

Mycotoxins in animal nutrition - problems and solutions

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Introduction

Mycotoxins are secondary metabolic products of fungi (moulds) and they are toxic to animals or humans. It is estimated that there are about 300 mycotoxins harmful to humans or animals. With improvements in analytical methods, the list is certainly going to be increased in the future.

Mycotoxins are responsible for diseases called mycotoxicoses. The toxicity of these compounds depends on the amounts ingested, time-span of exposure, type of animal, their breed, age, sex, health status, but also other parameters such as density of animals, diseases, temperature, etc...

The fungi responsible for the production of mycotoxins grow on plants and commodities. Mycotoxins can be produced before harvest (by so-called field fungi) or during storage (by the storage fungi). They can also be produced on the finished feed when storage conditions are not correct. The fungi not only produce mycotoxins, they also damage the crop. Food and Agricultural Organization (FAO) estimates that about 25% of the world's crops contain mycotoxins.

Typical symptoms caused by major mycotoxins in poultry and swine

Aflatoxins are the most well known mycotoxins and extensive research has been done about these mycotoxins. There are 4 major aflatoxins observed in feedstuffs: Aflatoxin B₁, Aflatoxin B₂, Aflatoxin G₁, and Aflatoxin G₂.

Aflatoxicosis was first clearly described in the 1960s when more than 100,000 young turkeys in England died in a few months from an apparently new disease that was called "**Turkey X disease**". This disease also affected ducklings and young pheasants with heavy mortality levels. Investigations showed that the cause of the outbreaks was a Brazilian peanut meal infected by a fungus, *Aspergillus flavus*. The toxin was named *Aflatoxin* because of its origin (*A. flavus*). Today, it is agreed that only four species of fungi produce aflatoxins. They are, namely, *A. flavus*, *A. Parasiticus*, *A. nomius* and *A. pseudotamarii* (Ito et al., 2001; Kurtzman et al., 1987; Payne, 1998). However, only *A. flavus* and *A. Parasiticus* are economically important.

Aflatoxins are produced when adequate substrate and favourable environmental conditions are present (tropical and subtropical climates, humid storage conditions). They usually appear on cereals (barley, corn, millet, oats, rice, sorghum, wheat) cottonseeds, peanuts, tree nuts and soybeans. Aflatoxins can be formed before harvest, but also during storage if the grain is improperly stored (Tangendjaja, 2002).

Aflatoxins typically affect liver. When ingested in large quantities, they can cause liver cancer, chronic hepatitis, jaundice and cirrhosis. Exposure to lower doses over a long period of time can also present a risk.

Liver weight in broilers is normally 2% of total body weight. In case of aflatoxicosis, the liver is pale and its weight, relative to body, increases. For example, when given a feed contaminated with 5 ppm aflatoxin, the weight of the liver is 4 to 5 % of total body weight.

In poultry, ducks are the most sensitive to aflatoxins, followed by turkey, broiler and layers. Duration of exposure, as well as age, is as important as the level of aflatoxins in feed (see Figures 1 and 2).

The following symptoms have been observed following contamination with aflatoxins in poultry: fatty liver, kidney disorders, leg and bone problems, pigmentation problems (carcasses, egg yolk), reduced hatchability, smaller eggs and reduced eggshell quality, coccidiosis, vaccine failure, reduced immunity, lower resistance to diseases, bacteria, viruses... and of course reduced performances (weight gain, FCR, mortality).

In pigs, aflatoxicity can be observed in all stages: suckling piglets, growing and finishing swine and breeder stock. Some of the symptoms observed are reduced feed intake, increased FCR, reduced weight gain, toxic hepatitis, nephrosis and systemic haemorrhages. Aflatoxins can also be transferred in uterus from sows to the piglet and affect the new born pigs.

The group of trichothecenes contains about 150 structurally related compounds (Grove, 1988). They are usually produced by fungi from the group of *Fusarium* but also from the genera *Trichoderma*, *Stachybotrys*, *Verticimonosporium*, *Cephalosporium* and *Myrothecium*.

Trichothecenes are divided in 2 groups. Group A trichothecenes include T-2 toxin, HT-2 toxin and Diacetoxyscirpenol (DAS). Common group B trichothecenes are Deoxynivalenol (= DON = vomitoxin), Nivalenol (NIV), and Fusarenol X.

Trichothecenes are found in plants grown in warm and moderate climates, because *Fusarium* is a large and complex group of fungi adapted to a wide range of habitats all over the world (Summerell et al. 2001). They are observed on cereals (barley, corn, rice, oats, wheat) and soybeans. There are also found in other commodities such as sorghum, potatoes, bananas, mustard seed, groundnuts, mangoes, sunflower seeds and cassava. The trichothecenes can be produced under field conditions but also during storage. For example, optimum temperature for production of DON is 20 to 30°C.

The group of *Fusarium* fungi include important plant pathogens causing serious diseases in growing crops. For example, *Fusarium* causes a disease called head scab or head blight, affecting cereals in most of the world, provided that rainy or humid weather happens during host flowering and early kernel-fill stages (Abramson 1998). In the People's Republic of China, wheat scab is estimated to affect more than 7 million hectares (28% of total area), with yield losses up to 20 to 40% (Bai and Shaner 1994).

In poultry, trichothecenes can be very harmful. Indeed, T-2 toxin and other type A trichothecenes are the most dangerous *Fusarium* mycotoxins for poultry. These mycotoxins usually cause lesions at the edges of the beaks and in the intestine. They result in decreased feed intake, weight loss, abnormal feathering in chicks, a severe and sudden drop in egg production, eggs with weaker shells, and mortality. Turkeys are also very sensitive to T-2 toxin (reduced growth, beak lesions, reduced immunity). Group B trichothecenes seem less harmful to poultry than they are to pigs for example. Chickens and turkeys are apparently not very susceptible to the effects of DON. Kubena *et al.* (1987) reported that DON at 18 ppm in feed of Leghorn chickens did not affect their weight gain. However, nivalenol was reported to have negative effects in poultry. For example Hedman *et al.* (1995) studied the effect of different doses of NIV in feed of 7-day old male broiler chickens. Body weight gain was reduced by 11% for levels of 6 and 12 ppm, while at lower doses no effect was observed. Feed intake and feed conversion ratio were also affected. Gizzard erosions were found in

one third of the birds given a feed containing 12 ppm NIV and in 8% of those fed 3 or 6 mg/kg. Such damages were not observed in the control group. Also, absolute and relative liver weights were reduced with levels of 6 and 12 ppm. In 2002, Garaleviciene *et al.* studied the effects of NIV in laying hens. For 7 weeks, White Leghorn hens (55 weeks old) were given access to diets containing 0, 1, 3 or 5 ppm NIV. Feed intake was reduced by NIV, but there were no apparent effects on body weight, egg production and egg quality. However, pathological examination of the birds at the end of the trial revealed that 40 to 75% of hens fed a diet containing NIV (3 and 5 ppm) had gizzard lesions, haemorrhages in the duodenum and swollen cloaca and oviducts with immature eggs. Some of the birds in the 1 ppm NIV group had light and fragile livers.

Deoxynivalenol and T-2 toxin appear as the most harmful trichothecenes in pigs. T-2 toxin (and other type A Trichothecenes) causes reduced productivity at feed concentrations of 200 ppb or less. In sows, infertility with some lesions in the uteri and ovaries can be observed after a feed contaminated with 1 to 2 ppm of T-2 toxin has been consumed. DON is causing a disease called “moldy corn toxicosis of swine”. The grain is unpalatable to pigs, feed intake is reduced, and results in poor weight gain or even weight loss, increased incidence of infectious diseases and digestive disorders (diarrhoea). In severe cases vomiting is observed (DON is also called vomitoxin). In the farrowing house, DON causes failure of mature sows to return to oestrus, reduced efficiency, but also intestinal tract inflammation and acute diarrhoea of suckling piglets, resulting in high mortality.

Zearalenone is also called ZON, Zea, or ZEN. It is produced by the *Fusarium* fungi, mainly by *Fusarium graminearum*. This fungus is regularly found in high-moisture corn and in mouldy hay or pelleted feeds. The production of zearalenone is possible in warm and moderate climates, all over the world. High humidity is necessary and the optimum temperature range is 18 to 30°C. A drop in temperature stimulates production of the toxin (Cheeke *et al.* 1985).

Zearalenone appears to be well tolerated by poultry. Chi *et al.* (1980) observed that a single oral ingestion of 15 g/kg body weight was not toxic. Data suggest that up to 800 ppm of ZON in feed from 6 to 9 weeks of age does not affect performances of broilers (Allen *et al.*, 1981). However it also appears that zearalenone can be detrimental at lower levels in turkey: for example, reduced egg production (-20%) was observed with 100 ppm for 56 days (Allen *et al.*, 1983). In contrast, different trials indicate that levels up to 800 ppm did not affect laying hens.

Pigs are very sensitive to zearalenone. ZON (above 0.05 to 0.25 ppm in feed) typically affects sows and gilts, causing the “estrogenic syndrome”: ZON interacts with oestrogen receptors and results in swollen vulva, enlarged mammary glands and disturbed reproduction. In severe cases it can cause rectal or vaginal prolapse. In boars, ZON causes enlargement of mammary glands and atrophy of sperm cells. The effects of ZON are not always spectacular on the farm but it can significantly affect the reproductive performance and cause severe financial losses to the farmers. Levels of 1 ppm can cause feed refusal. It appears that zearalenone can also be ingested by piglets in sow’s milk, causing estrogenic syndrome. Splay legs in newborn piglets have also been associated with ZON contamination.

Ochratoxins are produced by *Aspergillus* fungi (example: *A. ochraceus*) and some *Penicillium*. The most known (and most toxic) mycotoxin in this group is called Ochratoxin A (= OTA or OA). There are other compounds in this group, but they are

less toxic. *Aspergillus ochraceus* produces OTA in hot climates, while *Penicillium verrucosum* produces it in temperate countries. *A. ochraceus* is for example found in Brazil, Chile, Egypt, Senegal, Tunisia, India and Indonesia. *Penicillium verrucosum* is observed in Canada, US, Europe, and South America (Source FAO).

In poultry, OTA is often reported to have damaging effects, for example in the United States. Symptoms are increased mortality, reduced growth and increased feed conversion ratio, and feed refusal. At higher doses, one can observe diarrhoea, tremors and other neural malfunctions. In laying animals, OTA also reduces egg production and quality: weaker eggshell and eggs with blood and meat spots are typical indicators of ochratoxin contamination.

Gentles *et al.* (1999) have demonstrated the negative effect of 2.5 ppm of OTA in feed of young broiler chickens. After 3 weeks, their body weights were reduced by 23% when compared to controls, while feed conversion ratio was comparable. Stoev *et al.* (2002) observed a dramatic effect of 5 ppm on body weight of chicks (- 61% after 42 days) while a level of 1 ppm resulted in a loss of 18%.

In pigs, OTA causes a typical disease called porcine nephropathy (kidney damage). This can result in rejection of carcasses at the slaughter house. Indeed, OTA has an affinity with serum proteins (it is bound to them), which makes it quite stable. It can be found in pig meat and meat products. OTA also affects fertility of boars. It crosses the placental barrier, and can affect the development of foetuses. Tail necrosis sometimes observed in newborn piglets is often associated with OTA.

In the group of fumonisins, there are fifteen mycotoxins commonly found in corn. The most significant are called Fumonisin B₁ and Fumonisin B₂. Fumonisin were identified only recently (in the 1980s) but their effects have been described (in horses) since the 1890s. Fumonisin are produced by *Fusarium verticillioides* (syn., *moniliforme*) and *Fusarium proliferatum*. They often happen jointly with other mycotoxins, for example, aflatoxins, DON and ZON. Like trichothecenes, fumonisin are produced under warm and temperate climates. They are reported on corn worldwide, on wheat and other cereals in Asia and South America, and on rice in Asia. For example, *Fusarium* ear root is the most common disease of corn in the Corn Belt in US. The fungi growing on corn can also survive on plant residues after harvest and contaminate the next crop. Also, it is common that grains show no visible damage while more than 50% of them are spoiled. As a general status, it can be said that larger quantities of fumonisin are required to observe their toxicity, when compared to other mycotoxins. But it is also true that they are often present in much greater quantities in the commodities.

In poultry, relatively high levels are required to observe negative effects of fumonisin. Broomhead *et al.* (2002) report that feed intake, body weight gain, and feed conversion of chicks were not affected by fumonisin B₁, despite levels of 25 or 50 ppm were used. Ledoux *et al.* (1992) observed that levels of 100 to 400 ppm were detrimental to the performances in the case of day-old chicks. Kubena *et al.* (1997) studied the effects of fumonisin B₁, combined or not with Diacetoxyscirpenol and OTA in turkey. Reduced weight gain was observed with 300 ppm of fumonisin B₁. The reduction was 30% after 3 weeks when compared to control. The feed conversion ratio was also affected. Since the toxicity of fumonisin and DAS (or fumonisin and OTA) appeared to be additive, the authors stated that even if a level of 300 ppm is very unlikely under practical conditions, combinations of different mycotoxins at lower levels might put poultry at risk.

In pigs, the main symptom of Fumonisin B1 exposure is called PPE (porcine pulmonary oedema) which affects lungs and heart. At lower levels, liver and pancreas damage can be observed, as well as immunosuppression. Production parameters can also be affected: reduced weight gain (above 2 ppm), increased FCR, and reduced performances. They result in economic losses (fatty meat, lungs and liver damaged).

Economic impact of mycotoxins – the case of swine production

Even before mycotoxins are produced, the fungi generating them can have a negative impact. Indeed, when moulds develop on grains, they will use them as a substrate and reduce their nutritive value. Also, the colour and odour of the raw materials can be affected. Table 1 shows an example comparing the use of good quality corn with that of mouldy corn. In this case, the combination corn + oil brings 2450 kilo calories of metabolizable energy (ME) per kg of feed. Because mouldy corn has a lower ME value (in this case, 3250 vs. 3400 kcal/kg) than normal corn, it is necessary to adjust the levels of both corn and oil to achieve the same total ME value. The cost increase due to the lower metabolizable energy in corn is, in this example, \$ 215.35 - \$ 204.41 = \$ 10.94 per tonne of feed = 5%.

Dersjant-Li et al (2003) studied the impact of low concentrations of aflatoxin, deoxynivalenol or fumonisins in diets on growing pigs and poultry. In this review, they used simple linear regression to summarize different trials from the literature and estimate the relationship between mycotoxin level and, for example, growth rate. The authors estimated that for each additional part per million (ppm) of aflatoxin in the feed, the growth rate of pigs is depressed by 16 %. For instance, a level of 0.3 ppm aflatoxin in feed would result in a 5% reduction in daily weight gain when compared to a non contaminated feed. For deoxynivalenol, the reduction in daily weight gain was estimated at about 8% for each additional ppm. Similarly, it was calculated that growth performance of pigs is reduced by 0.4% for each additional ppm of fumonisins in the diet.

In 1991, Blaney and Williams studied the impact of different levels of DON in feed on performance parameters of growing pigs (Table 2). They also estimated the value of the wheat that should be used in the feed formula to maintain the same profitability on the farm. According to their calculation, when DON level in feed is 4 ppm, it is necessary to reduce the value of the wheat by 8%. Similarly, when the feed contains 11 ppm of DON, it is necessary to use a wheat that is 62% cheaper in order to keep the same profitability for the farm.

Etienne *et al.* (2006) have observed the negative effect of DON in sow feed during lactation. When comparing a control group to a group of sows fed with a diet containing 2 ppm of DON, they observed that the feed intake was depressed by 21% on the whole lactation period (see Figure 3). As a result, daily weight gain of the piglets was less in the contaminated group (237 vs. 274 g/day) even if the difference was not significant due to high variability. The sows eating the feed containing the mycotoxin lost more body weight (25.8 kg vs. 17.5 kg) during lactation than their counterparts. This is of course primarily due to their lower feed intake.

One of the mycotoxins having a major impact in swine production is zearalenone. In young gilts before puberty (less than 6 months of age), levels of 1 to 5 ppm in feed

result in swelling and reddening of the vulva and enlargement of the teats and mammary glands. Rectal and vagina prolapses can also be observed with higher contamination levels. In mature gilts, 1 to 3 ppm will affect the length of the oestrus cycle. In sows, a level of 5 to 10 ppm can cause anoestrus, and pseudo pregnancy can also occur. Generally, zearalenone does not cause abortion. However, if sows ingest this mycotoxin during the time of implantation of the embryos, the number of piglets born may be reduced. Based on various published or unpublished data we can estimate that for each additional ppm of zearalenone, the number of fetuses decreases by 0.15 (see Figure 3). This is true for a level below 60 ppm. If the contamination is higher, we can expect that no fetus will survive. Together with a reduced litter size, zearalenone is also usually associated with lighter piglets and symptoms such as play-leg. In lactation, piglets may develop enlarged vulva.

Figure 5 describes the impact mycotoxins can have on the different factors influencing the number of live born piglets per sow per year. Charmley et al. (1995), stated that “Based on 1991 prices, it was calculated that a 10 or 20% reduction in farrowing rate combined with a 10 or 20 % reduction in growth (as may occur if deoxynivalenol and zearalenone contaminated feed was consumed) would result in a 17 to 44% reduction in profit margins, due to increased feeding and veterinary costs per head, and a decline in the number of pigs marketed.”

Mycotoxins can also affect the immune system of pigs. This means a reduced resistance to diseases caused by pathogens or viruses and, of course, an economic impact.

The negative effect of mycotoxins on resistance to pathogenic bacteria has been described as early as in the late seventies. Outbreaks of salmonellosis were reported in the United States following the appearance of high concentrations of aflatoxins in corn harvested in the region (Miller et al. 1978). Aflatoxin also increased susceptibility to swine dysentery (Joens et al., 1981).

Stoev and collaborators observed that ingestion of ochratoxin A contaminated feed increases susceptibility to salmonella infection in pigs (Stoev *et al.*, 2000). Different authors (Müller *et al.*, 1999; Verma and Mathew, 1998; Baudrimont *et al.*, 1994) have reported other effects of Ochratoxin A on immunity: decreased lymphocytes (immune cells), increased apoptotic phagocytes (death of neutrophils and macrophages), increased eosinophils (cells involved in immune response), increased leukocytes and neutrophils (white blood cells) and reduced phagocytosis (ability to kill micro organisms in the body).

Oswald *et al.* have studied in 2003 the interaction between Fumonisin B1 and *Escherichia coli*. The results clearly indicate a correlation between the oral administration of Fumonisin B1 and the colonization of the small and the large intestine of piglets by the inoculated *E. coli* (see Figure 6). The counting of the bacteria revealed that 400 to 700 times more CFU of the inoculated strain were present in the intestines of treated animals compared to piglets that had received no Fumonisin.

Evaluating the economic impact of mycotoxins is a challenging task, since several production parameters such as feed intake, growth rate, feed conversion, or reproduction can be affected at the same time. As mentioned above, mycotoxins can

also have a negative impact on the immune defence of animals, reducing their resistance to pathogenic bacteria for example. Also, the severity of the problems caused by these compounds depends on the quantity ingested, time-span of exposure, type of animal, their breed, age, sex, health status, but also other parameters such as density of animals, diseases, temperature, etc... Finally, it has been mentioned by various authors that when more than one mycotoxin is present, the toxins can produce additive, and sometimes synergistic, effects in animals.

In an attempt to calculate the economic impact, we can use the example given in Table 3. In this case which is based on the conditions of October 2007 in the Philippines, we estimate that feed conversion ratio is increased by 2% (3.06 vs. 3.00), that the live weight of the pig sold is reduced (88.20 kg vs. 90.00) by another 2% (as a consequence of reduced weight gain and in order to empty the buildings for the next batch of pigs). At the same time, we estimate that the number of pigs sold per year is depressed by 2% (16.66 vs. 17.00). If the farm has 1,000 sows, the total profit is reduced by 8.1%, which means a loss of more than 70,000 USD or 16.28 USD per MT of feed. In this very simplified example, we did not calculate the impact on meat quality, or the increased medication costs.

Prevention and control of mycotoxins

If favourable growth conditions are met for the fungi, it is very difficult to avoid the production of mycotoxins. However, effective prevention strategies will certainly limit the incidence of mycotoxins. Prevention can be implemented before harvest with a good management of preceding crop residues, a correct crop rotation, the selection of seeds (quality of seeds, resistant varieties), an appropriate plant density, the correct use of fertilizers and of course the prevention of insects and fungi. Harvesting at the right time, in good conditions to avoid damaging grains and removing spoilt and moist grains, then storing good grain as soon as possible, will certainly help. Finally, during storage, the control of temperature, humidity, insects and rodents and the use of effective mold inhibitors will help to prevent mycotoxins. It must be noted, however, that prevention does not remove existing mycotoxins!

Many methods have been tested to remove mycotoxins from commodities. The problem is that they are costly, usually generate high losses and can reduce the palatability and the nutritional value of the raw materials. Among the methods that have been experienced, the following can be mentioned: treatment with ammonia, together with heat and pressure (effective against aflatoxins and to a lesser extent fumonisins, but generates toxic compounds), treatment with ozone, chlorine gas, ammonium hydroxide, hydrogen peroxide, hydrochloric acid and sulphur dioxide gas (against DON), formaldehyde (against zearalenone), roasting, heating (useful against DON), colour sorting with UV (against aflatoxins). Also, dehulling, polishing, sieving have been experimented. However, today, most of these methods are not used because of their drawbacks.

Use of mycotoxin binders

About twenty years ago, use of so-called “mycotoxin binders” has given a new perspective to the control of mycotoxins. One of the first scientific studies on binding

properties of clays is the one published by Phillips *et al.* (1988). They tested 38 different adsorbents from the major chemical class of aluminas, silicas and aluminosilicates, and showed that a type of phyllosilicate clays, called hydrated sodium calcium aluminosilicates (HSCAS) has high affinity for aflatoxin B1. Indeed, the good stability of the aflatoxin-HSCAS complexes over a wide pH range (2-10) and up to 37°C supports the in vivo efficacy of such binders (Sarr *et al.*, 1990). Further studies have demonstrated that HSCAS can be very helpful to prevent aflatoxicosis in different species such as chickens, turkeys, goats, cows, pigs, or lambs. However the efficacy of HSCAS seems to be only partial against zearalenone and ochratoxin A, while they appear totally ineffective to tackle mycotoxins from the group of trichothecenes (for example, T-2 toxin, diacetoxyscirpenol or deoxynivalenol, also known as vomitoxin).

Zeolites, another type of hydrated aluminosilicates, have given inconsistent results against aflatoxins. While some in vitro studies have been promising, high levels of zeolite in feed have given disappointing results (Fukal *et al.* 1990, Sova *et al.* 1991).

Ramos *et al.* (1996) also studied the possible benefits of sodium bentonite, a natural sealant used to treat porous soils. It is also used as a binding agent when producing pelleted feeds. Based on their findings, as well as data from other researchers, it appeared that bentonite is not effective against zearalenone, ochratoxin A or nivalenol while contradictory results have been obtained for aflatoxins.

Research has also been performed on the use of activated carbon, an insoluble powder formed by pyrolysis of different kinds of organic materials. Although activated carbon has proven to be effective at binding mycotoxins in vitro, for example, fumonisin B1 (Solfrizzo *et al.*, 2001) or ochratoxin A, it did not show clear positive effects when tested in vivo. Additionally, the concern is that activated carbon can indiscriminately bind other dietary components, such as vitamins, minerals and drugs.

Cholestyramine is a resin used to lower high cholesterol levels in the blood. It works by binding to bile acids in the intestine, which results in cholesterol being converted to bile acids in the liver. Ramos *et al.* (1996) observed that this resin is able to bind zearalenone while other researchers demonstrated a positive effect against fumonisins. However, relatively large quantities are needed (for instance more than 10 kg/MT of feed in the case of zearalenone) which makes its use economically prohibitive. Finally, polyvinylpyrrolidone (a vinyl polymer), used at 2 kg per ton of a swine feed contaminated with deoxynivalenol, did not improve the situation, as reported by Ramos *et al.* (1996).

Stanley *et al.* (1993) reported that *Saccharomyces cerevisiae* was helpful in the case of aflatoxin contamination, and their conclusion was that the cell wall was binding with the mycotoxins. Santin *et al.* (2003) studied the effects of yeast cell wall against ochratoxin in broilers. Their results indicate that ochratoxin impaired the feed intake, weight gain and feed conversion of the birds. The yeast cell wall could not improve these parameters. Yiannikouris *et al.* (2004) studied the interaction of yeast cell wall with zearalenone in vitro. Their conclusion was that weak non-covalent bonds are involved in the complex-forming mechanisms, and that the chemical interactions are therefore more of an adsorption type than a binding type.

Limitations of mycotoxin binders

Based on the different publications available, we can observe that the main limitations of the “mycotoxin binders” are:

1) Their efficacy is limited to a few mycotoxins.

Generally speaking, binders are effective against so-called polar mycotoxins, such as aflatoxins. This is due to the fact that these mycotoxins have a chemical structure which allows an efficient binding. In the case of other mycotoxins, such as trichothecenes, binding efficacy is generally very poor, if not zero.

2) Their efficacy *in vitro* does not guarantee their performance *in vivo*.

Because *in vitro* tests are performed under specific and rather simple conditions, they are not representative of what happens in the digestive tract. When parameters such as pH variation or interaction with feed or enzymatic secretions are not taken into account, the risk is to draw false conclusions. Indeed, when weak non-covalent bonds are formed between the binder and the mycotoxin, a change in the conditions of the “environment” can lead to a release of the mycotoxin.

3) Some of them are not specific to mycotoxins.

In such a case, the binder will interact with other dietary components, such as vitamins, minerals and drugs. This will limit the efficacy against the mycotoxin(s) and also affect the performance of the animals.

Biotransformation of mycotoxins

Therefore, binding of mycotoxins is a reversible process, the efficacy of which depends on the conditions of the media. Its practical application is also limited to a few mycotoxins. As a consequence, other strategies had to be found.

Recent research indicates that the biotransformation of mycotoxins, using live microorganisms or enzymatic preparations, gives promising results.

Shima *et al.* (1997) have for example reported the case where a bacterium belonging to the *Agrobacterium-Rhizobium* group was able to transform deoxynivalenol into a less toxic compound called 3-keto- deoxynivalenol, and suggested that the biotransformation was caused by an extracellular enzyme excreted by the organism (Figure 7). Similarly, Völkl *et al.* (2004) observed that a mixed culture of microorganisms was able to transform deoxynivalenol into two chromatographically separable products, the main one being identified as 3-keto-deoxynivalenol. Again, they stated that an extracellular enzyme was involved. Other trichothecenes such as 15-acetyl- deoxynivalenol, 3-acetyl- deoxynivalenol and fusarenon-X were also transformed.

Zearalenone can be converted into a far less oestrogenic product, called 1-(3,5-dihydroxyphenyl)-10'-hydroxy-1'-undecen-6'-one (Takahashi-Ando *et al.*, 2002). The enzyme responsible for the detoxification appears to be a hydrolase that cleaves the lactone ring (Figure 7). Zearalenone affects the reproduction cycle of animals when it interferes with oestrogen receptors. Since the structure of the mycotoxin is modified by the enzymatic reaction, it loses its toxic effect.

The application of such enzymatic transformations to the feed sector gives new opportunities. Indeed, enzymes can have a specific action and their reaction, compared to binding, is not reversible. With this new approach, we can talk about “mycotoxin eliminators” in contrast to “mycotoxin binders”. The combination of mycotoxin binders and enzymes is of course possible. In the development of a product to counteract the effects of mycotoxins in feed, Belgian company Impextraco screened many products, including binders and enzymes, in a system designed to simulate the digestive tract.

Gut simulation model

Simple measurements in the feed (*in vitro*) are not sufficient to reveal the real binding or inactivation of mycotoxins, since it is not clear whether reactions in the animal itself would influence the binding or the enzymatic reaction. For example, if the toxin is bound in the feed, but later released in the animal, the binder is not effective. Similarly, if the product only binds the toxin in the animal, but not in the feed itself, it is effective, but efficacy will be difficult to verify.

Analyzing the toxin inactivating effect in live animals is very difficult. Most of the studies look at performance of the animals. However, this parameter is influenced by many other factors difficult to control, so large and expensive tests are necessary. Some studies measure the serum levels. This is not possible for all mycotoxins and the serum levels are not always a good indicator of the amount of mycotoxin absorbed. Most toxins are rapidly metabolised or stored in the animal. Consequently, the serum level drop very fast. The time between absorption in the gut and sampling of the animal is very important, but differs due to individual variations like feed intake, retention time in the gut, etc. Also other factors like genetics, bodyweight, water intake etc will differ between the individual animals and influence the serum levels.

A perfect *in vitro* model of the animal would eliminate individual variation and control all other factors. Of course, there is no such thing as a perfect model, but the digestive tract has been well studied and several factors are easy to simulate. Dr. H. Clarijs's research group at the HAS in the Netherlands has developed a small intestinal model that can be applied to mycotoxin tests. The following factors are simulated: anaerobic environment, constant (body) temperature, several subsequent environments at different pH, retention times and the correct subsequent addition of bile, pepsin and gut enzymes and the correct moisture : feed (digestive bolus) ratio.

The gut simulator mimics the digestive tract and allows studying interactions between feed, mycotoxins and mycotoxin-deactivating substances in “real” conditions. This offers a clear advantage when compared to the classical in-vitro tests where only pH is controlled and other parameters are not taken into account.

Immense differences were found between the classical in vitro tests and the gut simulation model. The best toxin binder for aflatoxin B1 that emerged from this screening (a combination of several special types of HSCAS) was then combined with other, different products. Addition of more substances showed an additive effect. Another substance selected was a special type of chitosan, a biopolymer derived from the exoskeleton of insects and crustaceans. This biopolymer was selected for its mycotoxin binding properties, but also because it has been proven to have

antibacterial effects (No *et al.*, 2002). Helander *et al.* (2001) stated that chitosan appears to bind to the outer membrane of gram-negative bacteria and disrupts the barrier properties of the said membrane. Various enzymes were also tested. Several enzymes showed detoxification effects on a variety of toxins. A combination of HSCAS, biopolymer and enzymes was then obtained (Elitox, Impextraco NV, Belgium).

Conclusion

Mycotoxins are harmful to animals and can greatly affect their performances and productivity. Because there is a wide range of different mycotoxins, with different chemical structures, a simple approach cannot efficiently solve the problem.

Prevention is important but cannot guarantee the absence of mycotoxins. When commodities are contaminated, the use of several strategies is required. A correct combination of mycotoxin binders with toxin-degrading enzymes and a biopolymer gives a new approach and can be defined as a “mycotoxin eliminator”.

Table 1: Economic impact of mouldy corn (Situation Philippines – August 2007)

Raw material	ME (kcal/kg)	% in formula	ME contribution (kcal/kg)	Raw material cost (USD/MT)	Cost in formula (USD/MT)
Corn	3400	65.00	2210.00	277	180.05
Oil	8000	3.00	240.00	812	24.36
TOTAL			2450.00	TOTAL	204.41
Mouldy corn	3250	63.00	2047.50	277	174.51
Oil	8000	5.03	402.40	812	40.84
TOTAL			2449.90	TOTAL	215.35

Table 2: Effect of DON level on growth performance of pigs from 20-50 kg and relative value of the wheat that maintains the same profitability (Adapted from Blaney and Williams, 1991)

	DON level in feed (ppm)			
	0	4	8	11
Daily Feed Intake (kg)	2.05	1.76	1.47	1.28
Daily Weight Gain (kg)	0.89	0.80	0.58	0.45
Feed Conversion Ratio	2.32	2.22	2.63	2.94
<i>Relative wheat value</i>	<i>100%</i>	<i>92%</i>	<i>81%</i>	<i>38%</i>

Table 3: Simulation of the economic impact of mycotoxins (situation Philippines, October 2007)

	UNIT	CURRENT PERFORMANCE	Case A	Difference %
PRODUCTION DATA				
Feed Conversion	kg/kg	3.00	3.06	2.0
Feed price	USD/T	318.00	318.00	
Live Weight	kg	90.00	88.20	-2.0
Live Weight Price	USD/kg	1.86	1.86	
Number of Sows		1000	1000	
Number Sold / Sow / Year		17.00	16.66	-2.0
Sales - No. of Animals		17,000	16,660	-2.0
Sales - Kg of meat		1,162,800	1,116,753	-4.0
OVERHEAD COSTS	USD/animal	28.64	28.64	
COSTS AND RETURNS				
Market Value	USD/animal	167.40	164.05	-2.0
Feed Cost	USD/kg Lwt	0.95	0.97	2.0
Feed Cost	USD/animal	85.86	85.83	-0.0
FINANCIAL PERFORMANCE				
Cost of Production	USD/animal	114.50	114.47	-0.0
Cost of Production	USD/kg Lwt	1.27	1.30	2.0
Profit	USD/animal	52.90	49.59	-6.3
Total Profit	USD	899,300	826,108	-8.1
<i>Loss in USD</i>			-73,192	
<i>Loss in USD/MT of feed</i>			-16.28	

Figure 1: Effect of duration of exposure to a contaminated feed (5 ppm aflatoxin) on body weight of broilers: difference when compared to the control group, no aflatoxin (Adapted from Mariani, 1998).

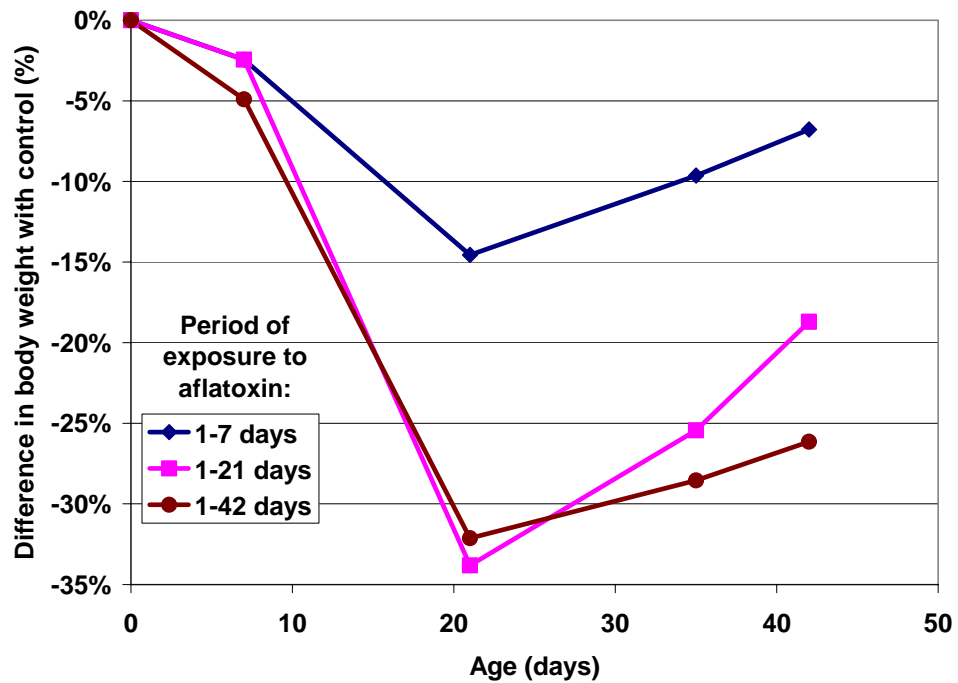


Figure 2: Effect of age of broilers, exposed for 7 days to a contaminated feed (5 ppm aflatoxin) on their body weight: difference when compared to control group, no aflatoxin (Adapted from Mariani, 1998).

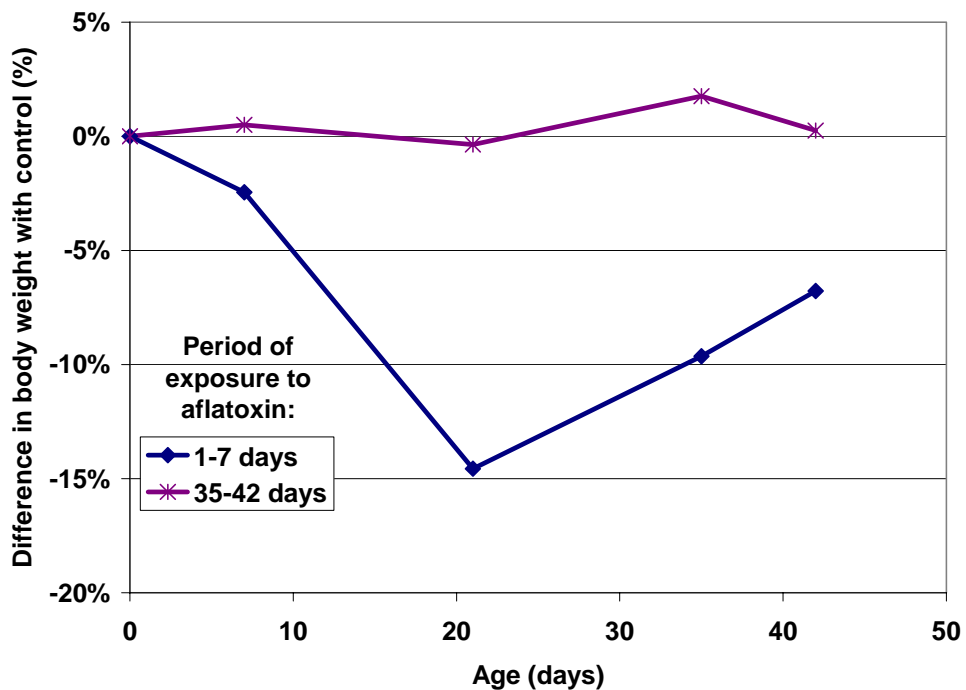


Figure 3: Effect of DON at 2 ppm in sow feed during lactation on feed intake of sows (kg/day). Adapted from Etienne *et al.*, 2006.

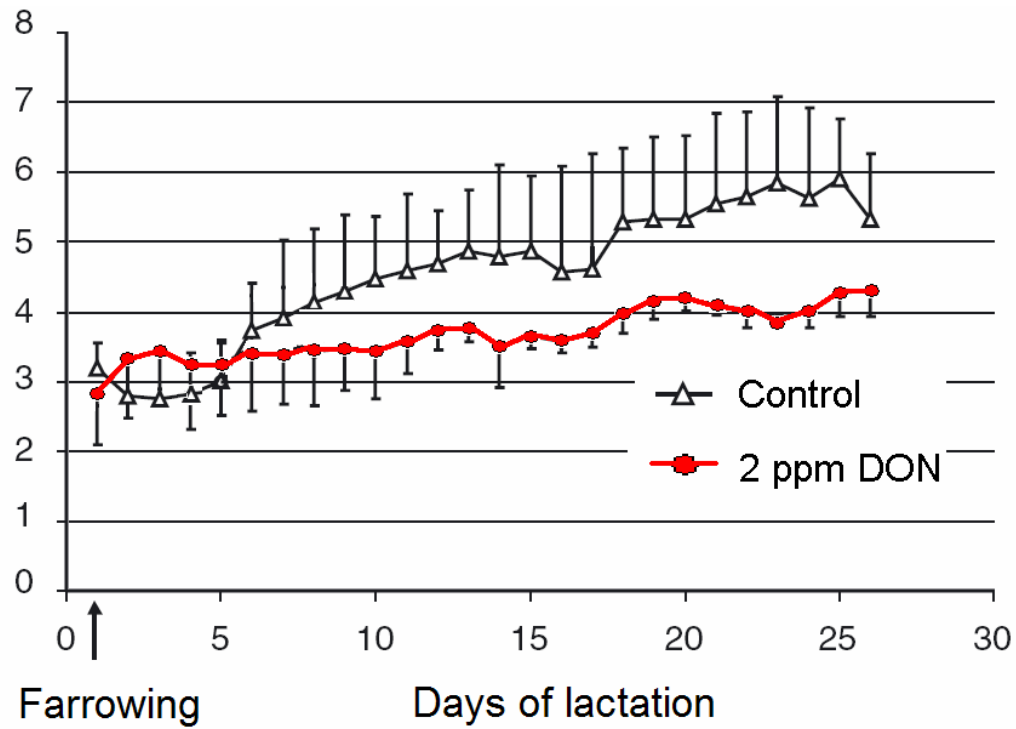


Figure 4: The impact of zearalenone level in sow feed on the number of fetuses

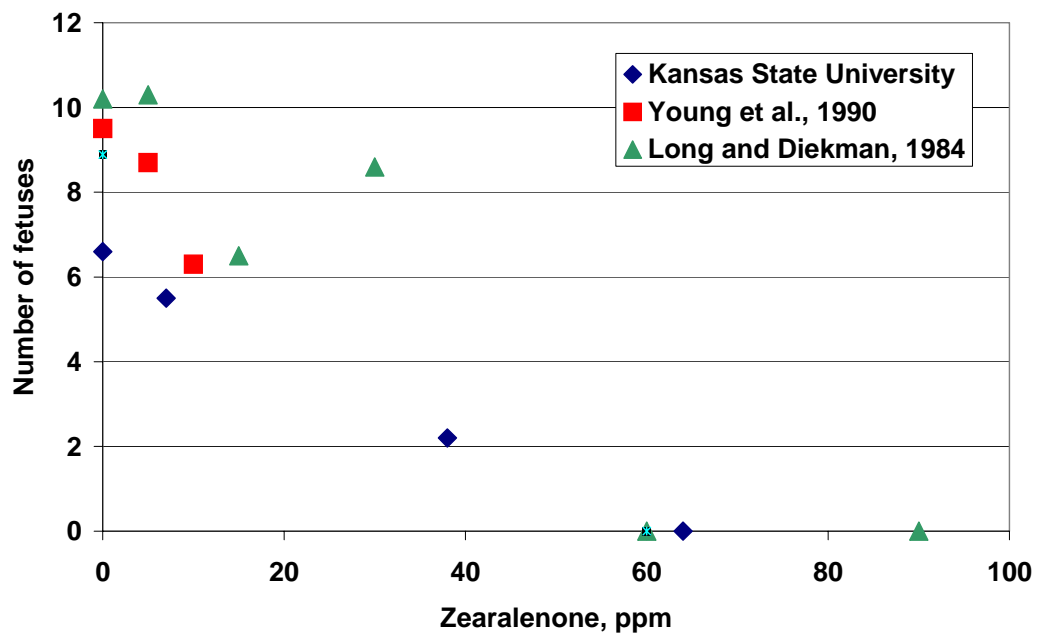


Figure 5: Mycotoxins and factors influencing the number of live born piglets/sow/year (Adapted from Cameron, 1998)

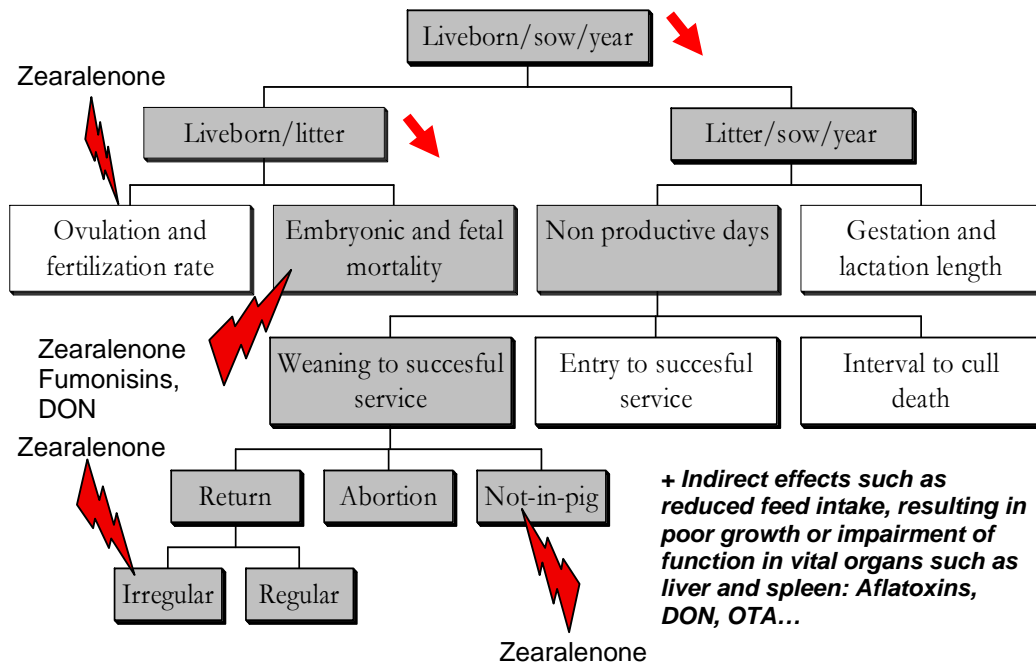


Figure 6: Effect of oral administration of FB1 on bacterial colonization of piglet intestines by E. coli strain 28CNalr (Adapted from Oswald et al., 2003).

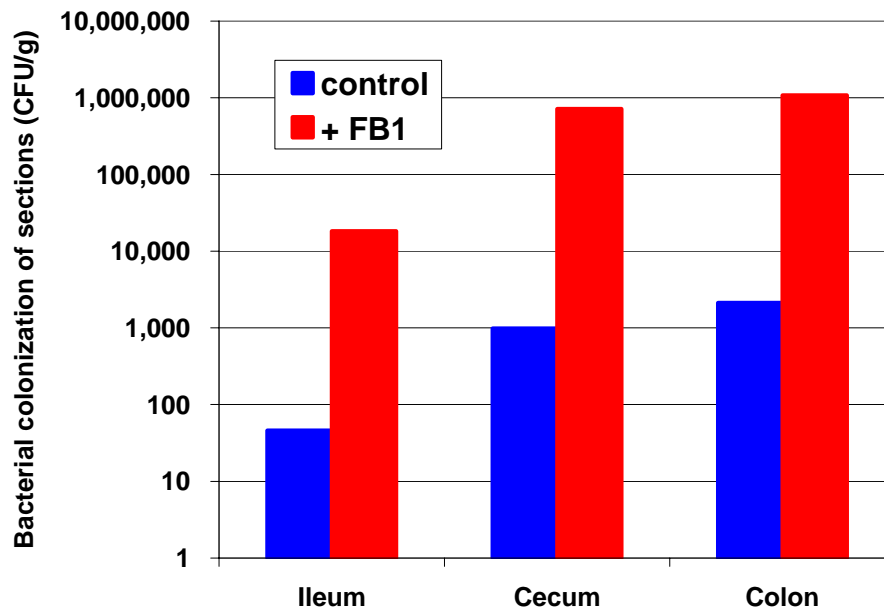
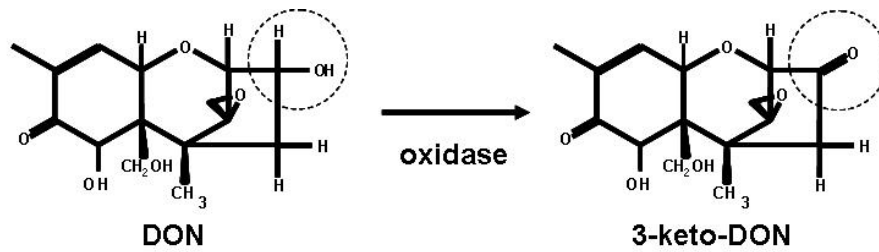
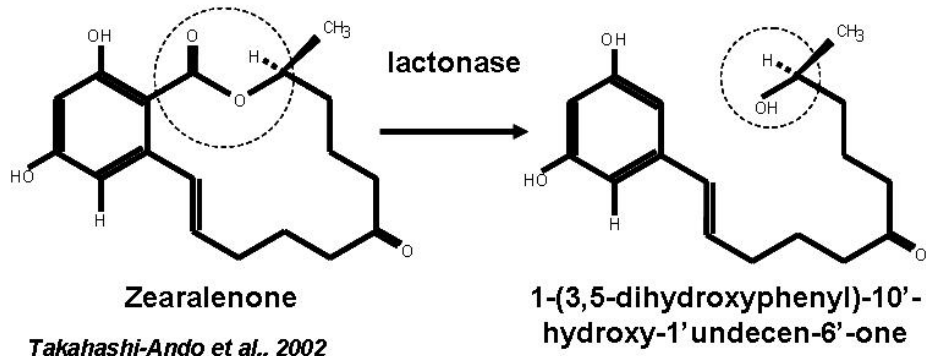


Figure 7: Examples of mycotoxin transformation with enzymes.



Shima et al., 1997



Takahashi-Ando et al., 2002

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