Nutrition CRSP Research Brief Number: 6

Nutrition CRSP Aflatoxin Research Note

Gerald Shively, Celeste Sununtnasuk and Jing Hao

Introduction

Aflatoxin, produced by one of the major classes of mycotoxin-producing fungi, deserves special attention as a naturally occurring toxic contaminant of food and animal feed. Aflatoxin poses a particular threat in developing countries because detection is difficult and relatively costly, and the ways in which susceptible grains (such as maize and groundnuts) are harvested, handled and stored create conditions favorable to the growth of mycotoxins, especially among smallholder farmers. On the one hand, the presence of aflatoxin can lead to substantial grain losses if contaminated grain is detected and removed from the food chain. On the other hand, if contaminated grain remains available for consumption, exposure can have very serious human health implications. Once present in food, aflatoxin cannot be removed by cooking or processing. Decontamination is very costly.

Aflatoxin's health effects have led to the establishment in numerous countries of guidelines for acceptable levels of aflatoxin in various foods. The U.S. Food and Drug Administration (FDA) has long recognized aflatoxin as an unavoidable contaminant. Rather than implement regulations to ensure zero contamination, the FDA has set minimum threshold levels. The amount of aflatoxin permitted in human food in the United States (which is similar to standards in many other countries in the world), is a limit of 20 parts per billion (ppb). Table 1 shows the US tolerance levels of aflatoxin in various human foods, animal feeds, and animal feed ingredients.

Table 1 FDA maximum aflatoxin levels

Level	Commodity/Food
300 ppb	Corn and peanut products intended for finishing beef cattle
300 ppb	Cottonseed meal intended for beef cattle, swine or poultry
200 ppb	Corn and peanut products intended for finishing swine
100 ppb	Corn and peanut products intended for breeding cattle, swine or mature poultry
20 ppb	Corn, peanut products and other animal feeds intended for immature animals
20 ppb	Corn, peanut products, cottonseed meal and other animal feeds & ingredients intended for dairy animals; for animal species or uses not specified, or when intended use is not known
20 ppb	Peanuts, peanut products, other nuts
20 ppb	Foods
0.5 ppb	Milk (M_1)

Source: US FDA (2000).

Mycotoxins are secondary metabolites and the toxic chemical products of fungi. Of the main classes of mycotoxins, *Aspergillus flavus* and *Aspergillus paraciticus* are the prominent producers of aflatoxins. Aflatoxin B_1, the most toxic of the four major aflatoxin groups B_1, B_2, G_1 and G_2, is a natural carcinogen and a common contaminant of foods. Kenya experienced an outbreak of acute aflatoxin poisoning (aflatoxicosis) in April of 2004, resulting in over one hundred deaths. The source of aflatoxin during that outbreak was traced to contaminated maize which had been locally grown and stored in conditions conducive to aflatoxin-producing mold. In 2005, another outbreak followed. The situation in Kenya highlighted the seriousness of aflatoxin to food security, in terms of both quantity and quality, and redirected international efforts to addressing contaminants and toxins within the food chain. With nearly a quarter of the world's crops susceptible to mycotoxins, implementing preventative measures is seen as essential to controlling the spread of aflatoxin within the food chain and protecting public health.

The occurrence of aflatoxin is heavily influenced by conditions favorable to mold growth during pre- and post-harvesting processing and storage. These conditions include high temperatures (36 to 38°C) and high humidity (above 85%) (Hell & Mutegi, 2011). Contamination can occur in the field before harvest or soon thereafter, although a recent analysis of more than 300 home-stored food samples in Uganda found no evidence of aflatoxin presence above threshold levels in maize, groundnuts or cassava that had been in storage for less than one month (Shively, pers. comm.). More commonly, growth of the molds during storage occurs if conditions are favorable. Periods of drought and insect damage also increase a plant's vulnerability, as weakened seed kernels of plants are more susceptible to fungal growth. Dietary staples such as maize, peanuts, cassava, and rice are some of the more common agricultural commodities that have been found to be contaminated with the toxins, and poor processing and storage leave developing countries particularly vulnerable to contamination (Farombi, 2005). In countries where food is scarce and diets lack diversity and rely heavily on staple foods, aflatoxin exposure presents an even higher risk. Other commodities and foods that can be affected include spices, tree nuts, dried fruit, meat, and milk (aflatoxin M_1).

Human and animal health and economic consequences

The health implications of aflatoxins on humans have not been thoroughly studied, but evidence to date suggests that liver damage, liver cancer, and impaired growth are the main consequences of exposure. Exposure to aflatoxin can be both acute and chronic. Chronic exposure in small amounts over extended periods of time places those exposed at risk of liver cancer and immune suppression. In children, growth deficiency may occur. Aflatoxicosis, a result of acute aflatoxin poisoning, can result in hemorrhage, hepatitis and liver damage, edema, and death. In addition to strong evidence that aflatoxin B_1 contributes to high incidences of liver cancer (particularly in individuals with Hepatitis B or C), exposure to aflatoxin in children ages nine months to five years has been associated with stunting and being underweight (Bryden, 2007; Gong et al., 2002). Among children in Africa, aflatoxin has also been associated with kwashiorkor, a form of malnutrition characterized by protein deficiency (see Hendrickse et al., 1982; Lamplugh & Hendrickse, 1982; and Ramjee et al., 1992).

Livestock animals which consume high quantities of contaminated feed may experience a weakened ability to absorb nutrients, slower growth, lower productivity (such as reduced milk production in cows), and a suppressed immune system leaving the animal more vulnerable to disease and less responsive to vaccinations. As with humans, high exposure to aflatoxin can result in hemorrhagic necrosis and cancer of the liver. Mature animals are more resistant to the adverse effects of aflatoxin than young animals, and certain species are more sensitive to the toxin than others. It has been suggested that animals affected by aflatoxin should be supplemented with high quality protein and vitamin E (Osweiler, 2005).

The economic consequences of aflatoxin contamination include crop losses, reduced fertility and feed efficiency and utilization in animals, and the costs of monitoring, testing, and dealing with contaminated supplies. Developing countries have a more difficult time preventing the occurrence of aflatoxin, as applications of various control strategies can be costly and application of appropriate technologies and correct agricultural practices may be weak. Where testing materials are available, traveling expenses are incurred to move samples to a laboratory (if a laboratory is accessible) to determine the level of contamination. More recently, rapid field tests have been developed, but these remain prohibitively expensive for most uses. Commodities which have been contaminated with aflatoxin may have lower market values or may be banned from international trade.

What can be done?

Pre-harvest strategies to reduce mycotoxin levels include the use of fungicides and insecticides, irrigation to prevent moisture stress when water is limited, and proper timing of harvesting. To protect commodities from the growth of fungus or production of toxins before or during storage, grains must be dried quickly after harvest and aerated properly, and moisture content and temperature must be regularly measured. Sodium bisulfite, ozone, and ammonia are chemical treatments that may be used to detoxify contaminated grain, and hydrated sodium calcium aluminosilicate (HSCAS) has been identified as a dietary additive that can prevent the toxin's absorption when ingested (Bryden, 2007).

Unfortunately, many of the methods used to reduce contamination are costly and therefore out of reach in most developing country settings. Furthermore, regulations on the use of ammonia and other chemicals for treatment vary, and may restrict trade and shipments across borders. Hazard Analysis and Critical Control Points (HACCP) is a system devised to control various contaminants and hazardous conditions in food through prevention and the monitoring of the contaminants throughout the food system. Table 2 presents a range of possible HACCP applications for the prevention and control of mycotoxins.

Table 2 Possible application of HACCP to agricultural commodities, food and animal feed

Stage	Commodity	Hazard	Corrective Action
Preharvest	Cereal grains, oil seeds, nuts, fruits	Mold infestation with subsequent mycotoxin formation	- Use crop resistant varieties - Enforce effective insect control programs - Maintain adequate irrigation schedules - Perform good tillage, crop rotation, weed control, etc.
Harvesting	Cereal grains, oil seeds, nuts, fruits	Increase in mycotoxin formation	- Harvest at appropriate time - Maintain at lower temperature, if possible - Remove extraneous material - Dry rapidly to below 10 percent moisture
Postharvest, storage	Cereal grains, oil seeds, nuts, fruits	Increase and/or occurrence of mycotoxin	- Protect stored product from moisture, insects, environmental factors, etc. - Store product on dry, clean surface
Postharvest, processing and manufacturing	Cereal grains, oil seeds, nuts, fruits	Mycotoxin carryover or contamination	- Test all ingredients added - Monitor processing/manufacturing operation to maintain high-quality product - Follow good manufacturing practices
Animal feeding	Dairy, meat and poultry products	Transfer of mycotoxin to dairy products, meat and poultry products	- Monitor mycotoxin levels in feed ingredients - Test products for mycotoxin residues

Source: Park, D., Njapau, H., & Boutrif, E. (1999). "Minimizing risks posed by mycotoxins utilizing the HACCP concept." Third Joint FAO/WHO/NEP International Conference on Mycotoxins, Tunis, Tunisia.

Evidence and situation in Nepal

The first examination of mycotoxin levels in Nepal began in 1978 as part of the Regional Monitoring of Food Contaminants Project underneath the FAO and United Nations Environmental Programme (UNEP). Food samples were taken from four countries (Nepal, India, Pakistan, and Sri Lanka) and tested for aflatoxin between 1980 and 1987 (Karki &

Sinha, 1991). Results showed that corn and peanuts were the two commodities most prone to aflatoxin contamination, and the plain area of the Terai provided the most favorable environment for the growth of *A. flavus* given the region's high temperatures and humidity. Karki and Sinha (1991) note that the process in which corn is harvested in Nepal, at high moisture content (18-21%) and then dried and stored without being shelled for six months, provides ample opportunity for the growth of molds. The transport of grains from the Terai region to food-deficit areas in the Hills and Mountains provides further opportunity for aflatoxin contamination. Another 832 samples were randomly collected between 1995 and 2003 from 16 districts in eastern Nepal to test for aflatoxin (Koirala et al., 2005). High levels (greater than 30 ppb) were detected in peanuts, corn flakes, peanut butter, and vegetable oil. One-third of all samples (32.8%) were contaminated with aflatoxin B_1 or B_2.

Although corn and peanuts are two of the most commonly reported food commodities for contamination of aflatoxin, rice represents an equally important Nepalese staple food at risk for growing aflatoxin producing fungi. Lack of proper storage facilities, particularly in the storage of rice grains that must be dried in the sun during the wet season, may result in higher moisture content making the grains more prone to fungi and bacteria. A study conducted by Reddy, Reddy, and Muralidharan (2008) detected levels of *Aspergillus* species in nearly all 1,200 rice samples collected across twenty states in India. The most frequent toxin detected was *A. flavus,* the producer of aflatoxin. Degrees of contamination were heavily influenced by storage conditions and environmental factors in each state, with levels of aflatoxin B_1 most prominent where storage conditions of rice were open and exposed to rain. This finding from India suggests aflatoxin problems in rice likely extend to Nepal, although specific evidence is lacking.

Evidence and situation in Uganda

With a mild and humid climate where the average temperature is 25°C and bimodal rainfall is 500 to 2000 mm a year, Uganda has favorable conditions for the growth of aflatoxin producing fungi (Kaaya & Warren, 2005). The earliest attempt at estimating the aflatoxin contamination in food was conducted in 1996 on groundnuts sold throughout the country. Fifteen percent of the sample was estimated to contain more than 1000 ppb of aflatoxin B_1, dramatically exceeding the FDA's maximum level of 20 ppb. An additional study in which 480 different food samples were analyzed found beans to be the most seriously contaminated, followed by maize and sorghum. Rice was found to have no aflatoxin contamination. In 54 samples collected from the districts of Kampala and Jinja, Sebunya and Yourtee (1990) found that poultry feeds and corn contained 20 ppb of aflatoxin B_1. Peanuts were the most powerful carrier of the aflatoxigenic fungi producing aflatoxin B_1, B_2, G_1 and G_2. Another study of Ugandan maize suggested that seven of eight samples obtained from shops and markets were contaminated by the aflatoxin B group at a level between 0 and 50 ppb during the storage period and more than 30% exceeded the 20 ppb level.

Studies have also shown that imported baby foods contained fewer fungi than domestically manufactured food, although both categories were *aspergillus flavus*. According to Kaaya and Warren (2005), consumption of aflatoxin contaminated food was highly correlated with hepatoma (the most common type of liver cancer) in Uganda.

Factors associated with aflatoxin contamination in Uganda include slow drying techniques, inappropriate storage methods on farm and retail levels, high moisture content and insect damages in grains, and physical damage during harvest that makes maize and groundnuts vulnerable to fungal infection (Kaaya & Warren, 2005). Strategies aimed at controlling aflatoxin include preventing the infection process, taking control of environmental factors, and enhancing management both pre-harvest and post-harvest. This can be achieved through the use of atoxigenic fungi to compete over toxigenic fungi, timely harvest, rapid and adequate drying, packaging and harvesting, and discouraging the consumption of "high risk" food (Hell and Mutegi, 2011). Few reports address aflatoxin prevention strategies in Uganda, though several institutions have research on aflatoxin underway. These institutions include Makerere University, the Government Chemist Laboratories, and the Uganda National Bureau of Standards. Some institutes in the National Agricultural Research Organization (NARO) also work with Makerere University on mold and aflatoxin issues (Kaaya and Warren, 2005).

This note was made possible through support provided by the Bureau of Economic Growth, Agriculture and Trade, U.S.A.I.D. through the Global Nutrition Collaborative Research Support Program. Opinions expressed herein belong to the authors and do not necessarily reflect the views of the sponsoring agency.

References

Bryden, W. L. (2007). Mycotoxins in the food chain: Human health implications. *Asia Pacific Journal of Clinical Nutrition*, 16: 95-101.

Farombi, E. (2006). Aflatoxin contamination of foods in developing countries: implications for hepatocellular carcinoma and chemopreventive strategies. *Afr. J. of Biotechnology*, 5(1): 1-14.

Gong, Y., Egal, S., Hounsa, A., Turner, P., Hall, A., Cardwell, K., & Wild, C. (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: The critical role of weaning. *Int'l. J. of Epidemiology*, 32: 556-562.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African J. of Microbiology Research*, 5(5): 459-466.

Kaaya, N. A., & Warren, H. L. (2005). A review of past and present research of aflatoxin in Uganda. *African J. of Food and Agriculture and Nutritional Development*, 5(1).

Karki, T. B., & Sinha, B. P. (1989). Mycotoxin contamination of foods and feeds in Nepal. In R. Semple, A. Frio, P. Hicks, J. Lozare (eds.), *Mycotoxin prevention and control in foodgrains,* 282-287. Bangkok: UNDP/FAO/ REGNET.

Koirala, P., Kumar, S., Yadav, B. K., & Premarajan, K. C. (2005). Occurrence of aflatoxin in some of the food and feed in Nepal. *Indian J. of Medical Sciences*, 59(8): 331-336.

Osweiler, G. (2005). Aflatoxins and animal health: Occurrence of aflatoxins in Iowa and neaby states. *Veterinary Diagnostic Laboratory.* Iowa State University, Ames, IA, USA.

Park, D., Njapau, H., & Boutrif, E. (1999). Minimizing risks posed by mycotoxins utilizing the HACCP concept. Third Joint FAO/WHO/NEP Int'l Conference on Mycotoxins, Tunis, Tunisia.

Reddy, K., Reddy, C., & Muralidharan, K. (2009). Detection of *Aspergillus* spp. and aflatoxin B_1 in rice in India. *Food Microbiology,* 26(1): 27-31.

Sebunya, T. L., & Yourtee, D. M. (2007). Aflatoxigenic Aspergilli in foods and feeds in Uganda. *Journal of Food Quality*, 13(2): 97-107.

US FDA (2000). Guidance for industry: Action levels for poisonous or deleterious substances in human food & animal feed. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm.

PURDUE
UNIVERSITY

Tufts
UNIVERSITY
Gerald J. and Dorothy R.
Friedman School of Nutrition
Science and Policy

The Global Nutrition CRSP
Nutrition Collaborative Research Support Program

USAID
FROM THE AMERICAN PEOPLE