Options for Mycotoxin Analysis in Corn and Feed

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

This publication is divided into five sections:

- Sampling recommendations
- Mycotoxin testing options
- Laboratories that provide mycotoxin testing services
- Commercially available mycotoxin test kits
- Additional resources

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 sampling harvested grain, a recommended sampling approach is to collect at least ten probefuls from a number of locations throughout the lot, or at least ten collections from a moving stream of grain. Do not collect a sample from a single location in the lot, as it is highly unlikely that it will be representative of the lot. A ten-pound sample is commonly recommended, especially for aflatoxins[1]. Two useful publications on sampling produced by the USDA Grain Inspection, Packers and Stockyards Administration (GIPSA), Federal Grain Inspection Service (FGIS), are:

- *Practical Procedures For Sampling Grain At Farm Sites And Remote Locations*, at
- http://www.gipsa.usda.gov/Publications/fgis/ref/practical_sampling.pdf
- *Inspecting Grain: Practical Procedures for Grain Handlers*, at http://www.gipsa.usda.gov/Publications/fgis/ref/primer.pdf.

Sampling ear corn is not as accurate as sampling shelled grain. However, circumstances sometimes require testing of ears. 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.

Shelled corn must always be ground before the sample is actually tested 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 that to about the consistency of flour (to pass through a No. 20 sieve)), and test that finely ground subsample for mycotoxins. The *Aflatoxin Handbook* (http://www.gipsa.usda.gov/publications/fgis/handbooks/afl_insphb.html), published by the USDA-GIPSA-FGIS, provides additional information on processing samples.

Recognize that mycotoxins can still accumulate if samples is held under warm, moist conditions. Storage or shipment of the sample at a moisture content below 12-13% essentially prevents the continued

[1] Some aflatoxin protocols call for smaller samples for trucks or wagons (2-5 lb),. As a rule, larger samples are likely to give more accurate results.

development of mycotoxins. Dried samples should be shipped in cloth or paper containers. If the sample is moist, freeze it immediately and ship it in a frozen state or hand-deliver frozen to the laboratory.

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 wet feeds (15+% moisture), sample as for dry feed, but maintain two-pound sub-samples frozen in plastic bags with the air squeezed out. Ship in a frozen state or hand-deliver frozen to the laboratory. For samples with intermediate moisture contents, the safest way to handle the sample is as if it were a wet sample.

Sampling silage poses special challenges. See the following Extension publication for suggested practices, http://learningstore.uwex.edu/assets/pdfs/A2309.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 (often <$30 per mycotoxin), 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 results or false negatives. 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 (such as 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 (LC-MS or LC-MS/MS); and gas chromatography for a few select mycotoxins. Other analytical methods are available but much less common.

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. Prices given are for October, 2012.

University of Kentucky Veterinary Diagnostic Lab
1490 Bull Lea Road
Lexington, KY 40511
(859) 257-8283
Analysis with HPLC for accurate quantification
$30.00 for aflatoxin only or $100 for complete mycotoxin panel
www.vdl.uky.edu

Breathitt Veterinary Diagnostic Laboratory
P.O. Box 2000 – 715 North Drive
Hopkinsville, KY 42241-2000
(270) 886-3959

www.breathitt.murraystate.edu
$44.50 for mycotoxin panel (TLC)

A & L Labs
2790 Whitlen Road
Memphis, TN 38133
(800) 264-4522
www.allabs.com
$55.00 for qualitative aflatoxin at 20 ppb

Barrow-Agee Lab
1555 Three Place 2

Memphis, TN 38116-3507
(901) 332-1590
www.balabs.com
$75.00 for aflatoxin on HPLC

Cumberland Valley Analytical Lab
P. O. Box 669
Maugansville, MD 21767
(800)CVASLAB
www.foragelab.com
$25.00 for aflatoxin (ELISA method)

Dairyland Laboratories
217 E. Main Street
Arcadia, WI 54612
(608) 323-2123
www.dairylandlabs.com
2012 special of $21.00 for aflatoxin
Normal 48-hour turnaround

Dairy One Lab
730 Warren Road
Ithaca, NY 14850
(800) 496-3344
www.dairyone.com
$65.00 for complete mycotoxin panel

Eurofins
2200 Rittenhouse St. Suite 175
Des Moines, IA 50321
(515) 280-8378
www.eurofins.com
Contact for analyses and pricing

Holmes Laboratory, Inc.
3559 US 62
Millersburg, OH 44654-8834
(800) 344-1101
www.holmeslab.com
Aflatoxin $53.00 for aflatoxin only, $35.00 if included with forage analysis

Midwest Laboratories
13611B Street 3

Omaha, NE 68144
(402) 334-7770
www.midwestlabs.com
$35.00 aflatoxin (LC/MS)

Romer Labs
1301 Stylemaster Drive
Union, MO 63084-1156
(635) 583-8600
www.romerlabs.com
Contact for analyses and pricing

Waters Agricultural Laboratory, Inc
2101 Calhoun Road, Hwy 81
Owensboro, KY 42301
(270) 685-4039
www.watersag.com/Ownesboro.htm

Commercially Available Mycotoxin Test Kits
Commercial kits for on-site mycotoxin testing can be purchased from a number of manufacturers. Important USDA-GIPSA web sites include the following:

- Chapter 1 of the *Aflatoxin Handbook*, at http://www.gipsa.usda.gov/publications/fgis/handbooks/aflatoxin/aflatoxin-ch01.pdf, which lists the aflatoxin test methods approved by USDA-GIPSA.
- *GIPSA Performance Verified Rapid Test Kits for Analysis of Mycotoxins*, at http://www.gipsa.usda.gov/fgis/tech-servsup/metheqp/testkits.pdf, which lists rapid mycotoxin test kits for mycotoxins that have been performance-verified by USDA-GIPSA.

A Partial List of Vendors of USDA-Approved Test Kits for Mycotoxins
Charm Science, Inc.
800-343-2170
http://www.charm.com/

Diachemix
414-902-6770
http://www.diachemix.com/

Envirologix Inc.
866-408-4597
http://www.envirologix.com

Neogen Corporation
800-234-5333
http://www.neogen.com

R-Biopharm, Inc.

877-789-3033
http://www.r-biopharm.com

Romer Labs
800-769-1380
http://www.romerlabs.com

Strategic Diagnostics, Inc.
800-544-8881
http://www.sdix.com

Vicam
800-338-4381
http://www.vicam.com

Additional Resources

For more information on mycotoxins, including sample collection and preparation, refer to the following University of Kentucky publications available at your county Extension office, as well as on the Internet.

- Aflatoxins in Corn, ID-59, http://www.ca.uky.edu/agc/pubs/id/id59/id59.pdf
- Fumonisin, Vomitoxin, and Other Mycotoxins in Corn Produced by *Fusarium* Fungi, ID-121, http://www.ca.uky.edu/agc/pubs/id/id121/id121.pdf