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Microfungi in grain and grain feeds and their potential toxicity

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Abstract. The aim of the research work was to study microfungi in grain (spring and winter wheat) and grain feeds of domestic origin and determine their composition with special attention on toxigenic and allergenic species.

The total number of fungi was estimated on wort agar or on the nutrient substratum of Czapek. The species and number of *Fusarium* were defined on the selective medium of Nash & Snyder. For a mycological survey of grain samples, the moist chamber method was used in the year of 1992. The fungi were determined by microscopy, using corresponding nominators (Raper et al., 1949; Raper et al., 1965; Arx, 1970; Bilai et al., 1988). The classification of *Fusarium* has been made according to Gerlach & Nirenberg (1982). The toxicity of isolated fungi was defined by means of a test organism, *Bacillus stearothermophilus* (Watson & Lindsay, 1982). Spring wheat of the years 1992, 1993, 1994 and winter wheat of the years 1992, 2002 and 2003, and spoilt grain feeds of the years 1997–2002 were investigated.

About half of the identified 63 fungi species are either potentially toxigenic or allergenic. In 1992–1994; on average *Alternaria* spp. occurred on 72% of spring wheat seeds and on 45% of winter wheat seeds, *Cladosporium* spp. on 20% and 8% of the seeds, *Aspergillus* spp. on 6% and 9% of the seeds, *Verticillium* spp. on 13% and 23% of the seeds, *Fusarium* spp. on 23% and 64% of the seeds, respectively. *Penicillium* spp. was represented very differently: in 1992 and 1994 on 10%, in 1993 on 80–90% of the seeds. The species known as toxicants were also from the genera *Chaetomium, Cochliobolus, Gliocladium, Mortierella, Mucor, Rhizopus, Stachybotrys,* and *Trichothecium.* In spoilt grain feeds the potential toxicants were represented from the genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Rhizopus,* and *Trichothecium.* Allergenic species were represented by the genera *Epicoccum, Penicillium, Aspergillus,* and *Ulocladium.* The toxicity of fungi isolated from grain, on the basis of the growth inhibition zone of *B. stearothermophilus,* was 0–7 mm, whereas on fungi isolated from spoilt feeds it was 0–18 mm. The most toxic fungi were *Penicillium cyclopium, Penicillium* sp., *Trichothecium roseum, Aspergillus terreus, Paecilomyces varioti, Rhizopus nigricans,* and *Acremonium* sp.

Key words: grain, grain feeds, mycoflora, toxins, toxigenic, allergenic, *Bacillus stearothermophilus*

INTRODUCTION

During vegetation period, several microorganisms develop on grain seeds, and their number in dry and cleaned grain can reach millions per 1g. It is mainly epiphytic microflora that has originated from plants' rhizosphere, from microorganisms

spreading by dust and insects. Epiphytes are not parasites on plants but grow at the cost of plant excretions and organic matter (dust, pollen, etc.) on plant surface. On harvesting soil, microbes get on seeds with dust from the soil. Epiphytes usually do not penetrate the inside of the seed and cause no harm to its quality. Under certain conditions they can go over from a saprophytic way of life to parasitic feeding type and, by penetrating inside of the seed, damage its quality and vitality. Most epiphytes are usually bacteria (800–850 thousand pieces per 1g), the number of fungi is less (1–6 thousand pieces per 1g) (Mishustin & Trisvyatskii, 1963). The main epiphytes in fungus flora are the species of *Penicillium, Aspergillus, Cladosporium, Alternaria,* and *Mucor*.

Several typical plant pathogens also occur on seeds: the microfungi *Tilletia, Urocystis, Ustilago, Fusarium, Cochliobolus, Pyrenophora, Drechslera, Leptosphaeria,* and other genera. On seeds there can be found some bacteria causing diseases on people and animals (*Bac. anthracis, Bact. mallei, Bact. melitensis* etc.) (Mishustin & Trisvyatskii, 1963).

The aim of our research was to study the composition of mycoflora occurring in grain according to all requirements treated and for comparison of mycoflora in spoilt grain feeds, paying the main attention to potentially hazardous species which can produce toxins and cause allergy on people or farm livestock.

Toxic microfungi in cereals have already for centuries been a very actual problem for crop growing and livestock farming and for processing industry. By decreasing the productive value of grain for food, feed and seed, the toxins caused by fungi in the grain result both chronic and acute toxications on animals and people, and also allergic appearances. Therefore, great attention is being paid to their study and prevention in the whole world. The majority of the EU states participated, for example, in studying the toxigenic fungi also in a programme initiated by the European Commission: "Agriculturally important toxigenic fungi. COST Action 835", where one of the main problems were *Fusarium* species (Annual Report 1998. Directorate-General for Research. 2000. EUR 19694).

In the session of a grain workshop of the European Commission on 4–5 December, 2003, it was concluded that grain within the EU was extensively polluted with mycotoxins (toxins of microfungi), especially with *Fusarium* toxins. Therefore, grain in tens of millions of tons stays unused, waiting for analyses, according to which its consumption purpose could be determined. It is possible that some part of the lot is not suitable for either food or feed any more. At the same time, 10% of the required grain is missing.

The mycotoxins do not decompose significantly in the digestive tract of animals but go over to production – eggs, meat and milk, jeopardizing thus people's health (Schachermayr & Fried, 2000). Most of them are also rather thermostable and do not decompose by thermal processing like steaming, boiling, cooking and can reach our table by bread and white bread products, to which special attention has been paid by German scientists (Obenauf, 2002).

To avoid the development of very hazardous microfungi on cereals already in the field and harvested grain or grain feeds in storehouses, it is essential to know their species composition, which also enables to plan and apply corresponding preventive and control methods.

MATERIALS AND METHODS

Seed samples of domestic spring wheat (Satu, Luja, Heta, Tjalve, Planet, Arkas) and winter wheat (Sani, Mironovskaya 808, Shirvinta) of the harvest of 1992–1994 and 2002–2003, also grain feeds with spoilt signs of the years 1997–2002 were investigated. The wheat samples (64) originated from the trials of the Estonian Research Institute of Agriculture and high-multiplication seed fields of seed breeding farms (Võhma, Vambola, Jõgeva, Tartu, Rannu), and the feed samples (32) were collected from animal breeding farms.

The preparation of the samples and analyses were carried out according to the methods described by Harrigan & McCance (1976), and later according to the standard of EVS-EN ISO 6887-1:2001 (Preparation of test samples, initial suspensions and decimal dilutions for microbiological examination). The total number of fungi was estimated by a sowing from the first, second or third dilution on wort agar or on the nutrient substratum of Czapek, for identifying *Fusarium* species on the Nash & Snyder selective medium. On mycological analysing of grain for developing microfungi, the moist chamber method was used: 50 seeds were placed in three replications in petri dishes (diameter 10 cm) on a moist filter paper at 20°C for 7 days. After that the fungi were assessed by microscoping, using corresponding nominators (Raper et al., 1949; Raper et al., 1965; Arx, 1970) and manuals (Bilai et al., 1988). *Fusarium* spp. was assessed according to Gerlach & Nirenberg (1982). The toxicity of isolated fungi was determined by the method of Watson & Lindsay (1982) with the biotest *Bacillus stearothermophilus* according to the range of the growth inhibition zone (0–1 mm – no toxicity; 2–5 mm – toxic; 6–10 mm or more – very toxic).

The mycological analysis of 1992 was made by concluding a contract with the researcher Peeter Soobik from the laboratory of microbiology of the Estonian Research Institute of Agriculture and Land Improvement.

Dispersion analysis was used in data processing.

RESULTS AND DISCUSSION

In the wheat yield of the seed breeding farms and trial fields of the Estonian Research Institute of Agriculture there were represented 58 fungus species from 40 genera. The typical epiphytic fungi (*Penicillium, Aspergillus, Cladosporium, Mucor, Alternaria, Trichothecium*), the so-called storage fungi – xerophytes (*Penicillium, Aspergillus, Rhizopus, Mucor*) and the pathogens (*Cochliobolus, Pyrenophora, Drechslera, Leptoshaeria, Mycosphaerella, Fusarium, Gibberella, Verticillium*) were represented. About half of the ascertained fungus species are under certain conditions able either to produce toxins or excrete substances causing allergy. Such are *Acremonium* spp. (toxin-oosporein or chaetomidin), *Alternaria alternata* (Fr.) Keissl., *A. tenuissima* (Fr.) Will. (alternariols, altenuenes, altertoxins), *Aspergillus niger* Tiegh., *A. sulphureus* (Fres.) Thom et Church. (aflatoxin B_1, ochratoxin A), *Chaetomium globosum* Kunze ex Fr. (hetomin), *Cladosporium* spp. (ATA toxins), *Cochliobolus sativus* (Ito et Kuribay) Drechs. et Dastur (helmintosporol, helmintosporal), *Gliocladium* spp. (*G. fimbriatum* – gliotoxin), *Mucor mucedo* Fr. (aflatoxin), *Penicillium expansum* Link ex Fr. (aflatoxin), *P. decumbens* Thom. (decumbin), *Rhizopus nigricans* Ehrenb. (aflatoxin), *Aspergillus fumigatus* (Fres.)

(fumigacin, fumigatin, fumigallin, spinulosin), *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes (satratoxin H), *Trichothecium roseum* Link. (trichothecenes), (*Verticillium* spp.(verticillin A). The production of the toxins of the mentioned species is discussed by Bilai & Pidoplitshko (1970), Ciegler et al., (1971), Kadis et al., (1972), Miller & Trenholm (1997), etc.

Of representatives of the genus *Fusarium*, the species occurring more often on the seeds were *F. oxysporum* (Schlecht) Snyd. et Hans., *F. semitectum* Berk. et Rav. and *F. sporotrichioides* Sherb. var minus Wr., and less *F. culmorum* (W.G.Sm.) Sacc., *Gibberella avenacea* R. J. Cook *(F. avenaceum)*, *G. baccata* (Wr.) Sacc. (*F. lateritium*), *G. fujikuroi* (Saw.) Wr. *(F. moniliforme*), *G. gordonii* C. Booth (*F. heterosporum*), *G. pulicaris* (Fr. ex Fr.) (*F. sambucinum*). In the wheat yield of the earlier years 1973–1981, the dominating fungi were *F. avenaceum* (Fr.) Sacc., *F. oxysporum*, *F. poae* (Pk.) Wr., and *F. ventricosum* App. et Wr. (Lõiveke et al., 2003). The mentioned species are capable of producing many toxins – DON, 3-ADON, 15-ADON, NIV, ZEN, HT-2, fumonisins, moniliformin, fusarin C, wortmannin, sambutoxin, fusarenon X, T-2, DAS, etc. (Bilai, 1977; Kadis et al., 1971; Miller & Trenholm, 1997).

Of allergic species, *Epicoccum purpurascens* Ehrenb., *Ulocladium consortiale* (Thüm.) Simmons, *Cladosporium herbarum* Link ex Fr., several representatives of the genera *Penicillium* and *Aspergillus* occurred.

The typical epiphytic fungi from the genera *Alternaria*: *A. alternata* and *A. tenuissima*, were most frequent on the seeds, occurring on 72% of spring wheat and 45% of winter wheat seeds. Saprophytes from the genera *Cladosporium: C. cladosporioides* (Fres.) de Vries and *C. sphaerospermum* Penz., occurred on 20% and 8% of the seeds, respectively. *Aspergillus* species: *A. niger*, *A. fumigatus* (Fres.) and *A. sulphureus*, were represented undemonstratively – only on 6% of spring and 9% of winter wheat seeds. The phytopathogenic *Verticillium* species *V. albo-atrum* Rke. et Bert., *V. dahliae* Kleb., *V. nigrescens* Pethybr. were found on 13% of spring and 23% of winter wheat seeds, *Fusarium* spp. on 23% and 64% of the seeds, respectively. The *Penicillium* species *P. expansum* and *P. decumbens* occurred in the samples of 1992 and 1994 only on 10% of spring wheat seeds but in the wheat of 1993 in masses – on 80–90% of the seeds (80–85% of the total number of fungi). In Finland (1976 and 1977) the most frequent species on wheat seeds were *Cladosporium* (46–73% of seeds) and *Alternaria* (17–71% of seeds), which were followed by the genera *Penicillium* (18–45%) and *Fusarium* (14–34%). Differently from our results, the species of *Verticillium*, *Aspergillus* and *Stachybotrys* were missing in Finnish wheat completely (Ylimäki et al., 1979). Considering the fact that in Estonian wheat *Aspergillus* (6–8% of seeds) and *Stachybotrys* (less than 0.1% of seeds) occurred to a small extent there is no big difference in the wheat contamination of Estonia and Finland with toxicants.

To estimate the microbiological quality of grain, the indicators of feed (Schmidt-Lorenz, 1980) and food grain (Baumgart & Firnhaber, 1993), established in Germany, were taken as the basis. According to these, in feed grain of normal quality the total number of either yeasts or moulds per 1g of dry grain must not exceed $4x10^4$–$8x10^4$, in food grain (wheat, rye) $3x10^4$, respectively. The data of the table 1 show that only one of the 6 grain lots does not meet the requirements of feed grain but already 4 of the lots do not meet the requirements of food grain (in 1994 Satu, in 2002 Sani and Shirvinta, in 2003 Sani).

Table 1. Number of microfungi per 1g of dry grain in 1993–1994 and 2002–2003.

Crop Variety	Year	Microfungi		Yeasts on maltagar	*Fusarium* sp. on Nash & Snyder medium	pH_{KCl}	Moisture %
		on maltagar	on Czapek medium				
*SW Satu	1993	$1.3x10^4$	$1.3x10^4$	-	110	5.20	10.0
SW Satu	1994	$1.4x10^5$	$1.7x10^5$	-	780	5.94	10.2
**WW Sani	2002	$2.1x10^4$	-	$8.7x10^4$	0	7.00	9.7
WW Shirvinta	2002	$2.5x10^4$	-	$3.7x10^4$	0	6.90	10.0
WW Sani	2003	$4.0x10^4$	-	$3.8x10^4$	440	7.18	9.6
SW Satu	2003	$1.7x10^4$	-	$1.2x10^4$	500	6.66	9.8

*SW – spring wheat, **WW – winter wheat

Table 2. Toxicity of microfungi on *Bacillus stearothermophilus* by the growth inhibition.

Microfungi	Crushed grain, 2001	Meal feed, 2002	Wheat Sani, Shirvinta , 2003
	Growth inhibition on *B. stearothermophilus* in mm		
Acremonium sp.	9	-	0
Alternaria sp.	-	3	4–5
Aspergillus oryzae	5	-	-
A. terreus	10	-	-
Cladosporium sp.	-	0	-
Geotrichum candidum	6	-	-
Paecilomyces varioti	-	10	-
Penicillium sp.	2	5–7	-
Rhizopus nigricans	-	10	-
Rhizopus sp.	3	-	-
Mucor sp.	3	10	-
Yeasts	0–5	0–5	2
F. culmorum	-	-	5–6
F. verticillioides	-	-	4–5
F. tricinctum	-	-	3–5
Fusarium sp.	-	-	7

To study the danger of fungus species occurring in grain, the total toxicity of some more-spread fungus genera was assessed according to the range of the growth inhibition zone of *Bacillus stearothermophilus* (Table 2). In the case of *Alternaria* spp. it was 4–5 mm, i.e. they were moderately toxic, whereas yeasts, on the other side, had low toxicity (growth inhibitor 2 mm). Of the *Fusarium* genus, *Fusarium* sp., *F. culmorum* and *F. verticillioides* (4–7 mm) had higher toxicity. The toxicity of *F. tricinctum* isolates was lower (3–5 mm). Thus, in properly dried grain there were also *Fusarium* isolates with toxicity above the average. *Acremonium* sp. turned out to be non-toxic in this case.

Table 3. Contamination of spring wheat varieties (%) with *Fusarium* species in the trials of the ERIA at Saku, 1992.

Fusarium sp. by Gerlach and Nirenberg (1982)	Satu	Luja	Heta	Tjalve	Planet	Arkas	Average
	Duration of growth period, days						
	100-102	100-102	100-102	106	>108	>108	
F. culmorum	1.3	1.0	1.2	1.3	2.0	0.6	1.2
F. oxysporum	11.3	5.3	10.7	14.7	18.0	14.0	12.3
F. semitectum	4.0	12.6	8.0	12.6	21.3	14.7	12.2
F. sporotrichioides	0.6	0	3.3	6.8	8.7	13.3	5.5
Gibberella avenacea	0	1.0	1.2	2.0	10.7	4.0	3.0
G. baccata	1.3	0	0	0	0	1.3	0.4
G. fujikuroi	0	1.3	1.3	0.6	0.6	5.3	1.5
G. gordonii	0.6	0	1.3	0	1.3	4.7	1.3
G. pulicaris	0	1.3	0	0.6	0	0.6	0.4
Total	19.1	22.5	27.0	38.6	62.6	58.5	38.1
Total Sd	for earlier varieties – 0.554			for later varieties – 2.383			-
Total LSD 95%	for earlier varieties – 1.356			for later varieties – 5.831			-

Whether potentially toxic species are going to produce toxins or not, depends on many ecological parameters (temperature, moisture, composition of the substrate, spore load of toxigenic strains, existence of competing microflora, microbial interaction, insect infestation, mechanical damages of harvested and stored grain, the ratio of CO_2 and O_2). A more determinative factor is time, i.e. how long these factors have been operating (Ominski et al., 1997). The appearance of toxins in grain already in the field can be expected with varieties susceptible to toxicants, at applying too much nitrogen for fertilisation, in the case of a rainy growth period and delayed harvest. It is favoured by the more intensive development of toxicants and the whole mycoflora under such conditions. In 1973–1981 in Estonia the biggest infection of grain with *Fusarium* species was in 1978, the year with the rainiest growth period (Lõiveke et al., 2003).

The multitude of mycoflora on seeds also depends on characteristics of the varieties. In the trials of the ERIA, conducted in 1992, wheat varieties with different growth period durations were cultivated according to the same agrotechnology (sowing, plant protection measures, harvesting, etc. carried out at the same time). In the grain harvested on 20 August and dried equally to 13% of moisture, the greatest infection with *Fusarium* species occurred on later varieties (Planet – 62.6%; Arkas – 58.5%; Tjalve – 38.6%), less infection appeared on earlier varieties (Heta – 27.0%; Luja – 22.5%: Satu –19.1%) (Table 3).

The number of toxicants is decreased and the occurrence of toxins inhibited after immediate cleaning and fast drying of grain. According to the study of Finnish researchers (Ylimäki et al., 1979), drying of grain below 14% of moisture is an effective method for avoiding the occurrence of mycotoxins. In Finland grain for both seeds and consumption is dried to 13–14% of moisture. According to the data of 1989 of the company Bayer, the German authors generally consider harmless grain moisture of 10–13%. Russian authors (Jevseyeva, 1992; etc) also agree that grain at moisture of

10–13% has no more toxic problems even if it is with *F. graminearum* infection. Mishustin & Trisvyatskii (1963) claim that drying grain immediately after harvesting to moisture of 12–13% completely avoids the development of *F. graminearum* on the stored grain.

It is known that microorganisms do not develop in grain without unbound (free) water and are in a resting position or anabiosis. But Ominski et al. (1997) have observed that some storage fungi, most of all the species of *Penicillium* and *Aspergillus*, can develop in grain also without unbound water and produce toxins. Lillehoj & Elling (1983) confirm that *Penicillium* and *Aspergillus* sp. can develop in a storehouse also at 13–18% of grain moisture. But Christensen (1957, cit. by Detroy et al., 1971) suggests, in the case of *Aspergillus* sp., a moisture limit of even 13–14% and points out that moisture in grain should be taken to 13% within 24 hours after harvesting.

It is a general opinion that production of toxins in a storehouse starts on many fungus species when the grain moisture exceeds 13–16% (Bullermann et al., 1984). It happens in the case of a rather wide interval of temperature. For example, *Penicillium patulum* produces patulin at an interval of 0–24°C, *Aspergillus flavus* produces aflatoxin at 11–37°C. Thus the little fluctuation in temperatures is less essential for productoin of toxins than changes of moisture.

In Estonia feed for consumption is dried to 14–14.5%, seed yield to 13–13.5% of moisture. For more prolonged storing of grain it is dried at first to moisture of 12.5–13%, considering the later moisture increase in the grain at the cost of the air moisture. It is considered harmless when grain moisture does not exceed 14.5% at a temperature of 18–25°C (Maasik, 1999). When the moisture of the stored grain exceeds 15%, extra drying of the grain is required.

The data of the given references and our research results, where only 2 of the 6 wheat lots meet the requirements of food wheat, confirm that in Estonia the grain for consumption should also be dried more than it has been done so far (maybe to moisture of 12.5–13%).

In 1997–2002 in the mycoflora of feeds with spoilt signs (feed meal, crushed grain, concentrated feed, mixtures, etc.), mostly *Penicillium, Aspergillus, Cladosporium, Acremonium, Fusarium* and mycelial yeasts, often also the species of *Mucor, Alternaria* and *Trichothecium* occurred (Table 4). In the case of self-heating, also *Rhizopus nigricans* and *Paecilomyces varioti* Bain. were added. At the same time, the number of microfungi in feeds exceeded 10–100 times the standard of $4x10^4$–$8x10^4$ allowed in Germany, being often within $4x10^5$–$9x10^5$, even about $6x10^6$ in 2001 per 1g of dry grain. The relative importance of *Fusarium* species in the total number increased in some cases up to 6.8–8.2%, whereas also the toxic species *F. culmorum, F. tricinctum, F. verticillioides, F. sporotrichioides* etc. were presented (Lõiveke et al., 2003). Of *Penicillium* species, *P. cyclopium* Westling (cyclopenin, cyclopenol, citrinon, ochratoxin A) and *P. expansum, Aspergillus* from the genera *A. oryzae* (Ahlb.) Cohn (aflatoxin), *A. ochraceus* Wilhelm (aflatoxin, ochratoxin A), *A. fumigatus* (fumigacin, fumigatin, fumigallin, spinulosin) and *A. terreus* Thom (gliotoxin X, patulin, terrein, terreic acid), *Trichothecium roseum* Link (trichothecenes) were dominating. Such frequent occurrence of the named fungus genera on feeds becoming spoilt has also been found by Ylimäki (1981) in Finland.

Table 4. Number of microfungi per 1g of dry feed and dominating species in spoilt feeds.

Grain product	Year	Microfungi on maltagar	Main genus of microfungi	pH_{KCl}	Moisture %
Barley	1997	$9.1x10^5$	Yeasts, *Fusarium*	5.76	10.8
Barley	1997	$4.7x10^5$	Penicillium, Aspergillus, Fusarium	5.85	11.1
Oat	1997	$1.2x10^5$	Yeasts, *Penicillium, Aspergillus, Rhizopus, Fusarium*	6.72	13.9
Malt	1999	$4.8x10^4$	Yeasts, *Mucor, Cladosporium, Fusarium*	5.09	5.5
Meal	2000	$8.3x10^5$	Yeasts, *Penicillium, Alternaria, Cladosporium, Rhizopus, Fusarium*	6.08	11.2
Grain (dry original)	2000	$7.3x10^5$	Yeasts, *Penicillium, Acremonium, Fusarium*	6.65	17.1
Crushed grain	2001	$5.8x10^6$	Yeasts, *Aspergillus, Mucor, Penicillium*	4.51	36.0
Meal	2002	$3.3x10^4$	*Acremonium, Cladosporium, Penicillium*, yeasts, *Alternaria*	6.16	12.2

The toxicity of single fungus species found in feeds towards *B. stearothermophilus* was rather different (Table 2). In the years 2001 and 2002, the most toxic ones (growth inhibition 9–10 mm) were *Acremonium* sp., *Aspergillus terreus*, *Paecilomyces varioti*, *Rhizopus nigricans*, and *Mucor* sp. But *A. oryzae*, *Geotrichum candidum*, *Penicillium* sp. and some yeasts had moderate toxicity. The toxicity of *Fusarium* sp. varied, the growth inhibition being 0–10 mm (Lõiveke et al., 2003). The fungi of very high toxicity were ascertained in the spoilt feed samples of 1997 and 1998: in ensiled grain – *Trichothecium roseum* (growth inhibition 7–10 mm), in concentrated feed – *Penicillium oryzae* (8 mm), *P. cyclopium* (18 mm), in crushed grain – *Penicillium* sp. (13–15 mm).

Since the application of spoilt feeds caused health problems or death on livestock, we can claim that the toxins of microfungi are a real danger in both grain and feeds.

CONCLUSIONS

Mycoflora occurring in domestic grain (wheat) and grain feeds is rich and much similar to the one ascertained in Finland. 63 fungi species were found in all, including epiphytes of a saprophytic feeding type and phytopathogens of a parasitic feeding type. About half of all the fungus species are either potential toxicants or allergenic ones, which refers to bad quality of grain and feeds. The multitude of fungus flora indicates also possible agrotechnical mistakes in grain growing or post-harvest treating, as well as mistakes at storing grain and feeds. Although the occurrence of toxicants in grain does not always bring along the formation of toxins (Eskola et al., 2001), it is still the sign of a potential danger that was also confirmed by the biotests with *Bacillus stearothermophilus*. More attention should be paid especially to the final moisture at grain drying and storage conditions as otherwise it is impossible to obtain either qualitative feed or food grain.

On grain feeds becoming spoilt, the toxic species from the genera *Penicillium, Aspergillus, Acremonium, Cladosporium, Fusarium*, as well as toxic mycelial yeasts are becoming dominant. The monitoring of mycotoxins of the years 1998–2003 by the Agricultural Research Centre confirm that mycotoxins generated by toxic fungi can also occur in Estonian food and feed grain and also in grain products. According to this, in 1999 there were mycotoxins in 30.3% of domestic food grain and its product samples and in 58.1% of feed grain and feed mixtures samples. In 2000 the percentage of domestic food grain and products contaminated with mycotoxins was 51.6%, whereas the mycotoxins occurred also in feed mixtures, both in the domestic and imported ones.

According to the research work carried out to obtain high-class food and feed grain, measures for decreasing the number of toxic microfungi should be taken already at growing the grain in the field. Microfungi should be prevented from reproducing and producing toxins also in the following processes of grain treatment: transport, after-harvest cleaning, drying, storing, and processing.

In order to detect heavily contaminated lots with hazardous toxicants, a mycological and microbiological survey should be considered. For discovering contamination with mycotoxins, the pre-selection of samples analysed by the biotest on *Bacillus stearothermophilus* should be applied, and after that the specific toxins should be determined by accredited methods of analysing.

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