Mycotoxin Testing Information

Testing Options for Mycotoxin Analysis in Corn and Feed

Corn producers sometimes have a need to test for mycotoxins in their corn, corn silage crops, or corn-based feeds, especially when drought or flooding damage occurs. Mycotoxins periodically of concern in Kentucky corn include aflatoxins, fumonisins, vomitoxin (DON) and zearalenone, although others may occur sporadically.

Sampling Corn for Mycotoxins

The distribution of mycotoxins in a corn lot is usually highly variable, and it can be extremely variable for aflatoxins.

For harvested grain, a recommended sampling approach is to collect at least ten probes full from a number of locations throughout the lot, or at least ten collections from a moving stream of grain. A ten-pound sample is commonly recommended, especially for aflatoxins. Although some aflatoxin protocols call for smaller samples for trucks or wagons (2-5 lb), larger samples are likely to give more accurate results.

For sampling ear corn, ears from 30-50 locations throughout the field should be collected, shelled, and mixed. The sample should represent no more than five acres of corn. Sampling ear corn is not as accurate as sampling shelled grain, but is sometimes necessary.

Shelled corn must be ground before the sample is analyzed for mycotoxins. A recommended approach is to mix the sample well, crack the entire sample, mix it again, remove a 2- to 4-pound subsample, grind to about the consistency of flour (to pass through a No. 20 sieve), and analyze the finely ground subsample.

For sampling dry feeds (no more than 12% moisture), collect feed from 12-20 locations within the lot. Include places where molds are likely to accumulate, such as the sides of storage bins or feed bunks. Mix the sample well and collect two pounds for analysis or shipment. Place in a clean cloth or paper container for shipment and storage. For sampling higher-moisture feeds (12+% moisture), sample as for dry feed, but maintain two-pound sub-samples frozen in plastic bags with the air squeezed out. Ship frozen or hand-deliver to the laboratory. If the moisture content is unknown, best practice is to handle as a wet sample.

Two useful publications on sampling produced by the USDA Agricultural Marketing Service (AMS) are:

- *Practical Procedures For Sampling Grain At Farm Sites And Remote Locations*, at
  https://www.ams.usda.gov/resources/practical-procedures-sampling-grain
- *Inspecting Grain: Practical Procedures for Grain Handlers*, at
  https://www.ams.usda.gov/resources/practical-inspection-procedures-grain-handlers

Sampling silage poses special challenges. See the following Extension publication for suggested practices, http://learningstore.uwex.edu/assets/pdfs/A2309.pdf.

Mycotoxins can accumulate during storage if samples are held under warm, moist conditions. Storage or shipment of the sample at a moisture content below 12-13% essentially prevents the continued development of mycotoxins. Dried samples should be shipped in cloth or paper containers. Moist samples should be frozen immediately and shipped frozen or hand-delivered to the laboratory.

The USDA-AMS *Mycotoxin Handbook* provides additional information on processing samples:

https://www.ams.usda.gov/sites/default/files/media/MycotoxinHB.pdf

Mycotoxin Testing Options

Methods for mycotoxin analyses fall into two main categories: **(1) rapid screening methods**; and **(2) conventional confirmatory methods**. The most appropriate method depends on the intended use of the results – for example, is a qualitative ("yes/no") result sufficient, or is exact quantitation (concentration) needed? Brief summaries of the various testing methods are provided below.

Rapid Screening Methods

The benefits of rapid screening methods are generally lower cost, faster results (often the same day), less skilled technical requirements, portability, and more rapid throughput of large numbers of samples. The downside of rapid screening methods is generally increased cross-reactivity and matrix interference, co-extraction of other substances from the sample, and considerably greater chance of false positive or false negative results. Also, many methods provide a qualitative ("yes/no") result, indicating the presence or absence of a mycotoxin above a predetermined value, but they cannot give an actual concentration. Most rapid screening methods also require some degree of instrumentation for detection of results, such as a spectrophotometer or fluorometer, but these are less expensive than the instrumentation required for more specific confirmatory testing. And lastly, most rapid screening methods are only valid for specific sample types. Any positive results generated by a rapid screening method should be confirmed with a more selective/specific confirmatory method.

There are numerous technologies utilized in rapid screening methods, including:

- Immunoassay-based methods (e.g., enzyme immunoassay [ELISA], fluorescence immunoassay [FIA], flow-injection liposome immunoanalysis, and lateral flow devices)
- Sensors and biosensors such as molecularly imprinted polymers; and,
- Thin-layer chromatography (TLC).

Other indirect screening methods include Fourier transform infrared spectroscopy (FTIR), near-infrared spectroscopy (NIR), and detection of volatile metabolites of fungi by "electronic noses". Other new emerging rapid methods are becoming more available.

Confirmatory Methods

The standard confirmatory methods provide accurate, selective, and sensitive analyses and generally involve separation methods such as chromatography or electrophoresis. The benefits of these methods are high specificity, high sensitivity, and hence much less risk of false positive or false negative results.

Also, these methods can provide actual concentrations of the different mycotoxins, so that the suitability of various feeds for different species and classes of animal (i.e., growing vs. reproducing; dairy vs. beef; etc.) can be determined. The downside of these methods includes more expensive instrumentation, much more skilled technical requirements, higher cost per analysis (typically $30 or greater per mycotoxin, or $100 or more for a full panel), and a longer run time for analyses (typically several days to a week). The standard confirmatory methods include high performance liquid chromatography (HPLC) with various detection methods such as fluorescence or mass spectrometric (LCMS or LC-MS/MS); and gas chromatography for a few select mycotoxins. Other analytical methods are available but much less common.

Choosing a Method

When would you choose one method over another? When testing a load of shelled corn intended for human food production or for undeclared interstate transport, a *qualitative* ("yes/no") rapid screening method is often sufficient. In such a case, the aflatoxin level must be <20 ppb. In contrast, imagine a situation where a producer has an aflatoxin-contaminated lot and wants to feed it on-farm. A quantitative test would be best, since different species and classes of livestock tolerate different concentrations of aflatoxin. Additionally, any sample positive for mycotoxins using a rapid screening method should be confirmed with a standard conventional method.

What about black light tests for mycotoxins? The black light test looks for the presence of a fluorescent green/yellow color when a sample is evaluated under a black light. Fluorescence is caused by a substance co-produced by the fungus that produces aflatoxin--not by aflatoxin itself. This is only a presumptive screen, and false positives and false negatives are very common. Many other compounds in grains can fluoresce, and any positive result should be confirmed with a conventional method. This test is inappropriate for any mycotoxin other than aflatoxin. Because of the inaccuracy of this test, it is generally not recommended.

Laboratories That Test for Mycotoxins

Below is a list of some of the laboratories that test for aflatoxins or other mycotoxins in corn and other products. It is advisable to contact the lab prior to submitting samples to determine whether they are doing quantitative or qualitative tests, using approved methods. If you are planning on testing forages such as silage and TMR rations, make sure the laboratory can analyze these complex feeds. If testing for an insurance claim, make sure the laboratory used is acceptable to your insurance adjustor.

A sampling of laboratories that provide mycotoxin analyses include the following.

Breathitt Veterinary Diagnostic Laboratory
Hopkinsville, KY
(270) 886-3959
https://breathitt.murraystate.edu/

Waypoint Analytical Pennsylvania
Leola, PA
(717) 656-9326
https://www.waypointanalytical.com/Contact

Barrow-Agee Lab
Memphis, TN
(901) 332-1590
https://balabs.com/

Cumberland Valley Analytical Lab
Maugansville, MD
(800) CVASLAB
https://www.foragelab.com/

Dairyland Laboratories
Arcadia, WI
(608) 323-2123
https://www.dairylandlabs.com/

Dairy One Lab
Ithaca, NY
(800) 496-3344
https://dairyone.com/

Eurofins
Des Moines, IA
(515) 280-8378
https://www.eurofins.com/

Holmes Laboratory, Inc.
Millersburg, OH
(800) 344-1101
https://holmeslab.com/

Midwest Laboratories
Omaha, NE
(402) 334-7770
www.midwestlabs.com

Romer Labs
Union, MO
(635) 583-8600

https://www.romerlabs.com/en/products/analytical-service/mycotoxins/us/

Waters Agricultural Laboratory, Inc
Owensboro, KY
(270) 685-4039
https://watersag.com/service/mycotoxinaflatoxin-detection/

Commercially Available Mycotoxin Test Kits
Commercial kits for on-site mycotoxin testing can be purchased from a number of manufacturers.

Information about mycotoxin test kit evaluation is provided by USDA AMS at:
https://www.ams.usda.gov/services/fgis/standardization/tke

Along with a list of USDA AMS performance verified mycotoxin test kits:
https://www.ams.usda.gov/sites/default/files/media/FGISApprovedMycotoxinRapidTestKits.pdf

Contact Dr. Megan Romano, UKVDL clinical veterinary toxicologist, at (859) 257-8283 or megan.romano@uky.edu for additional information on mycotoxin testing.