<제2주제>

Managing the risk of mycotoxins in modern feed production

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Introduction

Every feed producer has to take best care of the production process, proper formulations and a well performing nutritional balance of the produced diets based on their economic merits. Yet as a central part of the feed to food chain each feed producer must also take measures to protect the entire chain from harmful substances. Media reports and consumer pressure groups react to incidences of contaminated foodstuffs or products of ill quality as an easy and well-received target that traditionally can ride high on waves of negative publicity.

Safety awareness has also risen due to the simple fact that the methods for testing residues have become increasingly sophisticated and more available at all points of the supply chain. Mycotoxin contaminated feeds impair the farm operations in various ways. Mycotoxins are invisible, odourless and cannot be detected through smell or taste. Due to the complex nature of these naturally occurring contaminants and the elaborate analytics a risk management concept must be installed in order to reduce the risk encounter to a defined and acceptable level.

Assessment and management of mycotoxin contamination

Assessment and evaluation of the defined risk is a prerequisite to a practical risk management plan. In order to assess the risk situation with regard to mycotoxins several questions need to be answered.

What are mycotoxins?

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by higher animals. Fusarium, Aspergillus, and Penicillium are the most abundant moulds that produce these toxins and contaminate human foods and animal feeds through fungal growth prior to and during harvest or as a consequence of improper storage following harvest (Bhatnagar et al, 2001). Due to modern methods and thanks to a growing interest in this field of research more than 300 different mycotoxins can be differentiated today. However for our practical consideration in the feed manufacturing process a small yet potential number of toxins is of relevance.

Which mycotoxins are of relevance in this context?

The most commonly mycotoxins known are the aflatoxins due to the fact that they represent one of the most potential carcinogenic substances known so far. They are rated as Class 1 human carcinogens by the IARC (International Agency for Research on Cancer). Aflatoxin B1 is the strongest natural carcinogen known so far and the main hepatocarcinogen in animals, although effects vary with species, age, sex, and general nutrition. Trout, ducklings, pigs are highly susceptible, ruminants less (Weidenborner, 2001). The fatty liver or pale bird syndrome and inhomogeneous flocks are the most typical symptoms for such a contamination in feed.

Trichothecenes are a large group of mycotoxins produced by various species of moulds, in particular by those belonging to the genus of Fusarium. Approximately 170 Trichothecene mycotoxins have been identified up to date, with a sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system in common (Krska et al., 2001). Epidemiological surveys have revealed that the predominant type-A and -B trichothecenes are widely distributed in cereals as natural pollutants, whereas the macrocyclic trichothecenes occur rarely in food or feed. The most prevalent occurring mycotoxins of these groups are

deoxynivalenol (DON, vomitoxin), nivalenol (NIV), 3- or 15-acetyl-deoxynivalenol (AcDON), Fusarenon X (FUS-X) in case of B-trichothecenes, and T-2 toxin and HT-2 toxin of the type-A toxins. An important issue is that some of these closely related compounds occur frequently simultaneously (Fuchs et al., 2004) and are proven to cause synergistic effects (Weidenborner, 2001). Different types of trichothecenes vary in their toxicity though all of them are highly acute toxic. They may cause haematological changes and immune suppression, reduced feed intake and skin irritations as well as diarrhoea and haemorrhages of internal tissues.

Zearalenone is also produced by Fusarium species and has strong hyperestrogenic effects, which result in impaired fertility, stillbirths in female and a reduced sperm quality in male animals. Beside the detrimental effects on fertility no acute toxicity is described so far, so that it is rather a non-steroidal fungal hormone (estrogen) rather than a "mycotoxin".

Ochratoxin A (OTA), which is produced by Aspergillus and Penicillium species in moderate and colder climates, has been listed as possible carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). It caused renal toxicity, nephropathy and immune-suppression in several animal species, which entails reduced performance parameters in animal production. As it occurs in many commodities and there is a certain carry-over by products of animal origin human intake in some northern countries can be high. Examples for estimated human intakes are: Germany - 1.2 to 1.3ng/kg body weight (bw) and day, Sweden - 0.4ng/kg bw, Switzerland - 0.7mg/kg bw, Canada 1.1ng/kg bw. Half-life of OTA in humans is 8 to 12 times higher than in rats, so that there is a certain risk of accumulation in human serum, milk, and fatty tissues in many areas with high OTA prevalence (Weidenborner, 2001).

The most recently described mycotoxins with relevance in human and animal nutrition are Fumonisins, which were first reported in South Africa in 1988 and belong also to the

Fusarium mycotoxins. Fumonisins cause severe animal diseases like equine leukoencephalomalacia (ELEM) in horses and porcine pulmonary edema in swine (PPE). Besides their hepatotoxicity and nephrotoxicity they affect also the immune system. In addition human oesophageal cancer has been observed in distinct areas of the world where high levels of fumonisins occurred on maize and maize-based food products.

Acute toxicity

LD50 levels of the most prevalent mycotoxins can be found in table 1. No acute toxicity is known for zearalenone and fumonisins so far. Ingestion of 20g zearalenone/kg bw (body weight) did not produce deaths in mice and rats, while 15mg of fumonisins per kg bw induced liver cancer in rats, but also did not have lethal effects. ELEM can be induced at a level of 10mg Fumonisin B1 and B2 per kg bw, while PPE is caused at an oral supply of 100mg fumonisins/kg bw.

Table 1: LD50 of selected mycotoxins (Weidenborner, 2001; CAST report, 2003)

Toxin	Animal	Route	LD50
Aflatoxin B1	Rats	po	5.5-10 mg/kg bw
Ochratoxin A	Rats	po	20-22mg/kg bw
DON	Mice	po	46mg/kg bw
Nivalenol	Mice	po	39mg/kg
Fusarenone X	Rats	po	4.4mg/kg bw
T-2 toxin	Rats	po	4mg/kg bw
T-2 toxin	Swine	po	4mg/kg bw
T-2 toxin	Chicken	po	1.84mg/kg bw
HT-2 toxin	Mice	po	9mg/kg bw
DAS	Rats	po	7.3mg/kg bw

Mycotoxins are not equally toxic in their effects to all species. For example broiler chickens show a higher resistance to aflatoxins than ducks, while waterfowls tend to be

more susceptible to their negative impacts than other poultry. Pigs are generally more affected by trichothecenes than poultry, while ruminants can cope quite well with the entire group of trichothecenes due to their rumen flora, which is able to degrade and thus decontaminate this group of fungal toxins to a wide extent.

Prevention of mycotoxins

Management practices to maximize plant performance and decrease plant stress can decrease mycotoxin contamination substantially. This includes planting adapted varieties, proper fertilization, weed control, necessary irrigation, and proper crop rotation. But even the best management strategies cannot eliminate mycotoxin contamination in years favorable for disease development. Some fungi, like several Fusarium species, are widespread colonizers of crop residues, where the pathogen survives during winter. Thus wheat stubble, corn stalks and rice stubble can be major sources of these moulds, which get powerful inocula as temperatures increase in spring. Airborne release of spores might peak during and after rainy periods, distributing the fungal sources over wide distances, and causing epidemics. There are two routes of entry for mould infection of grain in general, and corn in particular: first, fungal spores landing on emerged silks can infect the ear by the silk channel, and second, wounds caused by birds, insects or extreme weather can provide a good opportunity for fungal invasion. This infection through wounding is especially critical before the kernels have hardened significantly but are an attractive substrate for spore germination. During harvest it is important to prevent excess damage to kernels, which may predispose them to infection during storage. Too high moisture content is likewise a high risk factor for mycotoxin infestation, with the final "safe" moisture content depending on the crop and the climatic conditions where the commodity is stored, although drying to 15% moisture content or below is widely recognized as being suitable. It should be mentioned that when conditions are generally favorable for fungal

contamination it is not uncommon for more than one type of fungus to be involved. During storage grain is often colonized by a succession of fungi, depending on temperature and moisture levels. Due to these possible interactions of several fungal species, grain may be contaminated with a number of different mycotoxins (Cast, 2003).

The use of mould inhibitors or preservation by acids can only reduce the amount of mould but does not influence the prior treatment generated mycotoxins. These toxic compounds remain in the formerly infected commodity even if no more mould can be seen or detected. The only way to really assess the quality of raw materials is the specific testing of mycotoxins or certain groups thereof.

Multi-toxin occurrence

Although scientific literature offers a broad variety of information on the effects of individual mycotoxins in various animal species, it is the multiple mycotoxin contamination that matters the live-stock industry most, as it refers to the naturally occurring circumstances. For example, aflatoxin and fumonisin B1, as well as DON or other trichothecenes (one or even more of them) and zearalenone frequently occur together in the same grain. Additionally, in the feed manufacturing process different batches of different raw materials are mixed together thus producing a totally new matrix with a totally new risk profile in the due course of manufacturing. Poor livestock performance and/or disease symptoms observed in commercial operations may be due to the synergistic interactions between multiple mycotoxins. Scientific reports on synergistic effects of mycotoxins at acute toxicity levels describe combinations of aflatoxins with various trichothecenes, as well as with ochratoxins and fumonisins, but also combinations of fumonisins plus DON. Nevertheless it has to be pointed out, that far more work has to be done in this particular field of research, especially in the subacute contamination range as well as with combinations of more than two toxins. Nevertheless multi-toxin

occurrence is one important explanation for divergences in effect-levels described in scientific literature, where defined, mostly purified mycotoxins are used as source of contamination, in comparison to effective levels observed in practice, where apparently lower mycotoxin contaminations often cause more severe effects.

Safety levels

The establishment of safe levels of mycotoxins is a legal rather than a scientific exercise. Though analytical methods improved in sensitivity significantly over the years, guideline levels are still the outcome of economic and political considerations. Settings of safe levels for mycotoxins have several components, i.e. (1) legal requirements, (2) sensitivity and availability of the analytical methods, (3) presumed safety to animals and humans, (4) criteria of economics, like what level can be afforded. In any scientific attempt to establish a safe level of a mycotoxin it seems essential to define safety and criteria to be used in establishing it. As it is very difficult to set up worldwide valid and acknowledged levels of performance in animal production, it is even more difficult to produce numbers and correlations that refer directly to the impact of hazards. Should safe levels refer to lethal effects, growth depression, pathological findings, or rather to the grade of immunological changes, deviations of enzymatic parameters or haematological factors? What is the proper criterion for "safety"? Hamilton (1984) takes an idealised assumption of proportionality between risk and mycotoxins and a zero intercept without a threshold. He postulates that there are no truly safe levels of mycotoxins, as a prudent person might assume that any level carries a potential risk. The higher the concentration and frequency of mycotoxin exposure, the higher the risk, with taking consciously "risk" as the y-axis label, and not response. Thus a given amount of mycotoxins will not ensure a given response under field conditions, but certainly poses a certain risk, i.e. threat - especially in case of interacting factors, which even increase this threat. These

considerations suggest there is no magic number above that it is unsafe and below which it is safe, but direct the attention to reducing the risk (figure 1).

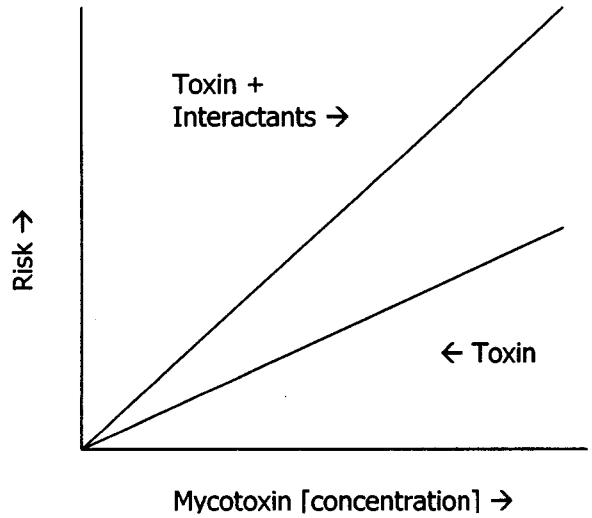

Figure 1: Illustration of prudent view about safe levels of mycotoxins (after: Hamilton, 1984)

But how should we act under practical conditions? Aside of the fact that there is no safety level we have to establish a risk level at which we can accept to expose the farm animals in a worst-case scenario. A proposal for such a list is listed in table 2.

It is again important to say that not the same level of mycotoxins affects all animals, ages and production stages the same. Diagnosis of animal mycotoxicosis is based on experimental studies with specific toxins and specific animals, very often under well-defined toxicological laboratory conditions, so that the results of such studies can be far from real-life or natural situations. Furthermore factors such as breed, sex, environment, nutritional status, as well as other toxic entities can affect the symptoms of intoxication. Diagnosis is very much dependent on receiving a sample of feed that was ingested prior to intoxication, but also on data from another representative group of animals of the facility and the results of a post-mortem examination (CAST, 2003).

Table 2: Estimated risk of mycotoxins in different animal species (Heidler, 2003, unpublished data)

Mycotoxin contamination in feed [ppb]	Low risk	Medium risk	Increased risk
A-Trichothecenes (T-2 toxin, HT-2 toxin, DAS)			
Swine (sow, piglet, fattener)	< 150	150 - 400	> 400
Poultry (breeder, layer, broiler)	< 150	150 - 400	> 400
Cattle (calves)	< 150	150 - 400	> 400
Cattle (dairy cows, beef cattle)	< 300	300 - 800	> 800
B-Trichothecenes (DON, AcDON, Nivalenol, Fusarenone X)			
Swine (sow, piglet, fattener)	< 250	250 - 1000	> 1000
Poultry (breeder, layer, broiler)	< 250	250 - 1000	> 1000
Cattle (calves)	< 250	250 - 1000	> 1000
Cattle (dairy cows, beef cattle)	< 500	500 - 2000	> 2000
Zearalenone			
Swine (sow, piglet)	< 50	50 - 250	> 250
Swine (fattener)	< 100	100 - 250	> 250
Poultry (breeder)	< 50	50 - 250	> 250
Poultry (layer, broiler)	< 80	80 - 300	> 300
Cattle (calves, dairy cows)	< 100	100 - 250	> 250
Cattle (beef cattle)	< 100	100 - 300	> 300
Ochratoxin A			
Swine (sow, piglet, fattener)	< 80	80 - 300	> 300
Poultry (breeder)	< 80	80 - 300	> 300
Poultry (layer, broiler)	< 100	100 - 400	> 400
Cattle (calves)	< 80	80 - 300	> 300
Cattle (dairy cows, beef cattle)	< 200	200 - 500	> 500
Aflatoxin B_1			
Swine (sow, piglet, fattener)	< 50	50 - 200	> 200
Poultry (breeder, layer, broiler)	< 80	80 - 300	> 300
Cattle (calves, dairy cows)	< 5	5 - 20	> 20
Cattle (beef cattle)	< 10	10 - 20	> 20

Regulations

Agreement and setting of international regulatory standards is very difficult, as not only

potential health benefits but also political and economical issues have to be considered. Some countries, with the United States, Argentina and China as the probably mostly affected ones, have to face unavoidable economic losses impacted by tighter mycotoxin regulations. In case that the EU proposed standards are adopted worldwide, total export losses from fumonisin in corn may exceed USD 300Million annually, threefold higher than if the less stringent US standards were adopted. Likewise estimated export losses from aflatoxins in peanuts may exceed USD 400Million under EU standards, which is fivefold higher than if the US standards were adopted (WU, 2004).

The economic costs of mycotoxins are impossible to be accurately determined, but the US Food and Drug Administration gives estimations based on a computer model: in the US only the mean economic annual costs of crop losses from the mycotoxins aflatoxins, fumonisins, and deoxynivalenol, are estimated to be USD 932million (CAST, 2003). Table 3 gives an overview about the mycotoxins considered the most relevant in the United States.

A survey on worldwide limits and regulations for mycotoxins was done in 2002/2003 and revealed that more than 100 countries have specific regulations or detailed guidelines for mycotoxins. The study was done under contract of the Food and Agriculture Organisation (FAO) and will be published in the course of the year 2004 (Egmond and Jonker, 2004).

Table 3. Mycotoxins of US interest (from: Bhatnagar et al., 2004)

Toxin	Commodities affected	Producing fungi
A. Major concern		
1. Aflatoxins	Corn, cottonseed, peanuts, treenuts	*Aspergillus flavus*
2. Trichothecenes	Corn, wheat, barley	*A. parasiticus*
3. Fumonisins	Corn	*Fusarium graminearum, F. verticillioides*
B. Significant concern		
1. Ochratoxin A	Wheat, barley, oats, corn, others	*A. ochraceus, Penicillium verrucosium*
2. Patulin	Apples, wheat straw residue	*Aspergillus spp., Penicillium spp.*
3. Zearalenone	Corn, hay	*F. graminearum*
4. Cyclopiazonic acid	Corn, kodo millet	*P. cyclopium, A. flavus*

Mycotoxins in Korea

Sohn et al. (1999) analysed obviously mouldy and obviously healthy corn from Kangwon province in Korea. Due to a period of prolonged rainy weather corn harvest was delayed throughout the province in the year 1997. In order to survey the occurrence of Fusarium mycotoxins including trichothecenes, zearalenone, and fumonisins in local corn, mouldy samples were compared to visibly healthy samples. Detailed results are given in table 4. 94% of mouldy corn were contaminated with DON, 91.7% with fumonisin B1. Additionally over 80% of samples were contaminated with 15-Acetyl-DON, nivalenol, zearalenone, and fumonisins B1 and B2. What is even more of interest is, that 22.9% of the visibly healthy corn was contaminated with DON, 20% with zearalenone and over 28% with fumonisin B1. The standard way of purchasing "good quality" raw material by pure visual check has herewith seen its limits.

Table 4: Natural occurrence of Fusarium mycotoxins in corn samples collected in 1997 (Sohn *et al.*, 1999)

Mycotoxins	% of positive samples		Mean level[μg/kg] of positive samples	
	mouldy	visibly sound	mouldy	visibly sound
DON	94.4	22.9	4.0	0.04
15-Acetyl DON	83.3	17.1	0.9	0.04
3-Acetyl DON	33.3	5.7	0.2	0.2
Nivalenol	88.9	17.1	1.7	0.08
Zearalenone	88.9	20.0	0.6	0.07
Fumonisin B1	91.7	28.6	23.2	3.2

Several studies on the contamination of foods from Korea have been published recently. Park et al. (2002) describe the co-occurrence of aflatoxin B1, fumonisin B1 and ochtratoxin A in barley and corn food, where the presence of one or more mycotoxins was proven in about 16% of tested barley and corn samples, with most incidences in roasted grains determined for making tea. According to the same authors (2002A) zearalenone might not be of major concern for human health in Korea, as in total 21% of analysed samples from corn and barley proved positive, but with relatively low contamination levels from 3.4 to 171 ppb. A survey conducted on fumonisins in Korean corn-based food products showed high incidence of fumonisins in corn flakes, corn snacks, corn starch, and popcorn (73.3%, 78.6%, 50%, and 58.3% respectively), with levels from 14 to 1210ppb. As corn is not a major crop in Korea but approximately 8.8mill tons are imported annually, mainly from China and the US, monitoring programs could be of relevance. The most recently published study deals with the estimation of the daily exposure of Koreans to aflatoxin B1 (Park et al., 2004). Based on surveying data published since 1997 the intake of aflatoxin B1 was calculated and compared with the provisional maximum tolerable daily intakes. Thus the main contributors to aflatoxin intoxication are rice and nuts (peanuts and cashew nuts).

Mycotoxin testing

It has been mentioned earlier that different fungi can produce different mycotoxins on the same commodity. Unfortunately there is nothing like a "leading toxin" as it was believed in the early days of research in this field. Therefore only if all the major naturally occurring toxins are tested a clear assessment can be made.

Testing for mycotoxins is a complicated process that generally consists of three steps:

(1) Sampling

(2) Sample preparation

(3) Analytical procedure.

Sampling consists of selecting a sample of a given size from a bulk lot. Sample preparation comprises the grinding of the sample and taking a representative sub-sample of ground material. The analytical step consists of several processes where the mycotoxin is solvent extracted from the sub-sample, the solvent is purified and the mycotoxin in the solvent is quantified. The mycotoxin value, measured in the analytical step is then used to estimate the lot concentration or is compared to a maximum limit in order to classify the lot as acceptable or unacceptable. This means that a very small quantity of the lot is finally used in the quantification step to estimate the mycotoxin concentration of the whole lot. An example for the dimensions involved: if the original lot is 25 tons, the bulk lot sample is probably 25kg, which means that the quantity inspected is reduced by a factor of 1000. Usually a sub-sample of 250g is taken for further quantification, thus reducing the inspected product by a factor of 100,000. Finally about 1g of product is represented in the solvent mixture from which the mycotoxin is quantified, so that in this example only 1g out of the original 25,000,000g is used to estimate the mycotoxin contamination of the whole lot (Whitaker, 2003).

Sampling is usually the largest source of error, which counts for up to 82% of variability and is mainly because usually only a small percentage of the kernels are contaminated,

and with small sample sizes it is difficult to get a representative amount of contaminated kernels into the analytical sample. It is easier to select a representative sample from a moving stream of product than from a static lot such as trucks or rail cars (Whitaker, 2003). This principle is used in sub-sampling mills like the Romer(r) Series II mill or the RAS mill (Romer Labs. Inc., MO), where sample grinding and continuous sub-sampling is done in parallel, thus providing a good profile of the total sample. The sample preparation error, which accounts to up to 9% of the total variability of a test procedure can further be reduced by increasing the sub-sample size and grinding into finer particles (Whitaker, 2003).

Analytical procedures for the determination of mycotoxins have improved continuously over the past years. Chromatographic methods have been used widely, including thin-layer chromatography (TLC), gas chromatography (GC) with electron capture detection (ECD) or mass selective detection (MS) as well as high-performance liquid chromatography (HPLC) with UV, fluorescence detection, and, described in more recent publications, also with mass spectrometry (Berger et al., 1999). The results of the most sophisticated chromatographic procedures are determined by the efficiency of the prior sample preparation, in particular by sampling, extraction and the further clean-up of the extract. As a large number of interfering compounds present in samples contaminate the primary sample extract, these components must be removed as completely as possible (Krska, 1999). Commonly used clean-up methods employ column chromatography, liquid-liquid extraction, solid phase extraction columns (SPE), as well as immuno-affinity columns (IAC), and one-step multifunctional clean-up columns (MFC, Mycosep(r)), which in particular comprise advantages like speed, simplicity, solvent efficiency, and, in some cases increased recovery and lower cost (Trucksess, et al., 1994; Fuchs et al., 2004). Without any rinsing steps required sample purification takes only 10 to 30 seconds. This one-step purification represents a very rapid and efficient alternative to conventional solid

phase extraction (SPE) or immunoaffinity (IAC) methods as both require usually three to four steps: precondition columns, retain extracted substances on packing material of the column, wash undesirable compounds, and elute analytes of interest (Fuchs et al., 2004).

In contrary to this, also a variety of immunological methods, like immuno sorbent assays (ELISAs) or radio immune assays (RIAs) are available, which require usually no further sample purification but have the major disadvantage, that only one toxin can be determined by each test, referring to the specifity of the antibodies. ELISA test kits are well favored as high throughput assays with low sample volume requirements and proceeding times of less than an hour, some even in less than 15 minutes (Agraquant(r)). However, although the antibodies have the advantage of high specifity and sensitivity to their mycotoxin target molecule, compounds with similar chemical groups would also interact with the antibodies. This so-called matrix effect is especially evident in case of high complexity of the test material, which is in particular the case with finished feed, and can lead to overestimates, underestimates, or even false negative or false positive results. Therefore it is critical that ELISAs are extensively studied on their accuracy and precision over a wide range of commodities. ELISA results of a certain material can be taken as trustworthy only if the kit was validated for the respective commodity (Zheng et al., 2004).

Which is the analytical system of choice for a practical feed mill operation? The most commonly used system for rapid testing is without doubt the ELISA since it is the fastest and most cost effective system, in case of high sample throughput and quick results requirements. Test kits based on the ELISA principle are not necessarily only in the microtiter format, thus requiring exact pipettes and photometric readers allowing quantitative determination, they can also come in qualitative formats like small cups (e.g. Aflacup), where an enzymatic colour reaction visualizes within five minutes whether

aflatoxins are present or not at a certain cut-off level.

For larger operations an HPLC could be feasible yet the time factor does not allow testing before unloading trucks delivering goods. The shortcoming of single analysis is overcome by parallel tests of the defined analytes. As most analytical procedures are complex procedures involving several steps where errors can occur, increasing the number of measurements made on the sub-sample extract can reduce these analytical errors, as well as using analytical methods with superior technology.

Quality assurance in mycotoxin analysis has become an important and critical issue. Check sample programmes, proficiency testing by means of laboratory intercomparison tests and the use of certified reference materials are the basis for proper quality management in the lab. The proficiency testing offered by the British FAPAS(r) programme follows the International Harmonised Protocol for Proficiency testing of Chemical Analytical Laboratories as well as the ISO guide and is probably the most elaborated and acknowledged in mycotoxin testing. Certified reference materials (CRM) of major mycotoxins and their carrier commodities are offered by the Standard, Measurements and Testing Program of the European Commission, which started in the early 1980s (CAST, 2003). In case of farming out analytical services to a contract lab make sure it is a lab that does mycotoxins on a routine base and uses the right confirmatory method and not only some rapid testing like ELISA as this might lead to certain problems with some commodities like finished feed.

Unfortunately until now there is no multi-toxin rapid test format in the market that suits the practical conditions of a feed mill. Therefore routine testing needs to be established for the most likely occurring toxins out of a specific area. That is viable only in case of known origin when the cereals are procured directly from the producing area. But what in case of traded goods? Since it is known by experience that different commodities are

more often contaminated with some mycotoxins an empirical testing plan could be suggested as described in table 5:

Table 5: Example of testing plan for different commodities

Commodity	aflatoxins	DON	zearalenone	ochratoxins	nivalenol	fumonisins
corn	+++	++	+	+	+	+++
wheat bran	---	++	++	++	---	---
wheat	+	++++	++	++	+	---
barley	---	++	+++	+++	---	---
Corn gluten	+++	+++	++	-	++	++
rice	+	--	--	---	-	---
tapioka	++	--	--	--	--	---
soybean	+++	++	++	---	---	---

+ testing frequency at every 1000 tons; ++ testing frequency at every 100 tons; +++ testing frequency at every truck load; - testing in case of doubtful unknown origin; -- testing once every month; --- testing only in case of reported clinical symptoms from feeds containing the commodity

In order to determine such a testing chart excessive testing of the major traded goods of a certain area is a precondition. But as terms of trade change quickly so must the chart for the testing. In case of Korea where the majority of raw material supply for feed production is imported, testing depends very much in the seasonal and geographical conditions of the country of origin. Continuous testing and adjustment may lead to the development of an efficient testing plan according to the special needs of an operation.

A mycotoxin sampling plan is defined by the mycotoxin test procedure (sample size, sample preparation method, and analytical method) and the respective accept/reject limit, i.e. the predefined threshold that separates acceptable lots from unacceptable lots. Because of the variability associated with each step of the mycotoxin test procedure, the true mycotoxin concentration of a bulk lot cannot be determined with 100% certainty. As a result, some lots can still be misclassified by the sampling program and the magnitude of

the risk associated with misclassification is directly related to the magnitude of the variability associated with the mycotoxin test procedure (Whitaker, 2003).

How to cope with the mycotoxin threat?

Most grain farmers are still paid on quantity of the commodity delivered but not in relation to the mycotoxin content of their grain. That is why the farmer has no incentive to prevent or monitor mycotoxin formation at this stage. The best prevention would be proper crop rotation and fungicide administration at the right time. One sometimes quite effective means in prevention of mycotoxin production is the choice of the right time to harvest - especially in corn - and the immediate drying of the grains when the moisture is above 15% at harvest. On storage grains must be kept free from infestation of bugs. Implementation of an HACCP concept with focus on fungal toxins could be outlined as it follows:

The first critical point of action is to conduct a hazard analysis. Prepare a list of steps in the process, where mycotoxin or mould infestation could occur and describe preventive measures. One could be the purchasing of raw materials. Many contracts do not mention mycotoxins at all and that is the first point to act. Add a clause with maximum acceptable levels of mycotoxin contamination in the contract. The second step in your HACCP system is to determine the critical control points, i.e. which are the materials, products or production steps that have to be monitored for fungal contaminants. One rule of the thumb could be the ratio of tests conducted on raw materials versus tests done on finished products, which is for example 9 to 1. Third step would be to establish critical limits, which means to determine the maximum tolerable toxin levels. What is the internal risk profile that is acceptable within an operation? Step number four is the establishment of procedures for monitoring the critical control points. This can include procedures for sampling, sample preparation and testing itself, or the outsourcing of parts of or even the

total analytical process. Step five covers the establishment of corrective actions, which could comprise the introduction of certain cleaning procedures for silos, bins, hoppers, and elevators into the maintaining plan, as repeated contamination could originate from bins containing materials like wheat bran that have never been cleaned so that contamination might originate from and spread within the same operation and not only from purchased raw materials. Step six comprises the verification procedures and step seven the documentation and record keeping.

The management of mycotoxin contamination in the daily operation.

The first and most practical approach so far has been the blending of low contaminated material with material above the limits thus lowering the average contamination levels to the accepted standards. In case that is not possible other methods need to be applied.

Since all mycotoxins are quite stable substances no physical or chemical treatment can be applied under practical field conditions, without altering the nutritive value of the grain or causing too high cost implications. Ammonia or strong oxidizing agents can reduce the contamination but reduce also the nutritive value of the feed at the same time. Based on that facts extensive research has lead to various products for the detoxification of feed.

The most commonly used strategy of reducing exposure to mycotoxins is the decrease of their bio-availability by the inclusion of various mycotoxin binding agents or adsorbents, which leads to a diminishing of mycotoxin uptake and distribution to the blood and target organs. Various substance groups have been tested and used for this purpose, with aluminium silicates, in particular clay and zeolitic minerals, as the most commonly applied groups. Froschl et al. (2000) investigated the aflatoxin-binding capacities of a large number of different aluminium silicates in relation to their physico-chemical properties. The tested materials were classified as bentonites (calcium

bentonites, sodium bentonites, organophilic (modified) bentonites, acid-treated bentonites, as well as some special forms), zeolithes, diatomites, and vermiculites. Most minerals tended to show higher adsorption of aflatoxins at higher pH levels, with sorption by vermiculites and zeolites being the most sensitive to pH alteration. An important criterion for evaluation of mycotoxin adsorbers is their effectiveness at high and low pH levels since a product must work throughout the gastro-intestinal tract, thus within a broad pH level. Since the mode of action has to start already in the stomach it must be effective at least at a pH level of 3. No correlation could be found between the cation exchange capacity and sorption of aflatoxins, while high specific surface and micro-pore volumina seemed to be related to better binding properties. An important aspect in the evaluation of potential mycotoxin binders is the stability of the sorbent-aflatoxin bond, in order to prevent desorption of the toxin.

The elimination of other mycotoxins than aflatoxins (e.g. trichothecenes, zearalenone, ochratoxins or fumonisins) from contaminated feedstuffs by the use of adsorbents did not lead to any satisfactory results so far, as most of the adsorbing agents bind them only weakly in vitro and are ineffective in vivo. In vitro experiments with natural and modified clay minerals showed little or no binding of DON and other trichothecenes, in contrast to the extensive binding of aflatoxins (Anantaggiato, et al., 2004; Thimm et al., 2000). As it is known that in the case of trichothecenes the 12,13-epoxide ring is responsible for their toxic activity and removal of this epoxide group entails a significant loss of toxicity, research focused on the identification of natural processes where this reaction occurs. Several authors described this de-epoxidation bioreaction of ruminal or intestinal flora (Kollarczik et al., 1994, He et al., 1992), but Biomin(r) researchers were the first to isolate a pure bacterial strain which is able to bio-transform the epoxide group of trichothecenes into a diene, thus detoxifying all relevant trichothecene toxins by this reaction.

The active isolate is a new species of the genus Eubacterium, named BBSH 797. For its

application as feed additive fermentation and stabilisation processes were established and optimized with respect to good and fast growth of the microbe, high biotransformation activity of the resulting product, and economic reasons. For enhancement of stability during storage and within the gastro-intestinal tract, a three-step encapsulation process was implemented. The additive' s efficiency in counteracting adverse effects of feed contaminated with trichothecenes was proven in piglet and broiler trials (Binder et al., 2001). Further research of the same group led to the isolation of a yeast strain, namely of the strain Trichosporum mycotoxinivorans, which can decompose and thus detoxify ochratoxin and zearalenone. Both, in vivo and in vitro trials have proven this additives ' high efficacy to counteract these mycotoxins under practical conditions (Schatzmayr et al., 2004).

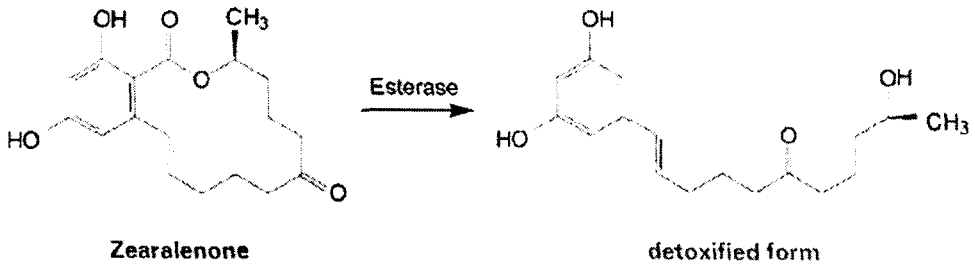

Figure 2. Mode of action of *Trichosporum mycotoxinivorans*: detoxification of Zearalenone

Based on the knowledge summarized above and information about the overall contamination levels the following mycotoxin detoxification strategy can be recommended:

For any contamination where aflatoxin occurs as the only contaminant a certified binder should be used for reduction of bioavailability of aflatoxins. The adsorber ' s certificate should comprise data about its efficacy, which means its guaranteed binding capacity at at

least two relevant pH levels (e.g. pH 3 and pH 6.5), as well as the absence of any potential hazard, in particular of dioxin. In case of trichothecene contamination of feedstuffs the addition of feed additive BBSH 797 would be a proper way to counteract the possible impacts due to this mycotoxin group, in case of positive indications of zearalenone or ochratoxins the addition of Trichosporum mycotoxinivorans should be considered.

Summary

In order to manage the risk of mycotoxins in a feed mill operation an internal risk profile has to be established. Based on continuous testing of raw materials as well as finished product the situation should be continuously monitored. In case that action is necessary to detoxify contaminated materials the several choices depending on mycotoxins and species shall be considered, in order to secure safety and performance of the farm animals in general and the whole food chain in particular.

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