

# Mycotoxin Characterization, Occurrence and Detection

L. W. Whitlow<sup>1</sup> and W. M. Hagler, Jr.<sup>2</sup>

<sup>1</sup>Animal Science Department and <sup>2</sup>Poultry Science Department  
North Carolina State University  
Raleigh, NC

## INTRODUCTION

A mycotoxin is a toxin produced by an actively growing mold. Some molds are not toxigenic and toxigenic molds may grow without elaborating a toxin. A feedstuff may thus be moldy without containing mycotoxins and conversely, a feedstuff may contain large amounts of mycotoxins without having a moldy appearance. The mistake of equating a moldy feed to a mycotoxin contaminated feed may have led to misconceptions about the toxicity of mycotoxins. The appearance of a feed or the presence of mold spores are not good indicators of mycotoxin presence or the toxicity of the feed (Hamilton, 1978 and Wyatt, 1991).

A priority list of mycotoxins was subjectively produced by a survey of mycotoxicologists worldwide and included: aflatoxin (**AF**), ochratoxin (**OT**), trichothecenes [primarily T-2 toxin (**T-2**), zearalenone (**ZEN**), deoxynivalenol (**DON**) and diacetoxyscirpenol (**DAS**)], citrinin, sterigmatocystin, patulin, and cyclopiazonic acid (Hesseltine, 1986). Fumonisin (**FB**) was identified after this list was compiled (Gelderblom et al., 1988), but undoubtedly it would be included in a current list.

The mycotoxicoses which may be most commonly associated with grazing cattle include, ergotism, paspalum staggers, fescue toxicity, sweet clover poisoning, facial eczema, and slaframine toxicity. These and other mycotoxicoses are important and have been reviewed by Lacey (1991).

This paper will concentrate on those mycotoxins which are of greatest concern for dairy cattle consuming stored feeds, and include: AF, fumitremorgens, and sterigmatocystin, which are primarily produced by *Aspergillus* molds; DON, ZEN, T-2, DAS and FB, which are produced by *Fusarium* molds; and OT, PR toxin and roquefortine, primarily produced by *Penicillium* molds. Several

other mycotoxins, produced by these and other molds, are known to be prevalent at times, including derivatives of those listed. It is probable that a lack of observation and simple analytical techniques have prevented us from more fully understanding the prevalence of these mycotoxins and their impact on animal production.

## MOLD GROWTH AND MYCOTOXIN FORMATION

Molds occur universally in a variety of feedstuffs, including roughages and concentrates and can produce mycotoxins under certain conditions. Molds can grow and mycotoxins can be produced pre-harvest or post-harvest, during storage, processing, or feeding. Mycotoxin production is often related to extremes in weather conditions (causing plant stress or excess hydration of stored feedstuffs), inadequate storage practices, low feedstuff quality, and faulty feeding conditions.

Conditions for mold growth and mycotoxin formation are dependent on the specific mold, but include the presence of fungal spores, an organic substrate and the proper levels of moisture, oxygen, temperature, and acidity (Moss, 1991). Temperatures may range from -5° to 60°C. Water activity must generally be above 0.7 a<sub>w</sub> (ratio of the vapor pressure of the product to that of pure water or equilibrium relative humidity as a percentage). Mold can begin growing when moisture exceeds about 12%. Higher levels of moisture will support mold growth up to the point where water excludes adequate oxygen. High levels of CO<sub>2</sub> can prevent mold growth even when O<sub>2</sub> is at levels high enough to support mold growth.

Oxygen as low as 0.5% can support mold growth, thus there can be pockets of adequate oxygen within silage and high moisture grain storage, within the feed mass and especially near the feed surfaces. A

fairly wide range of pH levels will support mold growth, although they do not grow well at extremely low or high pH levels. While silage pH is usually low enough to prevent most mold growth, yeasts are active at a lower pH and their activity can raise the pH to a point conducive for mold growth. Mold and mycotoxin occurrence in silage is documented by Woolford (1984) and more recently discussed by Gotleib (1997), Seglar (1997) and Whitlow and Hagler (1997).

The *Aspergillus* species grow at lower water activities and at higher temperatures than do the *Fusarium* species, which generally require higher water activities and grow at much lower temperatures. *Aspergillus flavus* and AF in corn are favored by the heat and drought stress associated with warmer climates. AF seems to be enhanced by insect damage before and after harvest. *Penicillium* species grow at relatively low water activities and low temperatures and are fairly widespread in occurrence. Since both *Aspergillus* and *Penicillium* grow at low water activities, they are considered the more likely storage fungi, with *Aspergillus* more likely in warm climates and *Fusarium* and *Penicillium* more likely in cooler climates.

The *Fusarium* species are generally considered to be field fungi and more likely to proliferate prior to storage. *Fusarium* commonly affects corn, causing ear and stalk rots, and small grains, causing field diseases such as head blight (scab). These field diseases are characterized by yield loss, quality loss and mycotoxin contamination. In wheat, excess moisture at flowering and afterward is associated with increased incidence of mycotoxin formation. In corn, *Fusarium* diseases are more commonly associated with insect damage, warm conditions at silking, and wet conditions late in the growing season. Joffe (1986) suggests that the toxic principle in the soil spreads to the plant, first affecting the vegetative parts and then the grain. The grain provides a favorable substrate for toxin accumulation. Trenholm et al. (1988) suggest that plowing in plant debris and crop residue left on the field after harvest may reduce fungal disease problems.

It should be noted that the conditions most suitable for mold growth are not necessarily the optimum conditions for mycotoxin formation. For example, the *Fusarium* molds associated with alimentary toxic aleukia (ATA) have been reported to grow prolifically at temperatures of 25 to 30°C without producing much mycotoxin, but at near freezing temperatures, large quantities of mycotoxins

are produced without much mold growth (Joffe, 1986). Field applications of fungicides may either increase or decrease mycotoxin production (Boyacioglu et al., 1992 and Gareis and Ceynowa, 1994). Reduction of the mold presence may reduce mycotoxins, however, the stress or shock to the organism may cause increased mycotoxin production.

## MYCOTOXIN OCCURRENCE

The warm, humid climate of the southern U.S. results in a considerably higher incidence of AF in feeds. From 1975 to 1980, 34% of corn grain in North Carolina contained more than 20 ppb of AF. Corn grain and peanut meal have been the primary sources of AF contamination in NC and are a likely source of aflatoxin across the South. Unless it is improperly stored, whole cottonseed has seldom been a problem source of AF for NC dairymen. On the other hand, in the Southwest, cottonseed may be an aflatoxin source in the diet. Corn samples from the Midwestern U.S. representing the 1988 season (severe drought) showed 8% with AF levels above 10 ppb, 3% positive for ZEN above 1 ppm, 3% positive for DON above 1 ppm and 7% positive for T-2 above 500 ppb (Russel et al., 1991).

Mycotoxin analysis results, from feed samples submitted by North Carolina farmers during a nine-year period and representing more than 2400 samples, were summarized (Whitlow, Hagler and Hopkins, 1998). Percentage of corn silage and corn grain samples testing positive were for aflatoxin  $\geq 10$  ppb, 8% and 9%; DON  $\geq 500$  ppb, 51% and 52%; ZEN  $\geq 300$  ppb, 17% and 3%; T-2  $\geq 200$ , 5% and 4% and FB  $\geq 1$  ppm, 37% and 60%, respectively. Occurrence was highly variable by year.

It is well documented that several mycotoxins may be found in the same feed (Hagler et al., 1984). Abbas et al. (1989) demonstrated that *Fusarium* species isolated from Minnesota corn produce an array of mycotoxins.

**Aflatoxin**, produced primarily by *Aspergillus flavus*, is a mycotoxin of major concern, because it is carcinogenic and is commonly found in the southern US. Major efforts are directed at eliminating food

residues. The FDA limits aflatoxin M<sub>1</sub> in milk to no more than 0.5 ppb. Since AF residues can be found in tissues, beef cattle should not be fed AF contaminated diets for three weeks prior to slaughter. Regulatory pressures and a widespread awareness have helped minimize AF problems. The GAO (1991) concluded that industry, federal and state programs are effective in detecting and controlling AF and that it is doubtful that additional programs or limits would reduce the risk of AF in the food supply. Thus, current surveillance programs aimed at reducing food residues make it very unlikely for AF to have significant production or health effects on dairy herds.

**Fumonisin** was isolated by Gelderblom et al. (1988). Fumonisin is thought to occur primarily in corn. A USDA, APHIS (1995) survey found an average of 6.9% of 1995 corn samples from Missouri, Iowa and Illinois to contain more than 5 ppm FB<sub>1</sub>.

**Deoxynivalenol** is the proper name for a commonly detected *Fusarium* produced mycotoxin often referred to as vomitoxin. Two independent Midwestern studies (Vesonder et al., 1978 and Côté et al., 1984) showed DON to be the primary mycotoxin associated with swine problems including feed refusals, diarrhea, emesis, reproductive failure, and deaths. DON is often found in corn and small grains. In some years, localized areas may have a majority of wheat grain contaminated. Two derivatives, 3-acetyl DON and 15-acetyl DON, are also common mycotoxins found in moldy feedstuffs.

**T-2 Toxin** is a *Fusarium* produced mycotoxin which has been associated with gastroenteritis, intestinal hemorrhages (Petrie et al., 1977 and Mirocha et al., 1976) and death (Hsu et al., 1972, Kosuri et al., 1970). Frequency of occurrence of T-2 is less than for DON, FB or ZEN. The hydroxyl derivative of T-2, HT-2, may also be found.

**Diacetoxyscirpenol** is a *Fusarium* produced mycotoxin. It may occur along with T-2 toxin and causes similar symptoms.

**Neosolaniols** are *Fusarium* produced mycotoxins which may be found along with T-2, HT-2 and DAS.

**Zearalenone** is a *Fusarium* produced mycotoxin which elicits an estrogenic response in monogastrics (Sundlof and Strickland, 1986). Several derivatives of ZEN have similar toxicity and include  $\alpha$ -zearalanol,  $\beta$ -zearalanol,  $\alpha$ -zearalenol, and  $\beta$ -zearalenol.

**Fumitremorgens** such as fumigaclavine A and B are produced by *Aspergillus fumigatus*, and are thought to be common in silages of the southeastern US. They can cause anorexia, diarrhea, unthriftiness and irritability (Cole et al., 1977).

**Sterigmatocystin** is primarily produced by *Aspergillus versicolor* and has been observed as a primary mycotoxin produced by *Aspergillus* on cereal grains in western Canada (Mills and Abramson, 1986). While toxic levels are thought to be infrequent in the U.S., sterigmatocystin was detected in a grain mixture and associated with bloody diarrhea and cow deaths in a field case in Tennessee (Vesonder and Horn, 1985).

**Ochratoxin** is produced primarily by a *Penicillium* mold, but also by certain *Aspergillus* molds. Ochratoxin has been reported to affect cattle (Vough and Glick, 1993). It is rapidly degraded in the rumen, and thus, thought to be of little consequence unless consumed by young pre-ruminant calves (Sreemannarayana et al., 1988). However, high-concentrate diets reduce ochratoxin degradation in the rumen.

**Patulin**, a *Penicillium* produced mycotoxin associated with aerobic deterioration of silage has been incriminated as a possible toxin in Europe and New Zealand (Lacey, 1991).

**PR toxin**, produced by *Penicillium roquefortii*, has been found in silage and was the suspected vector in a case study with symptoms of abortion and retained placenta (Still et al., 1972).

**Roquefortine**, produced by *Penicillium roquefortii*, is a tremorgen which has been found in silage.

Other mycotoxins such as rubratoxin, citrinin, cyclopiazonic acid, and ergotoxins may be of some importance. Many other mycotoxins may affect ruminants but are thought to occur less frequently, be less potent, or are unrecognized. Lillehoj et al. (1991) has reviewed toxin production in novel substrates, to include forages and fermentations.

## MYCOTOXIN TESTING

To determine toxicity, feeds should be analyzed for mycotoxins and not just mold content, however the type of mold present may suggest the mycotoxins most likely to be present. The amount or presence of mold or mold spore count is not very indicative of mycotoxin content (Wyatt, 1991). Molds may be present which do not produce, or are not currently producing mycotoxins. A mold may have produced mycotoxins and is no longer viable, resulting in mycotoxin levels without the obvious presence of mold. It is possible that opinions have been formed about the toxicity of mycotoxins, based on the presence of mold, which could lead to erroneous conclusions.

Analytical techniques for mycotoxins are improving (Chu, 1992). Several commercial laboratories are available and provide screens for a large array of mycotoxins. Scott (1990) has suggested that screening methods are needed for the *Fusarium* produced mycotoxins and that one approach is to test for DON, DAS, T-2 and nivalenol, because other *Fusarium* mycotoxins seldom occur without one of these four also present. Feeds could then be further tested for other mycotoxins. Our approach is to test for AF, DON, T-2, ZEN and FB.

Cost of analyses has been a constraint but the newer immunoassays have reduced current costs, which can be insignificant compared with the economic consequences of production and health losses related to mycotoxin contamination.

Collection of representative feed samples is a problem, primarily because molds can produce very large amounts of mycotoxins in small areas making the mycotoxin level highly variable within the lot of feed. Sampling of horizontal silos show mycotoxins to be highly variable throughout the silage; however, the silo face appears to have higher and more consistent levels. Because mycotoxins can form in the collected sample, it should be preserved and delivered to the lab quickly. Samples can be dried, frozen or treated with a mold inhibitor before shipping.

## CONCLUSIONS

There are many different mycotoxins in nature. Mycotoxins occur very frequently and in some cases at levels high enough to cause severe animal injury

and economic loss. Frequency and levels of occurrence are related to weather conditions as well as to agronomic, feed storage and feed handling practices. Different environmental conditions favor growth of different molds. Conditions favorable for mold growth may not be the most favorable for mycotoxin formation. Feed sampling and analysis are recommended when symptoms suggest possible mycotoxin involvement. Mycotoxins should be considered a possible primary cause of abnormal health, production or reproduction.

## LITERATURE CITED

- Abbas, H.K., C.J. Mirocha, T. Kommedahl, R.F. Vesonder, and P. Golinski. 1989. Production of trichothecenes and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. *Mycopathologia*. 108:55.
- Boyacioglu, D., N.S. Hettiarachchy, and R.W. Stack. 1992. Effect of three systemic fungicides on deoxynivalenol (vomitoxin) production by *Fusarium graminearum* in wheat. *Can. J. Plant Sci.* 72:93.
- Chu, F.S. 1992. Recent progress on analytical techniques for mycotoxins in feedstuffs. *J. Anim. Sci.* 70:3950.
- Cole, R.J., J.W. Kirksey, J.W. Dorner, D.M. Wilson, J.C. Johnson, Jr., A.N. Johnson, D.M. Bedell, J.P. Springer, K.K. Chexal, J.C. Clardy, and R.H. Cox. 1977. Mycotoxins produced by *Aspergillus fumigatus* species isolated from moldy silage. *J. Agric. Food Chem.* 25:826.
- Côté, L.M., J.D. Reynolds, R.F. Vesonder, W.B. Buck, S.P. Swanson, R.T. Coffey, and D.C. Brown. 1984. Survey of vomitoxin-contaminated feed grains in Midwestern United States, and associated health problems in swine. *J. Am. Vet. Med. Assoc.* 184:189.
- Council for Agricultural Science and Technology (CAST). 1989. Mycotoxins: Economic and Health Risks. Task Force Report No. 116. Ames, Iowa.
- Gareis, M. and J. Ceynowa. 1994. Influence of the fungicide Matador (tebuconazole/triadimenol) on mycotoxin production by *Fusarium culmorum*. *Lebensmittel-Untersuchung-Forsch.* 198:244.
- GAO. 1991. Food safety and quality. Existing detection and control programs minimize aflatoxin. Report RCED-91-109.
- Gelderblom, W.C.A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vlegaar, and N.P.J. Kriek. 1988. Fumonisin: Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* 54:1806.
- Gotlieb, A. 1997. Causes of mycotoxins in silages. *In: Silage: Field to Feedbunk*, NRAES-99, Northeast Regional Agricultural Engineering Service, Ithaca, NY. p. 213.

- Hamilton, P.B. 1978. Fallacies in our understanding of mycotoxins. *J. Food Prot.* 41:404.
- Hagler, W.M., K. Tyczkowska, and P.B. Hamilton. 1984. Simultaneous occurrence of deoxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from the Midwestern United States. *Appl. Environ. Microbiol.* 47:151.
- Hesseltine, C.W. 1986. Global significance of mycotoxins. *In: Mycotoxins and Phycotoxins*, P.S. Steyn and R. Vlegaar. (Eds). Elsevier Scientific Publishing Co., Amsterdam.
- Hsu, I.C., C.B. Smalley, F.M. Strong, and W.E. Ribelin. 1972. Identification of T-2 toxin in moldy corn associated with a lethal toxicosis in dairy cattle. *Appl. Microbiol.* 24:684.
- Joffe, A. Z. 1986. *Fusarium Species: Their Biology and Toxicology*. John Wiley and Sons, Inc., New York.
- Kosuri, N.R., M.D. Grave, S.G. Yates, W.H. Tallent, J.J. Ellis, I.A. Wolff, and R.E. Nichols. 1970. Response of cattle to mycotoxins of *Fusarium tricinatum* isolated from corn and fescue. *J. Am. Vet. Med. Assoc.* 157:938.
- Lacey, J. 1991. Natural occurrence of mycotoxins in growing and conserved forage crops. *In: Mycotoxins and Animal Foods*, J. E. Smith and R. E. Henderson (Eds.). CRC Press, Boca Raton, Florida.
- Lillehoj, E.B., T.E. Cleveland, and D. Bhatnagar. Mycotoxins in feedstuffs: Production in novel substrates. *In: Mycotoxins and Animal Foods*, J. E. Smith and R. S. Henderson (Eds.) CRC Press. Boca Raton, Florida. Pp. 399.
- Mills, J.T. and D. Abramson. 1986. Production of sterigmatocystin by isolates of *Aspergillus versicolor* from western Canadian stored barley and rapeseed/canola. *Can. J. Plant Pathol.* 8:151.
- Mirocha, C.J., S.V. Pathre, B. Schauerhamer, and C.M. Christiansen. 1976. Natural occurrence of *Fusarium* toxins in feedstuffs. *Appl Environ. Microbiol.* 32:553.
- Moss, M. O. 1991. The environmental factors controlling mycotoxin formation. *In: Mycotoxins and Animal Foods*, J. E. Smith and R. S. Henderson (Eds.) CRC Press. Boca Raton, Florida.
- Petrie, L., J. Robb, and A.F. Stewart. 1977. The identification of T-2 toxin and its association with a hemorrhagic syndrome in cattle. *Vet. Rec.* 101:326.
- Russel, L., D.F. Cox, G. Larsen, K. Bodwell, and C.E. Nelson. 1991. Incidence of molds and mycotoxins in commercial animal feed mills in seven Midwestern states, 1988-89. *J. Anim. Sci.* 69:5.
- Scott, P.M. 1990. General referee reports: Mycotoxins. *J. Assoc. Off. Anal. Chem.* 73:98.
- Seglar, B. 1997. Case studies that implicate silage mycotoxins as the cause of dairy herd problems. *In: Silage: Field to Feedbunk*, NRAES-99, Northeast Regional Agricultural Engineering Service, Ithaca, NY. p. 242.
- Sreemannarayana, O., A.A. Frohlich, T.G. Vitti, R.R. Marquart, and D. Abramson. 1988. Studies of the tolerance and disposition of ochratoxin A in young calves. *J. Animal Sci.* 66:1703.
- Still, P., R.-D. Wei, E.B. Smalley, and F.M. Strong. 1972. A mycotoxin from *Penicillium roqueforti* isolated from toxic cattle feed. *Fed. Proc.* 31:733.
- Sundlof, S.F. and C. Strickland. 1986. Zearalenone and zearalenol: Potential residue problems in livestock. *Vet. Hum. Toxicol.* 28:242.
- Trenholm, H. L., D. B. Prelusky, J. C. Young, and J. D. Miller. 1988. Reducing mycotoxins in animal feeds. *Agriculture Canada Publication 1827E*.
- USDA. APHIS. 1995. Mycotoxin levels in the 1995 midwest preharvest corn crop. *Veterinary Services Factsheet N195.1295*. The National Veterinary Services Laboratory, Ames, Iowa.
- Vesonder, R. F., A. Ciegler, R. F. Rogers, K. A. Burbridge, R. J. Bothast, and A. H. Jensen. 1978. Survey of 1977 crop year preharvest corn for vomitoxin. *Appl. Environ. Microbiol.* 36:885.
- Vesonder, R.F. and B. W. Horn. 1985. Sterigmatocystin in dairy cattle feed contaminated with *Aspergillus versicolor*. *Appl. Environ. Microbiol.* 49:234.
- Vough, L.R. and I. Glick. 1993. Round bale silage. *In: Silage Production from Seed to Animal*, NARES-67, Northeast Regional Agricultural Engineering Service, Ithaca, NY. pp. 117.
- Whitlow, L. W. and W. M. Hagler, Jr. 1997. Mycotoxins and spoilage; Effects of mycotoxins on the animal: The producer's perspective., *In: Silage: Field to Feedbunk*, Northeast Regional Agricultural Engineering Service, NRAES-99, Ithaca, NY. pp. 222.
- Whitlow, L.W., W.M. Hagler, Jr., and B.A. Hopkins. 1998. Mycotoxin occurrence in farmer submitted samples of North Carolina feedstuffs: 1989-1997. *J. Dairy Sci.* 81:(Abstr.)1189.
- Woolford, M.K. 1984. *The Silage Fermentation*. Markel Dekker, Inc., New York.
- Wyatt, R.D.. 1991. Measurement of mold growth and mycotoxins in feed: fallacies and innovations. *In: Proceedings 1991 Georgia Nutrition Conference*, University of Georgia, Athens.