NebGuide

University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources

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G1515

Sampling and Analyzing Feed for Fungal (Mold) Toxins (Mycotoxins)

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The purposes of this NebGuide are to provide information about sampling feeds to detect the presence of mycotoxins in them and about how feeds may be analyzed for mycotoxins.

Mycotoxins are chemicals produced by fungi (molds) under certain conditions. They are not essential for fungal growth or reproduction, and are toxic to animals or humans. They are naturally present in feed and cannot always be avoided.

Analysis of feeds for the presence of mycotoxins can help grain, feed and animal producers to minimize, if not eliminate, adverse health effects of mycotoxins in animals.

Mycotoxins commonly found in grains or feeds used in Nebraska are aflatoxins, ergot alkaloids, fumonisins, vomitoxin and zearalenone. Information about their health effects, diagnosis, and treatment may be found in NebGuide G1513, *Understanding Fungal (Mold) Toxins (Mycotoxins)*. Information about use of mycotoxins-contaminated feed may be found in NebGuide G1514, *Use of Feed Contaminated with Fungal (Mold) Toxins (Mycotoxins)*.

Sampling Feed for Mycotoxin Analysis

Sampling feed or grain for mycotoxins analysis is critical for their detection. Analysis of an inappropriate sample is a waste of resources. General recommendations for specimen collection are given in this section, but it is best to contact the laboratory you wish to use for mycotoxin analysis for its recommendation about collecting samples for analysis.

Mycotoxins are not evenly distributed in feeds or grains. Grain from individual plants or from individual seeds or kernels from one plant, may be highly contaminated with mycotoxins. Grain from other plants, or other seeds or kernels from those same plant may be mycotoxin-free. If the specimen collected for analysis includes contaminated grain, the mycotoxin will likely be detected. If not, the mycotoxin will not be detected. The objective of sample collection is to collect a specimen from as many parts of the grain or feed pile as possible. That way, the chance of collecting some of the contaminated grain is increased.

Correct sampling is especially critical for aflatoxin analysis. Aflatoxins can cause illness when present in relatively low concentrations (parts per billion, ppb). Other mycotoxins cause illness at higher concentrations (parts per million, ppm).

The size of the collected specimen depends on the size of the load or storage bin in which the grain or feed is located. Use these guidelines to estimate how much feed or grain should be collected for analysis.

- For a typical truck- or wagon-load size of grain or feed, collect at least 3 pounds.
- For a train-car load size of grain or feed, collect at least 10 pounds.

Samples are most conveniently collected when the feed is being moved from one container to another, such as when feed is being augered from truck to storage bin. Collect small amounts of sample at periodic intervals from the moving stream of feed. Space the sampling so that it occurs from start to finish of movement. Combine and mix all of the subsamples well. Submit all of the collected sample to the laboratory for analysis.

Sampling feed or grain when it is not moving is more difficult, for example when it is loaded on a truck, or already in a storage bin. It is best to collect small samples from as many different places in the load or storage bin as possible. Probes can be used to help collect samples deep into the grain. Use the same guidelines for sample size suggested above. Mix the collected sample well and submit it all for analysis.

Dry feed is better for shipment to the laboratory than wet grain because fungi may begin to grow on wet grain during shipment. If possible, oven dry the feed to less than 13 percent moisture before shipment. Known mycotoxins are not significantly degraded if feed is dried at moderate temperatures (e.g. 60 to 70°C). If wet feed cannot be dried, freeze and ship it so it remains frozen until it arrives at the laboratory where analysis will be performed.

It is better to ship feed in cloth or paper bags than in plastic bags to help prevent fungal growth during shipment. If fungi begin to grow during shipment, it may be difficult to determine if any mycotoxin detected was present before the sample was shipped.

Other Sampling Strategies

The presence or absence of visible mold growth in feed are not good predictors of presence or absence of mycotoxins. We often receive specimens collected from area(s) of feed visibly contaminated with mold, but rarely find detectable amounts of known mycotoxins in them.

If ill health due to mycotoxin exposure is suspected, it is best to collect specimens of feed from the bunks or feeders that best represent the feed the animals consumed just prior to or shortly after the problem was noticed. Samples taken from feed not yet offered to the animals may not contain any mycotoxins, even though they were present in the feed already consumed.

If you wish to compare results of mycotoxin analysis between laboratories, then the sample provided to each laboratory must contain the same amount of mycotoxin. Thoroughly mix a large sample, mill it, mix it again, and then split the sample, sending part of it to each laboratory. The objective of the sample preparation is to produce a sample as uniform with respect to mycotoxin concentration as possible. If a uniform sample is not provided to each laboratory and the laboratories find different concentrations, all results may be correct for the samples each laboratory was provided.

Market Grains or Feeds

If you wish to market your grain, the elevator to which you take it may screen your load for aflatoxins. Elevator staff can use most any method of analysis they desire, but most likely will screen for the presence of aflatoxin using a kit or black-light. Some elevators may use both methods. Then a decision will be made as to the value of your grain based upon the results obtained from the screen. Loads found to be contaminated may be rejected, or you may be offered a price below current market rates. Information about the methods of aflatoxin analysis may be found later in this NebGuide.

If your grain is found to be contaminated with aflatoxin by analysis conducted at the elevator, you have several options, depending upon circumstances. You may choose to:

- keep the grain,
- accept a lower price for it,
- find another purchaser, or
- request that the result be confirmed by a Grain Inspection Service laboratory.

Know the aflatoxin policy of the elevator which you wish to use **before** you take your grain to it.

If you want to market your grain or feed directly to animal producers or owners, it may be well worth the expense to collect a specimen from your product and have it analyzed for mycotoxins. The laboratory you select to perform the analysis should be carefully chosen to ensure its results will be acceptable should you be challenged about mycotoxin contamination in your product. Corn or feed containing corn or its products intended for consumption by horses should be analyzed for fumonisin.

Selecting a Laboratory for Mycotoxin Analysis

Select a laboratory to perform mycotoxin analysis carefully, because if you do not, you may expend more time and effort and spend more money than necessary.

Begin by clearly defining the purpose of the analysis. Is it to market your grain or feed? Is it to file for crop insurance benefits? Is it to assess health risk when fed to animals? Then select a laboratory that will provide you with results that are recognized as valid for your purpose.

For grain that is to be marketed, it is best to have the grain analyzed for mycotoxins at a grain inspection service laboratory approved by the USDA Grain and Plant Inspection Service (GPIS). Such laboratories comply with federal grain inspection requirements. Analytical services offered by these laboratories may not work very well for feeds other than grains, such as silages.

If you wish to file a crop insurance claim, select a laboratory for mycotoxin analysis acceptable to your crop insurance provider. Contact the insurer for information about laboratories they consider acceptable. Analytical services provided by those laboratories may not work for feeds other than grains, such as silages.

If you believe your animals are suffering from a mycotoxicosis, select a veterinary diagnostic laboratory to assist you. It is staffed to deal with suspected mycotoxicoses in animals. Analytical services provided by them may also work for feeds other than grains. Additionally, staff at those laboratories should be able to advise you about risk of mycotoxicoses based upon analytical results obtained from other laboratories.

Methods of Mycotoxin Analysis

There are several different methods of analysis available for mycotoxins available, and the one(s) chosen by a laboratory will vary. Some laboratories may use more than one method, depending upon its circumstances.

Cost of mycotoxin analyses depend partly upon the method used to detect their presence. When comparing cost between laboratories, it is important to know if the methods used by the laboratories are similar or different.

Some methods of mycotoxin analysis are able to detect several different mycotoxins with one test, others detect only one mycotoxin. Some are able to determine the concentration of mycotoxins present, others cannot.

Some laboratories may screen samples for the presence of mycotoxins, using a relatively fast, easy and cheap method of analysis. If that analysis finds a mycotoxin to be present, then the sample is re-analyzed using another method that can confirm the presence of the mycotoxins and determine the amount present. Consult with the laboratory you choose for information about its mycotoxin services.

Two methods of analysis commonly used to detect aflatoxins at grain elevators are black light and enzyme-linked immunosorbent assay (ELISA). Some general information about each follows.

The black light test for aflatoxin is based on the glow (fluorescence) of aflatoxin under ultra-violet light, commonly called black light. Research has demonstrated that results obtained using this method are rarely falsely negative, but often falsely positive. In other words, if "glowers" are observed in an appropriately collected sample, aflatoxin may be present but one cannot say that with certainty. Other chemicals present in the grain may also cause it to glow. If no glowers are found, then it is very likely that aflatoxin is not present in significant concentrations.

If glowers are found to be present, the concentration of aflatoxin present in a sample **cannot** be reliably determined by counting the number of glowers present under black light. Samples judged to be positive for aflatoxins by black light should be analyzed by a more reliable method to confirm the presence of aflatoxin and determine the concentration present.

Enzyme-linked Immunosorbent Assay (ELISA) test kits are available for aflatoxin, fumonisin, vomitoxin and zearalenone. The kits test for only one mycotoxin, so a separate kit is necessary for each. They are based upon the interaction between the mycotoxin and antibodies produced against the mycotoxin. Antibodies against the mycotoxin are bound to the sides and bottom of a plastic well. Mycotoxins in a sample of grain are dissolved into a solution, which is placed in the plastic well. If they mycotoxin which the kit is designed to detect is present in the solution, the mycotoxin binds to the antibody in the well. After a series of solutions are added and removed from the well, the presence of the mycotoxin is detected based on a color change in the final step. Some ELISA kits are designed to determine the concentration of mycotoxin in the grain. Others are designed to detect the presence of the mycotoxin above a specified concentration, but cannot be used to determine how much above that concentration is present.

More sophisticated methods of mycotoxin analysis may be used in laboratories for several reasons. They can detect mycotoxins in many different kinds of feeds, detect them more reliably, and allow the concentrations of mycotoxins present to be more precisely determined. Analytical services using such methods usually cost more and take longer than ELISA tests.

Each method has advantages or disadvantages, which are summarized in *Table I.* Consult with the laboratories about its analytical services.

Mold Culture and Identification, and the Detection of Mycotoxins

It is debatable whether culturing and identification of molds present in feed provides useful information for the assessment of mycotoxin contamination. The presence or absence of molds does not necessarily indicate the presence or absence of mycotoxins. At times, we find no detectable amounts of known mycotoxins in feeds that appear very moldy, and quite high concentrations of mycotoxins in feeds that appear to be very good.

Laboratories who culture molds usually report the numbers of colony forming units (CFUs) per gram or kilogram of feed. Mold counts in feeds are not considered an indicator of the presence of mycotoxins in feeds and are usually ignored in the assessment of possible mycotoxin contamination.

Table I. Analytical services for mycotoxins.

Service	*Method*	*Advantages*	*Disadvantages*	*Cost (current in April 2003)*
Mold culture and identification	Microbiological culturing; microscopic or biochemical identification tests.	Determines presence of molds, but not necessarily toxigenic strains.	Does not determine if molds found to be present can or are producing mycotoxins. May take a long time to complete test, so it may take a relatively long time to obtain results.	$30 to $50; the more specific the identification, the higher the cost.
Chemical analysis	Enzyme-Linked Immunosorbent Assay (ELISA)	Rapid, relatively inexpensive.	Only detects one mycotoxin at a time. Proof of mycotoxin presence usually requires additional testing.	$20 to $30.
	Thin layer chromatography (TLC)	Can detect more than one kind of mycotoxin.	Slower than ELISA. Proof of mycotoxin presence may require additional testing. May not work as well for feeds other than grain.	$25 to $50; may be more if quantitative analysis and confirmation are required.
	High performance liquid chromatography (HPLC)	Easier to provide quantitative results. May be able to detect more than one kind of mycotoxin.	May be more expensive than other methods of analysis. Proof of mycotoxin presence may require additional testing.	$25 or more, depending upon setup.
	Gas chromatography/mass spectroscopy (GC/MS)	Can identify the mycotoxin without additional testing; may be able to detect more than one kind of mycotoxin.	More expensive than other methods of analysis.	$50 to $100, or more.

Table II. Units of concentrations used to report mycotoxin concentrations in feeds.

Unit of concentration	*Definition*	*Remarks*
Parts per billion (ppb); May appear as ng/g or µg/kg.	1 part of the mycotoxin in 1,000,000,000 parts of feed	1 ppb equals 1 billionth of a pound of mycotoxin in a pound of feed.
Parts per million (ppm); May appear as ug/g, µg/g, or mg/kg.	1 part of the mycotoxin in 1,000,000 parts of feed.	1 ppm equals 1 millionth of a pound of mycotoxin in a pound of feed.

The identities of molds found in feeds may be of some value in assessing the possibility of mycotoxin contamination. Identification of the genus and species of a mold present in feed is of most use. However, it is difficult to determine if a particular mold found to be present in feed is capable of producing mycotoxins. Molds capable of producing mycotoxins are called toxigenic. Even if the genus and species of the mold are identified, it may not be possible to determine if that particular mold belongs to a toxigenic strain. If only the genus is identified, it is even more difficult to predict if the mold is toxigenic. If an identified mold is believed to be toxigenic, it is not possible to determine if the mold has or is actively producing mycotoxins in the feed. The presence or absence of mycotoxins can be determined only by chemical analysis for mycotoxins.

Cost of mold culture and identification is usually $30 to $50, but may be much more expensive if detailed identification is required. It may take several days to grow the molds, so results may take several days to obtain.

Units of Concentration

Quantitative results provided by chemical analysis consist of two parts, both equally important: a number and a unit of concentration. The numerical part, which may be a number or a range of numbers, tells how many "units of measure" are present. Simply providing the numerical part can lead to confusion. For example, if aflatoxin is said to be present at a concentration of "30," it makes a big difference if the result is 30 parts per billion (ppb) or 30 parts per million (ppm). Aflatoxin at a concentration of 30 ppm is present at a concentration 1,000 times higher than at 30 ppb. Such a difference in concentration can make a big difference in whether a feed is safe to use or not. When seeking advice how to use mycotoxin-contaminated feed, always provide both parts of quantitative results.

Units of concentration commonly used to report mycotoxin results are listed in *Table II.*

No Detectable Amounts (NDA)

No analytical method can determine if a sample is completely free of mycotoxins. Every method has a limit of detection. Concentrations present in the sample below the detection limit cannot be detected. A result reported as no detectable amount with a detection limit of 1 ppm means that the mycotoxin is not present at concentrations greater than 1 ppm, but it may be present at a concentration less than 1 ppm.

When no mycotoxin is detected in a sample, that may be reported as "none detected," "no detectable amounts" or "negative to test." An estimate of the detection limit should be provided. Any results reported as "0" should not be taken literally.

Failure to detect mycotoxins in the *sample provided for analysis* may be the result of sample selection. Such results do not prove mycotoxin are not present in other parts of the feed from which the sample was collected.

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