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STRICTLY CONFIDENTIAL **DUTCH DAIRY COMPANIES ONLY**

FACT SHEET
MYCOTOXINS IN DAIRY CATTLE FEED AND MILK

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NIZO food research

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Summary

This fact sheet summarizes current scientific knowledge and results of projects that were conducted by NIZO food research within the GRZ programme about the major mycotoxins in feedstuffs of concern to dairy cattle and dairy products.

The most common mycotoxins that can be present in the diet of dairy cows in The Netherlands are deoxynivalenol (DON) and zearalenone, which are produced by *Fusarium* moulds, and the silage-associated mycotoxins roquefortin C and mycophenolic acid, produced by *Penicillium roqueforti*. The carry-over of these mycotoxins from feed into milk is very low and therefore they are not of concern with respect to the food safety of dairy products. These mycotoxins are primarily of concern because of their potential detrimental effects on animal health (leading to immunosuppression and reduced reproductive efficiency) and productivity (leading to reduced feed intake and milk production). However, little information exists about the effects of these mycotoxins on animal health and productivity in Dutch dairying practice. Intake of silage-associated mycotoxins by cows can be prevented by application of good silage management at the farm. The only mycotoxin in feed of potential concern with respect to food safety of dairy products is aflatoxin B_1. This is due to its significant carry-over into milk, as aflatoxin M_1, and its carcinogenic effects. Aflatoxin B_1 in feed and aflatoxin M_1 in milk are effectively controlled in The Netherlands and additional control measures regarding this mycotoxin are not required. The overall conclusions is that the occurrence of mycotoxins in the diet of dairy cows in The Netherlands is not of significant concern with respect to the safety of dairy products for consumers.

Background

Mycotoxins belong to a large, diverse group of naturally occurring toxic metabolites produced by moulds. Currently, more than 300 mycotoxins have been identified. However, only a limited number of these form a potential risk to human and animal health.

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Mycotoxins can be found in a wide variety of crops all around the world, including crops that are commonly used as dairy cattle feeds, such as maize, cereals, soybean and grasses. It is estimated that one quarter of the world's production of agricultural crops is contaminated with mycotoxins to some extent. Some mycotoxins, such as deoxynivalenol (DON), zearalenone and roquefortin C frequently occur in dairy cattle feeds, whereas others are only found sporadically. From the perspective of dairying, mycotoxins in feed are of dual concern. Firstly, they can have detrimental effects on animal health and, consequently, cause production losses. Secondly, mycotoxins may jeopardize the safety of milk and milk products, because of the possibility of (partial) carry-over from feed to milk. Of the major mycotoxins in dairy cattle feeds this is only the case for aflatoxin.

This fact sheet summarizes information on the following topics:

- Major classes of toxinogenic moulds and mycotoxins
- Conditions leading to mycotoxin formation in feeds
- Occurrence of mycotoxins in feedstuffs of dairy cows
- Metabolism of mycotoxins in ruminants
- Toxic effect of mycotoxins in dairy cattle
- Carry-over of mycotoxins from feed into milk
- Prevention
- Decontamination and detoxification strategies
- Detection methods
- Legislation regarding mycotoxins for milk and milk products and for dairy cattle feed
- Aflatoxin M_1 concentrations in milk and milk products

Major classes of toxinogenic moulds and mycotoxins

The most important mycotoxin-producing mould species relevant to dairy cattle feeds are listed in Table 1 [1].

Aflatoxins are produced by *Aspergillus flavus* and, to a lesser extent, *Aspergillus parasiticus*. These mycotoxins are highly toxic and carcinogenic to man and animals. Aflatoxins are found worldwide, especially in warm and humid climates. Aflatoxins occur in several forms, of which aflatoxin B_1, B_2, G_1, G_2 are the most common. Reference is most frequently made to aflatoxin B_1, which is the most prevalent and most toxic form (genotoxic carcinogen). An important feature in dairying is the fact that aflatoxin B_1 shows a significant carry-over into the milk in its hydroxylated metabolized form aflatoxin M_1.

Trichothecenes constitute a family of more than 100 structurally related compounds produced by a number of fungal genera, of which *Fusarium* is the major genus implicated in food and feed. The trichothecenes are divided into four groups, type A, B, C and D, of which type A and B occur most frequently. The major type A trichothecenes are T-2 toxin and

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diacetoxyscirpenol. The major type B trichothecenes are deoxynivalenol (DON; synonym vomitoxin) and nivalenol. DON is the most prevalent trichothecene and is detected frequently in maize, wheat and barley. Its main toxic effect in animals is immunosuppression. The major trichothecene-producing *Fusarium* species are *Fusarium graminearum* and *Fusarium culmorum* (type B producers) and *Fusarium langsethiae, Fusarium poae* and *Fusarium sporotrichioides* (type A producers). These species are plant pathogens.

Zearalenone is another mycotoxin produced by *Fusarium* species, primarily by *Fusarium graminearum* and *Fusarium culmorum*, species that are also implicated in production of DON. Like DON, zearalenone is detected frequently in maize and wheat. Zearalenone has oestrogenic activity and can affect the fertility of animals.

Occurrence of **fumonisins** is specifically associated with maize. Fumonisin B_1 and B_2 are the most prevalent compounds. These mycotoxins are produced specifically by *Fusarium verticillioides* (syn., *Fusarium moniliforme*) and *Fusarium proliferatum*, plant pathogens of maize.

Ochratoxin A is produced by *Aspergillus* and *Penicillum* species, *Aspergillus ochraceus* and *Penicillum verrucosum* in particular. *Penicillium* tends to be more prevalent in cooler climates and *Aspergillus* in warmer climates. The significance of ochratoxin A in dairying is restricted to preruminant calves. In dairy cattle feed ochratoxin A is mainly associated with cereal grains and by-products of copra (coconut) processing.

Table 1. Major mycotoxigenic moulds and major mycotoxins produced by these moulds.

Genus	**Species**	**Mycotoxin produced**
Aspergillus	*flavus, parasiticus,* *ochraceus*	Aflatoxin B_1 (M_1), B_2, G_1, G_2 Ochratoxin A
Fusarium	*graminearum, langsethiae,* *culmorum,* *poae, sporotrichioides,* *verticillioides, proliferatum*	Deoxynivalenol (DON), nivalenol, zearalenone, T2-toxin, diacetoxyscirpenol, Fumonisins
Penicillium	*verrucosum,* *roqueforti*	Ochratoxin A Roquefortine C, mycophenolic acid
Claviceps	*purpurea*	Clavines, lysergic acid amide, ergot alkaloids
Neotyphodium	*lolii,* *coenophialum*	Lolitrem B Ergovaline

Ergot alkaloids are produced by *Claviceps purpurea*, a pathogen of grains and grasses, and *Neotyphodium* species that are endophytic symbionts of many grasses. Ergotism caused by grains containing high levels of *Claviceps* sclerotia is one of the oldest known mycotoxicoses. Syndromes such as rye grass staggers and fescue toxicoses are associated with the occurrence of *Neotyphodium* mycotoxins (e.g. lolitrem B and ergovaline) in areas where grazing of wild grasses in pasture is common. A number of mycotoxins relevant to dairy cattle are exclusively associated with moulds developing in poorly preserved silage.

Ensiled forage crops such as grass, maize and lucerne (alfalfa) form a major part of the feed ration of dairy cattle in many parts of the world. The most frequently detected **silage-associated mycotoxins** are roquefortin C and mycophenolic acid. Both compounds are produced by *Penicillium roqueforti*, an acid-tolerant species requiring low levels of oxygen for growth. Less frequently detected mycotoxins associated with silage include monacolin K, produced by *Monascus ruber*, and gliotoxin and the tremorgens fumigaclavine A and B, produced by *Aspergillus fumigatus*.

Other mycotoxins, such as rubratoxin, citrinin, PR-toxin, patulin, cyclopiazonic acid and sterigmatocystin, are occasionally detected in dairy cattle feeds and may in rare cases be important in dairying.

Conditions leading to mycotoxin formation in feeds

Growth of mycotoxigenic moulds and production of mycotoxins is influenced by environmental conditions (temperature, water activity, levels of oxygen and carbon dioxide, availability of nutrients). A distinction is made between mycotoxins that are formed during growth of a crop in the field and mycotoxins that are formed after harvesting, for instance during storage of grain with a high moisture content or during storage of ensiled feeds.

Aflatoxins can be either field-derived or storage-derived. These mycotoxins are mainly associated with crops grown in subtropical and warm temperate climates. Feedstuffs with a high risk of aflatoxin contamination include maize gluten and other maize by-products, products based on copra/coconut and groundnuts, cottonseed and sunflower cake. *Fusarium* mycotoxins (DON, zearalenone, fumonisins) are generally field-derived mycotoxins. *Fusarium* species are pathogens of maize, wheat, barley and grasses. Plant diseases associated with *Fusarium* infection include ear blight in wheat and ear rot and stalk rot in maize. Plants become contaminated via infection of kernels, leaves or stalks, or via 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. High levels of DON in wheat appear to be associated with high rainfall late in the growing season.

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Growth of moulds and production of mycotoxins in silage usually results from poor silage management practices. The extent of air infiltration during storage is an important factor as growth of moulds is not possible in absence of oxygen. Growth of moulds in silage is usually highest in surface layers, essentially because these layers have the highest exposure to oxygen. The most abundant mould in silage is *Penicillum roqueforti*. This species is capable of producing various mycotoxins, including roquefortine C, mycophenolic acid, PR-toxin and patulin. Roquefortine C (a neurotoxin) and mycophenolic acid (an immunosuppressant) are frequently occurring in silages. These mycotoxins have rather low toxicity for animals and humans. Low concentrations of roquefortine C and mycophenolic acid have also been detected in commercial blue-mould ripened cheeses. PR-toxin and patulin have higher toxicity but have only sporadically been detected in silages, presumably because they are unstable in the silage environment. *Penicillum roqueforti* is tolerant to acidic conditions, low oxygen levels and high carbon dioxide levels. It forms grey spots on silage surfaces, but is also responsible for the occasional occurrence of typical blue-green balls or lumps of mouldy silage in maize silage, which can be observed about 50 to 100 cm below the top surface (Figure 1).

Figure 1. Examples of maize silage with growth of *Penicillum roqueforti* on the surface (left) and in blue-green balls (right).

Other moulds that can frequently occur in silage are *Penicillum paneum* (closely related to *Penicillum roqueforti*), *Monascus ruber* (capable of producing monacolin K and citrinin) and *Aspergillus fumigates* (capable of producing gliotoxin, fumigaclavines and different other mycotoxins). Since the distribution of moulds in silage is highly heterogeneous, this is also the case for mycotoxins produced by these moulds. This is depicted in Figure 2, 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 [2].

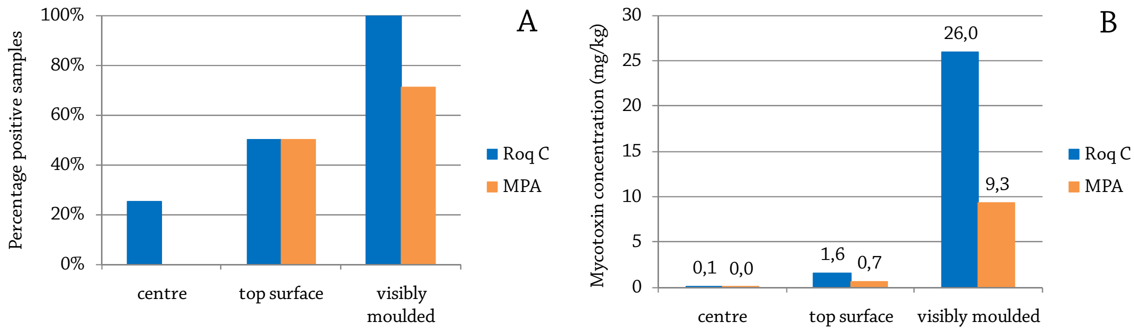

Figure 2. Incidence (A) and average concentrations (B) of roquefortin C (Roq C) and mycophenolic acid (MPA) in samples taken from the centre, top surface layer and visibly moulded areas of 16 maize silages at Dutch dairy farms [2].

Occurrence of mycotoxins in feedstuffs of dairy cows

The diet of Dutch dairy cows with a high milk yield consists of three categories of feedstuffs: forages, concentrates and wet by-products. Grass is the main forage and is fed fresh, as silage, or as hay (dried). Maize is the second forage and is fed as silage only. Concentrates are feeds high in energy or protein, usually dried and fed as blends of different ingredients (compound feeds) or as individual feeds. The composition of compound feed depends on market prices of the ingredients and on the needs of the animal. The third category, wet by-products, are used by about half of the Dutch farmers and are stored as silage at the farm. Examples are pressed sugar beet pulp, potato pulp, brewer's spent grains and maize gluten feed. Forages, concentrates and wet by-products constitute on average 70%, 25% and 5%, respectively, of the dry matter of the diet of lactating cows [2].

Due to the great influence of weather and storage conditions on development of mycotoxins, occurrence and concentrations are variable by year and geographical origin and also depend on farm management. Table 2 lists feedstuffs, commodities and forages that carry a high risk of contamination with mycotoxins.

A survey was conducted in 2005 to determine the occurrence of mycotoxins in feedstuffs of dairy cows in The Netherlands and to estimate total dietary intakes of mycotoxins [2]. Twenty-four randomly selected dairy farms were visited twice. A total of 169 feed samples were collected and analysed for 20 mycotoxins. DON, zearalenone, roquefortin C and mycophenolic acid were identified as the mycotoxins with the highest incidence. Fumonisins B_1 and B_2 were detected in three concentrate feed samples. Aflatoxins, ochratoxin A, T2-toxin and a number of less known mycotoxins were not detected in any of the samples. The results of this study are summarized in Table 3. As expected, roquefortin C and mycophenolic acid were detected in silages and ensiled by-products only, whereas DON and zearalenone were detected in concentrate feeds and in silages. Maize silage was found to be the most important source of all of the four mycotoxins by far: about 80% of the total dietary intake of DON and zearalenone and more than 95% of that of roquefortin C and mycophenolic acid originated from maize silage. The carry-over of DON, zearalenone, roquefortine C, and mycophenolic acid from feed into milk is negligible (see below). Therefore, their occurrence in the diet of dairy cows is not considered to be a hazard with respect to the safety of dairy products for consumers. Possible animal health effects were not monitored in the study. However, since none of the feedstuffs and complete diets analysed in the study exceeded the maximum concentrations of DON and zearalenone recommended by the EU, no acute effects on animal health are expected.

Table 2. High risk feedstuffs and forages for the major mycotoxins [1, 3].

Mycotoxin	High risk feedstuffs
Aflatoxin B_1	Copra/coconut, peanut/groundnut products, cotton seed, forage maize and maize products from warm climate areas
DON	Forage maize, maize products, wheat products
Zearalenone	Forage maize, maize products, wheat products, soy hulls
T2-toxin	Wheat products from cold climate areas
Fumonisins	Forage maize and maize products from warm climate areas
Ochratoxin A	High moisture cereal products
Neotyphodium alkaloids	Grasses from natural grasslands and extensively managed pastures
Silage-derived mycotoxins	All types of ensiled forages and wet by-products

Table 3. Incidence and the average and maximum concentrations of DON, zearalenone, roquefortin C and mycophenolic acid in the total diet of dairy cows in The Netherlands [2].

Mycotoxin	Incidence	Concentration (mg/kg)	
		Average	**Maximum**
DON	81%	0.32[1] (0.27[2])	0.97
Zearalenone	46%	0.04 (0.03)	0.20
Roquefortin C	23%	0.43 (0.11)	2.21
Mycophenolic acid	17%	0.27 (0.05)	1.84

[1] Average concentration of positive diets

[2] Average concentration of all diets

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Mycotoxins produced by *Aspergillus fumigatus* were not analysed in the Dutch survey in 2005 [2]. However, considering that *Aspergillus fumigatus* has a high prevalence in visually moulded silage, a high incidence of mycotoxins produced by this mould can be expected. This was confirmed in a recent German study, in which fumigaclavin C was detected in 46% of moulded maize silage samples [4].

Metabolism of mycotoxins in ruminants

The rumen has an important function in the metabolism of mycotoxins in ruminants. It contains a dense complex 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 (Table 4) [1, 5].

Table 4. Rumen detoxification of major mycotoxins and metabolites formed in rumen, blood or organs [1, 5, 6].

Mycotoxin	Detoxification in rumen	Major metabolites
Aflatoxin B_1	No	Aflatoxin M_1
DON	Yes	De-epoxy-DON
T2-toxin	Yes	HT2-toxin, acetyl-HT2, de-epoxy-T2
Zearalenone	No	α- and β-zearalenol, α- and β-zearalanol
Fumonisin B_1	No	None
Ochratoxin A	Yes	Ochratoxin α
Roquefortin C	No	None
Mycophenolic acid	No	None
Monacolin K	Unknown	Unknown
A. fumigatus mycotoxins	Unknown	Unknown
Claviceps alkaloids	No	Unknown
Neotyphodium alkaloids	No	Unknown

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 exposure to these mycotoxins via feed than non-ruminant animals such as pigs. Zearalenone is transformed in the rumen into different metabolites, with varying toxic activities. Fumonisins and aflatoxin B_1 are not metabolized in the rumen. Aflatoxin B_1 is transformed into aflatoxin M_1 in the liver of ruminants. Aflatoxin M_1 is less mutagenic and genotoxic than aflatoxin B_1, but the cytotoxicity of aflatoxin M_1 and B_1 is similar. Information concerning the metabolism of

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Claviceps and *Neotyphodium* alkaloid mycotoxins and *Aspergillus fumigatus* mycotoxins is lacking. Recent research by NIZO food research indicated that the silage-derived mycotoxins roquefortin C and mycophenolic acid are not metabolized in the rumen [6].

Toxic effect of mycotoxins in dairy cattle

Most mycotoxins are less toxic to cattle than to monogastric animals, such as pigs and poultry, which can partly be explained by the degradation of mycotoxins in the rumen. High levels of mycotoxins in feed can cause acute health problems. The major clinical signs or symptoms due to exposure to mycotoxins in dairy cattle and critical concentrations in feed are listed in Table 5. Cases of acute mycotoxicosis in dairy cattle are rare in The Netherlands and neighbouring countries. However, the impact of chronic health problems due to the frequent exposure of animals to low doses of a mycotoxin or multiple mycotoxins is probably much greater than that of acute health problems. These chronic health problems include reduced feed intake and milk production, increased incidence in diseases due to immune system suppression and reduced reproductive performance.

Table 5. Major toxic effects of mycotoxins in dairy cattle [1, 5].

Mycotoxin	Clinical signs or symptoms	Critical concentration (mg/kg)[1]
Aflatoxin B_1	Reduced feed intake and milk production, reduced reproductive efficiency, immunosuppression	0.1 - 0.2
DON	Reduced feed intake and fat corrected milk production, unthriftiness	2.7 – 6.5
T2-toxin	Reduced feed intake and milk production, diarrhoea, intestinal irritation	0.3 – 0.5
Zearalenone	Reduced conception rates, enlarged teats, vaginitis	13
Fumonisin B_1	Reduced feed intake and milk production, mild liver damage	100 – 150
Ochratoxin A	Reduced feed intake and milk production, diarrhoea	5 – 35
Roquefortin C	Reduced feed intake, reduced rumen fluid pH, ketosis, paralysis, abortion	25
Claviceps alkaloids	Reduced feed intake and milk production, reduced reproductive efficiency, diarrhoea, lameness, gangrene	Unknown
Neotyphodium alkaloids	Ryegrass staggers and other tremorgenic syndromes, fescue toxicosis syndromes, immunosuppression	Unknown

[1] Critical concentration in total feed ration (mg/kg dry matter)

Carry-over of mycotoxins from feed into milk

The extent of the transmission of mycotoxins or metabolites from feed to milk (carry-over) is of key importance with regard to food safety aspects of milk and milk products and compliance with legal standards. Excretion of mycotoxins and metabolites with milk in lactating animals can occur only when the mycotoxin or metabolite is able to pass the blood-milk barrier. Published carry-over rates of mycotoxins are listed in Table 6. Aflatoxin B_1 is the only mycotoxin with significant carry-over into milk (as aflatoxin M_1). Estimates vary between approximately 1 and 6 percent, with an average of approximately 2.5 percent.

Table 6. Carry-over rates of major mycotoxins into milk [1, 6, 7].

Mycotoxin	Carry-over rate	Substance(s) analysed
Aflatoxin B_1	1 to 6% (average 2.5%)	Aflatoxin M_1
DON	≤ 0.02%	DON plus metabolites
T2-toxin	≤ 0.02% [2]	T2-toxin
Zearalenone	≤ 0.01%	Zearalenone plus metabolites
Fumonisin B_1	< 0.01%	Fumonisin B_1
Ochratoxin A	≤ 0.03% [1]	Ochratoxin A
Ergovaline	< 0.02% [1]	Ergovaline
Claviceps alkaloids	< 0.01%	*Claviceps* alkaloids
Roquefortin C	0.002-0.004%	Roquefortin C
Mycophenolic acid	0.003-0.005%	Mycophenolic acid, plus mycophenolic acid glucuronide
Monacolin K	Unknown	Unknown
Aspergillus fumigatus mycotoxins	Unknown	Unknown

[1] Based on study with lactating goats;
[2] With measurements based on T2-toxin plus metabolites carry-over is ≤ 0.2%.

Figure 3 shows the appearance of aflatoxin M_1 in milk after ingestion of aflatoxin B_1-contaminated feed and the clearance of aflatoxin M_1 from milk after feeding of the contaminated feed was stopped [16]. Aflatoxin M_1 became detectable in milk already one day after the start of feeding the contaminated feed. About three days after feeding was stopped the milk was free of aflatoxin M_1. Also in an Italian study, in which cows were fed 10-times less aflatoxin B_1 and produced milk containing approximately 0.05 µg/kg of aflatoxin M_1 (the maximum level allowed in the EU; see below), milk was free of AFM1 three days after feeding of the contaminated feed was stopped [17].

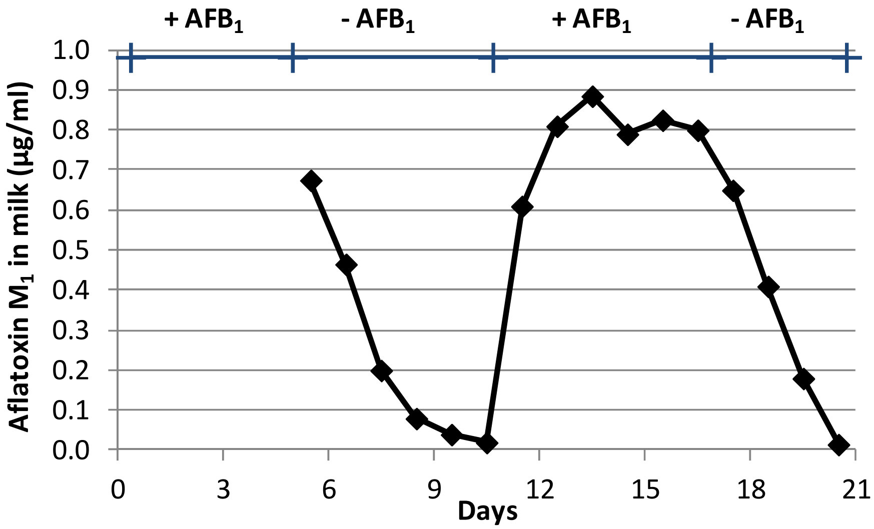

Figure 3. Appearance of aflatoxin M_1 in milk of dairy cows fed a diet with aflatoxin B_1 and clearance of aflatoxin M_1 from milk after the source of aflatoxin B_1 was removed from the diet. The concentration of aflatoxin B_1 in the diet was 55 ppb and the source was contaminated maize. The cows were the diet with aflatoxin B_1 for 5 days (day 1 through 5), then an aflatoxin B_1-free diet from day 6 to 11, then again the diet with aflatoxin B_1 from day 12 to 18, and finally again the aflatoxin B_1-free diet from day 18 to 21. Data adapted from Diaz *et al*. [16].

Carry-over rates of ochratoxin A, DON, T2-toxin, zearalenone, fumonisin B_1 and the *Neotyphodium* alkaloid ergovaline are at least about 100-fold lower. Experimental assessment of the carry-over rates of these mycotoxins is complicated by the fact that no measurable concentrations can be detected in milk of animals that have been fed a diet with naturally contaminated feedstuffs. In some studies the carry-over rate has been determined by adding high doses of purified mycotoxin to the diet. This approach has also been applied in recent research by NIZO food research to determine the carry-over of roquefortin C and mycophenolic acid (0.002-0.004% and 0.003-0.005%, respectively). Carry-over rates of *Claviceps* alkaloids, monacolin K and *Aspergillus fumigatus* mycotoxins have not been assessed experimentally. However, there are no indications that significant transfer of these mycotoxins to milk occurs.

Prevention

Strategies to prevent mycotoxin contamination of feed require a distinction between field-derived and storage-derived mycotoxins [1].

Prevention of **field-derived mycotoxins** focuses on reduction of the infection pressure of moulds and reduction of the susceptibility of the plant to fungal infections. Recommended measures to reduce the infection pressure are application of crop rotation and removal of crop residues from the field after harvesting, for instance by deep ploughing. Recommended measures to reduce plant susceptibility to fungi include the use of varieties that have been developed for their resistance to fungal infections, adequate fertilization, application of good agricultural practices to avoid plant stress from high temperatures and drought (irrigation, weed control, plant spacing) and prevention of mechanical and insect damage.

Prevention of **storage-derived mycotoxins** in dry feedstuffs primarily relates to adequate drying techniques and maintenance of uniform temperature and moisture regimes during storage. The most important factor to prevent mould growth and mycotoxin formation in silage is to limit exposure to oxygen during storage and to restrict infiltration of oxygen after opening the silo for feeding. Harvested crops should be ensiled as rapidly as possible. Creating a high packing density during filling of clamp or bunker silos is extremely important because it restricts air infiltration. Once filled, the silo should be sealed as quickly as possible with an air-tight cover. Measures should be taken to prevent damages of the seal by for instance birds or rodents. After opening the silo for feeding exposure to oxygen is inevitable at the silage face. However maintaining a high silage removal rate should minimize infiltration of oxygen into the silage behind the face. Finally, large spots of visibly moulded silage should be discarded before feeding, since these spots are likely to contain high concentrations of mycotoxins, as described earlier (more information on good silage making practices is given in the fact sheet Silage management).

Decontamination and detoxification strategies

Techniques have been developed for physical separation of 'clean' and mould-contaminated wheat grains or maize kernels based on differences in colour or density. In addition, techniques are available for degradation of mycotoxins by means of treatment with chemicals. However, these techniques are of little relevance at the level of individual dairy farms. Another approach to control the impact of mycotoxin contamination is the inclusion of additives in the animal diet that bind or degrade the mycotoxin in the intestinal tract of the animal. Mycotoxin binders and adsorbents include inorganic materials, such as clays, bentonites and aluminosilicates, and organic materials, such as activated charcoal and β-D-glucans from yeast cell walls. The objective of these substances is to reduce the bio-availability of mycotoxins in the animal digestive tract. However, the binding capacity of the materials varies. For instance, clays and other inorganic adsorbents have been shown to be effective binders of aflatoxins but have only limited or no effect on other mycotoxins. Another approach is the addition of enzymes or micro-organisms in the diet that are able to degrade mycotoxins. More research is required on the efficacy and possible side-effects of these additives in dairy cattle.

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Detection methods

Sampling is an important aspect of the determination of mycotoxins in feedstuffs as the distribution in raw materials and feed ingredients is often heterogeneous. To obtain representative samples, sampling methods consist of withdrawing a large number of incremental samples from a lot and mixing them to an aggregate sample. For analysis of silages an alternative approach is to sample after the feed has been mixed in a mixing wagon.

A variety of methods exists for the determination of mycotoxins [1, 8]. Rapid screening methods based on enzyme-linked immunosorbent assay (ELISA) are commercially available and are routinely used in the screening of for instance aflatoxin M_1 in milk and DON, zearalenone and total aflatoxins in raw materials for feedstuffs. Advantages of these methods are speed, ease of operation and high throughput. On the other hand, sophisticated chromatographical methods have been developed that allow simultaneous determination of different mycotoxins using liquid chromatography–mass spectrometry (LC-MS/MS). Using these so-called multi-analyte methods more than 20 mycotoxins can be determined in a single analysis run [2, 9].

Legislation regarding mycotoxins for milk and milk products and for dairy cattle feed

Regulations for aflatoxin M_1 in milk are in place in 60 countries. In the EU and EFTA (Switzerland, Norway, Iceland and Liechtenstein) member countries a maximum level of 0.05 μg/kg applies to raw and heat-treated milk and to milk for the manufacture of milk-based products. A maximum level of 0.025 μg/kg applies to infant milk and follow-on milk. A 10-fold higher maximum level of aflatoxin M_1 in milk (0.5 μg/kg) is allowed in the USA, MERCOSUR (Argentina, Brazil, Paraguay and Uruguay) and several Asian countries [1].

The difference between the two limits for aflatoxin M_1 has given rise to debates within Codex Alimentarius, leading to a re-evaluation of the human health risk of aflatoxin M_1 conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA concluded in 2001 that, with worst case assumptions, there is no significant health benefit of implementing a limit of 0.05 μg/kg milk compared with a limit of 0.5 μg/kg milk. Based on this result, the Codex Alimentarius established a limit concentration of 0.5 μg/kg for aflatoxin M_1 in milk [10]. The current EU position is that in practice the level of 0.05 μg/kg for aflatoxin M_1 in milk is achievable following the ALARA (As Low As Reasonably Achievable) principle and therefore the EU does not accept the higher level [11].

Table 7 gives an overview of maximum permitted concentrations of mycotoxins in different types of feedstuffs.

Table 7. Maximum levels of mycotoxins in EU legislation and products standards issued by GMP+ International.

Mycotoxin	Source	Feedingstuff	Maximum level (mg/kg)
Aflatoxin B_1	EU 2002/32	Complete and complementary feed	0.005
	GMP+	Complete, supplementary and feed materials for direct delivery to farms	0.005
DON	EU 2006/576	Complete and complementary feed	5
	EU 2006/576	Cereals and cereal products	8
	EU 2006/576	Maize by-products	12
	GMP+	Feed on full ration basis	3 (2.4)[1]
	GMP+	Feed material supplied to farms for immediate feeding	9 (3)
Zearalenone	EU 2006/576	Complete and complementary feed	0.5
	EU 2006/576	Cereals and cereal products	2
	EU 2006/576	Maize by-products	3
	GMP+	Feed on full ration basis	0.5 (0.4)[1]
	GMP+	Feed material supplied to farms for immediate feeding	1.5 (0.2)[1]
Fumonisin B_1+B_2	EU 2006/576	Maize and maize products	60
Ochratoxin A	EU 2006/576	Cereals and cereal products	0.25
Rye ergot (*Claviceps purpurea*)	EU 2002/32 & GMP+	Feed containing unground cereals/grains	1000

[1] Value between brackets is the action limit. According the GMP+ system, feed suppliers should undertake an investigation into the cause and should take corrective measures to remove or control that cause if the action limit is exceeded.

Aflatoxin B_1 and rye ergot (*Claviceps purpurea*) are listed in EU Directive 2002/32 as undesirable substances in animal feed [12]. For DON, zearalenone, ochratoxin A and fumonisins B_1 and B_2 the EU issued non-statutory, recommended maximum levels (guidance values) in animal feed materials (EU Recommendation 2006/576) [13]. Presumably, these guidance values will be transformed into statutory values, *i.e*. official maximum levels, in the future. Finally, suppliers of feed materials in The Netherlands are required to comply with products standards regarding mycotoxins issued by GMP+ International.

The maximum permitted level for aflatoxin B_1 in feedstuffs for dairy cattle of 0.005 mg/kg of EU Directive 2002/32 is identical to the level that was agreed between by the Dutch dairy industry and feed industry in the “Aflatoxine Convenant” in 1989. The maximum level for

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aflatoxin B_1 in feed materials in general and complete feedstuffs for other animals than dairy cattle is 0.020 mg/kg and 0.010 mg/kg for calves and lambs (EU Directive 2002/32).

Aflatoxin M_1 concentrations in milk and milk products

Analysis of aflatoxin M_1 in raw milk is part of the monitoring programme of the Dutch dairy industry on residues and contaminants in milk. In this programme, which is being conducted by Qlip, about 450 composite raw milk samples, composed of three raw milk samples from individual farms, are analysed annually. When a composite sample exceeds 0.020 µg/kg of aflatoxin M_1, the individual raw milk samples of the sample are also analysed and the sample with elevated aflatoxin M_1 is identified. Next, the farmer and suspect feed suppliers are informed and measures are taken to identify and eliminate the source of aflatoxin M_1 as soon as possible. In the great majority of composite samples the aflatoxin M_1 concentration is less than the detection limit of 0.010 µg/kg. Of 2713 composite raw milk samples analysed between 2006 and 2010 none contained aflatoxin M_1 in a concentration higher than 0.050 µg/kg and only three samples (0.1%) contained a concentration between 0.010 and 0.050 µg/kg (NZO database Residues and Contaminants, Zuivelweb). The Dutch Food and Consumer Product Safety Authority (nVWA) analyses aflatoxin M_1 in retail milk as part of a governmental testing programme ("Nationaal Plan") enforced by EU legislation. In none of 90 milk samples analysed between 2008 and 2010 was aflatoxin M_1 detected at levels above the EU maximum level of limit of 0.050 µg/kg. These data indicate that aflatoxin M_1 in milk is adequately controlled in The Netherlands.

Surveys conducted in 13 EU countries between 1992 and 2003, summarized by EFSA [15], indicated that 0.06% of a total of 11,831 raw milk samples (2 samples from Portugal and 5 from Greece) contained aflatoxin M_1 in a concentration higher than 0.050 µg/kg, whereas 3.8% samples contained aflatoxin M_1 in a concentration between 0.010 and 0.050 µg/kg. These data indicate that aflatoxin M_1 in milk is adequately controlled in most EU countries, but apparently less than in The Netherlands.

In the USA, in particular in the Southern States, reported levels of aflatoxin M_1 in milk are higher than in Europe. In surveys performed in the Southern and Midwestern States 4% to 40% of raw milk samples contained aflatoxin M_1 in a concentration higher than 0.050 µg/kg, whereas 0.1% to 18% samples contained aflatoxin M_1 in a concentration higher than the maximum level in the USA of 0.50 µg/kg [10]. The relatively high aflatoxin M_1 contamination level of milk in Southern States of the USA in comparison with EU countries probably relates to the high incidence of aflatoxin B_1 in maize and maize products in the USA. In south Asia, very high levels of aflatoxin M_1 can be found in milk. Surveys conducted in Indonesia, Thailand and the Philippines indicated that about 80% of the milk samples

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contained aflatoxin M_1 in a concentration higher than 0.050 µg/kg and about 20% samples contained a concentration higher than 0.50 µg/kg [10].

Main conclusions

- The most common mycotoxins in the diet of dairy cows in The Netherlands are the *Fusarium* mycotoxins DON and zearalenone and the silage-associated mycotoxins roquefortin C and mycophenolic acid;
- Maize silage is the most important source of mycotoxins;
- The carry-over of mycotoxins from feed into milk is very low for all relevant mycotoxins, except aflatoxin B1;
- Aflatoxin B_1 in feed and aflatoxin M_1 in milk are adequately controlled in The Netherlands;
- The occurrence of mycotoxins in the diet of dairy cows in The Netherlands is not of significant concern with respect to the safety of dairy products for consumers;
- Mycotoxins in the diet of dairy cows in The Netherlands potentially can have detrimental effects on animal health and productivity. However, these effects are not well understood;
- Aerobic spoilage of silage, in particular maize silage, is the main cause of silage-associated mycotoxins. Occurrence of these mycotoxins can be prevented by application of good silage management at the farm.

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