

OCCURRENCE OF MYCOTOXINS IN SILAGE

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INTRODUCTION

Mycotoxins are a large, diverse group of naturally occurring toxic metabolites of fungi. Currently, more than 300 mycotoxins have been identified. A number of mycotoxins have been implicated in diseases in animals and humans. Mycotoxins can be found in a wide variety of crops all around the world, including crops that are commonly fed as silage, such as maize, wheat and grasses. Some mycotoxins, such as trichothecenes, zearalenone and roquefortin C occur frequently in silage crops, whereas others are only sporadically found. Mycotoxins in silage are of dual concern. Firstly, they can have adverse effects on animal health and cause production losses. Secondly, they may jeopardize the safety of food products of animal origin. Of the major mycotoxins in silage crops, the second concern holds true for aflatoxin only, as is described later in this paper.

This paper summarizes scientific knowledge about the major mycotoxins occurring in silages, under which conditions they are formed and how they can be prevented. The metabolism of mycotoxins in ruminants and their carry-over into milk are briefly described. Toxic effects of mycotoxins in animals and man, analytical methods for detection of mycotoxins and legislative aspects are not described in this paper. Information about these topics can be found elsewhere (1-5).

MAJOR CLASSES OF TOXINOGENIC MOULDS AND MYCOTOXINS

The major feed crops that are conserved by ensiling are grasses, maize, lucerne (alfalfa), small grain cereals (wheat, triticale, rye, barley and other) and sorghum. Moulds and mycotoxins that are of relevance for silage produced from these crops are listed in Table 1. A distinction is made between mycotoxins that are formed before ensiling and those that are formed after ensiling. It is important to make this distinction because

different types of moulds, different types of mycotoxins and different types of agricultural factors influencing mycotoxin levels are involved. Mycotoxins that are formed before ensiling are associated with moulds that infect a crop during its growth in the field or by endophytic moulds that live as symbionts in for instance grasses or cereals (field-derived mycotoxins). These mycotoxins include trichothecenes, zearalenone, fumonisins and aflatoxins. Mycotoxins that are formed after ensiling are associated with moulds that develop in silage during storage or feeding-out (ensilage-derived mycotoxins), usually as a result of poor silage management practices. These mycotoxins include mycotoxins formed by *Penicillium roqueforti* and *P. paneum* and the diverse group of mycotoxins formed by *Aspergillus fumigatus*.

Field-derived mycotoxins

The major toxinogenic moulds capable of producing field-derived mycotoxins are *Fusarium* species, *A. flavus* and *A. parasiticus* and endophytic *Claviceps* and *Neotyphodium* species. Important factors influencing mould growth are moisture (water activity (a_w)), temperature and availability of nutrients and oxygen. Moulds are generally tolerant to low a_w . In contrast to most bacteria, many mould species are capable of growth at a_w values between 0.80 and 0.90. Mechanical damage or insect attack of plants or grain kernels plays an important role in mould infestation and subsequent mycotoxin contamination because these events cause disruption of the protective plant cell wall. This creates entry points for infective moulds. In addition, it causes release of nutrients from the plant endosperm that can be used by moulds for growth. The ability of a mould species to produce mycotoxins is genetically determined. However, the expression of these genes and the production of mycotoxins are influenced by environmental factors. Usually production of a mycotoxin occurs only over limited ranges of a_w and temperature.

Fusarium mycotoxins (trichothecenes, zearalenone and fumonisins)

Fusarium species are pathogens of various feed crops, including maize, wheat, barley and grasses. These moulds are occurring world-wide, but seem to be particularly prevalent in temperate climates. Infection of plants by *Fusarium* can take place via

kernels, leafs, the stalk or infected seeds. Soil and decaying plant residues in the field are the main sources of *Fusarium* spores and conidia. A high level of mechanical or insect damage of the plant increases the risk of infection and is often associated with higher mycotoxin levels. Weather conditions strongly influence development of *Fusarium* mycotoxins. Examples of plant diseases associated with *Fusarium* infection include ear rot and stalk rot in maize and ear blight in wheat. The predominant species causing these diseases are *F. graminearum* and *F. culmorum*. These species are capable of producing zearalenone and different types of trichothecenes, including deoxynivalenol (DON; synonym for vomitoxin), nivalenol, diacetoxyscirpenol and T-2 and HT-2 toxin. DON is the most commonly occurring trichothecene. DON and zearalenone are often co-occurring in contaminated crops. An important and often unrecognized feature of DON and zearalenone contamination of maize and wheat is that these mycotoxins occur not only in the grains and kernels but also in the green parts of the plant, *i.e.* the leafs and stalk. This is of significance because these crops often are fed as whole crop silage. The limited information that is available on this topic indicates that DON and zearalenone levels in leafs and stalk of maize can be even higher than in the cob (6). Fumonisins are formed by *F. verticillioides* (syn., *F. moniliforme*) and *F. proliferatum*, species associated with pink or white ear rot disease in maize. Fumonisins are found exclusively in maize.

It is generally assumed that DON, zearalenone and other *Fusarium* mycotoxins are not produced in silage. *Fusarium* species do not survive the acidic and anaerobic conditions of silage and usually have a low prevalence in comparison with *Aspergillus*, *Penicillium* and *Monascus* species. However, few studies report development of *Fusarium* mycotoxins in silage. An example is a study conducted in Italy in which zearalenone concentrations in highly aerobically deteriorated peripheral areas of maize silage were detected that were up to 40 times higher than in non-deteriorated central areas of the silage. The concentration in the central areas was similar to the concentration of the forage at ensiling (7).

Aflatoxins

Aflatoxins are produced by *A. flavus* and, to a lesser extent, *A. parasiticus*. Aflatoxins are highly toxic and carcinogenic to man and animals. Aflatoxin B₁ is the most

prevalent and most toxic form. Aflatoxin B₁ is transformed in the liver of cattle into aflatoxin M₁, the form in which it is (partially) excreted into milk. With respect of the risks of mycotoxins in feed in relation to safety of food products to consumers aflatoxin M₁ is the only mycotoxin of concern. This relates to its significant feed-to-milk carry-over rate and high toxicity. Though *Aspergillus* is generally classified as a mould associated with mycotoxin production during storage of commodities, it can infect crops in the field under favourable conditions, especially in subtropical and warm temperate climates. *A. flavus* and *A. parasiticus* are associated with aflatoxin production in a number of crops, including maize, sunflower, peanut and several tree nuts. Maize plants can become infected by *Aspergillus* conidia from the environment, usually soil or insects. A high level of insect damage increases the risk of infection. If conditions are favourable the mould colonizes the cobs and penetrates into the kernels. Aflatoxin development in the kernels occurs within narrow ranges of moisture content and temperature. Drought stress generally increases aflatoxin development in maize.

Alkaloid mycotoxins

Alkaloid mycotoxins are produced by *Claviceps purpurea* in rye and barley and some grasses and by endophytic *Neotyphodium* moulds in perennial grasses. *Claviceps purpurea* infects the plant when flowering. It produces a resting structure about the size of grain kernels, called sclerotia or ergots, which allow the mould to survive adverse conditions. These ergots contain high concentrations of alkaloids (e.g. clavines and lysergic acid amide). Several grasses, such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), harbor endophytic *Neotyphodium* species capable of producing alkaloid mycotoxins (e.g. lolitrem B and ergovaline). Benefits for the plant from this symbiosis include increase in drought tolerance and resistance to insects. Endophytic *Neotyphodium* are highly prevalent in 'wild' grass populations in natural or extensively managed pastures in the US, Australia, New Zealand and Europe. The prevalence of mycotoxin-producing endophytes in intensively managed pastures is generally low. Grass cultivars selected for grazing or silage production often do not contain these types of endophytes.

Ensilage-derived mycotoxins

The majority of mould species are aerobic micro-organisms. Therefore moulds do not develop in well-preserved, anaerobic silage. However, in practice silages are not completely anaerobic. Firstly, because silage covering materials are generally not fully airtight. Secondly, because of unintended damages to the silage covering during storage (for instance caused by rodents, birds). Moreover, exposure to air becomes inevitable after the silo is opened for feeding. Growth of moulds and development of mycotoxins in silage are associated with the duration and extent of air infiltration. The extent of infiltration of air into the silage mass is mainly dependent on the porosity and density of the silage and the rate of silage removal after opening. The occurrence of moulds in silage is usually highest in surface layers. Commonly detected moulds in silage are *Penicillium roqueforti* and *P. paneum*, *Monascus ruber*, *A. fumigatus*, *Byssochlamys nivea*, *Mucoraceae* (in particular *Rhizopus nigricans*) and *Chrysonilia sitophila* (8, 9). No mycotoxins from *Mucoraceae* and *C. sitophila* are documented. The predominant mould species in silages is *P. roqueforti*, which is tolerant to acidic conditions and able to grow at oxygen levels as low as 0.1% (v/v). At silage surfaces it usually forms white to grey coloured spots or layers. Occasionally, particularly in maize silage, the species forms typical green to blue coloured balls or lumps of mouldy silage approximately 50 to 100 cm below the top surface. *P. paneum* is closely related to *P. roqueforti* and these species cannot be differentiated visually on silage. *P. roqueforti* and *P. paneum* isolates from silage are capable of producing a wide range of mycotoxins *in vitro* under laboratory conditions, including for instance different roquefortins, mycophenolic acid, PR-toxin, festuclavine and agroclavine (10, 11). *P. paneum* additionally produces patulin. However, a number of these mycotoxins are probably not formed in silage or may not be stable under conditions prevailing in silage (as discussed later in this paper). *A. fumigatus* is a heat-tolerant mould species that is particularly detected in heavily moulded parts of silage and is capable of producing a large number of different toxic metabolites, including the potent toxin gliotoxin, verruculogen, fumitremorgens, fumigaclavines and trypacidin (12, 13). Apart from production of mycotoxins, the occurrence of *A. fumigatus* in silage is considered a health risk because inhalation of spores of this mould can cause lung disease (aspergillosis) in animals and man. An increasing amount of research in the area of

mycotoxins is dealing with *A. fumigatus* mycotoxins. *M. ruber* forms red-purple spots on silage surfaces. Mycotoxins produced by this species are monacolin K and citrinin. *B. nivea* is a producer of patulin.

STABILITY OF MYCOTOXINS IN SILAGE

Information about the stability of mycotoxins in silage is not fully conclusive. There are data indicating that certain field-derived and ensilage-derived mycotoxins are degraded in silage. However, reports in the literature about this subject are contradictory. This possibly relates to the fact that the conditions in silage are not homogeneous and not constant. In particular exposure of silage to oxygen leads to conditions that are continuously changing (for instance pH, temperature and chemical composition). As a result the composition of the microflora changes accordingly and a succession of different microbial groups occurs. In advanced stages of aerobic deterioration of silage high concentrations moulds and bacilli are present. To some extent this situation has similarity with composting processes. Under such conditions, probably most mycotoxins will be degraded.

Zearalenone is generally regarded as being stable in silage. No effect of ensiling on the zearalenone concentration was detected in studies in which the level of zearalenone was monitored during up to nine months of ensilage (14, 15). This finding is consistent with data showing that the average and range of zearalenone concentrations in maize silage and unfermented maize products used as feed ingredient are similar (16). With respect to the stability of DON in silage there is contradictory information. In a study investigating DON stability in wheat and maize silage it was concluded that ensiling induced a strong reduction of DON (17). However, in other studies no effect of ensiling on DON concentration was detected (15, 18). Furthermore, the average and range of DON concentrations in maize silage and unfermented maize products used as feed ingredient are similar (16). The conclusion is that under most conditions DON is stable in silage or may be degraded to a limited extent. Aflatoxin B₁ produced in maize in the field has been found to be degraded slowly in maize silage (19). This observation was confirmed in a recent French study, in which a 3-fold decline of aflatoxin B₁ was detected during nine months storage of maize silage (15). Likewise, partial degradation of

ochratoxin A, a mycotoxin that is associated with small grain cereals, has been observed in ensiled barley (20). No information is available about the fate of fumonisins in silage, but probably these mycotoxins are stable. Contradictory information is available about the fate of ergot alkaloids produced by *Claviceps* and *Neotyphodium* species in silage. Health problems of cattle have been associated with high concentrations of ergovaline in silage from endophyte infected perennial ryegrass (21) and with high concentrations of ergocryptine in silage from maize that was contaminated with a weed containing *Claviceps* ergots in the field (22), indicating that these substances were stable (at least partially) in silage. On the other hand, the concentration of *Claviceps purpurea* ergot alkaloids (ergometrine, ergotamine and ergocryptine) in extensively managed grasslands strongly reduced when the grass was ensiled (23).

The *Penicillium* mycotoxins roquefortin C and mycophenolic acid are stable in silage, whereas PR-toxin and patulin are presumably unstable. In contrast to roquefortin C and mycophenolic acid, PR-toxin and patulin are seldom detected in silage. Experiments with blue-veined cheeses manufactured with *P. roqueforti* strains showed that PR-toxin was degraded and detoxified as a result of a chemical reaction with ammonia and free amino acids (24). Many silage types contains relatively high concentrations of ammonia and free amino acids, so reaction with these compounds may be the reason that PR-toxin is often undetectable. For patulin a similar mechanism may apply. Patulin is known to react with SH-groups of cysteine and other sulphur containing amino acids in protein rich environments and to be inactivated in fermented foods, such as wine, beer and cheese (25, 26).

OCCURRENCE OF MYCOTOXINS IN SILAGE

There is considerable information about the occurrence and concentrations of mycotoxins in commodities and feed ingredients (1, 27, 28). But, despite the fact that silage usually constitutes a major fraction (often more than 50%) of the diet of dairy cattle in many parts of the world, information on the incidence and concentrations of mycotoxins in silages is relatively scarce, in particular for silages other than maize silage. DON, zearalenone, roquefortin C and mycophenolic acid appear to be the most frequently occurring mycotoxins in silage.

Table 2 gives an overview of results from surveys in Europe and the USA for DON and zearalenone in silage, conducted between 1989 and 2007. The data show that the incidence of DON in maize silage was high: in six out of seven surveys the incidence was 72 to 100%. In one survey the incidence was 42%, but the quantification limit of the detection method was relatively high in this study (0.5 mg/kg). The average DON concentration of positive samples in these surveys varied between 0.60 and 1.85 mg/kg. The average of the seven surveys for DON in maize silage is 1.1 mg/kg. The incidence of zearalenone in maize silage was high too, but generally lower than that of DON: in five out of six surveys the incidence was 32 to 59%, in one it was 96%. The average zearalenone concentration of positive samples varied between 0.05 and 0.45 mg/kg, and the overall average of the surveys was 0.17 mg/kg. Worth mentioning is that the average DON and zearalenone concentrations detected in samples of maize collected in a worldwide survey of commodities and feed ingredients between 2003 and 2005 (28) were similar to averages of the maize silage surveys. This is consistent with the view that DON and zearalenone are stable in silage. Information about the occurrence of DON and zearalenone in other silages than maize silage are scarce. In a Dutch survey between 2002 and 2004, DON was not detected in 120 grass silage samples and in 3 of 30 (10%) wheat silages, whereas zearalenone was detected in 7 of the grass silages (6%) and none of the wheat silages (32).

Fumonisin contamination of maize is widespread, as indicated by the high incidence of fumonisins in maize and maize by-products intended for use in animal feed (27, 28). Incidence of fumonisins in maize silage is likely to be high too, since evidence indicating degradation of fumonisins in silage is lacking. This is confirmed by the results of a survey in Midwestern USA in 2001 and 2002, in which fumonisin B₁, fumonisin B₂, and fumonisin B₃ were detected in, respectively, 97%, 72% and 57% of maize silages and average concentrations in positive silages were, respectively, 0.615, 0.093, and 0.051 mg/kg (35). In contrast, in the Dutch survey described earlier fumonisin B₁ and B₂ were detected only in 1.4% of the maize silages (32). This low incidence probably reflects that the environmental conditions of forage maize growth in the Netherlands are not favourable for infection by fumonisin producing moulds (*F. verticillioides*).

The occurrence of ensilage-derived mycotoxins produced by *P. roqueforti* and *P. paneum*, *A. fumigatus* and *Monascus ruber* usually relates to the preservation quality of silage. Growth of these moulds in silage is dependent on infiltration of oxygen during storage or during feeding-out. The predominance of *P. roqueforti* in silage can be explained by its tolerance to high concentrations of carbon dioxide and acetic acid and its ability to grow at low oxygen levels (9). Growth of moulds is usually highest in surface layers, essentially because these layers have the highest exposure to oxygen. Consequently, the distribution of ensilage-derived mycotoxins in silage is highly heterogeneous. The highest levels are occurring in visibly moulded areas and in the typical green-blue lumps of mouldy silage occasionally occurring in maize silages just below the top surface.

The heterogeneity of roquefortin C and mycophenolic acid in maize silage is depicted in Figure 1, which shows the incidence and average concentration of roquefortin C and mycophenolic acid in samples taken from the centre, surface layer and surface areas with visible moulds of maize silage at 16 Dutch dairy farms (33). Incidence and average concentration of both mycotoxins were highest in visibly moulded silage. For example, the average roquefortin C concentration in samples of visibly moulded silage was 16 times higher than that in silage surface samples and 270-fold higher than that in silage centre samples. Similar observations were made in a recent German study, not only with respect to the occurrence of roquefortin C and mycophenolic acid but also with respect to the *M. ruber* mycotoxin monacolin K and a number of *A. fumigatus* mycotoxins, including gliotoxin, verruculogen and fumigaclavin C (13). A selection of the results of this study is shown in Table 3.

Very low incidences of roquefortin C and mycophenolic acid in maize and grass silages were found in a Dutch survey, in which samples were analyzed that were taken relatively shortly after ensiling (3 to 6 weeks) from completely sealed silages that were not yet in use for feeding purposes. Roquefortin C was detected in none of 140 maize silages and in one of 120 grass silages at a low concentration (0.08 mg/kg). Mycophenolic acid was detected neither in maize nor in grass silages (32). These observations emphasize the effect of storage conditions and storage time on the occurrence of this class of mycotoxins in silage. Remarkably, low levels of roquefortin C,

mycophenolic acid and patulin were detected in freshly harvested maize prior to ensiling in a recent study conducted in Pennsylvania, USA (36). This finding indicates that *Penicillium* mycotoxins may also be formed in maize in the field, at least under the environmental conditions of maize growth in Pennsylvania, USA.

In a survey of mycotoxins occurring in the total diet of high-yielding dairy cows conducted in 2005 in the Netherlands DON, zearalenone, roquefortin C and mycophenolic acid were identified as the mycotoxins with the highest incidence (33). As expected, roquefortin C and mycophenolic acid were detected in ensiled feeds only. DON and zearalenone were detected in compound feed, feed commodities and ensiled feeds. Maize silage was found to be the most important source of all of these four mycotoxins in the diet. Maize silage represented on average 30% of the total daily feed intake of the animals, but contributed about 80% of the total dietary intake of DON and zearalenone and more than 95% of that of roquefortin C and mycophenolic acid. The greater contribution of maize silage to the intake of ensilage-derived mycotoxins in comparison with grass silage (which represented about 45% of the daily feed intake) is an indication that problems with aerobic deterioration were more serious in maize silage than in grass silage.

PREVENTION

Regarding prevention strategies, a distinction is made between field-derived and ensilage-derived mycotoxins. Prevention of field-derived mycotoxins focuses on two areas: reduction of the infection pressure of moulds and reduction of the susceptibility of the plant to fungal infections (1). Codex Alimentarius issued codes of practice for the reduction of mycotoxins in cereal crops (37). Recommendations in these codes of practice are summarized in Table 4.

The most important factor prevention strategy for ensilage-derived mycotoxins is to prevent exposure of silage to oxygen. Oxygen has a detrimental effect on silage quality because it enables growth of different groups of acid-tolerant aerobic microorganisms, which eventually is leading to complete deterioration of the silage. This process is usually initiated by yeasts, which oxidize the preservative acids present in silage. As the process

proceeds, temperature and pH rise and bacilli, moulds and other aerobic micro-organisms start to proliferate (9).

At ensiling, air is trapped in the ensiled mass. This oxygen is rapidly consumed (within hours) by respiratory activity of plant material and (facultative) aerobic microorganisms. Once filled, the silo should be sealed as quickly as possible, for instance with sheets of plastic or foil. Where appropriate, additional measures should be taken to prevent damages of the seal by, for instance, birds or rodents. However, since in practice the seal of a silo is never completely airtight, it is inevitable that surface layers will be exposed to air and some air will infiltrate the silage during the storage period. A high packing density of the silage is important because it restricts air infiltration into the silage during storage and after opening of the silo for feeding, when exposure to air becomes inevitable at the silage face. Another factor of importance is the silage removal rate during feeding. Maintaining a high silage removal rate minimizes infiltration of air into the silage behind the face. Finally, when preventive measures have not been successful, visibly moulded silage should be discarded before feeding, since these areas are hot-spots of ensilage-derived mycotoxins, as discussed previously.

METABOLISM OF MYCOTOXINS IN RUMINANTS AND IMPACT ON FOOD SAFETY

The significance of a mycotoxin occurring in feed with respect to animal health and the safety of animal food products for consumers is dependent on its metabolism in the animal, its toxicological effects in man and animals, and its carry-over from feed into milk, meat or organs. After intake via silage or another feed, mycotoxins, like other xenobiotics, follow the typical pharmacokinetic cascade of uptake from the gastrointestinal tract to the blood, internal distribution, metabolism, storage/remobilization and excretion. The rumen has an important function in the metabolism of mycotoxins in ruminants. It contains a complex and dense microflora with a high biodegradative power. Some mycotoxins are rapidly metabolized in the rumen into less toxic metabolites, some are transformed into equally toxic or more toxic metabolites and some are not transformed at all (5, 38). DON and ochratoxin A are examples of mycotoxins that are transformed into less toxic metabolites in the rumen. For that reason cattle are less

sensitive to these mycotoxins than non-ruminant animals such as pigs. Zearalenone is transformed in the rumen into different metabolites, with varying toxic activities. Fumonisin and aflatoxin B₁ are not metabolized in the rumen. Aflatoxin B₁ is transformed into aflatoxin M₁ in the liver of ruminants. Aflatoxin M₁ is less mutagenic and genotoxic than aflatoxin B₁, but the cytotoxicity of aflatoxin M₁ and B₁ is similar. Information concerning the metabolism of *Claviceps* and *Neotyphodium* alkaloid mycotoxins and *A. fumigatus* mycotoxins is lacking. Research on the metabolism of roquefortin C and mycophenolic acid in cattle is currently in progress.

Aflatoxin B₁ is the only mycotoxin with significant carry-over into milk: between 1 and 6 percent is excreted in milk (as aflatoxin M₁). Carry-over rates of DON, zearalenone, fumonisin B₁, ochratoxin A and the alkaloid ergovaline appear to be at least about 100-fold lower (5, 38). Carry-over rates of other mycotoxins frequently occurring in silage are not experimentally assessed. However, there are no indications that significant transfer of these mycotoxins to milk occurs.

CONCLUSIONS

Silage can be contaminated with a wide range of mycotoxins, originating from infection of the crop in the field or from growth of moulds in silage during storage or feeding-out. Prevention of contamination of silage by mycotoxins requires different strategies. Field-derived mycotoxins can be reduced by application of proper agricultural practices in crop production. Ensilage-derived mycotoxins can be reduced by application of adequate silage management, with emphasis on prevention of aerobic spoilage. Relatively little information is available about the effects that the ensilage-derived mycotoxins produced by *Penicillium* species and *A. fumigatus* can have on animal health and productivity. This topic should receive priority in future investigations.

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Table 1. Major mycotoxigenic moulds and mycotoxins in silage crops and silages.

Mycotoxin group	Major toxin(s)	Mould species	Crop(s)	Field- or ensilage-derived
Aflatoxins	Aflatoxin B ₁ (M ₁), B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>	Maize	Field
Trichothecenes	Type A: T ₂ , diacetoxyscirpenol	<i>Fusarium langsethiae</i> , <i>F. poae</i> , <i>F. sporotrichioides</i>	Maize, Sg cereals ¹	Field
	Type B: DON, nivalenol	<i>F. graminearum</i> , <i>F. culmorum</i>	Maize, Sg cereals, grass	Field
Fumonisin	Fumonisin B ₁ , B ₂	<i>F. verticillioides</i> , <i>F. proliferatum</i>	Maize	Field
Resorcylic acid lactones	Zearalenone	<i>F. graminearum</i> , <i>F. culmorum</i>	Maize, Sg cereals, grass	Field
Ochratoxins	Ochratoxin A	<i>A. ochraceus</i> , <i>Penicillium verrucosum</i>	Sg cereals	Field
Alkaloids	Clavines, lysergic acid amide, ergotamine	<i>Claviceps purpurea</i>	Sg cereals	Field
	Lolitrems B, ergovaline	<i>Neotyphodium lolii</i> , <i>N. coenophialum</i>	Grass	Field
<i>P. roqueforti</i> toxins	Roquefortin C, mycophenolic acid	<i>P. roqueforti</i> , <i>P. paneum</i>	All types of silages	Ensilage
<i>A. fumigatus</i> toxins	Gliotoxin, fumigaclavines	<i>A. fumigatus</i>	All types of silages	Ensilage
<i>M. ruber</i> toxins	Monacolin K, citrinin	<i>Monascus ruber</i>	All types of silages	Ensilage

¹ Sg cereals: Small grain cereals (wheat, triticale, rye, barley).

Table 2. Incidence and average and maximum concentrations of the *Fusarium* mycotoxins DON and zearalenone (ZEA) in silage in different surveys.

Mycotoxin	Silage crop	Location	Year(s)	Percentage positive (total number) ¹	Concentration (mg/kg) ²		Reference
					Average (of positive samples)	Maximum	
DON	Maize	North Carolina, USA	1989-1993	76% (106)	1.85	-	29
DON	Maize	Austria	1995-1999	91% (418)	0.75	2.8	30
DON	Maize	Germany	1998	79% (24)	1.61	9.86	16
DON	Maize	Pennsylvania, USA	2001-2002	42% (62)	0.6	3.7	31
DON	Maize	Netherlands	2002-2004	72% (140)	0.85	3.14	32
DON	Maize	Netherlands	2005	100% (16)	0.93	2.39	33
DON	Maize	Denmark	2007	100% (20)	1.06	5.09	34
DON	Wheat	Netherlands	2002-2004	10% (30)	0.62	1.17	32
ZEA	Maize	North Carolina, USA	1989-1993	32% (93)	0.45	-	29
ZEA	Maize	Germany	1993-1995	38% (44)	0.05	0.17	16
ZEA	Maize	Austria	1995-1999	59% (149)	0.07	0.6	30
ZEA	Maize	Germany	1998	96% (24)	0.13	1.07	16
ZEA	Maize	Netherlands	2002-2004	49% (140)	0.17	0.94	32
ZEA	Maize	Netherlands	2005	50% (16)	0.15	0.48	33
ZEA	Grass	Netherlands	2002-2004	6% (120)	0.09	0.31	32
ZEA	Grass	Netherlands	2005	13% (16)	0.13	0.21	33

¹ The percentage of positive samples and total number of samples analysed.

² Concentration in dry matter.

Table 3. Incidence and average and maximum concentrations of the *A. fumigatus* mycotoxins gliotoxin, verruculogen and fumigaclavin C and the *M. ruber* mycotoxin monacolin K in maize silage samples of different quality (normal versus visibly moulded), reported by Richter *et al.* (13).

Mycotoxin	Silage quality	Percentage positive ¹	Concentration (mg/kg)	
			Average (of positive samples)	Maximum
Gliotoxin	Normal	2.5%	0.01	0.01
Gliotoxin	Moulded	13.9%	0.09	0.51
Verruculogen	Normal	5.9%	0.01	0.02
Verruculogen	Moulded	13.9	0.05	0.25
Fumigaclavin C	Normal	3.4%	0.03	0.04
Fumigaclavin C	Moulded	46.3%	0.92	18.8
Monacolin K	Normal	6.8%	0.13	0.45
Monacolin K	Moulded	14.8%	7.32	54.6

¹ The percentage of positive samples; 118 samples of normal quality and 108 visibly moulded samples were analysed.

Table 4. Recommended agricultural practices for the prevention of development of field-derived mycotoxins (37).

Apply crop rotation, to reduce infection pressure

Remove crop residues from field, for instance by deep ploughing, to reduce infection pressure

Use seed varieties developed for resistance to fungal infections

Apply fertilization in conformity to crop demand, to avoid plant stress

Apply good agronomic practices (irrigation, weed control, plant spacing) and avoid plant stress from high temperatures and drought

Apply proper phytosanitary measures on seeds and crops, to avoid insect damage and fungal infections

Minimize mechanical damage, to avoid plant stress and fungal infections

Figure 1. The incidence (graph A) and average concentrations (graph B) of roquefortin C (Roq C) and mycophenolic acid (MPA) in samples taken from the centre, top surface layer and visibly moulded areas of 16 maize silages at dairy farms, reported by Driehuis *et al.* (33).

