FOOD CHAIN MYCOTOXINS 2010: THREATS AND SOLUTIONS

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Mycotoxins are produced by fungi found in both animal feedstuffs and human foods. These naturally-occurring poisons can cause kidney and liver damage, cancer, suppress the immune systems, induce malnutrition and interfere with the reproductive system among other acute and chronic disease states The reproductive effects include malformation of the genitals, infertility, feminization of males and early puberty and breast development in a variety of mammals, including humans.

Aflatoxins (found mostly in corn, peanuts, soy, cottonseed and nuts) are the best known mycotoxins and cause liver damage and liver cancer along with immune suppression and disruption of absorption and metabolism of essential nutrients.

Aflatoxins are produced by Aspergillus flavus and A. parasiticus which grows at 14-30% moisture, grows best around 25^{o}C and doesn't grow much <12^{o}C or >41^{o}C. Since only 20ppb total aflatoxins are allowed in US human food and dairy feeds and US milk must be less than 0.5ppb, aflatoxin is well-monitored by most feed companies. Those that produce feed for use on their own stock farms often lack the resources and motivation to test each bin, tank or silo for this known carcinogen and immunosupressant. And there is no widespread systematic monitoring of US dairy products for the M1 form of aflatoxin produced by animals fed mycotoxin-contaminated feed.

Zearalenone, found in grains (primarily corn), is one of the most powerful environmental estrogens known and, in contrast to aflatoxin, is not as frequently monitored at any step of the food chain, except in the case of some hog feeds. DON (vomitoxin) is produced by fusarium mold and causes reduced feed intake and a range of adverse symptoms in infected corn as well.

These bad actors have been followed by emerging mycotoxins such as citrinin, ochratoxin, fumonisins, and others, with various effects including severe damage to the kidney, the brain and give dairy producers false positive field tests for antibiotics in the milk. The purpose of this presentation is to discuss the threat, the detection and the means to stop the penetration of these mycotoxins into the human food at the retail, wholesale and production levels. This would include feeds and foods offered in commerce, as well as those produced, stored and processed on the farm.

Pre-emptive systematic characterization of what is going on throughout the food chain should help prevent outbreaks of animal and human disease rather than merely explain them in a post-disaster assessment.

AFLATOXINS SUPPRESS THE IMMUNE SYSTEM

Gambian children with detectible circulating aflatoxin adducts had lower IgA concentrations than those that did not and also had weaker responses to challenges by pneumococcal antigen (Turner et al. 2003). In their recent review of aflatoxicosis, Williams, et al. (2004) anticipated publication of results showing suppression of a variety of immune cell types and functions for Ghanaians with above average aflatoxin albumin adducts. Williams et al (2004) also infer that more rapidly turning over biomarkers, such as urinary AFM1, might be better matched to the pace of immune system modulation. Cusumano et al. (1996) found human monocytes were functionally impaired by AFB1.

In animal models, where prospective controlled dosage experiments are possible, the full extent and nature of damage to the immune system is better characterized. Hatori et al. (1991) found decreased CD4 cell numbers and associated drops in IL-2 when mice were dosed with AFB1. And a wide variety of animal models (mice, chickens, rats and swine) have shown that aflatoxin not only wrecks havoc on thymic and splenic T-lymphocytes (Pier at al. 1986, Ali et al. 1994), but also compromises the macrophages that envelope and present challenges to the lymphocytes (Richard and Thurston, 1975, Neldon-Ortiz 1991, Moon 1999).

It is little wonder that the most common acquired immune deficiencies studied in our food animals are caused by mycotoxins. Preliminary work in Haiti and Kenya has given us reason to believe that Haitian citizens have more than HIV to worry about in regards to immune competence, as well.

Stunts growth, anti-nutritive

Long known to impede growth in farm animals (Shane 1993) in the range of acute exposures reported for humans, aflatoxins have recently been implicated in stunting of children in Benin and Togo (Gong et al 2002, 2003, 2004) and perhaps causing kwashiorkor but certainly delaying recovery from the that condition (Adhikari, et al. 1994). Zinc, selenium, and vitamin A and levels are cut by half or more in animals fed aflatoxin, but less information is available for humans. None of this is surprising since aflatoxin binds the DNA responsible for the synthesis of proteins that represent the growth of young animals and the proteins responsible for the absorption of minerals and the binding and transport of vitamins.

Cirrhosis, liver cancer

These conditions are the usual focus of discussions of aflatoxin toxicology, but not here. Suffice to say that the livers of Third World peoples are no less susceptible to a given dose of aflatoxin (Gorelick et. al, 1993) and the connections among hepatitis B, aflatoxin and both cirrhosis and liver cancer are well documented for children and adults (Egner et. al 2001) in developing countries .

Role of mycotoxins in enhanced HIV infection.

Williams et al. (2010) have discovered strong associations between infection with HIV, the incidence of AIDS developing from those HIV infections and the inclusion of maize in the diets of Africans. Although the role of aflatoxin (commonly found in maize) in the suppression of the immune system is well known, the newly elucidated association seems to be more closely related to the presence of fumonisin. So in addition to checking horse feeds for the fumonisin that causes brain damage in horses, the time has arrived to assess the human food chain as well.

Mycotoxins as endocrine disruptors

Zearalenone (and related compounds and isomers) is produced by Fusarium molds that grow best at 20-25^{o}C at an optimum moisture of 45% but can grow at anything above 25%. Optimal toxin production requires a cool (15^{o}C) period after the fungi has established itself. These toxins are powerful environmental estrogens and reproductive toxins. These reproductive effects include malformation of the genitals, infertility, feminization of males and early puberty and breast development in a variety of mammals, including humans. Tomaszewski and others reported that women had elevated circulating zearalenone associated with hyperplastic endometria (47.8 ng/ml) and actual endometrial cancer (167.0 ng/ml) compared to women with normal uteri (below detection). Szuetz found early thelarche in Hungarian girls was associated with elevated zearalenone in the sera and food of these patients. Similar suggestions were made concerning an outbreak of precocious puberty in Puerto Rico (Saenz 1985), but in the latter case, no serum measurements were taken.

Improvements in animal nutrition have permitted US livestock to be bred earlier than was possible 50 years ago. Increased knowledge and application of nutritional sciences coupled with higher intakes of calories and reductions in physical activity have been accompanied by increased growth, increased obesity and decreased age at thelarche and first menstruation for US girls as well.

But, the dramatic increase among American girls for early puberty, early breast development and early development of adult secondary sex characteristics signal the presence of estrogen in these children's environment. A powerful estrogen like zearalenone bears examination as a contributor to this problem. New York's climate is ideal for producing zearalenone, and although many commercial swine feeds have been tested privately for this powerful environmental estrogen (commonly disrupts reproduction in both male and female pigs), most of our other livestock feeds and human foods are not.

DETECTION OF MYCOTOXINS

Dramatically increased awareness of the hazards of mycotoxins has led to the development and marketing of a wide variety of rapid detection methods, although the

quality varies. Part of the ongoing work in Cornell Animal Science Department is evaluating the various methods.

Visual examination of samples is the oldest and, for some commodities (e.g. peanuts) can be surprisingly effective. Counting the proportion of broken, soft, light, insect infested and (of course) moldy peanuts in the shell is quite predictive of the presence of aflatoxin, but is fairly subjective. This can be done with other commodities, but not as well: corn, cottonseed meal, etc.

Ultraviolet light can be used to look for aflatoxin, since it glows blue or green under such a lamp. Unfortunately, kojic acid, a common mold product, glows even brighter blue than aflatoxin, resulting in false positive tests for aflatoxin that frustrated many in the feed production and processing industry.

While HPLC, TLC and LC/MS methods are useful for precise, sensitive research and commercial lab services, they are not really viable on-farm methods. Fortunately, several companies have developed lateral diffusion immunoassays (dip sticks) and small ELISA columns available to pull a wide variety of mycotoxins from sample extracts for inexpensive fluorometry. The UN began work on well plate immunoassays which are evolving into useful techniques for commercial use.

ENTEROABSORBANTS

A recurring vision for those working in feed protection is an additive that can bind to mycotoxins and prevent their absorption by the animals fed contaminated feed. Unfortunately, there have been few successes in this area, and they tend to be of rather narrow application. For example there are hydrated sodium calcium alumino-silicates (HSCAS) that can selectively bind aflatoxin B1 without depleting micronutrients and are widely used in animal feeds (Williams et al. 2004). A few other clays of similar chemistry and mineral lattice architecture have some efficacy as well.

CHEMICAL TREATMENT

Once mycotoxins are formed in feed, there is not much one can do to get rid of them. In theory, a combination of heat with ammonia can irreversibly detoxify aflatoxins. Although what that does to feed texture and palatability is another story. Just heat or just a base other than ammonia can make the blue glow go away, but the feed is not protected. Ammonia can help prevent mold growth some, but not as well as propionic acid.

Propionic acid can help inhibit mold growth (and thereby prevent the production of mycotoxins). So if one finds themselves having to transfer high moisture corn or other fermented material from one place to another for subsequent storage or remote and delayed feeding, adding this silage preservative can prevent the mold growth that often happens under those circumstances.

Propionic acid is also the rare exception to the general lack of post synthesis destruction of mycotoxins: it can destroy citrinin at propionate concentrations used for general silage preservation.

BLENDING

Diluting an adulterated feed with clean feed to bring the total level below regulatory or toxic thresholds is tempting and often practiced. But the FDA frowns on this practice except in dire regional emergencies. In the view of some, mixing an adulterated feed with a clean feed produces a larger amount of contaminated feed. While that view is a non-quantitative way of looking at the world, giving problems inherent in getting representative samples of feeds for mycotoxins, it may have more merit than it seems.

Currently, to legally blend feeds contaminated with aflatoxin, one must get the permission of the regional FDA authorities and if clean feed is hard to find in your region, then one will usually be allowed to blend down anything with less than 500ppb total aflatoxins. So if you plan to meet the 300ppb standard for finishing beef cattle and your contaminated elevator is at 700ppb, you are out of luck. Similarly, if you are in a region where blending is OK'd by the FDA because of an emergency situation and you are trying to make dairy cattle feed (20ppb) by blending down a tank of 300ppb feed, then you will need a lot of clean feed.

Table 1. FDA action levels for aflatoxins (Food and Drug Administration, 2000)

Commodity	**Action Level ppb**
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)	300
Corn and peanut products intended for finishing swine of 100 pounds or greater	200
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20
Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20
Brazil nuts	20
Foods	20
Milk	0.5 (aflatoxin M1)
Peanuts and Peanut products	20
Pistachio nuts	20

The most current FDA DON advisory levels were updated on July 7, 2010 and are as follows:

1. "1 ppm DON on finished wheat products, e.g. flour, bran, and germ, that may potentially be consumed by humans. FDA is not stating an advisory level for

wheat intended for milling because normal manufacturing practices and additional technology available to millers can substantially reduce DON levels in the finished wheat product from those found in the original raw wheat. Because there is significant variability in manufacturing processes, an advisory level for raw wheat is not practical.

2. 10 ppm DON on grains and grain by-products (on an 88% dry matter basis) and 30 ppm in distillers grains, brewers grains, and gluten feeds and gluten meals derived from grains (on an 88% dry matter basis) destined for ruminating beef and feedlot cattle older than 4 months and ruminating dairy cattle older than 4 months, with the added recommendations that the total ration[2] for ruminating beef and feedlot cattle older than 4 months not exceed 10 ppm DON, and the total ration for ruminating dairy cattle older than 4 months not exceed 5 ppm DON. For chickens, 10 ppm DON on grains and grain by-products with the added recommendation that these ingredients not exceed 50% of the diet of chickens.
3. 5 ppm DON on grains and grain by-products destined for swine with the added recommendation that these ingredients not exceed 20% of their diet.
4. 5 ppm DON on grains and grain by-products destined for all other animals with the added recommendation that these ingredients not exceed 40% of their diet.

[2] The total ration includes grains, all grain by-products including distillers and brewers grains, hay, silage, and roughage."

Table 2. FDA Guidance for Fumonisins (June 18, 2009)

Class of Animal	Feed Ingredients & Portion of Diet[1]	Levels in Corn & Corn By-products[1]	Levels in Finished Feeds
Equids and Rabbits	Corn and corn by-products not to exceed 20% of the diet **	5 ppm	1 ppm
Swine and Catfish	Corn and corn by-products not to exceed 50% of the diet**	20 ppm	10 ppm
Breeding Ruminants, Breeding Poultry and Breeding Mink*	Corn and corn by-products not to exceed 50% of the diet**	30 ppm	15 ppm
Ruminants >=3 Months Old being Raised for Slaughter and Mink being Raised for Pelt Production	Corn and corn by-products not to exceed 50% of the diet**	60 ppm	30 ppm
Poultry being Raised for Slaughter	Corn and corn by-products not to exceed 50% of the diet**	100 ppm	50 ppm
All Other Species or Classes of Livestock and Pet Animals	Corn and corn by-products not to exceed 50% of the diet**	10 ppm	5 ppm

[1] Food and Drug Administration, 2009

FDA has not yet committed to advisory, guidance or action levels for citrinin, ochratoxins, or zearalenone.

INCINERATION

Burning corn directly for to generate electricity to power vehicles is far more efficient than converting it to ethanol and burning it in a stove for heat is even more efficient. Unlike conversion of contaminated corn to ethanol, burning it does not leave a toxic byproduct feed behind. And burning “red-tagged” corn does not remove food from the economy, if it is too toxic to feed or eat anyway.

RECENT MYCOTXIN WORK AT CORNELL ANIMAL SCIENCE

In 2006, Dr. Patricia Wolf from Meds and Food for Kids (NGO active in Haiti) sent samples to our laboratory and the initial levels we found in Haitian peanuts were alarming: 380-1567ppb total aflatoxins with an average of 797.5 +/- 218.5ppb by ELISA and fluorometry. HPLC analysis showed that January harvest samples were 88.5% B1, 11.5% B2 and May samples were 77.9% B1, 11.4 %B2, 8.9 % G1, and 1.7% G2, indicating that in May there were probably two species of Aspergillus (both *Aspergillus flavus* and *Aspergillus parasiticus*) making the toxin in Haiti.

On our advice, MFK began sorting the peanuts visually and removing kernels that floated in water. In September 2006, MFK found market peanuts which we analyzed at 412.5 +/- 32.1ppb. In November 2006 some farmer-stored, were found that had 125 +/- 7.1 ppb aflatoxin. The same month after stringent bulk selection on a farm in Port Margot, MFK found peanuts with 26.8 +/- 7.0 ppb. Still above US standards but much better. In January 2007, the PI directed some trials of sorting and floating procedure that resulted in a peanut supply that tested at 0.20 +/- 0.10 ppb, showing that Haitian manufacturers can eliminate most of the threat of aflatoxin by manual methods.

We have been successful in receiving long term funding for similar work in Haiti and Kenya and anticipate beginning to apply much of what we have learned overseas to the intake, metabolism and transmission of mycotoxins by New York livestock this month (October 2010).

The 2009 harvest year has been wet, cool, and delayed in many places and provided us with some moldy grains and byproducts to practice on and we are engaged in making sure we know, before contaminated foods arrive at the market, what is going on in the mycotoxin world on New York farms.

It is hoped that programs such as this will help producers regain control of food chain mycotoxin levels.

OBJECTIVES FOR CORNELL FEED MYCOTOXIN RESEARCH

To create a useful, annotated database that provides planners and extension personnel with the following:

1) Measurements of the incidence of aflatoxin and zearalenone incidence in corn produced and stored for use on 30-100 New York State animal production units per year for three years.
2) Measurements of the incidence of aflatoxin and zearalenone in commercially available animal feed from 30-100 companies of various sizes.
3) Measurements of citrinin, DON and ochratoxin in those above feeds destined for dairy stock and fumonisin in those destined for horses and human populations.
4) Published summary of practices and seasonal climate variables associated with these findings.
5) Measurements of the incidence of aflatoxin and zearalenone incidence in snack foods, milled grains, dairy and meat products produced in New York from 30-100 retail outlets across the state.
6) Investigation of how much of the mycotoxin that reaches livestock is transferred to food products. We plan to apply Wang's blood aflatoxin-lysine methods to dairy cattle so they can be used to sample feed over time, avoiding the problems of mold infection heterogeneity.

The following three populations will benefit from this work:

1. Consumers of New York State foods made from crops susceptible to aflatoxin contamination.
2. Animals and their owners that consume feeds grown and processed in New York.
3. The feed and food producers themselves that will find themselves ahead of the when public concern demands low mycotoxin foodstuffs.

In particular, there are sub-populations of New York citizens that will benefit from this work. Individual farmers, small processors and consumers lack the resources to fully and frequently test their foods for these contaminants. With exception of aflatoxins, Federal and State requirements for routine testing of these mycotoxins are entirely inadequate to follow these poisons through the food chain. Even in the case of aflatoxins, on-farm testing is rarely applied as regulatory focus falls on feeds and foods offered for sale after off-farm storage and processing. For this reason, public funding is needed to establish ways to fix this problem that can later be shared with private mycotoxin monitoring enterprises. We expect that systematic surveys of grains produced and processed in New York and the products made from them by both factories and livestock will show us where the critical points can be found to turn off the flow of mycotoxins that would otherwise harm our domestic animals and ourselves. We can't test all foods produced here, but examining a few representative product pathways can tell us a great deal about the rest of them. Because New York has both the warm humid summers molds need to establish themselves and the cool storage periods that Fusarium molds need to manufacture zearalenone and fumonisin, its small farms and

moderate sized feed and food processors are particularly vulnerable to this kind of threat.

We expect to have a database of information concerning the incidence of aflatoxin and zearalenone in a large sample (30+ enterprises at each level) of homegrown feeds, commercially sold feeds, and retail stock feed and New York food grain products including snack foods, dairy and meat. (and possibly a smaller base for citrinin and ochratoxin in dairy feeds and fumonisin in feeds destined for horses and humans). When they go on line, we plan to sample distillers' grains from ethanol producers, as well.

With this information, we can trace contamination forward and back through the food chain (in full consultation of actors at each level). The first impact will be awareness on the part of the feed and food industry, the second will be reductions based on recommendations of known steps to reduce contamination and the final step will be experimental implementation of new techniques.

Additional updates regarding approved detection methods and allowed strategies for reducing the impacts of mycotoxins will be presented at the oral presentations. FDA mycotoxin guidance and variance policies change regionally and with each harvest season.

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