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Occurrence and Significance of Mycotoxins in Forage Crops and Silage: a Review

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Abstract: Study of mycotoxins in animal feeding stuffs has concentrated on the occurrence of aflatoxins and, to a lesser extent, other mycotoxins in cereals, raw materials and concentrate feeds. However, ruminant diets contain a high proportion of forage crops such as grass or maize silage, hay and straw. Under adverse growing, production or storage conditions, fungal spoilage is likely to occur with some degree of mycotoxin contamination. The mould flora of forage crops is likely to differ significantly from that of cereals and mycotoxin contamination, should it occur, could differ qualitatively and quantitatively. Information relating to forage crops as a potential source of mycotoxins is reviewed. Some field incidents and animal disease which may be mycotoxin related are discussed and analytical methods are reviewed. Information on dose and effect of candidate mycotoxins is given where available. The review suggests areas which the authors consider merit further study. Crown Copyright 1998.

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INTRODUCTION

During growth, forage crops are at risk in the field from infection by a number of different fungi, some of which may produce mycotoxins. These fungi include species of *Fusarium*, *Alternaria*, *Cladosporium*, *Claviceps* and endophytic infections of grass. Consequently, forage crops at harvest or when directly grazed could contain a number of potential toxins. Good quality silage is prepared from grass, maize or other crops and by-products under anaerobic conditions and, if oxygen is successfully excluded, further mould growth and mycotoxin development is unlikely. These conditions also tend to reduce levels of some mycotoxins should they already be present at ensilement. If air subsequently gains access, silage will be at risk from storage moulds such as *Penicillium* and *Aspergillus*. However, moulds may be aerobic or anaerobic and this means that, even if oxygen is excluded, some moulds may be able to develop and metabolism proceed.

Stored hay and straw, if not dried effectively, could give rise to similar or additional moulds and toxins. Surveillance for mycotoxins in cereals and animal feeds for instance (Scudamore *et al* 1986a, 1997) has shown that, where mycotoxins are identified, mixtures of these toxins can also occur. Since the use of silage and similar feeding stuffs is increasing, it is important to assess whether mycotoxin contamination is occurring and, if so, whether it is a potential threat to human or animal safety.

Silage making in the UK, for example, has developed into the biggest preservation operation carried out on farms. Approximately 40 million tonnes of crops are harvested, transported and stored for later use each

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year and in 1990, about 90% of the total silage was prepared from grass, with maize, legumes, whole-crop cereals and by-products such as brewers grains playing only a minor role (Wilkinson 1990). Maize is also used widely in Europe. Silos may be stacks, bunkers or pits fitted with roofs to prevent rain damage. Big bale silage is increasing in popularity. There is a huge choice of additives available including 45% sulphuric acid, formic and propionic acids, enzymes and cultures of lactic acid bacteria.

When crops are harvested and stored, most of the toxic compounds present will remain stable under aerobic conditions. However, the field-derived fungi will, in time, be replaced by storage fungi, particularly with inadequate drying or if the moisture content is not maintained below about 15%.

The range of materials likely to be sources of mycotoxins include pasture grass grazed directly, dried grass, husks or chaff used as components of compound feeds, by-products such as sugar pulp, brewers grains and distillers grains, hay and straw (both barley and wheat), grass (which may also contain leguminous plants such as clovers) and maize silage subject to spoiling following access of oxygen (Anon 1995). Mycotoxins would not be expected in properly prepared and maintained silage although some 'field' mycotoxins such as zearalenone have been reported to survive ensilage.

Considerable research has been carried out on the acute and chronic toxicity of some single pure mycotoxins to a range of laboratory and domestic animal species, but sub-clinical toxicological effects (such as immunosuppression) and possible synergistic influence of mycotoxin mixtures has received little attention.

There have been few cases in which a mycotoxicosis has been proven as the cause of disease in livestock. However, this does not preclude the existence of mycotoxin-associated animal disease problems. In addition, mycotoxins at sub-clinical levels in animal feeds might be translocated to human food through meat and animal products (eg Allcroft 1969; Hult *et al* 1980). Forage crops and silage have received little attention. In a major review commissioned by the EC (Smith *et al* 1994), less than two pages included mention of forage crops or silage.

This paper reviews existing information on mould spoilage of forage crops and possible mycotoxin problems.

FORAGE CROPS AND SILAGE AS SOURCES OF FUNGI AND POTENTIAL MYCOTOXIN CONTAMINATION

Infection in the field

Animal fodder and silage are prepared from harvested growing crops such as grass and cereals. These crops are susceptible to infection in the field by a range of fungi and some may also produce toxic metabolites should appropriate conditions arise. This can occur in conditions ranging from cool, damp weather, to drought when crops become stressed. A number of moulds are also plant pathogens and cause disease in the crop.

Grass is grazed directly by ruminants and several mycotoxicoses are known, or suspected to arise, as a result of moulds or fungal endophytes present in the grass (eg Lewis and Clements 1986). Any such moulds or toxic metabolites will be present by the beginning of the silage or hay making processes.

Cereals such as wheat, barley and maize may be similarly infected with *Fusarium*, *Alternarium*, *Cladosporium* and other fungi (Niles 1980). The formation of mycotoxins in cereal grains has been studied extensively and is not considered further. The production of maize silage entails incorporation of the whole plant, seed and green matter. Therefore, the extent and type of infection affecting all parts of the maize plant, rather than solely in the grain, must be considered. Whole barley and wheat plants are sometimes used in the same way. Straws arising from harvested barley or wheat contain few seeds although hay will contain grass seeds themselves subject to fungal infection.

Fungal growth during ensilation

Silage making is based on the principle of preservation under anaerobic conditions together with the growth of lactic acid bacteria which promote a natural fermentation, lowering the pH to a level at which clostridial growth is prohibited. The conditions associated with well preserved silage, ie low pH and anaerobiosis, are also unfavourable for the growth of most moulds. Exclusion of air should ensure that few fungi are able to grow or survive under these conditions. Indeed, their presence in silage is unwelcome since they not only break down sugars and lactic acid, but also metabolise cellulose and other cell wall components and may also produce mycotoxins. The acidic anaerobic fermentation of good silage may also metabolise some of the mycotoxins already present at harvest. Damaglou *et al* (1984) inoculated rye grass with *Fusarium roseum* and *F tricinctum* prior to ensiling in laboratory experiments. Although *F tricinctum* survived longer than *F roseum*, there was only 1% remaining after 15 days. Pre-formed zearalenone only remained stable for a very short time in grass or after ensiling (Hacking 1979). However, silage made from corn cob maize infected with *F culmorum* and containing naturally produced zearalenone was studied over a 12 week period. By 11 days, no *F culmorum* could be isolated but zearalenone levels remained unchanged for the whole 12 week period (Lepom *et al* 1988).

Fungal development during storage of hay and straw

Fungi present at harvest in hay and straw are likely to persist during storage even if further fungal growth is prevented by drying. If the crops become damp and fungal growth occurs, field species of fungi are likely to be gradually replaced by storage fungi such as species of *Penicillium* and *Aspergillus*. The rate at which this happens depends on the conditions of storage. Poor storage conditions, caused by insufficient drying, condensation, leakage of rain water or insect infestation, can lead to further mould growth and heating. Livestock may then be faced with the dual hazard of mycotoxins and high levels of fungal spores in the atmosphere. Farm workers are also at risk from mould spores disturbed through handling of hay and straw.

THE PRESENCE OF MYCOTOXIGENIC FUNGI IN FORAGE CROPS AND SILAGE

Grass

Pasture swards may be of single or mixed species. Commercially used species include Italian rye grass (*Lolium multiflorum*), perennial ryegrass (*L perenne*), cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*) and fescue grasses (*Festuca* spp). It also is common practice to mix species of grass with legumes such as clovers (*Trifolium* spp).

Some of the most extensive studies of the fungi and their mycotoxins in pastures have been carried out in New Zealand where the production of grazing animals is a major industry and pasture related problems occur on a regular basis (New Zealand Pastoral Agriculture Research Institute 1995). Towers (1993a) discussed the European situation vis à vis that prevailing in New Zealand. He concluded that a similar combination of factors such as temperature, humidity and fungus etc. can occur in parts of Western Europe. The mould *Pithomyces chartarum*, which is implicated in facial eczema, is distributed throughout the world and has been reported from UK (Gregory and Lacey 1964) and other European countries. Endophyte-infected (*Acremonium lolii*) ryegrass has also been reported (Lewis and Clements 1986) and symptoms of a condition similar to ryegrass staggers is reported from time to time (Clegg and Watson 1960; Davies and Farmer 1961). *Fusarium* species occur world-wide and are commonly present in grasses.

Mycotoxin-producing *Fusarium* species such as *F graminearum* and *F culmorum* are common moulds in New Zealand grass (Di Menna and Parle 1970) and these moulds often occur in soil worldwide. They also infect cereals (Osborne *et al* 1988) and other growing crops and are likely to be found in grass.

Sclerotia (ergot bodies) are commonly produced by several species of *Claviceps* and may be found in pasture grasses and cereals. Langdon (1954) listed at least 30 different species of *Claviceps*. Fortunately, ergotism in man is no longer prevalent but remains a potential risk for animals. Ergots do occur but the dose of the toxic compounds associated with them is usually at a subclinical level (Osborne *et al* 1988).

Silage

During a survey of moulds infecting stored maize silage in France and Italy, Pelhate (1977) achieved a non-exhaustive count of 70 fungal species. He drew particular attention to the difficulty of quantifying the species, number and prevalence, when most species cultured *in situ* by partial aerobic techniques do not sporulate. This factor makes identification of the full range of species difficult and may not have been considered in earlier surveys such as those carried out by Bonner and Fergus (1959), Burmeister *et al* (1965) and Le Bars and Escoula (1973) and could explain why a less extensive range of moulds was found.

A few anaerobic moulds and yeasts are able to develop steadily in silage. Most *Fusarium* spp associated with maize and grass in the field, are aerobic and are unable to grow in silage. In contrast, *Geotrichum candidus* was found to occur commonly (Pelhate 1974). This fungus has been suspected as causing mycoses (Morquer *et al* 1955) although no toxic metabolites have been identified. It gives off a rancid odour which tends to repel animals and may thus prevent them feeding on the spoiled product. *Aspergillus fumigatus* is commonly reported in or on silage and is often associated with spoilage and heating. This mould can produce a number of toxic metabolites such as clavine alkaloids and tremorgenic compounds which may diffuse into the silage (Cole 1976; Cole *et al* 1977). Other species noted by Pelhate were *Monascus purpureus* responsible for a red coloration in silage, several *Mucor* spp, *Penicillium roqueforti*, *Trichoderma* spp, *Byssochlamis nivea* and *Paecilomyces varotia*.

Penicillium roqueforti was the predominant fungus found during a study of spoiled maize silage carried out in the Netherlands during 1986–1990 (Nout *et al* 1993). No PR-toxin, a metabolite often reported for this fungus, was detected although lumps of infected silage contained several unidentified fluorescent fungal metabolites.

The numbers of fungi and the proportion of *Alternaria* species in silage maize before harvesting and in stored hay were determined by Muller (1991). *Alternaria* accounted for 18% of total fungi present in growing maize and occurred mainly on leaves and husks. *Alternaria* species were also still found on hay stored for 8 months.

Hay and straw

In the field, grass contains a range of microorganisms. Cool, wet weather during cropping and field drying may result in further extensive growth of fungi such as *Fusarium*, *Alternaria* and *Cladosporium* (Christensen and Kaufmann 1965). After harvest these field fungi gradually die or are outgrown by storage moulds. Thus, hay and straw will be colonised by storage fungi if not sufficiently dry. Although *Fusarium* spp colonise grass and straw prior to harvest, they do not appear to be a major contaminant in store (Burmeister *et al* 1972). *Aspergillus* and *Penicillium* species are the major fungal infections likely to develop during storage although the particular species found will depend on the temperature and water activity. Lacey (1975) found, in hay samples, that 75% contained *A fumigatus*, 61% *A nidulans*, 37% *A versicolor* and 80% members of the *A glaucus* group. Storage fungi of the genus *Aspergillus* can grow over a wide range of water contents in feeds and mould counts can therefore serve as biological indicators of the storage conditions of hay (Lacey 1980). *Aspergillus flavus* does not appear to be a frequent mould in hay. In a study on storage of hay carried out in Sweden, Kaspersson *et al* (1984), found *Fusarium* and *Cladosporium* dominant together with *Alternaria*, *Acremoniella*, and a range of other mould species in the days immediately after harvest. These were soon displaced by *Aspergillus flavus*, *A glaucus*, *A candidus*, *A niger* and *Penicillium* spp. Some fungi such as Mucoraceae, *Alternaria* and *Acremoniella* were detected throughout the 92 days storage. In that work no *A fumigatus* was found.

Straw and hay may also be colonised by *Stachybotrys atra*, a mould that attacks cellulose and whose growth is favoured by damp conditions (Forgacs 1965). It can cause the disease stachybotryotoxicosis particularly in horses, although this has also been reported in calves (Ghergariu *et al* 1990), cattle (Bordas *et al* 1987), pigs (Danov and Baichev 1987), sheep and poultry (Forgacs 1965; Ivanics *et al* 1990). In France (Le Bars and Le Bars 1991), 15% of the strains of *S atra* were found to be highly toxigenic. Le Bars and Le Bars (1993) have suggested that straw may become mouldy due to upward percolation of moisture from damp ground, roof leaks, harvesting straw wetted with dew, water condensing under plastic covered bales or penetration of water into poorly compressed bales during storms.

Wallemia sebi is a xerophilic mould which has been shown to occur widely on food products as varied as pulses (Christensen 1987), cereals, dried fruit (Pitt and Christian 1968) and dried fish (Johan-Olsen 1887). It requires a highly selective medium to enable it to compete against other fungi and is thus frequently overlooked. Some isolates have been shown to be very toxic (Moss 1993). In the author's laboratory (Scudamore K A unpublished), mycological examination of hay from an incident which involved the death of ponies showed the predominant fungal species isolated to be *W sebi*, although no proven link with the deaths was established.

OCCURRENCE OF MYCOTOXINS AND POTENTIALLY IMPORTANT MYCOTOXINS, DOSE AND EFFECT OF SELECTED TOXINS

Some of the effects caused by mycotoxins are shown in Table 1. There are few references in the literature to metabolic or pharmacokinetic studies, but those that have been published are related to the 'well-studied' toxins.

The main fungi considered to be potential producers of mycotoxins in silages and forage crops are given in Table 2. Each mycotoxin listed has been reported as a metabolite of the associated fungus but has not necessarily been identified in silage or forage.

Aflatoxins

Four related aflatoxins may occur in animal feeding stuffs: B_1, B_2, G_1 and G_2. Aflatoxin B_1 is one of the most potent hepatocarcinogens known to man (Fishbein 1979). In cattle and other ruminants, aflatoxin B_1 is metabolised and its derivative, aflatoxin M_1, is excreted in milk. However, surveillance for aflatoxin in silage and forages has rarely been reported. In the UK, results of surveillance during 1977 (MAFF 1978) and 1981–1983 (MAFF 1984), found no aflatoxin in the 358 samples examined even though some of these samples were associated with known problems. Results for mould counts imply that the majority of the feeds, which included silage, had undergone significant fungal deterioration.

To ensure that aflatoxin B_1 levels are kept to a minimum, it is vital to identify all possible sources in animal feeding stuffs. The acidic conditions in silage are reported as unfavourable for growth of *Aspergillus flavus*, and hence for the development of aflatoxin (Gregory *et al* 1963; Lacey 1975; Clevström and Ljunngren 1984). It would thus seem unlikely that aflatoxin would be a significant contaminant. However, high concentrations are reported from time to time, such as in Mexico (Rosiles 1978) and the USA (Haggblom *et al* 1979). In addition, instances have been reported (Clevström *et al* 1981) where preservatives such as formic acid or other organic acid, added to hay or silage containing a high percentage of water, have produced levels of aflatoxins up to 1 mg kg^{-1}. Two further cases in which this occurred were barley (Hacking and Biggs 1979) and oats in Sweden (Pettersson *et al* 1978).

Aflatoxin formed in maize prior to ensilage has been shown to break down slowly in stored silage (Kalac and Woodford 1982). Aflatoxin in maize grown for silage would most likely occur in hot dry weather leading to

TABLE 1
Summary of some toxic effects of selected mycotoxins

Mycotoxin	*Effect*
Aflatoxin B_1	Carcinogenic, hepatotoxic, DNA damage
Aspergillus fumugitus toxins	Tremorgenic, haemorrhagic, many others
Sterigmatocystin	Carcinogenic, liver damage
Ochratoxin A	Carcinogenic, nephrotoxic, teratogenic, immunosuppresent
Citrinin	Nephrotoxic
Xanthomegnin and viomellein	Photosensitisation, liver and kidney damage?
Zearalenone	Oestrogenic
Patulin	Carcinogenic?, pulmonary oedema, haemorrhaging
Ergot alkaloids	Gangrene, reduced fertility
Trichothecenes	Haemorrhaging, diarrhoea, dermatitis, food refusal
Satratoxins	Necrosis, scouring, rhinitis
Penitrem A	Tremorgenic
PR toxin	Liver and kidney damage, mutagenic?
Gliotoxin	Hematuria, anti-varal immunosuppressant?
Aspergillus fumigatus toxins	Haemorrhaging, diarrhoea, tremorgenic
Tenuazonic acid	Haemorrhaging, convulsions, anorexia
Alternariol, alternariol monomethyl ether	Cytotoxic, faetotoxic
Sporidesmin	Photosensitisation, liver disease
Lolitrem B	Staggers

drought stress, seed damage and subsequent mould contamination.

There is a large accumulation of literature on the toxic effects caused by aflatoxins which are well known and will not be reviewed here.

Field derived mycotoxins

In a study carried out in Germany, a total of 87 strains of *Alternaria* isolated from maize and hay were examined to determine their ability to produce tenuazonic

TABLE 2
Moulds of silage and forage crops and potential mycotoxins

Fungal species	*Mycotoxins*
Acremonium lolii	Lolitrem B, paxilline
Alternaria spp	Tenuazonic acid, alternariol, alternatiol methyl ether, altenuene, iso-altenuene, altertoxins I and II
Aspergillus clavatus	*A clavatus* toxins
Aspergillus flavus	Aflatoxins
Aspergillus fumigatus	Fumigaclavine A, fumigaclavine C, fumitoxins A, B, C and D, gliotoxin, helvolic acid
Aspergillus versicolor	Sterigmatocystin
Byssochlamis nivea	Byssochlamic acid, patulin
Claviceps spp	Ergotamine, ergostine, ergocrystine, ergocryptine ergocornine, ergopeptide pyrrolizidine and lysergic acid derivatives
Fusarium spp	Deoxynivalenol, nivalenol, HT-2 toxin, moniliformin, T-2 toxin, other trichothecene compounds, zearalenone, moniliformin, fumonisins? (maize silage)
Paecilomyces varotia	Patulin
Penicillium aurantiogriseum group	Verrucosidin, viomellein vioxanthin, xanthomegnin
Penicillium roqueforti	Festuclavine, fumiclavine C, roquefortines A, B, C and D, PR toxin
Penicillium verrucosum	Ochratoxin A, citrinin
Penicillium viridicatum	Viomellein vioxanthin, xanthomegnin
Pithomyces chartarum	Sporodesmin
Stachybotris atra	Satratoxins
Wallemia sebi	Walleminol A

acid, alternariol, alternariol monomethyl ether and altenuene (Muller 1992). All strains produced tenuazonic acid and alternariol monomethyl ether, while 77% produced alternariol. *Alternaria alternata* and *A tenuissima* produced the largest amounts of toxins. The author did not report whether any of these mycotoxins were present in the silage.

Sporidesmin is produced in pasture grasses by the mould *Pithomyces chartarum*, and is responsible for cholestatic liver disease and secondary photo sensitisation which causes the condition known as facial eczema in sheep and cattle and other ruminants especially under conditions of high humidity and warmth.

Lolitrem B and paxilline are produced by *Acremonium lolii* which grows within the ryegrass plant. Evidence suggests that lolitrem B can occur in Europe (Lewis and Clements 1986) although quantitative assessment has not been carried out.

The sclerotia of *Claviceps* spp have been reported to produce more than 100 diverse biologically active compounds (Lorenz 1979). These include ergotamine, ergopeptide pyrrolizidine and lysergic acid derivatives. The principal ergot alkaloids of *C purpurea* are ergotamine, ergostine, ergocrystine, ergocryptine and ergocornine. Sclerotia of *C purpurea* may or may not contain alkaloids and, when present, the proportion of the various alkaloids may differ considerably (Mantle and Shaw 1977).

Mycotoxigenic *Fusarium* moulds capable of producing mycotoxins are widespread and are ubiquitous in soil, decaying vegetation and grass, often infecting growing crops especially during cool, damp conditions (eg Parry 1990). Some common species produce zearalenone, and trichothecenes such as deoxynivalenol and nivalenol. There is little information on their occurrence in standing crops or in grass but, in New Zealand, zearalenone in pasture is a recognised cause of infertility in sheep (Towers and Sprosen 1993). Similar *Fusarium* species occur widely and there is no reason why the same problems should not occur elsewhere. The presence of zearalenone in whole plants and parts of maize used for silage making has been investigated (Oldenberg 1993). Zearalenone was detected at concentrations up to 300 μg kg^{-1} and mainly accumulated at the end of the ripening process, with subsequent contamination of the silage. The highest concentrations were observed in the leaves, especially at the base of the plant. These mycotoxins tend to be unstable in grass and maize silage although zearalenone has been shown to survive ensilage (Lepom *et al* 1988). They are likely to be much more persistent in grass or plant material used to produce straw or hay.

The ability of zearalenone to cause hyperestrogenism, particularly in swine, has been known for many years. Two comprehensive reviews were published by Mirocha *et al* (1971) and Mirocha and Christensen (1974). Several closely related metabolites of zearalenone produced by *Fusarium* spp also possess similar properties, although few have been proven to occur naturally. Gareis (1993) reported that swine fed a diet containing 50 mg kg^{-1} of pure zearalenone suffered abortion and stillbirths, while levels above 10 mg kg^{-1} reduced the litter size and reduced the weight of piglets. Trial feeding of sows demonstrated that a concentration of 0·25 mg kg^{-1} or less, produced distinct redness and swelling of the vulva, slight swelling of the mammae with numerous vesicular follicles and some cystic follicles on the ovaria (Bauer *et al* 1987). These effects could be produced by lower levels of zearalenone in swine fed naturally contaminated feed. Although swine are the most sensitive domestic animal to zearalenone, calves show earlier sexual maturity, dairy cows have vaginitis, prolonged oestrus and infertility (Palti 1978) and sheep become sterile (Towers and Sprosen 1993). The effective dose for sheep may be approximately 1 mg kg^{-1}.

Oestrogenic activity has been found in feed and pastures, which may be due to components of clover, interactions of fungi with clover constituents or *Fusarium* metabolites (Drane and Saba 1978). Oestrogenic activity was demonstrated in mouldy feeds but the causative agent was not identified.

Effects of stachybotryotoxin/satratoxins G and H are usually seen in horses. However, Forgacs and Carll (1962) reviewed work carried out in small animals including dogs. Symptoms were anorexia, depression and death in 4–6 days for dogs fed a single dose of 1 ml of unpurified extract prepared from fungal cultures of *S atra*. Daily use of 0·25 ml of the extract resulted in development of leukocytosis in 10–20 days, and leukopenia and thrombocytopenia by 45 days. The dogs became paralysed and died by day 45.

In very mild toxicosis of horses, symptoms are limited to hyperesthesia or decreased performance in race horses, eg refusal to jump. In this situation, a few animals present rhinitis, conjunctivitis and a mild desquamation on the lips as a result of contact with trichothecenes (Servantie *et al* 1985). In more serious cases, necrosis of the oral mucosa appear. The blood coagulation time increases and sometimes bleeding occurs from small injuries. In severe cases, there is haemorrhaging in many tissues, particularly the lungs, muscles and into the peritoneal cavity. More than eight hundred donkeys, mules and horses died in an outbreak in Morocco (Le Bars and Le Bars 1993), most of them exhibiting such gross pathology. Lastly, in hyperacute cases, animals can die within approximately 15 h of ingestion through heart failure (Le Bars *et al* 1977), but without obvious clinical symptoms. Cardiac arrest in systole was reported.

Storage derived mycotoxins

The occurrence of ochratoxin in stored grain and other products has been widely reported (eg MAFF 1993;

Jiao *et al* 1994; Jorgensen *et al* 1996). Ochratoxin A is produced in temperate climates almost exclusively by *Penicillium verrucosum* (Frisvad 1989). This fungus also produces citrinin and both mycotoxins are found together, although citrinin is not often sought because of difficulties with its analysis. Ochratoxin A damages mainly the proximal tubules of the kidneys. Extra-renal effects are observed only after exposure to high levels, and include enteritis, necrosis of the lymphoid tissues, hepatic necrosis and fatty changes, and foetal malformation (Krogh 1976). Cattle are relatively resistant to the acute effects of ochratoxin and the difficulty in obtaining the quantity of toxin required for toxicological studies has meant that few have been carried out. However, single oral doses of 25 and 11 mg kg^{-1} have been found to be lethal to calves of several weeks old. For comparison the lethal dose for swine is 1 mg kg^{-1}, so it may be that the sensitivity of calves before having developed a functional rumen approximates to the sensitivity of swine. However a major problem occurred in the USA (Lloyd 1980; Lloyd and Stahr 1980) in which the kidneys of a large number of cattle were affected and 63 died. High concentrations of ochratoxin A and citrinin were found in the feed. In general, it seems likely that the major concern from ochratoxin A is chronic disease in livestock and the possibility of transfer of residues to humans in meat and animal products. Ochratoxin A is now considered a genotoxic carcinogen.

The *Penicillium* genus is the source of a number of different mycotoxins such as citrinin, penicillic acid, xanthomegnin, viomellein, verrucosidin, a potent nephrotoxin associated with *Penicillium aurantiogriseum*, and mycophenolic acid, some of which have been shown to occur in cereals and other foods and feeds.

Citrinin causes kidney damage and mild liver damage in the form of fatty infiltration. A review of citrinin toxicity is given by Scott (1977). It often co-occurs with ochratoxin and has been implicated in mycotoxic nephropathy of pigs (Krogh 1973).

Studies by Scudamore *et al* (1986a, b) have shown that, in a number of mouldy cereal samples, a group of structurally related naphthaquinone mycotoxins: xanthomegnin, viomellein and vioxanthin, can also co-occur. All five toxins have nephrotoxic properties. The naphthaquinones are not produced by *P verrucosum* but by several other different *Penicillium* species (Frisvad and Lund 1993). In practice, these are often found together with *P verrucosum*, which explains the occasional co-occurrence of the mycotoxins (Scudamore and Hetmanski 1995). Similar *Penicillium* species are reported to infect mouldy hay and straw, the possibility exists that the same cocktail of mycotoxins might occur.

There are few references to the effects of these toxins in large animals. However, the effects of xanthomegnin and viomellein have been studied in the guinea pig (Carlton and Tuite 1970a) and swine (Carlton and Tuite 1970b; Zimmermann *et al* 1979a, b) by feeding cultures of *P viridicatum* and *A ochraceus* which were shown to contain these toxins. In the guinea pig the main target organ was the liver but for swine it was the kidneys. The principle findings in the swine were lethargy, weight loss and death. Most suffered subcutaneous oedema, effusions into the abdominal and thoracic cavities, pulmonary atelectasis, oedema of the abdominal viscera and haemorrhages in the kidneys. Histologic examination showed changes in the kidneys. The obviously contrasting pathology in guinea pigs and swine demonstrate the limitations of modelling toxicity to man from other species. The toxicity of most substances show inter-species variation.

Aspergillus versicolor was isolated from 14·5% of 69 samples of hay and straw collected in Germany during the winter of 1984/5. These strains were inoculated onto a cracked maize substrate and examined for sterigmatocystin production. Most were highly toxigenic and 53% produced greater than 500 mg kg^{-1} (Lepom and Kloss 1988). The authors suggest that maize is a very good substrate for sterigmatocystin, particularly in the surface aerobic layers of feed stocks and maize silos. *A versicolor* and other *Aspergillus* species readily produce sterigmatocystin in culture, but despite this, it has rarely been reported in cereals or feeding stuffs.

The main damage to albino rats when treated with the toxin was necrosis of the liver and kidneys, and eventual peritonitis. There appear to be few toxicological studies with large animals. The acute toxicity, carcinogenicity and metabolism of sterigmatocystin has been compared with those for aflatoxin and several other hepatotoxic mycotoxins by Wannemacher *et al* (1991).

Many fungal isolates obtained from silage produce patulin in laboratory culture. However, patulin has rarely been found in the silage from which these moulds have been isolated, or when silage has been implicated in suspected mycotoxicoses. Hacking and Rosser (1981) isolated 26 *Paecilomyces* strains from ten silage clamps of which 21 strains produced patulin when cultured in potato dextrose broth. Patulin was not detected in any of the silage samples. This may be due to the instability of patulin, the differences between conditions in the field and the laboratory or the culture substrate favouring patulin formation. It reportedly reacts with sulphydryl-containing amino acids or proteins *in vivo*. The cysteine adduct was not acutely toxic but was teratogenic to chick embryos (Ciegler *et al* 1976).

Dutton *et al* (1984) considered the interactions of additives, yeasts and patulin production in grass silage. Both laboratory-prepared and sterile farm silage were found to support growth of *Paecilomyces* spp and to result in patulin production. However, the formation of patulin was affected by the levels of yeasts present in the silage, and there was an inverse relationship between yeast population levels and patulin concentration.

Out of 34 silage samples drawn in France from

cutting fronts, 59% contained patulin at levels between 1·5 and 40 mg kg^{-1}. Its presence was closely associated with *Byssochlamys nivea* (Escoula 1975), although not all samples infected by this species contained the toxin. The levels of patulin occurring naturally in silage were very high and caused little problem in its measurement. This contrasts with the difficulty reported by other workers in detecting patulin, despite the presence of a fungus able to produce patulin. In other studies, Escoula (1974) sought two other fungal species which are able to produce patulin, *Aspergillus terreus* and *Aspergillus clavatus*, but neither of these fungi were found.

For patulin, the LD_{50} for the rat has been reported as 15 mg kg^{-1} body weight (Broom *et al* 1944) and 25 mg kg^{-1} after subcutaneous injection (Katzman *et al* 1944). Death was usually caused by pulmonary oedema. Lungs were oedematous, with the alveoli filled with protein-rich fluid and many leukocytes. The pulmonary vessels were congested but haemorrhages were few. Hepatic and intestinal blood vessels were congested and in sections of the kidneys there was slight congestion, mild degeneration of the tubular epithelium, and a few loci of haemorrhages. Patulin injected in large amounts over a 2 month period was carcinogenic, resulting in induction of sarcomas at the injection site (Dickens and Jones 1961). Patulin was implicated in the death of 100 cows fed a dry malt feed (Rodricks *et al* 1977).

In view of its apparent toxicity to animals and its occasional occurrence in very high concentrations, patulin should be regarded as a mycotoxin associated with silage which may contribute to problems in livestock.

Ohmomo and Kitamoto (1994) isolated a number of strains of *Penicillium roqueforti* from maize silage. One strain was able to produce a number of toxic indole alkaloids when re-inoculated into silage. Five of these were identified as roquefortines A, B, C and D together with festuclavine. The authors considered this mould to be the principal fungus isolated from mouldy silage although other reports suggest that alternative fungi are as, or more, important. Roquefortine C has also been reported in moulded feed grain. A toxic metabolite, fumigaclavine C, was found in the culture broth of *A fumigatus* isolated from maize silage (Cole *et al* 1977) and is structurally very similar to roquefortine A. Wei *et al* (1973) produced PR-toxin from isolates of *Penicillium roqueforti* obtained from mouldy silage.

Acute toxicity of roquefortines in mice has been reported. That for roquefortine C was 20 mg kg^{-1} (IP) (Ohmomo 1982) and that for roquefortine A 340 mg kg^{-1} (IP) (Ohmomo *et al* 1975). From these data, the intake of mouldy silage required to cause acute toxicity in cattle is likely to be large. However, in the practical situation, other mycotoxins such as PR toxin might co-occur and ruminants may be more susceptible than mice. Little information is available on the chronic effects of these compounds.

PR toxin is one of the most acutely toxic compounds known to be formed by *Penicillium roqueforti*. LD_{50} values in mice ranged from 1 to 5·8 mg kg^{-1} (IP) and 58 to 100 mg kg^{-1} (oral). Degenerative changes were observed in the rat liver and kidney. It is not known whether it is carcinogenic but it is mutagenic to *Salmonella typhimurium*. Further information on biological activity has been given by Scott (1984). There appears to be no confirmed report of PR toxin occurring naturally in animal feeds. However, Vesely *et al* (1981) fed maize silage infected with *Penicillium roqueforti* to dairy cows. This resulted in loss of appetite, cessation of rumen activity and gastroenteritis. Abortion of first calvers in the 7th and 8th months was observed. Caution is required in attributing these symptoms to PR toxin as this fungus is known to be capable of forming a number of other toxic metabolites. However, isolates of the fungus produced up to 900 mg $litre^{-1}$ of PR toxin in liquid culture.

The ubiquitous occurrence of *Aspergillus fumigatus* means that it can be found on almost any spoiled forage crop and presents a dual hazard from the ingestion of pathogenic spores and from potential mycotoxin production. There are several sub-species of *A fumigatus*, and thus the possibility of a range of toxins being formed. The relationship between the sub-species and the metabolites has not been fully explored. A number of different toxic metabolites have been produced in fungal cultures, but identified less frequently in spoiled feeds. Cole *et al* (1977) isolated fumigaclavine A, fumigaclavine C and several tremorgens belonging to the fumitremorgen group from *A fumigatus* strains isolated from silage. Although those laboratory investigations were carried out in connection with an acute toxic syndrome in beef cattle, none of these mycotoxins were found in the silage. *A fumigatus* Fres was found to produce four UV-absorbing toxins designated as fumitoxins A, B, C and D by Debeaupuis and Lafont (1978), and the authors regarded these metabolites to be quite different from those previously described.

Cole *et al* (1977) reported that young calves dosed with crude extracts of *A fumigatus* cultures experienced severe diarrhoea, irritability, loss of appetite, serious enteritis and interstitial changes in the lungs. The clavine alkaloids, fumigaclavine A and C, and several tremorgens were identified from cultures of *A fumigatus* isolated from moulded corn silage. The LD_{50} for fumiclavine C was approximately 150 mg kg^{-1} orally in day old cockerels.

Some isolates of *A fumigatus* produce a steroid like antibiotic, helvolic acid (Wakeman *et al* 1943). *Aspergillus fumigatus* has also been reported as being able to synthesise dicoumarol and 4-hydroxy coumarin in cultures containing *o*-coumaric acid (Bye *et al* 1968; Bye and King 1970). 3,3′-Methylenebis(4-hydroxycoumarin) has anti-coagulant properties and has been identified as

the cause of fatal haemorrhagic disease of cattle and could also be implicated in the sporadic outbreaks of haemorrhagic disease in the UK although there is no direct evidence to support this (see haemorrhagic disease in cattle).

There are reports of gliotoxin being confirmed in silage, hay and straw and a number of studies of the metabolites formed by strains of *A fumigatus* isolated from these feeding stuffs have been shown to produce gliotoxin in culture. Kurbatskaya and Trotstanetskii (1987) inoculated hay, maize stalks and other cereals with *A fumigatus* and studied the formation of gliotoxin. Oats and hay were the most favourable substrates for gliotoxin synthesis. Optimal temperatures for production were 30–36°C suggesting that gliotoxin would most likely be formed during heating of mouldy products.

Gliotoxin has antiviral activities and inhibits the multiplication of RNA viruses such as polio virus, herpes simplex virus and influenza virus in tissue culture systems. The LD_{50} in rats was between 50 and 65 mg kg^{-1} body weight (Johnson *et al* 1943).

A toxic compound named walleminol A, together with other biologically active metabolites, were formed by the mould *Wallemia sebi* during a toxicological screen of fungi isolated from moulded foods (Wood *et al* 1990). Its toxicological effect on animals is uncertain.

FIELD INCIDENTS AND SOME ANIMAL DISEASES WHICH MAY BE RELATED TO THE PRESENCE OF MYCOTOXINS

Establishing firm links between mycotoxins and field incidents is difficult and few have been conclusively proven. Some of the disease syndromes which may be mycotoxin related are listed in Table 3 together with the fungal species and/or mycotoxin which may be associated with the condition.

Mycotoxicoses associated with grassland

New Zealand currently recognises three major grass-associated mycotoxicoses occurring principally in sheep: facial eczema, the result of photosensitisation caused by sporidesmin, zearalenone induced infertility and rye grass staggers.

In the USA fescue toxicosis, caused by ergopeptine alkaloids, is responsible for severe livestock losses. Similar toxins have also been found in New Zealand grasses (Towers 1993b) but the environmental conditions presumably do not encourage sufficient mycotoxin production to cause disease. Rye grass staggers and fescue toxicosis result from endophyte fungal infections of grasses.

Munro (1985) reported an outbreak of secondary photosensitisation resembling facial eczema and associated with possible liver damage in sheep grazing Italian ryegrass leys. There are other possible causes of photosensitisation including ingestion of primary photodynamic agents, inherited defects in porphyrin metabolism and liver damage causing accumulation of phyllorythrin in the photodynamic end product of chlorophyll metabolism (Anon 1990a). Cases occurred over a 3 year period and also affected cattle. Although only ergot was found on the grass, it was suggested that the nervous symptoms of these outbreaks resembled ryegrass staggers, caused by a tremorgenic mycotoxin, although none was identified.

Ryegrass staggers in cattle and sheep has been recognised to occur occasionally eg in New Zealand, the USA (Galey *et al* 1991; Di Jonge 1984) and in the UK

TABLE 3
Disease syndromes which may be linked to the presence of mycotoxins in feed

Disease	*Possible toxin or fungi implicated*
Fescue toxicosis	Ergopeptine alkaloids
Facial eczema	Sporodesmin
Ryegrass staggers	Lolitrem B, paxilline, *Acremonium lolii*
Secondary photosensitisation/liver damage	Tremorgenic toxins?
Ergotism	See table 2, *Claviceps* spp
Oestrogenic effects in pigs and sheep	Zearalenone and related compounds, α-zearalanol?
Haemorrhagic syndrome	Di-coumarol?, 3,3′-methylenebis(4-hydroxycoumarin)?
Pyrexia, pruritis and haemorrhagic syndrome	Unknown toxin(s)
Immunosuppression and abomasal ulceration	*P roqueforti* toxins/*Fusarium* toxins?
Grass sickness in horses	Unidentified neurotoxin
Congenital spinal stenosis	Mouldy straw
Stachybotryotoxicosis	Satratoxins
Neurodegeneration	*Aspergillus clavatus* toxins
Mycotic abortion	*Aspergillus* spp (fumigatus?)
Aflatoxicosis	*Aspergillus* spp

(Clegg and Watson 1960; Davies and Farmer 1961; Pritchard and Lewis 1995). Tremorgenic disease is associated with drought conditions in Europe (Sorgdrager 1978; Huyben and Sol 1992). In 1994, approximately 60 horses were affected by a staggers syndrome (Pritchard and Lewis 1995) and the presence of a grass endophyte identified. Tremorgens and fumigaclavin A have also been associated with a syndrome in silage-fed cattle which caused ill-thrift, irritability and some mortality (Cole *et al* 1977).

Ergotism, caused by the mixture of mycotoxins found in the sclerotia of *Claviceps* spp, occurs widely and is encouraged by warm, wet summers. Although ergot-infected pasture has occasionally caused disease (Woods *et al* 1966; Loken 1984), this is usually associated with the larger doses possible with heavily contaminated grain based diets. Ingestion of small quantities of ergots cause no apparent ill-effects. Disease symptoms include gangrene of the extremities, which is the common form in cattle, and neurotoxicity with convulsions, more common in sheep. The toxins can also cause hyperthermia (Anon 1990b).

Field cases of an oestrogenic syndrome in pigs associated with mouldy feed were recognised early in the 20th century (McNutt *et al* 1928). Zearalenone is now well recognised as causing well-described reproductive toxicity in pigs and other livestock, usually associated with contaminated cereals (Mirocha and Christensen 1974). It is not entirely clear if disease is the result of zearalenone acting alone or in combination with other mycotoxins. Some authors claim that ruminants are not affected by dietary contamination with zearalenone, possibly as a result of reduction of zearalenone to the more readily excreted zearalanol in the rumen (Smith and Henderson 1991). However, Towers and Sprosen (1993) demonstrated that *Fusarium* contaminated pastures in New Zealand can produce sufficient concentrations of zearalenone in herbage, especially in the summer, to cause reproductive toxicity in sheep. The effective dose of zearalenone in sheep may be as little as 1 mg day^{-1} for 7–10 days and the levels found in herbage ranged as high as 24 mg zearalenone kg^{-1} dried herbage.

α-Zearalanol, a metabolite of zearalenone, has been found in the urine of pasture-fed ruminants (Erasmuson *et al* 1994). Zearalanol, also called zeranol, has been used as a growth promoter but is now banned throughout the EU. If residues of zearalanol are detected in meat or animal products, the farmer may be liable for prosecution. The UK National Surveillance Scheme for Residues in Meat recorded two out of a total of several hundred samples of bile and urine positive for zearalanol in 1994. In neither case was there evidence of illegal use.

It is possible that zearalanol is a primary fungal metabolite or a metabolic product of zearalenone metabolism in ruminants. In either case the possibility of naturally occurring zearalanol contamination in meat or animal products should be investigated. Mouldy hay has also been reported to have caused oestrogenic effects in cattle (Khamis *et al* 1986). Apart from the possibility of grass contamination, zearalenone occurs commonly in maize and there is evidence that it survives ensilage (Lepom *et al* 1988).

There are no reports of infertility associated specifically with contaminated maize silage. However it is unlikely that the expected symptoms of extended calving conception interval, anoestrus and delayed oestrus would immediately be attributed to zearalenone contamination by farmer or veterinarian. At sufficiently high doses, zearalenone may be teratogenic (Becci *et al* 1982). The investigating veterinarian may not immediately associate sporadic birth defects with mycotoxicosis. Retrospective investigation of feed contamination would be impossible because the exposure to mycotoxin would have been several months prior to the observed congenital defect.

A haemorrhagic syndrome was described and reproduced by Cranwell (1983) using spoiled sweet vernal grass which contained dicoumarol. No mycotoxin has ever been found to explain the death and disease observed and Williams (1981) suggested that this might be interaction between sweet vernal grass in hay and fungal growth to produce a chemical similar to that investigated in USA and Canada. In the 1920s (Schofield 1922; Roderick 1929) many cases of fatal haemorrhagic disease occurred and this was quickly linked to feed containing improperly cured sweet clover hay. This followed an earlier outbreak in which similar symptoms were observed (Paulmann 1923). The clover itself was not toxic but it was suggested that mould growth found in the haystack was involved in the toxic condition of the hay. The research to determine the toxic principle is described by Scheel (1978) who concluded that the fatal haemorrhagic disease of cattle and sheep fed on mouldy hay or silage prepared from the common sweet clovers *Melilotus alba* and *M offinalis* is caused by 3,3′-methylene-bis-(4-hydroxycoumarin) in the forage. This toxicant is formed in the plant tissue by the reaction of formaldehyde with mould-produced 4-hydroxycoumarin. The source of the formaldehyde was most likely exogenous. An outbreak of mycotoxicosis reported among cows and new born calves fed mouldy silage was characterised by liver, kidney and blood coagulation disorders together with foetal malformations (Gerisch *et al* 1981).

Suspected immunosuppression and abomasal ulceration has been associated with *Penicillium roqueforti* and *Fusarium* contamination of silage (Sproat 1987). This refers to a single case of severe silage contamination where the affected animals were primarily ewes. Presenting symptoms were non-specific but included significant mortality. Post-mortem examinations revealed severe abomasal ulceration with lymphadenitis

and hepatitis as a consequence. Keratoconjunctivitis and listeria meningo-encephalitis were also diagnosed in the affected group. The problem was corrected by adding gentian violet at the rate of 1 kg tonne^{-1} to the concentrate supplement. *Penicillium roqueforti* and *Fusarium moulds* were consistently isolated from the silage but no mycotoxins were identified.

Grass sickness in horses is currently thought to be caused by a neurotoxic mycotoxin. The hare apparently suffers from a similar problem (Bonner 1995).

Mycotoxicoses associated with other forage crops in addition to grass

Haemorrhagic disease has been associated with a variety of mycotoxins, including aflatoxin and trichothecenes, which cause blood dyscrasias. Shreeve *et al* (1975) investigated 73 cases of suspected mycotoxicosis over a 1 year period. Twelve were haemorrhagic disease incidents in ruminants but no diagnosis was confirmed. Other cases of blood dyscrasias have been reported (Jeffers and Lenghaus 1986; Mansfeld *et al* 1989) where mycotoxicosis has been suspected but remained unconfirmed. Petrie *et al* (1977) associated T2 toxin in brewers grains with a haemorrhagic syndrome in dairy cows. Very recently Cockcroft (1995) associated haemorrhagic disease in adult cattle with high concentrations of aflatoxin in concentrate feed from a storage bin although the evidence for the link is tenuous.

Pyrexia, pruritis and haemorrhagic syndrome are a group of conditions of unknown aetiology which occur sporadically in cattle fed conserved feeding stuffs. It has a variable morbidity but a high mortality in clinically affected animals. The presenting symptoms vary considerably. Haemorrhages may be a major feature (Hall 1976; Breukink *et al* 1978; Thomas 1979, 1981; Griffiths and Done 1991).

Congenital spinal stenosis (CSS) has occurred in calves along with mortality of dams. It was apparently associated with mouldy cereal straw though specific mycotoxins were not isolated. In affected groups of calves the prevalence of CSS varied between 29 and 100%. The congenital abnormalities in the calves included limb and skull deformities. Dams in three of the four herds were affected by allopecia with or without pruritis and 25% of cows in one herd died. The description of this condition has some similarities to the pyrexia pruritis and haemorrhagic syndrome described above, especially the incident reported by Griffiths and Done (1991) which affected eight animals from a herd of 175 cows. The animals affected all came from a group whose diet included visibly mouldy citrus pulp pellets although both groups were also fed silage. The clinical signs, gross pathology and histopathology are described and compared with other outbreaks. The authors suggested that mycotoxins, particularly citrinin, were strongly implicated although no mycotoxins were detected. The possibility of *Fusarium* mycotoxins being implicated was not considered. Citrus pulp is susceptible to invasion by many different fungi including *Fusarium*, moreover, symptoms reported in this case resemble those that can result from trichothecene poisoning.

Le Bars and Le Bars (1993) have summarised the occurrence of stachybotryotoxicosis and have suggested that a chronic form occurs more often than is generally realised. Most cases occurred in horses, but stachybotryotoxicosis also occurs in deer, goats and has been suspected in cows.

Neurodegeneration has been reported in ruminants associated with *Aspergillus clavatus* contamination of a distillery by-product. The forage portion of ruminant diets is routinely supplemented with high cellulose by-products such as sugar beet pulp, brewers grains and distillers grains. It was reported by Gilmour *et al* (1989) that fungal spoilage of distillers grains by *Aspergillus clavatus*, a natural and common contaminant of malting barley, caused a mycotoxicosis in both fattening cattle and lambs. The mycotoxin(s) caused neuromuscular symptoms with posterior ataxia stiffness and 'knuckling' which progressed to recumbency with some signs of central nervous aberration. Histopathology of the CNS revealed acute degenerative changes in a variety of sites including both grey and white matter. The identity of the mycotoxin or mycotoxins responsible was uncertain and the presence of toxins was confirmed by use of a non-specific tissue culture assay (Robb *et al* 1982). *Aspergillus clavatus* can produce a number of mycotoxins (Tiang *et al* 1982; Flannigan 1986). Similar syndromes have been reported in France (Moreau and Moreau 1960), Bulgaria (Tomov 1965; Nikov *et al* 1965) and Israel (Schlosberg *et al* 1991).

Mycotic abortion probably accounts for up to 15% of all abortions in housed cattle. The pathogenesis of the condition is incompletely understood. A wide range of fungi have been associated including *Mucor*, *Aspergillus* and *Mortierella* species. These probably gain entry via inhalation or via the digestive tract. Foetal death results from placentitis and/or fungal infection of the foetus; placentitis is not always present (Anon 1990a). Over a 4 month period, approximately 1500 young turkeys out of a flock of 3500 died from symptoms characteristic of aspergillosis (Hacking and Blandford 1971). The source of infection was barley straw used for litter. High concentrations of spores of *A fumigatus* were measured in the vicinity of the straw bales. Post-mortem examination revealed extensive lesions in the lungs and air sacs. *Aspergillus fumigatus* is often found in mouldy silage and forages where disease problems have arisen. The possibility of a mixture of mycotoxins contributing to the overall problem through their production *in vivo* or carried on spores should not be dismissed.

If spores are transferred directly to the foetus, it is probable that its immature immune system is unable to cope with the challenge. The extracellular development of mycosis in maternal tissues is more difficult to explain. Perhaps immunosuppressive mycotoxins are released by the developing mycosis.

ANALYTICAL METHODS FOR DETECTION OF MYCOTOXINS IN FORAGE CROPS AND SILAGE

The quality control applied to any analytical procedure is of little value unless a sound sampling protocol is followed, ideally carried out following a statistically sound plan. This is particularly difficult for forages and silage. A procedure for silage, recommended by Wilkinson (1990), is to take core samples from several different places in a silo or from as many bales as possible, avoiding sites which may be unfit due to surface wastage. The core should travel through the full depth of the silo or bale. This recommendation would be of limited value in the investigation for suspected mould related incidents as the fungal spoilage is usually restricted to the surface or where air has gained access.

Most analytical methods described have been developed for cereals, stored food commodities, human food and animal feeding stuffs, especially where regulations have been introduced. The determination of aflatoxins, ochratoxin A and zearalenone have become simpler, more rapid, reliable and sensitive due to the availability of commercially marketed immunoaffinity columns. This new technology has not yet been applied to the analysis of forages. Sensitive methods are not generally available for those mycotoxins most likely to occur in silage or forage.

Methods for mycotoxins such as patulin, byssochlamic acid, roquefortines, PR-toxin, gliotoxin, satratoxins and toxic metabolites of *Aspergillus fumigatus* are insensitive or poorly developed and often based on TLC. Despite this, it should be possible to detect gross contamination by a mycotoxin, particularly if the nature of the toxin is suspected. Where the detection of potentially important sub-clinical levels of a toxin is required, few of the methods currently available would be of sufficient sensitivity. Table 4 lists details and references for selected methods found in the literature. For more general information the reader should consult Shotwell (1986) and Pomeranz *et al* (1990).

In some instances, such as with contamination by zearalenone, metabolites rather than the parent compound may be of greater public health significance. For most mycotoxins, reliable data on their metabolism and disposition together with appropriate analytical methods for their detection is rudimentary.

In summary, few of the methods reported for forage crops and silage are sensitive or have been fully tested and validated. Hence, only a few examples of analytical methods for some of the less studied mycotoxins are cited here. An alternative to attempting to analyse the feed, is to identify and quantify the presence of a mycotoxin or a related metabolite in a target animal. This might involve analysis of blood, urine or tissues. Hence, detection of aflatoxin M_1 in ruminant milk would indicate exposure to aflatoxin. Ultimately, it might be possible to correlate levels found within the animal with those of B_1 in the feed to obtain an indication of exposure using aflatoxin B_1 to aflatoxin M_1 ratios. This approach may prove more effective than attempting to monitor all sources of feed.

The method reported by Ohmomo and Kitamoto (1994) enabled the presence of 340 and 20 mg kg^{-1} of

TABLE 4
Selected analytical methods for determination of mycotoxins in forage crops or silage

Mycotoxin/feed	*Reference*	*Comments*
Aflatoxins and other mycotoxins in silage and forages	Roberts and Patterson (1975)	TLC Recovery and detection limits not given
Patulin in silage and forages	Coxon and Price (1978)	GC/MS Recovery and detection limit not given
Roquefortines A and C in maize silage	Ohmomo and Kitamoto (1994)	TLC Detection limit better than 100 μg kg^{-1}
Mycophenolic acid in hay and forage	Kahsai and Matthees (1994)	HPLC Recovery 85–95%, detection limit 500 μg kg^{-1}
Satratoxins G and H in straw	Harrach (1988)	TLC, detection limits poor
Sterigmatocystin in straw and hay	Lepom (1986)	HPLC
Zearalenone in maize silage and feeds	Fankel and Blutsch (1979)	HPLC. Recovery 80–90% detection limit 10–20 μg kg^{-1}
Zearalenone in maize and maize silage	Lepom (1988)	HPLC or TLC. Recovery 81%, detection limit 10 and 100 μg kg^{-1}

roquefortine A and C respectively to be detected in silage inoculated with *P roqueforti*. These concentrations represent very high levels of contamination but such methods are useful for demonstrating the natural occurrence of these mycotoxins. How this relates to formation under typical field conditions is unknown. Until evidence of natural occurrence is forthcoming, it is difficult to justify the resources necessary to develop more sensitive analytical methods. In clear cases of mycotoxicoses, the concentration of mycotoxin is likely to be high. Studies of potential transmission to human food arising from sub-clinical animal contamination require the development of specific and reliable methods operating at the low ppb level. The method published by Lepom (1988) used HPLC for determination of zearalenone in maize and maize silage.

UNRESOLVED PROBLEMS AND POTENTIAL AREAS FOR STUDY

Most research targeting mycotoxins in forage crops appears to have been of an *ad hoc* nature, usually arising as a result of a feed-related incident. Some valuable studies have been carried but have tended to address an individual toxin or mould species (eg Hacking and Rosser 1981). While this review has considered the mycotoxins thought most likely to occur, the possibility exists of less common moulds occurring from time to time and one must always be alert to the presence of unexpected or unrecognised toxins. Even when familiar fungi are present, some strains may produce unusual toxins. For example, Mantle *et al* (1991) found that some strains of *P aurantiogriseum* (a well-characterised mould) synthesised a potent nephrotoxin, the effects of which might have been casually attributed to ochratoxin A. Investigations showed that this was due to one or more unidentified polypeptide-like compounds.

There are few cases in which a link between animal disease and mycotoxin contamination of feed has been established even when mouldy feed was implicated. If cases are to be investigated, a clearly defined protocol is required, early recognition of possible mycotoxin involvement needed and the rapid response of expert scientists in several different disciplines. To this end, specific and rapid methods for the detection of mycotoxins and the fungi responsible need to be developed.

Study of the potential for transfer of mycotoxins to animal is only needed if they are sufficiently toxic (ie are suspected carcinogens or have oestrogenic properties). For many of the less well known mycotoxins, sensitive and reliable methods of analysis need to be developed for their determination in the feed and for their detection in meat and animal products. Further research is desirable on sampling, development of analytical and immunological methods, occurrence, development of biomarkers for detection of exposure, and risk assessment.

CONCLUSIONS

The potential problems posed by mycotoxins stemming from infection of forage crops and silage have, in general, been poorly researched. World-wide, but especially in temperate climates, grass, silage and forage crops are reported to cause occasional problems in livestock. Thus, while it is not considered that mycotoxins represent a major animal health problem in developed areas of the world, effects ranging from reduced productivity to occasional deaths may occur from time to time. The recognition of mycotoxicoses is extremely difficult. This problem is exacerbated through the absence of structured protocols for investigation of suspect cases.

If an occasional clinical disease problem exists, it is likely that sub-clinical exposure is much more frequent. Where evidence exists that a mycotoxin or its metabolites may persist in meat or animal products, the potential for transfer to man must be considered.

For aflatoxins, sufficient data and methods have been developed to assess and manage risk. For most other mycotoxins, this is not so although it is likely that the risks to animals and to man via meat and animal products are minimal, largely because of the small number of confirmed mycotoxicoses diagnosed.

Priority for future investigation should target identification of those mycotoxins which produce residues of the parent compound or metabolites in meat or animal products which may be bioavailable to man. Mycotoxins which may introduce residues into meat or animal products even if only present at sub clinical levels in livestock, should receive first priority.

The development of an algorithm (model) to assess and manage associated risks with mycotoxins and other causes of feed contamination would enable a consistent and focused approach to the investigation and risk assessment of field incidents, prioritisation of research and surveillance, management of risks identified and justification for allocation of resources. Effective management of any risks can only follow accurate risk assessment.

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