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Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany

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Abstract

A total of 220 samples comprising cereals, cereal byproducts, corn plants and corn silage as well as non-grain based feedstuffs was randomly collected during 2000 and 2001 from sources located in Germany and analysed for 16 *Fusarium* toxins. The trichothecenes scirpentriol (SCIRP), 15-monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), T-2 tetraol, T-2 triol, HT-2 and T-2 toxin (HT-2, T-2), neosolaniol (NEO), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivealenol (15-ADON), nivalenol (NIV) and fusarenon-X (FUS-X) were determined by gas chromatography/mass spectrometry. Zearalenone (ZEA) and α- and β-zearalenol (α- and β-ZOL) were analysed by high performance liquid chromatography with fluorescence and UV-detection. Detection limits ranged between 1 and 19 μg/kg. Out of 125 samples of a group consisting of wheat, oats, corn, corn byproducts, corn plants and corn silage only two wheat samples did not contain any of the toxins analysed. Based on 125 samples the incidences were at 2–11% for DAS, NEO, T-2 Triol, FUS-X, α- and β-ZOL, at 20–22% for SCIRP, MAS, T-2 tetraol and 3-ADON, at 44–74% for HT-2, T-2, 15-ADON, NIV and ZEA, and at 94% for DON. Mean levels of positive samples were between 6 and 758 μg/kg. Out of 95 samples of a group consisting of hay, lupines, peas, soya meal, rapeseed meal and other oilseed meals, 64 samples were toxin negative. DAS, T-2 triol, NEO and FUS-X were not detected in any sample. The incidences of DON and ZEA were at 14 and 23% respectively, those of the other toxins between 1–4%, mean levels of positive samples were between 5 and 95 μg/kg.

Key words: A-type trichothecenes, B-type trichothecenes, cereals, feedstuffs, zearalenol, zearalenone

Abbreviations:3-ADON – 3-acetyldeoxynivalenol; 15-ADON – 15-acetyldeoxynivealenol; DAS – 4, 15-diacetoxyscirpenol; DON – deoxynivalenol; FUS-X – fusarenon-X; HT-2 – HT-2 toxin; MAS – 15-monoacetoxyscirpenol; NEO – neosolaniol; NIV – nivalenol; SCIRP – scirpentriol; T-2 – T-2 toxin; α-ZOL – α-zearalenol; β-ZOL – β-zearalenol; ZEA – zearalenone

Introduction

Fusarium species play an important role as plant pathogens, causing a wide range of diseases in a diversity of host plants such as vascular wilt, pre- and post-emergence blight as well as root and stem rots [1, 2]. Most pathogenic strains occur on a worldwide basis and a variety of species and strains were found to be toxigenic, with a significant diversity of toxins formed under laboratory conditions [3]. Trichothecenes constitute the largest group of *Fusarium* toxins with more than 170 substances isolated. Out of four subgroups characterised by different functional groups within the molecule, the A- and B-type trichothecenes are of major importance [4]. Scirpentriol (SCIRP),

15-monoacetoxyscirpenol (MAS), 4,15-diacetoxyscirpenol (DAS), T-2 and HT-2 toxins (T-2, HT-2), T-2 triol, T-2 tetraol and neosoloaniol (NEO) belong to the subgroup of A-type trichothecenes, deoxynivalenol (DON) and its derivatives 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) as well as nivalenol (NIV) and fusarenone-X (FUS-X) to the subgroup of B-type trichothecenes (Figure 1). Furthermore zearalenone (ZEA) is ranking among the *Fusarium* toxins of major importance [5], derivatives are, amongst others, α- and β-zearalenol (α-, β-ZOL).

The natural occurrence of NIV, T-2, HT-2 and especially of DON and ZEA in grain has been examined worldwide [5–10]. A range of studies dealt with the occurrence of 3- and 15-ADON, FUS-X and DAS [11–18], whereas relatively few reports exist about the occurrence of SCIRP, MAS, T-2 triol, T-2 tetraol, NEO, α- and β-ZOL [6, 11–16, 18–24].

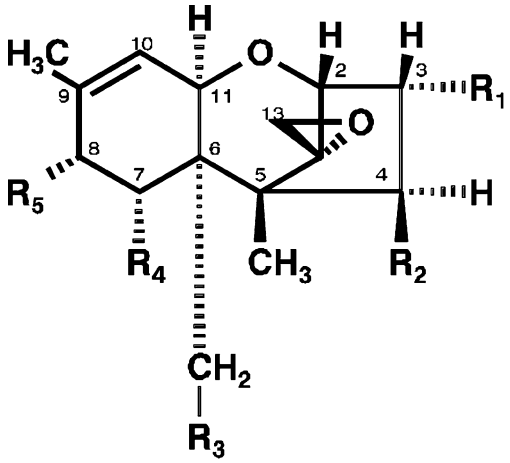
For the assessment of risks posed by *Fusarium* infected plants and plant products to animal and human health it is essential to determine the occurrence of a broad spectrum of toxins. In this context it is of interest that as a class, the scirpenols (DAS, MAS and SCIRP amongst others) appear to be at least as potent as the better-studied T-2 toxin [25], and that α-ZOL shows an at least four times higher estrogenic activity than ZEA [26].

The present study therefore investigated the occurrence of a total of 16 *Fusarium* toxins in a variety of plant derived commodities, attention was payed to the ranking of these toxins based on incidence and level. Commodities analysed included cereals, cereal byproducts, corn plants and corn silage, as well as non-grain based products such as hay, legumes and oilseed by products which serve as feedstuffs or mixed feed components in Germany. The investigation of this latter group was prompted by the fact that *Fusarium* infestation has been described for a broad variety of plants, whereas their exposure and that of outcoming feedstuffs to *Fusarium* toxins and their relative toxicological risk have been studied only to a limited extent [10]. For the present study commodities were selected for which the isolation of toxin producing *Fusarium* species or in some cases the natural occurrence of toxins in diseased plant materials has been described (for literature see the Discussion). Importance was attached to the investigation of a broad spectrum of commodities and to their collection preferably from different sources, in order to contribute to our knowledge of the variability of natural *Fusarium* toxin occurrence.

Materials and methods

Sample materials

A total of 220 samples was taken at random in 2000 and 2001. The following commodities were collected: Wheat (n = 41), oats (n = 17), corn (n = 41), corn byproducts (n = 13) (corn gluten and corn gluten feed (n = 7), screenings (n = 2), bran (n = 2), oil meal (n = 2)), corn whole plants



Trichothecene	R_1	R_2	R_3	R_4	R_5
Scirpentriol	OH	OH	OH	H	H
15-Monoacetoxyscirpenol	OH	OH	OAc	H	H
4, 15-Diacetoxyscirpenol	OH	OAc	OAc	H	H
T-2 Tetraol	OH	OH	OH	H	OH
T-2 Triol	OH	OH	OH	H	$OCOCH_2CH(CH_3)_2$
HT-2 Toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
T-2 Toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
Neosolaniol	OH	OAc	OAc	H	OH
Deoxynivalenol	OH	H	OH	OH	=O
3-Acetyldeoxynivalenol	OAc	H	OH	OH	=O
15-Acetyldeoxynivalenol	OH	H	OAc	OH	=O
Nivalenol	OH	OH	OH	OH	=O
Fusarenon-X	OH	OAc	OH	OH	=O

Figure 1. Chemical structure of trichothecenes investigated.

harvested in wax-ripe stage, prior to ensilage ($n = 8$), whole plant corn silage ($n = 5$), hay ($n = 28$) (first cut ($n = 16$), second cut ($n = 12$)), lupine beans ($n = 9$), pea beans ($n = 25$), soya meal (extracted) ($n = 13$), rapeseed meal (extracted) ($n = 12$), other oilseed byproducts (extracted) ($n = 8$) (sunflower meal ($n = 3$), linseed meal ($n = 3$), palm kernel expeller ($n = 2$)).

Samples were collected by the Governmental Advisory Board from German oil mills (rapeseed meal and soya meal), by staff of store houses and feed mills located in southwest Germany (wheat, oats, corn, corn byproducts, oilseed byproducts) as well as of a commercial corn processor located in Northern Germany using dry-milling procedure (corn, screenings, bran and oil meal). Further samples were collected by members of the University of Hohenheim from farms located in Southwest Germany (oats), and from experimental stations of this university (wheat, oats, corn, corn byproducts, corn plants, corn silage, hay). Sampling was performed either manually or using automatic stream samplers out of the moving stream of a product, or out of static lots to gain bulk samples which were divided manually or automatically. Samples were obtained also from plant breeding firms (peas and lupine) without details about sample collection. These samples were included in the study because they fitted into its screening purpose. All samples were of usual trade quality. Subsamples were milled (particle size about 1.5 mm) and stored at −20 °C prior to analyses. Bulk samples of corn plants and corn silage were dried at 65 °C and milled prior to subsampling. In these two commodities dry matter was determined according to Naumann and Basler [27] and toxin content was based on dry matter. For all other samples toxin content was based on original sample.

Toxin analyses

All standard substances were bought at Sigma (Deisenhofen, Germany). For trichothecenes analysis was carried out as described in detail previously by Schollenberger et al. [28, 29]. In brief, extraction was performed with a mixture of acetonitrile and water followed by liquid/liquid extraction with hexane. Clean up was carried out by solid phase extraction using a florisil and a cation exchange cartridge. Derivatisation was with trifluoroacetic anhydride, verrucarol was used to control derivatisation efficiency. Separation and quantitation was by GC-MS using a Magnum Ion Trap system in the chemical ionisation mode with isobutane as reactant gas. Detection limits were assessed at a signal to noise ratio of 3:1 and were 8, 3, 14, 6, 5, 7, 3, 4, 7, 7, 9, 14 and 19 μg/kg for SCIRP, MAS, DAS, NEO, T-2 triol, T-2 tetraol, HT-2, T-2, DON, 15-ADON, 3-ADON, NIV and FUS-X respectively. Quantitation limits were at a signal to noise ratio of 6:1. Toxin contents between detection and quantitation limit were calculated as the average.

Repeatability and recovery were determined using different matrices and spiking level of 200 μg/kg. Mean recovery rates for corn, oats and sunflower seed were between 72 and 115% as reported elsewhere [29]. For soybean and hay mean recovery rates were between 68 and 111%, standard deviations of the trichothecenes varied between 2 and 19% ($n = 4$).

To avoid false positive results due to carry over out of the preceeding sample during measurement, samples were injected twice in reverse order. Preferably samples of one commodity were measured within one series, grain based samples and non-grain based samples were not measured within the same series. To control results, selected samples were derivatised twice on different days, selected samples were completely analysed twice.

Determination of ZEA, α- and β-ZOL was carried out as described previously by Schollenberger et al. [17] slightly modified. In brief, after extraction with a mixture of acetonitrile and water, phosphate buffered saline was applied to the mixture. Samples other than grain kernels were cooled at 4 °C overnight to precipitate interfering matrix components. After filtration sample clean-up was carried out using an immunoaffinity column. For silage the clean-up of the immunoaffinity column was disturbed by matrix components. Therefore the sample size was diminished from 25 to 2.5 g to improve recovery rates. This measure additionally facilitated extraction step as silage material adsorbed extraction solvent to a high degree, thus strongly diminishing volume of extract obtained. Identification and quantification of ZEA , α- and β-ZOL was carried out by HPLC. Reversed-phase chromatography was used with two solvents as mobile phases, water-acetonitrile (52 + 48, v/v)

(A) and acetonitrile (B) in gradient mode with a flow rate of 1.5 ml per minute. The gradient run conditions were as follows: 0–8 min 100% A, 8 to 9 min 0 to 80% B, 8 to 11 min 80% B, 11–12 minutes from 80 to 0% B. Fluorescence detection was performed at 235 nm (excitation) and 450 nm (emission). Additionally UV detection at 280 nm or diode array detection respectively was used to control toxin identity for toxin levels exceeding 5 μg/kg. For fluorescence detection limits at a signal to noise ratio of 3:1 were at 1, 1 and 8 μg/kg for ZEA, α-ZOL and β-ZOL respectively. For silage these values were 10, 10 and 80 μg/kg respectively. For whole plants of corn as well as for corn bran α- and β-ZOL were not quantifiable because of matrix interference. Repeatability and recovery were determined using different matrices and spiking level of 10 μg/kg for ZEA and α-ZOL, 20 μg/kg for β-ZOL. Mean recovery rates for corn, oats and sunflower seed were between 43 and 101% as reported elsewhere [29]. For soybean and hay mean recovery rates ranged between 62 and 106% respectively, standard deviations ($n = 4$) were between 5 and 13%. For silage spiking levels of 100 μg/kg for ZEA and α-ZOL, 200 μg/kg for β-ZOL in combination with a sample size of 2.5 g were used, recovery rates were at 88, 96 and 93% for ZEA, α-ZOL and β-ZOL respectively standard deviations were at 6, 7 and 7% respectively.

Results for both groups of toxins investigated were not corrected for recovery.

Results

Cereals and cereal byproducts, corn plants and corn silage

Out of a total of 125 samples of a group consisting of wheat, oats, corn, corn byproducts, corn plants and corn silage (Table 1), 123 samples were positive for at least one of the toxins analysed, with two samples of wheat being toxin negative. The positive samples contained up to 13 toxins, with an average number of 5 co-occurring toxins. Marked differences existed between the incidence and in part also level of single toxins or toxin groups between commodities (Table 1). Nine, nine and eleven different toxins were detected in wheat, corn silage and corn plants, respectively, twelve toxins in oats, 13 toxins in corn kernels, and a maximum of 16 toxins in corn byproducts.

In the total of 125 samples DON was predominant for all commodities with the exception of oats, with an incidence at 71–100%, a mean content at 170–2919 μg/kg and a maximum content at 720–6682 μg/kg. Based on 125 samples the corresponding values were 94%, 758 and 6682 μg/kg.

On the other side, a low degree of contamination was found for DAS, NEO, T-2 triol, FUS-X, α-and β-ZOL. These toxins were detected in two or three out of six commodities, at incidences per commodity at 2–35% and mean as well as maximum levels of 3–494 μg/kg based on 125 samples. A somewhat higher degree of contamination was found for SCIRP, MAS, T-2 tetraol and 3-ADON. These toxins were detected in five or six commodities, at incidences per commodity at 2–94%, mean contents at 6–301 μg/kg and maximum contents at 6–916 μg/kg based on 125 samples.

The toxins HT-2, T-2, 15-ADON, NIV and ZEA ranked between this second group and DON. Their incidences per commoditiy were at 5–100%, mean contents at 6–1612 μg/kg, maximum contents at 6–6640 μg/kg based on 125 samples (see Table 1).

Non-grain based feedstuffs

Out of a total of 95 samples of non-grain based feedstuffs (Table 2) 64 samples were toxin negative. These were samples of hay, lupines, peas, soya meal, rapeseed meal, sunflower meal, linseed meal. No toxin was detected in peas, two toxins were found in rapeseed meal, three toxins in other oilseed byproducts, four toxins in hay and lupine, eight toxins in soya meal (Table 2). The number of samples positive for at least one toxin varied between commodities. It was at 50, 22, 0, 85, 8 and 38% for hay, lupine, peas, soya meal, rapeseed meal and other oilseed byproducts.

Based on incidence and level DON and ZEA ranked at about the same position. DON was detected in four, ZEA in three commodities, the incidence per commodity was at 8–54% and 13–69%, the maximum content at 42–237 μg/kg and 4–211 μg/kg; respectively (Table 2).

The other toxins mostly were found in one or two commodities. Based on 95 samples, the incidence was at 1% and 3% for SCIRP and MAS, at 3, 1 and 1% for HT-2 and T-2 and T-2

Table 1. Fusarium toxins in cereals and cereal byproducts, corn plants and corn silage

	SCIRP	MAS	DAS	NEO	T-2 triol	T-2 tetraol	HT-2	T-2	DON	3-ADON	15-ADON	NIV	FUS-X	ZEA	α-ZOL	β-ZOL
Wheat (n = 41)																
Incidence (%)[a]	5	2	0	0	0	5	54	5	95	2	7	20	0	63	0	0
Mean (μg/kg)[b]	17	6				38	9	6	309	24	11	33		15		
Max (μg/kg)[b]	22	6			62	61	6	1810	24	11	68		77			
Oats (n = 17)																
Incidence (%)	53	29	0	18	35	94	100	100	71	18	0	71	6	24	0	0
Mean (μg/kg)	48	11		18	19	150	181	73	170	32		155	62	21		
Max (μg/kg)	161	27		28	41	577	494	310	720	51	900	62	48			
Corn (n = 41)																
Incidence (%)	15	22	5	2	0	2	76	51	100	39	100	61	22	85	0	0
Mean (μg/kg)	45	24	49	9		13	21	16	849	66	160	291	62	48		
Max (μg/kg)	97	51	76	9		13	68	108	3820	322	680	1388	211	860		
Corn byproducts (n = 13)																
Incidence (%)	15	15	8	8	8	23	85	69	100	46	85	54	31	92	23	8
Mean (μg/kg)	31	31	21	9	8	39	55	29	1626	44	496	694	195	369	3	17
Max (μg/kg)	38	39	21	9	8	56	99	70	6682	114	1780	2050	494	1362	3	17
Corn plants (n = 8)																
Incidence (%)	75	75	0	0	13	38	88	75	100	13	100	100	0	100	n.q.	n.q.
Mean (μg/kg)	207	29			76	301	233	70	598	57	166	1312		159		
Max (μg/kg)	916	85			76	790	1469	363	818	57	550	6640		553		
Corn silage (n = 5)																
Incidence (%)	20	60	0	0	0	0	100	0	100	0	100	100	0	100	20	20
Mean (μg/kg)	124	30					18		2919		59	1612		432	15	116
Max (μg/kg)	124	49					26		3944		127	2809		1790	15	116

[a] Percentage of positive samples, based on the number of samples per commodity.
[b] Mean and maximum toxin content of positive samples (μg/kg).

Table 2. Fusarium toxins in non-cereal feedstuffs

	SCIRP	MAS	DAS	NEO	T-2 triol	T-2 tetraol	HT-2	T-2	DON	3-ADON	15-ADON	NIV	FUS	ZEA	α-ZOL	β-ZOL
Hay (n = 28)																
Samples positive	0	0	0	0	0	0	0	0	4	1	0	2	0	12	0	0
Mean (μg/kg)[a]									41	20		131		24		
Max (μg/kg)[a]									69	20		222		115		
Lupines (n = 9)																
Samples positive	0	2	0	0	0	0	1	1	0	0	0	1	0	0	0	0
Mean (μg/kg)		5					5	6				23				
Max (μg/kg)		5					5	6				23				
Peas (n = 25)																
Samples positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Soya meal (n = 13)																
Samples positive	1	1	0	0	0	1	1	0	7	0	0	0	0	9	4	2
Mean (μg/kg)	21	5				21	5		64					51	12	7
Max (μg/kg)	21	5				21	5		237					211	25	11
Rapeseed meal (n = 12)																
Samples positive	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Mean (μg/kg)									144		47					
Max (μg/kg)									144		47					
Other oilseed byproducts[b] (n = 8)																
Samples positive	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
Mean (μg/kg)							5		42					4		
Max (μg/kg)							5		42					4		

[a] Mean and maximum toxin content of positive samples.
[b] Sunflower meal, linseed meal, palmkernel expeller.
n.q. not quantifiable.

tetraol, at 14, 1, 1 and 3% for DON, 3-ADON, 15-ADON and NIV and at 23, 4 and 2% for ZEA, α-ZOL and β-ZOL.

SCIRP or its derivative MAS were detected in soya meal and lupine, T-2 or at least one of its derivatives; HT-2, T-2 triol and T-2 tetraol in lupine, soya meal and sunflower meal, DON in hay, soya meal, rapeseed meal and linseed meal, 3-ADON in hay, 15-ADON in rapeseed meal, NIV in lupine and hay, ZEA and or its derivatives α- and β-ZOL in soya meal, palm kernel expeller and hay.

Some difference was found between hay samples of the first and second cut which all were collected from an experimental station of the University of Hohenheim. DON, 3-ADON and ZEA were found in 1, 1 and 2 samples out of 16 first cut, NIV, DON and ZEA in 2, 3 and 10 out of 12 samples of the second cut, respectively.

Discussion

Cereals and cereal byproducts, corn plants and corn silage

Data reported in the literature regarding the occurrence of so far rarely studied A-type tri-

Table 3. Literature data about some trichothecenes in cereals and cereal byproducts[a]

Toxin	Commodity	Country	*n*[b]	%[c]	DL[d]	QL[e]	Range[f]	Ref.
SCIRP	Wheat	Norway	169	0.6	< 20			[19]
SCIRP	Wheat	Poland	248	1	10		10–30	[21]
SCIRP	Oats	Norway	178	3.4	< 20		–49	[19]
SCIRP	Oats	Poland	99	8	10		10–43	[21]
SCIRP	Cereals	Lithuania	159	–	< 20		–30	[30]
MAS	Wheat, oats	Norway	347	0.3	< 20		traces	[19]
MAS	Cereals	Lithuania	159	–	< 20		–27	[30]
MAS	Wheat	France	52	0	30	60		[31]
MAS	Corn	France	54	0	30	60		[31]
MAS	Corn products	U.K.	27	22	10		10–20	[16]
MAS	Corn gluten	U.K.	40	2.5	10		10	[16]
DAS	Wheat	Germany	721	0	3			[11, 14]
DAS	Oats	Germany	395	0	3			[13],unpubl.
DAS	Oats	Poland	99	12	10		10–118	[32]
DAS	Wheat, oats	Norway	347	0.3	< 20		traces	[19]
DAS	Cereals	Lithuania	159	0.5	< 20		< 5	[30]
DAS	Wheat	Finland	134	0	5–25			[31]
DAS	Oats	Finland	42	0			5–25	[31]
DAS	Wheat	France	52	0		20		[31]
DAS	Corn	France	54	0		20–30		[31]
DAS	Corn products	U.K.[g]	70	0		10		[16]
DAS	Wheat products	U.K.	95	0		10		[16]
DAS	Corn products	U.K	27	0	10			[16]
DAS	Corn gluten	U.K.	40	5	10			[16]
T-2 triol	Wheat	France	225	0	30	60		[31]
T-2 triol	Corn	France	25	0	40			[31]
T-2 triol	Corn products	U.K.	70	0		10		[31]
T-2 triol	Wheat products	U.K.	95	0		10		[31]
NEO	Wheat	France	225	0				[31]
NEO	Corn	France	25	0				[31]
NEO	Corn products	U.K.	70	0		10		[31]
NEO	Wheat products	U.K.	95	1		10	11	[31]

[a] Only reports based on methods with a detection or quantification limit ≤ 40 μg/kg were included.
[b] Total number of samples analysed.
[c] Percentage of positive samples.
[d] Detection limit (μg/kg).
[e] Quantification limit(μg/kg).
[f] Range of toxin contents detected (μg/kg).
[g] United Kingdom.

chothecenes in cereals and cereal byproducts are summarised in Table 3. Only those data were considered which were based on methods with a detection or quantification limit comparable to that of the present study.

According to Table 3 SCIRP and MAS were found at an incidence of 0.6–8% and 0–3.4%, respectively, in wheat, oats, cereals and corn from Norway [19], Poland [21], Lithuania [30] and France [31], with contents up to 49 μg/kg. In contrast, in the present study the incidence of SCIRP and MAS in wheat, oats and corn was at 5–53% and 2–29%, with a maximum content up to 161 μg/kg and 51 μg/kg, respectively (Table 1). DAS was not or at low incidence and level detected in small cereals, corn, and corn byproducts of European origin. A total of 2235 samples did not contain this toxin, including 721 wheat and 395 oats samples collected in southwest Germany [11–14]. It was only in oats from Poland [32], that this toxin was detected at a somewhat higher incidence of 10% and a level up to 118 μg/kg. Langseth and Rundberget [19] suggested a very limited production of acetoxyscirpenols in grain in Norway contaminated under natural conditions and a main occurrence of the deacetylated form, scirpentriol. This is consistent with the results of the present study concerning DAS, for the occurrence of SCIRP and MAS in cereals and cereal byproducts however results of both studies are not in accordance to each other.

A rare occurrence was also described for T-2 triol and NEO [31]. For these two toxins the findings agree with the results of the present study (Table 1).

Overall, the present results together with literature data suggest that DAS, T-2 triol and NEO are of minor importance in grain-based feeds. The occurrence of SCIRP and MAS may vary due to amongst others the geographic origin of samples. For wheat, oats, corn, corn byproducts as well as for corn plants and corn silage analysed in the present study SCIRP, MAS and T-2 tetraol ranked between DAS, T-2 triol and NEO on the one and T-2 and HT-2 on the other side (Table 1). Data about the occurrence of T-2 tetraol analysed with sensitive methods are very scarce in literature.

It is of interest that DAS, T-2 triol and NEO as well as SCIRP, MAS and T-2 tetraol were not or rarely found in cereal-based foods and other foods of plant origin, with levels not above 35 μg/kg [29].

Low incidences and levels of α- and β-ZOL were found in grain material in the present study (Table 1) are consistent with findings in the literature: Both toxins were detected only in seven and one out of a total of 721 wheat samples, respectively, and in none of 388 barley and 395 oat samples collected during six or five years in southwest Germany, although the detection limit was at 1 μg/kg for α-ZOL and 5 μg/kg for β-ZOL [11–14]. Furthermore both toxins were not found in any of 60 samples of wheat flour [18] 64 cereal-based foodstuffs and 85 vegetables and fruits [29]. Oldenburg et al. [33] detected α-ZOL, β-ZOL and ZEA in corn silage grown in northern Germany at a mean content of 20, 30 and 390 μg/kg, respectively.

Relatively high incidences and levels of the trichothecenes analysed were found in corn byproducts (Table 1). This is consistent with findings of Scudamore et al. [16] who reported high trichothecene levels in corn screenings, bran and germ among others of the British market. In that study the toxins DON, 3- and 15-ADON, NIV, FUS-X, DAS, MAS, HT-2 and T-2 were detected in at least one out of 40 samples of corn gluten [16] and except DAS also in samples of corn screen.

In Germany whole plant corn silage is a popular feedstuff for cattle and is prepared out of whole plant of corn harvested in the middle or end of wax-ripe stage [34]. The contamination of the basic raw material as well as of the silage with DON and ZEA (Table 1) is consistent with findings in the literature as reviewed by Oldenburg et al. [33]. In the present study corn plant material contained not only DON and ZEA but also NIV, 3- and 15-ADON, SCIRP, MAS, T-2 triol, T-2 tetraol, HT-2 and T-2 (Table 1). The distribution of DON in whole plant material was investigated by Oldenburg et al. [35]. Prior to harvest (wax-ripe state) DON was preferably located in rudimentary, unfertilised cobs placed below the mature cob of a plant, stem and mature cob did not significantly contribute to entire contamination. The detection of different *Fusarium* toxins in silage indicates an at least partial stability of these substances during fermentation. This is in accordance to Lepom et al. [36] who reported that the DON content of corn silage did not decrease during production of silage.

Non-grain based feedstuffs

With regard to hay, Engels and Krämer [37] found fusaria in 41–100% of freshly harvested grass samples (*Lolium perenne, L. multiflorum*) and isolated a variety of toxigenic strains from these samples. These authors found ZEA in grass at comparable incidence but markedly higher content than determined in hay in the present study. They detected also T-2 and DAS in 25% and 21.6% of samples using ELISA technique whereas these toxins were not found by us. B-type trichothecenes were not investigated in that study. These results and other reports cited by Engels and Krämer [37] suggest that grass in Germany is one of the feedstuffs with the highest incidence of fusaria and may be heavily contaminated with their toxins. The lower contamination of hay in the present study may have resulted from the grass varieties involved [37] and/or the date of harvest. Thus the higher incidence of *Fusarium* toxins in second cut compared to first cut hay observed may have been due to differences in the composition of the basing plant material as well as in date of harvest. Concerning this point no consistent trend was reported by Engels and Krämer [37]. These authors reported that during one year the ZEA content of grass increased from the first to the fourth cut, whereas during the following year the trend was inverse.

Out of the non-cereal based commodities analysed, soya meal was positive for the highest number of toxins (Table 2). *Fusarium* rot of soybeans has been described and a variety of *Fusarium* species have been isolated from this source [2, 38, 39]. In vitro formation of ZEA, T-2, T-2 tetraol, HT-2 and NEO by *Fusarium* species with soybeans as substrate has been reported [40], as well as the natural occurrence of T-2, DON, NIV and ZEA in soybeans [8–9, 41–44]. *Fusarium* toxins may be formed in soybeans not only in the field, amongst others, at favourable conditions such as wet weather [41], but also after harvest. T-2, DON and NIV were detected in soybean samples sent in by Hungarian feed mills at a mean content of 249, 253, and 259 μg/kg, respectively [44]. In contrast, in the present study T-2 and NIV were not detected in soya meal samples and their mean DON content was at 64 μg/kg (Table 2). This indicates a relative good mycological quality of these samples regarding their *Fusarium* infestation and toxin content.

With regard to the occurrence of *Fusarium* toxins in non-grain based commodities NIV, DON and T-2 were detected in sunflower seed for feed use [44], HT-2, T-2 and ZEA were found in sunflower seed for food use [29]. *Fusarium* strains were reported to infect oilseed rape [45], linseed [46], oil palm [47] and lupine [48] and different legumes [1]. Though *Fusarium* strains were isolated from different sorts of peas by several authors [1–3, 10] none of the toxins investigated were found in 25 pea samples in the present study.

It is well known that fungal growth and the ability to produce mycotoxins is greatly influenced by the complex interaction of several factors such as aggressiveness of *Fusarium* species [49], host susceptibility [50], climatic factors [51] as well as edaphic and agrotechnical factors [50, 52]. Furthermore *Fusarium* species differ in the spectrum of toxins produced and also within one species different toxigenic potential exists [2, 3]. Several different *Fusarium* species often can be found in one commodity [2]. Substrate influence was reported in the literature [53, 54] and may also contribute to differences in toxin contamination of commodities. Thus Castillo et al. [54] isolated *Fusarium* species from beans from Argentina and investigated these strains for their ability to biosynthesise trichothecenes and zearalenone either on rice grains or beans. These mycotoxigenic species produced several toxins when grown on rice but none or little amount when cultured on beans. Besides the limited number of samples analysed differences between results of the present study and literature data may be attributed to a variety of these factors.

The present study suggest a stronger contamination with *Fusarium* toxins of grain-based compared to non-grain based feedstuffs in Germany. An expanded pool of occurrence data will be a precondition for complete evaluation of this question.

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