

Screening for Mycotoxins in Silage

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Abstract

Mycotoxins are chemicals produced by fungi that can seriously affect the health of dairy cattle. Mycotoxin-producing fungi do not compete well with the microbes responsible for silage fermentation. However, if proper moisture content is not maintained or oxygen is allowed into the silage, these fungi can grow and potentially produce mycotoxins. In addition, mycotoxins formed in the field can persist in the silo. The presence of mycotoxins in silage can only be determined by chemical analysis. Mycotoxins are analyzed by several methodologies, including immunochemical (**ELISA**) assays, thin-layer chromatography (**TLC**), gas chromatography (**GC**) and high-performance liquid chromatography (**HPLC**). Analyses can be obtained from commercial and State labs for aflatoxins, DON, T-2, DAS, zearalenone, fumonisins, and ochratoxin. Other mycotoxins can occur in silage, however testing for these is not currently available or difficult to obtain.

Mycotoxins in Silage

Mycotoxins are toxic chemicals produced by fungi. The most common mycotoxin found in silage is deoxynivalenol, also known as DON or vomitoxin (Whitlow and Hagler, 1997). DON is produced by several species of *Fusarium* including the most common producer *F. graminearum*. When present in dairy cattle feeds, DON does not appear to significantly af-

fect milk production, milk quality, feed intake or animal health. Feeding studies utilizing DON contaminated feeds with early lactation (Ingalls, 1996), mid-lactation (Charmley et al, 1993) and non-lactating cows (Trenholm et al, 1985) all support this conclusion. Nonetheless, many producers have observed a correlation between DON in rations and problems with reduced milk production, feed intake and herd health. Thus DON appears to be an indicator for the presence of other possible toxins in feeds. Recommendations vary for the maximum level of DON in dairy cattle feed. Our search of the literature and Internet indicate a range as low as 300 micrograms per kilogram of silage (300 ppb) to as high as 6,000 ppb.

Other *Fusarium* mycotoxins that have been found in silage include T-2 toxin, diacetoxyscirpenol (**DAS**), zearalenone, and fumonisins. T-2 toxin and the related mycotoxin DAS are potent mycotoxins produced by *F. sporotrichioides* and *F. poae*, which cause severe mycotoxicoses in animals including dairy cattle. Extreme cases can result in death. Fortunately, these two mycotoxins are not commonly found in silage produced in the Midwest. The maximum recommended levels of T-2 and DAS in dairy cattle feed range between 100 micrograms per kilogram of silage (100 ppb) to 250 ppb. Zearalenone is produced by *F. graminearum* and is often present in DON-contaminated silage. Zearalenone has estrogenic effects in animals meaning that it can disrupt the reproduc-

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tive system. Large doses of the mycotoxin also may cause reduction in milk production. Zearalenone levels exceeding 500 micrograms per kilogram of silage (500 ppb) are of concern. Fumonisin is a group of mycotoxins produced by *F. verticillioides* and *F. proliferatum*. The most common fumonisin, FB1, has a variety of effects in animals many stemming from damage to the liver and kidney. The FDA has suggested that dairy cattle should not be fed more than 30,000 microgram total fumonisin (= FB1 + FB2 + FB3) per kilogram of feed (30 ppm) (<http://vm.cfsan.fda.gov/~dms/fumongui.html>).

In addition to the *Fusarium* mycotoxins, aflatoxin, ochratoxin, and ergot also occur in silage. Aflatoxins are potent liver toxins and carcinogens produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are of concern to dairy producers in particular because the FDA regulations require aflatoxin residues in milk to be less than 0.5 ppb. To prevent the carry over of aflatoxins into milk, silage and other feed components such as cottonseed should not contain greater than 20 micrograms aflatoxin per kilogram (20 ppb). Ochratoxin A is a nephrotoxin produced by several species of *Penicillium* and *Aspergillus* (CAST, 1989). Although this is a fairly toxic compound, concern for dairy cattle is somewhat moderated by the knowledge that rumen microorganisms are capable of metabolizing ochratoxin A (Hult et al., 1976). Ochratoxin levels in dairy cattle diet should not exceed 250 ppb. Ergot alkaloids are a complex group of mycotoxins produced by *Claviceps purpurea* and other related fungi (Kuldau and Bacon, 2000). *C. purpurea* infects nearly all grasses including barley, rye, wheat. This fungus infects through the flower and produces a structure called a sclerotium in the location where the seed would have formed. Ergot contamination is more common in haylage, however infected grassy weeds can be a source of contamination in corn silage. *Penicillium roqueforti* is a fungus commonly found in the acidic, low oxygen tension environment of si-

lage. This fungus produces at least four mycotoxins (PR toxin, roquefortine C, patulin and mycophenolic acid) all of which have been documented in silage. The effects of these mycotoxins on dairy cattle are not currently well understood.

Screening for Mycotoxins

Producers will certainly think of mycotoxins as a contaminant in their silage when they observe spoilage or when their herds are showing reduced feed intake, reduced milk production or an appearance of poor health. However, the presence of mold does not mean mycotoxins are present and other chemicals such as nitrates can cause similar animal symptoms to those caused by mycotoxins (Adams et al., 1992). The only means of determining their presence is by analysis. Mycotoxins are analyzed by several methodologies, including ELISA assays, TLC, GC and HPLC. One advantage of the TLC method is that more than one mycotoxin can be analyzed at once. With the immunochemical assays, GC, and HPLC separate analyses must be performed for each mycotoxin or class of mycotoxin. Analyzing mycotoxins in silage can be a challenge due its complex nature. If proper protocols are not followed interfering compounds can be extracted from the silage leading to false positives for the presence of mycotoxins. This is especially true for the ELISA assays. ELISA tests are useful for screening samples and to indicate which samples warrant further attention. It is best to have positive results verified by other methods such as TLC, HPLC, or GC. For this reason, one should have a professional laboratory do the analysis. Most veterinary schools at State universities have diagnostic labs that routinely test for mycotoxins. There are also several private companies, many having Internet sites. Routine analyses can be obtained for aflatoxins, DON, T-2, DAS, zearalenone, fumonisins, and ochratoxin. Currently, one or two labs analyze for ergots, and none analyze for PR toxin, roquefortine C, patulin and mycophenolic acid.



As with mycotoxin analysis of any commodity, sample collection and preparation are an important source of error when testing silage. One must provide the analytical lab with a representative sample. Such a sample is routinely obtained by combining numerous small subsamples taken from the silage mass. Because mycotoxin production will occur in the area of silage exposed to air, samples from moldy silage should give an indication of the mycotoxins present. If sampling moldy silage for analysis, it is important to take a separate sample from an area that is not moldy. Care should be taken with handling samples to assure that mycotoxins do not accumulate in the sample during shipping or while in wait for analysis. Drying the sample at moderate temperature (60°C or less) will best assure that the fungus stops further growth and mycotoxin production. Freezing the sample and shipping on ice by a one-day delivery service is another option.

More Information

For more information regarding mycotoxins in silage, visit the Internet site for the NC129 North Central Regional Research project Mycotoxins in Cereal Grains at <http://www.btny.purdue.edu/nc129>. Links are provided to many sources of information related to mycotoxins and silage.

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