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 available. The primary classes of mycotoxins are aflatoxins of which aflatoxin B$_1$ (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.

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, 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 (Coulombe, 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 and above 0.7 aw (water activity). Molds can grow on feeds containing more than 12-15% moisture. 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, 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., 1992). 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 postharvest. In corn, Fusarium molds are associated with ear rot and stalk rot, and in small grains, they are associated with diseases such as head blight (scab). 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. 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).

Rodrigues (2008) reviewed mycotoxin contamination of
diverse feedstuffs 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. 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 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 (Coulombe, 1993). Although mycotoxins occur frequently in a variety of feeds 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.

Because cattle consume forages and byproduct feeds, they may be exposed to a broader array of mycotoxins than are monogastric animals. Reviews are available on mycotoxins in forages (Lacey, 1991; Scudamore and Livesay, 1998) and byproduct feeds (Lillehoj et al., 1991).

**Mycotoxin effects**

The potentially harmful effects of feeding moldy grain and foods has been known for many years (Matossian, 1989), yet mycotoxicology, the study of mycotoxins, really began in 1960 with the outbreak of Turkey-X disease in the U.K. This mycotoxicology, the study of mycotoxins, 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 (Sargeant et al., 1961). Because of an intensive multidisciplinary research effort, a blue-fluorescent toxin was isolated (Sargeant et al., 1961). Because of an intensive multidisciplinary research effort, a blue-fluorescent toxin was isolated (Sargeant et al., 1961).

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Many mycotoxins have been linked to outbreaks of human disease. Mycotoxins also have been involved in outbreaks of human diseases (CAST, 1989; Hayes, 1980; Joffe, 1986). 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. This research led to 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). Mycotoxins 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, unthriftness, 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. Immuno toxic effects of mycotoxins are reviewed (Oswald et al., 2005 and Bondy and Pestka, 2000). The progression and diversity of symptoms can be confusing, making diagnosis difficult (Hesseltine, 1986; Schiefer, 1990). Diagnosis is further complicated by limited research, lack of feed analyses, nonspecific symptoms, few definitive biomarkers and interactions with other stress factors.

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 by animal species, imprecision in sampling and analysis, the large number of potential mycotoxins and interactions among mycotoxins and with stress factors (Hamilton, 1984; Schaefer 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 test animal species used, and it resulted in a toxic metabolite in milk of dairy cows (Allcroft and Carnaghan, 1962; 1963). 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. This research led to 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). Mycotoxins 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.

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**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)**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Sample</th>
<th>n</th>
<th>Mean ± s.d.</th>
<th>%</th>
<th>Mean ± s.d.</th>
<th>%</th>
<th>Mean ± s.d.</th>
<th>%</th>
<th>Mean ± s.d.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin &gt;10 ppb</td>
<td>Corn silage</td>
<td>461</td>
<td>8</td>
<td>28 ± 19</td>
<td>778</td>
<td>66</td>
<td>1,091 ± 2,878</td>
<td>219</td>
<td>11</td>
<td>206 ± 175</td>
</tr>
<tr>
<td>DON &gt;50 ppb</td>
<td>Corn grain</td>
<td>231</td>
<td>9</td>
<td>170 ± 606</td>
<td>362</td>
<td>70</td>
<td>1,504 ± 2,550</td>
<td>219</td>
<td>11</td>
<td>206 ± 175</td>
</tr>
<tr>
<td>Fumonisin</td>
<td>Corn silage</td>
<td>461</td>
<td>8</td>
<td>28 ± 19</td>
<td>778</td>
<td>66</td>
<td>1,091 ± 2,878</td>
<td>219</td>
<td>11</td>
<td>206 ± 175</td>
</tr>
<tr>
<td>ZEN &gt;70 ppb</td>
<td>Corn silage</td>
<td>1,617</td>
<td>7</td>
<td>245 ± 15</td>
<td>1,708</td>
<td>58</td>
<td>1,079 ± 4,080</td>
<td>1,694</td>
<td>18</td>
<td>445 ± 669</td>
</tr>
<tr>
<td>T-2 toxin &gt;50 ppb</td>
<td>All feeds</td>
<td>487</td>
<td>30</td>
<td>525 ± 799</td>
<td>717</td>
<td>7</td>
<td>1,504 ± 2,550</td>
<td>1,769</td>
<td>18</td>
<td>445 ± 669</td>
</tr>
</tbody>
</table>

n = number of samples. % = percentage of samples positive above given concentrations. mean of the positive samples plus and minus the standard deviation.

**September 16, 2009, Feedstuffs**
2. FDA action levels for total aflatoxins in food, feed*

<table>
<thead>
<tr>
<th>Food or feedstuff</th>
<th>Concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All products, except milk, designated for humans</td>
<td>20</td>
</tr>
<tr>
<td>Corn for immature animals and dairy cattle</td>
<td>20</td>
</tr>
<tr>
<td>Corn and peanut products for breeding beef cattle, swine and mature poultry</td>
<td>100</td>
</tr>
<tr>
<td>Corn and peanut products for finishing swine (&gt;200 lb.)</td>
<td>200</td>
</tr>
<tr>
<td>Corn and peanut products for finishing beef cattle</td>
<td>300</td>
</tr>
<tr>
<td>Cottonseed meal (as a feed ingredient)</td>
<td>300</td>
</tr>
<tr>
<td>All other feedstuffs</td>
<td>20</td>
</tr>
<tr>
<td>Milk</td>
<td>0.2</td>
</tr>
</tbody>
</table>


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). Toxicogenic *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 southwestern 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 preharvest 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 nontoxigenic *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. Nontoxigenic *Aspergillus* isolates are also available for use in peanuts (Horn et al., 2001) and corn (Dormer and Cole, 2002).

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 mycotoxocoses (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 (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.

Wolzak 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).

Depending on interactions with other factors, aflatoxin concentrations as low as 100 parts per billion 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 it 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 (Hellerich 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. (1985) 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. Ruminal alfalfa digestibility was depressed 50 and 67% with aflatoxin doses of 1 and 10 µg/ml, respectively (Westlake et al., 1989) but ruminal digestibility was not affected (Cheeke and Shull, 1985).

The mechanism by which aflatoxins reduce growth rate is related ZEA to an estrogenic response in ruminants, some effects (Weaver et al., 1986a). Several case reports have 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, some

Zearalenone

ZEA and zearalenol are estrogenic metabolites of several species of Fusarium. Chemically, ZEN is a resorcylic acid lactone. Fusarium 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 (Hagner 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. Swine appear to be most susceptible to ZEA (Diekmann and Green, 1992). In prepubertal gilts, swollen vulvae appear; this can progress to vaginal or rectal prolapse (Friend et al., 1990). Internally, enlarged, swollen, distorted uteri and shrunken ovaries are observed (Friend et al., 1990). Litter size may also 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). In rats, ZEA was fed at 1, 2, 4 and 8 mg/kg bodyweight, 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.

Boiler 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 has been shown to induce immunotoxicity in mice with decreases in lymphocytes, IgG, IgM, B cells, T-cell subsets (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).

ZEA is rapidly converted to alpha- and beta-zearalenol in rumen cultures (Kissling 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 (Hagner 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 (Kailela and Vasenius, 1982). A controlled study with nonlactating cows fed up to 500 mg 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, some.
Nutrition & Health  Dietary allowances for mycotoxins

times reporting abortions as a symptom (Kallala 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; Sprouse and Towers, 1995; Smith et al., 1995) later 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-zearalenol. 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. Cows also carry a coat. Several aspects of reproductive problems in dairy cattle were associated with ZEA-M concentrations estimated at 10 ppb.

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, which are commonly found in agricultural commodities (Desjardins 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 aspects of Fusarium and related genera produce trichothecenes.

DON (vomitoxin) is produced primarily by F. graminearum and F. culmorum (Rotter et al., 1996). Wet, rainy and humid weather at flowering promotes infection by Fusarium. The result is 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.

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, 2004). DON appears to affect serotonergic activity or serotonin receptors (Rotter et al., 1996). One part per million or more DON results in reduced食欲 and intake in swine, resulting in lower weight gains. Two independent midwestern field studies (Vesonder et al., 1978; Côté 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.

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 observed concentrations of mycotoxins.

Chickens and turkeys apparently are not very susceptible to the effects of DON. Leghorn chickens showed no effect on weight gain from dietary levels of DON at 18 ppb (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 pouls fed 75 ppb 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).

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). DON has been associated with reduced feed intake in nonlactating dairy cattle (Trenholm et al., 1985). There was a trend (F. graminearum) 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 0.27 and 6.5 ppm in treatment diets (Charmley et al., 1993). Danicke et al. (2005) demonstrated a reduction of approximately 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). 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 (1996) feeding containing cows diets containing 0, 3.6, 10.9 and 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.

The FDA had provided an advisory for DON in wheat and wheat-derived products (Table 3; Henry, 2006).

T-2 toxin is produced primarily by F. sporotrichioides and F. poae but is also produced by other species of Fusarium (Marasas et al., 1984). T-2 (and DAS) is often found in barley, wheat, millet, sorghum seed and in mixed feeds. The toxicity of T-2 toxin is best documented in laboratory animals (Wannemacher et al., 1991). Toxicity is a function of inhibition of protein synthesis, reduced immunity, 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 gain, low milk production, reproductive failure, diarrhea, gastrointestinal hemorrhage and increased incidence of disease. 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., 1980). Weaver et al. (1970) 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).

Fumonisins

Fumonisin B1 was first isolated in South Africa where Fusarium moniliforme — a species that is almost ubiquitous in corn and F. proliferatum are the main species producing high yields of fumonisins. Fumonisins 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, 2001), causes leucoencephalomalacia (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 and Diaz et al., 2000). Fumonisin inhibits ceramide synthesis, reduces the production of complex sphingolipids and results in accumulation of sphinganine, which has been used as a biomarker for fumonisin exposure (Norred et al., 1997; Marasas et al., 2004; Riley et al., 2001). The resulting effects are changes in cellular growth, differentiation, morphology, permeability and apoptosis.

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 corn-based feed with 37.12 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.

Fumonisins 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 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., 1995a,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

<table>
<thead>
<tr>
<th>Class of animal</th>
<th>Feed ingredients, portion of diet</th>
<th>DON levels (ppm) in: Grains, grain byproducts</th>
<th>Finished feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminants beef and feedlot cattle older than 4 months</td>
<td>Grain and grain byproducts not to exceed 50% of the diet</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Chickens</td>
<td>Grain and grain byproducts not to exceed 50% of the diet</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Swine</td>
<td>Grain and grain byproducts not to exceed 20% of the diet</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>All other animals</td>
<td>Grain and grain byproducts not to exceed 40% of the diet</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

3. FDA advisory levels for DON (vomitoxin) in livestock feed, (Henry, 2006)

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### 4. FDA guidance for industry on fumonisins levels in foods and animal feeds

<table>
<thead>
<tr>
<th>Human foods</th>
<th>Total fumonisins (FB1+FB2+FB3) Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degenerated dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of &lt; 2.25%, dry weight basis)</td>
<td>2</td>
</tr>
<tr>
<td>Whole or partially degenerated dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of &gt;2.25%, dry weight basis)</td>
<td>4</td>
</tr>
<tr>
<td>Dry milled corn bran</td>
<td>4</td>
</tr>
<tr>
<td>Cleaned corn intended for masa production</td>
<td>4</td>
</tr>
<tr>
<td>Cleaned corn intended for popcorn</td>
<td>3</td>
</tr>
<tr>
<td>Animal feeds</td>
<td>Feed Ingredients (ppm)</td>
</tr>
<tr>
<td>Corn and corn byproducts intended for:</td>
<td></td>
</tr>
<tr>
<td>Equids and rabbits (no more than 20% of diet)</td>
<td>5</td>
</tr>
<tr>
<td>Swine and cattle (no more than 50% of diet)</td>
<td>20</td>
</tr>
<tr>
<td>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)</td>
<td>30</td>
</tr>
<tr>
<td>Ruminants more than 3 months old being raised for slaughter and mink being raised for pelts production (no more than 50% of diet)</td>
<td>60</td>
</tr>
<tr>
<td>Poultry being raised for slaughter (no more than 50% of diet)</td>
<td>100</td>
</tr>
<tr>
<td>All other species or classes of livestock and pet animals (no more than 50% of diet)</td>
<td>10</td>
</tr>
</tbody>
</table>

*Limits on ingredients are on a dry weight basis.

**Ergot alkaloids**

One of the earliest recognized mycotoxicosis is ergotism caused by a group of ergot alkaloids. They are produced by several species of *Claviceps*, which 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 reduced weight gains, milk production and agalactia (Robbins et al., 1986). 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 toxins cause vasoconstriction resulting in elevated respiration rate, and gangrene that can result in loss of hooves, tails and ears. Cattle are intolerant 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.

**Other mycotoxins**

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 mycotoxic porcine nephropathy (Krogh, 1979). The primary toxic effect is inhibition of protein synthesis (Creppy et al., 1984) and is considered to be genotoxic (WHO, 2002). OTA can reduce weight gains and performance in swine (Cook et al., 1986) and poultry (Huff et al., 1988). Other symptoms include diarrhea, increased water consumption, diuresis and dehydration (Krogh et al., 1979). In cattle, OTA is rapidly degraded in the rumen and thus thought to be of little consequence unless consumed by young pre-ruminant calves (Seemannarayana et al., 1988); however, chronic exposure and acute toxicities are thought to occur in cattle. Moldy hay containing OTA has been implicated in cattle deaths and abortions. FDA provides no guidelines for OTA in feed (Henry, 2006).

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). Patulin is produced by *Penicillium*, *Aspergillus* and *Byssochlamys* (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 VFA 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;
Myco-phenolic Acid (MPA) is produced by a number of fungal fungal species, but is most commonly 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 suppression in organ transplant patients. Its toxicity to animals is 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 post-harvest as hay or silage (Lacey, 1991). Some mycotoxins have been reviewed (Lacey, 1991; Gottlieb, 1997; Schudamore and Livesay, 1998; Seglar, 1997; Whittow, 1997). 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, Mansfeld 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 Macaroneaeae, Mucor 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. 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 major concern. Mansfeld, Jones and Kuldau (2008) investigated the presence of four Penicillium-produced mycotoxins (roquefortine C, myco phenolic acid, 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.

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 CPA, DON, FB, PRT 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 lumi- tremorgens. Animal symptoms included generalized deterio-ration typical of protein deficiency, malnutrition, diarrhea, ir-ritability, 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 infection (Puntenney et al., 2003). Such mycoses occur in immunosuppressed ani- mals, and dairy cows are immune suppressed in early lacta- tion. When cellular immunity is an important mechanism for disease resistance, interactions with trichothecene myco- toxins may be an issue. Niyo et al. (1988a, b) showed that rabbits exposed to T-2 toxin had a decrease in phagocyto- sis of A. fumigatus conidia by alveolar macrophages and an increase in severity of experimental aspergillosis. Gliotoxin produced by A. fumuis has immunosuppressive, antibacterial and apoptotic effects. Gliotoxins is shown to affect rumen fer- mentation, reducing digestibility and VFA production in vitro (Morgavi et al., 2004).

Moldy alfalfa hay containing A. ochraceus was implicated as producing OTA associated with aboritions in cattle (Still et al., 1971). OTA in moldy forage has also been implicated in cattle deaths (Vough and Glick, 1950). Studies of grain and corn silage in Europe have found an occurrence of P. roqueforti in as many as 40% of samples (Auerbach, 2003). Penicillium produced mycotoxins in silages, such as roque- fortine C, myco phenolic acid, and PR toxins have been asso- ciated with herd health problems (Auerbach, 1998; Seglar et al., 1999; Sumarah et al., 2005). Seglar et al. (1999) suggested that PR toxin is a good marker for silages associated with dairy herds with cow health problems.

The most important pasture-induced toxicosis in the U.S. is tall- fescue toxicosis caused by endophytic alkaloids (Ba- con, 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 management

A quality assurance program for mycotoxin management has been reviewed by CAST (2003). The program is based on information from Romer Labs of Union, Mo. Elements include prevention, sampling, sample preparation, extraction, evaluation of testing requirements, testing, result validation, documentation, supplier involvement and removal.

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 (Whittaker, 2003). Because mycotoxins are not evenly distributed 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. Once collected, samples should be handled properly 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 accurate 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

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may be by thin-layer chromatography plates, high-performance liquid chromatography, gas-liquid chromatography, enzyme-linked immunosorbent assays, 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, ochratoxin, DON, ZEA, fumonisin and T-2 toxin. Minimum detection levels may limiting diagnosis because these levels are often directed at finding high levels that cause acute toxicity and serious animal disease rather than low levels associated with chronic effects such as production losses, impaired immunity and significant economic losses. 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 provides excellent background materials for the feed industry (at 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 (Truckssess and Pohland, 2000). Kriska et al. (2008) provided an update on mycotoxin analysis focusing on recent developments including multi-mycotoxin methods and quick tests.

**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. The implications of mycotoxins on agricultural trade has been reviewed (Dohlman, 2003). Van Egmond (2002) has reviewed the worldwide mycotoxin regulations. 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 nontransgenic corn, *Bacillus thuringiensis* (Bt)-transgenic corn had less corn borer damage, less *F. verticillioidei* infection and lower fumonisin contamination. Fungicides have shown little efficacy in controlling preharvest 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-toxigenic 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. Addition of 0.25 or 0.5% of calcium propionate to diets for detoxification may reduce the effects of aflatoxin (Bintvihok and Kositha-reenkul, 2006). A microbial detoxification method has been identified where a species of rumen bacterium (BBSH 797) was isolated that has the ability to biotransform trichothe-ncenes, rendering them nontoxic (Binder et al., 2000). While field studies have suggested that the microbial product used as a feed additive can protect growing pigs from the effects of DON, more controlled research using naturally contaminated feeds have produced imperfect results (Doll and Danicke, 2004). Ammoniation of grains can destroy some mycotoxins, but there is not a practical method to treat forages. Galvano et al. (2001) have reviewed dietary strategies to counteract mycotoxins. Increasing nutrients such as protein, energy and antioxidant nutrients may be advisable (Bruca et al., 1986; Galvano et al., 2001; 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 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 vivo 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). Fermentative bacteria may have potential of binding some mycotoxins (Niderkorn et al., 2007). A few reviews of mycotoxin binders are published (Avantaggiato et al., 2005; Bingham et al., 2005; Doll and Danicke, 2004; Hug et al., 2001; Ramos and Hernandez, 1997; Varga and Toth, 2005).

**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.