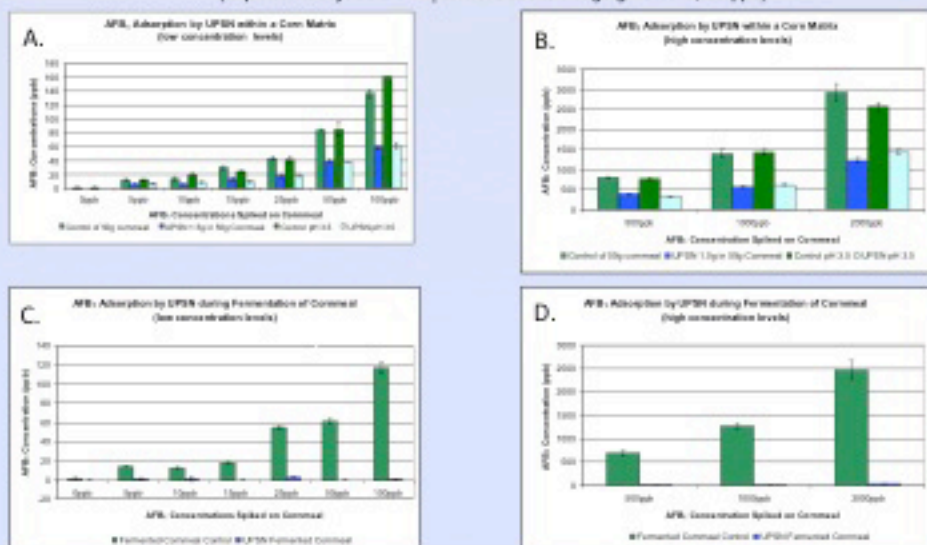
Figure 3. Intervention Trial Design (Crossover)



UPSN or placebo (calcium carbonate) was added to participant's food (above right) during the breakfast and dinner meals for 5 days. After 5 days participants were switched to the opposite treatment for an additional 5 days. Urine samples were collected daily and palatability questionnaires, along with adverse events monitoring, were performed after each meal.

RESULTS:

Figure 4. *In Vitro* Evaluation of UPSN/Cornmeal Mixtures and Controls (Triplicate Analysis for AFB₁ Concentrations Ranging from 5-2,000 ppb)

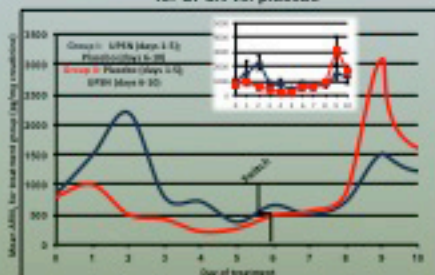


Each bar represents the mean concentration \pm standard deviations. (A) Lower concentration levels of AFB₁ (5 ppb-100 ppb) in controls and UPSN/cornmeal mixtures. UPSN/cornmeal mixtures had approximately 88% less AFB₁ in solution than controls. (B) Higher levels of AFB₁ (500-2,000 ppb) in controls and UPSN/cornmeal mixtures. This represents worst case scenario of aflatoxin exposure, yet UPSN samples still averaged 50% less AFB₁ in solution compared to controls. (C) Cornmeal with and without UPSN was allowed to ferment for 72 h and binding was determined. Low levels of AFB₁ (5-100 ppb) were bound between 56-100% by UPSN when it was added before fermentation. (D) High levels of AFB₁ (500-2,000 ppb) were bound approximately 30% by UPSN during fermentation.

Table 1: Comparison of food palatability between placebo and UPSN treated foods

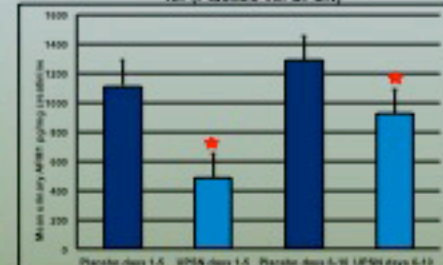
	Palatability of foods containing up to 3 g of calcium carbonate or UPSN clay				YES	
	Taste	Aroma	Texture	Eat Again?		
Good	84.8%	78.3%	78.3%	65.2%	80.4%	69.6%
Acceptable	15.2%	21.7%	21.7%	34.8%	19.6%	30.4%
Unacceptable	No food samples were deemed to be unacceptable or poor					
Poor						

Figure 5: Urinary AFM, Levels Per Day for UPSN vs. placebo



Based on a half-life of 24-48h, urinary AFM is a reliable short-term biomarker for AFB₁ exposures. In this study, the mean AFM concentration is lower in UPSN treated participants than the mean AFM concentration in placebo treated participants. Crossover of treatments is delineated in the graph.

Figure 6: Cumulative Mean AFM, Values for (Placebo vs. UPSN)



When all data is grouped according to treatment arms, the UPSN groups had significantly lower AFM than the placebo groups during both arms of the study. This indicates that the UPSN treated groups had diminished bioavailability of AFB₁ from their diet during the treatment period.

CONCLUSIONS:

- The production of organic acids, alcohols, and CO₂ during fermentation does not affect binding of AFB₁ to UPSN.
- UPSN sequestered 88-100% of AFB₁ in fermented maize samples at AFB₁ levels varying from 5ppb-2ppm.
- In a short-term study; participants consuming up to 3g of UPSN per day did not report having any adverse health events.
- Addition of UPSN clay in foods does not alter the aroma, taste, or texture.
- UPSN significantly ($p < 0.01$) reduced AFM, urinary biomarkers when compared to placebo controlled groups.
- Urinary AFM biomarkers can be used to assess efficacy during short-term intervention trials in human populations.
- Food inclusion of UPSN would be more sustainable and increase the use of the material within Ghana.