TROUBLESHOOTING MYCOTOXIN PROBLEMS

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Whether frankly spoken or just a lingering doubt in the mind, the central question from those raising livestock and poultry is, "are these things called mycotoxins real and do they really cause me any problems?" That is often followed closely by, "what can I do that will fix the problem but won't break my bank account nor take up all my time?" Between those two is the real question, "how do I know I have it (them) and what should I being doing about it (them)?" In dealing today with the topic of *Troubleshooting Mycotoxin Problems*, a little of the first question will be discussed; however, the emphasis will be on how to recognize the problem is there and how to approach such problems in a routine and systematic fashion. One caveat, however; protocols often used today for determining and addressing mycotoxin problems exist on several levels. Those approaches needed for the research laboratory for the conduct of controlled mycotoxin experiments may differ substantially from those appropriate for a veterinarian encountering a field question. This paper and presentation will focus on the simpler and more practical directives that animal producers may find useful.

Mycotoxins are relatively simple, small organic compounds which are synthesized as secondary metabolites during the normal life cycle of a group of fungi we call the filamentous fungi, or molds. A mycologist might offer a very long list of genera and species that fall into that category. All likely undergo secondary metabolism and produce secondary metabolites; some of these metabolites, by virtue of being toxic to man or animals, are included in the group called mycotoxins. Of that much larger list of molds, there are, perhaps, seven or eight major genera and a few minor ones that seem most often to be at work when mycotoxins of practical significance crop up (Table 1). *Aspergillus* and *Fusarium* are usually the first molds

Table 1. Molds often associated with production animal mycotoxicoses

Major Genera	Minor Genera
Alternaria	*Absidia*
Aspergillus	*Chaetomium*
Fusarium	*Pithomyces*
Myrothecium	Etc.
Penicillium	
Stachybotrys	
Etc.	

considered when a problem arises. Certainly *Aspergillus flavus*, which is one of two molds that produce aflatoxin, is commonly recognized, at least by name. Dairy producers probably hear more often about *Fusarium* molds occurring in their forage. What causes the problem to become truly complex is the fact that, while we often mention one mold in association with one particular toxin (e.g., *A. flavus* and aflatoxin), these molds can and do produce more than one toxic

secondary metabolite. Continuing the *A. flavus* example, aflatrem (a toxin producing spasms and seizures in livestock), cyclopiazonic acid (a compound underlying the spontaneous hemorrhaging seen in Turkey "X" disease in the early 60's), and others are produced by this single mold. To complicate the story further, *A. flavus* produces two forms of aflatoxin, aflatoxins B1 and G2, both highly toxic to many species but structurally a bit different from each other. With *Fusarium*, we can find even worse situations, with large numbers of chemically similar compounds with unique actions in poultry and livestock.

A group of species in this genus is primarily responsible for a family of mycotoxins called 'trichothecenes' of which DON (vomitoxin) and T-2 toxin are among the best known. There are dozens of trichothecenes, each one of which generally exists in several forms. And then, some of the same organisms can also contaminate feeds and forages with fumonisins, fusarins, fusaric acid, zearalenone, and many others. So from a seemingly manageable number of important molds (Table 1) we quickly reach a point where we are dealing with over four hundred possible mycotoxins that can be found in animal feeds, pastures, bedding, and forages either as a single entity (rare!) or in various combinations.

There are many factors that determine when and if a toxigenic strain of mold will produce a mycotoxin. Often these are cultural in nature: substrate, form or matrix characteristics of that substrate (hard wheat vs. soft, e.g.), stage in life cycle, nutrient availability, nutrient ratios, water, heat, competitors, environmental cycles, etc. The lead-in sentence implies that some strains of a given mold are not toxigenic. That is true. Isolates can be found of *A. flavus* or other molds which do not produce the mycotoxins for which the genus and species are so famous. Of those, which do synthesize a toxic metabolite, the conditions listed above may determine whether that synthesis takes place or not. In simpler terms, not every isolate of a suspect mold produces toxin. Not every isolate of a normally toxin-producing mold does so under all conditions or circumstances. For the producer this means that textbook expectations may not be reliable as predictors or diagnostic tools. And, for those who have those doubts about the reality of mycotoxins, it is just such features of these molds, which play up to those doubts. Moldy feed that tests negative for mycotoxins? Of course, happens all the time! But some feeds which look, smell, feel really good may harbor acutely toxic levels of mycotoxins. This doesn't mean that mycotoxins aren't real, only that troubleshooting mycotoxin problems is never going to be an easy task.

Demonstrating the reality of mycotoxin effects experimentally is not too difficult. Perhaps the one group that still has doubts is the dairy industry, since some still believe that rumen microflora consistently and completely degrade fungal metabolites such that mycotoxins should not be of any major consequence in ruminants. The issue of the complex interactivities of rumen microflora and mycotoxins was recently reviewed (Doerr, 2003). Karlovsky (1999) showed that during the early, aerobic period of ensiling, mycotoxin synthesis is active but generally plateaus a short time following the attainment of anaerobiosis . With limited oxygen availability some molds may even metabolize some of the pre-formed toxins with a possible decline in the concentrations of those chemicals. In short, many mycotoxins [e.g., aflatoxins, *Fusarium* toxins, patulin, mycophenolic acid , etc. (Karlovsky, 1999)] are synthesized and found in silage regardless of concentration dynamics. Further, Diaz et al. (1997) showed that the dairy cow responds very predictably to sequential feeding of aflatoxin-contaminated and non-contaminated

feeds as measured by concentration of aflatoxin M1 in the milk. These reports should amply demonstrate that mycotoxins exist in the real production animal world and that they can and do cause problems for producers.

Ask a veterinarian about infectious bursal disease virus in chickens or an organism causing dysentery in swine and you will likely get a fairly uncomplicated description with a list of signs and symptoms. E.g., if you see this array of conditions, then it is that particular disease process. Dealing with mycotoxins, however, does not present so clear a picture. First of all, many of the disease signs they cause are not pathognomonic; that simply means that a given symptom may be caused by agents or factors other than, or in addition to, mycotoxins. The best-known feature of aflatoxicosis in most species is fatty infiltration of liver tissues. An enlarged, yellow, fatty liver is often distinctive during aflatoxicosis; however, a diet too high in energy, other poisons, etc. may also result in an enlarged, yellow, fatty liver. But, we can usually deal with the fact that a given sign may have several possible causes. What is almost impossible to resolved, however, is the fact that those few signs, which we can use as hallmarks, can be altered or disappear when mycotoxins interact with one another (or with other factors).

Three examples should demonstrate the real nature of the problem we face with mycotoxins in farm animal production. First, a now classic study of mycotoxin interaction in poultry (Huff and Doerr, 1980) looked at two primary responses by baby chicks fed two different toxins at growth inhibitory levels. Young broilers fed aflatoxin exhibit enlarged, pale or yellow, fatty livers, since aflatoxin is primarily a hepatotoxin. Young broilers fed ochratoxin A, which is primarily a nephrotoxin (affecting the kidneys), exhibit enlarged, pale (often with uric acid deposits) kidneys. In the experiment, aflatoxin chicks presented typical livers. Liver weight relative to body weight was significantly increased; liver lipid was elevated. Ochratoxin chicks also presented typical signs. Kidneys were enlarged on a relative weight basis and plasma uric acid levels were elevated. The group of chicks fed both toxins simultaneously responded quite differently. Livers were of normal color, although they were large. Liver lipid was not elevated. Kidneys were excessively enlarged; the extent of relative weight change was significantly greater than for the ochratoxin birds. Urate deposits were evident throughout the internal organs (visceral gout). The results of the interaction from a lab perspective did not cause any real problem. But consider the plight of the producer or the veterinarian trying to assess a field problem. Fatty livers, which could serve as indicators of aflatoxicosis, were absent. No real reason to suspect aflatoxin was at work at all. Kidneys were so grossly affected that analysis of the feed for ochratoxin would be warranted. But, the result (e.g., ochratoxin A concentration) would be too low to be consistent with the extent of damage produced. So, the diagnostician is left to try to decide what other factors were responsible. Mycotoxin interactions often result in a "new" syndrome that is not easily related to conditions described during single toxin experiments.

Example 2. Another classic interaction study with aflatoxin combined this fungal toxin with a disease agent responsible for dysentery in swine. Jones et al. (1981) showed that the presence of both toxin and organism halved the time for onset of diarrhea, doubled the time that the episode of diarrhea lasted, and doubled the expected mortality. How many production animals are free from exposure to primary or opportunistic pathogens? When the infectious disease is clearly the

proximate cause of death or production problems, how many producers or veterinarians will consider that one or more mycotoxins may have also been involved?

Example 3. This is not a "classic" interaction experiment. It is a real farm example that points to the huge amount of "unknown" we face in trying to deal with mycotoxins. A northern mid-west dairy operation was trying to troubleshoot problems. Including one or more mycotoxins as possibilities, the producer sent samples of the dairy rations for both chemical and biological analysis. The chemistry revealed the presence of DON (vomitoxin) and Zearalenone. Nothing else was found (in fairness, that statement is made without certain knowledge of how many toxins were requested to be included in the screen, if any nor of the sensitivities of the tests employed). On the biological side, only three mold genera were identified: *Mucor, Absidia*, and *Penicillium*. *Mucor* hasn't really been associated with any particular mycotoxin issue; Absidia has some suspicious qualities and field reports of various troubles, but isn't known for any of the "major" mycotoxins. The *Penicillium* species found in this case is documented to produce four or five very potent mycotoxins and two highly toxic alkaloids. No *Fusarium* was found despite the chemical analysis, which showed that only Fusarium-produced toxins were present. What conclusions should you draw? How many mycotoxins were really at work? How many molds were present, either at the time of the sampling or at the time the cows were eating the feed or even earlier? How do you troubleshoot this episode.

So, as many have already come to know, feed-borne mycotoxins present an inherently complex problem given the nature of the multiplicity of molds and their mycotoxins, the virtually limitless array of cultural factors and conditions which suppress or enhance toxin formation, and the exceptional capacity of mycotoxins to react with each other or with other stressors denying producers an 'easy' set of diagnostic patterns on which to base assessments of these problems. How, then, to troubleshoot if you don't have a solid chance of even identifying the total nature of the problem?

As with other factors affecting poultry and livestock, some diseases have greater prominence or impact than others (at least to the extent we know!). Usually, then, we base the extent of our vigilance on the risk expected. One can argue that a similar approach is effective when dealing with the elusive mycotoxin. Certainly many understand that geographic region and climatic conditions may influence which of the mycotoxins tends to occur with greater frequency. For example, while aflatoxin may be found throughout the world (as a matter of natural formation, not just because of the importation or regional transportation of feed ingredients), highest exposures tend to occur in those areas with moderately hot temperatures and relatively high humidities, especially when coupled with wide day-to-night fluctuations. Such climatic regions must be more watchful for *Aspergillus* toxins that, perhaps, cooler, drier climate in which *Fusarium* may predominate.

Less well understood, perhaps, is the fact that the geographic distributions of some toxins may be localized down to the farm level. In other words, one farm unit may consistently or repeatedly face one combination of toxins more often than a neighbor down the road who may be seeing slightly different fungal problems. One guideline for troubleshooting mycotoxin problems, therefore, is to be prepared to establish the toxin(s) of local prevalence and monitor for it. Although other contaminants may get into the system, it is likely more productive to concentrate

on those fungal metabolites that might be considered more routine for a given farm. In essence, this is a form of statistical process quality control. Producers need to establish their own historical record of mycotoxin exposure and monitor for deviations from that pattern.

Many mycotoxicologists will confirm that when there is regular monitoring of a production unit's feed and ingredients, the occasional high test result for some particular mycotoxins is of much less concern than a frequent finding of low to moderate levels. Chronic exposure is apt to take its toll much more than a mild, acute dose. The reason for this simply reflects the way in which animals metabolize these toxins and the mechanisms of action of many of these fungal poisons. Various enzyme systems, usually in the liver, act to modify foreign compounds to accelerate their elimination from the body. With transient exposure, and otherwise good health, the animal can accommodate to some exposure without serious effect. As for the toxins, often they do their damage by interrupting some feature related to synthesis of proteins in the animal. This may be at the level of the gene or beyond at the actual assembly of proteins by the cell. Regardless, a transient "hit" is typically sustained and repaired quickly, while continuous exposure results in cumulative damage that eventually shows up in reduced performance or even more serious results.

The producer, then, needs to be in a position to monitor for one or more 'marker' toxins that will allow some reasonable conclusions to be drawn as to the overall mycotoxicologic quality of the feeds and forages in use. The concept of marker toxins is not new and derives from the fact that since many molds produce more than one toxin, and that some toxins are far easier to assay than others, using simple means to deduce the extent of contamination is most efficient. It is beyond the scope of this presentation to review methods of analysis. However, rapid, antibody-based tests that can be performed at the farm level may be more than adequate for this kind of quality control. For descriptions of current best-method approaches to mycotoxin analyses, one can consult with FDA for copies of the proceedings of a summer mycotoxin analytical workshop held last year. In particular reports by Richard et al. (2002) and Stack (2002) may be of use. Emerging technologies may give producers both accuracy and precision, and a means for assessing multiple toxins in one sample. Maragos (2002) has described optic sensors, bioprobes, and microbead assays that are now under investigation.

Some will immediately question the reliability of such tests. Certainly the research laboratory with access to expensive and sophisticated instrumentation may be able to do a more consistent job of achieving accurate, highly reliable data. However, it is the shift from one relatively broad category of exposure to another that heralds an incipient problem in the dairy barn. For example, a herd with good production records and herd health that has a periodic record of exposure to, say, 400 ppb DON and 200 ppb zearalenone, is likely not at great risk until significantly higher levels are seen, e.g., 1,000 ppb DON plus 400 ppb zearalenone[1]. In one major poultry operation, screening of incoming corn for several mycotoxins is a regular part of the quality assurance program. While DON, per se, does not affect young broiler chickens, it has been the experience of this company that when corn tested above a certain level (on the order of 900 ppb) of DON, sufficient other fungal metabolites were present to cause very discernible and costly performance losses. The simple expedient of establishing their internal baseline allowed

[1] These values are used for the sake of illustration and do not in any way suggest "safe" levels for any animal species.

decision makers to improve their control of potential *Fusarium* mycotoxin problems. A similar anecdote reflects the experiences of two neighboring broiler integrators. For one, the management policies for growing birds, the health program, the overall nutrition program, etc. were such that the company could routinely accept corn testing as high as 85 ppb aflatoxin without any detectable loss of performance. Down the road, company B, with a different feeding program (lower protein rations) found to its dismay that corn with as little as 25 – 30 ppb aflatoxin caused noticeable loss of pigmentation, a significant economic parameter for these birds. Therefore, the regular testing of feeds to meet a locally derived standard for mycotoxin contamination is a major troubleshooting guideline.

And when faced with questions of accuracy (how closely the test reveals the actual level of contamination) versus precision (how closely repeated tests of the same material will agree), in this kind of quality control, precision wins hands down! Of course, testing requires time and expense. Some may opt not to use this important troubleshooting step. To those I would urge consideration of the fact that most producers have in their operations the most reliable test system yet devised. It is the bioassay, which, for animal producers is the cow, gilt, or broiler. Do you really want to wait until you get a "positive" in your bioassay before using less costly tests?

Troubleshooting also raises the issue of what actions to take once a problem is detected. This is real catch question because if producers attempt to go after each mycotoxin individually, the approaches to solve problems are as numerous and complex as was earlier described for those mycotoxins themselves. Procedures, then, to be most useful also need to be most generic. What can be done with minimal knowledge about the specifics of the threat that will offer the greatest resolution?

The first approach recommended to producers is to make maximum use of steps likely to reduce the risk of serious mold infestation and subsequent mycotoxin formation. Table 2 lists some of the more effective methods in use today. Others might be added, such as fumigation of some feed facilities; however, cost, safety concerns, and other factors tend to make such procedures relatively impractical. It needs to be realized that application of all preventive measures does not provide for foolproof protection. Molds, like other successful organisms, adapt to a wide variety of conditions. "Breaks" will occur and even the most diligent producer should expect to have a problem now and then. Furthermore, what appears to be a uniform environment is likely far from it. Pelleted poultry rations in an on-farm storage tank might may seem like a relatively constant product. However, just within the tank, temperature differentials created by rise and fall of the sun create very discreet microenvironments, some of which having moisture changes sufficient to support germination and growth of mold spores.

The next step to ensure that animals are provided with adequate nutrition and health care. Two major negative interactions occur with mycotoxins – sub-optimal plane of nutrition and infectious disease. Actually the latter is more reflective of the fairly universal capacity of mycotoxins to impact immune function in one way or another. This stems from several factors, among which are the increased energy expenditure required to overcome pressures on the immune system, the vulnerability to opportunistic pathogens, the adverse changes likely to affect the gastrointestinal tract (and the mucosal immune system associated with it), and the indirect

effects on immunity in terms of impaired protein synthesis (e.g., acute phase proteins in the liver) and cell division (e.g., negative impact on proliferation of immune-competent cells). It has been repeatedly demonstrated that simple provision of sufficient resources to give animals the best advantage in a stress situation and careful attention to prevention of infectious disease are indicated at all times, but certainly when there is risk of mycotoxin exposure.

Table 2. Preventive Measures

Minimize moisture	General rule of thumb for feeds and ingredients is to control moisture to about 12 - 12.5% moisture[a]
High quality feed ingredients	Cracks, fines, damage, off-color, low protein, foreign material, etc. all tend to correlate with mycotoxin risk
Sanitized equipment	Storage tanks, trucks, mixers, feed troughs, etc.
Short feed residence time on-farm	The longer a rich nutrient source remains in storage, the greater the toxin risk
Crop selection	Seek appropriate cultivars. Caution: Some varieties bred to resist one mold may be more susceptible to another. See, for example: Duvick, 2001
Prevent insect damage	See, for example: Dowd et al., 2000; Paster and Bullerman, 1988
Use appropriate anti-fungal compounds at sufficient 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.

[a] Over drying of grains results in damage which can increase risk of mold infestation.

Unfortunately, even with best precautions mycotoxins will find their way into the feed chain. What, then, can a producer do? Again, the best advice to look for solutions that are relatively generic (not toxin-specific). One exception may be the use of selective adsorption for 'detoxification' of feed. There are many advocates of the use of various clay products (aluminosilicates, zeolites, etc.). While there are some precautions to be observed, primarily dealing with the plethora of products offered today under this category that have not been subjected to complete lab and field testing, many experimenters have shown utility in using certain adsorbents when aflatoxin is the major intoxicant. Consistently, aluminosilicates which sport good binding data in the lab and subsequent field trials, do so with aflatoxin and during aflatoxicosis provide a legitimate sparing effect on animals exposed to acutely toxic doses of that mycotoxin. That same statement cannot be made, however, with respect to other mycotoxins.

While many product manufacturers make claims for multi-toxin efficacy, it is essential to require that they validate those claims with controlled experiments in vitro *and* in vivo for each toxin. Keep in mind that a compound which will sequester a toxin in a beaker of aqueous methanol in the lab may not do so in the animal's intestinal tract. And, an agent which successfully binds adequate amounts of aflatoxin to offer relief to the exposed animal may not do so at all for another toxin such as diacetoxyscirpenol (DAS).

Finally, and this is certainly still an area under exploration, there has been some progress made in application of direct-fed microbial (DFM) products for the sparing of mycotoxicoses. If one looks just at the possibilities with regard to intestinal immune function and control of inflammatory processes, it makes sense to expect that some beneficial organisms can do much to enhance the animal's capacity to deal with mycotoxin exposure itself. Dalloul et al. (2003a, b) have demonstrated numerical and functional changes in intra-epithelial lymphocyte subpopulations in broiler intestinal tracts in their studies of probiotic reduction of coccidial infection. Up regulation of immune function could be expected to assist animals exposed to dietary mycotoxins in combating both the toxin effects and the potential secondary pathogens that often become the cause of the animal's demise.

However, another microbial product has been tested specifically for efficacy in a mycotoxin challenge situation. Wu (1997) reported highly significant sparing effects when a modified *Lactobacillus*-based microbial product was administered to chicks fed diets contaminated with *F. proliferatum*, an organism which produces at least two toxins, moniliformin and fumonisin B1. Using culture material assayed for both toxins as a feed additive at levels sufficient to kill a majority of birds, the DFM reduced toxin effects by about half. Using a water delivered form for poultry makes this an ideal strategy since only specific farms undergoing an actual problem need put it to use. This is, of course, consistent with the earlier recommendation that producers do their mycotoxin control assessments down to the individual farm level. But, with respect to this type of DFM, field trials, primarily with dairy cows exposed to levels of mixed mycotoxins (DON, zearalenone, DAS, aflatoxin, fumonisins, and others) that shut down milk production, interfere with estrus, and cause morbidity and mortality in the herd, have shown even better responses with a similarly formulated DFM. While additional work remains with these products, the number of field cases resolved this way and the few confirming research trials suggest that this may prove to be a useful, toxin-non-specific approach to preventing serious economic loss from exposure to mycotoxins.

In summary, troubleshooting mycotoxicologic problems on poultry and livestock farms starts with a fundamental approach to quality assurance at the farm level that includes basic preventive measures and the development of a unit's history of marker toxin exposures and animal performance. A deviation in either should be taken as a warning that mycotoxin exposure may have begun. Here the recommendations may diverge widely. One school will say that increasing sampling and testing is now warranted. The sporadic nature of mycotoxin contamination often means that when animals are showing distress the feed sampled then may no longer contain the toxins which caused the distress in the first place. However, if testing is regular and consistent, increase in positives or steady rise of concentrations are more realistic clues that a problem is underway. In any event, once that occurs, only a few remedies seem appropriate. If the responsible, or at least major, toxin is known, then a reasonable decision can

be made with respect to a feed additive such as a clay-based adsorbent. Confirming the animal's nutritional status and health are always warranted. In some cases there may be advantage in trying the application of more recent DFM approaches which offer the advantages of working without regard to the particular toxin or its mode of action. Finally, success comes usually to those that recognize that no single approach will solve all mycotoxin problems. Fungal infestation, metabolism, and synthesis of toxic products goes on all the time. Those who assume it can be there and have a regular program to control it will ultimately succeed in animal productivity and economic return over those who chose to wait until disaster strikes.

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