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FUSARIUM MYCOTOXINS IN LITHUANIAN CEREALS FROM THE 2004-2005 HARVESTS

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Abstract: *Fusarium* mycotoxins deoxynivalenol (DON), T-2 toxin, and zearalenone (ZEN) contamination in 5 kinds of cereal grain harvested in 2004 and 2005 in different regions of Lithuania was examined for their occurrence frequency and level. In all cereal species DON was the most frequently detected mycotoxin with an incidence rate of 98.0 - 100% and range in positive samples from traces to 691 µg kg^{-1} in 2004 and 62.5-94.0%, range from traces to 1,121 µg kg^{-1} in 2005, respectively. All the tested oat samples collected in 2004-2005 were found to be contaminated with the T-2 toxin. In one sample from the year 2004 the level of T-2 toxin (121.5 µg kg^{-1}) exceeded the allowable level. In 2004, ZEN contamination was more frequent in spring wheat, barley and oats grain, whereas in 2005 this toxin was identified at higher levels only in barley grain (68.0%). In one barley grain sample from 2004, ZEN content (193.4 µg kg^{-1}) exceeded the allowable level. Variation in the relative air-humidity exerted some effect on the incidence of *Fusarium* spp. fungi and mycotoxin content in wheat grain. The weather conditions at harvesting contributed to an increase in the contents of *Fusarium* fungi and DON and ZEN mycotoxins produced by them in winter wheat grain. This risk factor increases the threat to human and animal health.

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INTRODUCTION

The most prevalent mould fungi in cereal grain in temperate climate countries are the fungi genera of *Fusarium culmorum* (W. G. Smith) Sacc. Schwabe as well as several other species [9]. In Lithuania, the weather conditions are also favourable for the occurrence of *Fusarium* species, where the frequency of identification of these fungi is as high as 93.5% [14]. The most frequently isolated *Fusarium* species are *F. culmorum*, *F. avenaceum* (Fr.) Sacc., *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb. and others [4, 14]. As a result of mould growth, the grains are often contaminated by mycotoxins. According to the chemical composition and mode of action, *Fusarium* toxins are divided into 5 groups: zearalenone (ZEN), trichothecene mycotoxins deoxynivalenol (DON) and T-2 toxin, moniliformin, fusarine and fumonizine [2, 8]. Temperature and moisture conditions during the growing season and insect infestations are critical factors affecting fungal infection and toxin synthesis [5, 17]. More mycotoxins are produced during the warm, dry summers than in rainy and cool ones [11].

According to Muller *et al.*, during 5 years in an area of southwest Germany the incidence and levels of toxins varied from year to year [17]. DON was the major toxin with incidences at 49-85% and mean levels in positive samples of 52-302 µg kg^{-1}. Incidences of ZEN and T-2 were at 20-37% and 27-61%, respectively.

Mycotoxin contamination intensity in grain and its pro-ducts depends on the cereal species. Comparison of data from each harvest year in Denmark showed higher

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contents of DON in samples of wheat flour (range 20-527 μg kg^{-1}) than in rye (20-257 μg kg^{-1}) [20].

In Lithuania, *Fusarium* toxin DON was most frequently detected in the wheat and rye samples and nivalenol in the barley samples harvested in 1998 [12]. The noted concentrations were lower than those causing acute or chronic toxic effects in livestock or humans. However, mycotoxins can enter the food chain in the field, during storage, or at later points. There are differences in concentrations of mycotoxins ZEN and ochratoxin A in grain stored in different granaries [1].

Mycotoxins are capable of causing disease and death in humans and animals [10, 15, 21]. In order to reduce the levels of biogenic toxicants, European authorities are discussing further regulations on mycotoxins. EU directives (Commission regulation (EC) No. 856/2005) restrict the maximum allowable levels of the *Fusarium* mycotoxins DON to 1,250 μg kg^{-1} in unprocessed cereals and 1,750 μg kg^{-1} in unprocessed oats, ZEN to 100 μg kg^{-1} in unprocessed cereals. There is a proposal to limit T-2 toxin to 100 μg kg^{-1} [6]. In July 2006, new EU legislation came into force setting regulations for *Fusarium* mycotoxin content of food and raw grain.

The aim of the present study was to investigate the occurrence of mycotoxins DON, T-2, ZEN, and the incidence of *Fusarium* spp. fungi in the grain of different cereal species grown for food and feed in Lithuania during 2004-2005.

MATERIALS AND METHODS

Samples. Grain samples of winter and spring wheat (*Triticum aestivum* L.), winter and spring barley (*Hordeum distichon* L.), winter triticale (x*Triticosecale* Wittm.) and rye (*Secale cereale* L.), and spring oats (*Avena sativa* L.) collected at harvest during 2004 and 2005 from 29 districts of Lithuania were analysed for contamination by DON, ZEN, and T-2 toxin. The number of samples analysed is given in Table 1.

Part of each sample was subjected to mycological contamination, and the other part (about 50 g) was air dried, milled in a mill IKA A11 Basic and kept at -20ºC until analysis. Fifteen grain samples from the 2005 harvest taken after the rainy period were analysed for DON contamination twice: at the beginning of September, shortly after harvesting and after 10 weeks' storage in the following conditions: moisture 50-52%, temperature 16-18ºC.

Table 1. Number of samples tested in experimental years.

Mycotoxin	Number of samples					
	Wheat	Barley	Oats	Rye	Triticale	Total
2004						
DON[a]	62	25	5	9	5	106
ZEN[b]	51	16	5	5	5	82
T-2 toxin	-	-	5	-	-	5
2005						
DON	88	30	9	7	7	141
ZEN	49	50	2	5	7	113
T-2 toxin	-	-	5	-	-	5

[a]Deoxynivalenol; [b]Zearalenone

Analysis of mycotoxins. The wheat, rye, oats and barley samples were analysed by the ELISA (enzyme-linked immunosorbent assay) method [23]. The method is based on the antibody antigen interaction and provides sensitive, rapid and accurate monitoring of mycotoxins and is suitable for screening large numbers of samples. The Veratox test kits (Neogen Corporation, USA), approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. Mycotoxins extraction and tests were performed according to manufacturer's instructions. The optical densities of samples and controls from standard curve were estimated by multichannel programmable photometer Multiskan MS (Labsystems, Finland), using a 650 nm filter and calculation mode Point to Point. Measured absorbances were automatically converted to the mycotoxins concentration units – μg kg^{-1}.

While assessing our data with regard to food and forage safety we referred to the EU document No. 856/2005 for deoxynivalenol, zearalenone, and global research recommendations for T-2 toxin [6].

***Fusarium* spp. analysis.** Incidence of *Fusarium* spp. fungi on grain was evaluated following Mathur and Kongsdal methodology [16]. The fungi were isolated and identified according to cultural and morphological properties of the colonies. Identification was carried out using Nelson *et al.*, and Lugauskas *et al.* descriptors [18, 13].

Meteorological conditions. The weather conditions of the 2 harvest years differed. In 2004, the lack of precipitation was appreciable until the middle of June. At the end of June and July cool (13-19ºC) and wet weather prevailed. The weather became warmer (16-23ºC), periodically rainy at the end of July which persisted until the first half of August. During the 2004 growing season the weather conditions were favourable for the occurrence and development of fungal diseases in cereal stands.

The summer of 2005 was noted for exceptional weather conditions. The grain ripened relatively early because July was dry and warm. During the cereal flowering stage (second-third 10-day periods of June and first 10-day period of July), dry weather also prevailed, and the conditions during this important stage for grain storage were not conducive to the development and spread of *Fusarium* fungi. Rainy weather settled in at the beginning of harvesting. During the 4 rainy days some regions of Lithuania received 200-230 mm of rainfall. Only very few winter cereal crops were harvested before the rainy period, and no spring cereal crops were harvested before the rainy period.

RESULTS AND DISCUSSION

Our experimental evidence suggests that on average 19.0% (from 4.1%-44.3%) of the grain samples from the harvest year 2004 were affected by the fungi of *Fusarium* spp. The prevalent species were *F. poae*, *F. sporotrichioides*, and *F. avenaceum.*

Grain sample contamination by DON was 98.0-100%, the concentration in positive samples ranged from traces to 691 µg kg^{-1} and did not exceed the allowable rates (Tab. 2). A small number of the samples (7 of 106) were contaminated with DON levels higher than 300 µg kg^{-1}. The highest concentrations of this mycotoxin were determined in the grain of spring wheat (642 µg kg^{-1}) and winter rye (691 µg kg^{-1}).

The frequency of ZEN-contaminated grain was lower than these contaminated by DON and subject to cereal varied from 0-60%. This fact is supported by the results found in literature [17]. ZEN was detected in the concentration range from traces to 193.4 µg kg^{-1}. In 3 of the 82 grain samples of cereals tested mycotoxin concentration was higher than 30 µg kg^{-1}, and in one, i.e., in spring barley, the concentration of toxin exceeded the allowable limits and reached 193.4 µg kg^{-1}. ZEN-contamination was more frequent in spring than winter cereal grain.

All 5 oat samples of 2004 harvest tested were found to be heavily contaminated with mycotoxins (17.2-121.5 µg kg^{-1}), and the concentration in one of the samples exceeded the safe level [6]. This is consistent with high incidence of *Fusarium* spp. especially of *F. poae*, *F. sporotrichioides* (Tab. 3) those are the main producers of T-2 toxin in oats grain and can further initiate metabolite biosynthesis during the storage period [19]. Results discussed above indicate the necessity to control oats grain contamination with this mycotoxin in Lithuania.

In the grain of the 2004 and 2005 harvest years DON was the most prevalent *Fusarium* mycotoxin. DON was identified in 94.0% wheat grain samples with a range in positive samples from traces to 1,121 µg kg^{-1}, in 93.0% of barley samples with a range from traces to 372 µg kg^{-1} (Tab. 4). The lowest incidence of this mycotoxin was determined in the samples of winter triticale (62.5%, range from traces to 370 µg kg^{-1}). The highest DON concentration was identified in winter wheat grain (1,121 µg kg^{-1}). No samples were found with the mycotoxin concentration exceeding the allowable level.

ZEN was more prevalent in barley (68.0% of contaminated samples) and oats grain (50.0% of contaminated samples), though the mycotoxin concentrations were very low and did not exceed the allowable concentrations.

T-2 toxin infection was identified in 100% of oats grain, however, the contents were also lower than the maximum level, despite the heavy contamination of *Fusarium* spp. in oats grain of the 2005 harvest (Tab. 3). The contents of T-2 toxin were lower than in 2004. This might have been influenced by the species composition of fungi and fungal

Table 2. Mycotoxin contamination in grain harvested in 2004.

Cereals	No. of samples	% of positive samples	Levels in positive samples µg kg^{-1}	No. of positive samples with the following content of mycotoxins (µg kg^{-1})		
DON[a]				<100	100-300	>300
Wheat winter	47	97.9	Tr[c]-242	32	14	0
Wheat spring	15	100	Tr-642	4	8	3
Barley	25	100	Tr-198	16	8	0
Oats	5	100	122-204	0	5	0
Rye	9	100	Tr-691	3	4	2
Triticale	5	100	168-427	0	3	2
ZEN[b]				<10	10-30	>30
Wheat winter	39	25.6	Tr-16	7	3	0
Wheat spring	12	50.0	Tr-95.6	2	2	2
Barley	16	25.0	10-193.4	0	3	1
Oats	5	60.0	Tr-16.3	1	2	0
Rye	5	20.0	28.8	0	1	0
Triticale	5	0	0	0	0	0
T-2 toxin				<7.5	7.5-30	>30
Oats	5	100	17.2-121.5	0	2	3

Table 3. *Fusarium* species distribution (%) and composition in the grain of oats.

Fusarium spp.	2004		2005	
	Range	Average	Range	Average
F. avenaceum	0-1.0	0.2	8.7-44.4	34.0
F. sporotrichioides	1.0-5.0	2.6	14.3-33.3	25.8
F. poae	28.0-64.0	42.2	2.2-35.1	20.2
F. culmorum	0-2.0	0.4	0-6.5	2.5
F. tricinctum	0	0	5.7-11.1	7.5
Other *Fusarium*	0-4.0	2.0	2.7-17.1	10.0

Table 4. Mycotoxin contamination in grain harvested in 2005.

Cereals	No. of samples	% of positive samples	Levels in positive samples µg kg^{-1}	Number of positive samples containing the following content of mycotoxins (µg kg^{-1})		
DON[a]				<100	100-300	>300
Wheat	88	94.3	Tr-1,121	62	18	3
Barley	30	93.3	Tr-372	25	2	1
Oats	9	89.0	Tr-131	7	1	0
Rye	7	71.4	Tr	5	0	0
Triticale	7	57.1	Tr-370	3	0	1
ZEN[b]				<10	10-30	>30
Wheat	49	32.6	Tr-33.4	11	4	1
Barley	50	68.0	Tr-16.5	28	6	0
Oats	2	50.0	11	0	1	0
Rye	5	0	0	0	0	0
Triticale	7	28.6	Tr	2	0	0
T-2 toxin				<7.5	7.5-30	>30
Oats	5	100	10.8-41.8	0	3	2

[a]Deoxynivalenol; [b]Zearalenone; Tr – traces

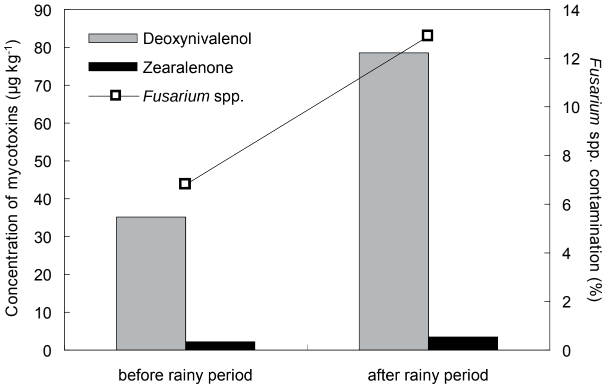

Figure 1. Mean DON (deoxynivalenol), ZEN (zearalenone) concentration in positive samples and *Fusarium* spp. contamination in wheat grain harvested before and after rainy period 2005.

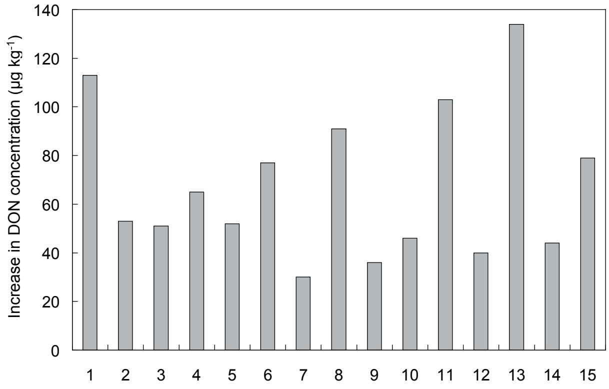

Figure 2. Increase in DON (deoxynivalenol) concentration µg kg^{-1} in grain during 10 weeks storage.

disease infection period, which is reported to be related to weather conditions during a specific growth stage [7, 11, 20, 17]. In the oats from the 2004 harvest year the prevailing species was identified to be *F. poae*, with an average infection level of 42.2%, and up to as much as 64.0%, depending on the sample. This species is one of the main producers of T-2 toxins [6]. Besides, the occurrence of *Fusarium* head blight in 2004 was determined by the rainy cereal flowering period. However, the grain of the 2005 harvest was much more severely infected by *Fusarium* spp. fungi. A grain moisture content of 17-19% is necessary for the development of various fungi of this genus [22]. In 2005, such conditions occurred at the beginning of harvesting, i.e. upon complete maturity of grain. The most prevalent fungus in oats in 2005 was *F. avenaceum*. Similar data were also obtained in other countries: heavy rainfalls in the summer and autumn of 1998 caused abundant *Fusarium* mould infection in Finnish cereals, and *F. avenaceum* was the most common *Fusarium* species found in their grains [7]. However, the mycotoxin concentrations in grain were very low.

Due to the extreme weather conditions, mycotoxin testing in grain harvested in 2005 was carried out in 2 stages: in the grain harvested before and after the rainy period. Variation of average DON and ZEN concentrations in relation to the weather conditions was more pronounced in wheat grain. Trends of mycotoxins content increase were noted for the grain harvested after the rainy period (Fig. 1), however, the differences identified were minor with regard to food or forage safety. An increase in average DON concentration was especially obvious.

Variation in mycotoxins content is related to the level of grain damage by *Fusarium* fungi. Heavy rainfalls during the harvest of 2005 caused the occurrence of *Fusarium* infection in cereals, particularly in wheat. Only 6.5% of wheat grain harvested before the rainy period was affected by the mould fungi, and after the rainy period the content of affected grain doubled to 12.9% (Fig. 1).

The weather conditions in 2005 were not conducive to the occurrence of head blight. The rainy period started before complete ripening of cereals. The incidence of the main producers of these mycotoxins (*F. culmorum*, *F. graminearum*) was very low on grain, and therefore very low concentrations of their metabolites (DON and ZEN) were identified. Cereals are most susceptible to primary infection and contamination with DON if there are rainfalls during the flowering period of crops [20]. However, depending on storage conditions, the level of mycotoxins can vary during the storage period [19].

Results from the analysis of 15 different cereal grain samples collected after the rainy period of 2005 and stored for 10 weeks, generally showed that the concentration of toxin deoxynivalenol became higher by 30-134 µg kg^{-1} or 67.6 µg kg^{-1} on average, than in the samples collected shortly after harvest (Fig. 2). The fact that levels of *Fusarium* mycotoxins increase with the time of grain storage has also been noticed by other researchers: results from analysis of 98 maize samples received between 1 November – 3 January generally showed that the concentration of trichotecenes A and B became progressively higher in the samples received later [19].

The trends of DON and ZEN variation in wheat grain might have been determined not only by the weather conditions but also by other factors such as soil peculiarities in different districts, application of plant protection products, choice of varieties, among others [3, 5, 10, 11].

CONCLUSIONS

The results of the present study show that DON was the most frequently detected *Fusarium* mycotoxin in Lithuanian cereals with an incidence rate of 62.5-100% and with a range of concentration from traces to 1,121 µg kg^{-1} over all samples for both years of the study. The frequency of ZEN-contaminated grain was lower than those contaminated by DON and subject to cereal and year varied from 0-68%; however, in one barley grain sample from the year 2004, ZEN content (193.4 µg kg^{-1}) exceeded the allowable level. T-2 toxin was detected in all the oats samples, and in one sample the concentration was 121.5 µg kg^{-1} which exceeded the safe level. The data of re-analysed grain samples collected after the rainy period of 2005 and stored for 10 weeks, showed a distinct tendency of DON accumulation during storage.

The weather conditions at harvesting exerted some effect on the accumulation of *Fusarium* spp. fungi and mycotoxins produced by them, since when winter wheat grain had been thrashed after a rainy period, a higher content of *Fusarium*-affected grain was identified, and trends of DON and ZEN increasing were revealed. This risk factor increases a threat to human and animal health.

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