THE CARRY-OVER OF AFLATOXINS IN DAIRY FEED TO MILK OF MODERN HOLSTEIN DAIRY COWS

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Aflatoxins are hepatotoxic and carcinogenic secondary metabolic products from the fungal species *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 (AFM1) is the major metabolite of Aflatoxin B1 (AFB1) present in mammalian milk. The US Food and Drug Administration (FDA) dictates a maximum allowable concentration of 20 μg/kg total aflatoxin in food and feed intended for dairy consumption, and 0.5 μg/kg AFM1 in milk and milk products. The European Commission dictates a maximum allowable concentration of 4 μg/kg total aflatoxin and 0.05 μg/kg AFM1 respectively. The carry-over of AFB1 (the amount of AFB1 in the feed that is excreted as AFM1 in the milk) is a major factor used to create regulations for acceptable AFB1 concentrations in dairy cattle feed. It has been observed that higher producing dairy cows (30-40 kilograms of milk per day) have a higher carry-over rate, but current regulations use older studies using low-producing dairy cows (10-20 kilograms of milk per day) as a reference for risk. The objective of this project was to measure the carry-over rate of AFB1 from feed to AFM1 in the milk of modern, high-producing US Holsteins milked three times a day to provide a more relevant assessment for current regulations. Corn naturally infected with aflatoxin-producing fungi was used to imitate a real-world contamination scenario, an approach applied in only one previous study (Frobish et al., 1986). Three replications of a feeding trial to test carry-over in high-producing dairy cows were completed; each using

12 high-producing dairy cows in early- to mid-lactation, fed naturally contaminated corn meal top-dressed on their daily total mixed ration. Cows in each replication were assigned to: control (0 μg/kg), low (10 μg/kg), or high (20 μg/kg) AFM1 groups. Feed and milk samples were taken for seven (replicates 1 and 2) or two (replicate 3) days and analyzed with a VICAM fluorometer. Using linear regression, the direct carry-over rate was 6.5%, much higher than the 1 to 2% estimated by previous researchers using low-producing dairy cows. These findings suggest that the US regulatory limit of 20 μg/kg of total aflatoxin in the feed is not a guarantee of protection against violating the regulatory limit of 0.5 μg/kg of AFM1 in milk of high-producing dairy cows.

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LITERATURE REVIEW

Anti-Quality Components of Forage

Anti-quality components of forage are defined as any factor that diminishes the degree to which a type of forage meets the nutritional requirements of a specific kind and class of animal. These components affect the ability of grazing animals to reach their potential for growth and reproduction. As the definition is quite inclusive, there are a large variety of anti-quality components affecting plants including phytochemicals in plant tissues, insect damage, secondary metabolites of microbes living on the plant, and also structural inhibitors in leaf and stem arrangement reducing intake (Allen and Segarra, 2001). Palatability, rate of passage, digestibility, nutrient density and balance, and intake are all factors determining the degree to which the forage is able to meet the nutritional demands of the animal (Fryxell, 1991).

Contemporary livestock production systems are increasing focus on the use of forage in animal diets for economic efficiency, environmental concerns, and animal well-being (Howarth and Goplen, 1983). Research focused on minimizing anti-quality components, thus helping maximize forage quality, is essential to efficient and successful use of forages in animal production systems.

This review summarizes types of anti-quality components and their effects on forage quality, animal effects these components may have, and explores specifically the effects of aflatoxin-producing *Aspergillus* sp on the forage quality of corn with respect to animal production.

Most of the chemical anti-quality components of forages belong to a group of related compounds with similar modes of actions. There are about 8,000 polyphenols,

270 non-protein amino acids, 32 cyanogens, 10,000 alkaloids and several saponins that have been reported to occur in various plant species (Kumar, 2003). Anti-quality attributes can reduce the digestibility of forage nutrients, produce toxic effects, or cause illness (Provenza et al., 1992). Studying any of these factors is quite difficult for a wide variety of reasons. Detection can be achieved through chemical evaluation or by using animal performance as a measure of anti-quality components. Chemical analysis can be difficult when there are multiple substances present and testing is only directed at known compounds. Furthermore, lesser known fodder trees and shrubs may contain unknown anti-quality components and their presence would only be revealed through feeding trials, not by chemical analysis directed at known compounds. It is also hard to quantify these chemicals, as there have been a wide variety of reported concentrations of certain compounds even in the same species of plant. It is also difficult to assess biological effects of different chemicals as there are such a variety of effects between species of animals and forages often contain more than one chemical anti-quality component (Kumar, 2003).

Economic Impacts of anti-quality components in forages

Economic consequences of anti-quality components of feeds can be severe if the loss is even a single animal with high economic value. The economic effect is much less obvious when the result is a subtle decrease in potential performance like growth or milk production. The greatest economic impact of anti-quality components is diminished forage quality with lowered potential for gain. If the impediments to quality were identified and eliminated, it might be more cost effective than shifting management focuses to expect reduced animal performance from lower quality feed. If even a small

proportion of these expected losses were eliminated through research, the potential economic impact would be very positive. This takes on added importance with increased recognition of forage as a feed resource (Allen and Segarra, 2001).

Forage Anti-Quality components effect on Animals

Because animals differ in nutritional needs and their ability to handle various toxins, high quality forage for one animal may be low quality for another. For example, forage that meets the nutritional needs for dry cows would be a high quality dry cow feed, but may not meet the requirements for milking cows and would be a low quality milking diet. Also, a chemical toxin or a physical inhibitor to intake for one species or class of animal may have little effect on another species or class of animal (James et al., 1992).

Some plant compounds reduce forage quality because they are nearly indigestible or have chemical effects that limit the digestibility of other plant compounds. For example, lignin and tannins can reduce forage digestibility by tying up nutrients. High content of indigestible compounds, such as lignin, silica, or waxes, can also decrease the digestive benefits of a plant and reduce animal preference for that species. Tannins also bind proteins and can decrease digestibility by deactivating digestive enzymes. Plant compounds, such as essential oils and tannins, have anti-microbial effects that kill microbes in the digestive system, thereby reducing forage digestibility (Provenza et al., 1992). Some of these compounds do not have overtly toxic symptoms, but cause the animal to feel ill or nauseous. This aversive post-ingestive feedback causes herbivores to decrease intake of foods containing toxins such as alkaloids in larkspur (*Delphinium spp*.) and tall fescue (*Festuca arundinaceae*), condensed tannins in blackbrush (*Coleogyne*

ramosissima), essential oils in big sagebrush (*Artemisia tridentata*) and juniper (*Juniperus sp*), and phytotoxins in mesquite (*Prosopis glandulosa*) (Provenza et al., 1992).

Animal behavior and adaptation are increasingly recognized as important aspects of anti-quality components. Animals, especially ruminants, have accrued behavioral and biological adaptations to combat anti-quality components of forages just as the plants have gained chemical and physical anti-quality components to combat being consumed by herbivores (Allen and Segarra, 2001). Selective grazing is one adaptation that grazing animals have developed. This is the first line of defense against the negative effects of plants with toxic or anti-quality components. Animals will select diets of higher quality than the average forage available. They also select plants and plant parts of relatively low toxin concentration (Provenza et al., 1992). Animals make these decisions by relating plant flavor to positive or negative digestive consequences. The consequences form the basis for dietary likes and dislikes, so the animal then seeks highly palatable foods and avoids aversive foods. The resulting behavior patterns generally lead to increased consumption of nutritious foods and limited consumption of toxic or low quality plants (Provenza, 1995). When foraging endeavors include several new types of plants, plants that dominate the diet may influence the importance of digestive or other feedback more than less-consumed plants, even if the less-consumed foods were responsible for the positive or negative feedback. Still, grazing animals have a strong natural tendency to select diets composed of several plant species and sample available plants on a regular basis. This behavior may increase the likelihood of ingesting necessary nutrients and reduce the potential of over-ingesting toxins (Provenza et al., 1992).

A plant's chemical and structural attributes dictate the potential digestible energy, nutrient yield, or toxicity of a plant. The digestion and detoxification abilities of ruminants and their rumen microbes, determine the actual yield of nutrients, energy, or toxins from the plant. The toxic effects of a plant are determined largely by the amount eaten, but rate of digestion is also important. Grazing animals can avoid excessive toxic effects by limiting their consumption of a specific toxic plant over time to allow sufficient time for detoxification, and to limit potential cumulative effects of specific toxins (Smith, 1992).

Usually, the liver primarily, and secondarily the kidney, intestinal mucosa, lungs, and skin contain enzyme systems that metabolize or alter toxic compounds, rendering them inert. The ability to metabolize or reduce sensitivity to specific phytotoxins varies by herbivore species and between individuals. Diarrhea also aids in rapid elimination of toxins from the gut thus reducing absorption from the intestines (Launchbaugh, 1996). The most important adaptive attribute of ruminants for ingesting toxic plants is the massive number of rumen microbes that transform most phytotoxins into inert or less-detrimental compounds. However, in some cases, such as nitrates or cyanogenic glycosides, the rumen microbes convert a harmless compound into a deadly toxin (Provenza et al., 1992). Rumen microbes facilitate the ability of animals to adapt to diets high in phytotoxins. Microbial populations can change rapidly depending on the substrates available for degradation. But, ruminants cannot always adapt to available toxic forages. The effects of many toxins are cumulative and animals may get progressively more poisoned as they continue to ingest plant material containing these toxins (Launchbaugh, 1996).

Fescue Toxicosis

Tall fescue (*Festuca arundinacea*) is one of the most important cool-season grasses grown in the United States occupying over 30 million acres. The attributes of tall fescue make it an attractive forage species because of its ability to withstand drought, poor soil conditions, and intensive defoliation from grazing. However, much of this fescue is infected with the endophyte-fungus *Neotyphodium coenophialum*. The endophyte lives in intercellular spaces of sheath, stem, leaf, and seed tissues. There is no invasion of plant cells nor does the endophyte become pathogenic. It is passed from generation to generation via seed, so infected plants create infected offspring and perpetuate the association (Thompson et al., 2001). In a survey of over 1500 pasture samples obtained throughout the United States, more than 70% of the samples had 60% or more endophyte infection rates (Shelby and Dalrymple, 1987). The endophyte protects the plant by discouraging grazing. Protection from defoliation suggests that solar energy capture and retention is a priority in this association. Reduced insect or livestock grazing results in more photosynthetically active leaf area, resulting in greater energy capture, greater energy reserves, and greater re-growth capacity when infected with the endophyte. Endophyte-infected tall fescue has greater forage and seed productivity than the endophyte-free form and is more drought tolerant (Hill et al., 1991). Hence, it is in the plant's interest to provide the needs for the endophyte; and the endophyte to provide protection to the plant against climatic and biological forces that maybe threatening.

The animal effects of fescue toxicosis can be severe. One symptom is heat intolerance. Outward signs of heat intolerance in cattle include standing in water, excessive use of shade, and rough hair coats. Heat intolerance affects the majority of the

herd when temperatures exceed 86^{o} F. The primary cause of heat intolerance appears to be vasoconstriction by ergot alkaloids produced by the endophyte. As a result, the animal loses its ability to dissipate heat through the skin and ears. Increased respiration rates are often observed as animals seek alternative methods to dissipate heat. In addition, cortisol also increases in the blood with increased intake of endophyte-infected tall fescue (Thompson et al., 2001).

Fat necrosis is another symptom. This occurs when blood flow to the body core decreases. Dead adipose cells are usually found interspersed with healthy cells in necrotic fat lesions. These hard, necrotic lesions can cause constriction of intestines, reproductive problems, and kidney failure in cattle (Thompson et al., 2001). Fescue foot is the most well known negative effect associated with grazing endophyte-infected tall fescue. Researchers estimate 20% of a herd grazing endophyte-infected tall fescue will be affected with this condition (Emile et al., 2000). Early clinical signs of fescue foot may appear three to seven days after cattle graze endophyte-infected fescue. These signs include a red line forming at the coronary band of the hind feet and skin discoloration and swelling, which will worsen if animals are allowed to remain grazing endophyte-infected tall fescue. Death of peripheral tissues can occur as a result of vasoconstriction and subsequent inadequate blood flow to the periphery. Fescue foot occurs more commonly in cool periods because cattle have normal vasoconstriction to conserve body heat compounded with vasoconstriction by ergot alkaloids. When lameness is first observed, cattle must be immediately removed from endophyte-infected tall fescue and fed an alternative feed (Thompson et al., 2001).

Decreased production is the most costly adverse effect caused by endophyte-infected tall fescue. Decreased production can result in significant economic losses to the livestock producer, because of lower cow and calf weights at the end of the grazing season due to decreased feed intake. Decreased calf weights have also been reported for calves grazing endophyte-infected compared to endophyte-free tall fescue and other forage grasses. Decreased weaning weights are caused in part by decreased milk consumption because cows grazing endophyte-infected fescue experience reduced milk production. Decreased milk production appears to be a result of decreased prolactin secretion in cows grazing endophyte-infected tall fescue (Peters and Grigsby, 1992).

It was also demonstrated that immunosuppression due to fescue toxicosis is long-lasting, and was measurable throughout the stress of cross country transportation and throughout a 150-day feedlot finishing period. The lowered immunity is likely to contribute to added costs of medications and labor in treating animals that are less-tolerant to stress and disease (Mayland and Cheeke, 1995).

Tannins

Temperate forages grazed in the leafy vegetative state have high concentrations of metabolizable energy and total nitrogen. Rumen digestion of readily fermentable and structural carbohydrate is efficient on such diets, but with nitrogen digestion, duodenal flow of non-NH_3 nitrogen (NAN) is only about 65 % of the nitrogen consumed (Ulyatt and Macrae, 1974). This is due to the extent of degradation of forage proteins to NH_3 by rumen micro-organisms (70–80 %) being much faster than the rate that NH_3 can be incorporated into microbial protein, resulting in high absorption of nitrogen as NH_3 from the rumen (Ulyatt, 1973). Subsequent research, using post-ruminal infusions of proteins

and amino acids or dietary supplementation with undegraded proteins, identified absorption of essential amino acids from the small intestine as limiting productivity in ruminants fed entirely on diets of high quality fresh forages ad libitum (Barry, 1981).

It was first shown in a laboratory study in 1977 that reactivity between condensed tannins and forage protein was pH dependent, with stable complexes being formed at pH 3.5–7.5, but the complexes dissociating and releasing protein at pH < 3.5. It was examined in animal studies that this protein reactivity could be the basis for increasing undegradable protein and essential amino acid absorption in ruminants fed entirely on diets of fresh forages (Jones and Mangan, 1977). Some browsing animals like deer have evolved production of condensed tannin-binding proline-rich salivary proteins, as a means of counteracting the plants' chemical defense against defoliation and of reducing the anti-nutritional effects of high condensed tannin concentrations (Austin et al., 1989). So far as we know, domesticated sheep and cattle do not produce condensed tannin-binding proteins in their saliva. This means dietary condensed tannins can be used to manipulate nitrogen and essential amino acid digestion in sheep and cattle fed on fresh forages to combat protein digestion-related issues. This is achieved through using condensed tannins to bind forage proteins in the less acidic rumen environment, avoiding microbial degradation to NH_3, and then the compound dissociating in the more acidic environment of the small intestine to be absorbed (Barry and McNabb, 1999).

Ruminants grazing forage diets are subject to a number of diseases, some of which have a nutritional component. Two of these conditions are rumen frothy bloat in cattle and internal parasite infections in grazing sheep, cattle, deer and goats. Regular oral administration of detergents in the case of bloat to disperse the foam and anthelmintic

drenches in the case of internal parasites to kill the parasites are used to combat these issues. Parasitism of the abomasum and small intestine causes extensive protein losses in sheep (Kimambo et al., 1988) and re-directs protein synthesis away from skeletal muscles and into repair of gut tissues, leading to reduced nitrogen retention (MacRae, 1993). Increasing dietary protein intake and abomasal infusion of protein results in the animal being much better able to tolerate these infections and improves nitrogen retention (Coop and Holmes, 1996) with the main effect of increased protein supply being to increase the rate of acquisition of immunity. In this way, condensed tannins could also be used to hasten the rate of immunity acquisition to internal parasites without external sources of antihelmintics, reducing increased costs of labor and medicine.

Because anti-quality components of forages differ in effects depending on the species and production status of an animal among other things, targeted research has been able to show that condensed tannins in forage can be used to improve the efficiency of nitrogen digestion in ruminants fed on fresh forage diets. Studies have observed increased wool growth, milk protein secretion, ovulation rate and aided the development of more nutritionally-based and ecologically-sustainable systems for disease control in grazing animals (Barry and McNabb, 1999).

Glycosides

Glycosides are naturally occurring compounds found in many rangeland plants and forages. These compounds can serve an important function in the life cycle of certain plants by attracting pollinators or seed dispersers or repelling herbivores and microorganisms, but they can also be highly toxic to grazing animals. Glycosides are a chemically diverse group of compounds that bear little resemblance to each other and

they can form toxic compounds upon hydrolysis. The variations on this chemical theme yield a variety of powerful toxic effects when animals eat plants containing glycosides. These toxic effects can be observed as restlessness, uncontrolled bleeding, convulsions, or rapid death (Majak et al., 2001).

Glycosides containing a toxic nitro-group (NO_2) are observed in several species of legumes. Acute clinical signs of toxicosis caused by nitro-containing glycosides include incoordination, distress, labored breathing, bluish skin or tongue, muscular weakness, and collapse. Death may occur within a few hours after ingestion of the toxin. In chronic poisoning, animals lose weight and develop respiratory distress, a poor hair coat, hind limb paralysis, and nasal discharge. Protein supplementation can enhance the activity of unique rumen bacteria capable of detoxifying this group of glycosides. These bacteria reduce the nitro group to the much less toxic amino group (Majak et al., 2001).

Hydrocyanic acid (HCN), is released from plant cells when the cell walls are disrupted during chewing and digestion. The cyanide is extremely toxic because it blocks the vital cellular process of aerobic respiration, which yields energy for cell and tissue function. Clinical signs of subacute and acute poisoning in cattle include rapid heart rate, rapid breathing, recumbency, darkening of the mucous membranes around the eyes and mouth, and convulsive contractions. Administration of nitrite-thiosulfate is the preferred treatment, especially if it is supplemented with oxygen (Cheeke and Shull, 1985).

Cardiac glycosides have a long history as medicines and poisons because of their powerful effect on the heart. Membrane bound proteins in the heart are the major receptors for cardiac glycosides. The presence of these glycosides results in more forceful contractions of the heart. Sub-acute to acute signs of poisoning in cattle and sheep

include restlessness, labored breathing, frequent urination and defecation, and irregular or rapid heartbeat. Treatments include administering activated charcoal, potassium chloride, atropine, digoxin-specific antibodies, beta-adrenergic blocking agents, procainamide and phenytoin (Cheeke and Shull, 1985).

Saponins are complex glycosides that are widely distributed throughout the plant kingdom. Saponins are noted for their ability to destroy red blood cells, even at low concentrations. Because of their low degree of absorption from the gastrointestinal tract, only a few species containing saponins yield toxic effects. Toxic saponin effects usually begin in the mouth and throat, causing permeability changes or loss of membrane-bound enzymes in mucosal membranes. These effects can result in intestinal lesions and severe inflammation of the digestive tract. Under these conditions, saponins may be absorbed from the stomach and intestines and produce liver damage, respiratory failure, violent convulsions, and coma. The adverse effects of saponins can be reversed by the addition of dietary cholesterol, presumably because saponins form insoluble complexes with cholesterol (Cheeke and Shull, 1985).

Bioactivation and toxicity of the glycosides mainly depends on the: 1) rate of digestion and hydrolysis by rumen microbes 2) rate of detoxification and 3) degree of absorption from the gastrointestinal tract. It's important to maintain animals with healthy rumen populations. The microbes in an herbivore's gut are the first line of defense against ingested toxins. Ruminants often graze or browse lightly on toxic plants, and rumen organisms may adapt to detoxify many toxins (Majak et al., 2001).

Combating effects of anti-quality components on forage quality

The difficulties of quantification and the complexity of the biological effects of

anti-quality components impede the development of methods to alleviate their effects. The simplest approach of dilution may reduce the risk of toxicity but the relative nutrition of the feed may not be optimal. Also, the required degree of dilution is difficult to recommend because of uncertain quantification (Kumar, 2003). The usefulness of management practices involving harvesting tree leaves at times when the concentration of anti-quality components are lowest is limited because of patterns of changes in concentration of various chemical anti-quality components (Vaithiyanathan & Singh, 1989). For a particular anti-quality component, the effect of season may also vary between plant species. It has also been noted that, as leaves mature, both the anti-quality components and nutrient contents decrease (Singh, 1982).

Many chemical anti-quality components are heat sensitive. Destruction through heat can be used by the feed industry but not by farmers. This is because heating would substantially increase the cost due to the energy involved both in the treatment and transport of the feed. Simple washing with water removes the soluble chemical anti-quality components but nutrients also leach out. Since anti-quality components usually play a major role in plant defense, genetic selection for low anti-quality component lines may have undesirable effects on the plant (Kumar, 2003).

From the animal side, there have been some studies looking at manipulating rumen microbe populations to combat certain anti-quality components of forages. The rumen environment is slightly acidic provides reductive and hydrolytic reactions which can decrease the biological activity of chemical anti-quality components before their absorption from the digestive tract. Rumen bacteria and fungi capable of degrading lignin have been isolated. Anaerobic degradation of flavonoid and hydrolyzable tannins by

mixed rumen microbes has also been demonstrated. Such rumen microbes are present in small numbers and their growth rate is slow. Anaerobic microbial degradation of condensed tannins has also been demonstrated (Kumar, 2003). Future research could be drawn towards identification of the various anaerobic and rumen microbes capable of destroying chemical anti-quality components, testing the survivability of organisms in the rumen and seeing whether the destruction is plasmid encoded, so that genetic manipulation of rumen bacteria can be used to ferment chemical anti-quality components (Russell and Wilson, 1988).

Anti-quality components of maize

Maize (Zea mays L.) is a vital feed and food grain worldwide. It responds well to irrigation and fertilizer to produce a large amount of consumable calories per ha. Being a cross-pollinated crop, and relatively easy to genetically manipulate, great advances have been made during the last half-century in improving the plant's architecture, pest resistance and overall yield characteristics (Bruns, 2003). In the United States, the current rate of increase in yield is about 1.0% per year, with most of this increase being a result of improved yields per unit of land area (Cassman and Duvick, 1999). One important anti-quality component affecting maize species is fungal infection. The biggest problem with this is not with loss of nutritional value, but with secondary metabolites called mycotoxins that several species are capable of producing which are extremely toxic to animals and humans. Mycotoxins can cause sudden death in poultry and livestock when concentrations are high. At lower levels in feed they can cause animals to become unthrifty, gaining at slow rates or making no weight gains at all (Cheeke and Shull, 1985). In dairy animals, on top of potential decreases in milk yields, feed intake and

immune function, certain mycotoxins can be passed through the animal into the milk and become a human health hazard.

Plant stress is a major factor in the infection of maize with certain anti-quality components. Root rot diseases are considered a disease complex involving a number of different fungi, nematodes, root-feeding insects and even some bacteria (White, 1999). Foliar diseases, such as blights and rusts, are also serious pests of maize and can greatly weaken the plant. Stalk rots, which can be caused by a number of different pathogens, may result in the premature death of the maize plant just before the developing kernels have reached physiological maturity. All such diseases are referred to as primary pathogens (Bruns, 2003).

One key to combat anti-quality components affecting forage quality is to minimize plant stress. High plant populations require lots of water and fertility, especially nitrogen. Irrigation is a requirement under these production conditions, particularly in the Mid South and Southeastern United States. Maize crops not supplied with sufficient amounts of these essentials throughout the growing season will become stressed very quickly and severely, thus becoming subject to fungal infection and mycotoxin production (Bruns, 2003). The management practices that have been found effective at reducing the incidence of mycotoxin contamination in the field include timely planting, proper plant nutrition, especially adequate amounts of nitrogen, avoiding drought stress, particularly during kernel filling, controlling certain insect pests and proper harvesting (Jones, 1979; Lisker and Lillehoj, 1991).

As part of adequate plant nutrition, sufficient levels of nitrogen are known to be important in reducing the risks of fungal infection and the development of mycotoxins

(Jones, 1979). Nitrogen is the central element in structural and metabolic proteins as well as nucleic acids. Maize plants suffering from nitrogen deficiencies during reproductive growth, will often translocate nitrogen from older leaf tissue to the developing grain and eventually abort the older leaves. Plant stress resulting from low nitrogen-fertilization rates was found to increase the incidence of mycotoxin contamination in maize (Lillehoj and Zuber, 1974). Jones (1979) stated that maize might be predisposed to mycotoxin contamination and other anti-quality factors like insect infestation due to insufficient uptake of nutrients associated with drought stress or leaching of mineralized nitrogen from the root zone due to excessive rain.

Phosphorus deficiency in maize during the early weeks of growth can result in a poorly developed root system, which in turn can reduce the plant's ability to take up adequate levels of other essential nutrients and water (Stoloff and Lillehoj, 1981). This could logically lead to the early onset of drought stress, which is an important prerequisite to fungal infection and mycotoxin development as well as other opportunistic anti-quality components. Phosphorus is also important to plant growth as it is incorporated into a number of vital biological compounds. It is a key element in nucleotides by virtue of being a component of the phosphate sugars found in DNA and RNA. It is also the key component in energy transfer compounds like ATP. This compound serves a number of vital functions, one of which is facilitating peptide bonds between certain amino acids in the formation of proteins (Bruns, 2003).

Also with regard to stress upon developing maize, weeds rob the crop of water, nutrients and sunlight. Certain weed species are also known to exude chemicals via their roots into the soil that stunt crop development, a process known as allelopathy (Rice,

1984). Heavy weed infestations in maize place the crop under considerable stress due to the competition they create.

Aflatoxin effects on animal performance

The two most common and toxic mycotoxin compounds are aflatoxin, produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, and fumonisin produced by *Fusarium moniliforme*. Fumonisins are known to cause leukoencephalomalacia in horses and pulmonary edema in swine (Harrison et al., 1990) and aflatoxins are both hepatotoxic and carcinogenic (Binder et al., 2007). Fungi that cause ear rots such as those caused by *Aspergillus spp*. and *Fusarium spp*. are opportunists, infecting and contaminating maize grain with mycotoxins after the plants have been stressed by some other factor, including other plant diseases. Any of these events increase the chances for infection of the grain by mycotoxin producing fungi, which are referred to as secondary pathogens or infections (Bruns, 2003).

Aflatoxins are the most dangerous of the mycotoxins. The most toxic of the aflatoxins is aflatoxin B1 (AFB1) that shows high carcinogenic properties to both humans and animals (Liu and Wu, 2010). In both humans and animals, aflatoxin B1 is metabolized by the liver creating, among others, a hydroxylated metabolite called aflatoxin M1. Both AFB1 and AFM1 are considered group 1a carcinogens (IARC, 2007). Aflatoxin M1 is of particular importance to the dairy industry as it is the major aflatoxin metabolite present in mammalian milk.

The amount of any mycotoxin produced by a fungal species will depend on physical factors (moisture, relative humidity, temperature and mechanical damage), chemical factors (carbon dioxide, oxygen, composition of substrate, pesticide and

fungicides), and biological factors (plant variety, stress, insects, spore load) (Bryden, 2012). Direct consequences of consumption of mycotoxin-contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression), and reduced reproductive capacities (Fink-Gremmels, 2008). However, the major problem associated with mycotoxin-contaminated animal feed is not acute disease episodes but low level toxin ingestion which may cause a variety of metabolic disturbances resulting in poor animal productivity. In studies with pigs and poultry it has been shown that low level mycotoxin intake can result in reduced feed intake, poor growth rate, lower egg production, changes in carcass quality, reduced fertility and hatchability of eggs and immunosuppression. It is concluded that mycotoxins constitute a significant problem for the animal feed industry and an ongoing risk to feed supply security (Bryden, 2012). Various mycotoxins even have the ability to modify the rumen flora due to their antimicrobial activity. This may decrease the degrading capacity of the rumen resulting in an unexpected passage rate of intact toxins from other sources. A comparable effect can be also be expected in cases in which the rumen flora is affected in the course of metabolic diseases like rumen acidosis (Fink-Gremmels, 2008).

Scientific literature offers a variety of information on the effects of individual mycotoxins in various animal species, but concurrent exposure to multiple mycotoxins is more likely in the livestock industry. Additionally, in the feed manufacturing process, various batches of different raw materials are mixed together and produce a new matrix with a new risk profile. Poor livestock performance and disease symptoms observed in commercial operations may be due to the synergistic interactions between multiple

mycotoxins. Scientific reports on synergistic effects of mycotoxins at acute toxicity levels describe combinations of aflatoxins with various other mycotoxins (Binder et al., 2007). Far more work has to be done in this particular field of research, especially in the sub-acute contamination range as well as with combinations of more than two toxins.

Conclusion

Anti-quality components of forages are an increasingly important point of research as the economical importance of forage quality becomes more apparent. Maize crops have become an immense commodity in the United States and are a significant component of most livestock production systems. Anti-quality components specific to these cash crops are more important now than ever. Chemical, biological and physical aspects of forage can all contribute to diminishing quality of forages so there is a wealth of information on occurrence, co-occurrence, toxicological effects, biological effects and economic impacts among others that need further investigation.

INTRODUCTION

Aflatoxin are highly oxygenated, heterocyclic, difuranocoumarin compounds produced by the fungi *Aspergillus flavus, Aspergillus parasiticus* (Diaz et al., 2008). Aflatoxins are hepatotoxic and carcinogenic secondary metabolic products from these fungal species. More than 20 aflatoxin-like secondary metabolites have been identified. Aflatoxin B1 (AFB1) was shown to possess the most toxic and carcinogenic properties to humans and animals (Binder et al., 2007). Once aflatoxins are produced by the fungi, they are heat, cold, and light stable. They persist to some extent in food even after the inactivation of the fungi by food processing methods, such as ultra-high temperature, due

to the significant chemical stability of aflatoxins. Aflatoxins are colorless, odorless, and tasteless, and because concentrations are often low and unevenly distributed in a commodity, they are difficult to detect accurately (Peraica et al., 1999).

Aflatoxins are natural contaminants in cereals (such as maize, sorghum, pearl millet, rice, wheat, corn), oilseeds (such as peanut, soybean, sunflower, cotton), spices, fruits, hazelnuts and tree nuts (Veldman et al., 1992). Major sources of exposure are corn and peanuts as they are the species most susceptible to contamination and consumed in the greatest amounts by humans (Sharma et al., 2014).

Aspergillus flavus and *A. parasiticus* develop mainly in tropical and subtropical climates but they have also been detected in more temperate areas with summers similar to subtropical regions (Binder et al., 2007). While fungi are a normal part of the microflora of standing crops and stored feeds, the production of the secondary metabolites, such as aflatoxin B1, are promoted by physical and biological factors during harvesting, handling, and storage (Bryden, 2007). Physical factors include moisture, humidity, and mechanical damage of the crops. Biological factors include plant variety, stress from pre-harvest drought, insect damage and spore load. The fungal species will also dictate optimum factors for toxin production and total toxin production is not necessarily linked to total fungal biomass (Magan, 2006).

The timing of harvest of Aspergillus susceptible crops interacts with these physical and biological aspects to promote growth of aflatoxins and other secondary mycotoxins. Heavy rains at the time of harvest or post harvest can create excessive wet spots in the collected crops. This makes adequate drying before storage much more difficult for avoiding incubation of existing mycotoxin-producing species. The humidity

range, temperature range and aeration during drying and storage are extremely important (Sharma et al., 2014). Hot spots perfect for mycotoxin producing fungal species can be created in storage by migrating moisture due to temperature changes when grain is cooling. Methods to prevent infection by mycotoxin-producing fungi in stored feed include storing the grain below 130g/kg moisture content, regularly inspecting grain for insect activity and wet spots, and maintaining a high turnover of feed to reduce time available for fungal growth (Bryden, 2012).

Metabolism and health effects

The extent of mycotoxin toxicity or carcinogenicity is related to the proportion of mycotoxin that is converted to metabolites that bind to critical cellular macromolecules. Aflatoxin is metabolized by the cytochrome p450 group of enzymes in the liver. It may be converted to aflatoxin 8,9 epoxide that can induce mutations in DNA leading to hepatic carcinoma (Fink-Gremmels, 2006).

Following ingestion of aflatoxin-contaminated feeds, a part of the ingested aflatoxin B1 is degraded in the rumen, resulting in the formation of aflatoxicol. The remaining fraction is absorbed in the digestive tract by passive diffusion and is hydroxylated in the liver to aflatoxin M1. Aflatoxin M1 is either conjugated to glucuronic acid, and subsequently excreted via bile, or enters the systemic circulation. Circulating aflatoxin M1 can be excreted in the urine or appear in milk (Fink-Gremmels, 2006).

Both Aflatoxin B1 and Aflatoxin M1 are classified as group 1a carcinogens (IARC, 2002). Large doses are lethal and chronic exposure can result in cancer and immune suppression. The primary target of these toxins is the hepatic system with acute effects of hemorrhagic necrosis and bile duct proliferation, and chronic effects of

hepatocellular carcinoma, immune suppression and growth retardation (Sharma et al., 2014).

In cattle, acute effects include reduced feed consumption, depressed milk yield, and liver damage. Chronic effects include immunosuppression that can be exacerbated by other stressors such as high production and poor management (Bodine and Mertens, 1983).

Economic Impacts of Mycotoxins

There are economic losses from mycotoxins due to effects on crop production, animal productivity and overall industry costs. While it is difficult to estimate the incidence of mycotoxin infection of crops and the associated economic impact, a FDA computer model estimated $932 million annual crop losses from aflatoxins, fumonisins, and deoxynivalenol (CAST, 2003). Animal losses due to death are easier to determine but losses due to morbidity may be of greater economic importance due to effects of immunosuppression and decreased reproductive performance (Charmley et al., 1993). Overall industry costs related to mycotoxin contamination of crops include research, monitoring and extension work, extra handling and distribution costs, increased processing costs, and loss of consumer confidence in the safety of food products (Robens and Cardwell, 2003).

Occurrences

It is estimated that 25% of world crops are infected with mycotoxins (Sharma et al., 2014). Approximately 5 billion people in developing countries worldwide are at risk of chronic exposure to Aflatoxin B1 through food. Aflatoxin may have a causative role in up to 28% of all global cases (550,000-600,000 new each year) of

hepatocellular carcinoma (Liu and Wu, 2010). The greatest risk for human health is in developing countries in tropical regions where aflatoxin-affected crops are food staples. Food insufficiency in these areas exacerbates this risk due to high temperature, moisture, unseasonal rains, and flash floods. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to mycotoxin production (Sharma et al., 2014).

Accumulation of aflatoxin in crops is associated with high temperatures, insect damage and prolonged drought conditions (Payne, 1998). Because Aspergillus can tolerate lower water activity than some other mycotoxins, such as those from Fusarium *sp.*, it is more likely to contaminate commodities both pre- and post-harvest (Abramson, 1998).

META-ANALYSIS OF PREVIOUS STUDIES

In order to design a relevant and dynamic animal study to look at the carry-over of aflatoxin from feed to milk, a preliminary picture of the occurrence and apparent carry-over of aflatoxin B1 to aflatoxin M1 in milk in the field was be created.

We collected individual aflatoxin data and specific methods information from 12 studies from 1967 to 2014 (Figure 1). Some aflatoxin intake data were inferred from methods and other information provided in the papers. Consolidation of these historical works provided an overall picture based on the limited number and scope of past studies. These data are presented together with our field carry-over data in Figure 1 with trend lines added to show approximate carry-over percentages.

Many of the studies represented in Figure 1 used cows with relatively low milk production (10-30 kilograms of milk per day). The apparent carry-over percentage of 1.2% for these studies is in line with the understanding that lower-producing cows excrete less AFM1 into the milk as a percentage of AFB1 consumed.

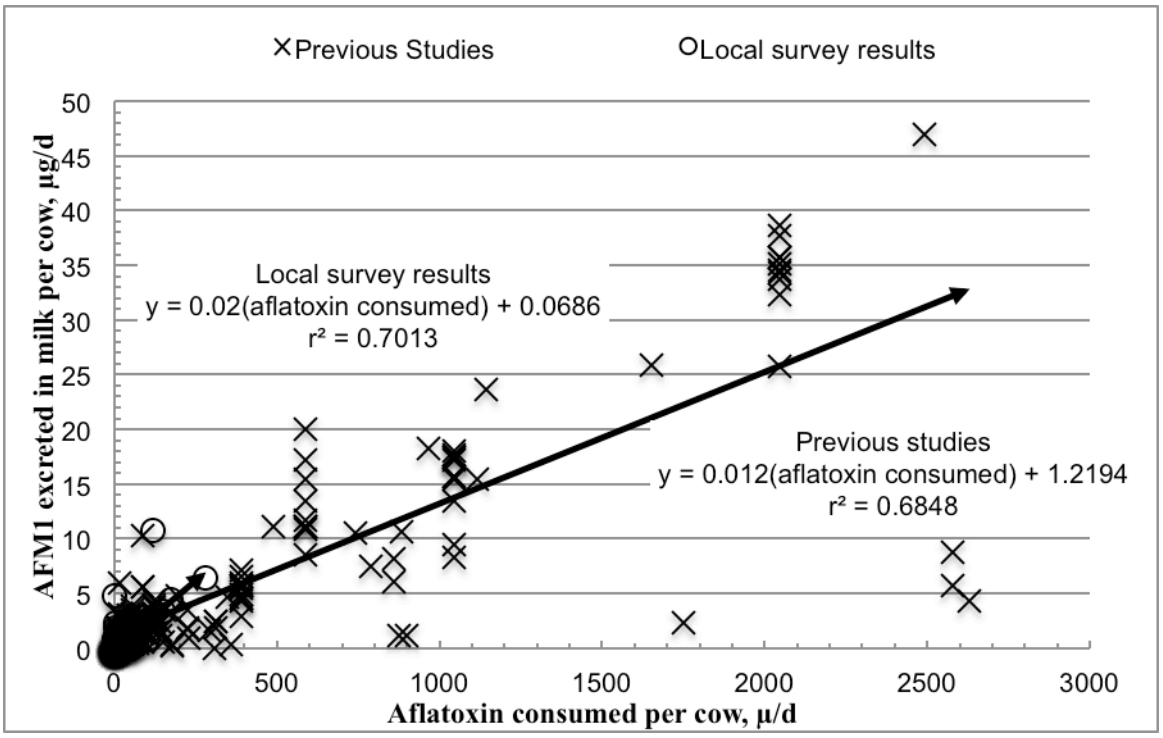

Figure 1: Average mass of AFM1 excreted in milk vs average mass of aflatoxin consumed from field survey data and data collected from previous carry-over experiments (Allcroft et al., 1968; Lafont et al., 1980; Patterson et al., 1980; Applebaum et al., 1982; Price et al., 1985; Frobish et al., 1986; Munksgaard et al., 1987; Veldman et al., 1992; Chopra et al., 1999; Masoero et al., 2007; Britzi et al., 2013).

FIELD CARRY-OVER STUDY

Thirty-eight farms were surveyed in upstate NY, taking feed and milk samples to establish a general occurrence level of mycotoxins and formulate a field data carry-over rate for aflatoxin from feed to AFM1 in milk. None of the included farms were pasture-based operations.

On farms with cows fed a TMR, samples of TMR were collected by hand from all the bunkers from which the animals on each diet were fed and stored in 4-liter Ziplock bags. If more than one diet was fed on the farm, samples were taken from all feeding areas for each diet separately. The bags were stored in a cooler for transit and refrigerated for no longer than one week until testing.

On farms with cows not fed strictly a TMR, samples were taken of each type of feed from where they were stored. Multiple handfuls were taken from all accessible storage areas for each ingredient and collected in 4-liter Ziplock bags. If certain ingredients were only given to a particular milking group, they were labeled as such.

Samples were ground with a BODUM Bistro electric blade coffee grinder (Bodum co., Switzerland) and then tested using a VICAM (Waters, inc., Milford, MA) Series 4 Fluorometer using the VICAM Aflatest procedure for Animal Feeds manual (pg 30, 2012).

Milk samples were collected from groups or individual cows from each of the feeding groups on the same day as feed samples were taken. Almost all of the sampled farms had 1 to 2 feeding groups with only one having 3 feeding groups. Collection methods varied according to farm and milking logistics and farmer amenability.

For some farms, different feeding groups were not milked separately. Samples from these farms were collected by hand stripping individual cows' udders into sample containers or milking an individual cow fully with a portable milking machine and a milk pail and sampling from there.

For farms with only one feeding group, samples were taken from the bulk tank following one of the milkings for that farm. For farms milking each feeding group

separately, samples were taken from the bulk tank following the milking of each feeding group. This resulted in a milk sample from strictly one feeding group followed by a milk sample including both groups as logistics would dictate.

All milk samples were collected in 60 mL centrifuge tubes and stored in a cooler for transit followed by refrigeration until testing. Milk samples were tested using a VICAM (Waters, inc., Milford, MA) Series 4 Fluorometer using the VICAM Aflatest M1 Fl+ procedure for liquid milk.

Results and discussion

It is difficult to get a representative sample for mycotoxins in feeds because of their heterogeneous distribution (Bryden, 2012). Because of the quick excretion rate of aflatoxins in milk, it is hard to collect a sample of the feed that became part of the milk sample (Decastelli et al., 2007). While we expected farms with unregulated homegrown feed sources to have high milk mycotoxin levels, that was not the case. However, we did find that over 14% of all milk samples collected would violate EU regulations for AFM1. We put together a field carry-over graph using 120 points of aflatoxin and AFM1 measurement data (Figure 2). There was a positive relationship between feed and milk aflatoxin levels (Figure 2). This required the assumption that the specific feeds sampled in the bunkers and troughs represented the feed consumed by the cows, but the feed to make the milk was obviously fed before the samples were taken.

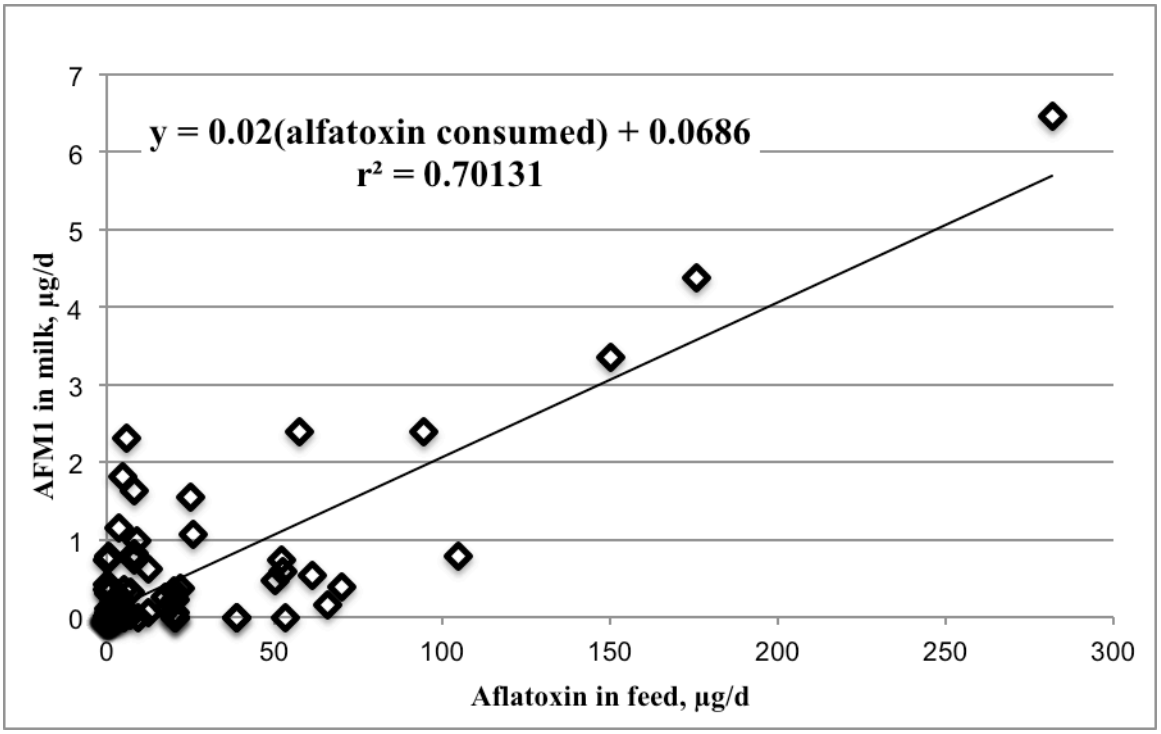

Figure 2: Local field survey data for mass of aflatoxin M1 in milk vs mass of aflatoxin in feed.

FEEDING TRIAL

Methods

There were three replications, each using 12 high-producing Holstein cows in early- to mid-lactation. The cows were purposely not treated with bST so that the results would be applicable world-wide. For each replication, cows were assigned to experimental groups by lactation number and by random selection. Cows of each lactation number were assigned by random selection to each level of dietary aflatoxin so that there was an equal number of cows of each lactation number fed each aflatoxin level. Corn meal for treatment groups was contaminated with aflatoxin through natural means by inoculating the corn plants with *Aspergillus* sp. This naturally contaminated corn meal was sourced from an experimental corn project at Texas A & M University.

Replicate 1 cows. There were three 1st lactation cows with average days-in-milk (DIM) of 41.3 and an average milk production level of 36.7 kg per day. There were six 2nd lactation cows with average DIM of 41.2 and an average milk production level of 45.1 kg per day. There were three 3rd lactation cows with an average DIM of 39.3 and average milk production level of 53.8 kg per day.

Replicate 2 cows. There were six 1st lactation cows with average DIM of 49.3 and an average milk production level of 38.2 kg per day. There were three 2nd lactation cows with average DIM of 52.3 and an average milk production level of 48.0 kg per day. There were three 3rd lactation cows with average DIM of 50.6 and an average milk production level of 52.4 kg per day. One 3rd lactation cow was removed from the trial due to health issues unrelated to the experiment.

Replicate 3 cows. There were nine 1st lactation cows with average DIM of 47.2 and an average milk production level of 38.1 kg per day. There were three 2nd lactation cows with average DIM of 36.3 and an average milk production level of 49.6 kg per day.

Cows were housed in tie stalls with individual feeding bins. For each replicate, there was a 2.5-week adjustment period to these stalls to avoid stress as a factor that might affect dry matter intake or milk production. For replicate 1 and 2, the experimental period during which cows were fed experimentally formulated diets of different aflatoxin levels lasted 7 days. Due to supply issues for aflatoxin-contaminated corn meal, the experimental feeding period for replicate 3 was 2 days.

Cows were fed at 8 am. A Roto Mix VX-515 mixer was used to deliver the feed from the feed bunker to the barn housing the experimental cows. The feed was delivered to each individual feeding bin using a weight-tracking Calan Super Data Ranger. The

TMR was corn silage based and did not include monensin or mycotoxin binders that are commonly fed in commercial upstate NY dairies.

Feed samples of the base fed TMR diet were taken each day of the experimental feeding period. Samples were collected by taking multiple small handfuls of TMR from each individual cow's feeding bin and collecting them in plastic bags that were then stored in the refrigerator for up to three days until testing. Samples of the refusals for each cow were taken each day of the experimental period. These were pooled and tested at the end of each replicate. After sampling, the refusals were removed and weighed to record total dry matter intake for each cow.

For the experimental period of each replicate, one kilogram of cornmeal was top-dressed on the TMR. Cornmeal for the experimental groups was procured from Texas A & M University from experimental plots of corn artificially infected with Aspergillus sp. to create cornmeal with a natural contamination of aflatoxin.

One-kilogram cornmeal bags were prepared for each cow for each day. Control group cows were given only cornmeal purchased from Agway that tested at 0 ppb total aflatoxin. Cows assigned to the low aflatoxin level were given a mix of clean (0 ppb) cornmeal and cornmeal naturally contaminated with aflatoxin procured from Texas A & M University to make the estimated total ingested feed for that day contain approximately 10 ppb total aflatoxin. Cows assigned to the high aflatoxin level were given a mix of clean (0 ppb) cornmeal and cornmeal naturally contaminated with aflatoxin to make the estimated total ingested feed for that day contain approximately 20 ppb total aflatoxin. Samples from each top-dressing cornmeal bag were taken each day and tested in the lab for total aflatoxin concentrations.

Samples were tested using a VICAM (Waters, inc., Milford, MA) Series 4 Fluorometer using the VICAM Aflatest procedure for Animal Feeds manual (pg 30, 2012). Total fed aflatoxin consumed by each cow, each day, was calculated by adding any aflatoxin found in the base fed TMR diet to the measured aflatoxin level in the top-dressed cornmeal and subtracting any aflatoxin found in the refusals.

The cows were milked at approximately 10 am, 6 pm, and 2 am during the trial period and all milk was disposed of until milk samples tested 0 ppb for aflatoxin M1 for each cow. Milk weights were recorded at each milking. Milk samples were taken at all three milkings starting the morning after the first experimental cornmeal top-dressing was fed, and then again at all three milkings on day 4 and day 6 of the 7-day experimental feeding for replicates 1 and 2 of the trial. Then milk samples were then taken once every other day from day 8 until the milk tested 0 ppb Aflatoxin M1 at which point the cows were released back into the general milking herd. There was enough contaminated corn meal for only 2 days of experimental feeding for replicate 3. Therefore, milk samples were taken at all three milkings starting at the first milking after the first experimental cornmeal top-dressing was fed and continued through the second and final day of experimental feeding and then on until the milk tested at 0 ppb Aflatoxin M1 at which point the cows were released back into the general milking herd.

Milk samples were taken using the DeLaval Fat Sampler CPL and then stored in 60 mL centrifuge tubes at refrigerator temperature until testing. Milk samples were tested using a VICAM (Waters, inc., Milford, MA) Series 4 Fluorometer using the VICAM Aflatest M1 Fl+ procedure for liquid milk.

The number of days of feeding AFB1 levels within blocks was too short for any meaningful conclusions on the effect of day on dry matter intake (DMI) or milk production. Therefore, average DMI was calculated for each cow and analyzed with the effect of block and AFB1 level in the analysis of variance model.

Test-day milk yield and composition were obtained from farm records for three dates surrounding the experimental period of each block (Table 1). The statistical model included main effects of Block and AFB1 level with cow within Block and AFB1 level as a random effect to test AFB1 level. DIM and the quadratic value of DIM were included as covariates.

Table 1: Experimental dates and milk test dates

Block		Experimental dates	Test dates	DIM
1	Start	2/22/16	2/5/2016	29
	End	3/19/16	3/5/2016	58
			4/19/2016	103
2	Start	4/6/16	3/5/2016	20
	End	5/2/16	4/19/2016	65
			5/25/2016	101
3	Start	5/3/16	4/19/2016	30
	End	5/22/16	5/25/2016	66
			7/29/2016	132

Results and discussion

Average levels of aflatoxin ingested for each feeding group in each trial are presented in Table 2 as well as average peak levels of AFM1 recorded in the milk for each group in each trial.

Table 2: Average daily dietary aflatoxin concentration and maximum concentration of AFM1 in milk for each level of aflatoxin within each replicate. Numbers in italics violate the US action levels for aflatoxin and AFM1.

Diet	Replicate 1 (7d)	Replicate 2 (7d)	Replicate 3 (2d)
Aflatoxin ingested (µg/kg feed DM)			
Control	0	0	0
Low	5.2	12.3	9.4
High	*21.7*	*21.9*	16.0
AFM1 excreted (µg/kg milk)			
Control	0	0	0
Low	0.278	*0.543*	0.174
High	*1.010*	*0.966*	*0.504*

Total aflatoxin ingested and total AFM1 excreted were calculated using measured AFM1 levels in the milk, measured aflatoxin levels in ingested feed, DMI, and milk yield at each milking. Using the total aflatoxin ingested and the total AFM1 excreted instead of a daily average of each provides a better overall picture of the carry-over effect without omitting or averaging data during the time to steady-state conditions and the time to 0 µg/kg AFM1 in the milk following the cessation of dosing (average of 3 days). Linear regression was used to calculate the direct carry-over into milk as 6.5 µg/100 µg consumed (Figure 3).

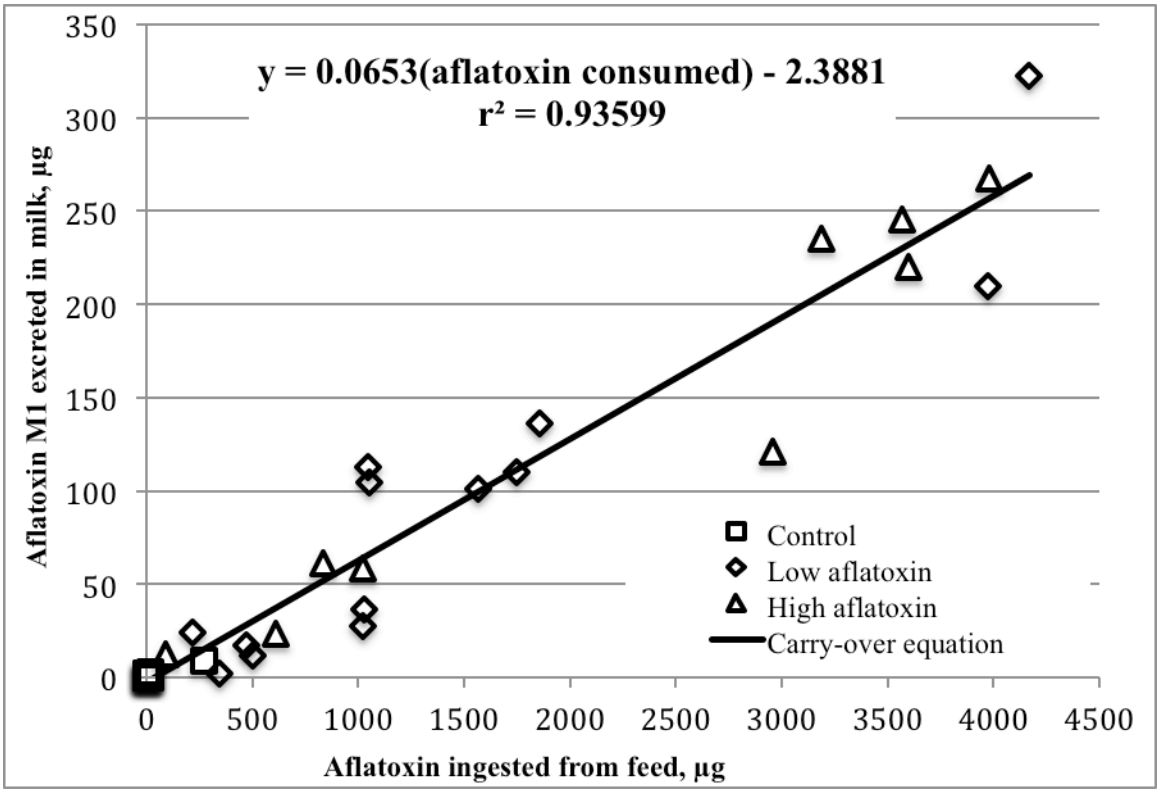

Figure 3: Individual cow data for total mass of AFM1 excreted in milk vs mass of aflatoxin ingested from the feed over the trial period.

Average concentrations of ingested aflatoxin in feed and average concentrations of excreted AFM1 in milk were calculated for each cow in the low and high groups for the experimental feeding period (Figure 4). The vertical and horizontal red lines mark the US regulatory limits for total aflatoxin in feed (20 µg/kg) and aflatoxin M1 in milk (0.5 µg/kg) respectively. Linear regression was used to calculate the relationship between ingested and excreted concentrations of aflatoxin and AFM1. The linear regression line crosses the line marking the US regulatory limit for AFM1 in milk at an aflatoxin level of 15 µg/kg (ppb) in the feed suggesting that this level of aflatoxin in the feed is the maximum likely to produce milk below the US regulatory limits.

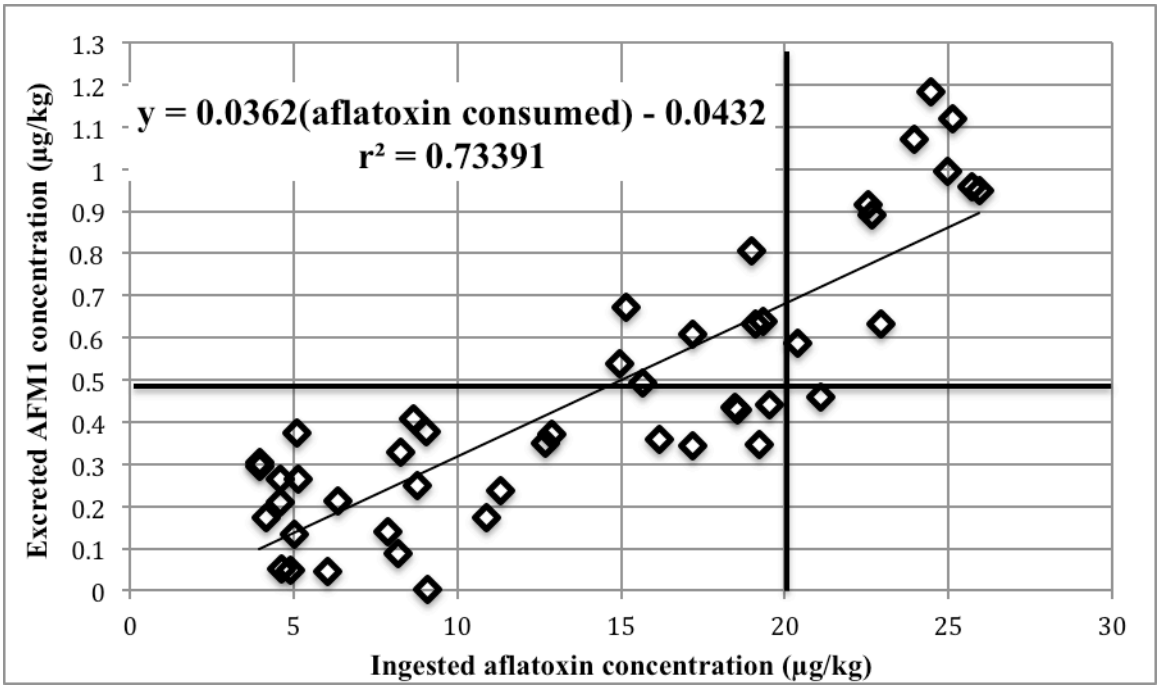

Figure 4: Average concentration (µg/kg) of excreted AFM1 for each cow over the experimental feeding period vs average concentration (µg/kg) of ingested aflatoxin for each cow. Emboldened vertical and horizontal lines represent current US regulatory limits for Aflatoxin B1 in feed and AFM1 in milk, respectively.

Sufficiently high AFB1 levels could damage the liver and possibly other organs if fed for long enough. In this experiment, however, assigned AFB1 levels were not fed for a sufficiently long enough to observe such an effect. Mean DMI values were 24.8, 24.7, and 23.8 ± 1.03 kg/d and not significantly different for Control, Low, and High assigned AFB1 levels.

As shown in Table 3, test-day milk yields of cows were high. After adjustment for DIM, AFB1 level had no effect on test-day milk, fat, or protein for test dates surrounding the experimental periods.

Table 3: Effect of level of dietary AFB1 on yield and composition of test-day milk and composition adjusted for DIM[1]			
AFB1 level	**Test-day milk, kg/d**	**Test-day fat, %**	**Test-day protein, %**
Control (0 ppb)	44.6	3.8	3.0
High (16-22 ppb)	44.9	3.8	3.0
Low (5-12 ppb)	45.7	3.7	2.9
SEM	6.08	0.29	0.17
p-value	0.552	0.909	0.785
DIM intercept	34.8	4.96	3.15
SE	1.5	0.224	0.0736
p-value	< 0.001	< 0.001	< 0.001
DIM	0.23	-0.02834	-0.00797
SE	0.491	0.00712	0.00235
p-value	< 0.001	< 0.001	0.001
DIM^2	-0.00109	0.000155	0.000060
SE	0.000329	0.000048	0.000016
p-value	0.002	0.002	< 0.001

[1]Average DIM: 67 ± 36.9 (SD).

FUTURE IMPLICATIONS AND RECOMMENDATIONS

Feed producers and some dairy farmers screen for a range of mycotoxins, but only aflatoxin is subject to action levels by the FDA. In the US, milk is not regularly screened for mycotoxins. This experiment tested the safety of feeding high-producing dairy cows at the legal limit of aflatoxin concentration. Thus, the current "safe" limits of aflatoxin concentration allowable in feed for dairy cows do not protect against violating the current regulations for AFM1 residue in the resulting milk. The carry-over percentage of 6.5% in our study demonstrates that high-producing dairy cows have a higher carry-over percentage than the 1 to 2% that has been suggested by previous studies (Allcroft et al., 1968; Lafont et al., 1980; Patterson et al., 1980; Applebaum et al., 1982; Price et al., 1985; Frobish et al., 1986; Munksgaard et al., 1987; Veldman et al., 1992; Chopra et al., 1999; Masoero et al., 2007; Britzi et al., 2013) using low-producing cows.

These results indicate that the current US maximum allowable level of 20 µg/kg total aflatoxin in dairy cow feed is not protective to avoid violation of the 0.5 µg/kg AFM1 regulatory levels for milk in high-producing cows. It must also be considered that the maximum allowable levels for the US are in no way protective against EU regulatory levels of AFM1 in the milk limiting possible trade or exportation of milk products. Farmers should be vigilant about proper harvesting, storing, and regular testing of feedstuffs for dairy cows to ensure the safety of the animals and the humans consuming their products.

REFERENCES

Abramson, D. 1998. Mycotoxin formation and environmental factors. In: Sinha, K. K., and D. Bhatnager, editors, Mycotoxins in agriculture and food safety. Marcel Dekker, New York, NY. p. 255-277.

Akin, D. E. 1989. Histological and Physical Factors Affecting Digestibility of Forages. Agron. J. 81(1): 17-25.

Allcroft, R., B. A. Roberts, and M. K. Lloyd. 1968. Excretion of aflatoxin in a lactating cow. Food Cosmet. Toxicol. 6(5): 619-625.

Allen, V. G., and E. Segarra. 2001. Anti-quality components in forage: Overview, significance, and economic impact. J Range Manage. 54(4): 409-412.

Applebaum, R. S., R. E. Brackett, D. W. Wiseman, and E. H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. J Dairy Sci. 65: 1503-1508.

Austin, P. J., L. A. Suchar, C. T. Robbins, and A. E. Hagerman. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. J. Chem. Ecol. 15(4): 1335-1347. doi:10.1007/BF01014834.

Barry, T. N. 1981. Protein metabolism in growing lambs fed on fresh ryegrass (Lolium perenne)–clover (Trifolium repens) pasture ad lib. Br. J. Nutr. 46(3): 521-532. doi:10.1079/BJN19810061.

Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. of Nutr. 81(4): 263-272. doi:10.1017/S0007114599000501.

Binder, E. M., L. M. Tan, L. J. Chin, L. Handl, and J. Richard. 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Technol. 137(3-4): 265-282. doi:10.1016/j.anifeedsci.2007.06.005.

Bodine, A. B., and D. R. Mertens. 1983. Toxicology, metabolism, and physiological effects of aflatoxin in the bovine. In Diener U. L., Asquith. R. L, and J. W.

Dickens, editors, Aflatoxin and Aspergillus flavus in corn. Alabama Agric Exp Sta, Auburn Univ, AL. p. 46-50.
Britzi, M., S. Friedman, J. Miron, R. Solomon, O. Cuneah, J. A. Shimshoni, and A. Shlosberg. 2013. Carry-over of aflatoxin B1 to aflatoxin M1 in high yielding Israeli cows in mid- and late-lactation. Toxins (Basel). 5(1): 173-183. doi:10.3390/toxins5010173.
Bruns, H. A. 2003. Controlling Aflatoxin and Fumonisin in Maize by Crop Management. Toxin Rev. 22(2-3): 153-173. doi:10.1081/txr-120024090.
Bryden, W. L. 2007. Mycotoxins in the food chain: human health implications. Asia Pac. J. Clin. Nutr. 16: 95-101.
Bryden, W. L. 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Anim. Feed Sci. Technol. 173(1-2): 134-158. doi:10.1016/j.anifeedsci.2011.12.014.
CAST (Council for Agricultural Science Technology). 2003. Mycotoxins: Risks in plant, animal, human systems. Task Force Report, Ames, Iowa. 139: 1-199.
Campbell, J. B. 2001. Anti-Quality Effects of Insects Feeding on Rangeland Plants: A Review. J. Range Manage. 54(4): 462-465.
Cassman, K. G., and D. N. Duvick. 1999. Post-Green Revolution Trends in Yield Potential of Temperate Maize in the North-Central United States. Crop Sci. 39: 1622.
Charmley, E., H. L. Trenholm, B. K. Thompson, D. Vudathala, J. W. Nicholson, D. B. Prelusky, and L. L. Charmley. 1993. Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. J. Dairy Sci. 76(11): 3580-3587.
Cheeke, P. R., and L. R. Shull. 1985. Natural toxicants in feeds and poisonous plants. AVI Pub. Co, Westport, CT.
Chopra, R., A. Chhabra, K. Prasad, A. Dudhe, T. Murthy, and T. Prasad. 1999. Carryover of Aflatoxin M1 in Milk of Cows Fed Aflatoxin B1 Contaminated Ration. Indian J. Anim. Nutr. 16(2): 103-106.
Coop, R. L., and P. H. Holmes. 1996. Nutrition and parasite interaction. Int. J. Parasitol. 26(8–9): 951-962.
Decastelli, L., J. Lai, M. Gramaglia, A. Monaco, C. Nachtmann, F. Oldano, and C. Bandirola. 2007. Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005. Food Control. 18(10): 1263-1266. doi:10.1016/j.foodcont.2006.08.006
Diaz, G. J., E. Calabrese, and R. Blain. 2008. Aflatoxicosis in chickens (Gallus gallus): an example of hormesis? Poult. Sci. 87(4): 727-732. doi:10.3382/ps.2007-00403
Emile, J. C., S. Bony, and M. Ghesquiere. 2000. Influence of consumption of endophyte-infested tall fescue hay on performance of heifers and lambs. J. Anim. Sci. 78(2): 358-364.
Fink-Gremmels, J. 2006. Moulds and mycotoxins as undesirable substances in animal feeds. In D. Barug, D. Bhatnagar, H. P. van Egmond, J. W. van der Kamp, W. A. van Osenbruggen, and A. Visconti, editors, The Mycotoxin Factbook. Wageningen Academic Publishers, The Netherlands. p. 37-50.
Fink-Gremmels, J. 2008. Mycotoxins in cattle feeds and carry-over to dairy milk: A review. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.

25(2): 172-180. doi:10.1080/02652030701823142.
Frobish, R. A., B. D. Bradley, D. D. Wagner, P. E. Long-Bradley, and H. Hairston. 1986. Aflatoxin Residues in Milk of Dairy Cows after Ingestion of Naturally Contaminated Grain. J. Food Prot. 49(10): 781-785.
Fryxell, J. M. 1991. Forage Quality and Aggregation by Large Herbivores. Amer. Nat. 138(2): 478-498. doi:10.2307/2462483.
Harrison, L. R., B. M. Colvin, J. T. Greene, L. E. Newman, and J. R. Cole. 1990. Pulmonary Edema and Hydrothorax in Swine Produced by Fumonisin B1, a Toxic Metabolite of Fusarium Moniliforme. J. Vet. Diagn. Invest. 2(3): 217-221. doi:10.1177/104063879000200312.
Hill, N. S., D. P. Belesky, and W. C. Stringer. 1991. Competitiveness of Tall Fescue as Influenced by Acremonium coenophialum. Crop Sci. 31(1): 185-190. doi:10.2135/cropsci1991.0011183X003100010042x.
Howarth, R. E., and B. P. Goplen. 1983. Improvement of forage quality through production management and plant breeding. Can. J. Plant Sci. 63(4): 895-902. doi:10.4141/cjps83-113.
IARC. (2002). Aflatoxins. IARC Monograph Evaluation of Carginogenic Risks to Humans. 82: 225-248.
James, L. F., D. B. Nielsen, and K. E. Panter. 1992. Impact of Poisonous Plants on the Livestock Industry. J. Range Manage. 45(1): 3-8. doi:10.2307/4002517.
Jones, R. K. 1979. Epidemiology and management of aflatoxins and other mycotoxins. In J. G. Horsfall and E. B. Cowling, editors, Plant disease : An Advanced Treatise (Vol. 4). Academic Press, New York, NY. p. 381-392.
Jones, W. T., and J. L. Mangan. 1977. Complexes of the condensed tannins of sainfoin (Onobrychis viciifolia scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28(2): 126-136. doi:10.1002/jsfa.2740280204
Kimambo, A. E., J. C. MacRae, A. Walker, C. F. Watt, and R. L. Coop. 1988. Effect of prolonged subclinical infection with Trichostrongylus colubriformis on the performance and nitrogen metabolism of growing lambs. Vet. Parasitol. 28(3): 191-203.
Kumar, R. 2003. Anti-nutritive factors, the potential risks of toxicity and methods to alleviate them. http://www.fao.org/DOCREP/003/T0632E/T0632E10.htm. Accessed 10 December 2015.
Lafont, P., J. Lafont, S. Mousset, and C. Frayssinet. 1980. Contamination of cow's milk during ingestion of small quantities of aflatoxin. Ann. Nutr. Aliment. 34(4): 699-707.
Launchbaugh, K. L. 1996. Biochemical aspects of grazing behavior. In J. Hodgson and A. W. Illius, editors, The Ecology and Management of Grazing Systems. CAB International, Wallingford, Oxon, UK. p. 159-184.
Lillehoj, E. B., and M. S. Zuber. 1974. Aflatoxin problem in corn and possible solutions. Presented at the Proceedings of 30th Annual Corn & Sorghum Research Conference, Chicago, IL. p. 230-250.
Lisker, N., and E. B. Lillehoj. 1991. Prevention of mycotoxin contamination (principally aflatoxins and Fusarium toxins) at the preharvest stage. In J. E. Smith, and R. S.

Henderson, editors, Mycotoxins and Animal Foods. CRC Press, Boca Raton, FL. p. 689-719.
Liu, Y., and F. Wu. 2010a. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ. Health Perspect. 118(6): 818-824.
MacRae, J. C. 1993. Metabolic consequences of intestinal parasitism. Proc. Nutr. Soc. 52(1): 121-130. doi:10.1079/PNS19930044.
Magan, N. 2006. Mycotoxin contamination of food in Europe: early detection and prevention strategies. Mycopathologia. 162(3): 245-253. doi:10.1007/s11046-006-0057-2.
Majak, W., J. W. Hall, and T. A. McAllister. 2001. Practical Measures for Reducing Risk of Alfalfa Bloat in Cattle. J. Range Manage. 54(4): 490-493.
Masoero, F., A. Gallo, M. Moschini, G. Piva, and D. Diaz. 2007. Carryover of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts. Animal. 1(9): 1344-1350. doi:10.1017/S1751731107000663.
Mayland, H. F., and P. R. Cheeke. 1995. Forage-induced animal disorders. In R. F. Barnes, D. A. Miller, C. J. Nelsen, and M. E. Heath, editors, Forages: An Introduction to Grassland Agriculture. 5th ed., Vol. 2. Iowa State University Press, Ames, Iowa. p. 121-136.
Moore, K. J., and H-J. G. Jung. 2001. Lignin and Fiber Digestion. J. Range Manage. 54(4): 420-430.
Munksgaard, L., J. Larsen, H. Werner, P. Andersen, and B. Viuf. 1987. Carry over of aflatoxin from cows' feed to milk and milk products. Milchwissenschaft, 42(3): 165-167.
Patterson, D. S. P., E. M. Glancy, and B. A. Roberts. 1980. The 'carry over' of aflatoxin M1 into the milk of cows fed rations containing a low concentration of aflatoxin B1. Food Cosmet. Toxicol. 18: 35-37.
Payne, G. A. 1998. Process of contamination by aflatoxin-producing fungi and their impact on crops. Mycotox. Agri. Food Safe. 9: 279-306.
Peraica, M., B. Radić, A. Lucić, and M. Pavlović. 1999. Toxic effects of mycotoxins in humans. Bull. World Health Organ. 77(9): 754-766.
Peters, C. W., and K. N. Grigsby. 1992. Performance, Forage Utilization, and Ergovaline Consumption by Beef Cows Grazing Endophyte Fungus-Infected Tall Fescue, Endophyte Fungus-Free Tall Fescue, or Orchardgrass Pastures. J. Animal Sci. 70(5): 1550-1561.
Price, R. L., J. H. Paulson, O. G. Lough, C. Gingg, and A. G. Kurtz. 1985. Aflatoxin conversion by dairy cattle consuming naturally-contaminated whole cottonseed. J. Food. Protec. 48(1): 11-15.
Provenza, F. D. 1995. Postingestive Feedback as an Elementary Determinant of Food Preference and Intake in Ruminants. J. Range Manage. 48(1): 2-17. doi:10.2307/4002498
Provenza, F. D., J. A. Pfister, and C. D. Cheney. 1992. Mechanisms of Learning in Diet Selection with Reference to Phytotoxicosis in Herbivores. J. Range Manage. 45(1): 36-45. doi:10.2307/4002523.
Rice, E. L. (1984). In Allelopathy. 2nd ed. Academic Press, Orlando, FL.

Robens, J., K. Cardwell. 2003. The Costs of Mycotoxin Management to the USA: Management of Aflatoxins in the United States. J. Toxicol: Toxin Rev. 22(2-3): 139-152. doi:10.1081/txr-120024089.
Russell, J. B., and D. B. Wilson. 1988. Potential Opportunities and Problems for Genetically Altered Rumen Microorganisms. J. Nutr. 118(2): 271-279.
Sharma, L., B. Srivastava, S. Rana, A. Sagar, and N. K. Dubey. 2014. Aflatoxins as Serious Threats to Economy and Health. In A. G. Faulner, editor, Aflatoxins: Food Sources, Occurrence and Toxicological Effects. Nova Science Publishers, Inc., New York, NY. p. 259-286.
Shelby, R. A., and L. W. Dalrymple. 1987. Incidence and distribution of the tall fescue endophyte in the United States. Plant Dis. 71(8): 783-786.
Smith, G. S. 1992. Toxification and Detoxification of Plant Compounds by Ruminants: An Overview. J. Range Manage. 45(1): 25-30. doi:10.2307/4002521.
Stoloff, L., and F. B. Lillehoj. 1981. Effect of genotype (open-pollinated vs hybrid) and environment on preharvest aflatoxin contamination of maize grown in southeastern united states. J. Am. Oil Chem. Soc. 58(12): A976-A980. doi:10.1007/BF02679305.
Thompson, F. N., J. A. Stuedemann, and N. S. Hill. 2001. Anti-Quality Factors Associated with Alkaloids in Eastern Temperate Pasture. J. Range Manage. 54(4): 474-489. doi:10.2307/4003119.
Ulyatt, M. J. 1973. The feeding value of herbage. In G. W. Butler and R. W. Bailey, editors, Chemistry and Biochemistry of Herbage. Vol. 3. Academic Press: London. p. 131-178.
Ulyatt, M. J., and J. C. Macrae. 1974. Quantitative digestion of fresh herbage by sheep: The sites of digestion of organic matter, energy, readily fermentable carbohydrate, structural carbohydrate, and lipid. J. Agric. Sci. 82(2): 295-307. doi:10.1017/S0021859600059190.
Vaithiyanathan, S., and M. Singh. 1989. Seasonal changes in tannin contents in some top feeds in arid region. Indian J. Anim. Sci. 59: 1565-1567.
Veldman, A., J. A. C. Meijs, G. J. Borggreve, and J. J. Heeres-van der Tol. 1992. Carry-over of aflatoxin from cows' food to milk. Anim. Prod. 55(2): 163-168. doi:10.1017/s0003356100037417.
Watts, J. G., E. W. Huddleston, and J. C. Owens. 1982. Rangeland Entomology. Annu. Rev. Entomol. 27(1): 283-311.
White, D. G. 1999. Root Rots. In D. G. White, editor, Compendium of corn diseases 3rd ed. APS Press, St. Paul, Minnesota. p. 11.