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FUNGI RECOVERED FROM SLOVAKIAN POULTRY FEED MIXTURES AND THEIR TOXINOGENITY

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Abstract: To contribute towards the knowledge of microbiology of feeds, more than 100 samples of poultry feed mixtures from Slovakia were mycologically investigated in terms of the overall fungal diversity and toxicological potential of isolated fungi. The study revealed that out of 22 genera recovered, *Penicillium* was the most frequent and diverse genus, followed by *Aspergillus* and *Mucor* being found in 89% (34 spp.), 69% (11 spp.) and 50% (4 spp.), respectively. The most frequently encountered taxa were *Fusarium proliferatum*, followed by *Penicillium aurantiogriseum*, *Mucor racemosus*, *Penicillium crustosum* and *Aspergillus flavus*. In addition, the following genera were recorded (in descending order) *Rhizopus* (44%, 3 spp.), *Eurotium* (42%, 5 spp.), *Fusarium* (42%, 3 spp.), *Cladosporium* (31%, 1 sp.), *Alternaria* (22%, 3 spp.), *Absidia* (16%, 3 spp.), *Acremonium* (12%, 2 spp.), *Scopulariopsis* (10%, 2 spp.), *Paecilomyces* (4%, 1 sp.), *Ulocladium* (3%, 1 sp.), *Trichoderma* (2%, 1 sp.), *Zygorrhynchus* (2%, 1 sp.), and finally *Emericella*, *Epicoccum*, *Geosmithia*, *Monascus*, *Stachybotrys*, *Syncephalastrum* and *Wardomyces*, all were encountered in 1% of the samples being represented by a single species. The mean value counts of total fungi ranged from 1×10^3 to 200×10^5 cfu/g. Outcomes from mycotoxin screening within the appropriate potentially toxinogenic species showed a number of mycotoxin producers, namely those forming aflatoxin B_1 (n=3), citrinin (17), cyclopiazonic acid (76), fumonisin B_1 (86), griseofulvin (42), moniliformin (18), ochratoxin A (5), patulin (56), penitrem A (30) and sterigmatocystin (10).

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INTRODUCTION

The relatively high intake of cereal material in the diet of poultry may have adverse effects on animal health and on productivity when mycotoxin-contaminated feeds are consumed [12]. Moreover, mycotoxins – through their potentially toxic residues in tainted poultry products, such as meat, liver or eggs - may also be harmful to human [5, 15, 40]. In contrast to an extensive range of reports monitoring significant *Aspergillus*, *Penicillium* and/or *Fusarium* mycotoxins in feeds including poultry feed mixtures [11, 13, 27, 28, 39, 48, 49], there is only a limited number of reports dealing with an overall fungal diversity in the feeds available, especially those mapping the situation in Europe. The main goal of this study therefore, was to investigate poultry feed mixtures used in chicken breeding in Slovakia from the mycological point of view, and thus contribute towards the knowledge of microbiology of this kind of feed. Special emphasis was laid on the ability of isolated fungi to produce some significant toxic extrolites – mycotoxins.

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MATERIAL AND METHODS

The samples. The samples of feed mixtures were sent to the State Veterinary Institution in Nitra from different poultry farms located in the south-western region of Slovakia (Nitra County) to be inspected by a preventive mycological examination in terms of enumeration and generic identification of the fungi recovered. To carry out a more detailed mycological investigation, a total of 108 samples, representing those for chickens, were simultaneously analysed at the department of microbiology (Slovak University of Agriculture, Nitra) during a 3-year-long period from May 2001 to June 2004. The samples (ca. 4-5 samples per month) were usually analysed immediately upon arrival or, if necessary, they were stored for 2-3 days in paper sacks at room temperature (22-25ºC). There was no closer specification available concerning the individual farms and conditions under which the feeds concerned were stored and/or handled.

Isolation of the fungi. Dilute plate technique was used for isolation of fungi from the samples according to Samson *et al.* [50]. A part of feed sample weighing 20 g was mixed with 180 ml of saline solution (0.85% sodium chloride) with 0.05% Tween 80 on a horizontal shaker for ca. 30 minutes. Then, 1 ml of appropriate dilutions made up to 10^{-5} was applied on Dichloran Chloramphenicol Peptone agar (DCPA, [4]) for *Fusarium* species, and *Aspergillus flavus*/*A. parasiticus* medium (AFPA) for potentially aflatoxinogenic species. Czapek-Dox agar (Imuna, Šarišské Michaľany) with 100 mg of Rose Bengal and malt extract agar (Imuna) with 100 mg of chloramphenicol were used for isolation of the other fungi and overall quantitative enumeration of fungal propagules referring to cfu (colony forming units) per gram of sample. After 5-7 days of incubation at 25ºC in dark, resulting colonies were counted and transferred onto appropriate identification media. All formulae used here are those given in Samson *et al.* [51].

Identification of *Aspergillus* and *Eurotium* species. Conidial suspensions were inoculated at 3 equidistant points, both on Czapek-yeast extract agar (CYA), Czapek-yeast with 20% Sucrose (CY20S), and malt extract agar (MEA), and incubated in the dark at 25ºC for 7-14 days. Species identification was carried out according to Klich and Pitt [30], Klich [29], Pitt and Hocking [46] and Samson *et al.* [51].

Identification of *Penicillium* species. The penicillia belonging to *Aspergilloides*, *Furcatum* and *Biverticillium* subgenera were inoculated at 3 equidistant points, both on Czapek-yeast extract agar (CYA) and malt extract agar (MEA), and incubated in the dark at 25ºC. Sub-cultivation on CYA at 37ºC was also used. In addition, Creatine Sucrose agar (CREA) was used for isolate belonging to *P. glabrum*, *P. spinulosum* and *P. purpurascens*. The penicillia representing subgenus *Penicillium* were inoculated on CYA, MEA, CREA and Yeast Sucrose agar (YES), and incubated at 25ºC in the dark. Species identification was carried out after 7 days according to Pitt [43, 44], Pitt & Hocking [46], Samson *et al.* [51], and Frisvad & Samson [22].

Ehrlich test. This method was use for the identification of the penicillia belonging to the subgenus *Penicillium* producing cyclopiazonic acid or related alkaloids [35]. An agar plug was cut from the colony centre of 1-week old cultures of CREA-positive isolates growing on CYA and then placed, mycelium side up, on a Petri dish lid. A strip (12 × 6 mm) of Whatman No. 1 filter paper was dipped in Ehrlich reagent consisting of 4-dimetylaminobenzaldehyde 2 g, 96% ethanol 85 ml, 10 N HCl 15 ml, and placed across the agar plug, with an agar plug in the centre of the strip. The arrangement was then placed in an air stream in a fume hood and allowed to dry. A violet ring in the strip within 10 min indicated a positive response.

Identification of *Fusarium* species. Potato Dextrose agar (PDA) was used for observation of colony characteristics. Synthetic nutrient agar (Synthetischer nährstoffarmer agar, SNA) was used for micro-morphological observation. Cultures were incubated at 25ºC in dark (PDA) and UV-light 365 nm (SNA). Species identification was carried out after 7 days according to Burgess *et al.* [10], Nelson *et al.* [38], Pitt and Hocking [46], and Samson *et al.* [51].

Identification of *Alternaria* species. Potato-carrot agar (PCA) was used for examination of morphological traits (sporulation pattern and conidia morphology). Cultures were incubated under alternating light/dark cycle consisting of 8 h of white-cool light, followed by 16 h darkness at 25ºC. Species identification was carried out according to Andersen *et al.* [2] and Simmons [54, 55].

Identification of other fungi. CYA and MEA were used for species identification of all other fungi. As additional media, PDA was used for *Epicoccum nigrum* identification. Cultures were incubated at 25ºC for 7 days. The MEA cultures of *Geosmithia putterillii* were incubated at 20ºC. Species identification was carried out according to Domsch *et al.* [16], Ellis [17], Pitt and Hocking [46], and Samson *et al.* [51].

Mycotoxins screening by a modified agar plug method. The *Aspergillus* and *Penicillium* mycotoxins, i.e. aflatoxin B_1 and G_1 (AB_1, AG_1), citrinin (C), cyclopiazonic acid (CA), griseofulvin (G), ochratoxin A (OA), patulin (P), penitrem A (PA) and sterigmatocystin (S) were screened by a method adapted from Samson *et al.* [52].

To detect AB_1, AG_1, C, G, OA and P, 3 small pieces (each ca. 3 × 3 mm) were cut from the colony growing on YES at 7 and/or 14 d and placed into a small 4 ml screw vial. To detect CA, PA and S the plugs were removed

Table 1. Fungi recovered from Slovakian poultry feed mixtures.

Fungi	Samples	Frequency %	Isolates*
Zygomycetes			
Absidia corymbifera	9	8	36
A. glauca	1	1	1
A. sp.	6	6	9
Mucor circinelloides	20	18.5	42
M. hiemalis	14	13	64
M. racemosus	36	33	208
Mucor sp.	15	14	126
Rhizopus oryzae	8	7	47
R. stolonifer	29	27	55
R. sp.	15	14	63
Syncephalastrum racemosum	1	1	1
Zygorrhynchus moelleri	2	3	11
Ascomycetes			
Emericella nidulans	1	1	1
Eurotium amstelodami	14	13	78
E. chevalieri	11	10	25
E. repens	19	18	177
E. rubrum	1	1	3
E. sp.	10	10	34
Monascus ruber	1	1	2
Mitosporic fungi			
Acremonium strictum	2	2	2
Ac. sp.	9	8	36
Alternaria alternata	5	5	8
A. tenuissima	1	1	1
Al. sp.	20	19	51
Aspergillus candidus	30	28	325
A. clavatus	5	5	10
A. flavus	32	30	188
A. fumigatus	24	22	174
A. niger	8	7	14
A. ochraceus	6	6	31
A. sp.	19	18	45
A. sydowii	5	5	9
A. terreus	9	8	20
A. ustus	3	3	4
A. versicolor	13	12	33
Cladosporium cladosporioides	33	31	195
Epicoccum nigrum	1	1	3
Fusarium oxysporum	2	2	2
F. proliferatum	42	39	1,720
F. subglutinans	12	11	395
Geosmithia putterillii	1	1	1
Paecilomyces variotii	4	4	4
Penicillium subg *Aspergilloides*			
P. capsulatum	1	1	2
P. glabrum	1	1	3
P. purpurascens	7	6.5	8
P. spinulosum	7	6.5	20
Penicillium subg *Furcatum*			
P. citrinum	5	5	8
P. corylophilum	7	6.5	30
P. janthinellum	1	1	3
P. jensenii	2	2	2
P. fellutanum	4	4	36
P. mandriti	1	1	1
P. oxalicum	3	3	5
P. raistrickii	1	1	1
P. waksmanii	1	1	2
Penicillium subg *Penicillium*			
P. aurantiogriseum	37	34	202
P. brevicompactum	12	11	19
P. chrysogenum	24	2	60
P. commune	4	4	13
P. coprophilum	1	1	5
P. crustosum	34	6.5	210
P. dipodomyicola	1	1	1
P. expansum	7	6.5	7
P. griseofulvum	12	11	51
P. italicum	2	2	1
P. olsonii	1	1	1
P. palitans	1	1	8
P. polonicum	2	2	10
P. solitum	7	6.5	118
P. verrucosum	1	1	2
P. viridicatum	2	2	5
Penicillium subg *Biverticillium*			
P. funiculosum	1	1	1
P. islandicum	1	1	3
P. minioluteum	1	1	1
P. purpurogenum	2	2	5
P. variabile	6	6	8
Scopulariopsis brevicaulis	8	7	13
S. sp.	3	3	5
Stachybotrys chartarum	1	1	1
Trichoderma sp.	2	2	2
Ulocladium sp.	3	3	4
Wardomyces anomalus	1	1	1

* a total of 5,132 isolates were recovered

from the colonies growing on CYA or CY20S. Then, 0.5 ml of extraction solvent (chloroform:methanol, 2:1, v/v) was added to the vial containing the agar plugs and shaken on a vortex at least for 1 min. Twenty microliter aliquots, along with the standards, were applied after–wards to the TLC plate (Silicagel 60, Merck, Germany) being spotted 1 cm apart. Consequently, the spots were dried, and the plates developed in solvent system toluene:ethylacetate:formic acid (5:4:1 v/v/v); this gave an average Rf value of 0.3 for AB_1, 0.2 (AG_1), 0-0.68 (C), 0.53 (G), 0.45 (OA), 0.58-0.90 (CA), 0.48 (P), 0.7 (PA) and 0.6 (S). The *Fusarium* mycotoxins, i.e. fumonisin B_1 (FB_1) and moniliformin (M) were screened according to Mubatanhema *et al.* [37].

The procedure was essentially the same as above except the YES plugs were extracted with 1 ml of acetonitrile:water (1:1, v/v) and butanol:acetic acid:water (20:10:7, v/v/v), giving an average Rf value of 0.64 for FB_1 and 0.68 for M was used as a solvent system.

Mycotoxin visualisation. Under UV-light (365 nm), some of the mycotoxins were directly detectable as coloured spots, namely AB_1 (blue), AG_1 (green), C (yellow green-tailed), G (blue), OA (bluish-green) and S (reddish). CA was visualized by spraying with the Ehrlich reagent [35] and after drying detected as a violet-tailed spot in daylight. P was visualized by spraying with 0.5% methylbenzothiazolone hydrochloride (MBTH, Merck, Germany) in methanol, heated at 130ºC for 8 min and then detectable as a yellow-orange spot. PA was visualized by spraying with 20% $AlCl_3$ in 60% ethanol, heated at 130ºC for 8 min and then viewed under daylight as a dark green to black spot. FB_1 was visualized by spraying with 0.5% p-anysaldehyde in methanol:acetic acid:conc. sulfuric acid (17:2:1 v/v/v), heated at 120ºC for 2-3 min and then appeared as a reddish-purple spot under daylight. M was visualized by spraying with the MBTH, heated at 120ºC for 2-3 min and appeared as very faint yellowish-orange spot under daylight, and showing orange fluorescence under UV.

In addition to the screening technique, isolates of the *Aspergillus flavus* group were simultaneously tested for their ability to produce aflatoxins using the method by Abarca *et al.* [1] in liquid medium consisting 20% sucrose and 2% yeast extract. After 14 d incubation at 28ºC in dark, 20 µl of the broth were directly applied onto the plate and let to elute, as described above.

Mycotoxin standards. The mycotoxin standards, except patulin (Calbiochem, USA), were obtained from Sigma (Germany).

RESULTS AND DISCUSSION

Fungal counts observed on malt extract agar ranged from 1×10^2 to 8.2×10^4 cfu.g^{-1} of sample, with an average 1.8×10^3 cfu.g^{-1} of sample. Similar results concerning the fungal counts in poultry feed mixtures from Turkey have been reported by Heperkan and Alperden [26], from Spain by Bragulat *et al.* [9], and from Argentina by Dalcero *et al.* [12, 14]. On a single occasion the overall fungal counts exceeded 30×10^3 cfu/g of sample, which is the maximal allowable amounts of the cfu for the poultry feed in general in Slovakia. Altogether, 5,132 isolates belonging to 84 fungal species (including 12 fungi determined to a genus level only) representing 22 genera were recovered during this study. The incidence and total number of isolated fungi in Slovakian poultry feed mixtures are presented in Figure 1 and Table 1, and the ability to produce appropriate mycotoxins in Table 2.

From the systematic point of view, 5 genera belong to *Zygomycetes* (i.e. *Absidia*, *Mucor*, *Rhizopus*, *Syncephalastrum* and *Zygorrhynchus*), 3 genera belong to *Ascomycetes* (i.e. *Emericella*, *Eurotium* and *Monascus*); the majority, within so-called mitotic fungi (formerly *Deuteromycetes*), encompassed 12 genera (i.e. *Acremonium*, *Alternaria*, *Aspergillus*, *Fusarium*, *Geosmithia*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma*, *Ulocladium* and *Wardomyces*). The most frequent fungi were those from the genus *Penicillium*, recovered from 96 samples (89%) and representing 34 taxa in all. Of the all fungi encountered, *Fusarium proliferatum* (found in 39%), *Penicillium aurantiogriseum* (34%), *Mucor racemosus* (33%), *Penicillium crustosum* (32%) and *Aspergillus flavus* (30%) appeared to be the most prevalent. Along with results from the similar studies reported by Heperkan and Alperden [26], Bragulat *et al.* [9], Dalcero *et al.* [12], it may be stated that *Aspergillus* (including *Eurotium*), *Penicillium* and *Fusarium* are the typical fungal genera inhabiting poultry feed mixtures. In fact, they are very important contaminants being renowned for their ability to form a huge number of various types of toxic extrolites-mycotoxins [22, 24]. The isolates belonging to the subgenus *Penicillium*, so-called terverticillate penicillia, were by far the most dominating fungi among the penicillia in the studied samples, which is in accordance with Frisvad *et al.* [21] who linked their primary ecological habitats with animal feed and/or excrements. These species are the main contaminants of stored cereals as well as feeds with worldwide occurrence [18, 33, 45] being producers of toxicologically significant mycotoxins [20, 23, 24]. Representatives of all 4 *Penicillium* subgenera were detected, namely *Aspergilloides* in 15% of samples represented by 4 species, *Furcatum* in 20% of samples by 9 species, *Penicillium* in 80% by 16 species

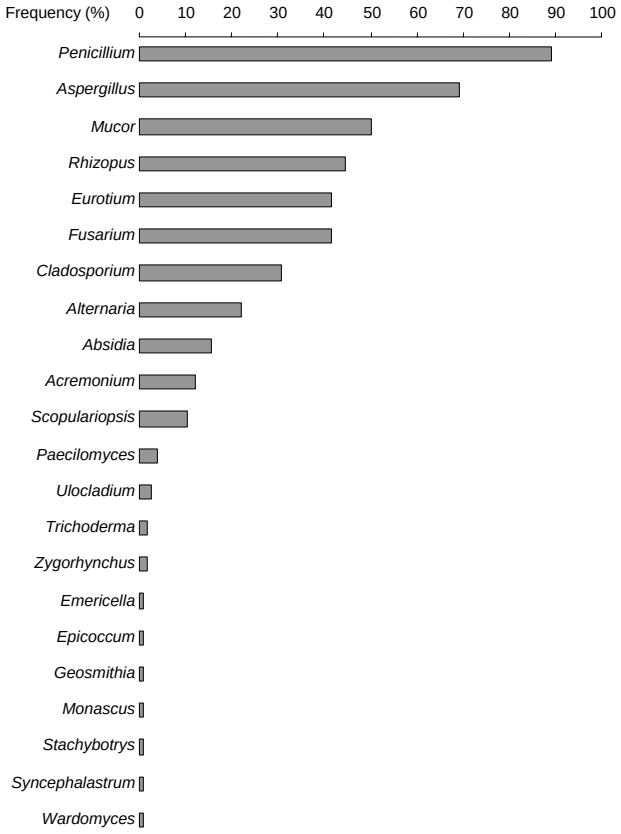

Figure 1. Frequency of fungal genera recovered from Slovakian poultry feed mixtures.

and *Biverticillium* in 12% by 5 species. In their work dealing with Turkish poultry feed mixtures and feed ingredients, Heperkan and Alperden [26] found that *P. aurantiogriseum* was the main fungal contaminant, being encountered in 59% of samples, followed by *Aspergillus flavus* (48%) and *A. niger* (23%). Likewise, in a study reported by Leistner [33] dealing with toxinogenic penicillia in feed and food, *P. aurantiogriseum* was the most widely isolated species. According to this author, *Penicillium* isolates determined in feed or food-related habitats should be considered as potential mycotoxin producers. Actually, a great number of the *Penicillium* isolates tested during this study were proved to be producers of significant mycotoxins including patulin, citrinin, cyclopiazonic acid, ochratoxin A or griseofulvin. An overall list of mycotoxin-producing fungi recovered from the feed samples during this study is given in Table 2. The high occurrence frequency of *P. crustosum* should be taken into consideration for the sake of its ability to produce powerful neurotoxin penitrem A [19, 22, 51]. According to Pitt and Hocking [46], the presence of *P. crustosum* in food or feed should be regarded as a warning signal, since nearly all investigated isolates of the species showed the ability to produce the mycotoxin in high levels. Indeed, each of *P. crustosum* isolates (n=30) tested produced penitrem A here as well.

The commonest mycotoxin produced by the penicillia during this study appeared to be griseofulvin, cyclopiazonic acid and patulin being detected in all 40 *P. griseofulvum* isolates tested. Likewise, all isolates of *P. expansum* (n=7) proved to be toxinogenic for patulin and citrinin, as well as for some non-specified indole metabolites, as indicated by positive reaction with the Ehrlich [35]. Within the alkaloids produced by *P. expansum* belong chaetoglobosins and/or communesins [4]. The patulin production was observed in all 6 *Aspergillus clavatus* isolates. In addition, *A. terreus* is considered as a producer of both citrinin and patulin [51], yet only patulin was detected in 2 out of 9 strains screened here. Citrinin along with ochratoxin A was detected in 2 *Penicillium verrucosum* isolates recovered. *P. verrusosum* is so far the only species within the genus *Penicillium* know as being the producer of both citrinin and ochratoxin A [23]. Citrinin production was also observed in all 8 *P. citrinum* isolates. The ochratoxin A and citrinin along with other significant nephrotoxins potentially produced by species within *P. aurantiogriseum* complex, such as vioxanthin, viomelein or xanthomegnin [22] may play a crucial role in nephropathy syndrome in both animals and humans [24, 44]. In addition to *P. verrucosum*, ochratoxin A was also found in 3 out of 7 *Aspergillus ochraceus* isolates, while in none out of 10 *A. niger* isolates. The

Table 2. Mycotoxin production by fungi recovered from Slovakian poultry feed mixtures.

Fungi	AB_1	AG_1	C	CA	FB_1	G	M	OA	P	PA	S
Aspergillus flavus	*3/187**	0/187		20/20							
A. clavatus									6/6		
A. ochraceus								3/7			
A. niger								0/10			
A. sydowii											2/2
A. terreus			0/9						2/9		
A. versicolor											5/5
Emericella nidulans											3/3
Eurotium amstelodami											0/15
E. chevalieri											0/10
E. repens											0/17
E. rubrum											0/2
Fusarium proliferatum					86/86		0/86				
F. subglutinans					0/36		18/36				
Monascus ruber			0/2								
Penicillium citrinum			8/8								
P. commune				10/10							
P. crustosum										30/30	
P. dipodomyicola				1/1		1/1			1/1		
P. expansum			7/7						7/7		
P. griseofulvum				40/40		40/40			40/40		
P. palitans				5/5							
P. raistrickii						1/1					
P. verrucosum			2/2					2/2			

AB_1 aflatoxin B_1, AG_1 aflatoxin G_1, C citrinin, CA cyclopiazonic acid, FB_1 fumonisin B_1, G griseofulvin, M moniliformin, OA ochratoxin A, P patulin, PA penitrem A, S sterigmatocystin; * positive isolates; ** only isolates of the appropriate potentially toxinogenic species were screened.

identification of the black aspergilli within *A. niger* complex, based solely on the traditional morphological approach, is difficult due to taxonomic changes in the course of the past 20 years [53]. A population of the black aspergilli found in the samples analysed here fits perfectly with the species concept of *A. niger* sensu Klich [29]. Considering other significant *Aspergillus* species, the main contaminating fungus appeared to be *A. flavus*, a potentially toxinogenic species for the aflatoxins. Likewise, the species has showen high occurrence frequency in the studies of Heperkan and Alperden [26], Magnoli *et al.* [36], and Dalcero *et al.* [14]. However, another aflatoxin producer, *A. parasitisus*, a very frequently encountered fungus in the feed mixture samples of 2 later studies from Argentina, has not appeared at all in the Turkish samples studied by Heperkan and Alperden [26], nor in the present study with Slovakian samples. As for the ability to produce carcinogenic aflatoxins, only 3 out of 187 *A. flavus* isolates (1.6%) appeared to produce aflatoxin B_1 by the screening methods used here. Likewise, Piecková and Jesenská [42] tested 136 *A. flavus* isolates recovered from various maize products, including maize grains of Slovakian origin, for their ability to produce aflatoxin B_1, but all with negative results. In contrast, a high percentage of aflatoxin-producing strains of *A. flavus* and/or *A. parasiticus* were found in Argentinean feed mixture samples [36], along with the levels of aflatoxins directly occurring in the samples reported by Cespedes and Diaz [11] and/or Dalcero *et al.* [14]. On the other hand, all *A. flavus* isolates (n=20) tested in the course of this study produced cyclopiazonic acid, which with the other cyclopiazonic acid producers encountered, such as *P. commune* (n=10), *P. griseofulvum* (n=40), *P. palitans* (n=5), or even the rare *P. dipodomyicola* (n=1), may represent a higher concern from the toxicological point of view then for aflatoxin. Vaamonde *et al.* [59] recently reported 13% aflatoxinogenic *A. flavus* isolates recovered from wheat samples, while 93% of them produced cyclopiazonic acid. Mycotoxin has been detected in naturally contaminated feeds, corn, peanuts, and other foods and feeds [47]. Also, sterigmatocystin, as another significant mycotoxin of carcinogenic moiety [56], was detected in all *Aspergillus versicolor* (n=5), *A. sydowii* (n=2) and *Emericella nidullans* (n=3) isolates encountered. In their reviews, Frisvad [19] as well as Smith and Ross [56], mentioned also some common species within the genus *Eurotium* and *Aspergillus terreus* as being sterigmatocystin producers. However, neither *A. terreus* (n=9) or *Eurotium* species (n=44) isolates appeared to produce the toxin during this study even after 30 d incubation on both CYA and CY20S. On the other hand, the production of sterigmatocystin has not been convincingly confirmed by any of the claimed *Eurotium* species (Frisvad, personal communication). In order, the third mostly encountered fungi were representatives of the genus *Mucor*. Within the genus, 4 taxa were identified, of which *M. racemosus* predominated. With the lesser frequency of 44%, the representatives of the genus *Rhizopus* were found, with the most common *R. stolonifer* presented in 27% of samples. Very interestingly, in their study, Varga *et al.* [60] reported the ability of *Mucor* and *Rhizopus* strains to degrade some significant mycotoxins, such as ochratoxin A, patulin or zearalenone, under laboratory conditions. The *Eurotium* and *Fusarium* genera were also widespread through the samples occurring with the same frequency of 42%. Among the *Eurotium* spp., *E. repens* was the most frequent, being found in 18% of samples. Contrary to this, the following genera *Emericella*, *Geosmithia*, *Monascus*, *Stachybotrys*, *Syncephalastrum* and *Wardomyces* were found only in 1% of samples and represented by a single species. Of the mentioned fungi, *Monascus ruber* has been reported as being a citrinin producer [25], yet none of two *M. ruber* isolates recovered from the samples produced this toxin. A matter for concern is undoubtedly the findings of fumonisin B_1 producing *Fusarium proliferatum* isolates detected in all 86 isolates tested (100%), even though no moniliformin was detected within these strains. The later mycotoxin was detected in half of 36 *F. subglutinans* isolates tested. Of the fusaria, and also even among all the fungi encountered, *F. proliferatum* was by far the most frequent (39% of samples) and numerous fungus recovered here. The species is renowned for its role as an important maize ears pathogen in Europe [34, 41], including Slovakia [57], during the vegetation period and therefore contamination of maize or maize-based products by appropriate mycotoxins may be expected. Likewise, the same situation is in the case of wheat grains, as reported by Bottalico [7], Bottalico and Perrone [8], Tomczak *et al.* [58], and Birzele *et al.* [6]. The incidence of significant *Fusarium* mycotoxins, namely fumonisins, A and B trichothecenes, zearalenone and moniliformin in 50 samples of poultry feed mixtures of Slovakian origin were analysed by Labuda *et al.* [31, 32], being found with comparatively high frequency, albeit in relatively low concentrations. The outcomes of this study clearly show that feed mixtures used for poultry feeding in Slovakia represent a rich source of significant mycotoxin producers, especially those from the *Penicillium*, *Fusarium* and *Aspergillus* genera.

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