Mycotoxin Testing in the Feed Chain: A Risk Prevention Strategy for Raw Material Suppliers, Grain Storage Facilities and Processors, and Feed Manufacturers

INTRODUCTION

For feed chain stakeholders in an increasingly competitive marketplace, the bottom line can rise or fall with the contaminant levels in grain and other raw materials. The repercussions of feedstuffs that fail to meet the highest safety and quality standards can spread across the entire value chain, jeopardizing livestock health and the reputation and economic performance of multiple businesses and entire growing regions. Ultimately, toxic contaminants that slip through quality control checks put the safety, abundance, and affordability of the animal proteins in our food supply at risk. The price of undetected contamination can prove particularly high when the toxic threat in question comes from a class of fungal contaminants known as mycotoxins. The reasons lie in the very nature of these toxic mold metabolites and their numerous biological effects on animals.

THE NATURE OF MYCOTOXINS: PREVALENT, PERSISTENT, AND POTENT

Feed grains are grown all around the globe, with the midwestern grain belt of United States dominating corn and soybean production and China and India occupying the top two spots for wheat. Mycotoxins not only occur in every grain-growing region of the world, but can also inhabit virtually every feed ingredient from whole grains to milling byproducts, oil meals, and ethanol co-products. A global survey program conducted between 2004 and 2013 uncovered mycotoxin contamination in over 76 percent of the 25,900 feed and feed ingredient samples that were tested.[1]

The molds that produce mycotoxins are commonly occurring soil- and airborne species that can infect grains and other raw materials in the field as well as during storage, transportation, and processing. Factors that fuel the spread of these molds and their toxic byproducts range from unavoidable environmental conditions to faulty processes:

- High temperatures
- Extremes in water activity (e.g., excessive moisture levels, drought)
- Severe storms
- Insect damage
- Inadequate drying
- Improper storage conditions (e.g., poor ventilation, leaks, high-humidity)

While mold reduction strategies, such as drying and irradiation, can help prevent the formation of additional mycotoxins in contaminated commodities, they're less successful at eliminating the mycotoxins that are already there. Heat processing may also leave a significant proportion of these highly stable chemical compounds intact. Some forms of processing actually increase mycotoxin content. For instance, the processes that create distillers' dried grains with solubles (DDGS) and many other grain byproducts tend to concentrate mycotoxins in the feed components.[2] Detoxification methods such as ozone and organic acid treatments as well as feed additives that bind to or degrade mycotoxins in an animal's gut also come with limitations, including their cost. Some of these methods may also reduce nutrient content or form toxic residues.

FDA regulations generally prohibit grain dealers from blending contaminated feedstuffs with clean grain to dilute concentrations of mycotoxins that exceed its current guidelines. Consequently, companies faced with unsafe mycotoxin levels often have no real alternative to paying the price of detoxification or discarding or diverting contaminated lots to less profitable market outlets. If suppliers, storage facilities, or millers have to repeatedly resort to these strategies, their ability to offer competitively priced products or services will eventually suffer. Regional outbreaks of contamination in grain crops have more far-reaching economic implications. Widespread shortages of acceptable raw materials can result in region-wide price increases. Feed manufacturers are then left to choose between passing the higher cost on to their customers or substituting less expensive ingredients that could potentially reduce their product's quality and functionality. For some manufacturers, the only practical option may be to rebuild their supply chain in a different geographical area, a strategy that entails risks and uncertainty for them as well as for the regional economy.

The costs of mycotoxin control escalate not only as the contamination becomes more extensive, but also as it flows downstream. The greatest risk lies in the failure to detect and deal with contamination before products reach feed customers. The urgent need to address that risk is rooted in the most significant characteristic of mycotoxins. As a group, they include some of the most potent toxins found in nature. For instance, the most prevalent mycotoxin, aflatoxin B_1, is the most carcinogenic naturally occurring substance known and can cause severe toxic effects in every animal species. The other highly potent mycotoxins that most frequently occur in feedstuffs include ochratoxin A (OTA); fumonisins; deoxynivalenol (DON, vomitoxin); zearalenone (ZEA); and the T-2 toxin. Concentrations of these mycotoxins low enough to be measured in parts per million (ppm) or even parts per billion (ppb) can seriously compromise livestock health and performance.

Susceptibility to the harmful effects of mycotoxins is heightened in young animals as well as in those that are immune-compromised or stressed. Sensitivity to a particular mycotoxin also varies according to the animal's sex, breed, nutritional status, weight, and species. Swine are highly sensitive to all six of the major mycotoxins, while other species may exhibit some degree of resistance to one or more of these contaminants. (See Table 1.) Poultry, for instance, are fairly resistant to ZEA;[3] while cattle have a higher tolerance for OTA than poultry and swine.[4] The effects of other mycotoxins on cattle also tend to be milder in comparison to the response of other species; however, acute aflatoxicosis does occur in cattle when they consume contaminated feed for long periods. Because very young calves lack the ability to degrade mycotoxins in their upper gastrointestinal tract, they are at greater risk for all forms of mycotoxin disease than more mature calves and adult cattle.

Although mycotoxin action and guidance levels set by the FDA (see Table 1) and other government agencies factor in the species and age of the animal, they make no allowances for various real-world stresses such as infections, temperature extremes, and poor management practices that can increase the mycotoxin sensitivity of livestock in the field. Nor do they fully account for another risk factor: feed typically contains a mixture of mycotoxins. Because interactions between different mycotoxins can dramatically increase their toxicity, doses of two or more mycotoxins that would be harmless if consumed individually can cause serious symptoms when they're combined. Examples of highly toxic combinations include pairs of chemically similar mycotoxins such as DON and nivalenol and the T-2 and HT-2 toxins. These additional hazards point to the importance of striving to not only keep mycotoxin levels as low as possible, but also to gain a detailed picture of the mycotoxin content of feedstuffs.

Mycotoxin-contaminated feed also has serious health and economic implications for the human food supply. Laboratory studies have shown that animal products can retain traces of the mycotoxins that livestock have consumed. Milk that contains more than 0.5 ppb of the aflatoxin B_1 metabolite aflatoxin M_1 is banned from sale in the United States. An even lower legal limit of 0.05 ppb prevails in the European Union. To reduce the risk of contaminated milk, regulators generally set much lower aflatoxin limits for dairy cows than for other mature animals. The presence of aflatoxins, OTA, and other mycotoxin residues in meat and eggs is also a target of increasing consumer and regulatory concern.

THE EFFECTS OF MYCOTOXINS ON LIVESTOCK HEALTH AND PRODUCTIVITY

The high price of mycotoxin contamination first came to light in 1960 when an epidemic of acute aflatoxin poisoning killed over 100,000 turkeys. Industry awareness of the problem has grown in the wake of subsequent outbreaks of acute aflatoxicosis and other types of potentially fatal toxicity such as the fumonisin diseases that attack the lungs of swine (porcine pulmonary edema) and the brains of horses (leleukoencephalomalacia). As costly as these lethal poisonings are, livestock producers incur even heavier losses as a result of the more subtle effects of mycotoxins on animal health and performance. (See Table 1.) Mycotoxin contamination that causes chronic organ damage and diminished productivity, reproductive capacity, and resistance to disease not only increases overhead expenses, such as veterinary care and discarded feed, but also steadily erodes profits. Farmers and ranchers who raise livestock for meat may face revenue losses as a result of lower birth rates or the decreased market value of animals that weigh less because of digestive ailments and reduced feed consumption. These same health issues can also lead to declines in the quantity and quality of milk or eggs for farmers whose income depends on the yields and marketability of these products.

Feedstuffs that expose livestock producers to these costly risks can have equally devastating consequences for manufacturers and their supply chain partners. Depending on the extent of the potential or actual damage and the company's liability, the fallout can range from product recalls and FDA interventions to lawsuits. The ramifications of these events can extend beyond their immediate financial impact on the company, casting a lingering shadow of mistrust over its brand.

THE COSTS OF MYCOTOXIN-CONTAMINATED FEED AND HOW TO CONTROL THE DAMAGE

Frequent monitoring of raw materials ay every stage of production can stop the spread of mycotoxins before the costs of managing the problem become too steep. In addition minimizing the risk of contaminated raw materials and finished feed, this approach can lead to significant process improvements in storage facilities and feed mills. Timely discovery of an uptick in mycotoxin levels may point to problems such as a leaky water pipe, dirty storage bins, or inadequate attention to rotating stock that indicate the need for internal practices that will help prevent future outbreaks. Now that the FDA Food Safety Modernization Act (FSMA) holds companies responsible for preventing contamination, this proactive approach to hazard control is not only financially smart but also legally prudent.

For feed industry businesses and the laboratories that test their products, the quality of the data that a test system provides is paramount. Inaccurate or inconsistent analytic results compound the costs of misinformed decisions with the budgetary impact of a misspent technology investment. Sampling plans based on principles developed by GIPSA address the most frequent cause of misleading test data. The substantial risk of under- or overestimating the overall contamination level of bulk shipments stems from the tendency of mycotoxins to accumulate in tiny, widely scattered pockets of damp or damaged grain. To obtain a statistically representative picture of the lot's contamination level, incremental subsamples should be taken from multiple locations and pooled into a larger aggregate sample. GIPSA recommends collecting a minimum of 2 to 10 pounds of grain, depending on the size of the load and using proper sampling equipment, such as a hand or mechanical probe for stationery grain or a pelican or diverter-type sampler for a moving stream.[5] These procedures should be formally documented and followed to the letter by every employee. To further minimize the opportunities for error, correct sampling techniques should be combined with a technology solution that's officially validated for the commodity that's under scrutiny.

To qualify as practical and cost-effective, this solution must deliver data that advance the user's most urgent goals. Easy, economical access to reliable real-time mycotoxin information is as vital to laboratory efficiency as it is for onsite quality checks and effective storage management. Exceptionally robust test methods that confirm all relevant mycotoxin levels conform to official standards are equally essential for high-stakes buying decisions and evidence-based compliance with supplier verification programs. Although recommended limits lack the force of law, mycotoxin levels that exceed them can be used as evidence that products are adulterated and unfit sale, raising the specters of regulatory penalties, breach of contract suits, and disrupted supply chain relationships. The complexity and strictness of these standards vary depending on the market where a laboratory or feed business operates. In the EU, safety standards span seven mycotoxins and include maximum limits as low as 5 ppb for aflatoxin B1.[6] A growing number of suppliers and processors in the United States need equally comprehensive and exacting mycotoxin profiles to satisfy the increasingly stringent quality and safety demands of today's feed manufacturers. These data not only help ensure that raw materials and finished products command the best prices, but also minimize the risk that those commodities will expose animals to the increased toxic potential of combined mycotoxins.

RAPID MYCOTOXIN TEST METHODS

Massachusetts test developer VICAM offers a complete line of GIPSA- and AOAC-approved mycotoxin testing options that meet all these diverse needs. Widely recognized by government regulators for its superior performance capabilities, VICAM's patented monoclonal antibody technology ensures fast, accurate, reliable detection and measurement of the most significant mycotoxins that occur in feedstuffs.

QUANTITATIVE STRIP TESTS

VICAM's quantitative strip tests combine the benefits of speed, affordability, and ease of use with the assurance of accurate numerical results. No special skills are required for sample preparation or interpreting the test. The test solution develops in as little time as 5 minutes, and results are clearly displayed on the digital screen of a portable optical reader.

Strip tests are the most economical choice for situations where immediate onsite decision support is critical, including:

- determining the acceptability of shipments at buying points
- high-throughput laboratory screening
- routine monitoring and quality control checks at mills and silos

The line enables untrained users to detect and measure four highly significant mycotoxins at levels that meet rigorous safety standards:

- Aflatoxins: Afla-V® (ppb levels)
- DON: DON-V® (ppm levels)
- Fumonisins: Fumo-V® (ppm levels)
- Ochratoxin A: Ochra-V™ (ppb levels)

IMMUNOAFFINITY COLUMNS

The most powerful, versatile, and comprehensive solution set in VICAM's portfolio is its line of immunoaffinity (IA) columns. The company offers a variety of columns that are officially approved for determining mycotoxins in a wide range of feed ingredients, including corn, barley, corn bran, condensed distillers solubles, corn flour, corn gluten feed, corn gluten meal, corn meal, corn screenings, corn/soy blend, distillers' dried grains, DDGS, sorghum, soybeans, and wheat. Used to purify and concentrate samples for analysis by fluorometry or laboratory instruments, IA columns optimize the value of representative sampling by addressing two other frequent causes of inaccurate test results:

1. Matrix interferences: Grain and other feedstuffs contain a variety of components with properties similar to those of mycotoxins. IA sample cleanup effectively removes these components from the test sample, reducing the risk of false negatives and false positives.
2. Human error: The benefits of a purified sample can be offset by a complex, error-prone cleanup process. By simplifying sample preparation, IA columns minimize the chance of procedural missteps.

Test methods that incorporate IA sample cleanup detect lower concentrations of mycotoxins and measure them more precisely than strip tests. Their cost varies according to their technical sophistication and performance level. The most economical option uses a fluorescence detection device that can be easily operated by onsite users as well as laboratory technicians. Methods that require more specialized analytical services offer the greatest degree of accuracy and precision for a tradeoff in price.

Coupled with a portable fluorometer, VICAM's IA columns can be used in laboratories to prescreen samples for instrumental analysis and to check mycotoxin levels in grain elevators, incoming shipments, and processing facilities. This cost-effective rapid method requires no special training and provides accurate ppb measurements in less than 15 minutes.

For results that ensure highest degree of certainty, VICAM recommends combining IA column cleanup with liquid chromatography (LC). The exceptional sensitivity of sophisticated instrumental techniques such as high-performance and ultra-performance liquid chromatography (HPLC and UPLC) with optical detection and LC with mass spectrometry (MS) detection enables laboratory analysts to obtain exact measurements of very low levels of mycotoxins in complex matrices. These methods provide the precise, reproducible data needed to satisfy manufacturers who require third-party certification that ingredients are fit for use in their products. These certifications also play a vital role in documenting the geographical source of feed ingredients and verifying the observance of industry-standard safety practices across the supply chain.

There's an IA solution for every major mycotoxin of concern:

- AflaTest®
- DONtest™ HPLC
- FumoniTest™
- OchraTest™
- T-2test™ HPLC
- ZearalaTest™

IA columns are also available in fast-flow wide-bore versions to accelerate sample throughput. To help laboratories further boost their efficiency while responding to their clients' concerns about highly potent mycotoxin mixtures, VICAM offers several kits that simultaneously determine ppb levels of two or more mycotoxins in a single test run:

- AflaOchra™ HPLC (aflatoxin/OTA)
- AOZ® HPLC (aflatoxin/OTA/ZEA)
- DON-NIV™ WB (DON/nivalenol)
- T-2/HT-2™ HPLC (T-2/HT-2)
- Myco6in1+® for LC/MS/MS (six major classes of mycotoxins)

These multi-analyte kits offer multiple advantages:

- Faster time-to-results
- Higher throughput
- Reduced spending on lab consumables and hazardous waste disposal
- Decreased hands-on time
- Smaller environmental footprint

The multi-analyte method that delivers the greatest efficiency gains and the most detailed and authoritative mycotoxin data is Myco6in1+ for LC-MS/MS. Validated by the European Committee for Standardization (CEN), this high-powered instrumental method accurately detects and quantifies 12 different mycotoxins, including aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone, the T-2 and HT-2 toxins, and nivalenol at or below EU guidance levels.

THE LONG-TERM VALUE OF MYCOTOXIN CONTROL: A GLOBAL PERSPECTIVE

Widespread adoption of today's most advanced mycotoxin test methods at every stage of feed production promises a wealth of benefits for not only industry stakeholders but also society as a whole. As a global strategy, a robust, agile mycotoxin control system is a key element of worldwide efforts to sustain the livelihoods of thousands of agribusiness employees, the economic health of countries in every region of the world, and the strength and value of a vital link to the food chain.

Table 1. Health and performance effects of major mycotoxins of concern for susceptible livestock and current FDA feed safety guidelines[7, 8, 9, 10, 11, 12, 13, 14, 15]

Swine				
Mycotoxins	Commodity	FDA level	Health effects	Performance effects
Aflatoxin	Corn/peanut/other ingredients excluding cottonseed meal	200 ppb*	Liver damage Liver cancer Lower immunity Embryo death Birth defects Hemorrhage	Reduced reproductive performance Feed refusal/weight loss
	Cottonseed meal	300 ppb†		
DON	Grain/byproducts	5 ppm	Vomiting/intestinal symptoms Lower immunity	Feed refusal/decreased weight gain
Fumonisins		20 ppm	Porcine pulmonary edema (PPE) Heart/liver/pancreas damage Lower immunity	Reduced feed intake/growth
OTA§			Liver/kidney damage	Decreased productivity Decreased feed intake/weight loss Contaminated meat
T-2			Intestinal symptoms Blood disorders Lower immunity Intestinal lesions	Reduced feed intake/lower weight gain
ZEA			Embryo death Vomiting/diarrhea Hemorrhage Hormonal disorders	Reduced reproductive performance Feed refusal/weight loss

Poultry				
Mycotoxins	Commodity	FDA level	Health effects	Performance effects
Aflatoxin	Corn/peanut/other ingredients excluding cottonseed meal	100 ppb*	Liver damage Liver cancer Lower immunity Embryo death Birth defects Hemorrhage	Reduced egg production Contaminated eggs/poultry
	Cottonseed meal	300 ppb†		
DON	Grain/byproducts	10 ppm	Lower immunity Intestinal disorders	Impaired performance/productivity
Fumonisins	Corn/byproducts	100 ppm‡	Diarrhea Liver damage	Decreased egg production/quality
OTA			Liver/kidney damage	Reduced egg production Decreased feed intake/growth Contaminated eggs/poultry
T-2			Intestinal symptoms Oral lesions Bruising Lower immunity	Reduced feed intake/weight loss Reduced egg production

Dairy cattle				
Mycotoxins	Commodity	FDA level	Health effects	Performance effects
Aflatoxin	Corn/peanut/ other ingredients	20 ppb*	Liver damage Embryo death Birth defects Hemorrhage Diarrhea	Reduced reproductive performance Contaminated milk Lower milk production
DON	Grain/byproducts	10 ppb	Digestive symptoms	Feed refusal Lower milk prodution Reduced reproductive efficiency
	DDG/brewers grains/gluten	30 ppm		
Fumonisins	Corn/byproducts	60 ppm‡	Liver/kidney damage	Weight loss Lower milk production
OTA§			Depression Dehydration	Feed refusal/weight loss
T-2			Intestinal symptoms/ hemorrhage Lower immunity	Decreased milk production Feed refusal Reduced reproductive performance
ZEA			Hormonal disorders Abortions	Reproductive problems Reduced feed intake Lower milk production

Beef cattle				
Mycotoxins	Commodity	FDA level	Health effects	Performance effects
Aflatoxin	Corn/peanut/other ingredients excluding cottonseed meal	300 ppb*	Liver damage Embryo death Birth defects Hemorrhage Diarrhea	Reduced reproductive performance Contaminated meat Feed refusal
	Cottonseed meal	300 ppb†		
DON	Grain/byproducts	10 ppm	Digestive symptoms	Reduced feed intake Impaired reproductive performance
	DDG/brewers grains/ gluten	30 ppm		
Fumonisins	Corn/byproducts	60 ppm‡	Liver/kidney damage	Weight loss
OTA§			Depression Dehydration	Feed refusal/weight loss
T-2			Intestinal symptoms Lower immunity	Decreased feed intake Reduced reproductive performance
ZEA			Hormonal disorders Abortions	Reduced reproductive performance

Horses				
Mycotoxins	Commodity	FDA level	Health effects	Performance effects
Aflatoxin	Corn/peanut/ other ingredients	20 ppb	Liver damage Seizures Lower immunity Embryo death Birth defects Hemorrhage	Reduced reproductive performance Weight loss
DON	Grain/byproducts	5 ppm		Feed refusal/decreased intake
Fumonisins	Corn/byproducts	5 ppm	Equine leleukoencephalomalacia	
OTA			Kidney damage	Reduced growth/performance
T-2			Intestinal symptoms Lower immunity	Decreased feed intake
ZEA			Hormonal disorders	Reduced reproductive performance

**Action level for immature animals is 20 ppb; guidance level for breeding swine and cattle is 100 ppb.*

† All animals regardless of age or breed.

‡Advisory level for breeding animals is 30 ppm.

§Very young (preruminant) calves.

References

1. G. R. Murugesan, et al., "Prevalence and Effects of Mycotoxins on Poultry Health and Performance, and Recent Development in Mycotoxin Counteracting Strategies," *Poultry Science*, 2015. doi: 10.3382/ps/pev075
2. Luciano Pinotti, et al, "Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts," *Toxins*, February 15, 2016.
3. "Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Zearalenone as Undesirable Substance in Animal Feed," *EFSA J*, 2004.
4. Gianni Battacone, Anna Nudda, and Giuseppe Pulina, "Effects of Ochratoxin A on Livestock Production," *Toxins* (Basel), 2010 July; 2(7): 1796–1824.
5. USDA Grain Inspection Handbook—Book I Grain Sampling, https://www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx (accessed March 26, 2016).
6. The Merck Veterinary Manual, Merck & Co., rev. May 2012, http://www.merckvetmanual.com/mvm/toxicology/mycotoxicoses/fumonisin_toxicosis.html (accessed March 7, 2016).
7. "Background Paper in Support of Fumonisin Levels in Animal Feed: Executive Summary of this Scientific Support Document," U.S. Food and Drug Administration, November 9, 2001, http://www.fda.gov/Food/FoodborneIllnessContaminants/NaturalToxins/ucm212900.htm (accessed March 7, 2017).
8. D. Dhanasekaran, S. Shanmugapriya, N. Thajuddin, and A. Panneerselvam, "Aflatoxins and Aflatoxicosis in Human and Animals," Bharathidasan University, 2P.G. & Research Department of Botany & Microbiology, A.V.V.M. Sri Pushpam College, http://cdn.intechopen.com/pdfs-wm/20393.pdf (accessed February 27, 2016).
9. "Understanding and Coping with Effects of Mycotoxins in Livestock Feed and Forage," North Carolina State University and A & T University Cooperative Extension, https://www.cals.ncsu.edu/an_sci/extension/animal/nutr/Understanding_mycotoxins.pdf (accessed February 24, 2016).
10. W. Awad, K. Ghareeb, J. Böhm, J. Zentek, "The Toxicological Impacts of the Fusarium Mycotoxin, Deoxynivalenol, in Poultry Flocks with Special Reference to Immunotoxicity," *Toxins* (Basel), April 29, 2013; 5(5): 912–25. doi: 10.3390/toxins5050912.
11. X. Chen, B. Grenier, and T. J. Applegate, "Aflatoxins in Poultry," Purdue Extension, https://www.extension.purdue.edu/extmedia/AS/AS-615-W.pdf (accessed February 24, 2016).
12. L. W. Whitlow, W. M. Hagler, Jr., "Mycotoxin Contamination of Feedstuffs – An Additional Stress Factor for Dairy Cattle, North Carolina State University," https://www.cals.ncsu.edu/an_sci/extension/dairy/mycoto~1.pdf (accessed February 27, 2016).
13. "Molds, Mycotoxins and Their Effect on Horses," Ontario Ministry of Agriculture, Food, and Rural Affairs, http://www.omafra.gov.on.ca/english/livestock/horses/facts/info_mycotoxin.htm (accessed March 1, 2016).
14. "FDA Regulatory Guidance, A Guide for Grain Elevators, Feed Manufacturers, Grain Processors, and Exporters," *National Grain and Feed Association*, August 2011.

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