MYCOTOXINS

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Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity (D'Mello and Macdonald, 1998). The diverse effects precipitated by these compounds are conventionally considered under the generic term "mycotoxicosis", and include distinct syndromes as well as non-specific conditions. A list of the principal mycotoxins occurring in feeds and forages is given in Table 1, which also indicates the fungal species associated with the production of these contaminants. Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Contamination may also occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi. Moisture content and ambient temperature are key determinants of fungal colonization and mycotoxin production. It is conventional to subdivide toxigenic fungi into "field" (or plant-pathogenic) and "storage" (or saprophytic/spoilage) organisms. *Claviceps*, *Neotyphodium, Fusarium* and *Alternaria* are classical representatives of field fungi while *Aspergillus* and *Penicillium* exemplify storage organisms. Mycotoxigenic species may be further distinguished on the basis of geographical prevalence, reflecting specific environmental requirements for growth and secondary metabolism. Thus, *Aspergillus flavus*, *A. parasiticus* and *A. ochraceus* readily proliferate under warm, humid conditions, while *Penicillium expansum* and *P. verrucosum* are essentially temperate fungi. Consequently, the *Aspergillus* mycotoxins predominate in plant products emanating from the tropics and other warm regions, while the *Penicillium* mycotoxins occur widely in temperate foods, particularly cereal grains. *Fusarium* fungi are more ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereals from warm countries.

Table 1. Origin of principal mycotoxins occurring in common feeds and forages

Mycotoxins	**Fungal species**
Aflatoxins	*Aspergillus flavus; A. parasiticus*
Cyclopiazonic acid	*A. flavus*
Ochratoxin A	*A. ochraceus; Penicillium viridicatum; P. cyclopium*
Citrinin	*P. citrinum; P. expansum*
Patulin	*P. expansum*
Citreoviridin	*P. citreo-viride*
Deoxynivalenol	*Fusarium culmorum; F. graminearum*
T-2 toxin	*F. sporotrichioides; F. poae*
Diacetoxyscirpenol	*F. sporotrichioides; F. graminearum; F. poae*
Zearalenone	*F. culmorum; F. graminearum; F.*

	sporotrichioides
Fumonisins; moniliformin; fusaric acid	*F. moniliforme*
Tenuazonic acid; alternariol; alternariol methyl ether; altenuene	*Alternaria alternata*
Ergopeptine alkaloids	*Neotyphodium coenophialum*
Lolitrem alkaloids	*N. lolii*
Ergot alkaloids	*Claviceps purpurea*
Phomopsins	*Phomopsis leptostromiformis*
Sporidesmin A	*Pithomyces chartarum*

An emerging feature is the co-production of two or more mycotoxins by the same species of fungus (Table 1). This observation has enabled a fresh interpretation of the causes of well-known cases recorded in the history of mycotoxicoses.

Aflatoxins

This group includes aflatoxin B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2, respectively). In addition, aflatoxin M_1 (AFM_1) has been identified in the milk of dairy cows consuming AFB_1-contaminated feeds. The aflatoxigenic *Aspergilli* are generally regarded as storage fungi, proliferating under conditions of relatively high moisture/humidity and temperature. Aflatoxin contamination is, therefore, almost exclusively confined to tropical feeds such as oilseed by-products derived from groundnuts, cottonseed and palm kernel. Aflatoxin contamination of maize is also an important problem in warm humid regions where *A. flavus* may infect the crop prior to harvest and remain viable during storage.

Surveillance of animal feeds for aflatoxins is an ongoing issue, owing to their diverse forms of toxicity and also because of legislation in developed countries (D'Mello and Macdonald, 1998). In the United Kingdom, analysis conducted during the 1987-1990 period indicated that all imported feedstuffs complied with legislation in force for AFB_1 levels. Elsewhere, however, aflatoxin levels in certain feeds still pose serious risks to animal health. Thus, in India total aflatoxin levels of 3 700 g/kg were detected in a sample of groundnut cake. Of potentially greater significance is the contamination of maize samples in China and northern Viet Nam with combinations of AFB_1 and *Fusarium* mycotoxins. In China, 85 percent of maize samples were contaminated with both AFB_1 and fumonisin B_1 at levels ranging from 8 to 68 g/kg and 160 to 25 970 g/kg, respectively. Feed-grade maize in northern Viet Nam had AFB_1 levels ranging from 9 to 96 g/kg, and fumonisin B_1 levels in the range of 271 to 3 447 g/kg (Placinta, D'Mello and Macdonald, 1999). Between 1988 and 1989, analyses of farmgate milk in the United Kingdom showed low levels of AFM_1 contamination, but more than 50 percent of milk samples in the United Republic of Tanzania were found to contain the mycotoxin (D'Mello and Macdonald, 1998). The importance of aflatoxins in animal health emerged in 1960, following an incident in the United Kingdom in which 100 000 turkey poults died from acute necrosis of the liver and hyperplasia of the bile duct ("turkey X disease"), attributed to the consumption of groundnuts infected with *Aspergillus flavus*. This event marked a defining point in the history of mycotoxicoses, leading to the discovery of the aflatoxins. Subsequent studies showed that aflatoxins are acutely toxic to ducklings, but ruminants are more resistant. However, the major impetus arose from epidemiological evidence linking chronic aflatoxin exposure with the incidence of cancer in humans.

Ochratoxins

The *Aspergillus* genus includes a species (*A. ochraceus*) that produces ochratoxins, a property it shares with at least two *Penicillium* species. Ochratoxin A (OA) and ochratoxin B are two forms that occur naturally as contaminants, with OA being more ubiquitous, occurring predominantly in cereal grains and in the tissues of animals reared on contaminated feed. Another mycotoxin, citrinin, often co-occurs with ochratoxin. In recent Bulgarian wheat samples, OA and citrinin levels ranged from < 0.5 to 39 g/kg and from < 5 to 420 g/kg, respectively. In oats, higher levels of OA were detected (maximizing at 140 g/kg) while citrinin was below detection limits (D'Mello, 2001).

The ochratoxins and citrinin are nephrotoxic to a wide range of animal species. OA is frequently implicated in porcine nephropathy and in Balkan endemic nephropathy of humans. The role of citrinin in these syndromes has yet to be elucidated.

Fusarium mycotoxins

Extensive data now exist to indicate the global scale of contamination of cereal grains and animal feed with *Fusarium* mycotoxins (D'Mello and Macdonald, 1998). Of particular importance are the trichothecenes, zearalenone (ZEN) and the fumonisins. The trichothecenes are subdivided into four basic groups, with types A and B being the most important. Type A trichothecenes include T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS). Type B trichothecenes include deoxynivalenol (DON, also known as vomitoxin), nivalenol and fusarenon-X. The production of the two types of trichothecenes is characteristic for a particular *Fusarium* species. However, a common feature of the secondary metabolism of these fungi is their ability to synthesize ZEN which, consequently, occurs as a co-contaminant with certain trichothecenes. The fumonisins are synthesized by another distinct group of *Fusarium* species (Table 1). Three members of this group (fumonisins B_1, B_2 and B_3) often occur together in maize.

Virtually all the toxigenic species of *Fusarium* listed in Table 1 are also major pathogens of cereal plants, causing diseases such as head blight in wheat and barley and ear rot in maize. Harvested grain from diseased crops is therefore likely to be contaminated with the appropriate mycotoxins, and this is supported by ample evidence. Surveillance of grain and animal feed for the occurrence of *Fusarium* mycotoxins has been the subject of many investigations over recent years (Tables 2 and 3). The global distribution of these mycotoxins is a salient feature, but striking regional differences should also be noted. Another aspect worthy of comment is consistent evidence of the co-occurrence of various *Fusarium* mycotoxins in the same sample. These issues have been considered at greater length by Placinta, D'Mello and Macdonald (1999) who, for example, referred to a German study in which 94 percent of wheat samples analysed were contaminated by between two and six *Fusarium* mycotoxins and 20 percent of the samples were co-contaminated with DON and ZEN (Table 2). The most frequent combination included DON, 3-ADON and ZEN. T-2 and HT-2 toxins were detected at levels ranging from 0.003 to 0.250 mg/kg and 0.003 to 0.020 mg/kg, respectively, but these mycotoxins only occurred in combination with DON, NIV and ZEN.

Table 2 . Global distribution of deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) in cereal grains and animal feed (mg/kg)				
Country	**Cereal/feed type**	**DON**	**NIV**	**ZEN**
Germany	Wheat	0.004-20.5	0.003-0.032	0.001-8.04
Poland	Wheat	2.0-40.0	0.01	0.01-2.0
	Maize kernels	4.0-320.0		
	Maize cobs: axial stems	9.0-927.0		
Finland	Feeds and grains	0.007-0.3		0.022-0.095
	Oats	1.3-2.6		
Norway	Wheat	0.45-4.3	max 0.054	
	Barley	2.2-13.33	max 0.77	
	Oats	7.2-62.05	max 0.67	
Netherlands	Wheat	0.020-0.231	0.007-0.203	0.002-0.174
	Barley	0.004-0.152	0.030-0.145	0.004-0.009
	Oats	0.056-0.147	0.017-0.039	0.016-0.029
	Rye	0.008-0.384	0.010-0.034	0.011
South Africa	Cereals/animal feed		0.05-8.0	
Philippines	Maize		0.018-0.102	0.059-0.505
Thailand	Maize			0.923
Korea, Republic	Barley	0.005-0.361	0.005-0.361	
	Maize	mean 0.145	mean 0.168	
Viet Nam	Maize powder	1.53-6.51	0.78-1.95	
China	Maize	0.49-3.10	0.6	
Japan	Wheat	0.03-1.28	0.04-1.22	0.002-0.025
	Barley			0.010-0.658
	Wheat	0.029-11.7	0.01-4.4	0.053-0.51
	Barley	61.0-71.0	14.0-26.0	11.0-15.0
New Zealand	Maize	max 3.4-8.5	max 4.4-7.0	max 2.7-10.5
USA	Wheat	up to 9.3		
	Wheat (winter), 1991	< 0.1-4.9		
	Wheat (spring), 1991	< 0.1-0.9		

	Wheat, 1993	< 0.5-18.0		
	Barley, 1993	< 0.5-26.0		
Canada	Wheat (hard)	0.01-10.5		
	Wheat (soft, winter)	0.01-5.67		
	Wheat (soft, spring)	0.01-1.51		
	Maize	0.02-4.09		
	Animal feeds	0.013-0.2	0.065-0.311	
Argentina	Wheat	0.10-9.25		
Source: **Adapted from Placinta, D'Mello and Macdonald, 1999.**				

Table 3. Worldwide contamination of maize and animal feeds with fumonisins (g/kg)

Country	FB1	FB2	FB3	Total
Maize				
Benin	nd[1]-2 630	nd-680		nd-3 310
Botswana	35-255	nd-75	nd-30	35-305
Mozambique	240-295	75-110	25-50	340-395
South Africa	60-70	nd	nd	60-70
South Africa	max 2 000			
Malawi	nd-115	nd-30	nd	nd-135
Zambia	20-1 420	nd-290		20-1 710
Zimbabwe	55-1 910	nd-620	nd-205	55-2 735
Tanzania, United Republic	nd-160	nd-60	nd	nd-225
Honduras	68-6 555			
Argentina	85-8 791	nd-11 300	nd-3 537	85-16 760
Uruguay	nd-3 688			
Costa Rica	1 700-4 780			
Italy	10-2 330	nd-520		10-2 850
Portugal	90-3 370	nd-1 080		90-4 450
Viet Nam	268-1 516	155-401	101-268	524-2 185
China	160-25 970	160-6 770	110-4 130	430-36 870
Philippines	57-1 820	58-1 210		
Thailand	63-18 800	50-1 400		
Indonesia	226-1 780	231-556		
Animal feed				
South Africa	4 000-11 000			
Uruguay	256-6 342			
India	20-260			

[1] **nd = not detectable.**
Source: **Adapted from Placinta, D'Mello and Macdonald, 1999.**

In the Lublin region of southeastern Poland, type A trichothecene contamination of barley grain was linked with the natural incidence of fusarium head blight, in which the predominating organism was *F. sporotrichioides* (Placinta, D'Mello and Macdonald, 1999). Of 24 barley grain samples, 50 percent were positive for T-2 toxin, with a range of 0.02 to 2.4 mg/kg. In five of these samples, co-contamination with HT-2 toxin occurred, with a range of 0.01 to 0.37 mg/kg. Maize ears may also become naturally infected with *Fusarium* pathogens. The findings of one study in Poland indicated that infection with *F. graminearum* can result in contamination of cobs with DON (Table 2) and 15-ADON simultaneously (Placinta, D'Mello and Macdonald, 1999). Concentrations of DON and 15-ADON in *Fusarium*-damaged kernels ranged from 4 to 320 mg/kg and 3 to 86 mg/kg, respectively, but the axial stems of the cobs were more heavily contaminated, at 9 to 927 mg/kg (Table 2) and 6 to 606 mg/kg, respectively. Oat grains produced in Norway by commercial growers were found to be more heavily contaminated with DON than barley or wheat kernels (Table 2). In addition to NIV (Table 2), other contaminants included 3-ADON and fusarenon-X. For example, 56 percent of certain oat samples contained detectable quantities of 3-ADON at 0.03 mg/kg or more. Other notable examples of DON contamination include wheat and barley samples from Japan and the United States (Table 2). It should be stated, however, that even in samples with lower levels of contamination, high incidence rates have been recorded. Thus, 90 and 79 percent of cereal samples in the Netherlands were positive for DON and NIV respectively (Placinta, D'Mello and Macdonald, 1999).

Widespread contamination of maize and animal feed with fumonisins has recently been reported (Table 3). In most instances the predominant fumonisin was FB_1. The highest values for FB_1 were recorded from maize samples in China, where AFB_1 co-occurred in 85 percent of samples, and in Thailand. Multiple contamination of maize with fumonisins, DON, NIV and AFB_1 was also observed in northern Viet Nam. For FB_2, the highest values in maize were found in samples from Argentina. In the Philippines, Thailand and Indonesia, FB_1 and FB_2 occurred in more than 50 percent of maize samples, and these mycotoxins co-occurred with aflatoxins in 48 percent of samples (Placinta, D'Mello and Macdonald, 1999).

The *Fusarium* mycotoxins induce a wide range of effects in farm livestock (D'Mello, 2000). DON is a potent feed intake inhibitor in pigs; ZEN is associated with reproductive abnormalities in pigs and ruminants. Fumonisins have been linked with specific syndromes, namely porcine pulmonary oedema and equine leukoencephalomalacia. Fumonisin contamination of maize in South Africa has been correlated with the occurrence of oesophageal cancer in humans.

Endophyte alkaloids

The endophytic fungus *Neotyphodium coenophialum* occurs in close association with perennial tall fescue, while another related fungus, *N. lolii*, may be present in perennial ryegrass (D'Mello, 2000). Ergopeptine alkaloids, mainly ergovaline, occur in *N. coenophialum*-infected tall fescue, while the indole isoprenoid lolitrem alkaloids, particularly lolitrem B, are found in *N. lolii*-infected perennial ryegrass. The ergopeptine alkaloids reduce growth, reproductive performance and milk production in cattle, while the lolitrem compounds induce neurological effects in ruminants.

Phomopsins

In Australia, lupin stubble is valued as fodder for sheep, but infection with the fungus *Phomopsis leptostromiformis* is a major limiting factor because of toxicity arising from the production of phomopsins by the fungus. Mature or senescing parts of the plant, including stems, pods and seeds, are particularly prone to infection. Phomopsin A is considered to be the primary toxin, causing effects such as ill-thrift, liver damage, photosensitization and reduced reproductive performance in sheep (D'Mello and Macdonald, 1998).

Sporidesmin

Pithomyces chartarum is a ubiquitous saprophyte of pastures and has the capacity to synthesize sporidesmin A, a compound causing facial eczema and liver damage in sheep.