Clinical and Subclinical Events Related to the Presence of Mycotoxins in Cattle Feed

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Introduction

Mycotoxins are toxigenic metabolites of fungi which occur worldwide and are a potential risk for humans and animals when feeds and cereals are contaminated. Their importance and occurrence were recognized in the last three decades. Oral uptake of mycotoxin-contaminated foods and feeds cause mycotoxicoses in susceptible animals. These intoxications should be differentiated from mycoses, which are caused by active invasion of the growing fungi in living tissues resulting in mechanical destruction.

In cattle the course of mycotoxicoses may be acute or chronic, depending on the amount of these toxic substances in the feed. Some predispose for infectious diseases by decrease in the natural resistance and immune mechanisms. Some mycotoxin contaminated feedstuffs are a potential risk to the consumer because of residues in meat and milk products. Specific mycotoxins also affect certain organs or tissues as liver, kidney, brain, mucous membranes of the gastrointestinal and genital tracts.

In this paper an overview of the most important mycotoxins affecting cattle is given and cases which recently occurred in Austrian dairy herds and steer fattening units are described. Feeding studies with zearalenone and vomitoxin contaminated feed and their effect on dairy cows are reported.

Based on the climatic conditions the most important mycotoxins in Austrian feed-stuffs are fusarium toxins (deoxynivalenol = vomitoxin and other trichothecenes), affecting the mucous membranes of the digestive tract, and zearalenone that causes infertility. Aflatoxins (liver damage) occur in imported feed (peanut meal) and ochratoxin A (kidney problems) are of less importance in terms of economic losses in cattle.

Primary mycotoxicoses in cattle which affect the liver are caused by aflatoxins, tubratoxin and sporidesmin. Aflatoxins, which are very potent carcinogens, are synthesized by *Aspergillus flavus* and *parasiticus spp.* under subtropical conditions. At least 18 different chemical compounds of this highly toxic fungal metabolite are known and 4 of them (B1, B2, G1, G2) are detected in different feedstufs. Aflatoxin B1 is the most toxic and is metabolized in the liver to aflatoxin M1 and excreted in the milk. Clinically chronic aflatoxicosis can only be suspected, while an incorrect feeding ration, endoparasites and other diseases increase this intoxication. In dairy cows, 100 to 300 ppb and in fattening steers, 200 to 300 ppb total aflatoxin cause clinical symptoms. Adult dairy cows are not as sensitive as young or pregnant animals. Clinical symptoms of chronic aflatoxicosis include a decreased milk yield and weight gain. A rough coat, decreased appetite and occasional diarrhea are observed. In acute intoxication, clinical symptoms are depression, loss of appetite and fever. Histopathologic findings are cholangiectasis, loss of liver cell glycogen, fatty degeneration, fibroblastic proliferation and perivascular edema.

Ochratoxins (A - D) are nephrotoxic mycotoxins, formed by *Aspergillus* and *Penicillium spp.* and were found in Austrian feedstuffs in chronically effective concentrations. Experimental examinations of 30 day old calves, which received 0.1 to 0.5 mg/kg LM OA *per diem* over a period of 4 weeks showed polyuria, depression, decreased weight gain, low specific gravity of urine and dehydration. On necropsy, grey-coloured kidneys and slight enteritis were seen. Histopathological findings were slight tubular degeneration with abundant eosinophilic, hyalinic material as a sign of deposition of protein in the tubuli and Bowman capsule. Necrosis of the epithelium of proximal tubules and intestitial fibrosis later occurred. OA was also found combined with citrinin, a metabolic product synthesized by the same fungi as OA in concentrations of 1 to 2 ppm.

Trichothecenes cause irritation of the mucous membranes of the digestive tract. They comprise approximately 50 different toxins with various toxicities. In farm animals deoxynivalenol (vomitoxin), nivalenol, T-2 toxin, diacetoxyscirpenol (DAS) and the macrocyclic trichothecenes, such as roridin A and verrucarin A cause intoxications. These secondary metabolites are developed by Fusariumital species, such as *F. roseum* (Gibberella zeae), *F. granimearum* and *F. culmorum.*

In Austria, DON and the estrogenic-active zearalenone, cause important economic losses. Humid

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and cold weather, as well as abrupt changes in temperature between day and night, enhance the severity of fusarium toxin contamination of cereals and maize. T-2- toxin, DAS and the macrocyclic trichothecenes are more toxic than DON. The following clinical symptoms in affected animals are decreased feed intake, general weakness and severe bleeding, cardiovascular shock and apathy. Local cytotoxic effects such as inflammation of the muzzle, lips, tongue and pharynx are described. In calves after 6 weeks of feeding (4 ppm), T-2-toxin also causes atrophy of the thymus.

Little information is available on the effects of DON in cattle. Experiments with three dairy cattle, which were fed for 5 days with 43 and 83 ppm total DON, failed to exhibit any clinicopathological signs. Milk yield as well as minerals were in the normal range. DON was excreted in the feces and urine for a very short time but not in milk. It can therefore be concluded that DON is metabolized by the rumenal flora. A trichothecene toxicosis can only be diagnosed by positive detection of the toxin in the feed. Because of rapid metabolism in the rumen, T-2-toxin, DON and DAS could not be found in tissues, urine or liver respectively.

Zearalenone affects particularly the genital tract and is synthesized by *Fusarium spp.* This estrogen-mimicing fungal metabolite frequently occurs with DON and sometimes with its active alcohol, zearalenol. The optimal growing condition for fungus and toxin synthesis of these *Fusarium spp.* is a moisture content of 23% and an environmental temperature of 27°C are necessary. The optimum temperature range for the enzyme essential for zearalenone synthesis must be 12 - 24°C and changes in temperature are essential for toxin production. Structurally, zearalenone shows a similar configuration to estradiol, is bound by cytoreceptors and causes estrogenic effects such as abnormal estrus. Heifers display prolonged estrus as well as decreased conception and by non-return rates. Dairy cows, which were fed with fusarium contaminated grain (25 ppm) show vaginitis, extended estrus, decreased feed intake and milk yield. Corn silage, maize kernels and wheat were frequently contaminated and contain the highest zearalenone levels. In Great Britain decreased fertility in dairy cows was observed after feeding hay and grass silage containing zearalenone (14 ppm) The insemination ratio increased from 1.2 to 4.0.

Case Histories and Results

Case 1

In a dairy herd of 20 Brown Swiss cows nonspecific infertility problems were observed with clinical symptoms of anestrus, false estrus or nymphomania with gray-opaque vaginal discharge. To determine the cause of this disease the following examinations were carried out: clinical status, urine, rumen fluid, blood (enzymes, minerals, serological parameters), swab samples from cervix uteri and evaluation of the feed (composition of the ration, microbiological and mycotoxicological status).

The animals were in a good nutritional condition, but appeared slightly restless. While 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 to moderately decreased.

Blood samples were normal other than increased amounts of total bilirubin and decreased glucose levels. Blood phosphorous and magnesium were at the lower threshold levels. Serological tests for *Brucella bovis* antibodies, *leptospira spp* and IBR-IPV were negative.

In the cervix uteri swabs no specific bacteria responsible for infertility were found. Slight to moderate presence of hemolytic streptococci and a few nonspecific bacteria were detected.

The calculation of the feed ration and composition was balanced with nutritional requirements according to the milk yield.

The microbiological levels of the corn and grass silage samples were moderate, and only fungal slight contamination of the grass silage was found. The feed samples were checked for the infertility-causing fusariotoxin zearalenone. In corn silage, 50 ppb (µg/kg) and in grass silage 100 ppb were detected. Based on the feed intake of the animals (12 kg corn and 10 kg grass silage) the daily burden of zearalenone was 1600 µg or 1.6 mg zearalenone per animal.

Case 2

Ten lactating cows three weeks after a 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 for sale by law because of high concentrations of aflatoxins. This feed for high yielding dairy cows was analysed for aflatoxins (B1, B2, G1, G2) by thin layer chromatography and 11.6 ppm total aflatoxins were detected and 7 ppm aflatoxin B1. By law the threshold level of aflatoxins is 5 ppb.

Case 3

Diarrhea was observed in a fattening steer operation containing three units of 30 animals. The animals were fed with corn silage and concentrates and kept on a concrete slatted floor. An important fact in the case history was the new batch of corn silage that was fed. A distinct red discoloration of this feedstuff was noted and was suspected of causing the enteritis. Microbiological examination showed high contamination with *Monascus ruber* (>100 Mio colonies/g) in pure culture and

mycotoxicologically (14.5 ppm vomitoxin and 4.5 ppm T-2 toxin). To exclude other pathogens, blood and feces samples were checked for antibodies against prominent viruses and bacteria. None of the samples were positive serologically.

Case 4

In November 1993 in a beef cattle unit containing 130 animals, located in Lower Austria, sudden death occurred in 12 animals weighing 300 - 400 kg. Based on the finding of sudden death, a feed induced problem was considered. In the concentrates (prepared feed, mixed feed) and corn silage vomitoxin concentrations of 60 and 4,000 ppb, respectively were detected. Based on these results, detoxificants were added to the corn silage (Bolatox®, Fa. Likra, Linz; Mycofix®, Fa. Biomin and Fixatox®, Fa. Werfft). During December 1993, no further disease or mortality was observed. At the end of January 1994, several animals displayed symptoms of indigestion, severe fever and central nervous signs. To reach a diagnosis, two diseased steers were submitted to our clinic and organs from an emergency slaughtered animal (brain, kidney, liver, intestines) were examined. At the clinic the following symptoms were found: depression, increased body temperature (41.5°C), mucous membranes of the eyes were slightly reddened, cyanotic and were dirty red in colour. The oral cavity showed reddening as well as cyanosis, slight salivation, venous congestion, weak pulse rate of 80/min., no rumen activity. The neurological examination displayed a highly decreased or lack of sensitivity of the skin in the neck, both hind limbs and the thighs. Based on the clinical symptoms, bacterial infection of the CNS was suspected. Cerebrospinal fluid (CSF) was taken by lumbar puncture of the subarachnoid space and following parameters examined: total protein 2.2 g/L, glucose 1.2 mmol/L, creatine-kinase 45 U/L. Cytologically 50% lymphocytes and 50% segmented neutrophil granulocytes.

Serum samples were tested for antibodies to *Brucella bovis,* enzootic leucosis, IBR/IPV, BVD, RSV and adenovirus as well as BVD virus antigen. All samples were negative to these pathogens and therefore virus infection was excluded. The cerebrospinal fluid had a gelatinous appearance, and was fairly cloudy with fibrin clots. *Hemophilus sommus* could be isolated from the CSF, but not *Listeria monocytogenes,* and on examination of the brain, *H. somnus* was cultured.

Case 5

In a beef cattle unit, 150 steers were fed with a mixed ration consisting of sliced roots concentrates, soya, corn silage and barley. The feeding period was on the average 420 days, the body mass 630 kg. A nutritionist recommended a change of feed. Instead of barley, cracked maize could be fed because of its low price. He also stated that this compound was of high quality, sound and in normal commercial use. According to their weights, the fattening steers were fed with 1.2 - 2.4 kg cracked maize, sliced root feed, corn silage, soybean and mineral concentrate. After feeding them for 4 months, the farmer observed poor development of the animals, which showed a decreased feed intake, a long and rough coat, apathy and a significantly lower weight gain.

A low starch content of this cracked maize was first suspected, but it could not be confirmed by examination. No explanation for the poor growth of the animals was found. Six steers were slaughtered, the carcases displayed very low weight and the muscular tissues exhibited a poor fat layer. Blood samples were checked for enzymes; AP, SGOT, GGT, GLDH and total bilirubin, minerals (Ca, Mg, P) and blood status. Feces samples were tested for parasites. The blood and feces samples showed values within the normal range. It should be mentioned that the fattening steers did not show prior, during or after feeding the bruised maize, respiratory or gastrointestinal symptoms. On the base of the feed change using the suspect cracked maize, mycological and mycotoxicological examinations of the kernels were carried out with the following results:

> Microbial count 725,000 colonies/g, fungal count 18,000 colonies/g (60% Fusarium, Penicillium, Aspergillus), Fusariotoxins (in ppb): Vomitoxin - 1,300, Zearalenon - 560, Moniliformin - 420. A total of 2,280 ppb.

The farmer had fed 150 fattening steers for 4 months with the highly contaminated cracked maize which was injurious to health. These fusariotoxins caused a decreased feed intake, and daily weight gain (0.7 kg instead of 1.3 kg per day). Some of the animals were stunted with loss of weight as well as poor meat quality.

Discussion

Based on the case histories, the clinical symptoms and elimination of bacterial and viral pathogens, a correlation between the course of the disease and the feed was apparent, leading to diagnosis of intoxication of dairy cows and beef cattle by mycotoxin contaminated feed. The diagnosis was confirmed by positive microbiological and mycotoxicological examination of the suspected feed samples.

In case 1 a secondary ketosis connected with a slight disturbance of liver function and a slight phosphorus and magnesium deficiency were evident, in addition. These were caused by decreased feed intake and resorption problems by the mycotoxin burden. As therapeutic measures, a decrease of the amount of grass si-

lage and an increase of hay was recommended to attain a dilution effect of the zearalenone concentration in the total ration. The phosphorus and magnesium content in the ration was increased by adding them to the concentrate. Flushing of the uterus using Lotagen and antibiotics, parenteral application of vitamins, and prostaglandin treatment for anestrus were recommended. These measures caused a significant improvement of the herd within a few weeks.

In herds 2 and 3, the contaminated feed components were removed (coarse peanut) and by diluting the corn silage with a high quality feed at a ratio of 1:10, this economically important problem was solved. Based on the diagnosis of ISTME (case 4) hygienic as well as managemental measures in this beef cattle unit lead to improvement. The stall units were cleaned, disinfected and after being empty for 14 days, new animals were housed. As a prophylactic measure the purchased calves were treated with oxytetracycline hydrochloride at a dosage of 25 mg/kg LM/day calves up to a live weight of 300 kg, and no new infections or mortality occurred. ISTME is caused by *H. somnus,* a saprophyte of the respiratory tract mucosa. Predominantly beef cattle of 300 - 400 kg LM are involved. The conditions for a harmless saprophyte to change to an aggressive pathogen are still unknown. It can be presumed that other causes and, in particular, additional stress situations promote the outbreak of this infectious disease. ISTME also belongs to the so-called multifactorial diseases.

In this case predisposing factors such an immunosuppressive effect by the extremely high levels of DON detected (threshold level 1.000 ppb), stress caused by stall rotation and perhaps, weather changes are questioned. The examination of the CSF was very important because an increased pressure and amount are also typical of this disease. In the CSF of patient 1, the total protein and cell count, especially the granulocytes, were increased and characteristic of a CNS bacterial infection. Additionally, a highly increased creatine kinase activity of the CSF was detected, but the interpretation of this finding in terms of CNS-infections in cattle requires further investigation.

The last case caused tremendous financial loss (approximately Aus $600,000,--) to the farmer because of retarded growth of the animals, the extended fattening period, stunting and poor meat quality. After 4 months of feeding, the highly mycotoxic contaminated feed compound was changed to high quality barley and significant improvement occurred. The feed company sold 25 tons of this suspect feed component by declaring it as "healthy and sound" and in normal commercial usage. No compensation for the huge economic damage to the farmer by the feed company was admitted and for that very reason prolonged legal measures against the sales company were initiated.

High moisture maize was treated with propionic acid preservative and stored in a 40,000 bushel steel bin. The maize heated in storage and spoiled and was subsequently treated with the preservative. The poor quality maize was used in the ration for a herd of dairy cows and replacement heifers. The finished feed was cultured for fungi and analysed for mycotoxins. Results obtained were 750,000 Fusarium spp. colonies/g of feed, 1.5 mg zearalenone and 1.0 mg vomitoxin/kg of feed. *Candida, Mucor* and *Penicillium spp* and aflatoxin were also found in the feed. Frequent episodes of behavioural estrus of 2-5 days duration, that were not synchronized with the ovarian cycle, were observed. Cows in the second and third trimester of pregnancy also had episodes of behavioural estrus. Idiopathic vaginitis was diagnosed. Mammary development occurred in the prepubertal heifers. Cows bred in true estrus were found in true estrus 35-55 d later. All of the heifers with precocious mammary development were subsequently culled from the herd because of sterility.

Eighteen primiparous, Holstein cows were used in a 10 week lactation study, preceded by a 2-week covariate period, to determine the effect of deoxynivalenol (vomitoxin) in the diet on cow performance and transfer of deoxynivalenol and its metabolite, deepoxydeoxynivalenol, to the milk. Diets were formulated to contain DON at 0.6 and 12 mg/kg of concentrate DM, and daily intake of DON was 0.59, 42 and 104 mg respectively. Increasing DON in the diet did not affect intake of concentrate or forage. Total milk yield was not affected, however milk fat responded quadratically: cows given DON at 6 ppm of concentrate DM had the lowest milk fat content and fat output. Overall energetic efficiency was not influenced because reduced energy output in milk was compensated by increased body weight gains. No transfer of DON and DOM-1 in milk was observed and concentrations were below detectable limits (1 µg/ml) using HPLC-MS. It was concluded that diets containing DON up to 6 mg/kg of dietary DM did not reduce feed intake of cows in this study and that DON and DOM-1 were not excreted in the milk. Further studies are required to confirm the apparent lack of effect of DON on milk production.

Effects of Rumen Flora on the Metabolism of Mycotoxins

Aflatoxins, ochratoxin A and the fusarium toxins, zearalenone, T-2, DAS and DON are potential risk factors for cattle. The rumenal fluid of ruminants however represents a detoxifying barrier. Examinations show that these mycotoxins are metabolized to significant less toxic substances and protozoa are much more effective than bacteria. OA is metabolized to ochratoxin (and phenylalanine, zearalenone to α-zearalenol and ß-zearalenol, DAS and T-2 to monacetoxyscirpenol (MAS), and HT 2 toxin deacetylated. Such metabolic changes

in the rumen are natural defense mechanisms of ruminants against toxigenic feed-components. Aflatoxin B1 is metabolized in the liver to AM1, and excreted in the milk. In cattle with a completely developed forestomach, the rumenal fluid content represents for certain mycotoxins, such as ochratoxin A, zearalenone, T-2, DAS and deoxynivalenol, a detoxifying barrier and the protozoa are significantly more active than bacteria. DON is metabolized to deepoxymetabolite 1 (DOM 1) and excreted in the urine.

Diagnosis

It is quite difficult to diagnose mycotoxicoses in cattle. First, the case history containing clinical symptoms (indigestion, hemorrhagic diathesis, central nervous disturbances) and the feeding regimens are of utmost importance. Frequently an antibiotic therapy is ineffective, a seasonal occurrence of such intoxications (spring, fall) is observed, storage conditions of the different feed components should be checked and visible mold contaminated feedstuffs are suspicious for mycotoxin production. Other pathogens, such as bacteria, viruses and parasites must be excluded. By detection of the mycotoxins in the feed or residues in tissue samples (liver, kidney) and blood serum, respectively, the diagnosis of mycotoxicosis is confirmed.

Therapy

It should be emphasized that a causal therapy of mycotoxicosis is not available. Based on the clinical symptoms, a symptomatic therapy must be carried out. The following therapeutic measures are recommended:

- Hemorrhagic diathesis: blood transfusion, vitamin C, Ca-gluconicum.
- CNS disturbances: tranquilizers, sedatives, vitamins (B-complex).
- Indigestion: transmission of physiological rumen fluid, ruminantia (propionic acid)
- Application of antihistamines, glucocorticoids, infusion of glucose and change of feed.

Prevention

For preventive measures of mycotoxin contamination of feedstuffs, high moisture grain should be dried immediately after harvest. Proper management during harvest, transportation and storage can decrease mold growth and subsequent mycotoxin production. To preserve high moisture grains from mold invasion, organic acids (propionic acid 0.5% v/v) are used.

Many methods of detoxification 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 feeds.

A survey of fusariotoxin-contaminated feedstuffs in problem herds in 1994 is shown in Tables 1 and 2.

Table 1. Vomitoxin levels (ppb) from feed samples of problematic herds in 1994.

	number	pos.	%pos.	<100	100-1,000	>1.000
maize	246	219	89	6	132	81
corn silage	99	84	85	4	64	16
barley	38	30	79	4	25	1
oats	117	101	86	54	41	6
wheat	8	5	63	0	2	3
bran	7	7	100	1	4	2
soja	19	12	63	3	8	1
mixed feed	30	28	93	2	22	4
pellets	6	6	100	0	3	3
Total	**570**	**492**	**86**	**74**	**301**	**117**

Table 2. Zearalenone levels (ppb) of feed samples from problematic herds in 1994.

sample	number	pos.	%	<100	100-1,000
maize	72	25	35	20	5
corn silage	43	21	49	19	2
barley	36	2	6	2	0
oats	112	7	6	7	0
wheat	6	1	17	1	0
bran	9	0	0	0	0
soja	9	2	22	2	0
mixed feed	28	17	61	16	1
pellets	3	0	0	0	0
Total	**321**	**92**	**29**	**67**	**8**

References

1. Buchner, A, Hotter, H, Schilcher, F, und Baumgartner, W. 1995. Auftreten der infektiösen septikämisch-thrombosierenden Meningoencephalitis (ISTME) in einem niederösterreichischen Maststierbestand. *Wien. Tierärztl. Mschr.* 11: 351-354. 2. Charmley, E, Trenholm, HL, Thomplson, BK, Vudathala, D, Nickolson, JWG, Prelusky, SB and Charmley, LL, 1993. Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. *J. Dairy Sci.* 76: 3580-3587. 3. Copyscock, RW, Mostrom, MS, Sparking, CG, Jacobsen, B and Ross, SC. 1990. Apparent zearalenone intoxication in a dairy herd from feeding spoiled acid-treated corn. *Vet. Hum. Toxicol.* 32: 246-248. 4. Cote. LM and Buck, B 1984. Implications of mycotoxins in the bovine. *Proc. XIIIth World Congress on Diseases of Cattle;* Durban, Republic of South Africa. World Buiatric Assoc. 2: 702-707. 5. Gedek, B 1980. Kompendium der medizinischen Mykologie. Parey, Berlin und Hamburg. 6. Kiesling, KH, Petterson, K, Sandholm, K and Olsen, M. 1984. Metabolism of Aflatoxin, Ochratoxin, Zearalenone, and three Trichothecenes by intact rumen fluid, rumen protozoa and rumen bacteria, *Appl. Environ. Microbiol.* 47: 1070-1073. 7. Marasas, WFO, and Nelson, PE 1987. Mycotoxicology. The Pennsylvania State University Press, University Park and London. 8. Schuh, M 1981. Klinische Auswirkungen der in österreich vorkommenden Mykotoxine. *Wien. Tierärztl. Mschr.* 68: 308-312. 9. Schuh, M 1989. Bedeutung der Mykotoxinaufnahme für Leistung und Gesundheit der Tiere. *Dtsch. Tierärztl. Wschr.* 96: 353-355. 10. Schuh, M and Baumgartner, W. 1988. Mikrobiologisch und mykotoxikologisch kontaminierte Futtermittel als Krankheitsursache bei Rindern. *Wien. Tierärztl. Mschr* 9: 329-332. 11. Wyllie, DT and Morehouse, LG 1978. Mycotoxicosis of domestic and laboratory animals, poultry and aquatic invertebrates and vertebrates. Vol. 2 Dekker, Inc., New York and Basel.