

By D.E. DIAZ, L.W. WHITLOW and W.M. HAGLER JR.

Diaz is Feed Quality Technical manager at Novus International, Whitlow is professor emeritus in the department of animal science, and Hagler is professor emeritus in the department of poultry science at North Carolina State University.

**M**old growth is an inevitable consequence of feed production, as a result their harmful metabolites “MYCOTOXINS” are commonly found in livestock and poultry diets. In the last 40 years great advances in the field of mycotoxins have increased our knowledge on the detrimental effects of these toxins on animal production. It is still, however, a fairly new field of study with many unanswered questions. Mycotoxins are toxic secondary metabolites produced by fungi (molds). Only some molds produce mycotoxins, and they are referred to as toxigenic. The fungal toxins are chemically diverse — representing a variety of chemical families — and range in molecular weight from about 200 to 500. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis that are commercially available. The primary classes of mycotoxins are aflatoxins of which aflatoxin B1 (AFB1) is the most prevalent, zearalenone (ZEA), trichothecenes — primarily deoxynivalenol (DON) and T-2 toxin (T-2) — fumonisins, ochratoxins (OTA) and the ergot alkaloids.

A practical definition of a mycotoxin is a fungal metabolite that causes an undesirable effect when animals or humans are exposed. Usually, exposure is through consumption of contaminated feedstuffs or foods. Mycotoxicoses are diseases caused by exposure to foods

or feeds contaminated with mycotoxins (Nelson et al., 1993). Mycotoxins exhibit a variety of biological effects in animals: liver and kidney toxicity, central nervous system effects and estrogenic effects, to name a few. Some mycotoxins, i.e., aflatoxin, fumonisin and ochratoxin, are carcinogenic.

## Mold growth, mycotoxin formation

Perhaps the major mycotoxin-producing fungal genera, in terms of research in the U.S., are *Aspergillus*, *Fusarium* and *Penicillium*. Many species of these fungi produce mycotoxins in feedstuffs. Molds can grow and mycotoxins can be produced pre-harvest or during storage, transport, processing or feeding. Mold growth and mycotoxin production are related to plant stress caused by weather extremes, to insect damage, to inadequate storage practices and to faulty feeding conditions. In general, environmental conditions — heat, water and insect damage — cause stress and predispose plants in the field or feed in transit or storage to mold growth and mycotoxin contamination (Coulumbe, 1993). Computer models to predict mycotoxin concentrations in corn prior to harvest are based on temperature, rainfall and insect pressure (Dowd, 2004) and similarly for DON in wheat (Prandini et al., 2009). Molds grow over a temperature range of 10-40°C (50-104°F), a pH range of 4-8, aw (water activity) above 0.7 and moisture content greater than 13-15%. Most molds are aerobic, and therefore high-moisture concentrations that exclude adequate oxygen can prevent mold growth. However, in practical situations, molds will grow in wet feeds such as silage or wet byproducts, when oxygen is available.

*Aspergillus* species normally grow at lower water activities and at higher temperatures than the *Fusarium* species. Therefore, *Aspergillus flavus* and aflatoxin in corn are favored by the heat and drought stress associated with warmer climates (Klich et al., 1994). Aflatoxin contamination is enhanced by insect damage before and after harvest. The individual *Penicillium* species have variable requirements for temperature and moisture but are more likely to grow under post-harvest conditions, in cooler climates, in wet conditions and at a lower pH.

The *Fusarium* species are important plant pathogens that can proliferate pre-harvest, but continue to grow post-harvest. *Fusarium* molds are associated economically important diseases, causing ear rot and stalk rot in corn and head blight (scab) in small grains. In wheat, *Fusarium* is associated with excessive moisture at flowering and early grain-fill stages. In corn, *Fusarium graminearum* is referred to as a red ear rot and is more commonly associated with a cool, wet growing season and with insect damage. *Fusarium* ear rots that produce fumonisins are referred to as pink ear rots and vary in their environmental requirements. They are generally associated with dry conditions in mid-season followed by wet weather (CAST, 2003).

## Mycotoxin occurrence

Worldwide, approximately 25% of crops are affected by mycotoxins annually (CAST, 1989), which would extrapolate to billions of dollars of losses (Trail et al., 1995). Annual economic costs of mycotoxins to the U.S. agricultural economy is estimated to average \$1.4 billion (CAST, 2003). Economic losses are due to effects on livestock productivity, crop losses and the costs of regulatory programs directed toward mycotoxins. The implications of mycotoxins on agricultural trade have been reviewed (Dohman, 2003). Van Egmond (2002) has reviewed the worldwide mycotoxin regulations.

Rodrigues (2008) reviewed mycotoxin contamination of diverse feedstuff samples from throughout the world for six mycotoxins (aflatoxin B1, ZEA, DON, fumonisin, T-2 toxin and OTA). Fumonisin was the most frequent contaminant (58% of samples) occurring commonly in corn, distillers grains and finished feeds. The data suggest routine exposure of animals to mycotoxins. Feed byproducts derived from ethanol production can contain up to three times greater concentrations of mycotoxins than the original grain from which the byproduct is derived, because as starch is removed, mycotoxins become concentrated in the residue. The economic impact to the livestock industry resulting from mycotoxins in ethanol co-products has been modeled and estimated to be significant (Wu and Munkvold, 2008). In North Carolina (Table 1), feed samples submitted by North Carolina farmers over a nine-year period indicate that mycotoxins occur frequently at unsuitable concentrations in feeds, including corn silage and corn grain (Whitlow et al., 1998). Miller (2008) recently reviewed the challenges of mycotoxin contamination of small grains and corn grain and recommended greater preparedness to manage an expected increase in occurrence of mycotoxins associated with future climate and technological changes.

Occurrence and concentrations of mycotoxins are variable by year and associated with variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). In the 2009-10 crop year, several regions of the U.S. experienced higher concentrations and incidence of mycotoxins primarily due to a wet and delayed harvest season. Although mycotoxins occur frequently in a variety of feedstuffs and are routinely fed to animals, it is less frequent that mycotoxins occur at concentrations high enough to cause immediate and dramatic losses in animal health and performance. However, mycotoxins at low levels interact with other stressors to cause subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. To the animal producer, these subclinical losses are of greater economic importance than losses from acute effects and even more difficult to diagnose.

## Mycotoxin effects

In his book "Poisons of the Past: Molds, Epidemics & History," Matossian (1989) discusses the potentially harmful effects of moldy grain and foods throughout history. The study of mycotoxins (mycotoxicology) really began in 1960 with the outbreak of Turkey-X disease in the U.K. This outbreak was linked to peanut meal imported from Brazil (Sargeant et al., 1961). Because of an intensive multidisciplinary research effort, a blue-fluorescent toxin was isolated and mycelia of *A. flavus* were observed. *A. flavus* was shown to produce the same toxic compound(s) found in the toxic peanut meal. The toxin was characterized chemically and biologically and was given the trivial name aflatoxin. Aflatoxin was shown to be very toxic and carcinogenic in some of the test animal species used, and it resulted in a toxic metabolite in milk of dairy cows (Allcroft and Carnaghan, 1962; 1963). Additional incentives for mycotoxin research resulted from their potential use in biological warfare, the identification of fumonisin in the late 1980s, and the increased incidence of *Fusarium* produced mycotoxins (in small grains but also corn) following the unusually wet weather in the Midwest during the early 1990s. More recently, the application of molecular techniques to develop a new field of study — toxigenomics — has led to a greater understanding of mycotoxins (Afshari et al., 2011).

The discovery of aflatoxin and elucidation of some of its effects led to research on other livestock health and production problems linked with moldy feedstuffs and the discovery of additional mycotoxins produced by other fungi. In dairy cattle, swine and poultry, mycotoxin contamination of feeds affects growth, milk production, egg production, reproduction and immunity (Diekman and Green, 1992). Mycotoxins have also been involved in outbreaks of human diseases (CAST, 1989; Hayes, 1980; Joffe, 1986).

Mycotoxin effects may be acute, resulting from a high level dosage, or chronic, resulting from long-term, low-level exposure. Mycotoxins exert their effects through several mechanisms: (1) intake reduction or feed refusal, (2) alteration in nutrient content of feed and in nutrient absorption and metabolism, (3) effects on the endocrine and exocrine systems, (4) suppression of the immune system, (5) antibiotic effects and (6) cellular death.

In the field, animals experiencing a mycotoxicosis may exhibit a few or many of a variety of symptoms, including: digestive disorders, reduced feed consumption, unthriftiness, rough hair coat or abnormal feathering, undernourished appearance, low production, poor production efficiency, impaired reproduction and/or a mixed infectious disease profile. Mycotoxins can increase incidence of disease and reduce production efficiency. Some of the symptoms observed with a mycotoxicosis may therefore be secondary, resulting from an opportunistic disease, present because of mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins are reviewed (Oswald et al., 2005 and Bondy and Pestka, 2008). Immune suppression may result in chronic toxicity even with low levels of mycotoxins particularly if multiple mycotoxins are present (Xue et al., 2010). The progression and diversity of symptoms can be confusing, making diagnosis difficult (Hesseltine, 1986; Schiefer,

1990). Diagnosis has been further complicated by limited research, lack of feed analyses, non-specific symptoms, few definitive biomarkers and interactions with other stress factors. Although research on use of biomarkers has been limited, biomarkers are suggested for aflatoxin (Kensler et al., 1996), ochratoxin (Duarte et al., 2011), and for the *Fusarium* produced mycotoxins: fumonisins, deoxynivalenol, zearalenone and T-2 toxin (Cano-Sancho et al. (2010).

With few exceptions, a definitive diagnosis of a mycotoxicosis cannot be made directly from symptoms, specific tissue damage or even feed analyses. However, experience with mycotoxin-affected herds or flocks increases the probability of recognizing a mycotoxicosis. A process of elimination of other factors, coupled with feed analyses and responses to treatments can help identify a mycotoxicosis. More definitive diagnoses can be made for specific mycotoxins by detecting aflatoxin in milk or for fumonisin by induced changes in sphingolipid concentrations (Riley et al., 2001). Regardless of the difficulty of diagnosis, mycotoxins should be considered as a possible cause of production and health problems when appropriate symptoms exist and problems are not attributable to other typical causes (Schiefer, 1990).

## Safe levels of mycotoxins

Some of the same factors that make diagnosis difficult also contribute to the difficulty of establishing levels of safety. These include lack of research, sensitivity differences of animal species, imprecision in sampling and analysis, the large number of potential mycotoxins, interactions among mycotoxins and interactions with stress factors (Hamilton, 1984; Schaeffer and Hamilton, 1991). Field toxicities appear to be more severe than predicted from laboratory research.

Naturally contaminated feeds are more toxic than feeds with the same level of a pure mycotoxin supplemented into the diet. Aflatoxin produced from culture was more toxic to dairy cattle than pure aflatoxin added to diets (Applebaum et al., 1982). In swine, Foster et al. (1986) demonstrated that a diet containing pure added DON was less toxic than diets with similar concentrations of DON supplied from naturally contaminated feeds. Smith and MacDonald (1991) have suggested that fusaric acid, produced by many species of *Fusarium*, occurs along with DON to produce more severe symptoms. Lillehoj and Ceigler (1975) gave an example where penicillic acid and citrinin were innocuous in laboratory animals when administered alone, but were 100% lethal when given in combination. These studies strongly suggest the presence of other unidentified mycotoxins in naturally contaminated feeds and that mycotoxin interactions are extremely important. It is well documented that several mycotoxins may be found in the same feed (Hagler et al., 1984). Abbas et al. (1989) demonstrated *Fusarium* species isolated from Minnesota corn produced multiple mycotoxins. Because animals are fed a blend of feedstuffs and because molds produce an array of mycotoxins, many mycotoxin interactions are possible. Speijers and Speijers (2004) discussed the combined toxicity of mycotoxins and, therefore, suggest daily tolerable intake limits for groups of mycotox-

### 1. Occurrence of five mycotoxins in corn silage, corn grain and in all feed samples submitted for analysis by producers in North Carolina over a nine-year period (Whitlow et al., 1998)

Sample	--Aflatoxin >10 ppb--			-Fumonisin- ----DON >50 ppb----			----ZEN >70 ppb----			---T-2 toxin >50 ppb--			>1 ppm	
	n	%	Mean ± s.d.	n	%	Mean ± s.d.	n	%	Mean ± s.d.	n	%	Mean ± s.d.	n	%
Corn silage	461	8	28 + 19	778	66	1,991 + 2,878	487	30	525 + 799	717	7	569 ++ 830	63	37
Corn grain	231	9	170 + 606	362	70	1,504 + 2,550	219	11	206 + 175	353	6	569 + 690	37	60
All feeds	1,617	7	91 + 320	2,472	58	1,739 + 10,880	1,769	18	445 + 669	2,243	7	482 + 898	283	28

n = number of samples.

% = percentage of samples positive above given concentrations.

Mean of the positive samples plus and minus the standard deviation.

## 2. FDA action levels for total aflatoxins in food, feed<sup>a</sup>

Food or feedstuff	Concentration (ppb)
All products, except milk, designated for humans	20
Corn for immature animals and dairy cattle	20
Corn and peanut products for breeding beef cattle, swine and mature poultry	100
Corn and peanut products for finishing swine (>100 lb.)	200
Corn and peanut products for finishing beef cattle	300
Cottonseed meal (as a feed ingredient)	300
All other feedstuffs	20
Milk	0.5b

<sup>a</sup>Wood and Trucksess, 1998.

<sup>b</sup>Aflatoxin M1.

ins. Interactions of multiple mycotoxins are discussed in the CAST (2003) report.

Mycotoxin interactions with other factors make it difficult to determine safe levels of individual mycotoxins. Animals under environmental or production stress may show the more pronounced symptoms. There is a clear temperature interaction with fescue (ergot) toxicity, such that more pronounced symptoms are expressed during heat stress (Bacon, 1995). Fumonisin at 100 parts per million has been shown to reduce milk production in dairy cattle (Diaz et al., 2000) but in a separate study did not affect average daily gain in beef cattle fed 148 ppm (Osweiler et al., 1993). While this contrast may reflect a difference in factors such as duration of feeding, it may also suggest differences due to greater stress in early lactation dairy cattle compared with growing beef cattle.

Jones et al. (1982) demonstrated that productivity losses in commercial broiler operations occurred when aflatoxin concentrations were below concern levels determined by controlled research in laboratory situations. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals (Brucato et al., 1986; Galvano et al., 2001; Smith et al., 1971). Dietary ingredients such as clay pellet binders and beta-glucans adsorb some mycotoxins, reducing exposure of the animal. Thus, many factors and interactions make it difficult to relate field observations to those from controlled research.

Mycotoxin effects vary by species. Wannemacher et al. (1991) presented data showing that laboratory animals such as the mouse and rat have distinct species differences in response to T-2 toxin. Mycotoxin effects are also moderated by factors such as sex, age, duration of exposure and stresses of the environment and production.

Partial degradation of mycotoxins in the rumen helps to protect ruminants against acute toxicity but may contribute to chronic problems associated with long-term consumption of low levels of mycotoxins. Extent of ruminal degradation appears to be variable and perhaps reduced in feeding situations where ruminal turnover rate is increased or when rumen microbial population is altered. Ruminal degradation of mycotoxins appears to be more dependent on protozoal than bacterial activity (Kiessling et al., 1984; Hussein and Brasel, 2001). On the other hand, because of a broader array of mycotoxins found in forages and byproduct feeds, ruminants may be exposed to a greater potential toxicity than are monogastric animals. Effects of mycotoxins in ruminants are reviewed by Jouany and Diaz (2005).

### Aflatoxin

Aflatoxins are a family of 15-20 extremely toxic, mutagenic and carcinogenic compounds primarily produced by *A. flavus* and *A. parasiticus* (Deiner et al., 1987; Kurtzman et al., 1987). Aflatoxin contamination of corn, peanuts, tree nuts, cottonseed and other commodities is a continuing worldwide problem. Aflatoxin formation is affected by a large

number of biotic and abiotic factors in the environment of the fungus (Klich, 2007). Toxigenic *A. flavus* isolates produce aflatoxins B1 and B2, and toxigenic *A. parasiticus* isolates produce aflatoxins B1, B2, G1 and G2 (Cotty et al., 1994). *A. flavus* is the predominant fungus in aflatoxin-contaminated corn and cottonseed while *A. parasiticus* is probably more common in peanuts than corn (Davis and Diener, 1983).

Most of the aflatoxin problem in corn originates in the field although aflatoxin can be formed in stored grains. Field infection of corn with *A. flavus* (Wicklow, 1983) is expected when temperatures, including nighttime temperatures, are high and there is drought stress. Growth conditions in the southern U.S. result in routine aflatoxin contamination of crops, but aflatoxin can be found in crops grown in other regions in years when weather conditions are conducive. For example, 8% of samples of Midwestern U.S. corn grain from the 1988 drought season contained aflatoxin (Russell et al., 1991).

Corn is susceptible to *A. flavus* infection via the silks (Marsh and Payne, 1984), and stress conditions at the time of anthesis (pollination) lead to pre-harvest aflatoxin contamination in corn. *A. flavus* spores as inoculum are plentiful at this time. Insect activity is also important in the events leading to aflatoxin contamination of corn, but its importance varies by location (Payne, 1983).

Aflatoxin is a greater problem in cottonseed grown in the southwestern U.S. than in the southeastern U.S. (Ashworth et al., 1969). The complex effects of relative humidity, temperature, precipitation and their daily variations may interact to produce conditions conducive to *A. flavus* infection and aflatoxin production in the Southwest (Ashworth et al., 1969). Early harvest and decreased late-season irrigation may reduce contamination (Russell et al., 1976). Long- or full-season cotton production systems seem to favor high cottonseed aflatoxin levels. Experimentally, the use of spores of non-toxigenic *A. flavus* isolates in southwestern cotton fields has resulted in greatly reduced aflatoxin levels in cottonseed (Cotty et al., 1994). Improperly stored cottonseeds are susceptible to mycotoxin contamination if mold activity is allowed. Non-toxigenic *Aspergillus* isolates are also available for use in peanuts (Horn et al., 2001) and corn (Dorner and Cole, 2002).

Aflatoxin enters the cell and is either metabolized in the endoplasmic reticulum to hydroxylated metabolites that are further metabolized to glucuronide and sulfate conjugates or is oxidized to the reactive epoxide that undergoes hydrolysis and can bind to proteins resulting in cytotoxicity. The epoxide can react with DNA or protein or be detoxified by the glutathione S-transferase enzyme. Both the DNA adduct and the protein adducts have proven useful as biomarkers in humans and laboratory animals (Riley and Pestka, 2005).

Aflatoxin lowers resistance to diseases and interferes with vaccine-induced immunity in livestock (Diekman and Green, 1992). Suppression of immunity by aflatoxin B1 has been demonstrated in turkeys, chickens, pigs, mice, guinea pigs and rabbits (Sharma, 1993). Swine, turkeys, ducks and rainbow trout are very susceptible to aflatoxin. Broiler chickens are resistant compared to these but are much more susceptible to aflatoxin than layer-type chickens. Pale, friable, fatty livers may be evident in acute aflatoxicosis in poultry.

Symptoms of acute aflatoxicosis in mammals include: inappetence, lethargy, ataxia, rough hair coat and pale, enlarged fatty livers. Symptoms of chronic aflatoxin exposure include reduced feed efficiency and milk production, icterus and decreased appetite (Nibbelink, 1986). If problems are found and analysis shows aflatoxin, the feed should be immediately replaced with fresh feed (Nibbelink, 1986). Reduced growth rate may be the only clue for chronic aflatoxicosis and other mycotoxicoses (Raisbeck et al., 1991; Pier, 1992). The mechanism by which aflatoxins reduce growth rate is related to disturbances in protein, carbohydrate and lipid metabolism (Cheeke and Shull, 1985).

Several reports have shown that differences in resistance to aflatoxin exist in different breeds and strains of chickens

Naturally ahead

 **Biomini** 



# Biofix<sup>®</sup>

*More milk is the result of keeping the rumen healthy and systems functioning normally.*

**Biofix<sup>®</sup>** is the solution for your herd, especially when feed quality isn't what it should be.

**Biofix<sup>®</sup>**, to maintain milk production with more profitably.



## *up to 14 days faster.*

Mycotoxins decrease performance and interfere with the health status of your pigs.

**Biofix<sup>®</sup>** is the feed additive for mycotoxin risk management.

**BIOMIN America Inc.**  
1842 Lockhill Selma, Suite 102  
San Antonio, TX 78213 U.S.A.  
Tel: +1 210 342 9555  
Fax: +1 210 342 9575  
e-Mail: [info@biomin.net](mailto:info@biomin.net)

For trials and additional information email us at:

**[office.usa@biomin.net](mailto:office.usa@biomin.net)**

(Smith and Hamilton, 1970; Washburn et al., 1978; Lanza et al., 1982). Marks and Wyatt (1980), using Japanese quail, demonstrated the feasibility of breeding for resistance to aflatoxin. Manning et al. (1990) developed a line of chickens resistant to acute and chronic dietary aflatoxin exposure. This was accomplished after five generations of selecting for resistance to a single oral dose of aflatoxin.

Wolzack et al. (1985) found that hens consuming feed contaminated with more than 3,300 mg/kg of aflatoxin B1 over a 28-day period produced contaminated eggs. Contamination peaked in four to five days, and egg contamination cleared within a similar period after aflatoxin removal. Qureshi et al. (1998) also found that aflatoxin residues are transmitted into eggs; their experiments showed that the chicks hatched from eggs of dams exposed to aflatoxin were compromised both in immune function and performance.

In finishing pigs, 385 ppb of aflatoxin increased liver weights and decreased weight gains; liver lesions occurred with 480 ppb of dietary aflatoxin (Southern and Clawson, 1979). In pigs (14 kg original weight) AFB1 at 60 ppb combined with DON at 300 ppb fed for 33 days may reduce intake and growth rate while levels of AFB1 at 120 ppb combined with DON at 600 ppb may suppress immunity, increase systemic inflammation and cause liver damage (Chaytor et al., 2011). Rustemeyer (et al., 2011) demonstrated negative health and growth effects in growing barrows (14 kg original weight) fed 250 ppb AFB1.

Depending on interactions with other factors, aflatoxin concentrations as low as 100 ppb may be toxic to beef cattle. However, the toxic level is generally considered to be between 300 and 700 ppb. Garrett et al. (1968) showed an effect on weight gain and intake with diets containing 700 ppb aflatoxin, but if increases in liver weights are used as the criteria for toxicity, then 100 ppb would be considered toxic to beef cattle. Feed efficiency and rate of gain was depressed in steers consuming 600 ppb of dietary aflatoxin (Helferich et al., 1986). Guthrie (1979) showed a decline in reproductive efficiency when lactating dairy cattle in a field situation were consuming 120 ppb aflatoxin. When cows were changed to an aflatoxin-free diet, milk production increased more than 25%. Patterson and Anderson (1982) and Masri et al. (1969) also suggested 100 ppb may reduce milk production. Applebaum et al. (1982) showed that impure aflatoxin produced by culture reduced production while equal amounts of pure aflatoxin did not. Ruminant alfalfa digestibility was depressed 50% and 67% with aflatoxin doses of 1 and 10 µg/ml, respectively (Westlake et al., 1989), but ruminant digestibility was not affected by 9.5 ng/ml of aflatoxin (Auerbach et al., 1998).

There are few literature reports about aflatoxin toxicity of horses. Toxicity has been noted in laboratory research (Cyswski et al., 1982) and in field observations (Vesonder et al., 1991) with symptoms that included hepatic lesions, mild bile duct proliferation and death. A review of the toxicological effects of aflatoxins in horses suggests that toxicity is similar to that noted in other large animals with symptoms of inappetence, depression, fever, tremor, ataxia and cough (Caloni and Cortinovis, 2010). The liver is a primary target, with necropsy findings of a yellow-brown necrotic liver.

Humans are exposed directly to aflatoxin and other mycotoxins through consumption of contaminated foods. Handling contaminated feed can result in exposure of mycotoxins through the skin and by inhalation (Schiefer, 1990). Indirect exposure of humans to aflatoxins occurs through foods — primarily milk, liver and eggs — derived from animals that consumed contaminated feeds (Hayes, 1980).

Aflatoxin B1 is excreted into milk of lactating dairy cows primarily in the form of aflatoxin M1 with residues approximately equal to 1-3% of the dietary concentration (Van Egmond, 1989). Aflatoxin appears in the milk within hours of consumption and returns to baseline levels within two to three days after removal from the diet (Frobish et al., 1986). A concentration of 20 ppb of aflatoxin B1 in the total mixed ration dry matter of lactating dairy cattle will result in afla-

toxin M1 levels in milk below the Food & Drug Administration's action level of 0.5 ppb. However, the European Union and several other countries presently have an action level of 0.05 ppb in milk and milk products. Assumed safe feeding levels may result in milk concentrations above the FDA action level because absolute concentrations of mycotoxins in the feed are difficult to determine, concentrations may not be uniform throughout a lot of feed and concentrations can change over time.

FDA has established non-binding action levels as informal guidelines for its enforcement of aflatoxin in feedstuffs (Table 2; Wood and Trucksess, 1998). Blending contaminated ingredients with uncontaminated ingredients with the purpose of reducing aflatoxin concentrations is not allowed.

## Zearalenone

ZEA and zearalenol are estrogenic metabolites of several species of *Fusarium*. Chemically, ZEN is a resorcylic acid lactone. *F. graminearum* is the major ZEA-producing fungus of the *Fusarium* species that causes corn ear and stalk rots, but other species of *Fusarium* produce ZEA as well as other mycotoxins (Christensen et al., 1988). ZEA has been reported to occur in corn, other grains and silage in many areas of the world. Weathered soybeans have also been reported to be contaminated with ZEA (Hagler et al., 1989). ZEA is also found in wheat, barley, oats, sorghum, sesame seed, hay and silages. Conditions exacerbating ZEA accumulation in corn include weather that holds moisture contents at 22-25% or delayed harvest (Abbas et al., 1988).

ZEA competitively binds with estrogen receptor sites in the uterus, mammary gland and liver with an efficacy comparable to that of 17β-estradiol, the principle endogenous estrogen receptor ligand. ZEA passively crosses the cell membrane and competitively binds to the cytosolic estrogen receptor. The receptor-ZEA complex is rapidly transferred into the nucleus where it binds to specific nuclear receptors and generates estrogenic responses via gene activation resulting in the production of mRNAs that code for proteins that are normally expressed by receptor-estrogen complex binding (Riley and Pestka, 2005).

Swine appear to be most susceptible to ZEA (Diekman and Green 1992). In prepubertal gilts, swollen vulvae appear; this can progress to vaginal or rectal prolapse (Friend et al., 1990). The liver, kidney and spleen may be enlarged (Jiang et al., 2011). Internally, enlarged, swollen, distorted uteri and shrunken ovaries are observed (Friend et al., 1990). Litter size may be reduced.

Hyperestrogenism occurs when contamination of ZEA is as low as 0.1 ppm (Mirocha et al., 1977). Young male pigs exposed to ZEA undergo symptoms of "feminization," such as enlarged nipples, testicular atrophy and swollen prepuce (Newberne, 1987). Low oral doses for ZEA (20 ug/kg body weight) for 48 days to immature gilts induced hyperestrogenism, (Gajecka et al., 2011). In rats, ZEA was fed at 1, 2, 4 and 8 mg/kg bodyweight, and dose-related decreases were observed in feed consumption and fetal growth (Collins et al., 2006). The highest dosage reduced bone ossification, number of viable fetuses, number of litters totally resorbed and estradiol levels.

Broiler chicks and laying hens are less susceptible to ZEA, even at very high dietary concentrations. Turkeys, on the other hand, at the high dietary levels of 300 ppm, develop greatly enlarged vents within four days with no other gross effects noted (Christensen et al., 1988). While limited data are available with horses, recent studies have shown that mares are sensitive to the estrogenic effects of ZEA (Minervini et al., 2006). ZEA also has negative effects on reproduction in fish, affecting relative spawning frequency and relative fecundity (Schwartz et al., 2010).

ZEA has been shown to induce immunotoxicity in mice with decreases in lymphocytes, immunoglobulin G (IgG),

### 3. FDA advisory levels for DON (vomitoxin) in livestock feed

Purpose of use levels (ppm) including grain, forage, etc.	Feed ingredients,	DON levels (ppm) in:	
	Maximum diet inclusion	Feed ingredients, grains, grain byproducts*	Total diet
Ruminating beef and feedlot cattle older than 4 months	Grain and grain byproducts (88% DM basis) not to exceed 50% of the diet	10 or 30*	10
Ruminating dairy cattle older than 4 months	Grain and grain byproducts (88% DM basis) not to exceed 50% of the diet	10 or 30*	5
Chickens	Grain and grain byproducts not to exceed 50% of the diet	10	5
Swine	Grain and grain byproducts not to exceed 20% of the diet	5	1**
All other animals	Grain and grain byproducts not to exceed 40% of the diet	5	2**

\*30 ppm is the guidance level for distillers grains and brewers grains destined for use in ruminating (older than four months) beef and dairy cattle diets with the added stipulation that the total diet not exceed 10 ppm (beef) or 5 ppm (dairy).

\*\*Assumed guidance amount in the total diet calculated from the ingredient concentration guidance multiplied by the maximum diet inclusion.

immunoglobulin M (IgM), B cells, T-cell subtypes (CD3(+), CD4(+)) and CD8(+)) and natural killer and pro-inflammatory cytokines (Salah-Abbes et al., 2008). Data also suggest that exposure to ZEA likely increases the metabolism of co-administered drugs and potentially causes food-drug interaction in humans (Ding et al., 2006).

Several congeners of ZEA may be produced in cultures and contribute to observed toxicity (Pfeiffer et al., 2010). In rumen cultures, ZEA is rapidly converted to alpha- and beta-zearalenol (Kiessling et al., 1984). Alpha-zearalenol is about four-fold more estrogenic in rats than ZEA, while beta-zearalenol is about equal in strength to ZEA (Hagler et al., 1979). However, ZEA has been considered of less importance to ruminants. Ruminal conversion of ZEA was found to be about 30% in 48 hours (Kellela and Vasenius, 1982). A controlled study with non-lactating cows fed up to 500 mg of ZEA showed no obvious effects except that corpora lutea were smaller in treated cows (Weaver et al., 1986b). In a similar study with heifers receiving 250 mg of ZEA by gelatin capsule, conception rate was depressed about 25% with no other obvious effects (Weaver et al., 1986a). Several case reports have related ZEA to an estrogenic response in ruminants, sometimes reporting abortions as a symptom (Kellela and Ettala, 1984; Khamis et al., 1986; Mirocha et al., 1968; Mirocha et al., 1974; Roine et al., 1971). Other cattle responses may include vaginitis, vaginal secretions, poor reproductive performance and mammary gland enlargement of virgin heifers.

In a field study (Coppock et al., 1990), diets with about 750 ppb ZEA and 500 ppb DON resulted in poor consumption, depressed milk production, diarrhea and total reproductive failure. New Zealand workers (Towers et al., 1995a; Towers et al., 1995b; Sprosen and Towers, 1995; Smith et al., 1995) have successfully estimated intake of ZEA and its metabolites (ZEA+M) by measuring urinary ZEA+M, which include zearalanone, alpha- and beta-zearalenol and alpha- and beta-zearalanol. ZEA+M intake (predicted from urinary ZEA) was associated with reproductive disorders in sheep and dairy cattle. In sheep, ZEA+M was related to reduced ovulation and lower conception rates. With dairy cattle, herds with low fertility had higher levels of blood and urinary ZEA+M originating from pastures containing higher ZEA+M. Individual cows within herds examined by palpation and determined to be cycling had lower blood ZEA+M levels than did cows that were not cycling. The reproductive problems in dairy cattle were associated with ZEA+M concentrations estimated at 400 ppb.

Further review of zearalenone is available in Zinedine et al. (2007). Currently, FDA has established no guidelines for ZEA in feed (Henry, 2006).

#### Trichothecenes

Trichothecenes are a family of 200-300 related compounds including T-2 toxin, diacetoxyscirpenol (DAS) and DON that are commonly found in agricultural commodities (Desjar-

dins et al., 1993). They exert their toxicity through protein synthesis inhibition at the ribosomal level, are immunosuppressive, toxic to cell membranes and induce apoptosis (Sharma, 1993; Shifrin and Anderson, 1999). The toxic effects of trichothecenes include gastrointestinal effects such as vomiting, diarrhea and bowel inflammation. Anemia, leukopenia, skin irritation, feed refusal, reduced growth and reproductive failure are also common. Several species of *Fusarium* and related genera produce trichothecenes.

DON (deoxynivalenol or vomitoxin) is a commonly occurring mycotoxin produced primarily by *F. graminearum* and *F. culmorum* (Rotter et al., 1996). Wet, rainy and humid weather at flowering promotes infection by *Fusarium*. The results are ear rot in corn and scab or head blight in sorghum, barley, wheat, oats and rye (Tuite et al., 1974). DON occurs in cereal grains worldwide and can increase in stored grain with high kernel moisture contents. In some geographical regions such as China and Japan, *Fusarium* produces nivalenol in excess of DON (Placinta et al., 1999). Nivalenol is considered to be more toxic than DON (Eriksen et al., 2004).

Chronic effects of DON include reduced feed consumption, reduced growth (anorexia and decreased nutritional efficiency), immune function changes (enhancement and suppression) and reproductive effects (reduced litter size) (Pestka, 2007 and 2010). Schoevers et al. (2010) demonstrated that DON interferes with oocyte development by disturbing cytoplasmic maturation. DON appears to affect serotonergic activity or serotonin receptors (Rotter et al., 1996). DON levels greater than 1 ppm can result in reduced feed intake and lower weight gains in swine. The no observable adverse effect level (NOAEL) for swine is estimated at 0.03-0.12 mg/kg of bodyweight per day (1-3 ppm in feed) (Pestka and Smolinski, 2005). Two independent Midwestern field studies (Vesonder et al., 1978; Cote et al., 1984) showed DON to be the primary mycotoxin associated with swine disorders, including feed refusals, diarrhea, emesis, reproductive failure and deaths. Vomiting has been reported in some outbreaks with high DON concentrations. Diets containing pure DON decrease feed consumption on a dose-related basis (Marasas et al., 1984). Danicke et al. (2006) demonstrated that DON reduces protein synthesis in the kidney, spleen and ileum but not the liver, skeletal and heart muscle, mesenteric lymph nodes, duodenum, jejunum, jejunal mucosa cells, pancreas and lung of exposed pigs. However, Pinton et al., (2010) showed that DON inhibits claudin-4 protein in the intestine leading to impaired intestinal barrier function.

Foster et al. (1986) found that feeds naturally contaminated with DON were more toxic to pigs than equal amounts of pure DON added to diets. Smith and McDonald (1991) have indicated that fusaric acid interacts with DON to produce the symptoms previously attributed just to DON. Other mycotoxins such as 3- and 15-acetyl DON often co-occur with DON. Berthiller et al. (2005) reported on the occurrence of a glucoside of DON in corn and wheat samples. This conjugated DON escapes detection by routine analytical methods and may account for the toxicity associated with low

#### 4. FDA guidance for industry on fumonisin levels in foods and animal feeds<sup>a</sup>

	Total fumonisins (FB1+FB2+FB3) Concentration (ppm)	
	Feed ingredients (ppm)	Finished feed (ppm)
<b>Human foods</b>		
Degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of < 2.25%, dry weight basis)		2
Whole or partially degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of >2.25 %, dry weight basis)		4
Dry milled corn bran		4
Cleaned corn intended for masa production		4
Cleaned corn intended for popcorn		3
<b>Animal feeds</b>		
Corn and corn byproducts intended for:		
Equids and rabbits (no more than 20% of diet) <sup>b</sup>	5	1
Swine and catfish (no more than 50% of diet) <sup>b</sup>	20	10
Breeding ruminants, breeding poultry and breeding mink and including lactating dairy cattle and hens laying eggs for human consumption (no more than 50% of diet) <sup>b</sup>	30	15
Ruminants more than 3 months old being raised for slaughter and mink being raised for pelt production (no more than 50% of diet) <sup>b</sup>	60	30
Poultry being raised for slaughter (no more than 50% of diet) <sup>b</sup>	100	50
All other species or classes of livestock and pet animals (no more than 50% of diet) <sup>b</sup>	10	5

<sup>a</sup>Federal Register, 2001.

<sup>b</sup>Limits on ingredients are on a dry weight basis.

observed concentrations of mycotoxins.

Chickens and turkeys apparently are not very susceptible to the effects of DON and other type B trichothecenes apparently due to low absorption into plasma and tissue and because of rapid clearance (Prelusky et al., 1986). Leghorn chickens showed no effect on weight gain from dietary levels of DON at 18 ppm (Kubena et al., 1987). Layers appear to be more tolerant to DON than are broilers under the stress of rapid growth (Huff et al., 1986). A two-week study with poults fed 75 ppm DON revealed no effect on feed consumed or growth (McMillan and Moran, 1985). No residues of DON were found in meat or eggs in birds fed high levels of DON in several experiments (El-Banna et al., 1983; Kubena et al., 1987; Lun et al., 1986). Although poultry can be more resistant to the intake effects of these toxins, recent research points to alterations in blood chemistry associated with feeding of combinations of 3-8 ppm of both DON and ZEA (Faixova et al., 2010), and effects on immune function and gut health at levels below those that may cause clinical outbreaks. Birds fed DON levels of around 3 ppm from a naturally contaminated feed had decreased cell-mediated response (Girish et al., 2008), suggesting poultry exposed to DON-contaminated grains may be less resistant to a disease outbreak. In a similar study, feed naturally contaminated with *Fusarium* and containing 3 ppm DON resulted in reduced villus height and absorptive villus surface area in duodenum and jejunum but not in ileum (Girish and Smith, 2008). Girgis et al., (2010a) showed that feeds naturally with fusarium mycotoxins can adversely affect intestinal immunity and mucosal cells in poults and that such feeds modulate immune response to coccidial disease (Girgis et al., 2010b). Magnoli et al. (2011) suggests that dietary monensin may reduce the capacity of sodium bentonite to bind aflatoxin. Such effects could apply to other mycotoxins, binders and additives.

The impact of DON on dairy cattle is not established. However, clinical data appear to show an association between DON contamination of diets and poor performance in dairy herds but without establishing a cause and effect (Whitlow et al., 1994). DON may, therefore, be a marker for low-quality mycotoxin-contaminated feeds in these herds. Other field reports help substantiate an association of DON with poorly performing dairy herds, although it is clear that other mycotoxins are usually involved (Gotlieb, 1997; Seglar, 1997). There was a trend ( $P < 0.16$ ) for a 13% loss in 4% fat-corrected milk in a study utilizing 18 mid-lactation dairy cows (average 19.5 kg milk), consuming diets shown to contain no common mycotoxins other than DON, which was at levels of approximately 0, 2.7 and 6.5 ppm in treatment diets (Charmley et al., 1993). Danicke et al. (2005) demonstrated a reduction of approxi-

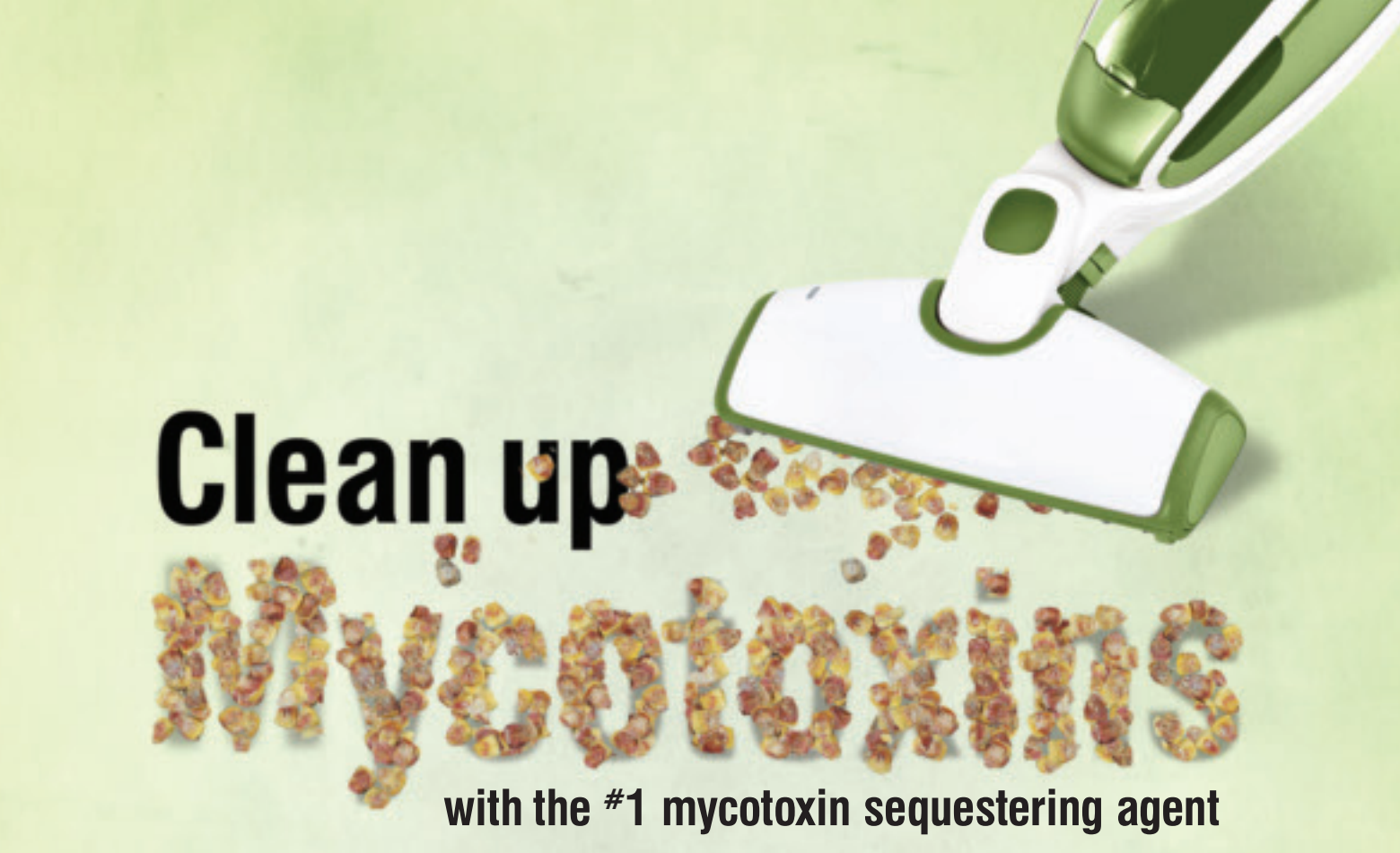
mately 20% in metabolizable protein associated with feeding 3.1 ppm DON from contaminated wheat. Reduced protein synthesis in the rumen was also indicated by higher rumen ammonia concentrations. Korosteleva and Smith (2006) also noted elevated concentrations of blood urea nitrogen in dairy cows consuming a mixture of fusarium mycotoxins including DON. Grain contaminated with DON has been shown to alter rumen volatile fatty acid concentrations (Keese et al., 2008). DON has been associated with reduced feed intake in non-lactating dairy cattle (Trenholm et al., 1985).

Noller et al. (1979) utilized 54 lactating dairy cows in a 3 X 3 Latin Square experiment with 21-day feeding periods. *Gibberella zeae* (*F. graminearum*)-infected corn was utilized to provide estimated concentrations of 0, 1,650 and 3,300 ppb DON and 0, 65 and 130 ppb of ZEA in three experimental diets. While neither intake nor milk production (22.9 kg per day) were affected, cows that received contaminated grain gained significantly less weight. Conversely, Ingalls (1994) fed lactating cows diets containing 0, 3.6, 10.9 or 14.6 ppm of DON for 21 days without an apparent effect on feed intake or milk production (30 kg per day). DiCostanzo et al. (1995), in a review of several individual studies, concluded that beef cattle and sheep can tolerate up to 21 ppm of DON without obvious deleterious effects.

Pets are affected by DON (Hughes et al., 1999). In dogs, feed consumption was reduced with 4.5 ppm of dietary DON and vomiting occurred with 8 ppm of DON. In cats, intake was reduced with 7.5 ppm of DON and vomiting was associated with 10 ppm of DON.

FDA provided on June 30, 2010, an updated non-binding guidance recommendation for DON in wheat products for human consumption and grains and grain byproducts for animal feed (Table 3). In general, the guidance values are similar to the last advisory provided by FDA in 1993; however, more details are provided. The new guidance doubles the maximum of DON for adult beef cattle and suggests higher maximum concentrations (30 ppm) allowed in distillers grains and brewers grains than for other feed ingredients ([www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/Guidanceforindustry/default.htm](http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/Guidanceforindustry/default.htm)).

**T-2 toxin** is produced primarily by *F. sporotrichioides* and *F. poae* and other species of *Fusarium* (Marasas et al., 1984). T-2 (and DAS) is often found in barley, wheat, millet, safflower seed and in mixed feeds. The toxicity of T-2 toxin is best documented in laboratory animals (Wannemacher et al., 1991). Li et al., (2011) in his review of T-2 toxin indicates that of the 190 identified members of the trichothecene family, T-2 is the most toxic and yet has received limited scientific study. Toxicity is a function of inhibition of protein synthesis, reduced immu-



# Clean up

# Mycotoxins

with the #1 mycotoxin sequestering agent

Feeding Mycosorb, from Alltech, reduces mycotoxin absorption within the animal, thereby negating the damaging effects of mycotoxins on the health of your animals.



**MYCOSORB®**

**gets the job done!**

#### Key Facts About Mycotoxins

- There are approximately 500 known mycotoxins.
- Animals are consistently exposed to multiple toxins.
- Interaction between toxins makes diagnosis difficult.
- Long term exposure to low levels of mycotoxins in the diet can reduce production and reproductive performance.
- **52 peer-reviewed papers, 17 PhDs and 19 masters, 14 years of research.**

Mycosorb® is not for sale in the USA and Canada.  
Integral™ is for sale in the USA and Canada.

**Alltech®**

Alltech.com



facebook.com/AlltechNaturally

@Alltech

nity, cellular necrosis and hematopoietic effects (primarily a decrease of circulating blood cells frequently associated with bone marrow failure). A significant effect is immunosuppression (Bondy and Pestka, 2000). T-2 also induces apoptosis in the gastric mucosa, gastric glandular epithelium and intestinal crypt cell epithelium (Li et al., 1997). Animal symptoms include unthriftiness, reduced feed intake, reduced production, reproductive failure, diarrhea, gastrointestinal hemorrhage and increased incidence of disease. T-2 is shown to induce degenerative articular changes in rodents (Wang et al., 2011), and is suspected as a factor in Kaschin-Beck disease in China which is a form of endemic osteoarthritis (Yang 1995).

Effects of T-2 on swine include infertility accompanied with some lesions in the uteri and ovaries. Feed intake was reduced when pigs received 0.5 ppm of dietary T-2. Drastic and sudden decreases in egg production in laying hens have been shown to be caused by T-2 toxin in the parts per million range. Other effects in chickens include decreased shell quality, abnormal feathering, mouth lesions and reduced weight gain. Pier et al. (1980) reported that egg production and shell quality were decreased at 20 ppm of dietary T-2 toxin. Turkeys fed T-2 exhibited reduced growth, beak lesions and reduced disease resistance (Christensen et al., 1988). Mouth lesions were caused by DAS and other trichothecene mycotoxins in broiler chickens (Ademoyero and Hamilton, 1991).

In cattle, T-2 toxin has been associated with gastroenteritis, intestinal hemorrhages in cattle (Petrie et al., 1977; Mirocha et al., 1976) and death (Hsu et al., 1972; Kosuri et al., 1970). Weaver et al. (1980) showed that T-2 was associated with feed refusal and gastrointestinal lesions in a cow but did not show a hemorrhagic syndrome. Pier et al. (1980) noted that 0.64 ppm dietary T-2 for 20 days resulted in death and bloody feces, enteritis and abomasal and ruminal ulcers. Kegl and Vanyi (1991) observed bloody diarrhea, low feed consumption, decreased milk production and absence of estrous cycles in cows exposed to T-2. Serum immunoglobulins and certain complement proteins were lowered in calves receiving T-2 toxin (Mann et al., 1983). Gentry et al. (1984) demonstrated a reduction in white blood cell and neutrophil counts in calves. A calf intubated with T-2 developed severe depression, hindquarter ataxia, knuckling of the rear feet, listlessness and anorexia (Weaver et al., 1980).

## Fumonisin

Fumonisin B1 was first isolated in South Africa where *Fusarium*-contaminated feed has long been associated with animal problems (Gelderblom et al., 1988). Fumonisin is a family of mycotoxins produced by the species of *Fusarium* in the Liseola section. *F. verticilloides* (formerly *F. moniliforme* — a species that is almost ubiquitous in corn) and *F. proliferatum* are the main species producing high yields of fumonisins. Fumonisin B1, B2 and B3 (FB1, FB2 and FB3) are fumonisins in fungal cultures or found in naturally contaminated corn samples (Cawood et al., 1991).

Fumonisin is carcinogenic in rats and mice (National Toxicology Program, 2002), causes equine leukoencephalomalacia (ELEM) in horses (Marasas et al., 1988), pulmonary edema in swine (Harrison et al., 1990) and hepatotoxicity in rats (Gelderblom et al., 1991) and cattle (Osweiler et al., 1993; Diaz et al., 2000). Fumonisin cause toxicity to animals primarily by disruption of lipid metabolism (Riley and Pestka, 2005), and of calcium homeostasis (Domijam and Abramov, 2011). Fumonisin are specific inhibitors of ceramide synthase (sphinganine and sphingosine N-acyltransferase), a key enzyme in the pathway leading to formation of ceramide and more complex sphingolipids. ELEM, porcine pulmonary edema, liver- and kidney toxicity that result from fumonisin exposure are closely correlated with the degree of disruption of sphingolipid metabolism (WHO, 2000).

A U.S. Department of Agriculture-Animal & Plant Health Inspection Service survey of 1995 corn from Missouri, Iowa and

Illinois found that 6.9% contained more than 5 ppm fumonisin B1 (Anon., 1995). Murphy et al. (1993) reported fumonisin concentrations in corn for the Iowa, Wisconsin and Illinois crops. Incidence of contamination was greater than 60%, and concentrations ranged from 0 to 37.9 ppm. Corn screenings contained about 10 times the fumonisin content of the original corn.

In horses, fumonisin is primarily associated with ELEM; however, ELEM is secondary to cardiovascular effects (Smith et al., 2002) and perhaps concurrent with liver damage (Wilson et al., 1992). ELEM symptoms are also preceded by elevations in serum sphinganine to sphingosine ratio (Wang et al., 1992). ELEM is characterized by facial paralysis, nervousness, lameness, ataxia and inability to eat or drink (Marasas et al., 1988). In a study conducted by Wilson et al. (1990), 14 of 18 horses fed with a corn-based feed with 37-122 ppm of FB1 died of ELEM. The macroscopic diagnostic lesion in ELEM is the liquefaction of the interior of the cerebral hemispheres; this lesion is not known to occur in other species exposed to fumonisin. It has been reported that if the fumonisin dose is high enough, horses will die of liver toxicity before the grossly observable lesion develops (Wilson et al., 1990; Wilson et al., 1992). Equidae are apparently the most sensitive species and can tolerate no more than about 5 ppm in corn. Coloni and Cortinovis (2009) have reviewed the effect of fusariotoxins in equine.

Fumonisin cause pulmonary edema in swine (Harrison et al., 1990; Ross et al. 1990). Lower doses of FB resulted in a slowly progressive hepatic necrosis; higher doses resulted in acute pulmonary edema coincident with hepatic toxicity (Haschek et al., 1992).

Poultry are apparently more resistant to fumonisins than are swine and equines. Relatively high doses are required to induce measurable effects. Chicks fed 450 and 525 ppm of fumonisin for 21 days exhibited lowered feed consumption and weight gains. At 75 ppm, free sphingosine levels were elevated (Weibking et al., 1993a). In another study, Weibking (1993b) found that day-old poults fed with rations containing 199 and 200 ppm of FB1 for 21 days had lower bodyweight gains and feed efficiency when compared to the controls. There were also differences in organ weights and blood parameters. He concluded that *F. moniliforme* culture material containing fumonisin is toxic to young turkey poults and that the poult appears to be more sensitive to the toxin than broiler chicks (Weibking et al., 1993a,b).

While FB1 is thought to be much less potent in ruminants than monogastrics, work by Kriek et al. (1981) suggested that fumonisin was toxic to sheep. Osweiler et al. (1993) fed young steers 15, 31 or 148 ppm of fumonisin in a short-term study (31 days). There were no effects on feed consumption or gain; however, there was a trend toward lower intake and weight gains for those fed 148 ppm. With the highest feeding level, there were mild liver lesions found in two calves, and the group had elevated liver enzymes indicative of liver damage. Lymphocyte blastogenesis was significantly impaired at the end of the feeding period in the group having the highest dose.

Dairy cattle (Holsteins and Jerseys) fed diets containing 100 ppm fumonisin for approximately seven days prior to freshening and for 70 days thereafter demonstrated lower milk production (6 kg per cow per day), which was explained primarily by reduced feed consumption. Increases in serum enzymes concentrations suggested mild liver disease (Diaz et al., 2000).

Fumonisin carryover from feed to milk is thought to be negligible (Richard et al., 1996; Scott et al., 1994), even though destruction of fumonisin in the rumen is low (Calino et al., 2000; Guring et al., 1999).

Fumonisin is carcinogenic to rats and mice (National Toxicology Program, 1999) and has been associated with esophageal cancer in humans in China (Chu and Li, 1994) and South Africa (Rheeder et al., 1992) and is considered a possible human carcinogen (International Agency for Research on Cancer, 2002). Fumonisin has also been associated with an increase in human neural tube defects (Missmer et al., 2006).

FDA released a guidance for fumonisin levels in human food and animal feed in late 2001 (Table 4; *Federal Register*, 2001).

## Ergot alkaloids

One of the earliest recognized mycotoxicosis was ergotism caused by a group of ergot alkaloids. They are produced by several species of *Claviceps* that infect the plant and produce toxins in fungal bodies called sclerotia or ergots. Ergotism primarily causes a nervous or gangrenous condition in animals. Symptoms are directly related to dietary concentrations and include lameness, gangrene, agalactia, reduced weight gain, abortion, hypersensitivity, ataxia, convulsions and, in sheep, intestinal inflammation. In cattle grazing tall fescue, syndromes known as fescue foot, summer slump and fat necrosis have been described (Robbins et al., 1985). Sclerotia concentrations above 0.3% are related to reproductive disorders. Sorghum containing 3% ergot (16 mg alkaloids per kilogram) was fed to sows resulting in reduced plasma prolactin, apparent reduction in milk production and lower weight gain of litters (Kopinski et al., 2008).

Fescue is a major pasture grass in the U.S., growing widely throughout the lower Midwest and upper South. Fescue infected with *Neotyphodium coenophialum* may contain toxic alkaloids, ergovaline, associated with "fescue toxicity" (CAST, 2003). More than half of the fescue is endophyte infected, making this a serious problem for cattle and horse producers. The absorption and metabolism of ergot alkaloids may be quite different in monogastrics and ruminants. The more water-soluble alkaloids appear to be better absorbed than the lipophilic ones, and ruminal microbes appear to play an important role in the release and intestinal availability of soluble alkaloids in the rumen making ruminants more sensitive to their effects (Riley and Pestka, 2005). The toxins cause vasoconstriction, resulting in elevated respiration rate, and gangrene that can result in loss of hooves, tails and ears. Cattle become less tolerant to heat, appear unthrifty, have reduced rate of gain and lower reproductive performance. In dairy cattle, fescue toxicity reduces prolactin, causes agalactia and lower milk production. In horses, mares have prolonged gestation, dystocia, agalactia and abortions (Roberts et al., 2005). Endophyte-free varieties are available, but they are not as hardy as infected varieties. Fescue infected with a nonpathogenic endophyte may be more field hardy and less toxic.

A thorough review of ergot toxicity is provided by Strickland et al., (2011).

## Ochratoxin

OTA is produced by species of *Penicillium* and *Aspergillus* and is a causative agent of kidney disease in pigs that has been referred to as mycotoxin porcine nephropathy (Krogh, 1979). The primary toxic effect is inhibition of protein synthesis (Creppy et al., 1984) and is considered to be a genotoxic carcinogen (Pfohl-Leskowicz and Manderville, 2007).

In pigs and poultry, the proximal tubules are mainly affected and the kidney can become discolored and grossly enlarged. Additionally, fatty liver can occur be a common occurrence in poultry. The most sensitive indicator of acute ochratoxicosis in chickens is the reduction in total serum proteins and albumin, and in pigs, a decrease in phosphoenolpyruvate carboxykinase in kidney is a sensitive and specific indicator (Marquardt and Frohlich, 1992; Krogh, 1992). In pigs, large increases in proteins excreted in urine and are correlated with histological observations of renal damage (Riley and Pestka 2005). Exposure to lower levels of OTA in poultry and pigs can result in altered performance including reduced feed consumption and weight gain and immunosuppression that can lead to higher susceptibility to infection (Stoev et al., 2000). Other effects in poultry include decreased egg production, increased susceptibility to bruising due to poor blood coagulation, compromised bone strength, intestinal damage, carcass

discoloration and glycogen accumulation in liver (Riley and Pestka, 2005). Clinically, beak lesions and increased water intake in birds can be seen even at low levels of OTA.

In cattle, OTA 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, chronic exposure and acute toxicities are thought to occur in cattle. The ochratoxin A molecule associates a methyl-isocoumarin unit (ochratoxin-alpha) with L-beta-phenylalanine via a peptide bond, which can be easily hydrolyzed by rumen microbes. Thus, OTA in the rumen is converted into phenylalanine and OTA-alpha, the latter being less toxic but retaining some genotoxicity (JECFA 56th, 2001). However, moldy hay containing OTA has been implicated in cattle deaths and abortions. The mechanism of action of OTA is unclear, but its structural similarity to phenylalanine and the fact that it inhibits many enzymes and processes that are dependent on phenylalanine strongly suggest that OTA acts by disrupting phenylalanine metabolism (CAST, 2003).

Currently, FDA provides no guidelines for OTA in feed (Henry, 2006). However, OTA regulations do exist or are proposed for food and animal feed in some 60 countries.

## Other mycotoxins

Citrinin can co-occur with OTA, is produced by both *Penicillium* and *Aspergillus* and, like OTA, targets the kidney (Kitchen et al., 1977). The toxicity of citrinin was reviewed, indicating that it is a parasympathomimetic agent, causes necrosis of tubular epithelial cells in the kidney and, in some cases, hepatotoxicity (Hanika and Carlton, 1994). Citrinin may also suppress immunity through inhibition of nitric oxide production (Liu et al., 2010).

Patulin is produced by *Penicillium*, *Aspergillus* and *Byssoschlamys* (Dutton et al., 1984; Hacking and Rosser, 1981). Patulin is most likely to occur in moldy fruits such as apples, but may also be found in grains, especially wet grains, and silage. Patulin is antibiotic against gram-positive bacteria. Added to rumen continuous cultures at 0, 20, 40 or 80 mg per day, patulin reduced volatile fatty acid production, fiber digestion and bacterial yield (Tapia et al., 2005). The potential for patulin toxicity of livestock is thought to be low, but there are reported case studies of toxicity (Sabater-Vilar et al., 2004).

PR toxin, produced by *P. roquefortii*, caused acute toxicity in mice, rats and cats by increasing capillary permeability resulting in direct damage to the lungs, heart, liver and kidneys (Chen et al., 1982). PR toxin has been found in silage (Hacking and Rosser, 1981) and was the suspected vector in a case study with symptoms of abortion and retained placenta (Still et al., 1972). Surveys of grass and corn silage in Europe have found an occurrence of *P. roquefortii* in up to 40% of samples (Auerbach, 2003). PR toxin may be a key mycotoxin associated with silage toxicity (Auerbach, 2003; Seglar et al., 1999; Sumarah et al., 2005; Scudamore and Livesay, 1998).

Mycophenolic acid (MPA) is produced by a number of fungal genera, but is of most concern because of production by *Penicillium* that occurs frequently in silages. Schneweis et al. (2000) found that 32% of silages collected in Germany contained MPA. MPA has antifungal, antibacterial and antiviral activities and is used for immune system repression in organ transplant patients. Its toxicity to animals appears to be low (Cole and Cox, 1981); however, MPA blocks the proliferative response of T and B lymphocytes and inhibits both antibody formation and the production of cytotoxic T cells (Eugui et al., 1991). Sheep were fed 0, 0.5, 1.2 or 5 mg per day of MPA per kilogram of bodyweight for six weeks with no effect on general health (Baum et al., 2005). The highest dose resulted in shrinkage of thymic lobules. The numbers of IgG or IgM positive plasma cells decreased in the ileum with increasing MPA doses. These results suggested that high levels of MPA that can be found in silage may affect the morphology of lymphatic organs of sheep.

## Mycotoxins in forages

Mycotoxins found in forages result in exposure of herbivores to a broad array of multiple mycotoxins. Many different mycotoxins have been found to occur in forages either in the field or in storage as hay or silage (Lacey, 1991). Some mycotoxicoses in cattle resulting from contaminated forages (Lacey, 1991; Gotlieb, 1997; Scudamore and Livesay, 1998; Seglar, 1997; Whitlow, 1997) and byproduct feeds (Lillehoj et al., 1991) have been reviewed. Mold grows in hay stored too wet or with damp spots. The limiting factors for mold growth in silage are pH and oxygen. Silages stored too dry or insufficiently packed and covered can allow air infiltration, resulting in growth of yeast, depletion of silage acids, an increase in pH and, thus, conditions conducive for mold growth and deterioration of the silage.

In Pennsylvania, Mansfield and Kuldau (2007) found multiple mycotoxigenic molds, including *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*, in corn silage samples at harvest and after ensiling, suggesting the possible presence of multiple mycotoxins. El-Shanwany et al. (2005) isolated 43 fungal species belonging to 17 genera from 40 silage samples collected in Egypt. The most prevalent genera were *Aspergillus* and *Penicillium* followed by *Fusarium* and *Gibberella*. Mycotoxins were found in 206 of 233 grass or corn silage samples collected in Germany during 1997-98 (Schneweis et al., 2000). *Penicillium* was the dominant genus followed by *Mucoraceae*, *Monascus* and *Aspergillus*. *Penicillium* is a major silage mold and may be a greater silage problem because it grows at a lower pH than do other molds.

Mansfield, Jones and Kuldau (2008) investigated the presence of four *Penicillium*-produced mycotoxins (roquefortine C, MPA, patulin and cyclopiazonic acid) in fresh and ensiled corn silage in Pennsylvania. The four mycotoxins were often found to co-contaminate freshly harvested corn and were generally found in greater frequencies and concentrations after ensiling. Auerbach et al. (1998) found *P. roquefortii* in 89% of visibly moldy forage samples and 85% of samples without visible mold. Surveys of grass and corn silage in Europe have found an occurrence of *P. roquefortii* in as many as 40% of samples (Auerbach, 2003). *Penicillium* produced mycotoxins in silages, such as roquefortine C, MPA and PR toxins have been associated with herd health problems (Auerbach, 1998; Seglar et al., 1999; Sumarah et al., 2005). Data from Boysen et al. (2000), Seglar et al. (1999) and Sumarah et al. (2005) point to the possibility that PR toxin is a silage mycotoxin of potential concern. Seglar et al. (1999) suggested that PR toxin is a good marker for silages associated with dairy herds with health problems.

It appears that *A. flavus* does not grow well in hay or silage; however, aflatoxin concentrations of up to 5 ppm have been reported (Kalac and Woolford, 1982). Table 1 shows that the frequency of aflatoxin in corn silage is not different from the frequency of aflatoxin in corn grain, but the concentrations are lower. The frequency and concentrations of some *Fusarium*-produced mycotoxins are also compared in Table 1. There was a trend toward a higher frequency of ZEA in corn silage than in corn grain, and the concentrations of DON were higher in corn silage than in corn grain (Table 1). Conversely, ZEA was not detected in 25 hay and silage samples collected in Minnesota, Wisconsin and Illinois, but there was a high incidence of low levels of cyclopiazonic acid, DON, FB, PR toxin and alternaria TA toxin (Yu et al., 1999).

*A. fumigatus* is thought to be a fairly common mold in both hay (Shadmi et al., 1974) and silage (Cole et al., 1977). Silage was found to contain fumigaclavine A and C and several fumitremorgens. Animal symptoms included generalized deterioration typical of protein deficiency, malnutrition, diarrhea, irritability, abnormal behavior and occasional death. The hay was fed to goats and rats and resulted in retarded growth and histopathological changes in the livers and kidneys. *A. fumigatus* has been proposed as the pathogenic agent associated with mycotic hemorrhagic bowel syndrome in dairy cattle, often attributed to clostridial infections (Puntunney et al., 2003). A variety of enzymes and toxins produced by *A. fumigatus* may

facilitate colonization of tissue and evasion of host immunity (Tekaiia and Latge, 2005). Gliotoxin, produced by *A. fumigatus*, inhibits platelet function (Bertling et al., 2010). Gliotoxin is also immunosuppressive, antibacterial and induces apoptosis. Gliotoxin is shown to affect rumen fermentation, reducing digestibility and volatile fatty acid production *in vitro* (Morgavi et al., 2004). *A. fumigatus* also produces fumagillin, which inhibits neutrophil function (Fallon et al., 2010).

Mycoses are observed to occur in immunosuppressed animals, and dairy cows are immune suppressed in early lactation. When cellular immunity is an important mechanism for disease resistance, interactions with trichothecene mycotoxins may be an issue. Niyo et al. (1988a, b) showed that rabbits exposed to T-2 toxin had a decrease in phagocytosis of *A. fumigatus* conidia by alveolar macrophages and an increase in severity of experimental aspergillosis.

Moldy alfalfa hay containing *A. ochraceus* was implicated as producing OTA associated with abortions in cattle (Still et al., 1971). OTA in moldy forage has also been implicated in cattle deaths (Vough and Glick, 1993).

The most important pasture-induced toxicosis in the U.S. is tall-fescue toxicosis caused by endophytic alkaloids (Bacon, 1995). Other forage toxicoses of fungal origin include ergotism, perennial ryegrass staggers, slobbers syndrome, a hemorrhagic disease associated with dicoumarol produced in fungal-infected sweet clover and sweet vernal grass and syndromes of unthriftiness and impaired reproduction associated with *Fusarium* (Cheeke, 1995).

## Mycotoxin testing

The accurate determination of mycotoxin concentrations in grain and feeds depends on accuracy from sampling to analytical techniques. A statistically valid sample must be drawn from the lot, which is not simple because mycotoxins are distributed unevenly in grains and other feedstuffs. Most of the error in a single analysis is due to sampling — as much as 90% of the error is associated with the taking of the initial sample (Whittaker, 2003). Once collected, samples should be handled to prevent further mold growth. Wet samples may be frozen or dried before shipment, and transit time should be minimized.

The second-largest source of error is inaccurate grinding and subsampling of the original sample. Finally, the subsample is extracted, the extract purified using one of several techniques, and then the toxin is measured. Toxin determination may be by thin-layer chromatography plates, high-performance liquid chromatography, gas-liquid chromatography, enzyme-linked immunosorbent assays (ELISA), spectrophotometer or by other techniques. New technologies are progressing rapidly.

Mold spore counts may not be very useful and are only a gross indication of the potential for toxicity, but mold identification can be useful to suggest which mycotoxins may be present. Blacklighting for bright-greenish-yellow fluorescence (BGYF) is often used as a screening technique for aflatoxin in corn, but it is very inaccurate. Newer and better methods should be used. As far as we are aware, blacklighting is completely inappropriate for other mycotoxins.

Generally, laboratories provide analysis for only a limited number of mycotoxins, perhaps including aflatoxin, OTA, DON, ZEA, fumonisin and T-2 toxin. Laboratory analysis may be directed toward detection of high levels of mycotoxins associated with acute toxicity and serious animal disease rather than low levels associated with chronic effects such as production losses, impaired immunity and significant economic losses. Therefore, minimum detection limits set by a laboratory may inhibit the diagnosis of a chronic mycotoxicosis.

Analytical techniques for mycotoxins are improving, costs are decreasing and several commercial laboratories are available that provide screens for an array of mycotoxins. The Federal Grain Inspection Service (USDA-GIPSA) provides a list on the internet of approved mycotoxin tests for grains and pro-

vides excellent background materials for the feed industry (at [www.usda.gov/gipsa/pubs/mycobook.pdf](http://www.usda.gov/gipsa/pubs/mycobook.pdf)). Laboratory methods can be found in "Official methods of analysis of AOAC International" (Horwitz, 2000). Analytical protocols for mycotoxins are published (Trucksess and Pohland, 2000). Krska et al. (2008) provided an update on mycotoxin analysis focusing on recent developments including multi-mycotoxin methods and quick tests. Maragos and Busman (2010) reviewed the rapid and advanced tools for mycotoxin analysis. Rasmussen et al. (2010) reported the multi-analysis for 27 mycotoxins and secondary metabolites in corn silage (15 were detected) using LC-MS/MS, which has also been used to quantitatively measure mycotoxins in urine (Ahn et al., 2010). Innovative methods such as a magnetoresistive immunoassay (Mak et al., 2010), and detection by STING sensors (Actis et al., 2010) have been investigated. Because analytical methods can be either qualitative or quantitative, done by inexpensive kits or by sophisticated analytical instruments and can be quick or time fairly time consuming, it may be difficult to determine and select the right method for the right need (Scudamore, 2005).

## Mycotoxin prevention, treatment

The Food & Agriculture Organization (2001) provides a manual on application of hazard analysis and critical control points (HACCP) techniques for mycotoxin prevention and control. A quality assurance program for mycotoxin management has been reviewed by CAST (2003). Elements include prevention, sampling, sample preparation, extraction, evaluation of testing requirements, testing, result validation, documentation, supplier involvement and removal.

Management of mycotoxins in the cereal supply chain may be improved by use of predictive mathematical models and regional occurrence data (van der Fels-Klerx and Booi, 2010), including models developed for the U.S. of which an example is one developed by de Wolf et al. (2003) that uses within-season weather data to predict risk of *Fusarium* head blight in wheat. Jornet et al. (2007) and Kabak et al. (2006) have reviewed methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds.

Pre-harvest control has involved using agronomic practices, which minimize plant stress, fungal invasion and, thus, mycotoxin accumulation in the field. These include proper irrigation, insect control, pesticide application in some cases, resistant or adapted hybrids, tillage type, proper fertilization, timely planting and avoiding delayed harvest. Unfortunately, breeding for mycotoxin-resistant hybrids has been only partially successful. Munkvold et al. (1999) have shown that compared with non-transgenic corn, *Bacillus thuringiensis* (Bt)-transgenic corn had less corn borer damage, less *F. verticillioides* infection and lower fumonisin contamination. Fungicides have shown little efficacy in controlling pre-harvest aflatoxin contamination in corn (Duncan et al., 1994), but may be helpful in the control of other mycotoxins. The stress or shock of the fungicide to the mold organism may reduce mold growth and yet not reduce the production of mycotoxins (Boyacioglu et al., 1992; Gareis and Ceynowa, 1994; Simpson et al., 2001). A major success in reducing aflatoxin is the use of non-toxicogenic fungi to competitively exclude toxigenic fungi.

The best strategy for post-harvest control of mycotoxins is proper storage and handling of feedstuffs to prevent conditions conducive to fungal growth. Temperature, water activity and insects are the factors most closely associated with mycotoxin formation in storage. Management strategies also include: mycotoxin analysis of feedstuffs, diversion of contaminated lots; treatments to reduce mold growth, dilution and treatments to reduce mycotoxin levels. Research is progressing in discovery of methods to protect animals from mycotoxin exposure and their toxicity. Physical separation by cleaning or screening grains can be helpful.

Certain chemical and biological processes may also be of value in reducing mycotoxin effects. Kabak and Dobson (2009)

have reviewed the biological strategies to counter the effects of mycotoxins. Mycotoxins may be degraded into non-toxic metabolites by use of microorganisms or enzymes. Fermentative bacteria may have potential of binding some mycotoxins (Niderkorn et al., 2007). Enzymes have been found to have potentially useful mycotoxin degrading activity, including protease A (Abrunhosa et al., 2006), pancreatin (Abrunhosa et al., 2006), carboxypeptidase A (Schatzmayr et al., 2006), epoxidase (Schatzmayr et al., 2006) and lactonohydrolase (Takahashi-Ando et al., 2002). Microorganisms with potential for mycotoxin detoxification include *Flavobacterium aurantiacum* (aflatoxin), *Enterococcus faecium* (aflatoxin and patulin), *Eubacterium*: BSSH 797 and LS 100 (trichothecenes) and *Trichosporon mycotoxiniuorans* (ZEA and OTA). Acetyltransferases produced by certain *Fusarium* may degrade DON (Khatibi et al., 2011). Certain subspecies of *Bacillus subtilis* have shown extensive degradation of zearalenone (Cho et al., 2010).

Ammoniation of grains can destroy some mycotoxins, but there is not a practical method to treat forages. Addition of 0.25 or 0.5% of calcium propionate to diets for detoxification may reduce the effects of aflatoxin (Bintvihok and Kositcha-roenkul, 2006). Galvano et al. (2001) have reviewed dietary strategies to counteract mycotoxins. Increasing nutrients such as protein, energy and antioxidant nutrients may be advisable (Brucato et al., 1986; Galvano et al., 2001; Mezes et al., 2010; Smith et al., 1971).

Research has demonstrated that adsorbent materials such as silicate clays (bentonites and others), activated carbons or beta-glucan polymers (extracted from yeast cell wall) can reduce the effects of mycotoxins (Diaz et al., 2004; Galey et al., 1987; Galvano et al., 1996; Phillips et al., 2002). *In vitro* methods for determining mycotoxin adsorption have been reviewed and results suggest that while *in vitro* methods may have potential in screening products, results are not always predictive of *in vivo* results (Lemke et al., 2001 and Doll et al., 2004). An *in vitro* gastrointestinal model is proposed to better simulate *in vivo* conditions and has been used to assess the mycotoxin binding efficacy of some additives (Avantaggiato et al., 2007). A few reviews of mycotoxin enterosorbents are published (Avantaggiato et al., 2005; Bingham et al., 2003; Doll and Danicke, 2004; Huwig et al., 2001; Ramos and Hernandez, 1997; Varga and Toth, 2005).

A scientific report entitled "A review of mycotoxin-detoxifying agents used as feed additives: Mode of action, efficacy and feed/food safety" was submitted to the European Food Safety Authority in 2009. Paraphrasing the concluding remarks, it was noted that inorganic absorbing agents seem to be effective at preventing adverse effects of many toxic agents. Organic absorbing agents have the ability to stimulate the immune system. Proven detoxifying agents may benefit animal health and indirectly humans. Potential risks or adverse effects must be considered. A risk/benefit ratio could not be estimated with current data. FDA has approved no products for binding mycotoxins. FDA's Center for Veterinary Medicine provides a position paper on mycotoxin binding claims at [www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm131675.htm](http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm131675.htm).

## Areas of needed information

More information is needed about why mycotoxins occur, when to expect them, how to prevent their occurrence and how to deal with their presence. More data are needed about animal toxicity and about interactions with other mycotoxins, nutrients and stress factors such as disease organisms or environmental stress and about the role of mycotoxins in immunosuppression. Improved screening techniques are needed for monitoring mycotoxin occurrence, including the detection of multiple toxins, diagnosing toxicities and prevention and treatment (CAST, 2003).

## References

The extensive list of references can be obtained by email from [tlundeen@feedstuffs.com](mailto:tlundeen@feedstuffs.com). ■