

Effect of mycotoxins in dairy cows

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Mycotoxins are toxigenic metabolites of fungi. They occur worldwide and are a potential risk to humans and animals when feeds and cereals are contaminated. Their importance and occurrence have been recognised for over 30 years. Following the eating of contaminated foods and feeds, so called mycotoxicoses are observed in animals.

These intoxications should be differentiated from mycoses, which are caused by active invasion of growing fungi into living tissues in a way that results in destruction of the tissue. In dairy cows the course of mycotoxicoses is acute or chronic, depending on the amount of mycotoxin ingested. Furthermore, mycotoxins depress the cow's immune system making her more susceptible to infections.

Mycotoxin contaminated feedstuffs are a potential risk to the consumer because of residues in meat and milk products. Specific mycotoxins affect certain organs, such as the liver, kidney and brain, or mucous membranes of the gastrointestinal or genital system. In this article a general view of the most important moulds and mycotoxins and their effects on dairy cows will be considered and cases studies from Austrian dairy herds are described.

Feeding studies with zearalenone and vomitoxin contaminated feedstuffs and their effect on dairy cows are also reported.

Based on the climatic conditions the most important mycotoxins in Austrian foodstuffs are as follows:

- Fusarium toxins (deoxynivalenol = vomitoxin and other trichothecenes). These

North Asia*	Aflatoxins	ZON	DON	FUM	T-2 toxin	OTA
No. of tests	414	414	414	324	387	111
No. of positives	23	228	310	188	3	31
Positive (%)	6	55	75	58	<1	28
Average (µg/kg)	51	592	731	1579	299	82
Maximum (µg/kg)	494	14105	10374	14714	399	82

*North Asia includes China, Japan, Korea and Taiwan

ZON = Zearalenone; DON = Deoxynivalenol; FUM = Fumonisin; OTA = Ochratoxin A

Table 1. Occurrence of mycotoxins in North Asia (Biomim Mycotoxin Survey Programme 2005-2006).

affect the mucous membranes of the digestive tract.

- Zearalenone, causing infertility.
- Aflatoxins (liver damage) occur in imported feedstuffs (peanut meal, soybean meal).
- Ochratoxin A (kidney problems) are of lesser importance in terms of economic losses in dairy herds.

Aflatoxicosis

Primary mycotoxicoses in dairy cows which affect the liver are caused by aflatoxins. These are potent carcinogens and are produced by *Aspergillus flavus* and *parasiticus* spp. under subtropical conditions.

Currently 18 different chemical compounds of this highly toxic fungal metabolite are well known and four of them (B1, B2, G1, G2) have been detected in different feedstuffs. Aflatoxin B1 is the most toxic compound and is metabolised in the liver to aflatoxin M1, which is eliminated via the

milk. Clinically a chronic aflatoxicosis can only be suspected as poor ration, endoparasites or other diseases will all compound the problem. In dairy cows 100-300µg/kg feed (88% dry matter (DM)) and 200-300µg/kg feed total aflatoxins will cause clinical symptoms in young stock.

Adult dairy cows are not so sensitive to aflatoxins as younger or pregnant animals. The predominant clinical symptoms of a chronic aflatoxicosis are decreased milk yield and finishing performance.

Roughened hair coat, decreased appetite and occasional diarrhoea are often also observed. In the case of acute intoxications

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Case history No. 1

Ten lactating cows, three weeks after feed change, showed the following clinical symptoms: decreased feed intake, slight emaciation and reduction of milk yield (30-50%).

Protein in the feed was provided by imported peanut meal, which was condemned from the market by law because of high contamination with aflatoxins.

This feed for high yielding dairy cows was analysed for aflatoxins (B1, B2, G1, G2) by means of HPLC (high performance liquid chromatography) and 11.6mg/kg total aflatoxins detected.

Aflatoxin B1, the most toxic compound of aflatoxins, was found at 7mg/kg. By law the threshold level for aflatoxins is 5µg/kg feed.

Table 2. The occurrence and highest levels of mycotoxins detected based on commodities and country (October 2003-May 2006; Chin, L. J., 2006).

Mycotoxin	Sample size	Percent positive	Average of positives (µg/kg)	Median (µg/kg)	Maximum level detected (µg/kg)	Commodity found	Country of origin
Aflatoxins	1487	17	39	<4	457	Corn	China
ZON	1488	37	494	<32	10374	DDGS	China
DON	1456	48	824	<50	18991	Wheat	China
FUM. B1	1435	44	869	<100	14714	Corn	China
T-2 toxin	1203	<1	258	125	494	Finished feed	Thailand
OTA	239	22	13	<2	143	Corn	Thailand

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clinical symptoms are depression, loss of appetite and fever.

Histopathological findings are cholangiectasis, loss of liver cell glycogen, fatty degeneration, fibroplastic proliferation and perivascular oedema – all of which are seen in the liver.

Ochratoxicosis

Ochratoxin A is a nephrotoxic mycotoxin, formed by *Aspergillus* and *Penicillium* spp. and was found in Austrian feedstuffs at concentrations that would cause chronic problems.

Experimental examinations of 30 day old calves, which received 0.1-0.5mg ochratoxin A/kg LM (life mass) daily over a period of four weeks showed polyuria, depression, decreased weight gain, low specific gravity of urine and dehydration. At necropsy greyish coloured kidneys and a mild enteritis were seen.

Histopathological findings were those of a slight tubular degeneration with abundant eosinophilic, hyaline material as a sign of deposition of protein into the tubules and Bowmann's capsules.

Furthermore, necrosis of the epithelium of proximal tubules and interstitial fibrosis occurred. Ochratoxin A was also found combined with citrinin, a metabolic product

produced by the same fungi as ochratoxin A at concentrations of 1-2mg/kg feed (88% DM).

Trichothecenes – Toxicosis

Trichothecenes cause irritations of the mucous membranes of the digestive tract and they are comprised of approximately 50 different toxins with varying toxicity.

In farm animals deoxynivalenol (vomitoxin), nivalenol, T-2 toxin and diacetoxyscirpenol cause intoxications.

These secondary metabolites of fungi are produced by *Fusarium* sp. The main producers being *Fusarium roseum* (*Gibberella zeae*), *F. graminearum* and *F. culmorum*.

On Austrian farms deoxynivalenol and the oestrogenic active zearalenone cause important economic losses.

Humid and cold weather conditions as well as abrupt changes of temperature between day and night enhance the severity of fusarium toxin contamination of different cereals and maize. T-2 toxin, diacetoxyscirpenol and the macrocyclic trichothecenes are more toxic than deoxynivalenol.

The following clinical symptoms were seen: decreased feed intake, general weakness and severe bleeding, cardiovascular shock and apathy. As local cytotoxic effects inflammation of muzzle, lips, tongue and pharynx were seen. T-2 toxin causes atrophy of thymus in calves after six weeks of feeding (4mg/kg feed).

Concerning the effect of deoxynivalenol in cattle only a small amount of information is available. Experiments using three dairy cattle, which were fed for five days with 43 and 83mg total deoxynivalenol did not exhibit any clinical or pathological symptoms.

Milk yield as well as minerals were in the normal range. Deoxynivalenol was excreted via faeces and urine in a very short period of time but not via milk.

Therefore, it can be concluded that deoxynivalenol is metabolised by rumen flora. A trichothecene toxicosis can only be diagnosed by confirming detection of the incriminating toxin in the feed.

Because of the rapid metabolism in the rumen T-2-toxin, deoxynivalenol and diacetoxyscirpenol could not be found in tissues (including the liver) or in serum and urine.

Hyperoestrogenism

Zearalenone particularly affects the genital system and is elaborated by *Fusarium* spp. in the field. Frequently, this oestrogenic effective fungal metabolite occurs with deoxynivalenol and sometimes its active alcohol zearalenol. The most favourable growth factors for this fungus and toxin production of these *Fusarium* spp are moisture content of 23% and environmental temperature of 27°C. The temperature optimum range of the enzyme activity which is essential for the elaboration of zearalenone should be 12-

24°C and changes of temperature are the basis for toxin production.

Chemically zearalenone shows a similar configuration to oestradiol and so it can connect to cytoceptors and causes oestrogenic effects as well as abnormal oestrus. Heifers display a prolonged oestrus as well as decreased conception – and non-return-rates. Dairy cows, which were fed with fusarium contaminated grain (25mg zearalenone/kg) showed vaginitis, extended oestrus, decrease of feed intake and milk yield as well.

Corn-silage, maize kernels and wheat were frequently contaminated and exhibited the highest zearalenone levels. In Great Britain a decreased fertility in dairy cows after feeding of hay and grass-silage containing zearalenone (14mg) has been observed. In this instance, the insemination ratio increased from 1.2 to 4.0.

Effects of rumen flora

Aflatoxins, ochratoxin A and the fusarium-toxins zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol are potential risk factors for dairy cows.

In these animals with completely developed forestomach-system the rumen fluid content is for certain mycotoxins as ochratoxin A, zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol a detoxifying barrier and the protozoa are significantly more active at detoxification than bacteria.

Examinations have shown, that mycotoxins mentioned above are metabolised to significantly lesser toxic substances and protozoas are more effective than bacteria.

Ochratoxin A is metabolised to ochratoxin α and phenylalanine, zearalenone into α -zearalenol and β -zearalenol, diacetoxyscirpenol and T-2 toxin into monacetoxyscirpenol and HT-2 toxin deacetylated. Such metabolic changes in the rumen are natural defence mechanisms of ruminants against toxigenic feed components.

Aflatoxin B1 is metabolised in the liver into AM1, and excreted via milk. Deoxynivalenol is metabolised into deepoxymetabolite I (deoxynivalenol I) and excreted via urine.

Diagnosis

Diagnosis of mycotoxicoses in dairy cows is difficult because of the great variety of clinical symptoms seen. Mycotoxicoses can occur at toxin concentrations below detection limit and also masked mycotoxins as well as synergistic effects of several mycotoxins have to be considered.

First of all the case history including clinical symptoms (indigestion, haemorrhagic diathesis, central nervous disturbances) and the feeding regimens are of utmost importance. Inevitably, an antibiotic therapy is ineffective and a seasonal occurrence of such intoxications (spring and autumn) is

observed. The storage conditions of the different feed components should be checked and visible mould contaminated feedstuffs should be tested for mycotoxins.

Other pathogens like bacteria, virus and parasites must be excluded. Masked mycotoxins may occasionally give false negative analytical results such as deoxynivalenol-glucoside on maize, zearalenone-4-sulphate on rice, hydroxylation and glucosylation of ochratoxin A in wheat and maize cultures.

In terms of synergism effects of one mycotoxin might be enhanced in the presence of other mycotoxins, for example, deoxynivalenol and zearalenone, T-2 toxin synergised the activity of deoxynivalenol with regard to several parameters including

weight gain and aflatoxins and T-2 toxin showed a synergistic effect on lethality.

By analytical detection of the mycotoxins in the feed or residues in tissue samples (liver, kidney) and/or blood serum samples a 'mycotoxicosis' diagnosis can be confirmed. Following preventive measures of mycotoxin contamination of feedstuffs should include:

- In the field (fungi resistant grains, high quality seed, balanced fertilisation, insect control, management of crop residues and optimised plant density).
- During harvest (appropriate time, careful harvesting procedure, appropriate harvesting equipment, removal of damaged por-

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tions and high moisture plant parts).

● Storage (without any delay, good hygienic conditions of storage facilities, moisture control, humidity control, insect control, chemical preservatives such as organic acids and antifungal agents).

High moisture grain should be dried immediately after harvest, proper attendance during harvest, transportation and storage can decrease mould growth and subsequent mycotoxin production. To preserve high moisture grains for mould invasion organic acids (such as, propionic acid 0.5 % v/v) are used. Prevention reduces, but does not eliminate, the risk of mycotoxin contamination.

According to FAO 25% of the annual world grain production is contaminated. Based on the occurrence of aflatoxins, doxynivalenol and fumonisins the cost of mycotoxin contamination of crops in the USA is approximately \$932 million.

Counteractive approaches to mycotoxin contamination of feedstuffs should include preventive measures during feed production, decontamination during feed processing and deactivation during feed digestion.

In terms of the disadvantages of decontamination procedures it should be mentioned that physical treatments are expensive, of uncertain outcome and connected with high feed losses and limited practical application. Chemical treatments are also expensive

Case history No. 2

In a dairy herd of 20 Brown Swiss cows unspecific infertility problems were observed with clinical symptoms of anoestrus, false oestrus or nymphomania with a grey-opaque vaginal discharge. On examination, the following results were found:

- The animals were in a good nutritional status, the general external appearance slightly restless; other clinical findings were in the normal range; three cows showed a slight increase of ketone bodies in the urine and the activity of rumen protozoa (ciliates and flagellates) was slightly decreased.
- Cervical/uterus swabs revealed no specific pathogens responsible for infertility. Moderate levels of α -haemolytic streptococci and non-specific bacteria were detected.
- The feed ration was balanced and capable of providing the nutritional requirements according to the milk yield of the animals.
- The microbiological state of the corn and grass-silage samples was moderate, only slight contamination with fungus of the grass-silage was found. The feed samples just mentioned were checked for the infertility causing fusariotoxin zearalenone. In corn-silage 50 μ g/kg and in grass-silage 100 μ g/kg were detected.

Based on the feed intake of the animals (12kg corn and 10kg grass-silage) the daily burden of zearalenone was 1600 μ g (1.6mg) per animal.

and time consuming, change the palatability and nutritive value and decrease feed quality with toxic byproducts and therefore not of real practical use.

Deactivation of mycotoxins in animal nutrition is performed by adsorption by use of clay minerals and mainly effective with aflatoxins, but in a lesser extent with fumonisins, ochratoxin A and zearalenone.

By means of enzymes and microbes mycotoxins are biotransformed to non-toxic metabolites. This biotransformation is specific, irreversible, usable for all mycotoxins

and the metabolites are non-toxic. The microbe *Eubacterium* BBSH 797 produces specific enzymes which detoxify trichothecenes in the intestinal tract of animals. Many methods of decontamination and deactivation of mycotoxin contaminated feedstuffs have been investigated, but they are very expensive and in general not applicable under practical conditions.

The experienced mycotoxicologist should make the final decision about use or destruction of fungal or mycotoxin contaminated foods and feeds. ■