“Down to Earth Science”

This paper is a paraphrased version of an original published research report entitled: NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: II. Reduction in biomarkers of aflatoxin exposure in blood and urine. Food Additives and Contaminants 25(5):622-34. This paper is meant to be used with school children in grades approximately 7 to 10, as part of a learning experience about the scientific method.

INTRODUCTION

The Kingdom Fungi includes many different organisms, such as mushrooms, yeasts and molds. While many of the members of this group are not harmful, such as eating mushrooms on pizza or bread made using yeast, many of the members can be toxic to animals and people. Some species make very toxic compounds. One group of fungi, known as Aspergillus, produces a group of toxins called aflatoxins (1). Aflatoxins can be found in corn and peanuts (2). Figure 1 shows Aspergillus fungi growing on corn. One toxic form of aflatoxin is aflatoxin B_1. This toxin has caused many problems for animals and people. Scientists and veterinarians found that dogs that ate dog food contaminated with aflatoxin B_1 developed very bad liver and kidney problems. Some of these dogs even died from eating this toxin (3). If people eat food contaminated with this toxin, sickness and other negative effects can develop. Over a long period, aflatoxin B_1 can cause cancer of the liver (4). Liver cancer is a major cause of death, particularly in developing countries (5). For some regions of the world, aflatoxins are unavoidable in food, especially in tropical areas where fungus grows well.

Figure 1. Aspergillus fungus on corn kernel

So, there is a real problem with aflatoxins. To solve the problem, our laboratory is using “down to earth science.” Did you know that animals and people have been known to eat clay? Clay is the finest particle in soil (6). Macaws found in South America can eat seeds that are toxic to many other animals, yet they don’t get sick. How is this possible, you may wonder? After the macaws eat the seeds, they flock to the banks of a river to eat clay. It is thought that this clay could play a role in keeping these parrots from getting sick (7). Figure 2 shows these Macaws eating clay. People in many cultures have also been known to eat clay. This led us to hypothesize that animals and humans regularly exposed to aflatoxins could be protected if they ingested clay at the same time.

Figure 2. Macaws in Peru. Munn, CA, 1994. Macaws: winged rainbows. Natl. Geograph. 185:118-140.

Not all clay is made equal though. Clays from all over the world have different structures. Our past research has shown that a specific clay, with a unique structure, can bind aflatoxin B_1 (8). This means, that aflatoxin B_1 fits tightly in the clay structure like a hand fits in a glove. If the toxin is bound to the clay, it is not able to cause its negative effects. The specific clay is known as NovaSil. In past experiments, NovaSil (NS) clay was added to animal feeds contaminated with aflatoxins and animals did not get sick (9). What’s more, in a study where 50 healthy humans volunteered to eat this clay (which was put into capsules) for 2 weeks, they remained healthy and showed no problems (10). This study made sure that the clay was safe for humans to eat before we could test to see it helped decrease aflatoxin levels. To test if the clay could decrease levels of aflatoxin that people were getting through their diet, we preformed the following study, using the scientific method to test our hypothesis.

To measure if a person has eaten aflatoxin B_1, scientists can measure markers in the blood and urine. For example, after someone eats aflatoxin B_1, enzymes in the liver metabolize (or change) aflatoxin B_1 to a new compound called aflatoxin M_1. Aflatoxin M_1 has been shown to be excreted in human urine and animal milk, for which the toxin was named. When dairy cows ate feed contaminated with aflatoxin B_1, the marker was found in the cow's milk (11); so, the name of the marker was called aflatoxin M_1 (for milk toxin). Scientists and farmers knew that if a cow had aflatoxin M_1 in its milk, that it had eaten feed contaminated with aflatoxin B_1. It has also been shown that aflatoxin M_1 is found in the urine from people who have eaten aflatoxin B_1 (12). Using laboratory analysis, scientists are able to measure the amount of aflatoxin M_1 in a person's urine to see if they have eaten aflatoxin B_1.

In this report, our lab analyzed urine samples collected from 180 participants who either took NS clay capsules or took no clay capsules (which was called the placebo control group). The purpose of this study was to see if aflatoxin M_1 levels were lower in people who took NS capsules for 3 months. If the levels were lower in the group of people who ate clay, then we could conclude that NS clay decreases aflatoxin exposure.

MATERIALS AND METHODS

Study population, design, and procedures

A study site in Ghana was chosen since studies have shown people there are exposed to aflatoxins from their diet (13). Ghana is located in western Africa. Overall, the climate of the region is tropical. Over 75% of the population in the study area is involved in agriculture. People grow and eat crops such as corn, peanuts and yams (14). Study participants were divided into three groups: high dose, low dose or placebo control. The high dose group took 3.0 g of clay each day, the low dose group took 1.5 g of clay each day, and the placebo control group took capsules that did not contain any clay for 3 months. Figure 3 shows the capsules used in the study. All of the capsules were the same size, shape and color. Participants provided urine samples at the beginning of the study (baseline), after 1 month and 3 months of treatment, and 4 months (1 month without treatment).

Figure 3. Capsules used in the study

Measurement of aflatoxin M_1 in urine

Aflatoxin M_1 in urine was measured using the following method (15). The sample was mixed with water and acid, and then passed through an antibody column. An antibody binds a very a specific partner, by forming a bond. The shape of the partner has to fit exactly for the antibody to bind it. The partner for this particular antibody column was aflatoxin M_1. By allowing the sample to slowly flow over the antibody column, we were able to pull the aflatoxin M_1 out of the urine. Next, we washed the column with water and added methanol. The methanol literally pulled aflatoxin M_1 off of the column. By adding methanol, we broke the bonds between the antibody and its partner, aflatoxin M_1. We were then able to measure how much aflatoxin M_1 was in the urine by using liquid chromatography. Chromatography, which means "separating colors," is used to separate compounds. In principle, this concept is similar to what you may have learned in a lab on paper chromatography.

By passing the sample through a liquid chromatography instrument, we were able to detect a peak that represented aflatoxin M_1. The bigger the peak, the more aflatoxin M_1 was in the sample, and the smaller the peak, the less aflatoxin M_1 in the sample. Figure 4 shows an example of the aflatoxin M_1 peak detected in a urine sample. Every compound has what is called a retention time, which is the time it takes for you to see the peak after the sample is inserted into the instrument. For aflatoxin M_1, the retention time was seen at ~15 minutes.

Urine can range from very concentrated to very dilute (think how much water you drink). To make sure that we did not misread our data, we divided the amount of aflatoxin M_1 (calculated from the peak) by the amount of creatinine in each urine sample. Creatinine is a protein that stays constant in a person's urine. St. Joseph's Laboratory provided the creatinine values for each urine sample. In figure 5, we show aflatoxin M_1 with the units picograms of aflatoxin M_1 per milligram of creatinine (pg/mg creatinine). One gram equals 1 trillion picograms. So, one package of sugar (which is ~1 gram) would equal 1 trillion picograms. One picogram is a very small amount!

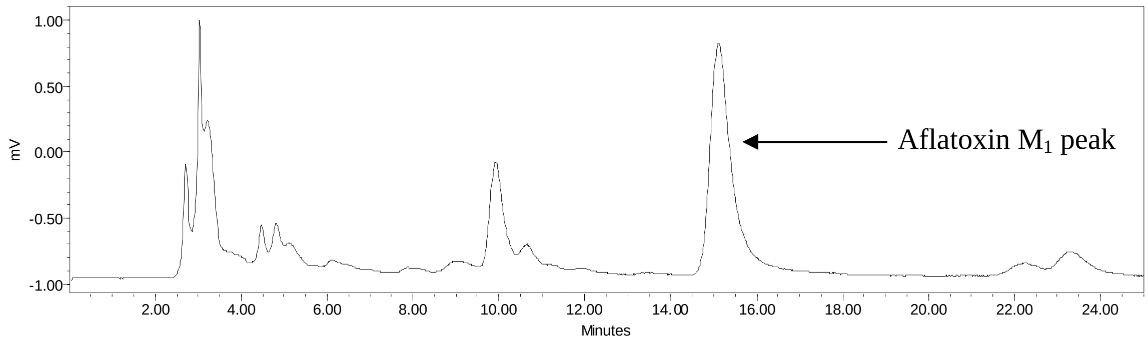


Figure 4. Aflatoxin M_1 peak in urine of study participant. Retention time equals about 15 minutes

RESULTS

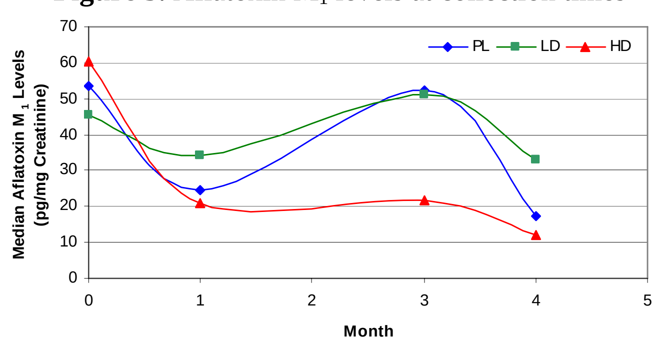
Out of the samples measured at baseline (before any treatment), 88.1% (140/159), had detectable levels of aflatoxin M_1 ranging from very small to very large (0.02–13,297.7 pg/mg creatinine). We calculated the median aflatoxin M_1 level for each group. Figure 5 shows the aflatoxin M_1 levels over the course of the study. To see if the clay decreased aflatoxin M_1 levels, our data analysis compared the different treatment groups: high dose (HD), low dose (LD), and placebo (PL). There was no significant difference in the aflatoxin M_1 levels among the three study groups at baseline (PL=53.4, LD=45.5, HD=60.3) or after 1 month (PL=24.6, LD=34.2, HD=21.0). There was a significant decrease in the aflatoxin M_1 levels after 3 months of taking clay, at the high dose level. While values for the PL and LD groups were similar (52.4 and 51.2), there was a 58.7% reduction between the PL and HD group (52.4 reduced to 21.6).

DISCUSSION

The main objective of this study was to see if our clay could decrease aflatoxin exposure in humans. Results showed that taking NS clay reduced aflatoxin M_1 levels. Aflatoxin M_1 in the urine represents a recent exposure to aflatoxin. So, after eating aflatoxin B_1, aflatoxin M_1 can be measured in urine about one to two days after. However, after one week of eating aflatoxin, you could not detect aflatoxin M_1 in the urine because it would have already been excreted. So, aflatoxin M_1 is only a short-term measure. This study was the first to test if clay could reduce aflatoxin M_1 levels in humans. Other studies have used different interventions to see if aflatoxin was reduced. One study used tested chlorophyllin, which is a food-coloring agent derived from chlorophyll, the green pigment found in most plants (16). Here, 188 healthy adults took either chlorophyllin or placebo over a 4 month period. Researchers measured an aflatoxin marker in urine samples collected from participants. They found that taking chlorophyllin at each meal led to a 55% reduction in median levels of the aflatoxin marker. This is similar to the decrease we observed in our study.

In summary, results from this study support the idea of using NS clay to decrease exposure to aflatoxins and to prevent the negative effects of aflatoxins in humans who eat contaminated foods. Future studies, for instance, 6 month and 1 year trials, will be required to test NS clay as a long-term solution to the problem with aflatoxins.

Figure 5. Aflatoxin M_1 levels at collection times



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