MYCOTOXINS IN FEEDSTUFFS PRODUCED OR IMPORTED IN EUROPE FOR ANIMAL FEEDS

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Introduction

Works on mycotoxins are numerous in the world and have been reviewed recently (CAST, 2003). However, it is important to point out the subject in focusing on the European commercial feedstuffs and feeds because the European context is changing. Consumers are more and more educated and are interested in the sanitary quality of foods and feeds. The European Commission has the will to manage a high quality sanitary policy for human beings as well as for animals. Finally, the feed industry and farmers who produce home feeds are responsible for the quality of their feed production.

The main mycotoxins

Mycotoxins are non proteic molecules produced by some microscopic fungi belonging to different species. They are not necessary for the life of the fungi. All the fungi species are not genetically able to produce mycotoxins and in a species, all the varieties are not genetically able to produce them. Moreover, in a genetically able variety, production depends on environmental factors which are not always understood.

Mycotoxins are very numerous in the world (more than 400) but European feedstuffs and feeds are concerned by only a few of these toxins.
Aflatoxins B1, B2, G1 and G2 are mainly produced by *Aspergillus* outside Europe. Ochratoxin A is produced by different *Penicillium* (mainly *P. verrucosum*) in feedstuffs during their storage. Fusariotoxins are produced by different *Fusarium* species on cereals during their growth in the field because of a high aw needed both for the growth of fungi but also for the production of toxins. Fusariotoxins are very numerous and may be classified into the trichothecens group, the zearalenone group and the fumonisines group. Trichothecens are themselves classified in groups A (comprising T2 and HT2 toxins) and B (comprising deoxynivalenol and its acetyl forms, and nivalenol). Deoxynivalenol (DON) and its acetyl forms, nivalenol and zearalenone (ZEN) are mainly produced by *F. graminearum* and *F. culmorum*, whereas fumonisines are mainly produced by *F. moniliforme, F. verticilloides and F. subglutinans.*

Other mycotoxins are evoked from time to time. Among them, the alkaloids from ergot (*Claviceps purpurea*) are not completely eliminated from cereal production even the risk of finding them is very low. Similarly, *Alternaria* toxins have been identified in different feedstuffs and especially in rapeseed, but it is not known if they can be deleterious for animals in the practical conditions.

The main effects of mycotoxins in animal feeding

The effects of mycotoxins are often studied in a short time period with high concentrations in feeds but in practice, mycotoxin effects concern low doses consumed over a long time.

Mycotoxins may cause a great diversity of effects. A single mycotoxin may have different effects on an animal. Some mycotoxins have more pronounced effects in one animal species and not in other ones. Some mycotoxins concern an organ and not another one (and not always the same in all the species). The nature of the pathology is varied: aflatoxins and ochratoxin mainly induce cancer, DON mainly reduces feed intake (especially in the pig) and slightly modulates the immune system, ZEN has estrogenic effects especially in the pig, fumonisins reduce immunity defence and efficiency of vaccination, T2 and HT2 toxins reduce immunity and lead to tissue necrosis, alkaloids from ergot have effects on nervous system and constrict blood vessels.
The effects of association of mycotoxins are questioned, especially when mycotoxins contents in the feed are low.

Mycotoxins consumed by animals may only concern the productivity, the health and the welfare of the animals but in some cases, they may concern meat, milk and eggs which are consumed by humans. The main problems concern aflatoxin M1 in the milk (coming mainly from the metabolism of aflatoxin B1), and deposition of ochratoxin in the kidneys, liver and muscle.

How to evaluate the mycotoxin contamination

Mycotoxin contents are characterized by heterogeneous distributions. In a wheat field, DON content may range from 1 to 15. After harvest, the range of variation is less (because the most contaminated grains are small and remain in the field, and because the harvest mixes grains) but is still important. In a silo, cereal contamination by ochratoxin is also heterogeneous because filling the silo may be done with different cereal batches with different moisture content, or due to the migration of moist air among grains.
So it is important before measuring a mycotoxin content to ensure that the sample on which the measurement will be done is representative. The European Commission has published directives concerning sampling before aflatoxins or ochratoxin measurement but these directives are not well adapted to huge batches for the feed industry.

After isolation of a sample, toxins must be extracted from it. Some of the tasks realized for this purpose as size of particles obtained by grinding or saturation of immunoaffinity columns in the case of purification of fumonisins play an important role (Raimbault et al, 2004).

Measurement of mycotoxins may be done by different techniques. Rapid methods using immunoenzymatic reactions are proposed by different commercial firms. An immunoenzymatic test concerns one mycotoxin and only in a specific range of content. So such tests may be useful to sort batches, but are not precise. Moreover they cannot be used by non-specialized persons. Precise measurements are based on chromatography, especially

high performance liquid chromatography (HPLC) or gas chromatography (GC), with or without mass spectroscopy. GC allows the measurement of different trichothecens in a simple operation.
New ways are investigated such as the infra red (Petterson, 2000) or indirect measurement as the evaluation of gene Tri5.
Interlaboratory tests are carried out to improve the quality of analyses, but important differences between laboratories remain.

Occurrence of mycotoxins
In spite of these differences, the European Commission has organized surveys on contamination of food for human beings. These surveys, called scientific cooperative tasks (SCOOP tasks) concerned aflatoxins (2000), ochratoxin (2002) and fusariotoxins (2003). They can be used for evaluating some feedstuffs (grains). Other surveys have been carried out but few are published.
DON is the most prevalent mycotoxin in European cereals. In French wheat, about 3% of the batches have more than 1000 µg/kg on average; but regional effects may be seen each year. DON content in maize is higher than in durum, than in wheat, than in barley in France.
ZEN may be found in all cereal species but more often in maize than in other grains.
Fumonisines are quite specific for maize, and especially in batches grown in the south of Europe.
T2 and HT2 toxins are less prevalent.
OTA have been found in a few cereal batches, in low amounts.
Aflatoxins are mainly found in imported feedstuffs coming from intertropical areas but they have also been founded in small amounts in maize samples coming from the south of Europe. It is not understood if this situation is occasional or if it reflects the greenhouse warming of the Earth.

Factors influencing the production of mycotoxins
Factors influencing the production of mycotoxins by fungi have begun to be estimated. DON content in wheat and durum depends principally on the climate between flowering and harvest of the cereal (Barrier-Guillot, 2004). But some other factors can modulate this important factor. The quantity of contaminated residues coming from the previous crop increases the level of DON. This occurs when wheat is grown after maize, especially with maize grown for grain comparatively to the whole plant for silage (Obst, 2000); this occurs also when no tillage is applied, and there is an interaction between these two factors. The level of resistance of the variety to Fusarium attack influences the DON content, but this role is limited; moreover, the level of the resistance of all the varieties to *Fusarium* attacks is not well known. At last, the phytosanitary protection of wheat may play a role. Only triazoles are efficient against *Fusarium* and lead to reduced DON content in wheat; strobilurines, which are efficient against *Microdochium* - a fungus responsible for the same symptoms on wheat as *Fusarium* - are unable to limit wheat DON content when wheat is concerned with Fusarium head blight.
DON content in maize depends on the same factors as for wheat, but may also be influenced by a late harvest or by delays between harvest and drying, or by storage in cribs.
Factors of variation of ZEN content have not been studied so well as for DON, and are considered as the same ones.
Fumonisines contents are thought to be influenced by hot summers and wounds on grains caused by insects, especially the European corn borer (*Ostrinia nubilalis*). The combat against this insect may be conducted by classical ways but it could be done by using Bt varieties (Bakan et al, 2002).
Ochratoxin A content depends on grain moisture, and more precisely on the aw of the part of the batch where *Penicillium* development is possible.

Means of decontamination
Means of decontamination of contaminated batches by mycotoxins are numerous. Unfortunately, the practical means are not numerous (for example, washing grains may be used to eliminate a part of the fusariotoxines which are soluble, but this technique is not appropriate in industry). Sorting is based on the fact that small grains and residues of crops contain more fusariotoxins than medium sized grains, but is this technique economically interesting? Different clays have been tested to adsorb mycotoxins but success depends on the considered mycotoxin: percentage of mycotoxin adsorbed is higher for aflatoxin than for OTA than for ZEN than for DON (Devegowda et al, 2000). Studies are in progress to degrade mycotoxins by enzymes but commercial applications are still disappointing for DON (Dänicke et al, 2004). Ammoniation is currently used to inactivate aflatoxins in tropical feedstuffs such as groundnut meal.

Legislation concerning mycotoxins
European legislation concerning mycotoxins in feedstuffs and feeds is limited. Aflatoxins and ergot are ruled by directives 2003/100 and 2002/32.
European legislation dealing with mycotoxins for food is useful to know because it has consequences on the quality of batches which are designed to animal feeds. This legislation is made of regulations concerning aflatoxins, ochratoxin A and patuline; it also concerns ergot for intervention in the case of wheat and durum.
Projects concerning fusariotoxins in foods and different mycotoxins for feeds are in progress.

Conclusion
The effects of mycotoxins on man and animals must be considered case by case, in considering couples ‘a mycotoxin/a target which is an organ or a function of a given animal with its age and its sanitary conditions’. They must not be taken globally: all the mycotoxins are not as dangerous as aflatoxins.
Mycotoxins do not generally present a sanitary risk for animals in the European conditions, but represent a decreased performance of animals and therefore economical losses.
The fight against mycotoxins must be supported by a global strategy. Decontamination of feedstuffs by the feed industry is limited. So the device must be: prevention of contamination is better than cure.