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Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.).
Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009

pages 205-224

Article available on line / Article disponible en ligne à l'adresse :

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To cite this article / Pour citer cet article

Jouany J.P., Yiannikouris A., Bertin G. **Risk assessment of mycotoxins in ruminants and ruminant products**. In : Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). *Nutritional and foraging ecology of sheep and goats*. Zaragoza : CIHEAM / FAO / NAGREF, 2009. p. 205-224 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85)



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Risk assessment of mycotoxins in ruminants and ruminant products

J.P. Jouany*, A. Yiannikouris** and G. Bertin**

*INRA, UR1213 Herbivores, Research Centre of Clermont-Theix, F-63122 Saint-Genès Champanelle (France)

**Alltech Inc., 3031 Catnip Hill Pike, Nicholasville, Ky 40356 (USA)

Abstract. Mycotoxins are secondary metabolites produced by several fungi, more specifically those of the genera *Aspergillus*, *Fusarium* and *Penicillium*. They were discovered in the early 1960s, when more than 100,000 young turkeys died in the United Kingdom from an apparently new disease, which was named "turkey × disease". The toxin-producing fungus was identified as *A. flavus* and the toxin was given the name aflatoxin by virtue of its origin. Forages, cereals and protein sources such as peanuts and cottonseed are potential mycotoxin vectors in ruminant diets. New techniques for preserving wet forages, such as silages or wrapped bales, are unsafe when anaerobic conditions are not strictly controlled. Fresh grasses can also be contaminated with mycotoxins including fungal endophytes that produce toxins such as ergovaline, lolitrem B, peramins and swainsonine. It is generally considered that ruminants are less sensitive to mycotoxins than other animals. This may be due to the capacity of the ruminal microbial ecosystem to degrade or convert mycotoxins into less toxic compounds. However, there is some evidence that ruminants can be poisoned by mycotoxins, causing lower animal production or even death of animals. Part of mycotoxins ingested by ruminants can be recovered in milk or animal tissues, either in the parent form or as metabolites. The transfer rate, usually very low, is estimated at less than 1% for the major toxins. Thus animals are considered as "filters" of mycotoxins in the food chain. However, there is a risk for infants fed only on a milk diet of being exposed to mycotoxin levels that exceed the regulated limits. Although not currently authorised in Europe, some mycotoxin inactivators, based on sequestering or degrading properties, have been tested *in vitro* and *in vivo*. The mode of action of yeast β -D-glucans against mycotoxins is presented, together with measures to limit the level of feed contamination by mycotoxins.

Key words. Mycotoxin – Feedstuff – Ruminant – Transfer in milk – Toxicity – Risk mitigation.

Gestion du risque mycotoxique chez le ruminant et dans les produits du ruminant

Résumé. Les mycotoxines sont des métabolites secondaires produits par les champignons, plus particulièrement par ceux appartenant aux genres *Aspergillus*, *Fusarium* et *Penicillium*. Elles ont été découvertes vers 1960, après une intoxication ayant entraîné la mort de plus de 100 000 dindons en Angleterre. La toxine impliquée a été identifiée comme provenant d'*A. flavus*, d'où son nom d'aflatoxine. Les fourrages, les céréales, les sources protéiques issus d'arachide et de graine de coton en particulier, sont des vecteurs potentiels de mycotoxines. La conservation de fourrages humides sous forme d'ensilage ou de balles enrubbannées constituent un risque majeur si les conditions anaérobies ne sont pas respectées. On peut également trouver certaines toxines issues d'endophytes qui colonisent les fourrages verts. Les ruminants sont considérés comme peu sensibles aux mycotoxines car ces dernières peuvent être métabolisées par les microbes du rumen. Toutefois, des cas fréquents d'intoxication chez la vache laitière sont imputés aux mycotoxines. Outre leur effet sur l'animal, les mycotoxines ou leurs métabolites peuvent se retrouver dans les produits animaux destinés à l'alimentation humaine, en particulier dans le lait. Le taux de transfert est faible, de l'ordre de 1%, ce qui amène le ruminant à être considéré comme un filtre efficace des mycotoxines présentes dans les produits végétaux. Le risque existe toutefois pour les nourrissons ou les jeunes enfants dont l'alimentation est essentiellement lactée, de dépasser les doses limites réglementaires. Certains additifs capables d'inactiver les mycotoxines ont été testés *in vitro* et *in vivo*. Le mode d'action des β -D-glucanes de levure sur les mycotoxines sera présenté, ainsi que les moyens de limiter la contamination des aliments.

Mots-clés. Mycotoxines – Aliments – Ruminant – Transfert dans le lait – Toxicité – Atténuation du risque.

I – Introduction

In the 1960s more than 100,000 young turkeys on poultry farms in the United Kingdom died in a period of a few months from an unidentified disease, which was named "turkey × disease". Ducklings and other poultry animals were also affected, and high mortalities were observed. A careful survey of the inputs and environment of the affected farms indicated that the disease was associated with feeds and specifically with peanut meal imported from Brazil. A disease with symptoms typical of turkey × disease was reproduced when animals were fed the same peanut meal. Intensive investigations were then carried out on the suspected ingredient to identify the nature of the toxin, which was soon found to be of fungal origin. The toxin-producing fungus was identified as *Aspergillus flavus* (Nesbitt *et al.*, 1962) and the toxin was accordingly called aflatoxin. There has since been a growing awareness of the potential hazards of toxins produced by fungi that contaminate food and feeds, and that might be life-threatening to animals and even humans.

Historically, the oldest recognized type of human mycotoxicosis is ergotism. It is caused by toxins from *Claviceps purpurea*, and became epidemic in the Middle Ages when it was known as Saint Anthony's fire. Early symptoms were hallucinations with burning sensations on the hands and feet, and subsequent necrosis leading to loss of appendages. Although mycotoxins have always existed, studies on these toxins started only 40 years ago. Since then, numerous mycotoxins other than aflatoxins have been discovered, many of which have now been found to cause intoxications. The total number of mycotoxins that exist is not yet known, but there are probably thousands. Mycotoxins do not only account for human and animal disease; they have also a considerable economic impact on crops. The plant pathogenicity of the majority of mycotoxin-producing fungi harms crop production and crop quality. The importance of regulatory control of mycotoxins in the international grain trade and marketing of food and feeds is increasing, and concerns mainly exports from developing countries (Wilson, 2006). Mycotoxins present in animal feeds can be transferred to animal products, mainly milk, and go on to contaminate dairy produce in the food chain. The risk is significant mainly for high-milk consumers such as infants and children, but is not a concern for adults.

Analytical difficulties arising from the complexity and diversity of the chemical structures of mycotoxins slowed progress until the 1980s. However, the development of chromatographic techniques coupled with tandem mass spectrometry has allowed a decisive advance in the study of mycotoxins over the last ten years.

This paper describes the complex world of mycotoxins, their effect on ruminants, their transfer into animal products (mainly milk) and the possible ways in which the level of contamination in the food chain can be controlled.

II – What are mycotoxins?

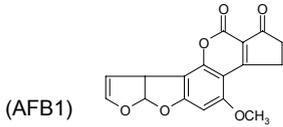
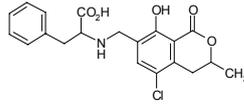
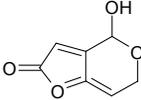
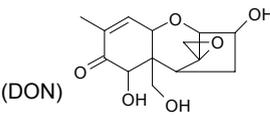
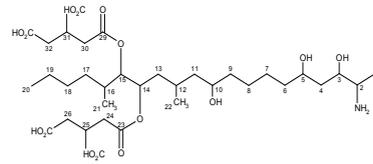
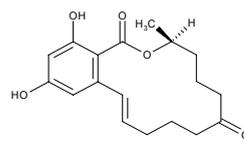
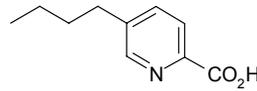
1. Definition

The word mycotoxin combines the Greek *mykos*, fungus and the Latin *toxicum*, poison. Mycotoxins are produced by fungi through their secondary metabolism. Mycotoxin concentration can therefore be independent of the growth of the fungi, which is associated with the primary metabolism. The diversity of the compounds formed and the specificity of the fungal strain for mycotoxin production result from the secondary metabolism, which is usually activated by signals from the environment (cold, heat, dryness, fungicide, etc.). Among the numerous mycotoxins, several groups have been identified, produced by the three major fungus genera *Aspergillus*, *Penicillium* and *Fusarium* (Table 1).

Toxigenic fungi have been roughly divided into two categories: (i) field fungi, which contaminate plants during their growth in the field, and are mainly represented by the genus *Fusarium*; and (ii) storage fungi, which grow after harvest during storage, transport, processing or distribution of

ingredients. *Aspergillus* and *Penicillium* are the most common fungi found after the plants have been cut.

Table 1. Major fungi and their associated mycotoxins

Fungi	Mycotoxins
<i>Aspergillus flavus</i> , <i>A. parasiticus</i> , <i>A. nomius</i>	Aflatoxins B1, B2, G1, G2 
<i>Penicillium verrucosum</i> , <i>Aspergillus clavatus</i> , <i>A. ochraceus</i>	Ochratoxin A 
<i>Penicillium expansum</i> , <i>P. urticae</i> , <i>Aspergillus clavatus</i> , <i>Byssoschlamys nivea</i>	Patulin 
<i>Fusarium sporotrichioides</i> , <i>F. graminearum</i> , <i>F. culmorum</i> , <i>F. poae</i> , <i>F. roseum</i> , <i>F. tricinctum</i> , <i>F. acuminatum</i>	Trichothecenes (DON) 
<i>Fusarium moniliforme</i> , <i>F. proliferatum</i>	Fumonisin B1, B2, B3 
<i>Fusarium graminearum</i> , <i>F. culmorum</i> , <i>F. crookwellense</i>	Zearalenone 
<i>F. moniliforme</i> , <i>F. crookwellense</i> , <i>F. subglutinans</i> , <i>F. sambucinum</i> , <i>F. napiforme</i> , <i>F. heterosporum</i> , <i>F. oxysporum</i> , <i>F. solani</i> , <i>F. proliferatum</i>	Fusaric acid 

The same mycotoxin can be produced by several different fungi, and the same fungus can generate several mycotoxins. For example, aflatoxins can be formed by *A. flavus*, *A. parasiticus* and some other *Aspergillus* species, while ochratoxin A (OTA) is thought to be produced mainly by *A. ochraceus* in tropical countries and *P. verrucosum* in temperate countries. This means that multi-contamination probably occurs in contaminated food and feeds. Also, the trends in global trading of feed grains increase the likelihood that blending grains may result in combinations of several mycotoxins in a particular diet.

Mycotoxins are not considered as a single chemical group; their molecular features are diverse and

include polyketides, terpenes, indoles, cyclopeptides and nitrogenous metabolites. The function of mycotoxins in their natural environment has not been clearly established, but they are assumed to play a role in regulating competition with other microorganisms, and protecting plants from herbivores.

2. Toxic effects of mycotoxins on humans and animals

Depending on their nature, the toxins may be carcinogenic, mutagenic, teratogenic, oestrogenic, neurotoxic or immunotoxic (Table 2). In animals, 5 mycotoxins [aflatoxins (AFB1 and AFM1), ochratoxin A (OTA), toxin from *F. moniliforme* and sterigmatocystin] have been demonstrated to be carcinogenic. Only aflatoxins are currently classified as carcinogenic for humans by the International Agency for Research on Cancer (IARC).

Table 2. The major toxic effects of mycotoxins

Toxic effects	Mycotoxins
Carcinogenic	Aflatoxins B1, B2, G1 (Group 1); aflatoxin M1 (Group 2B); Ochratoxin A (Group 2B); sterigmatocystin (Group 2B); fumonisin B1 (Group 2B); toxins from <i>F. moniliforme</i> (Group 2B)
Hepatotoxic	Aflatoxins B1, sterigmatocystin
Nephrotoxic	Ochratoxin A; citrinin
Immunotoxic	Aflatoxin B1; ochratoxin A; trichothecenes
Neurotoxic	Ergot; fumonisin B1; citreoviridin
Tremorgenic	Trichothecenes
Oestrogenic	Zearalenone
Cardiotoxic	Citreoviridin; penicillic acid
Diabetogenic	Terric acid
Teratogenic	Aflatoxin B1; ochratoxin A

In humans and susceptible animal species, aflatoxin B1 (AFB1) is metabolised by cytochrome P-450 enzymes into aflatoxin-8,9-epoxide, which binds to DNA and to albumin in blood, forming adducts and causing chromosome damage. The levels of the major AFB1 adducts (the *N*⁷-guanine and serum albumin adducts) identified in humans and animals are similar. The enzyme glutathione S-transferase (GST) can detoxify the 8,9-epoxide form of AFB1 through conjugation with glutathione, which makes the adduct soluble and able to be excreted in urine. There is a wide variability in the activity of GST between animal species and humans. Humans have lower GST activity than mice or rats, indicating that humans are more susceptible to AFB1 toxicity. Chromosome damage includes gene mutations, micronucleus formation, mitotic recombination and sister chromatid exchange. These changes have been identified as responsible for human liver tumors in geographic areas with high risk of aflatoxin exposure and in animals fed with contaminated feeds (IARC, 1993, 2002). Aflatoxin B1 is classified as a potent carcinogen, despite being naturally-occurring.

III – Environmental conditions for fungal growth and production of mycotoxins

Toxinogenic fungi can grow under a wide range of climatic conditions, on solid or in liquid media, provided they have access to substrates for energy, available water, proper temperature and oxygen (Samapundo *et al.*, 2007). Many species of insects, worms, rodents and birds can facilitate

the entry of mycotoxin-producing fungi into commodities such as seed, grains, nuts and fruits by damaging the external wall and also by carrying the fungal spores (Dowd, 2003; Mehrnejad and Panahi, 2006).

1. Temperature

Most fungi grow in the temperature range 10-30°C (Ribeiro *et al.*, 2006; Valero *et al.*, 2006). The growth slows down outside this range and will even cease for most fungi below 5°C or above 35°C. A few fungi, however, have adapted to either very low temperatures (cryophiles) or very high temperatures (thermophiles). The blue mould *Penicillium* spp. is frequently observed on oranges kept in a refrigerator. In contrast, *A. fumigatus* in hay harvested and stored in humid conditions is often responsible for a rise in temperature, which can reach over 150°C. It has been shown that the fungus can grow up to 50°C.

2. Moisture

Fungi require water for nutrient absorption and metabolic activity. Water requirements are expressed as water activity (a_w), defined as the pressure of water in the ingredient divided by that of pure water at the same temperature.

Optimal a_w values range widely according to the fungal species (Fig. 1). As *Aspergillus* spp. are the least water demanding fungi, they will occur mainly in countries with hot and rather dry climates. Conversely, *Fusarium* spp. will be found mainly in wet and cold countries, and *Penicillium* spp. will be present in between these extreme a_w values. For example, it has been shown that moisture conditions at anthesis and during summer periods are critical in *Fusarium* infection of ears.

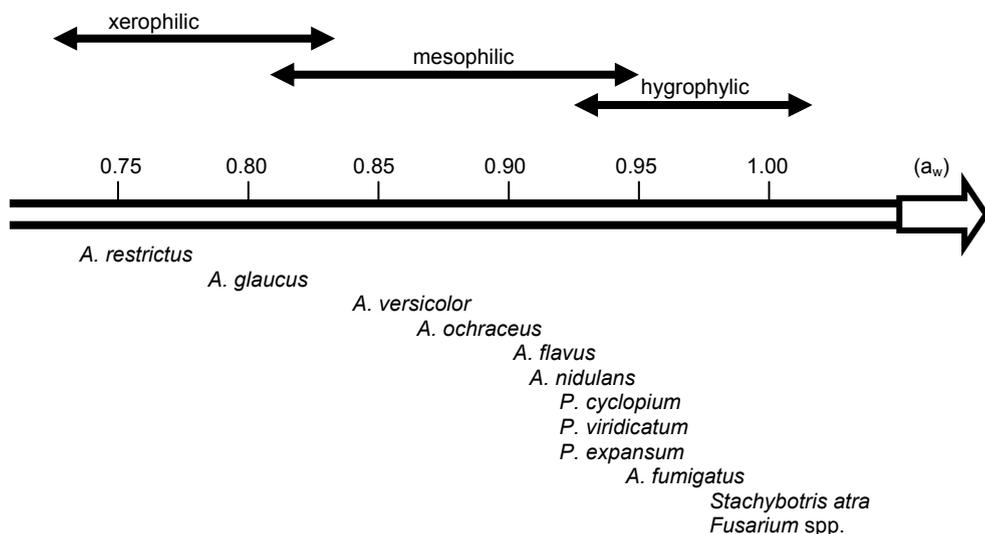


Fig. 1. Range of humidity (a_w) for the growth of toxinogenic fungi.

3. Production of mycotoxins by fungi

The growth (primary metabolism) and mycotoxin production (secondary metabolism) depend on two different types of metabolism of fungi and so are not closely related. Thus the presence of visible fungal biomass is not a proof of toxin contamination. Similarly, the absence of detectable fungi does not mean that toxins are absent. Fungi may have disappeared after producing mycotoxins.

The secondary metabolism is usually activated by environmental factors. Fungi produce mycotoxins in stress conditions such as cold or dryness, or to counter an external aggression such as a fungicide. Hope and Magan (2003) found that toxin production by *F. culmorum* occurred in the narrow a_w range 0.995-0.950, whereas growth persisted to 0.900 a_w . Interestingly, they noted that the optimal conditions for deoxynivalenol (DON) and nivalenol (NIV) production ($a_w = 0.981-0.995$ at 25°C) were inside the range of optimal fungal growth. These a_w levels correspond to water contents of 26% to 30% in grains, respectively, which are within the normal range for harvested corn silage. Toxin production was significantly higher at 25°C than at 15°C. Both toxins continued to accumulate with time during the 40-day period of the experiment.

Mould infection of grains can be partly controlled by the use of fungicides, but this treatment complicates matters as there is some evidence that fungicides may stimulate toxin production. Thus circumstances may arise where the obvious manifestations of *Fusarium* ear blight (FEB) have been reduced or even eliminated by fungicides, but high levels of mycotoxins are still present. In other work coordinated by the UK Home Grown Cereal Authority (HGCA), azoxystrobin reduced the fungal contamination of grain by *F. culmorum* and *Microdochium nivale*, while increasing the levels of DON (Nicholson *et al.*, 2003). The authors explain that the fungicide eliminates the non-toxigenic *M. nivale*, which is a natural competitor of toxin-forming *Fusarium* species. Other fungicides that stimulate toxin production include tridemorph, which increased T-2 toxin production by *F. sporotrichoides*, and tubiconazole and difenoconazole, which both stimulated monoacetyl DON production by *F. culmorum* (Magan *et al.*, 2002). The fungicides tebuconazole, metconazole and HGGA 2 worked in the opposite way by selectively inhibiting *F. culmorum*. Also, the dose level of the fungicide has to be considered. It has been suggested that sub-optimal doses may cause higher levels of mycotoxin production in grain, but fungicides such as tebuconazole, metconazole and HGCA 2 were able to inhibit toxin production at levels that failed to eradicate FEB completely in wheat and oats (Parkin *et al.*, 2006). The possible emergence of fungicide-resistant strains also needs to be considered, as evidenced in *Fusarium* and *Aspergillus* phytopathogens (D'Mello *et al.*, 1998). The issue of mycotoxins in organic agriculture also arises. The results of comparisons between conventional and organic agriculture are not fully conclusive (FAO, 2000). Doll *et al.* (2002) determined DON and zearalenone (ZEN) in conventionally and organically grown grain in Germany. They observed that ZEN contamination was rather low, whereas that of DON was higher in conventional than in organic wheat. They noted that 43% of the conventional wheat and 37% of the organic wheat had DON concentrations that exceeded the tolerable daily intake (TDI) established by the European Scientific Committee on Food for a mean body weight of 60 kg at a mean wheat consumption level.

IV – Level of contamination of ruminant feeds

According to the FAO (2001), around 20% of marketed food is contaminated by mycotoxins. In a large-scale survey, Pittet (1998) found a natural occurrence of mycotoxins in 40% of 25,000 food and feed samples collected in 30 countries around the world. Cereal grains and nuts were the most potentially hazardous ingredients in the human food chain and in monogastric animals (Table 3). The question is more complex for ruminants because their diets include both forages and concentrate, which increases the likelihood of multicontamination occurring.

1. Mycotoxin contamination of forages

The contamination of forage crops and silages with toxic fungal metabolites and its consequences have been largely ignored in the past (Smith *et al.*, 1994). Pastures have been shown to harbour fungi such as *Claviceps purpurea*, which is responsible for ergotism, *Pithomyces chartarum*, which produces sporidesmin which causes facial eczema, *Neotyphodium*, which causes dry gangrene, and *Rhizoctonia*, a producer of slaframin, which induces sialorrhoea in ruminants (Le Bars and Le Bars, 1996). Hay can be contaminated by fungi of three ecological types: field fungi during the pre-harvest period, intermediate fungi during harvesting, and storage fungi (Pelhate, 1987).

Intermediate and storage fungi are totally absent from hay harvested and stored in dry conditions, but become numerous and diverse in incompletely dried hay. Hydrophilic and heat-tolerant species, such as *A. fumigatus*, which produces gliotoxin, and *Stachybotrys atra*, which produces satratoxins G and H causing stachybotryotoxicosis, both predominate in hay harvested and stored in humid conditions. Several highly toxinogenic species of *Aspergillus* and *Penicillium* have been detected in damp hay and straw (Le Bars, 1976; Clevstroem *et al.*, 1981; Pelhate, 1987), in addition to *Fusarium* spp., which produce zearalenone (ZEN) (Scudamore and Livesey, 1998). As a consequence, a wide range of toxins, including the harmful patulin (PAT), AFB1 and sterigmatocystin can be found in insufficiently dried hay.

In theory, the preservation of ensiled high-moisture green grass or hay bales in an enclosed airtight system is safe with regard to the mycotoxin risk. However, the availability of oxygen plays a crucial role in the development of mycoflora with such techniques of preservation with humid forages. Depletion of oxygen during the initial stage of fermentation totally inhibits the growth of *Fusaria* (Damaglou *et al.*, 1984; Lepom *et al.*, 1988). Some fungi such as *A. fumigatus*, *Monascus ruber* and some *Penicillia* are tolerant to partial oxygen deprivation, while others such as *Byssochlamys nivea*, *P. varioti* and *P. roqueforti* are considered as micro-aerophilic or indifferent to oxygen presence. These fungi can survive in the silo, and then grow after the silo is opened or when anaerobiosis is not complete (lack of packing, too high DM content of forage, defective sealing, etc.). Organic acids such as formic, propionic and butyric acids have some antifungal properties (Poisson and Cahagnier, 1973). In a survey in France and Italy in three successive years, Pelhate (1975) analysed 1230 maize silage samples for 70 fungal species. The incidence of *P. roqueforti*, *Byssochlamys*, *Monascus*, *Aspergillus* and *Paecilomyces* was 76%, 41%, 31%, 21% and 27%, respectively. This result was confirmed by Escoula *et al.* (1972). *A. fumigatus* has frequently been evidenced in samples from Germany and the US. The fate of field-derived mycotoxins in silages is not completely known. Concentration of DON remains constant, whereas that of ZEN and AFB1 declines with storage time (Kalac and Woolford, 1982).

2. Mycotoxin contamination of grains

Many species of fungi cause moulding in grain, and most grain fungi are relatively non-specific. They become associated with the grain in the field, and can also grow during transport and storage if environmental conditions such as humidity and temperature are favourable. Insect damage is another factor that predisposes grain to fungal colonization, because insect herbivory creates kernel wounds that give fungi access to the endosperm, and insects themselves serve as vectors of fungal spores. Thus under some conditions, genetic engineering of grain for insect resistance may make it safer for animal and human consumption (Munkvold *et al.*, 1999). *Fusarium*, *Gibberella*, *Penicillium*, *Aspergillus*, *Cladosporium* and *Claviceps* are among the prevalent grain moulds found before harvest. *Aspergillus* and *Fusarium* species are likely to be the most significant mycotoxin-producing field fungi found in tropical developing countries. Several fungal diseases have been identified in grains: *Aspergillus* ear rot in corn, peanuts, cottonseed, sorghum, barley and oats; ergot due to *Claviceps* in wheat, rye, triticale and oats; *Fusarium* ear rot in corn; scab or head blight due to *Fusarium* (also named *Gibberella*) in wheat and barley. The fungi survive in crop residues after harvest, and the spores generated from residues contaminate the soil. Hence crop rotation is one way to reduce the risk of mycotoxin contamination of grains. Infection due to soil-borne inoculum can be lowered during harvest by a cutting height set at 7-10 cm above ground level. The postharvest period can also be critical for mould growth and mycotoxin production. To minimize the risk, grain moisture content should be reduced to less than 15% within 48 h after the grain has been harvested. This may be difficult in many storage facilities, especially in large silos. Good air circulation throughout the storage bin is important to dry the grain and avoid temperature rise. Rodents and insects must be eliminated to maintain the integrity of the pericarp of kernels, and prevent fungi gaining access to the endosperm. Grain moulds do not develop evenly throughout a storage unit, but generate several hot spots distributed randomly in the grain bulk.

Grain is considered as the main mycotoxin vector in food or feeds (Pfohl-Leskowicz, 2000a). The

most hazardous toxins are aflatoxins produced by *A. flavus* and *A. parasiticus* often found in cereals, peanuts, cottonseed and oilseed products from hot and humid countries, both before and after harvest. Ochratoxin A is mainly found in corn, barley, oats, rye and wheat, after harvest. Among fusariotoxins, trichothecenes such as DON, T-2 toxin and diacetoxyscirpenol (DAS), are the most common toxins found in grain. Unlike fumonisins, which are found only in corn, ZEN can contaminate all cereals. A study of wheat and corn harvested in France in 1996 and 1997 showed that the levels of contamination by fusariotoxins were variable and depended on the cereal and year (Richard-Molard, 1999).

V – Effects of mycotoxins on ruminants

Fungal contamination affects the organoleptic characteristics and the nutritional value of feeds. In addition, the presence of mycotoxins carries a risk of both animal toxicosis and toxin transfer to animal products entering the food chain (milk and meat). Most mycotoxins are absorbed through the intestinal tract by three processes: (i) simple diffusion of polar toxins in liquid phase; (ii) diffusion in lipid phase of non-ionic toxins; and (iii) active transport. Diffusion of the non-ionic forms of toxins across the lipid membrane of cells is the main route of mycotoxin absorption. Polar toxins are easily eliminated in urine and are less strongly retained in animal tissues. Increasing polarity by glycosylation of toxins during liver metabolism is a common way of protecting animal cells against xenobiotics.

The biological effects of a mycotoxin depend on the ingested dose, other toxins that are present because toxins can interact, duration of exposure and animal sensitivity. Mycotoxins have acute or chronic effects on animals, depending on the dose and time of exposure. Mycotoxins can induce disorders specific to each toxin, impair the immune system of animals and favour many other dysfunctions. This is the main reason why mycotoxicosis is difficult to diagnose.

1. Effects of individual mycotoxins

Ruminants are considered to be more resistant to the adverse effects of mycotoxins than monogastric animals. The main reason for this difference is that mycotoxins can be degraded or bioconverted by rumen microorganisms. However, significant cases of mycotoxicosis have been described in ruminants, adversely affecting animal production and farm profitability (Miller, 1979). The toxic effects will be described here only for the three main families of mycotoxins: aflatoxins, fusariotoxins, and OTA. Special references on goats will be pointed out.

Acute aflatoxicosis causes significant liver lesions, leading to congestion and bleeding, and is often fatal (Pier, 1992). They cause fatty acid accumulation in the liver, kidneys and heart, and may be responsible for encephalopathies and oedemas (Pfohl-Leszkowicz, 2000b). The animal may die within a few hours or days. Chronic toxicosis is, however, more frequent. In this case, aflatoxin binds to guanine base in liver DNA, leading to cell death or giving rise to a tumour. In goats fed with AFB1-contaminated feeds doses of 0.1-0.4 mg/kg body weight per day, Miller *et al.* (1984) noted a non-significant increase in mean concentration of γ -globulins and a decrease in β -globulins in serum. Microscopic changes included bile ductule proliferation, hepatocytic karyomegaly, hepatocellular degeneration, pneumonia, rhinitis, and proximal renal tubular nephrosis. Animals had ascites, pale liver, petechial haemorrhages, nasal discharge and icterus, confirming hepatic lesions. The authors consider goats to be sensitive enough to AFB1 to be used as a ruminant model to study ruminant aflatoxicosis. Similar clinical manifestations were described for the effects of AFB1 on goats by Maryamma and Sivadas (1975), and by Samarajeewa *et al.* (1975).

Fusariotoxins, and among them trichothecenes, are another important family of mycotoxins that cause weight loss, vomiting, severe skin problems, bleeding and, in some rare cases, death of animals. They have immuno-suppressive properties acting principally on the cellular immune system. Pigs are especially sensitive to DON, whereas ruminants seem to be less susceptible. No information is currently available on the behaviour of goats with respect to trichothecenes. The

major effects of ZEN are oestrogenic. It induces some feminisation signs in young male pigs such as testicular atrophy and mammary gland enlargement. In the most severe cases, it can progress to rectal and vaginal prolapse. In females, it can cause anoestrus, pseudopregnancy, teat enlargement and udder secretion. High concentration of ZEN can affect cycling, conception, ovulation and foetal development. Sensitivity of animals to ZEN toxicity has been demonstrated in several studies (see Hagler *et al.*, 2001), pigs being considered to be the most sensitive animal species (Olsen and Kiessling, 1983). As pigs and goats both have a high capacity to form both α -zearalenol and β -zearalenol in the microsomal fraction of the liver, goats may be as susceptible as pigs to zearalenone intoxication, as confirmed by Wolde-Michael *et al.* (1989). Fumonisin B1 (FB1) has been shown to affect weanling angora goats fed naturally contaminated corn at 95 mg/kg dietary DM for 112 days (Gurung *et al.*, 1998). Elevated concentrations ($P < 0.10$) of blood-borne enzymes such as aspartate aminotransferase, lactate dehydrogenase and γ -glutamyl transpeptidase, and increased concentrations of cholesterol and triglycerides indicated mild liver damage and kidney dysfunction in treated goats. A linear relationship was evidenced between these serum constituents and duration of FB1 exposure. Typical of FB1 toxicosis, an elevated free sphinganine/free sphingosine ratio in liver, kidney, and heart tissues was observed. However, no clinical signs of toxicosis appeared, and live weight gain of animals was not affected by the FB1 treatment. Thus intoxication can occur with no apparent change in animal behaviour, which makes diagnosis very difficult.

Ochratoxin A is a nephrotoxic mycotoxin that impairs proximal tubular function and urine excretion, and increases urine excretion of glucose (Krogh, 1976). Other organs can be affected, and enteritis, necrosis of lymphoid tissue and fat enrichment of the liver have been observed (Szczzech *et al.*, 1973). Bioconversion of OTA by cytochrome P450 resulted in the formation of carcinogenic metabolic intermediates (Fink-Gremmels *et al.*, 1995a,b). Pigs and poultry are sensitive to OTA, but ruminants seem to be fairly resistant to it. This difference can be explained by the ability of rumen microbes to actively degrade OTA to OT α , which is much less toxic than the parent substance.

2. Synergistic effects of mixed mycotoxins

A diet composed of mixed ingredients, each presenting a risk of contamination with toxinogenic fungi, is liable to harbour several toxins. This situation is quite common, and diets are rarely mycotoxin-free. Synergistic effects between mycotoxins in blends of toxin-contaminated diets have been described. For instance, the presence of fusaric acid increases FB1, DAS and DON toxicity in animals (D'Mello and McDonald, 1997; Smith *et al.*, 1997). Combinations of fusaric acid and ZEN increase the concentration of each toxin in milk by a factor of two to five (Porter *et al.*, 1996). An interaction between FB1 and DON increases the amount of blood protein, whereas an interaction between FB1 and T-2 toxin leads to an increased plasma calcium concentration (Kubena *et al.*, 1997a) or an increased haemoglobin and haematocrit concentration (Kubena *et al.*, 1995). Such combinations of toxins also affect the growth rate of chickens (Kubena *et al.*, 1997a) and turkeys (Kubena *et al.*, 1997b). Other mixtures containing DON and DAS, DAS and aflatoxins and FB1 also show interactive effects (Harvey *et al.*, 1995a,b). A synergistic toxic effect on three different cell lines (C6 glioma cells, Caco-2 cells and Vero cells) has been evidenced when FB1 and OTA are combined (Creppy *et al.*, 2004).

VI – The fate of mycotoxins in ruminants

1. Ruminal metabolism of mycotoxins

As the rumen microbes can degrade or metabolise some mycotoxins, ruminants are more resistant to most mycotoxins than monogastrics. The protozoal fraction of the rumen microbial ecosystem seems to be chiefly responsible for mycotoxin metabolism, together with some bacteria such as *Butyrivibrio fibrisolvens*, *Selenomonas ruminantium* and *Anaerovibrio lipolytica* (Westlake *et al.*, 1987a). These bacteria are able to use T-2 as a source of energy through two enzyme systems

(Westlake *et al.*, 1987b). Several strains of *B. fibrisolvens* isolated from the rumen are able to degrade DAS, DON, ZEN, OTA, and verrucaric A *in vitro* (Kießling *et al.*, 1984; Westlake *et al.*, 1989). T-2 toxin, HT-2, DON and DAS are metabolised by rumen microbes when present at a concentration of 10 µg/ml (Prelusky *et al.*, 1986), and can yield numerous metabolites as indicated in Fig. 2. The epoxy cycle of DON generates de-epoxy DON, commonly known as DOM-1, which is non-toxic (Côté *et al.*, 1986). HT-2 and neosolaniol produced from T-2 toxin, are about a tenth as toxic as the parent toxin. The toxicity of the metabolites of DAS has not been determined. About 90% of DAS and T-2 deacetylation is achieved by rumen protozoa (Hussein and Brasel, 2001). T-2 toxin can attach to eukaryotic ribosomes and can thus inhibit the growth of protozoa (Mackie and White, 1990). The antibiotic activity of DON and fusaric acid has been tested on the eubacterium *Ruminococcus albus* and the *Archaea* species *Methanobrevibacter ruminantium* (May *et al.*, 2000). Fusaric acid inhibited both microorganisms even at a low concentration (15 µg/ml), and DON had no effect up to 100 µg/ml.

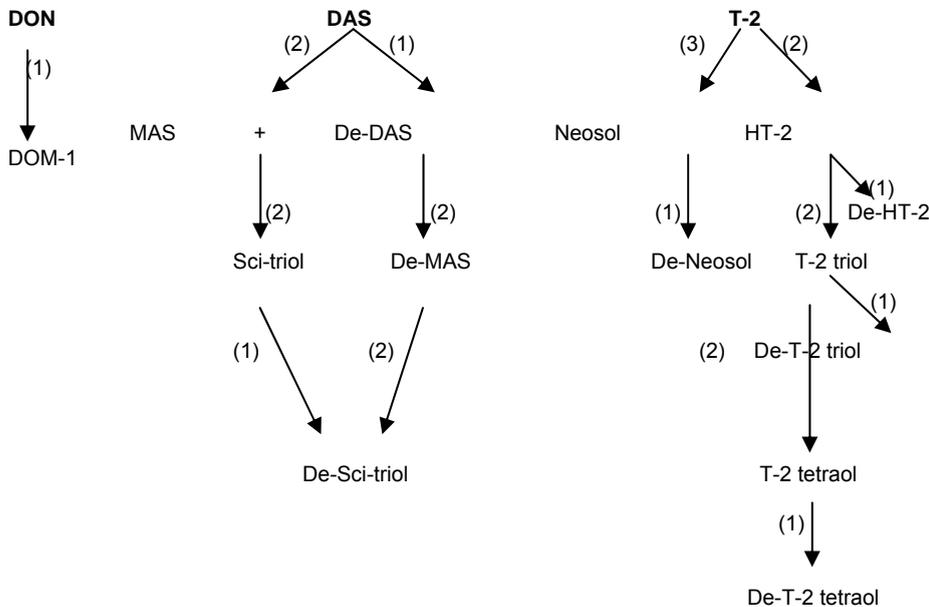


Fig. 2. Possible metabolic pathways of trichothecenes in the rumen (diagram from Mackie and White 1990 modified by Jouany). DON = de-oxynivalenol; DOM-1 = de-epoxy deoxynivalenol; DAS = diacetoxyscirpenol; MAS = monoacetoxyscirpenol; De-DAS = de-epoxy diacetoxyscirpenol; Sci-triol = scirpentriol; De-MAS = de-epoxy monoacetoxyscirpenol; De-Sci-triol = de-epoxy scirpentriol; Neosol = neosolaniol; De-HT-2 = de-epoxy HT-2; De-Neosol = de-epoxy neosolaniol; De-T-2 triol = de-epoxy T-2 triol; De-T-2 tetraol = de-epoxy T-2 tetraol. Reactions of de-epoxidation (1), de-acetylation (2), isovaleryl de-esterification (3).

The peptide bond of OTA is actively hydrolysed in the rumen to produce phenylalanine and ochratoxin α , which is much less toxic than the parent toxin (Hult *et al.*, 1976). It may also be esterified to produce ochratoxin C (Galtier and Alvinerie, 1976) of similar toxicity (Chu, 1974). Protozoa are thought to play a major role in OTA degradation (Galtier and Alvinerie, 1976). The effect of diet on the capacity of rumen microbes to degrade OTA is variable. Xiao *et al.* (1991a,b) showed that the half-life of OTA in the rumen decreased when the level of forage increased at the expense of cereals in the diet. Conversely, Özpınar *et al.* (2002) observed that adding starch ingredients up to the maximum level of 30% in a forage-based diet decreased the concentration of OTA in the rumen. Such divergent results may be explained by the evolution of the concentration of

protozoa in the rumen. Addition of concentrate in a diet stimulates the protozoal population up to about 30% starch in dietary DM, and may depress it when large amounts of easily fermented starch is supplied (Jouany, 1989). The toxicity of OTA in ruminal microorganisms has been tested in *B. fibrisolvens* (Westlake *et al.*, 1987a), since this bacterium is considered as essential in the microbial detoxification process (Mackie and White, 1990). The absence of any effect of OTA at 5 ppm on the main ruminal functions indicates that this toxin has no harmful action on the microbial ecosystem.

Zearalenone is rapidly converted to α -zearalenol, which is about five to ten times more toxic than the parent toxin, and to a lesser extent to β -zearalenol, which has a low toxicity. Protozoa are mainly responsible for this conversion (Kiesling *et al.*, 1984). Further hydrogenation of α -zearalenol gives zeranol, which has been detected in bovine bile after oral dosage of ZEN (Kennedy *et al.*, 1998). The authors observed that the α -zearalenol/zeranol ratio was always above 5. This index could be used to detect fraudulent use of zeranol to improve the growth of animals. All these metabolites use the same oestrogenic receptor site as ZEN, which explains their activity and their toxicity. Zearalenone has no toxic effect on the rumen bacterium *B. fibrisolvens*, and is considered not to alter the digestive rumen functions (Westlake *et al.*, 1987a), although Danicke (2002) noted a significant decrease in the *in sacco* degradability of wheat straw in wethers fed on a ZEN + DON contaminated diet, while the *in sacco* degradability of lucerne was not altered.

Aflatoxins are generally poorly bioconverted in the rumen, with less than 10% degraded for concentrations from 1.0 to 10.0 $\mu\text{g/ml}$ (Westlake *et al.*, 1989). Formation of aflatoxicol, a highly toxic hydroxylated derivative of AFB1 has been observed (Auerbach *et al.*, 1998). Many ruminal bacteria are completely inhibited by concentrations of AFB1 below 10 $\mu\text{g/ml}$. Digestive and fermentative functions of the rumen microbial ecosystem can thus be disturbed by aflatoxins.

2. Metabolism of mycotoxins in intestines and splanchnic organs

Intestinal epithelium, liver and kidneys are sites of bioconversion of nutrients and xenobiotics including mycotoxins. These bioconversions involve two reaction phases. The first phase includes reductive, oxidative and hydrolytic reactions. Microsomal cytochromes P450, mono-oxygenases, amino-oxidases and alcohol dehydrogenases are the main oxidative enzymes, while epoxide-hydrolases, aldehyde-reductases and ketone-reductases are the major enzymes responsible for reduction. In addition, animal tissues and body fluids contain a large number of non-specific esterases and amidases capable of hydrolysing xenobiotics (Galtier, 1999). The second phase involves conjugating reactions applied on the products of the first phase. These reactions decrease the toxicity of toxins and increase their solubility in water, thus facilitating their excretion in urine and milk, and protecting the animal (Dominguez-Bello, 1996). The major conjugating enzymes are microsomal glucuronosyl-transferases, cytosolic sulpho-transferases, methyl-transferases, aminoacyl-transferases, S-glutathione-transferases and N-acetyl-transferases (Galtier, 1999).

The absorbed fraction of AFB1 is extensively metabolised in the liver, resulting predominantly in AFM1, which enters the systemic circulation or is conjugated to glucuronic acid, and is then excreted in bile. Circulating AFM1 may be excreted in urine or milk. Synthesis of AFB1 8-9 epoxide in the liver is the prerequisite step in the formation of covalent bonds with nucleic acids in the subsequent acquisition of the mutagenic and carcinogenic character of AFB1.

Trichothecenes are detoxified chiefly by a glucuronidation reaction and by reduction of the epoxy group responsible for the reactivity of these toxins. The liver cytochrome P450 converts OTA to hydroxyl-OTA, which has immuno-suppressive properties similar to those of OTA (Creppy *et al.*, 1983). T-2 toxin is rapidly metabolised in two steps. First, T-2 is de-acetylated in the rumen and liver to give HT-2 and a small amount of T-2-triol (see Fig. 2). These metabolites are then conjugated with glucuronic acid, facilitating their excretion in bile. Two other hydroxylated metabolites are also produced, OH-HT-2 and diOH-HT-2. Other compounds such as de-epoxy T-2 or de-epoxy T-2-triol may be formed. Diacetylscirpenol is metabolised to de-epoxylated and de-acetylated products. Deoxynivalenol is converted to de-epoxy DON (or DOM-1), which is then glucuronidised, making it more hydrophilic and increasing its excretion in urine (Côté *et al.*, 1986).

Fumonisin B1 and DON are eliminated in bile and are weakly absorbed in the digestive tract. They mostly culminate in faeces (61% of FB1 and 54-75% of DON), and as trace contaminants in urine (1-3%). Liver hydrolases or intestinal enzymes may partly hydrolyse FB1 to produce a monoester and aminopentol, both of which are then excreted in faeces (Galtier, 1999).

Zearalenone is converted into α - and β -zearalenol by the liver ketone-reductases. Genetic differences among animal species in the reductase activities can account for differences in the sensitivity of animals to ZEN (Galtier, 1999).

3. Routes of mycotoxin excretion

A. Urinary and faecal excretion

As indicated previously, ruminal and hepatic bioconversions of mycotoxins change the polarity of toxins and consequently alter their affinity to water or lipids. Their absorption through the digestive tract, their fixation in animal tissues, or their excretion in urine, faeces, or milk, is therefore under the control of the metabolic pathways. Given that the rumen can play a major role in the bioconversion of toxins, ruminants are to be regarded as a special case in the metabolism of mycotoxins.

Urinary excretion after oral administration is most efficient for mycotoxins that are strongly absorbed and metabolised, such as AFB1, citrinin, OTA, PAT and ZEN. Conversely, faecal excretion results from a lack of absorption by the gastrointestinal tract or elimination of toxins or metabolites through the biliary system. T-2 toxin, DON and FB1 are excreted in faeces because of their low absorption in the digestive tract, whereas a significant proportion of AFB1, cyclopiazonic acid, OTA and ZEN are eliminated as conjugated metabolites in bile (Galtier, 1998).

B. Excretion in milk

The excretion of toxins and their metabolites in milk is another route by which they are eliminated to protect the animal. The transfer of toxins from blood to milk may involve intercellular filtration, passive diffusion across cellular membranes, or active transport via secretion vesicles. Transfer of AFM1, OTA, ZEN and their metabolites can be significant enough to represent a potential risk to milk consumers (Yiannikouris and Jouany, 2002). Milk concentration of AFM1 peaked two days after an oral administration in cows (Whitlow *et al.*, 2000). The same study showed that AFM1 disappeared 4 days after AFB1 was removed from the diet.

Although the rate of transfer of AFB1 is highest among mycotoxins, it is generally considered to be as low as 0.3-2.2% according to van Egmond (1989) and Spahr *et al.* (2000). However, the carry-over rate can range widely among animals, days, and from one milking to the next. It is greatly influenced by physiological factors such as diet and health status of animals, especially the status of the liver and its enzymatic activities. For high-yielding dairy cows producing up to 40 kg of milk per day, Veldman *et al.* (1992) found a carry-over percentage as high as 6.2%. Changes in the plasma-milk barrier and the consumption of large amounts of concentrated feeds in high-merit dairy cows may explain the increase in the carry-over rate of AFM1 in milk. Using data published since 1985 (10 observations from 5 controlled experiments), Pettersson (1998) proposed the following equation ($r^2 = 0.915$) to estimate the transfer of AFM1 in milk:

$$\text{AFM1 (ng/kg milk)} = 10.95 + 0.787 \times (\mu\text{g AFB1 intake per day}).$$

This equation indicates that the animals must ingest less than 50 and 25 μg AFB1 per day to comply with the European regulatory levels of contamination in milk set at 0.05 and 0.025 $\mu\text{g/kg}$ of milk for adults and infants, respectively. Thus cows must ingest less than 10 and 5 kg of feed contaminated at the maximum authorised level (5 μg AFB1/kg feed for dairy cattle) to maintain a safe level of AFM1 in milk. There is a risk of exceeding these levels in milk when dairy cows are fed diets based on contaminated corn silage, since they can ingest more than 15 kg DM of silage per day. Recent data indicated that in two regions of Italy, the prevalence of AFM1 in milk samples

exceeded the statutory limit after home-grown maize was incorporated into dairy cow feed (RASFF, 2003). Vallone and Dragoni (1997) conducted a study in Italy showing that samples taken from a corn silage trench for a 4-month period had levels of AFB1 ranging from 25 to 40 µg/kg, which is much higher than the maximum authorised level of 5 µg/kg in Europe (Table 3). The European Scientific Panel on Contaminants in the Food Chain noted that more precise estimates of mycotoxin exposure of sheep goats, camels and buffaloes are needed, and transfer rates have to be evaluated not only to comply with the maximum level established for aflatoxin M1 in milk, but also with the maximum level of 0.025 µg AFM1/kg in infant formulae (EFSA, 2004).

VII – Current legislation for mycotoxin content in feeds

Considering both the carry-over into milk and the associated risk for consumers of milk products, and the adverse effects on animal health, approximately 45 countries have set maximum levels for mycotoxin contamination of feeds (van Egmond and Jonker, 2004). Regulations on aflatoxins have been drafted by the European Commission (JOCE, 2006a), and recommendations have been proposed for maximum levels of DON, ZEN, OTA, FB1 + FB2 (JOCE, 2006b). The maximum levels of mycotoxins in feeds marketed in Europe, USA and Canada are given in Table 3. There are substantial differences in authorised concentrations between countries. For instance, 6 times more AFB1 is permitted in animal feeds in the US than in Europe, and the US allows 10 times more AFM1 in milk than Europe. This is due to imperfect scientific knowledge concerning mycotoxin toxicity, analytical and dosage difficulties, and the economic impact of such regulation.

Table 3. Maximum levels of mycotoxins in feeds in Europe, the United States and Canada (aflatoxins have legislated maximum tolerated levels in Europe, USA and Canada; fumonisins are under guidance levels in USA; the values for the other mycotoxins are considered as advisory levels)

Mycotoxin	Animal or class	Feed (12% humidity)	Maximum level (mg/kg)		
			EU	FDA	Canada
Aflatoxins	Non-dairy ruminants	Feeds and ingredients (except below)	0.05	0.30	NG [†]
		Peanut, cottonseed, corn, palm products	0.02	0.30	NG
	Dairy ruminants	Feeds and ingredients	0.005	0.02	0.02
	Immature ruminants	Feeds and ingredients	0.01	0.02	NG
Deoxynivalenol	Adult ruminants	Ingredients: grains, grain by-products (except corn by-products)	8	-	-
		Corn by-products	12	-	-
	Young ruminants (<4 months)	Feeds, complementary feeds	2	-	-
	Adult beef, feedlot cattle	Grains, grain by-products (<50% diet)	-	10(5) ^{††}	-
	Other ruminants	Grains, grain by-products (<40%)	-	5(2) ^{††}	-
	Non-dairy ruminants	Grains, grain by-products (<50% diet)	-	-	5
	Dairy ruminant	Grains, grain by-products (<40%)	-	-	1
Zearalenone	Non-dairy ruminants	Ingredients: grains, grain by-products (except corn by-products)	2	NG	-
		Corn by-products	3	NG	-
	Dairy, young ruminants	Feeds, complementary feeds	0.5	NG	10(1.5) ^{†††}
	Sheep	Feeds, complementary feeds	-	-	5
Ochratoxin A	All ruminants	Grains and derived products	0.25	NG	NG
Fumonisin (B1 + B2)	Adult ruminants (>4 months)	Corn and derived products	50	60(30) ^{††}	NG
	Young ruminants (<4 months)	Corn and derived products	20	10(5) ^{††}	NG
	Dairy cattle	Corn and derived products	-	30(15) ^{††}	NG
HT-2 toxin	Dairy cattle	Feeds and ingredients	NG	NG	0.025
Ergot	All ruminants	Feeds and ingredients	NG	NG	2

[†]NG = no guidance.

^{††}In finished feed.

^{†††}If other toxins are present.

VIII – Potential economic cost of mycotoxins

It is difficult to estimate the precise economic costs of mycotoxins. The Council for Agricultural Science and Technology (CAST, 2003) evaluated the sum of food and feed losses, and mitigation efforts to reduce the risk of mycotoxins, in the United States only, at \$932 million and \$466 million per year, respectively. The annual livestock costs amounted to \$6 million. Only aflatoxins, fumonisins and DON were considered in these assessments, and the human health costs were not taken into account.

IX – Decontamination and detoxification strategies

The control of mould contamination of plants needs the farmer and feed manufacturers to operate at the field level, as well as during harvest, transport and storage, processing and distribution. At the field level, it is advised to limit the spore contamination of soil by crop rotation and avoidance of cereal monoculture. Contamination of grain with soil matter must be avoided during harvest. Also, maintaining the physical integrity of cereal grains to limit the access of moulds to nutrients present in grains is strongly recommended. This implies eliminating insects and rodents that attack plants. During storage and preservation steps, environmental conditions (humidity, O₂, temperature) must be strictly controlled. If moulds or mycotoxins are already present, then chemical, physical and biological methods can be applied to reduce the risk of mycotoxins in food and feeds. However, it must be considered that the presence of mycotoxins is almost unavoidable and that practical solutions (mechanical separation of contaminated grain, density segregation, thermal inactivation, irradiation, solvent extraction, chemical or biological treatments) that totally eliminate mycotoxin contamination of feed are not currently available. Several chemical methods have been proposed to degrade aflatoxins (e.g., acids, bases, aldehydes, bisulphite, oxidising agents). Although some successfully destroy aflatoxins, they can generate toxic by-products and (or) alter the nutritional quality of feed ingredients. Ammoniation is currently used in Mexico, Sudan, South Africa, Senegal, and Brazil to degrade aflatoxins to less toxic products, including aflatoxin D1 and an unknown derivative with a molecular weight of 206. However, the initial step in the process can be reversible, the lactone group being reformed. It is necessary to ensure the reaction proceeds to completion.

The strategy of decreasing animal exposure to mycotoxins by including sequestering agents or sorbents in the diet has been given considerable attention during the last decade. These compounds either adsorb mycotoxins in specific internal and surface structures or form chemical bonds with mycotoxins, making them non-adsorbable in the digestive tract and thus protecting animals against any harmful effects. As early as 1979, Masimango *et al.* (1979) observed that clay minerals could bind AFB1 in liquids. The use of bleaching clays to process canola oil was shown to lessen the effects of T-2 toxin (Carson and Smith, 1983; Smith, 1984). The first enterosorbent study with aflatoxin was carried out with a calcium montmorillonite clay, a hydrated sodium calcium aluminosilicate (HSCAS) (Phillips *et al.*, 1988). It showed that HSCAS notably protected broilers and chickens from the toxic effects of 7.5 ppm AFB1 in the diet. Numerous studies were then conducted with clays, and HSCAS used at 1% of the diet was shown to reduce the absorption of radiolabelled aflatoxins, decrease the aflatoxin residues in animals, and lower the concentration of AFM1 in milk from dairy cows and goats (see CAST, 2003). Known for its adsorption properties, activated carbon (Piva *et al.*, 1995; Galvano *et al.*, 1996) and cholestyramine (Underhill *et al.*, 1995; Ramos *et al.*, 1996) were also used to bind AFB1, FB1 and ZEN, respectively. However, these inorganic materials showed only limited efficacy against mycotoxins other than aflatoxins. Also, they can reduce the biological value of some nutrients and may contain dioxins and heavy metals.

To overcome the relative inefficacy of clays towards mycotoxins other than aflatoxins, an organic product made of modified yeast cell wall has been proposed (Devegowda *et al.*, 1998) as an alternative solution to complex several mycotoxins in the gastrointestinal tract of animals without impairing nutrient bioavailability or harming the environment. Cell wall of lactobacilli, bifidobacteria and propionibacteria were also tested and showed a marked sequestering effect against aflatoxins both *in vitro* or *in vivo* (El Nezami *et al.*, 1998, 2004a,b). The mode of action of these microbial

components against mycotoxins has been recently described (Yiannikouris *et al.*, 2004a,b,c,d, 2006). The authors show that β -D-glucans of yeast cell walls are the main organic component involved in the formation of chemical complexes with ZEN used as a model of mycotoxin. They indicate that the 3D-structure of β -D-glucans involving a single and (or) triple helix structure is decisive for their efficacy since the alkali-insoluble fraction of β -D-glucans has a higher affinity for ZEN than the soluble fraction. Chitin has been shown to have a negative impact on the affinity rate by stiffening the β -D-glucan network, thus restricting mycotoxin accessibility to the binding sites of the β -D-glucan helix. *In silico* models have been produced showing that ZEN, OTA, PAT, and DON molecules can interact with β -D-glucans (Fig. 3). Using molecular mechanics, it has been shown that hydroxyl, ketone, and lactone groups of toxins are involved in both hydrogen bonds and van der Waals interactions with the hydroxyl groups and rings in glucans, respectively. Differences in the sequestering capacity of mycotoxins are due to their specific spatial molecular shapes and their chemical characteristics. Devegowda *et al.* (1998) showed *in vitro* that Mycosorb®, a commercial product based on yeast cell wall extracts, could bind 85% of aflatoxin, 67% of ZEN, and 67% of fumonisins. Moderate activity was seen with T-2 toxin (33%), whereas for OTA, DON, citrinin and DAS, the sequestering rate ranged between 12 and 18%. Nivalenol and fusariotoxin X were bound at 8% only. Several *in vivo* studies achieved with Mycosorb® showed a real efficacy in protecting horses (Raymond *et al.*, 2005) or pigs (Swamy *et al.*, 2002) against feeds naturally contaminated by *Fusarium* mycotoxin, which is only weakly fixed by clays.

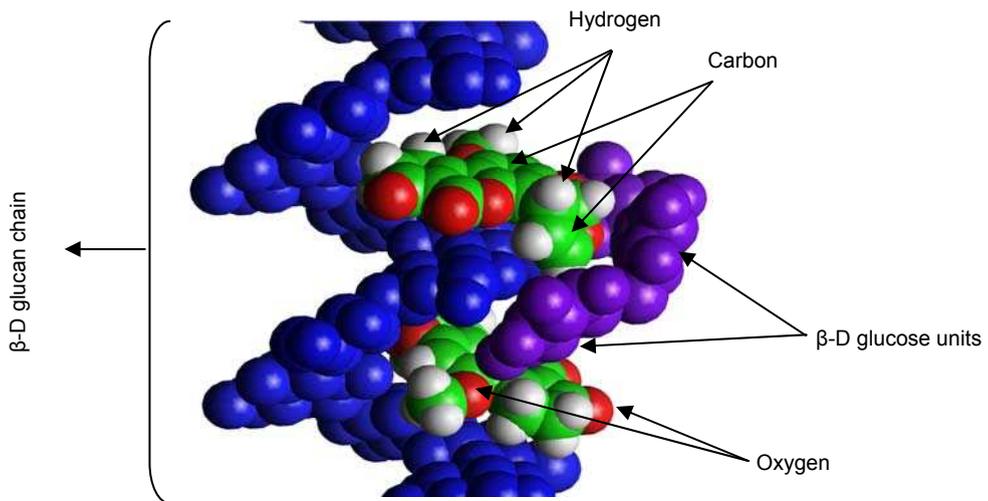


Fig. 3. *In silico* model of the docking of AFB1 into a (1-3)- β -D-glucan chain branched with three β -D-glucose units of a (1-6)- β -D-glucan side chain (Yiannikouris *et al.*, 2006).

X – Conclusions

Although mycotoxins have been discovered only recently, many studies have been conducted during the last two decades to assess their risk in the food chain. It is considered that the presence of mycotoxins in food and feeds of plant origin (mainly cereals, oilseeds and their by-products) is almost unavoidable, and no practical solution is currently available to eliminate them totally. The possible transfer of mycotoxins to animal products indicates that milk and meat may also be contaminated by mycotoxins and their metabolites. The risk of chronic intoxication of humans and animals cannot therefore be ignored. Acute intoxications in humans are rare, although some cases

have been revealed in Kenya (Ngindu *et al.*, 1982) and in India (Krishnamachari *et al.*, 1975a,b) with fatalities. Recent epidemiological studies indicated that about half of the European population is exposed to OTA (European Commission, 1997).

Chronic intoxication of animals is difficult to detect since no clear symptoms appear before significant damage of organs has occurred. Contrary to what is commonly assumed, ruminants are not indifferent to mycotoxins. Indeed some toxins can be degraded in the rumen by microorganisms, but this detoxifying activity is lowered in case of rumen dysfunction. Also, bioconversion of some mycotoxins by rumen microbes can increase their toxicity, as shown in the present paper for zearalenone.

Except for the ammoniation procedure applied on aflatoxins, mycotoxins are very difficult to eliminate once they have been produced. That is why prevention of contaminated must be privileged by grain producers and livestock producers. The use of binders to inactivate mycotoxins remains attractive once they have been detected in food and feeds. The basic research conducted in our laboratory to demonstrate the mode of action of yeast cell wall against mycotoxins must be pursued to develop a new generation of inactivators that are active on a large range of toxins but, owing to the diversity of the chemical structures of mycotoxins, this aim will be difficult to achieve.

References

- Auerbach H., Maas R.F.M., Op den Camp H.J.M., Pol A. and Fink-Gremmels J., 1998. Biodegradation of aflatoxin B1 by bovine rumen microorganisms *in vitro* and its effects on rumen fermentation. In: *Revue de Médecine Vétérinaire*, 149. p. 573.
- Carson M.S. and Smith T.S., 1983. Role of bentonite in prevention of T-2 toxicosis in rats. In: *J. Anim. Sci.*, 57. p. 1498-1506.
- CAST (Council for Agricultural Science and Technology), 2003. *Mycotoxins: Risks in plant, animal, and human systems*. Task Force Report No. 139. Ames, USA: CAST. p. 199.
- Chu F.S., 1974. Studies in ochratoxin. *CRC Critical Reviews in Toxicology*, 2. p. 499.
- Clevstroem G., Göransson B., Hlödversson R. and Pettersson H., 1981. Aflatoxin formation in hay treated with formic acid and in isolated strains of *Aspergillus flavus*. In: *J. Stored Products Research*, 17. p. 151-161.
- Côté L.M., Dalhem A.M., Yoshizawa T., Swanson S.P. and Buck W.B., 1986. Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy cows. In: *J. Dairy Sci.*, 69. p. 2416-2423.
- Creppy E.E., Chiarappa P., Baudrimont I., Borracchi P., Moukha S. and Carratù M.R., 2004. Synergistic effects of fumonisin B1 and ochratoxin A: Are *in vitro* cytotoxicity data predictive of *in vivo* acute toxicity? *Toxicology*, 201. p. 115-123.
- Creppy E.E., Störmer F.C., Roschenthaler R. and Dirheimer G. (1983). Effects of two metabolites of ochratoxin A, (4R)-4-hydroxyochratoxin A and ochratoxin α , on immune response in mice. In: *Infection and Immunity*, 39. p. 1015-1018.
- D'Mello J.P.F. and McDonald A.M.C., 1997. Mycotoxins. In: *Anim. Feed Sci. Techn.*, 69. p. 155-166.
- D'Mello J.P.F., McDonald A.M.C., Postel D., Dijkema W.T.P., Dujardin A. and Placinta C.M., 1998. Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. In: *European J. Plant Pathology*, 104. p. 741-751.
- Damaglou A.P., Shannon W. and Downey G.A., 1984. The interactions between Fusaria and their toxins in grass silage. In: *J. Sci. Food Agric.*, 35. p. 279-284.
- Danicke S., 2002. Effects of *Fusarium* toxin contaminated wheat grain and of a detoxifying agent on rumen physiological parameters and *in sacco* dry matter degradation of wheat straw and Lucerne in wethers. In: *J. Anim. Feed Sci.*, 11. p. 437-451.
- Devegowda G., Raju M.V.L.N., Afzali N. and Swamy H.V.L.N., 1998. Mycotoxin picture world-wide: Novel solutions for their counteraction. In: Lyons T.P. and Jacques K.A. (eds). *Biotechnology in the Feed Industry*. Nottingham, UK: Nottingham University Press. p. 241-256.
- Doll S., Valenta H., Danicke S. and Flachowsky G., 2002. *Fusarium* mycotoxins in conventionally and organically grown grain from Thuringia/Germany. In: *Landbauforschung Volkenrode*, 52. p. 91-96.
- Dominguez-Bello M.G., 1996. Detoxification in the rumen. In: *Annales de Zootechnie*, 45(suppl.). p. 323-327.
- Dowd P.F., 2003. Insect management to facilitate preharvest mycotoxin management. In: *J. Toxicology*, 22. p. 327-350.
- EFSA, 2004. Opinion of the scientific panel on contaminants in the food chain on a request from the Commission related to AFB1 as undesirable substance in animal feed. In: *The EFSA Journal*, 39. p. 1-27.
- El Nezami H., Kankaanpää P., Salinen S., Mykkänen H. and Ahokas J., 1998. Use of probiotic bacteria to reduce aflatoxin uptake. In: *Revue Médicale Vétérinaire*, 149. p. 570.

- El Nezami H., Mykkänen H., Haskard C., Salminen S. and Salminen E., 2004a.** Lactic acid bacteria as a tool for enhancing food safety by removal of dietary toxins. In: Salminen S., von Wright A. and Ouwehand A. (eds). *Lactic Acid Bacteria: Microbiology and Functional Aspects*, 3rd edn. New York, USA: Marcel Dekker. p. 397-406.
- El Nezami H., Polychronaki N., Lee Y.K., Haskard C., Juvonen R., Salminen S. and Mykkänen H., 2004b.** Chemical moieties and interactions involved in the binding of zearalenone to the surface of *Lactobacillus rhamnosus* strains GG. In: *J. Agric. Food Chem.*, 52. p. 4577-4581.
- Escoula L., Le Bars J. and Larrieu G., 1972.** Etudes sur la mycoflore des ensilages et de la mycoflore des fronts de coupe des ensilages de graminées fourragères. In: *Annales de Recherches Vétérinaires*, 3. p. 469-481.
- European Commission, 1997.** Reports on tasks for scientific cooperation. Assessment of dietary intake of ochratoxin A by the population of EU member States. Report EUR 17523 EN. Brussels, Belgium: European Commission, Directorate-General for Industry.
- FAO, 2000.** Food safety and quality as affected by organic farming. 22nd FAO Regional Conference for Europe, Porto (Portugal), 24-28 July 2000.
- FAO, 2001.** Safety evaluation of certain mycotoxins in food. JECFA document, FAO Food and Nutrition Paper 74. Rome, Italy: FAO.
- Fink-Gremmels J., Blom M.J., Woutersen van Nijnanten M.A., Jahn A. and de Groene E.M., 1995a.** Biotransformation process in the etiology of ochratoxicosis. In: Eklund M., Richard J.L. and Mise K. (eds). *Molecular Approaches to Food Safety: Issues Involving Toxic Microorganisms*. Fort Collins, Colorado, USA: Alaken Inc. p. 107-121.
- Fink-Gremmels J., Jahn A. and Blom M.J., 1995b.** Toxicity and metabolism of ochratoxin A. In: *Natural Toxins*, 3. p. 214-220.
- Galtier P., 1998.** Biological fate of mycotoxins in animals. In: *Revue de Médecine Vétérinaire*, 149. p. 549-554.
- Galtier P., 1999.** Biotransformation and fate of mycotoxins. In: *J. Toxicology – Toxin Reviews*, 18. p. 295-312.
- Galtier P. and Alvinerie M., 1976.** *In vitro* transformation of ochratoxin A by animal microbial floras. In: *Annales de Recherche Vétérinaire*, 7. p. 91-98.
- Galvano F., Pietri A., Bertuzzi T., Bognanno M., Chies L., de Angelis A. and Galvano M., 1996.** Activated carbons: *In vitro* affinity for fumonisins B1 and relation of absorption ability to physicochemical parameters. In: *J. Food Protection*, 60. p. 985-991.
- Garung N.K., Rankins D.L., Shelby R.A. and Goel S., 1998.** Effects of fumonisin B1-contaminated feeds on weanling Angora goats. In: *J. Anim. Sci.*, 76. p. 2863-2870.
- Hagler W.M., Towers N.R., Mirocha C.J., Eppley R.M. and Bryden W.L., 2001.** Zearalenone: Mycotoxin or mycoestrogen? In: Summerel B.A., Leslie J.F., Backhouse D., Bryden W.L. and Burgess L.W. (eds). *Fusarium: Paul E. Nelson Memorial Symposium*. St. Paul, Minnesota, USA: APS Press. p. 321-331.
- Harvey R.B., Edrington T.S., Kubena L.F., Elissalde M.H., Corrier D.E. and Rottinghaus G.E., 1995a.** Effect of aflatoxin and diacetoxyscirpenol in ewe lambs. In: *Bulletin of Environmental Contamination and Toxicology*, 54. p. 325-330.
- Harvey R.B., Edrington T.S., Kubena L.F., Elissalde M.H. and Rottinghaus G.E., 1995b.** Influence of aflatoxin and fumonisin B1-containing culture material on growing barrows. In: *American J. Vet. Res.*, 56. p. 1668-1672.
- Hope R. and Magan N., 2003.** Two dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by *Fusarium culmorum* on a wheat-based substrate. In: *Letters in Applied Microbiology*, 37. p. 70-74.
- Hult K., Teiling A. and Gettenbeck S., 1976.** Degradation of ochratoxin A by a ruminant. In: *Applied and Environmental Microbiology*, 32. p. 443-444.
- Hussein S.H. and Brasel J.M., 2001.** Toxicity, metabolism and impact of mycotoxins on human and animals. In: *Toxicology*, 167. p. 101-134.
- IARC, 1993.** Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines, and mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 56. Lyon, France: International Agency for Research on Cancer. p. 571.
- IARC, 2002.** Traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 82. Lyon, France: International Agency for Research on Cancer.
- JOCE, 2006a.** Règlement (CE) N° 1881/2006 de la Commission du 19 décembre 2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires. *Journal Officiel de l'Union Européenne*, 20/12/06, L364/5-L 364/24.
- JOCE, 2006b.** Recommandation de la Commission du 17 août 2006 concernant la présence de déoxynivalénol, de zéaralénone, d'ochratoxine A, des toxines T-2 et HT-2 et des fumonisines dans les produits destinés à l'alimentation animale. *Journal Officiel de l'Union Européenne*, 23/08/06, L 229/7-L 229/9.
- Jouany J.P., 1989.** Effect of diet on populations of rumen protozoa in relation to fibre digestion. In: Nolan J.V.,

- Leng R.A. and Demeyer D.I. (eds). *The Roles of Protozoa and Fungi in Ruminant Digestion*. Armidale, Australia: Penambul Books. p. 59-74.
- Kalac P. and Woolford M.K., 1982.** A review of some aspects of possible associations between the feeding of silage and animal health. In: *Brit. Vet. J.*, 138. p. 305-320.
- Kennedy D.G., Hewitt S.A., McEvoy J.D., Currie J.W., Cannavan A., Blanchflower W.J. and Elliott C.T., 1998.** Zeranone is formed from *Fusarium* sp. toxins in cattle *in vivo*. In: *Food Additives and Contaminants*, 15. p. 393-400.
- Kiessling K.H., Pettersson H., Sandholm K. and Olsen M., 1984.** Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. In: *Applied and Environmental Microbiology*, 47. p. 1070-1073.
- Krishnamachari K.A.V.R., Bhat R.V., Nagarajan V. and Tilac T.B.G., 1975a.** Investigations into an outbreak of hepatitis in Western India. In: *Indian J. Medical Research*, 63. p. 1036-1048.
- Krishnamachari K.A.V.R., Bhat R.V., Nagarajan V. and Tilac T.B.G., 1975b.** Hepatitis due to aflatoxicosis. In: *Lancet*, 1. p. 1061-1063.
- Krogh P., 1976.** Epidemiology of mycotoxic porcine nephropathy. In: *Nordic Veterinary Medicine*, 28. p. 452-458.
- Kubena L.F., Edrington T.S., Harvey R.B., Buckley S.A., Phillips T.D., Rottinghaus G.E. and Casper H.H., 1997a.** Individual and combined effects of fumonisin B1 present in *Fusarium moniliforme* culture material and T-2 toxin or deoxynivalenol in broiler chicks. In: *Poultry Science*, 76. p. 1239-1247.
- Kubena L.F., Edrington T.S., Harvey R.B., Phillips T.D., Sarr A.B. and Rottinghaus G.E., 1997b.** Individual and combined effects of fumonisin B1 present in *Fusarium moniliforme* culture material and diacetoxyscirpenol or ochratoxin A in turkey poults. In: *Poultry Sci.*, 76. p. 256-264.
- Kubena L.F., Edrington T.S., Kamps-Holtzapfle C., Harvey R.B., Elissalde M.H. and Rottinghaus G.E., 1995.** Influence of fumonisin B1 present in *Fusarium moniliforme* culture material and T-2 toxin on turkey poults. In: *Poultry Sci.*, 74. p. 306-313.
- Le Bars J., 1976.** Mycoflore des fourrages secs: Croissance et développement des espèces selon les conditions hydrothermiques de conservation. In: *Revue de Mycologie*, 40. p. 347-360.
- Le Bars J. and Le Bars P., 1996.** Recent acute and subacute mycotoxicoses recognized in France. In: *Vet. Res.*, 27. p. 383-394.
- Lepom P., Knabe O. and Baath H., 1988.** Occurrence of *Fusarium* species and their mycotoxins in maize. 3. The influence of silaging on the zearalenone content in CCM maize. In: *Arch. Anim. Nut.*, 38. p. 817-823.
- Mackie R.I. and White B.A., 1990.** Recent advances in rumen microbial ecology and metabolism. Potential impact on nutrient output. In: *J. Dairy Sci.*, 73. p. 2971-2995.
- Magan N., Hope R., Colleate A. and Baxter E.S., 2002.** Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. In: *European J. Plant Pathology*, 108. p. 685-690.
- Maryamma K.I. and Sivasdas C.G., 1975.** Aflatoxicosis in goats; An experimental study. In: *Indian Vet. J.*, 52. p. 385-392.
- Masimango N., Remacle J. and Ramaut J., 1979.** Elimination par des argiles gonflantes de l'AFB1 des milieux contaminés. In: *Annales de la Nutrition et des Aliments*, 33. p. 137-147.
- May H.D., Wu Q. and Blake C., 2000.** Effects of the *Fusarium* spp. mycotoxins fusaric acid and deoxynivalenol on the growth of *Ruminococcus albus* and *Methanobrevibacter ruminantium*. In: *Canadian J. Microbiol.*, 46. p. 692-699.
- Mehrnejad M.R. and Panahi B., 2006.** The influence of hull cracking on aflatoxin contamination and insect infestation in pistachio nuts. In: *Applied Entomology and Phytopathology*, 73. p. 39-42.
- Miller D.M., Clark J.D., Hatch R.C. and Jain A.V., 1984.** Caprine aflatoxicosis: Serum electrophoresis and pathologic changes. In: *American J. Vet. Res.*, 45. p. 1136-1141.
- Miller J.K., 1979.** Current mycotoxin studies at the Edinburgh veterinary investigation centre. In: *Proceedings of a Third Meeting on Mycotoxins in Animal Disease*, Edinburgh (UK). p. 40-42.
- Munkvold G.P., Hellmich R.L. and Rice L.G., 1999.** Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. In: *Plant Disease*, 83. p. 130-138.
- Nesbitt B.F., O'Kelly J., Sargeant K. and Sheridan A., 1962.** *Aspergillus flavus* and Turkey × disease: Toxic metabolites of *Aspergillus flavus*. In: *Nature*, 195. p. 1062-1063.
- Ngindu A., Kenya P.R., Ocheng D.M., Omonde T.N., Ngare W., Gatei D., Johnson B.K., Ngira J.A., Nandwa H., Jansen A.J., Kaviti J.N. and Siongok T.A., 1982.** Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. In: *Lancet*, 1. p. 1346-1348.
- Nicholson P., Turner J.A., Jenkinson P., Jennings P., Stonehouse J., Nuttall M., Dring D., Weston G. and Thomsett M., 2003.** Maximising control with fungicides of *Fusarium ear blight (FEB)* in order to reduce toxin contamination of wheat. Project Report No. 297, HGCA, London.
- Olsen M. and Kiessling K.H., 1983.** Species differences in zearalenone-reducing activity in subcellular fractions of liver from female domestic animals. In: *Acta Pharmacologica et Toxicologica*, 52. p. 287-291.

- Özpinar H., Bilal T., Abas I. and Kutay C., 2002. Umsatz von ochratoxin A in pansenflüssigkeit *in vitro*. In: *Facta Universitatis, Series Medecine and Biology*, 9. p. 66-69.
- Parkin C.S., Miller P.C., Magan N., Aldred D., Gill J. and Orson J.H., 2006. The deposition of fungicides on ears to control *Fusarium* ear blight and the mycotoxin contamination of grain. In: *Aspects of Applied Biology*, 77. p. 445-452.
- Pelhate J., 1975. Mycoflore des maïs fourrages ensilés. In: *Revue de Mycologie*, 39. p. 65-95.
- Pelhate J., 1987. La microbiologie des foin. In: Demarquilly C. (ed.). *Les Fourrages Secs: Récolte, Traitement, Utilisation*. Paris, France: INRA Editions. p. 63-81.
- Pettersson H. (1998). *Carryover of aflatoxin from feedingstuff to milk*. Report of Department of Animal Nutrition and Management. Swedish University of Agricultural Sciences, Uppsala, Sweden. p. 21.
- Pfohl-Leszkowicz A., 2000a. Ecologie des moisissures et des mycotoxines. Situation en France. In: *Cahiers de Nutrition et de Diététique*, 35. p. 379-388.
- Pfohl-Leszkowicz A., 2000b. Risques mycotoxicologiques pour la santé des animaux et de l'homme. In: *Cahiers de Nutrition et de Diététique*, 35. p. 389-397.
- Phillips T.D., Kubena L.F., Harvey R.B., Taylor D.R. and Heidelbaugh N.D., 1988. Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. In: *Poultry Sci.*, 67. p. 243-247.
- Pier A.C., 1992. Major biological consequences of aflatoxicosis in animal production. In: *J. Anim. Sci.*, 70. p. 3964-3967.
- Pittet A., 1998. Natural occurrence of mycotoxins in foods and feeds – An update review. In: *Revue de Médecine Vétérinaire*, 149. p. 479-492.
- Piva G.F., Galvano F., Pietri A. and Piva A., 1995. Detoxification methods of aflatoxins. A review. In: *Nut. Res.*, 15. p. 689-715.
- Poisson J. and Cahagnier B., 1973. Problème de la stabilisation de la microflore des grains humides par les acides organiques. In: *Annales de Technologie Agricole*, 22. p. 567-586.
- Porter J.K., Wray E.M., Rimando A.M., Stancel P.C., Bacon C.W. and Voss K.A., 1996. Lactational passage of fusaric acid from the feed of nursing dams to the neonate rat and effects on pineal neurochemistry in the F1 and F2 generations at weaning. In: *J. Toxicol. Environ. Health*, 49. p. 161-175.
- Prelusky D.B., Veira D.M., Trenholm H.L. and Hartin K.E., 1986. Excretion profiles of the mycotoxin deoxynivalenol, following oral and intravenous administration to sheep. In: *Toxicological Sci.*, 6. p. 356-363.
- Ramos A.J., Hernández E., Pla-Delfina J.M. and Merino M., 1996. Intestinal absorption of zearalenone and *in vitro* study of non-nutritive sorbent material. In: *Int. J. Pharm.*, 128. p. 129-137.
- RASFF (Rapid Alert System for Food and Feed), 2003. Information concerning the detection of AFM1 in raw milk from Italy. News Notification 03/87, 19 November 2003.
- Raymond S.L., Smith T.K. and Swamy H.V.L.N., 2005. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, metabolism, and indices of athletic performance of exercised horses. In: *J. Anim. Sci.*, 83. p. 1267-1273.
- Ribeiro J.M.M., Cavaglieri L.R., Fraga M.E., Direito G.M., Dalcero A.M. and Rosa C.A.R., 2006. Influence of water activity, temperature and time on mycotoxins production on barley rotlets. In: *Letters in Applied Microbiology*, 42. p. 179-184.
- Richard-Molard D., 1999. Mycotoxin occurrence, identification of *Fusarium* species and role of processing. In: *IRTAC Conference*, 1-2 December 1999, Paris, France (internal document: IRTAC, 16 rue Nicolas-Fortin, 75013 Paris).
- Samapundo S., De Meulenaer B., Atukwase A., Debevere J. and Devlieghere F., 2007. The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B1 production of *Fusarium verticillioides* and *F. proliferatum* on corn. Part II: The effect of initial headspace oxygen concentration. In: *International J. of Food Microbiology*, 113. p. 339-345.
- Samarajeewa U., Aseculleratine S.N. and Tennekoon G.E., 1975. Spontaneous and experimental aflatoxicosis in goats. In: *Res. Vet. Sci.*, 19. p. 269-277.
- Scudamore K.A. and Livesey C.T., 1998. Occurrence and significance of mycotoxins in forage crops and silage: A review. In: *J. Sci. Food Agric.*, 77. p. 1-17.
- Smith M.E., Lewis C.W., Anderson J.G. and Solomons G.L., 1994. A literature review carried out on behalf of the Agro-industrial division, E2, of the European Commission Directorate-General XII for scientific research and development. In: *Mycotoxins in Human Nutrition and Health*. Brussels, Belgium: EC-DG XII. p. 27.
- Smith T.K., 1984. Spent canola oil bleaching clays: Potential for treatment of T-2 toxicosis in rats and short-term inclusion in diets of immature swine. In: *Canadian J. Anim. Sci.*, 64. p. 725-732.
- Smith T.K., McMillan E.G. and Castillo J.B., 1997. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. In: *J. Anim. Sci.*, 75. p. 2184-2191.
- Spahr U., Walther B. and Sieber R., 2000. Transfert des mycotoxines dans le lait: Vue d'ensemble. In: *Revue Suisse Agricole*, 32. p. 75-78.
- Swamy H.V.L.N., Smith T.K., MacDonald E.J., Boermans H.J. and Squires E.J., 2002. Effects of feeding a

blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain regional neurochemistry and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. In: *J. Anim. Sci.*, 80. p. 3257-3267.

- Szczeczek G.M., Carlton W.W. and Tuite J., 1973.** Ochratoxicosis in beagle dogs. II. Pathology. In: *Veterinary Pathology*, 10. p. 219-231.
- Underhill K.L., Rotter B.A., Thompson B.K., Prelusky D.B. and Treholm H.L., 1995.** Effectiveness of cholestyramine in the detoxification of zearalenone as determined in mice. In: *Bulletin of Environmental Contamination and Toxicology*, 54. p. 128-134.
- Valero A., Farre J.R., Sanchis V., Ramos A.J. and Marin S., 2006.** Effects of fungal interaction on ochratoxin A production by *A. carbonarius* at different temperatures and a_w . In: *Int. J. Food Microbiol.*, 110. p. 160-164.
- Vallone L. and Dragoni I., 1997.** Investigation of mycotoxin (aflatoxin B1) occurrence in corn silage trench. In: *Atti della Società Italiana delle Scienze Veterinarie*, 51. p. 237-238.
- van Egmond H.P., 1989.** Aflatoxin M1: Occurrence, toxicity, regulation. In: van Egmond H.P. (ed.). *Mycotoxins in Dairy Products*. London and New York: Elsevier Applied Science. p.147-159.
- van Egmond H.P. and Jonker M.A., 2004.** Current regulations governing mycotoxin limits in food. In: Magan N. and Olsen M. (eds). *Mycotoxins in Food: Detection and Control*. Cambridge, UK: Woodhead Publishing Ltd. p. 49-68.
- Veldman A., Meijst J.A.C., Borggreve G.J. and Heeres-vander Tol J.J., 1992.** Carry-over of aflatoxin from cow's food to milk. In: *Anim. Prod.*, 55. p. 163-168.
- Westlake K., Mackie R.I. and Dutton M.F., 1987a.** Effects of several mycotoxins on specific growth rate of *Butyrivibrio fibrisolvens* and toxin degradation *in vitro*. In: *Applied and Environmental Microbiology*, 53. p. 613-614.
- Westlake K., Mackie R.I. and Dutton M.F., 1987b.** T-2 toxin metabolism by ruminal bacteria and its effect on their growth. In: *Applied Environmental Microbiology*, 53. p. 587-592.
- Westlake K., Mackie R.I. and Dutton M.F., 1989.** *In vitro* metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. In: *Anim. Feed Sci. Technol.*, 25. p. 169-178.
- Whitlow L.W., Diaz D.E., Hopkins B.A. and Hagler W.M., 2000.** Mycotoxins and milk safety: The potential to block transfer to milk. In: Lyons T.P. and Jacques K.A. (eds). *Proceedings of Alltech's 16th Annual Symposium*. Nottingham, UK: Nottingham University Press. p. 391-408.
- Wilson J.S., 2006.** Mycotoxin regulations and food safety: The international trade dimension. In: *The Proceedings of The World Mycotoxin Forum*, 4th edn, Cincinnati (OH, USA), 6-7 November 2006. p. 22.
- Wolde-Michael T., Miller H.M., Holmes J.H., McGregor B.A. and Galloway D.B., 1989.** Effect of supplementary feeding and zearanol on puberty in feral Cashmere goats. In: *Aust. Vet. J.*, 66. p. 124-126.
- Xiao H., Marquadt R.R., Frohlich A.A., Phillips G.D. and Vitti D.G., 1991a.** Effect of a hay and a grain diet on the rate of hydrolysis of ochratoxin A in the rumen of sheep. In: *J. Anim. Sci.*, 69. p. 3706-3714.
- Xiao H., Marquadt R.R., Frohlich A.A., Phillips G.D. and Vitti D.G., 1991b.** Effect of a hay and a grain diet on the bioavailability of ochratoxin A in the rumen of sheep. In: *J. Anim. Sci.*, 69. p. 3715-3723.
- Yiannikouris A., André G., Buléon A., Jeminet G., Canet I., François J., Bertin G. and Jouany J.P., 2004a.** Comprehensive conformational study of key interactions involved in zearalenone complexation with b-D-glucans. In: *Biomacromolecules*, 5(6). p. 2176-2185.
- Yiannikouris A., André G., Poughon L., François J., Dussap C.G., Jeminet G., Bertin G. and Jouany J.P., 2006.** Chemical and conformational study of the interactions involved in mycotoxins complexation with b-D-glucans. In: *Biomacromolecules*, 7(4). p. 1147-1155.
- Yiannikouris A., François J., Poughon L., Dussap C.G., Bertin G., Jeminet G. and Jouany J.P., 2004b.** Adsorption of zearalenone by β -D-glucans in the *S. cerevisiae* cell wall. In: *J. Food Protection*, 67. p. 1195-1200.
- Yiannikouris A., François J., Poughon L., Dussap C.G., Bertin G., Jeminet G. and Jouany J.P., 2004c.** Alkali-extraction of β -D-glucans from *S. cerevisiae* cell wall and study of their adsorptive properties toward zearalenone. In: *J. Agric. Food Chem.*, 52. p. 3666-3673.
- Yiannikouris A., François J., Poughon L., Dussap C.G., Jeminet G., Bertin G. and Jouany J.P., 2004d.** Influence of pH on model b-D-glucans complexation toward zearalenone. In: *J. Food Protection*, 67(6). p. 2741-2746.
- Yiannikouris A. and Jouany J.P., 2002.** Mycotoxins in feeds and their fate in animals: A review. In: *Anim. Res.*, 51. p. 81-99.