



Evaluation of mycotoxin-contaminated cereals for their use in animal feeds in Hungary

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(Received 18 June 1999; revised 21 February 2000; accepted 21 March 2000)

In the period between December 5, 1991 and September 17, 1998, 760 maize, 367 wheat, 119 soybean, 222 barley, 85 bran, 32 triticale, 60 oat, 14 rye and 22 sunflower samples were investigated for the presence and concentration of seven fusariotoxins (T-2 toxin, zearalenone, deoxynivalenol, nivalenol, diacetoxysciperol, HT-2 toxin, fusarenone-X) and OTA. The comparison of analytical data with those of the relevant literature revealed that although the incidence rate and/or concentration of Fusarium mycotoxins and OTA in Hungarian-grown cereals is occasionally considerable, the position of the country is not worse than the average of countries. Our findings indicate that soybean tends to be good substrate for trichothecene-producing fungi and the rate of contamination is regarded as substantial. The commodities were assorted into one of three quality categories. The proportion of objectionable samples was only 3.0, 2.2, 2.3 and 1.7% in maize, wheat, barley and soybean samples, respectively. However, this low rate of objection might still be a source of great economic loss. The proportion of objectionable samples was much higher in the case of bran, oat and triticale (7.1, 6.7, and 6.3%, respectively). The results of the present investigation indicate a need for regular screening for mycotoxins of importance and individual appraisal of each commodity from the point of their use in animal feeds.

Keywords: Fusarium toxins, advisory mycotoxin level, zearalenone, T-2 toxin, deoxynivalenol, nivalenol,

fusarenone-X, HT-2 toxin, T-2 toxin, diacetoxysciperol, ochratoxin A, grain, wheat, barley, maize, corn, rye, oat, bran, soybean, sunflower

Introduction

The multiple hazardous effects of mycotoxins on the health and production of livestock and poultry and their negative effects in the food chain have attracted increasing public concern for a long time. Many papers have been published on the occurrence of various mycotoxins in cereals and other commodities most of which, however, have been restricted to reporting the natural occurrence of different mycotoxins on variety of commodities. Detailed description of the analytical methods, major contaminants, averages, incidence rates and, sometimes, the upper and lower limit values have been reported. Excellent reviews have appeared on this subject (Jelinek *et al.* 1989, Pohland 1993, Patel *et al.* 1996, Pittet 1998).

Few, if any, attempts have been made to analyse the natural mycotoxin contamination of grains and other feed ingredients in respect of their suitability for processing into animal feeds.

Besides individual and herd-level sensitivity, the effects of mycotoxins depend on their dietary concentration, composition of contaminants and length of exposure. Sensitivity and duration of exposure are independent variables that at present cannot be included in risk assessment for animals.

Dietary concentration of mycotoxins and their composition are, however, measurable parameters. Therefore, risk assessment should be based on the maximum tolerable level of mycotoxins established for grains, other feed components and feed mixtures of livestock and poultry on the one hand, and, due to frequent co-contamination of grains with variety of mycotoxins, on the individual appraisal of grains and other commodities, on the other.

Most countries regulate the maximum tolerable levels of mycotoxins in food by law or codex standards for the maximum protection of consumers. Apart from

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aflatoxins, and in few countries ochratoxin A (OTA), such regulations are not existent for feeds or feed ingredients. Instead, advisory limit concentrations or guidelines have been established by relevant scientific bodies and applied in several countries.

For diagnostic and veterinary reasons the term of critical concentration has also been introduced (Meyer *et al.* 1993) for well-studied mycotoxins of frequent occurrence. The term embraces two levels of concentration. The higher one establishes clinical symptoms and pathological findings characteristic for the mycotoxin. The lower concentration fails to provoke recognizable symptoms but leads to a decline in production.

In Hungary the maximum tolerable level of aflatoxin B₁ and OTA in feed ingredients and feed mixtures is regulated by law (Anon. 1996). In respect of mycotoxins other than aflatoxin B₁ and OTA the Hungarian Feed Codex (Anon. 1990) sets advisory limit values, which are more or less equivalent with the aforementioned lower critical concentration.

In the continental climate of Hungary no home-grown cereals are contaminated with aflatoxins. Health and production of farm animals in this country is primarily affected by the secondary metabolites of the *Fusarium* species (trichothecenes, ZEA and fumonisins) and some *Penicillium* and *Aspergillus* species producing OTA. Although more than 30 trichothecenes are known, only a few (T-2 toxin, HT-2 toxin, DON, nivalenol [NIV], diacetoxyscirpenol [DAS], fusarenone-X) play a role in Hungarian animal production along with ZEA and OTA. These mycotoxins may contaminate the grains alone or in combination. It follows that reliable risk assessment requires simultaneous determination of mycotoxins of importance and each set of analytical data needs individual appraisal.

In the period between December 5, 1991 and September 17, 1998, 760 maize, 367 wheat, 119 soybean, 222 barley, 85 bran, 32 triticale, 60 oat, 14 rye and 22 sunflower samples were investigated for the presence and concentration of seven fusariotoxins (T-2 toxin, ZEA, DON, NIV, DAS, HT-2 toxin, fusarenone-X) and OTA.

Chemical analysis data were used for classification of each sample into one of three categories: A, B and C. Each of these categories stands for the quality of the commodity with respect to its further use as a feed ingredient (see later).

This paper reports the main findings of mycotoxin analysis, attempts to compare the data with those of the relevant literature and discloses the proportion of cereals and other feed ingredients allocated into one of the quality classes.

Materials and methods

HPLC and capillary gas chromatography (Bata *et al.* 1983, 1984a, b, 1986) have been used for regular analysis of grains and other commodities for the presence and concentration of the aforementioned seven fusariotoxins and OTA. Although the sensitivity of the method would have made it possible to measure trace concentrations, no data were recorded in the range of 1–50 µg/kg because we considered trace concentrations biologically meaningless. This, however, made the data somewhat biased, viz. the incidence rate of contamination is smaller, the average values, at the same time, are higher than if trace concentrations had been included.

Grains and other feed ingredients were sent in for analysis by major feed mills after harvest and during storage. Sampling of grain lots was performed by the employees of the feed mills according to Hungarian Standard No. 6962–84 (Anon. 1984). The results of the analysis were used by the feed mills at formulation of their feed mixtures.

On the basis of the advisory limit values set by the Hungarian Feed Codex (Anon. 1990) for mycotoxins (table 1) and also on our previous experiments and experiences, the mycotoxin contamination of cereals and other commodities was individually assessed with respect to the risk they pose to livestock when the grain is incorporated into animal feeds and all commodities were assorted into one of the following groups.

Group A: These samples are either free from the eight mycotoxins we examined or may be contaminated, but both compositions of mycotoxins and their peak concentration are considered harmless. Therefore, the lot of the commodity represented by the sample can be used unconditionally for processing into feed mixtures or direct feeding for all farm animals (examples: (a), in the case of 0 contamination the commodity may be fed to all species even in its pure form; (b), wheat with

Table 1. Advisory limit values of mycotoxin contamination in feeds (Anon. 1990).

Mycotoxin/commodity	Limit value (mg/kg)
Deoxynivalenol (DON)^a	
Compound feeds of pigs, geese, ducks, turkeys and pets	0.4
Compound feeds of not milking ruminants and poultry (laying and broiler) feeds	2.0
Zearalenone (ZEA)	
Feeds of non breeding ruminants and compound feeds of broilers, turkeys, geese, ducks, laying hens, other birds and fattening pigs	0.5
Feeds of breeding animals and replacements (cattle, sheep, pig, turkey, guinea fowl, goose and duck)	0.08
T-2 toxin, HT-2 toxin, DAS, NIV^b	
Feeds of adult ruminants	2.0
but over 5 kg daily intake	1.0
Feeds of broilers	0.5
Feeds of layers and fattening pigs	0.3
OTA	
Feeds of layers	0.01
Feeds of adult ruminants	0.1
Other compound feeds	0.025

^a Cereals contaminated with DON at and higher than 4 mg/kg concentration can only be used in feeds of ruminants. The resultant concentration of DON in the compound feed cannot exceed 0.4 mg/kg.

^b Mode of action and toxicity of T-2 toxin, HT-2 toxin, DAS and NIV is very similar, therefore concentrations should be summed.

0.14 mg/kg ZEA and 0.23 DON may be incorporated into feed mixtures at the usual proportion, etc.).

Group B: In this case the sample contains one or more mycotoxins at different concentrations. However, the grain may be used for compounding with certain care (e.g. use in decreased proportion, dilution with non-contaminated cereals, incorporated into feeds of animals that are not sensitive to the mycotoxins in question, etc.). The member of group B can be used conditionally. (Examples: (a), maize of 0.27 mg/kg OTA: the commodity might be used for compounding feeds only for grown up ruminants up to maximum 30 % proportion; (b), maize with 0.3 mg/kg DAS: the commodity can be used for compounding feed mixtures, however feeding in its grain form is not suggested).

Group C: Due to the extreme contamination, these commodities cannot be diluted as much with non-contaminated grains as to reduce the resultant mycotoxin concentration of the compounded feed below the critical (advisory) level. This may be valid either for all feed mixtures (e.g. mostly in case of massive contamination with OTA) or relevant for feeds of breeding animals (e.g. in the case of massive contamination with ZEA) or young, sensitive animals (e.g. high peak concentration of mycotoxins). This group, therefore, should be excluded from formulation of all feeds or from feeds of special sectors of livestock and poultry (e.g. breeding or young animals). (Examples: (a), maize with 1.9 mg/kg OTA is not suitable for further processing; (b), wheat or bran of 0.31 and 0.57 mg/kg ZEA, respectively: it should be excluded from formulating feed mixtures for all breeding animals and breeding replacements).

Discussion

In the first part of this discussion data from the present investigation (tables 2 and 3) are compared with those reported from other countries (table 4) in terms of the rate of incidence and concentration and in the second part the quality categories are discussed.

Maize

Hungary is situated in the Corn Belt, therefore maize is the most important component of feed mixtures of pigs and poultry and its quality has decisive importance in respect of animal production. Data from the present investigation indicated that somewhat more than 40 % of maize samples were free from the mycotoxins included in the analysis. The average incidence of trichothecene, ZEA and OTA contamination was 41.3, 18.4 and 9.3 %, respectively, in the 760 corn samples. Both average concentration and incidence of DON, NIV and ZEA in the Hungarian corn samples were less than the respective values reported by Tanaka *et al.* (1988), Scott (1997) and Scudamore *et al.* (1998). In French (Bakan *et al.* 1998) and Korean (Ryu *et al.* 1996) corn samples DON was the prevalent toxin while the incidence rate of T-2 toxin was extremely low (4 and 0 %, respectively), in

Table 2. Contamination of cereals and grains with trichothecenes (TR).

Commodity	Item	T-2	DON	NIV	All TR
Maize (n = 760)	Average, µg/kg ± SD	225.2 ± 125.1	191.0 ± 79.4	186.7 ± 12.2	259.5 ± 173.0
	Rate of incidence, %	28.2	10.8	0.4	41.3
	Range, µg/kg	50-980	50-870	130-260	50-1080
Wheat (n = 367)	Average, µg/kg ± SD	193.8 ± 52.7	298.9 ± 212.6	177.3 ± 62.4	328.6 ± 240.6
	Rate of incidence, %	6.5	78.2	9.0	82.0
	Range, µg/kg	80-370	70-1560	50-590	60-1560
Barley (n = 222)	Average, µg/kg ± SD	220.0 ± 59.3	268.0 ± 198.0	136.4 ± 40.7	287.1 ± 165.1
	Rate of incidence, %	7.2	72.5	6.3	76.6
	Range, µg/kg	50-310	50-1200	60-340	60-1200
Oat (n = 45)	Average, µg/kg ± SD	292.5 ± 85.3	188.6 ± 85.6	110.0 ± 55.4	274.8 ± 209.6
	Rate of incidence, %	8.9	62.2	13.3	64.4
	Range, µg/kg	120-360	50-870	50-180	50-870
Triticale (n = 32)	Average, µg/kg ± SD	160.0 ± 282.8	256.5 ± 149.5	100.0 ± 0	276.8 ± 161.6
	Rate of incidence, %	3.1	81.3	3.1	87.5
	Range, µg/kg	50-160	50-580	100-100	60-620
Rye (n = 14)	Average, µg/kg ± SD	150.0 ± 40.1	313.0 ± 175.9	120.0 ± 31.1	340.0 ± 199.0
	Rate of incidence, %	7.1	71.4	7.1	71.4
	Range, µg/kg	150-150	120-490	120-120	180-550
Bran (n = 85)	Average, µg/kg ± SD	234.7 ± 133.1	292.9 ± 193.5	137.3 ± 47.3	391.6 ± 279.1
	Rate of incidence, %	17.6	91.8	12.9	91.8
	Range, µg/kg	50-950	50-960	60-180	60-1230
Soybean (n = 119)	Average, µg/kg ± SD	249.1 ± 107.7	252.7 ± 168.1	258.7 ± 161.1	438.3 ± 276.0
	Rate of incidence, %	18.5	65.5	44.5	79.8
	Range, µg/kg	50-450	60-720	50-680	60-1090
Sunflower (n = 22)	Average, µg/kg ± SD	236.7 ± 83.2	150.0 ± 32.0	0.0 ± 0.0	292.5 ± 121.4
	Rate of incidence, %	13.6	4.5	0.0	18.2
	Range, µg/kg	230-250	150-150	0-0	150-370

contrast to our findings. Both the ZEA and OTA contents of our corn samples take an intermediate position among the data reported (table 4).

Of the trichothecenes DON was the dominant mycotoxin in the rest of the commodities we examined. This is in agreement with most reports, which indicate that DON is the mycotoxin most often detected in grain species (Chelkowski 1989, Langseth *et al.* 1995).

Wheat

Only 12.5 % of the samples we tested was found to be free of the eight mycotoxins. Rates of contamination with DON and OTA were similar and inferior, respectively, to those found in other countries (table 4). The ZEA contamination of wheat samples in the

present investigation was comparable to that found in Bulgaria (Vrabceva *et al.* 1996), but higher than reported from Germany (Müller *et al.* 1997a) and Switzerland (Bucheli *et al.* 1996, Noser *et al.* 1996).

Barley

The average incidence rate and concentration of DON were similar to those found in other countries (table 4). ZEA was present in 22 % of the 240 barley samples tested in Germany (Müller *et al.* 1997b) with a range of 2-311 µg/kg. Scott (1997) found considerably less contamination with ZEA in the Canadian barley (1 %, range: 4-21 µg/kg) in contrast with the present investigation. Only six (2.7 %) of our 222 barley samples contained OTA, which is considerably less than was found Germany and the UK (table 4).

Table 3. Contamination of cereals and grains with zearalenone and ochratoxin-A.

Commodity	Item	ZEA	OTA
Maize (<i>n</i> = 760)	Average, µg/kg ± SD	228.9 ± 112.8	320.0 ± 128.5
	Rate of incidence, %	18.4	9.3
	Range, µg/kg	60–1350	60–1850
Wheat (<i>n</i> = 367)	Average, µg/kg ± SD	210.3 ± 149.8	207.8 ± 33.4
	Rate of incidence, %	58.6	2.5
	Range, µg/kg	50–890	90–320
Barley (<i>n</i> = 222)	Average, µg/kg ± SD	139.8 ± 165.1	76.7 ± 22.1
	Rate of incidence, %	48.2	2.7
	Range, µg/kg	50–840	50–250
Oat (<i>n</i> = 60)	Average, µg/kg ± SD	101.5 ± 73.8	100.0 ± 61.1
	Rate of incidence, %	28.9	8.9
	Range, µg/kg	50–290	80–200
Triticale (<i>n</i> = 32)	Average, µg/kg ± SD	216.2 ± 160.4	120.0 ± 0
	Rate of incidence, %	65.6	3.1
	Range, µg/kg	50–580	120–120
Rye (<i>n</i> = 14)	Average, µg/kg ± SD	231.3 ± 152.7	250.0 ± 0
	Rate of incidence, %	57.1	7.1
	Range, µg/kg	80–520	250
Bran (<i>n</i> = 85)	Average, µg/kg ± SD	308.5 ± 239.3	0.0
	Rate of incidence, %	62.4	0.0
	Range, µg/kg	50–1560	0.0
Soybean (<i>n</i> = 119)	Average, µg/kg ± SD	181.1 ± 102.1	350.0 ± 45.2
	Rate of incidence, %	31.9	1.7
	Range, µg/kg	50–520	50–350
Sunflower (<i>n</i> = 22)	Average, µg/kg ± SD	0	160.0 ± 68.7
	Rate of incidence, %	0	18.2
	Range, µg/kg	0	100–260

Oat, triticale and rye grown in North America are thought to be resistant to *Fusarium* infections (Miller 1994). This is generally in contrast with the findings of the present investigation in which 64.4, 87.5 and 71.4% of the oat, triticale and rye samples were contaminated with trichothecenes and the ZEA contamination was also considerable (table 3).

Oat

The incidence rate of DON in the 45 oat samples tested was about 15 and 10% less than its occurrence in wheat and barley respectively. The DON content of the Norwegian oat samples (Langseth and Elen 1996) was found to be significantly higher than that of the wheat and barley. In this survey 97.1% of the oat, 66.3% of the wheat and 68.7% of the barley samples

contained 30 µg/kg DON or more. The difference in contamination level among grain species was attributed partly to agricultural factors and partly to variation in the susceptibility to different *Fusarium* species in interaction with climatic factors. Jørgensen *et al.* (1996) found a higher rate of incidence (50%) of OTA contamination in conventionally-grown Danish oat in comparison with our data.

Triticale

Very few, if any, data are available on the natural occurrence of *Fusarium* toxins and OTA in triticale, therefore no reliable comparison can be made between our data and published data. However, the high proportion of samples contaminated with trichothecenes and ZEA compared with the rate of

Table 4. Incidence of mycotoxins in different commodities.

Commodity	Mycotoxin	Country	Incidence, %	Source		
Maize	ZEA	Korea	7	Ryu <i>et al.</i> 1996		
		Argentina	30	Resnik <i>et al.</i> 1996		
		UK	52	Scudamore <i>et al.</i> 1998		
		Canada	69	Scott 1997		
	OTA	Virginia	0	Shotwell and Hesseltine 1983		
		Brazil	0	Hennigen and Dick 1995		
		UK	20	Scudamore <i>et al.</i> 1998		
		Russia	0.1	L'vova <i>et al.</i> 1992		
		Indonesia	1.9	Widiastuti <i>et al.</i> 1988		
		Bulgaria	9.0–27.3	Petkova-Bocharova and Castegnaro 1985		
		Turkey	36.2	Alp <i>et al.</i> 1997		
		Kenya	100	Muriuki and Siboe 1995		
		Wheat	DON	Argentine	80–93	Dalcerro <i>et al.</i> 1997
						Pacin <i>et al.</i> 1997
Bulgaria	67			Vrabceva <i>et al.</i> 1996		
Canada	33			Scott 1997		
OTA	Germany		71–98	Müller <i>et al.</i> 1997a		
	Switzerland		84.7	Noser <i>et al.</i> 1996		
	Denmark		29.3	Jørgensen <i>et al.</i> 1996		
	Switzerland		54	Noser <i>et al.</i> 1996		
	Barley		DON	Canada	100	Abramson <i>et al.</i> 1998
				Germany	81	Müller <i>et al.</i> 1997b
Norwegian		64.1–84.9		Langseth and Elen 1996		
OTA		Korea	76	Ryu <i>et al.</i> 1996		
		Germany	31	Thellman and Weber 1997		
		UK	6.5	Buckle 1981		
	UK	20	Scudamore <i>et al.</i> 1998			

contamination of other cereals tested may indicate genetic sensitivity to *Fusaria*. This view is partly supported by the findings that triticale showed 20 % greater sensitivity to *Fusarium* head blight symptoms than wheat grown in the same period (1985–1989) in Poland (Lew *et al.* 1993).

Rye

The occurrence rate of *Fusarium* head blight symptoms was also higher in rye than in wheat (Chelkowski *et al.* 1991). The world-wide screening of contamination of cereals with *Fusarium* mycotoxins made by Tanaka *et al.* (1988) revealed big variations in the *Fusarium* toxin contamination of rye samples from zero contamination in Nepal to 100 % contamination in Canada. DON contamination of the 14 rye samples we tested was intermediate between German (Tanaka *et al.* 1988) and Korean (Lee *et al.* 1985) samples.

Bran, soybean and sunflower

The overall trichothecene contamination of bran, soybean and sunflower samples decreases in the order: bran, soybean and sunflower. The high contamination rate of bran with trichothecenes and ZEA is not surprising in the light of studies made by Schnurer (1991), which indicated that 97 % of the fungal biomass was found in the bran fraction of wheat kernels. Szigeti *et al.* (1995) have also shown that 20–50 % of mould propagules was found on the kernels while the majority (50–80 %) contaminated the pericarp or inner side of the seeds of barley, wheat, triticale and oat.

While Mahmoud's investigation (1993) supports the view that soybean meal appears to be a poor substrate for mycotoxin formation, Nesheim and Wood (1995) demonstrated that soybean may assist the growth of many mould species, which can produce aflatoxins, trichothecenes and cytochalasins. However, the natural occurrence of these toxins in soybeans has not been a problem, since limited surveys of soybeans and

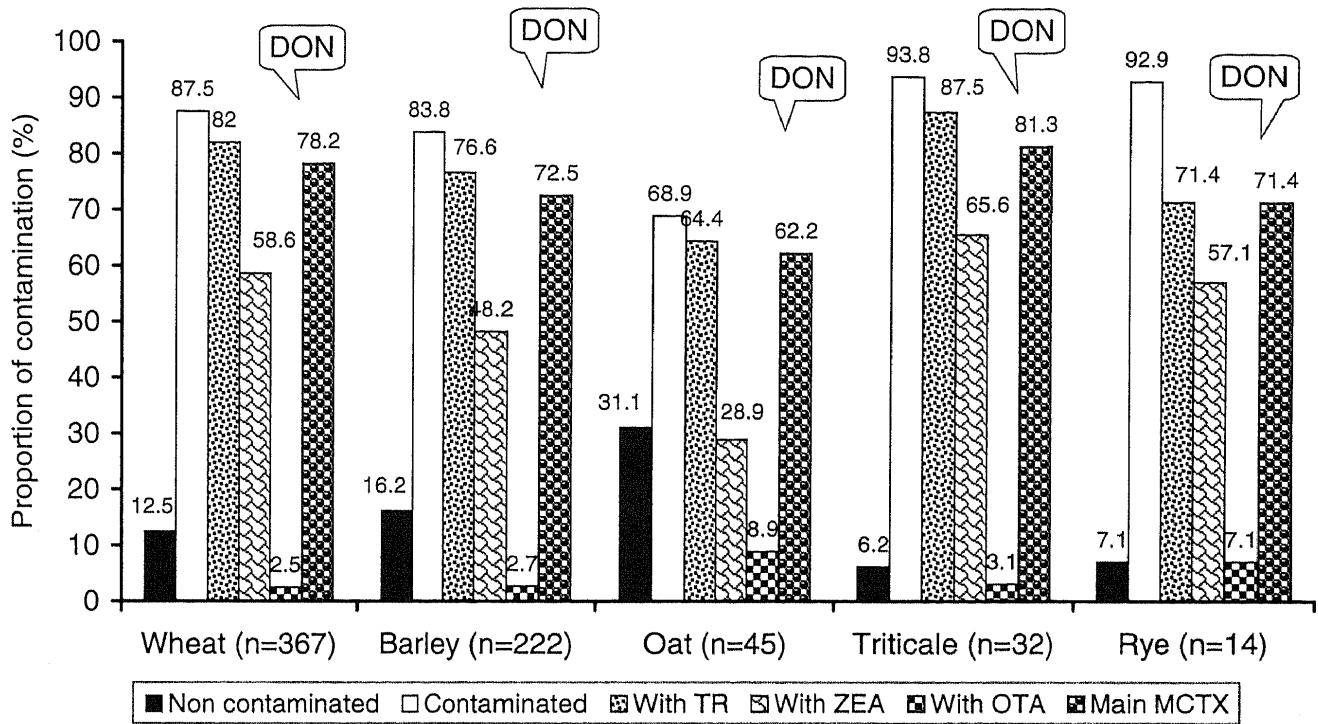


Figure 1. Mycotoxin contamination of grain samples.

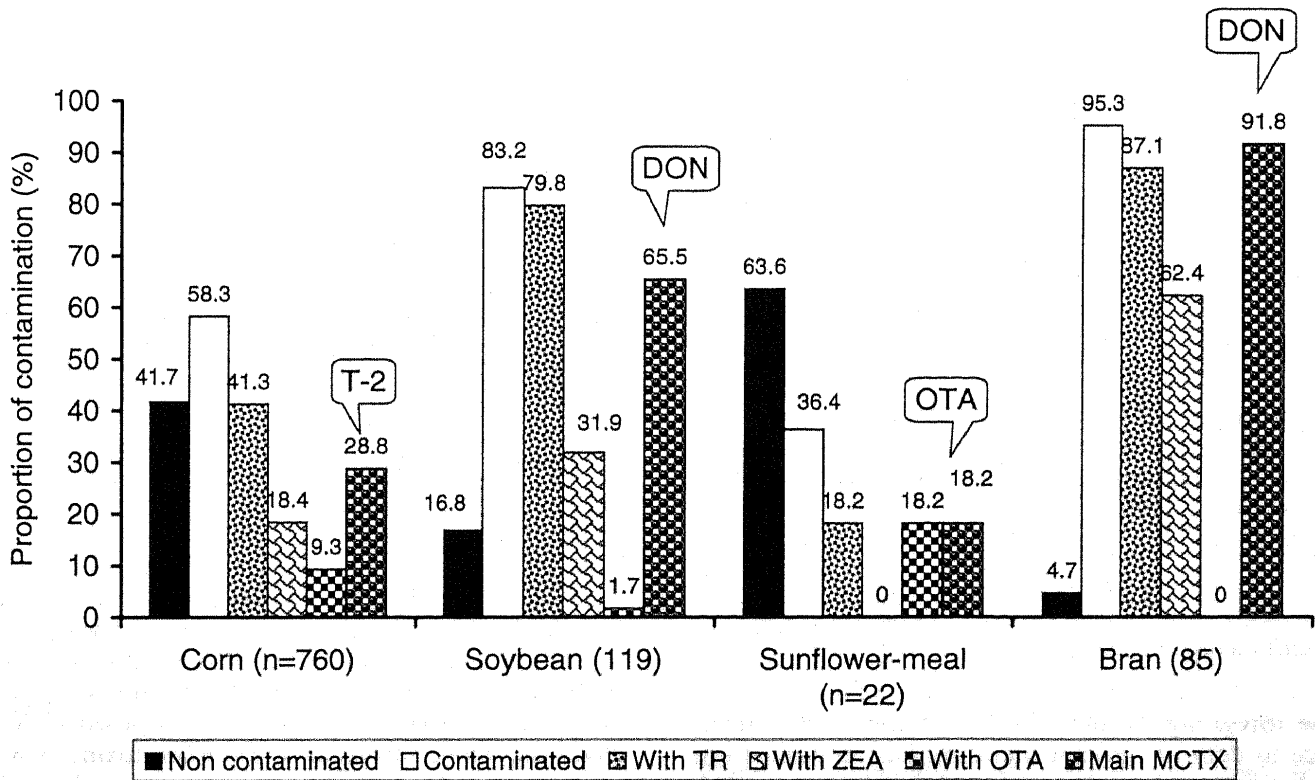


Figure 2. Mycotoxin contamination of grain samples.

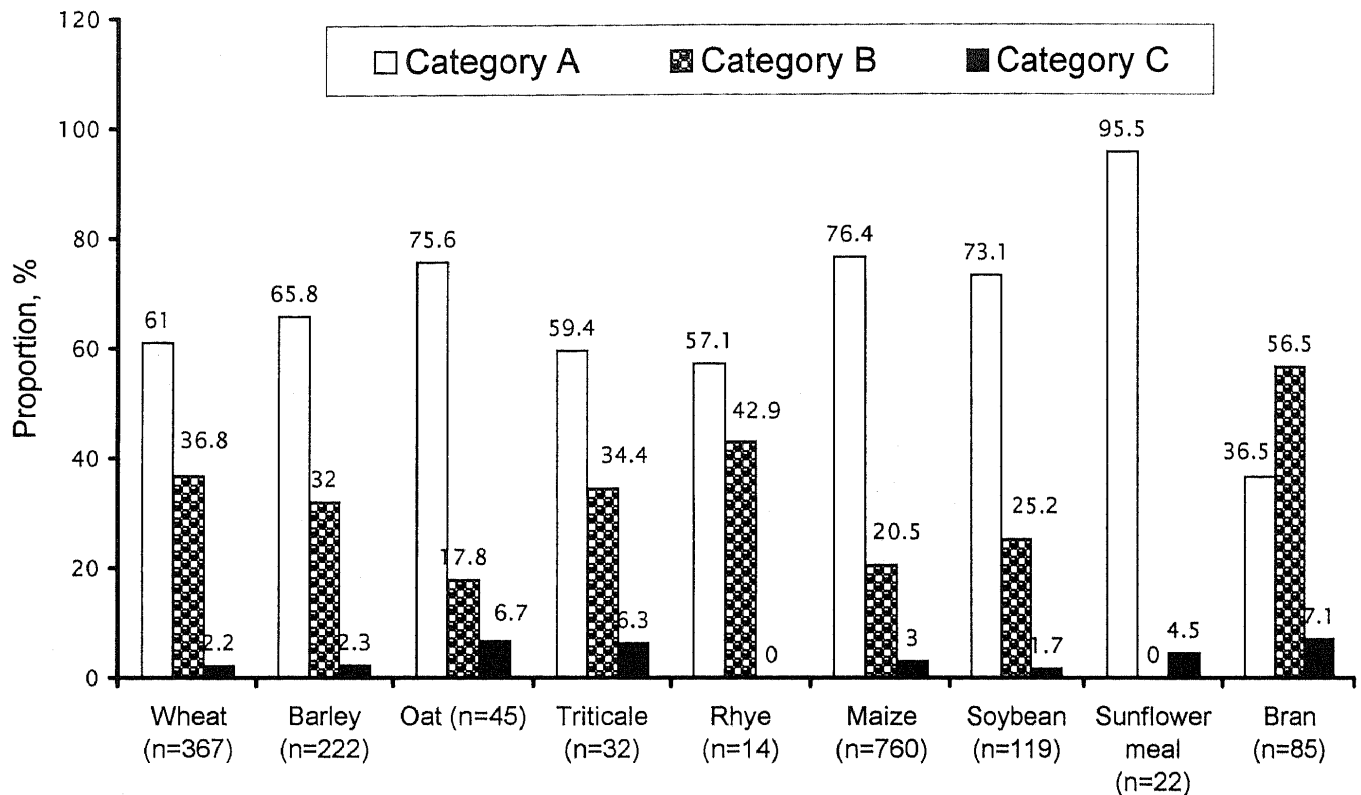


Figure 3. Grading of cereals.

soy-based infant formulas have not revealed significant contamination. Our findings indicate that soybean may be good substrate for trichothecene-producing fungi and the rate of contamination is considerable.

Sunflower

Trichothecene, ZEA and OTA contamination of the 22 sunflower-meal samples investigated was rather low (18.2, 0.0 and 18.2%, respectively). Scrutiny of the relevant literature has not shown comparable figures.

Quality considerations

The foregoing discussion of our findings with reference to data of relevant literature has reinforced our earlier impressions, that although incidence rate and/or concentration of *Fusarium* mycotoxins and OTA in

Hungarian-grown cereals is occasionally considerable, the position of the country is no worse than the average of countries.

In spite of the well documented, multilateral hazardous effect of feeding *Fusarium* toxin and/or OTA-contaminated feeds to livestock and poultry, very few attempts have been made to estimate the economic consequences. One of the few examples was reported by Müller *et al.* (1997a). Based on the Canadian guidelines for mycotoxin contamination Stratton *et al.* (1993) found that 9% of the grain harvested in 1987 in southwest Germany exceeded the 5 mg/kg limit set by the guideline for DON contamination in grains for ruminants and poultry. In other years between 1987 and 1993 the percentage of samples with DON exceeding the 5 mg/kg level varied between 0 and 3%. However, this risk assessment was based on only one mycotoxin. Due to frequent co-contamination of grains by mycotoxins (Müller and Schwandorf 1993) in evaluating risks posed to livestock the whole spectrum of *Fusarium* toxins as well as their interactions (additive or synergistic) should be considered (Müller *et al.* 1997a).

Figures 1 and 2 summarize the mycotoxin contamination of the grains samples analysed in the present investigation. Data of figure 3 indicate a rather low proportion of category C samples in the most important grains. However, these figures indicate that 3.0, 2.2, 2.3 and 1.7% of the maize, wheat, barley and soybean produced in Hungary should have been either excluded from further processing into compound feeds or used in animal feeds with extreme care. Careless use of category C commodities might lead to considerable economic loss in terms of decreased weight gain, depressed feed conversion rate, reproductive failures, increased rate of disease incidence, etc.

The high rate of category C bran samples (7.1%) is self-explanatory, because mycotoxins—among others OTA—accumulate on and directly beneath the epidermis of grain seeds (Osborne *et al.* 1996). Almost 7% of the oat samples were graded for exclusion. This finding accords well with that reported by Szigeti *et al.* (1995) who demonstrated that the average mould infection of oat samples was several times higher than that of the wheat and barley. This is explained by the fact that oat fields are hardly, if ever, treated with fungicides during the period of vegetation. Triticale is suspected as being genetically sensitive to *Fusarium* infections. It is also known that efficiency of treatments with fungicides in the period of vegetation depends on the genetic resistance of the plant (Mesterházy and Bartók 1996). This might be the explanation of the very high rate of C grading of the triticale samples.

In accordance with conclusions of similar studies (Müller *et al.* 1997a, b, Park *et al.* 1996) the results of the present investigation also urge regular screening for mycotoxins of importance and the necessity of individual appraisal of each commodity from the point of their use in animal feeds. This conclusion was adopted by the task force of the Hungarian Academy of Science, which elaborated the strategy of prevention and defence against mycotoxins for the Hungarian plant and animal production (Rafai and Mészáros 1998).

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