

Mycotoxin Interactions in Livestock

John A. Doerr, Ph.D.
Associate Professor of Animal Science,
Assistant Dean, Academic Programs
College of Agriculture and Natural Resources
University of Maryland
College Park, MD 20742

Abstract: Mycotoxins present a unique challenge to producers because they arise from common molds, occur in great variety and number, are sporadic or heterogeneous in their distribution, and because their actions in farm animals are often not diagnostic. Further, the producer does not have a catalog of mycotoxin-specific treatments; e.g., there are no vaccines for aflatoxin or fumonisin! There are preventive measures which should be applied in production animal settings. Included in 'prevention' is routine testing for one or more mycotoxins as a part of regular feed quality assurance. While there are a few signs that may be observed in poultry and livestock which point directly to one or more mycotoxins, most of the symptoms tend to be general: poor performance, increased morbidity, etc. Beyond prevention, the producer needs to have some remedy when mycotoxins already exist in animal feeds. There has been recent interest in the potential of selected direct-fed microbial (DFM) products to provide such remedy. While these are not yet fully established, there is sound basis for considering that they may have a place in production agriculture. As part of an overall control strategy, DFM may offer practical relief.

Introduction

To understand the nature of mycotoxin exposure, discovery, prevention, and/or treatment for livestock, one must first appreciate the simplicity of the source and the complexity of the problem. The fungi which produce the chemical residues which we call mycotoxins are common, simple organisms which have very simple requirements for existence. Nutritional needs are met by a source of carbon and nitrogen; they do require water; all need oxygen, although some require much less than others. For some, a specific mineral or two may be required to support the biosynthesis of those toxic secondary metabolites about which we are concerned. Add the dimension of very simple reproductive mechanisms (generally asexual; most molds exhibit multiple reproductive strategies) and durability under harsh, adverse conditions, and the organisms responsible for mycotoxicoses in farm animals become very capable opponents. They need little, they reproduce easily, and they have adapted highly successful survival mechanisms.

Complexity begins with the nature of animal feeds. Consider any component: forage, grain, oilseeds, supplements, pasture, etc. Each supplies the requisite carbon and nitrogen. Each has some amount of moisture (water activity). And, even for those items which we consider to be anaerobic (e.g., silage), the process is not perfect and low oxygen tension may exist at some or many points within the silage storage unit. Molds, then, should be considered to have the initial advantage in any animal feeding operation. However, the complexity does not stop there.

There are, perhaps, half a dozen or more mold genera with which we are usually most concerned (although there are many, many more that can adversely affect animals). *Fusarium*, *Aspergillus*, *Alternaria*, *Penicillium*, *Stachybotrys*, and *Helminthosporium* are representative of this group. For each genus there are numerous toxigenic species. Many of those species have the capacity to produce more than one kind of mycotoxin, which, in part, accounts for the more than 350 recognized toxins. And, each, in turn, may infest those several feed components

mentioned above and leave behind one or more toxic residues. What begins as our concept of a single mold and/or single mycotoxin is seen by the animal as a multitude of toxic challenges. And, consider, what is mentioned above refers primarily to problems in harvested feed components. Other issues, such as pre-formed toxin in fresh cut material destined for ensiling or endophytic toxins in pasture grasses, add just that much more to the complexity.

How do we sort these issues out? How do we assess risk? How do we intervene?

Prevention

Ideally, we would like to insure that components of animal feeds are of the best possible quality and are maintained by methods which will prevent the growth, metabolism, and toxin formation of molds. While it is unfortunate that this approach cannot succeed fully, it is, nonetheless, the first point of attack and one that should not be overlooked or minimized by producers. Some of the quality control measures which are useful are time consuming, or labor intensive, or bring some other cost. And, some producers will fail to perform such measures as a result. Their response may often be an appropriate one; after all, if the herd is doing well, why subtract from a decent bottom line by doing things that seem unnecessary. Unfortunately when animals become sick, or fail to produce, or their products are in some fashion contaminated and in regulatory violation, the comparative costs of preventive quality control measures will seem puny when placed against the devastating losses that mycotoxins are capable of causing.

Table 1 shows an abbreviated list of some actions designed to prevent the growth of molds and the formation of mycotoxins. Molds can thrive in small niches within a large lot of feed material. Therefore, the first limitation of most of these approaches is that they must be applied thoroughly and uniformly. Secondly, a long history of trying to control fungi has amply demonstrated that no single strategy works alone. Several, or even all, of these approaches may need to be in use in order to have a reasonable chance to thwart mycotoxigenic fungi. Third, each preventive measure has both explicit and implicit limitations. Take, for example, the issue of moisture. Some molds require more available water to germinate and grow than do others. Actively growing molds metabolize substrate to yield CO₂ and water! Metabolic water, therefore, is constantly being put back into the environment; stated another way, a feedstuff that was 12.5% moisture yesterday might have already been modified by microbial action to contain slightly more moisture. Furthermore, feedstuffs will try to equilibrate with atmospheric moisture. When humidity is up, feedstuffs take up water. And, circadian cyclical heating and cooling of feedstuffs in storage causes available water to migrate. While multiple tests may reveal a feedstuff with a mean moisture of 12.5%, some portions are drier; some, wetter. And the list of factors affecting just moisture can go on and on.

However, the two most important factors which impact the application of the preventive measures listed in Table 1 are cost and time. For many animal producers the days are not long enough nor the pocketbook fat enough to support year-round prevention. Emptying, cleaning, and sanitizing feeding equipment is not a trivial task. Alternatives, such as fumigation, are inherently dangerous (and still bear a monetary cost). Despite the wisdom of full time prevention, we are more likely to react as the typical homeowner in an older house: it seems easier to spray once in a while when some mildew appears on the shower tiles than to invest in improved ventilation and treat the tile walls after each shower! Preventive measures, then, are more often used as reactions to a mycotoxin break in a herd or flock.

If we choose, then, to be reactive rather than pro-active, what is the first step? While it suffers from a huge problem, the first course of action recommended by most mycotoxicologists to animal producers is to have a routine, regular testing program. Before determining what sort of test methodology should be used, one needs to consider 1) what the most likely important mycotoxin(s) will be for the region and 2) of the likely toxins, which ones will affect which particular feedstuffs most often. Southwest dairy operations, for example, might see greater frequencies of aflatoxin in oilseed meals or nut hulls than those in Canada; conversely, certain *Fusarium* toxins may be either in lower concentration or found less frequently during very hot months compared to the same times

in the northern tier states. So, the first call is to identify the two or three toxins likely to be of most concern. Second, one needs to carefully consider the kinds of feedstuffs and, therefore, how sampling will be done. While some people worry about the accuracy or sensitivity of specific mycotoxin tests, more erroneous toxin reports likely stem from improper sampling than from a poor test method! Then, one can begin to consider the kind of test.

Sending samples off to a commercial laboratory has merit in a couple of instances. First, when a break is occurring, it may be important to gain very accurate information on a variety of mycotoxins. Second, when issues of liability may become involved, it will be important to ensure that a laboratory using legally acceptable procedures is consulted for identification and quantitation of mycotoxins in specific feedstuffs. However, such tests will be expensive. For farms with sizeable herds or for cooperatives or other organized arrangements of producers, in-house programs may be both cost-effective and provide the needed background data with which to make rational decisions about exposure, risk, and control strategy.

Table 2 lists some of the methods currently employed for mycotoxin analysis. At the moment, the immunochemical procedures probably offer the best return on investment for producers. They permit rapid analysis for a variety of mycotoxins at reasonable cost and in 'laboratory' settings consistent with animal production. That is, some can be done from the back of a truck or in any convenient room. They do not require use of dangerous solvents or elaborate equipment (although one can increase the sophistication of such systems to gain additional accuracy). Once in place, routine use establishes the background level (positive test levels for which little or no adverse reaction is observed) for an operation and permits producers to spot deviations from those norms. Monitoring at least a couple of key toxins is a prudent feed quality assurance measure and can avoid more costly solutions. Sure, one can always use the 'cheap' alternative: a no front-end-cost bioassay. Of course, that is the herd, itself. The payout for that type of testing is, unfortunately, experienced by more producers than should ever be the case.

Signs

One of the difficult aspects of mycotoxicoses is that there are few signs or characteristics which are specific, diagnostic, or even sure clues that fungal toxins are at work. Consider the claim by one respected mycotoxicologist that apart from those episodes which are diagnosed and confirmed, perhaps as much as 35% of all idiopathic disease in poultry and livestock (disease for which no cause can be found) are likely the result of mycotoxin poisoning. I routinely urge clients to accept and understand that synergistic interactions are likely when several mycotoxins occur together. But, that just scratches the surface. Researchers who have undertaken interaction tests with many potential factors (heat, other chemicals, social stress, nutrition, etc.) generally have an easy time demonstrating that any ill effect of one of those factors may be made significantly worse by the added challenge of dietary mycotoxin. A classic interaction problem was demonstrated in poultry. In an experiment involving feeding of both aflatoxin and ochratoxin A to chickens (Huff and Doerr, 1981), it was found that nephritis and urate accumulation far exceeded that which would normally be associated with amount of ochratoxin fed. However, the livers of chickens fed both toxins looked essentially normal despite the well-known fatty accumulation produced by aflatoxin. In short, the primary characteristics associated with each toxin were not observed in the interaction group. Stated another way, the markers that would lead diagnosticians to a rapid conclusion were absent. One would not readily suspect mycotoxicosis in that study, although that was the sole etiology.

agents subsequent to observing these signs cannot be taken as proof of those agents as the sole cause. Opportunists flourish during mycotoxicoses in animals and can easily be assumed to be the sole etiologic agent.

Intervention?

The bottom line becomes this: animals are exposed to more mycotoxins that we can test for; they are also subject to the additional stresses of production which complicate the picture with regard to interactions. They ,

therefore, exhibit signs or symptoms which may not be very elucidating as far as responsible toxins may be concerned. Regardless, those animals are suffering and something must be done. But what?

Previously some preventive measures were listed (Table 1). Other measures after the fact have included treatment of feeds for decontamination (e.g., ammoniation), providing nutritional supplements to animals, using non-nutritive feed additives with real or claimed adsorption capacities (toxin binders, clays), and other approaches. Some of these are based on sound science and good experience. None seem to work universally. Some can, in fact, compound the problem. For example, clay products in use to-date deliver only aflatoxin binding despite claims for multi-mycotoxin efficacy. Check their data!

While not yet a fully vetted technology, some producers are moving toward using direct-fed microbials (DFM) as a means to 'correct' problems in the digestive tract of production animals and thereby remove a number of factors that influence the impact of mycotoxins. The initial use for DFM related solely to the reduction of unwanted microbial colonizers. However, in looking more closely at what that primary goal might truly accomplish, other mechanisms have been found that have direct bearing on how foreign compounds are 'handled' by animals. For example, Roth et al. (1997) suggested that during inflammatory responses in the alimentary tract "...otherwise nontoxic doses of xenobiotic agents can progress to overt toxicity...". What does this mean? Cellular changes at the level of intestinal epithelium which result from the biochemistry associated with the inflammatory process (which is induced by bacterial endotoxins, for example) alter the permeability of those cells, and subsequently, change the absorption rates of foreign compounds. Additionally, there may be some changes in the way in which the body metabolizes what it does absorb. This means that if an animal consumes a dose of mycotoxin that would normally not cause a problem, having an on-going inflammatory process has the effect of increasing the apparent toxicity of that dose. So, the reduction in Gram-negative organisms (generally includes pathogens and/or opportunistic pathogens and producers of bacterial endotoxins) reduces this cycle of inflammatory cytokines altering cell metabolism and, therefore *reduces* apparent foreign chemical toxicity.

However, those same changes to intestinal microflora (and microflora elsewhere? ¹) result in an overall improvement in gut "condition". This means that the intestinal epithelium, villus structure, and microvillar organization are improved or optimized. In turn this suggests that functions that would be associated with the normal, healthy animal, such as increased secretion of key enzymes at the microvillus level, improved absorption of desired nutrients, and, perhaps, better discrimination and reduced absorption of foreign compounds, would be occurring. The net result would logically seem to be an animal, which can tolerate a greater challenge from its environment without prejudice to health or product. This would work, then, whether one is talking about exposure to mycotoxins in feed or to pesticide residues!

There is substantial literature describing the capacity of many common bacteria to assimilate and degrade foreign chemicals, such as mycotoxins. However, recently specific laboratory investigations were conducted which revealed that a lactic acid producer, *Lactobacillus casei pseudopiantarum* cultured from a silage inoculant completely inhibited the synthesis by *Aspergillus flavus* of aflatoxins B₁ and G₁ in vitro. The factor responsible for the inhibition appears to be a protein (Gourama and Bullerman, 1997). Do some DFM have direct effects on the toxins or the molds which produce them?

There has been a great deal of research with direct-fed microbials, although that body of work barely scratches the surface of what is needed to understand and maximize the utility of this kind of technology. Metchnikoff (1907) alerted the world to the potential benefits of DFM in his descriptions of factors extending the life span of residents of the Balkan region of Eastern Europe. Regular consumption of large amounts of active lactic acid bacteria seemed to be a common link among those enjoying best health and longest life. Consider that Metchnikoff was one of the founders of the modern science of immunology (he, in fact, received the Nobel prize in 1908 for discovery of phagocytosis). Should this tell us something?

1 Many bacteria incorporated into direct-fed microbial preparations are lactic acid producers. Will the additional acidity modify rumen microflora? In an advantageous way?

Mycotoxins are consistently recognized for their capacity to interfere with or destroy immune functions in exposed animals. Intestinal immune activity is among the animal's first lines of defense and first factors in overall health. Apart from indirect interaction with immune function through the inflammatory process (Roth et al., 1997), could DFM up- or down-regulate intestinal immunity? Would such regulation influence the animal's susceptibility or resistance to dietary mycotoxins?

Recently we asked the first question: will a DFM with primary active cultures of lactic acid bacteria directly alter intestinal immunity? Does such interaction alter the response of the animal to a disease challenge? A synopsis of our results (Dalloul et al., 2003a, 2003b) in young broiler chickens offers some clues. One group was fed conventional pelleted rations throughout the study. The other ate the same ration to which a commercial product (containing several strains of *Lactobacillus*) was added. At 21 days of age, the two groups were subdivided and half of each was challenged with a high dose of an intestinal parasite, *Eimeria acervulina*, one of the agents responsible for avian coccidiosis. After 10 days, specific sub-populations of intestinal intra-epithelial lymphocytes, secretory antibody, a primary cytokine, and *E. acervulina* proliferation were measured. Basically these trials demonstrated that 1) T-cells (T-helper and cytotoxic T cells) increased in the intestinal epithelium in response to *Lactobacillus*; 2) secretory antibody increased but circulating (serum) antibody declined; 3) interferon-gamma (IFN- γ) which is known to rise in response to coccidial challenge, was reduced; and 4) the total shed oocyst counts of *E. acervulina* were reduced by as much as 75%. To state another way, 1) cells that participate in the identification and destruction of foreign invaders were up-regulated by *Lactobacillus*; 2) during *Lactobacillus* treatment, the host animal increased antibody protection excreted into the intestinal lumen but because it was so successful at combating the disease agent there, it required little development of circulatory antibody (humoral response), thus conserving defense mechanisms; 3) the cytokine typically associated with the infection was reduced indicating the reduced disease challenge; and 4) the disease agent failed to complete its reproductive cycle in the host to any extent resembling its normal efficiency! All of this was accomplished by the simple expedient of feeding a *Lactobacillus*-based DFM (Dalloul et al., 2003a, 2003b)

How about the second question, will this work for mycotoxins? There is very limited university controlled research with a modified *Lactobacillus* (a cell-wall deficient strain or "L-form") and a growing body of field evidence that animals may benefit substantially from the regular application of this L-form in a fermentation mixture. The results have been encouraging in dairy cattle, swine, and poultry (broilers and turkeys) trials. Is this the answer?

There is a huge list of strategies that have been offered to prevent and combat mycotoxins. Table 1 gave but a very few. In thirty years of working with fungal toxins, it has been my experience that no single approach ever solved the problem. If it had, I would not be delivering this report today! At the same time, producers need to understand that the nature of the mycotoxin problem is large but elusive. Individual producers have little hope of knowing at any given time exactly how much of what mycotoxin(s) their animals are consuming (except when an outright toxicosis occurs and herds or flocks go down!). It would seem logical, therefore, that measures which increase the ability of the animal itself to thwart this environmental onslaught can yield the greatest returns on investment. It would be irresponsible to suggest that a simple DFM product could solve all problems alone. Producers must still take time and energy to undertake preventive measures and improve overall quality of feedstuffs. But, to the extent that DFM demonstrate capacity to enhance the animal's native resistance, this should be an essential element of control. Unlike many other measures, it does not require knowing the absolute chemistry of the offending compounds; it does not require daily monitoring of toxin consumption; it does not require absolute perfection in the accomplishment of those other preventive/quality assurance measures.

Perhaps the best advice is to consider the approach now being taken very actively by food manufacturers, NASA, and others. Application of the principles of Hazard Analysis Critical Control Point (HACCP) philosophy is being proposed for control of mycotoxins (Boutrif et al., 2002). My take on this is that individual producers need to learn a bit about HACCP and develop plans unique to their individual operations. I would suggest that simple preventive measures (such as equipment hygiene) and feed quality control (including purchase decisions, regular

testing, and record keeping) and enhancement of animal resistance (through an appropriate *Lactobacillus* vehicle) be included. Mycotoxin problems are not apt to disappear anytime soon. Successful animal producers will avail themselves of the control measures that are recommended today but be constantly watching for new strategies on the horizon.

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TABLE 1. Preventive Measures

1	Minimize moisture	Other than ensiled feeds, dry feeds ¹ generally store best at 12 - 12.5% moisture ²
2	Sanitize feed handling equipment	From storage tanks, to trucks, to mixers, to feed troughs, etc.
3	Use only best quality feed ingredients	Cracks, fines, damage, off-color, low protein, foreign material, etc. all tend to correlate with mycotoxin risk
4	As appropriate, minimize on-farm residence time for feeds, ingredients, and supplements	Primarily for milled or chopped materials. The longer a rich nutrient source remains in storage, the greater the toxin risk
5	Plant appropriate cultivars and use best agronomic practices	Caution! Some seed stock bred to resist one mold may, in fact, be more susceptible to infection by another mold genus. See, for example: Duvick, 2001
6	Protect feed crops from insect damage [e.g., insecticide application] and provide for proper storage post-harvest	See, for example: Dowd et al., 2000; Paster and Bullerman, 1988
7	Use appropriate anti-fungal compounds at adequate application rates	As a rule, a given amount of mold inhibitor buys a given amount of preservation time. That protection is subject to variation due to moisture and other factors. See, for example: Paster et al., 1999.

¹ “feeds” includes those separately handled and/or fed ingredients.

² Over-drying of feeds/ingredients is also bad since damaged grain is more easily infected by mold.

TABLE 2. Analytical Methods for Mycotoxins

Test	Comment
<i>Conventional - Chemical Affinity</i>	
1 “mini” column	Some commercial, can be constructed in-house. Qualitative or semi-quantitative
2 TLC	Screening; quantitative. Reliable. Reasonably simple to perform (but requires laboratory). Relatively low cost; moderate turn-around time. See Richard et al., 2002.
3 HPLC, GC, GC-MS, etc.	Low detection limits, high accuracy & precision. Equipment, supplies expensive. Time factor. Sophisticated laboratory access. See Stack, 2002.
<i>Biotechnological - Immunochemical</i>	
4 ELISA	As conventional 96-well plate ELISA or in modifications (single or well-strip tests), dipstick, membrane, etc. Reasonably reliable (assuming quality antibody & adequate extraction/preparation). Relatively fast/inexpensive. Some adaptable to ‘field’ use.
5 Antibody affinity column	Rapid, quantitative. Fast, relatively inexpensive. Minimal (low-cost) instrumentation.
<i>Emerging</i>	
6 Biosensors, fiber optic probes, micro-bead assays, etc.	See Maragos, 2002.
7 Flow-thru membrane ELISA	Field kits for rapid detection. See De Saeger et al., 2002.