In recent years, mycotoxins seem to be getting more attention than ever. The risks of mycotoxins, a series of toxic chemical byproducts produced by mold in feedstocks, and their effect on animal health are actively combated in the feed and grain industries: Feed formulators are increasing their inclusion of additive technology to negate the negative effects of mycotoxins, testing equipment suppliers continue to invest in research and development to improve the process and its results, and feed mills are working to streamline their mycotoxin management programs.

However, depending on your position within the feed industry, your understanding of how mycotoxin detection works -- and which test best fits your needs -- may vary.

“Overall, we’re seeing mycotoxin analysis of finished feed and the various feed ingredients increasing,” explains John Reuther, president, Eurofins Central Analytical Laboratories. “Some years more than others, we see a large surge in testing. In those instances, it’s typically the buyer that is concerned, depending on conditions during the crop year, and is looking for more than a standard certificate of analysis for the feed.”

“YOU NEED TO FIND A SUPPLIER WITH A GOOD PRODUCT WHO IS WILLING TO WORK WITH YOU AND KNOW YOUR PRODUCT.”

— KATE PLAISANCE, GLOBAL ANALYTICS DIRECTOR, CARGILL ANIMAL NUTRITION

Here, at the onset of the 2014 planting season, the following article serves as a mycotoxin testing refresher, exploring technology developments and a general overview of when and how feed millers would test their products and ingredients.

What tests are available and why would we use them?

With hundreds of types of mycotoxins on the books, it’s no wonder different testing methodology would be required to best identify which toxins are present and at what levels. Here is a list of the most popular test types and their applications:
**Lateral flow:** Lateral flow tests or lateral flow immunochromatographic assays detect the presence or absence of “a target analyte in sample matrix.” In the grain and feed industry, these tests are relatively inexpensive and provide a quick, basic result for the grain being sampled at a grain elevator or feed mill’s receiving station.

“For the feed mill folks, there is still much need for rapid tests because they don’t have the time to wait a week for samples to be sent to a lab, analyzed and sent back to them,” says Steve Mobley, 37+ Lab manager, Alltech.

According to Jim Topper, Neogen’s market development manager, lateral flow tests have now evolved to the point where they can provide a quantitative result.

**ELISA:** Enzyme-linked immunosorbent assays (ELISA) provide a wet-lab analysis using antibodies to detect a substance in a liquid sample.

“ELISA methods are fast and the accuracy is good. These tests are validated for a number of standard feed ingredients and related byproducts,” Reuther says.

In addition to speed, ELISA tests are popular because they’re easy-to-use and offer a quantitative result; however, it is unlikely that they would be used to analyze finished feed.

**HPLC:** High performance liquid chromatography (HPLC) is an analytic technique used to separate, identify and quantify the components of material being tested.

According to Mobley, “Due to increased sensitivity, validated HPLC methods for mycotoxin testing allows the scientist to identify more mycotoxins. HPLC is sensitive enough that you can see parts per billion.”

This test requires samples to be sent off to a lab and costs more than a lateral flow test.

“At the low end, you have lower cost, quicker results, but you give up sensitivity and selectivity. So you’re going to see fewer mycotoxins and they’re going to be there in much higher concentrations to detect them,” Mobley explains. “HPLC is probably the most common commercial laboratory-based test. As you move up in sophistication, you have more sensitivity and more selectivity. You can see maybe a dozen or so mycotoxins at lower concentrations, offering a better picture of the total profile.”

**LC-MS-MS:** Liquid chromatography–mass spectrometry (LC-MS-MS) is the most sophisticated type of analytical considerations

While mycotoxin levels should be monitored and controlled before the raw ingredients are used in feed production, instances arise when a finished product would be tested. According to Eurofin’s Reuther, the most common circumstances for testing finished feed for mycotoxin content typically involves a buyer who wants the feed manufacturer to produce a certificate and less so because they have cause for alarm.

“Most of the testing we do is conducted prior to use to prevent problems rather than reactive testing,” he says.

Kyle Donovan, Envirologix’ business unit manager, feed and food safety, agrees: “Costs increase as contaminated product moves downstream. Finished feed is tested to ensure a safe product and reduce losses due to contamination. Another possibility, in preparation for upcoming FSMA requirements, customers may want to get a jump on their competition.”

However, testing for finished feeds is much more complicated than it is with validated ingredient testing. For this reason, ELISA tests are not recommended. Cargill Animal Nutrition’s Kate Plaisance prefers to use a reference wet chemistry method for finished product testing over lateral flow-based testing: “A rapid test would need to be validated to the specific formula of finished product and the formula would need to remain fixed for the test to remain valid.”

HPLC or LC-MS-MS are the preferred testing methods, which would be conducted off-site at a commercial lab.

“If we choose to test a finished product, accuracy is of the utmost importance and we’re less constrained by time,” Plaisance explains. For Cargill Animal Nutrition, “using the reference method, HPLC does take more time, but the accuracy is very important because particular test overcomes the matrix issues that may be present using a rapid test.”
testing available on the marketplace. It is the combination of the separation of liquid chromatography (LC) offered by HPLC, but includes the mass analysis of mass spectrometry. It provides higher levels of selectivity for distinguishing between multiple mycotoxins and higher levels of sensitivity for detecting low levels of the mycotoxins.

“The 37+ lab tested 5,000 samples of a variety of different feed matrices from all regions of the world and we are seeing that, consistently, we’re finding six to eight mycotoxins on average per sample,” says Mobley. “The LC-MS-MS allows us to see more mycotoxins at lower levels. We can detect up to 38 different mycotoxins with our method, and we can detect them at parts-per-billion and parts-per-trillion levels.”

If finished feeds were being tested, either the HPLC or the LC-MS-MS would likely be used. On the ingredient side, LC-MS-MS aids in creating a more accurate dilution of the mycotoxins levels.

“In a commercial lab, rather than validate an ELISA method for the finished feed, they would choose to use HPLC or LC-MS-MS because the sample will not suffer from the interferences the ELISA method would produce, e.g. generating false positives or false negatives,” Reuther explains. “LC-MS-MS can be a highly controlled, quantitative method that is more rugged and can simultaneously test for multiple toxins.”

In the end, choosing between HPLC and LC-MS-MS may come down to cost.

“There are some great LC-MS-MS methods out there, but HPLC still does a good job with the major isoforms of the toxin you would be looking for. HPLC is more cost effective; LC-MS-MS costs more because you’re paying for the improved sensitivity,” Kate Plaisance, global analytics director, Cargill Animal Nutrition, which holds select supplier agreements with Charm and one other company.

Aside from cost, the speed at which samples are analyzed is this method’s primary disadvantage.

**Mycotoxin management tips**

Beyond understanding the types of testing available and their best use, there are several internal actions that can be set in place to ensure the safety of the finished feed produced in your mill:

1. **Make sure you know your mycotoxin levels**
   Once the mycotoxin(s) is bound to the matrix of the material, there’s no way to remove it, so the first line of defense is to capture mycotoxin data early and mitigate the risks accordingly.

   “It’s important to have a good sampling procedure in addition to protocols when receiving the different grains,” says Topper. “Once you’ve accepted the grain, the burden falls on you with any issues that arise and there are definite methods for getting a representative sample. We’ve seen studies suggest that a single contaminated grain of corn in a railcar can provide readings too high for acceptance for most mycotoxins. GIPSA has a recommended sampling procedure that we suggest people use to ensure a representative sample.”

   Plaisance agrees: “There isn’t a good solution for mycotoxin detoxification in a grain. Our mycotoxin management strategy is to measure it before it hits the mill. If the supplier tests the grain, we don’t just accept it, we’re going to audit them. If we do that due diligence, then we will accept the proprietor’s certificate of analysis.”

2. **Mycotoxin test validation**
   “The No. 1 thing to do when testing for mycotoxins is to...
make sure that the test you choose is validated to the commodity being tested,” Plaisance says. “In the feed business, we are using a lot of byproducts as well as whole grains.”

For example, a mycotoxin test used for corn should not be used for a corn byproduct unless validated.

In choosing a mycotoxin testing vendor, seek out a partner who is transparent in their validation data to ensure the test is appropriate and accurate.

3 Detailed information provides balance

As the testing equipment and methodology becomes more sophisticated, feed mills have a better understanding of how many mycotoxins are present and at what levels.

“Testing advancements allow you to see more mycotoxins at lower levels that are present in the sample and have a better understanding of the potential for them to work cumulatively,” Mobley says. “You can have small amounts of half a dozen different types of mycotoxins that work together to affect the health of the animal.”

The ability to know what you’re working with allows for proper grain blending and the addition of mycotoxin binders.

He suggests quarterly, seasonal testing of finished feeds.

“If all you’re doing is finished product testing, that is not a quality control program because you’re testing too late. The goal is to keep mycotoxins out, so we want to push that testing up the supply chain to have good control,” Plaisance adds.

4 Manage your levels beyond the scale

According to Mobley, in-depth, periodic grain analysis gives the feed miller better information for monitoring to ensure “the mycotoxin profile isn’t worsening,” as they can develop during transportation and storage.

“Be proactive and anticipate that problems may arise,” Mobley says. “Address those potential problems by periodically testing your grains and product to assure that you are managing the total program: Are you’re rotating your stock properly? Are there areas -- or critical control points -- within the grain transfer or milling process where residue could accumulate and create pockets of mycotoxins? Are there areas where moisture might be getting into an area of the storage silo or mill? Identify those areas and change your process.”

5 Train your employees

According to Plaisance, 10 percent of the error of accurately describing the mycotoxin levels of a load comes from the actual analytical test used; the other 90 percent of the error is a question of how that truck or railcar was sampled.

“The responsibility of the sampling is the feed mill’s responsibility and that’s under our control,” Plaisance says.

For this reason, testing suppliers such as Neogen offer their clients test kit training and certification programs.

How to choose a mycotoxin test

Depending on whether you are testing incoming grains or finished feed, your testing needs will vary; however, there are several indicators that the vendor or commercial lab will produce the desired results.

“Testing labs need a lot of experience with the various feed types and technologies,” Reuther explains. “Any lab using LC-MS-MS methods there really need to be high-level scientists running the equipment and program quality controls into the run so the data is reliable.”

Lab certification, such as ISO-17205, and participation in mycotoxin check sample programs (AOCS, FAPAS, etc.), are a good indication you are working with a professional commercial lab.

“The feed mills and the test kit suppliers are in the same business of making sure the animals we feed are healthy, which means we both work to manage mycotoxins to the best of our ability. You need to find a supplier with a good product who is willing to work with you and know your product,” Plaisance advises. [FMI]
How do mycotoxins affect feed quality worldwide?

Tracking mycotoxin levels by country and their influence on animal production

It is not surprising mycotoxins continue to be found at high levels once again in the 2013 grains harvest. As worldwide demand for animal-based products continues to grow, demand for high-quality feed also will increase. Despite increasing awareness of the problems caused by mycotoxins, the impact that these toxic substances have on the animal industry has not yet been fully understood.

An overview of the world feed market

According to the International Feed Industry Federation (IFIF), world compound feed production is approaching 1 billion tons/year. Feed manufacturing generates a turnover of approximately more than US$370 billion/year. The poultry feed industry comprises 45 percent of the total market, followed by the ruminant and pig industries at 27 percent and 24 percent, respectively. Aquaculture is a growing market, which represents 4 percent of total feed.

Despite control strategies mycotoxins remain a global issue

Testing feed components or finished feed for mycotoxin content is a routine practice in many parts of the world. However, the main concern observed by the IFIF is that, out of the 1 billion tons of feed produced worldwide, around 300 million tons are produced in farms that mix their own compound feed and do not regularly test the raw materials used on a regular basis for possible human and animal health hazards, such as mycotoxins.

The latest BIOMIN Mycotoxin Survey focuses on some of the main global feed commodities (corn, wheat, soybean, rice and DDGS) analyzed between January and September. The most common mycotoxins in animal feed are produced by Aspergillus, Fusarium and Penicillium species, and include aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Mycotoxin levels were found to be above the detection limits in 73 percent of all samples tested. The highest
concentration of Afla (89 ppb on average) was observed in U.S. maize samples. At 89 ppb, Afla poses a medium risk for pigs and poultry, yet a very high risk for dairy cows. FUM levels were also highest in North American maize with a concentration of more than 6,000 ppb, a level that can affect all types of livestock.

In China, the highest DON levels overall were observed in wheat at more than 1,800 ppb, a concentration level that has a high impact on pigs and poultry. ZEN levels in Asia were also highest in maize samples and were calculated at 350 ppb. At high concentrations, ZEN is a high risk factor especially for pigs, but can also affect the performance of breeders and layers. The global values for OTA were relatively low in samples from 2013.

Mycotoxins affect profits

Feed is the most important component in animal production and can represent up to 70 percent of total costs. Producing high-quality feed is an indispensable but challenging task due to the unavoidable contamination of grains with mycotoxins.

The effects of mycotoxins in the animal depend on several parameters such as age, physiological state,
nutrition, and the type and quantity of mycotoxin uptake. Ingestion of these toxic compounds does not always lead to visual symptoms, which makes the problem very difficult to detect. Animals fed a mycotoxin-contaminated diet have reduced immune responses due to an alteration of the normal immune system, which leads to immunosuppression.

Fungal toxins have been shown to decrease the resistance of the animal to many pathogens, resulting in higher susceptibility to diseases. They can also affect the animal’s response to vaccination programs, leading to reduced antibody titers.

Aflatoxins target primarily the liver and have carcinogenic effects on animals. The highest sensitivity is observed in dairy cows, which metabolize aflatoxin B1 to aflatoxin M1, a metabolite found in the milk. Animals ingesting zearalenone exhibit various symptoms related to the reproductive system. Sows are known to have

MYCOTOXIN LEVELS WERE FOUND TO BE ABOVE THE DETECTION LIMITS IN 73 PERCENT OF ALL SAMPLES TESTED.

FIGURE 3. Mycotoxins by region, commodity and toxin

Occurrence of mycotoxins by region, commodity and type of toxin shown as percent of positive values above the limit of detection (% pos.) and average mycotoxin contamination levels (ppb) of all samples tested positive in the main producing regions (for maize and wheat) and worldwide (for soybean, rice and DDGS). The survey provides insights on the occurrence of the most important mycotoxins in agriculture and animal production and covers aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA).
the highest sensitivity to ZEN due to its estrogenic effects. Deoxynivalenol inhibits protein synthesis and leads to feed refusal, especially in piglets, which results in reduced weight gain. Fumonisins interfere with the sphingolipid metabolism leading to cell membrane damage that can affect the liver, kidney, brain and also the nervous system. The highest sensitivity to FUM is observed in pigs. Ochratoxins are carcinogenic and nephrotoxic compounds which greatly affect poultry, particularly layers and breeders.

The ingestion of mycotoxins leads to an overall decline in performance. The reduced fertility of sows due to ZEN increases costs for the farmer. Animal products affected by mycotoxins, such as decreased egg production and egg quality, and aflatoxin-contaminated milk ultimately result in economic losses. Preventing the disease in the first place is surely more cost effective than treating ill animals. Mycotoxin risk management is therefore crucial in order to eliminate the effect of fungal toxins. [FMJ]

**FIGURE 2. World feed production by region**

<table>
<thead>
<tr>
<th>Region</th>
<th>Million metric tons</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td>38%</td>
</tr>
<tr>
<td>Latin America</td>
<td>20%</td>
</tr>
<tr>
<td>Europe</td>
<td>14%</td>
</tr>
<tr>
<td>Middle East + Africa</td>
<td>6%</td>
</tr>
<tr>
<td>Asia</td>
<td>28%</td>
</tr>
</tbody>
</table>

Source: Biomin

Production of feed in each region of the world in million tons per year.

DR. PAULA KOVALSKY WORKS AS A PRODUCT MANAGER WITHIN BIOMIN’S COMPETENCE CENTER FOR MYCOTOXIN RISK MANAGEMENT.

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