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MYCOBIOTA AND MYCOTOXIC CONTAMINATION OF FEED CEREALS

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ABSTRACT

The cereals are a suitable substrate for the growth of microscopic filamentous fungi. Micromycetes are capable of reducing the nutritional value of feedstuff and they can produce several mycotoxins. The most frequent genera of microscopic filamentous fungi are *Fusarium*, *Penicillium*, *Alternaria* and *Aspergillus*. The contamination by microscopic fungi and mycotoxins was determinated in 56 samples of feed cereals originating from the Slovak Republic. The most common genera of fungi detected in the feed cereals included: *Alternaria* (67.8 %), *Fusarium* (44.6 %), *Penicillium* (39.2 %), *Mucor* (30.3 %), *Rhizopus* (28.5 %), *Cladosporium* (21.4 %), *Scopulariopsis* (8.9 %) and *Aspergillus* (1.7 %). Deoxynivalenol was present in 24 samples (42.8 %) and zearalenone in 15 samples (26.7 %). The values of both mycotoxins did not reach the regulatory limits and thus they do not pose a risk to livestock nutrition.

Key words: cereals; deoxynivalenol; ELISA; microscopic filamentous fungi; zearalenone

INTRODUCTION

Food and feed commodities are often contaminated by microscopic filamentous fungi. The most common substrates for microscopic filamentous fungi growth are cereals (wheat, corn, barley and other) (Fig. 1). Cereal grains may become contaminated during growth, storage or processing. The filamentous microscopic fungi have been classified into two groups: field fungi and storage fungi. The first group includes the genus *Fusarium* (Fig. 2), *Alternaria* and *Cladosporium*. The genera *Aspergillus* and *Penicillium* are known as storage fungi [18]. Approximately 20—45 % of the world cereal production is contaminated by storage fungi [30]. Some of these microscopic filamentous fungi produce metabolites — mycotoxins that have no biochemical significance in fungal growth or development [36]. The most critical environmental factors that determine mycotoxins production in a substrate are temperature, relative humidity and pH. As another environmental pollutant, mycotoxins also can adversely affect the health and productivity in animals [27, 45]. In farm animals, mycotoxin-containing feed can cause mycotoxicoses and may induce

Fig. 1. The wheat contaminated with *Fusarium* spp.

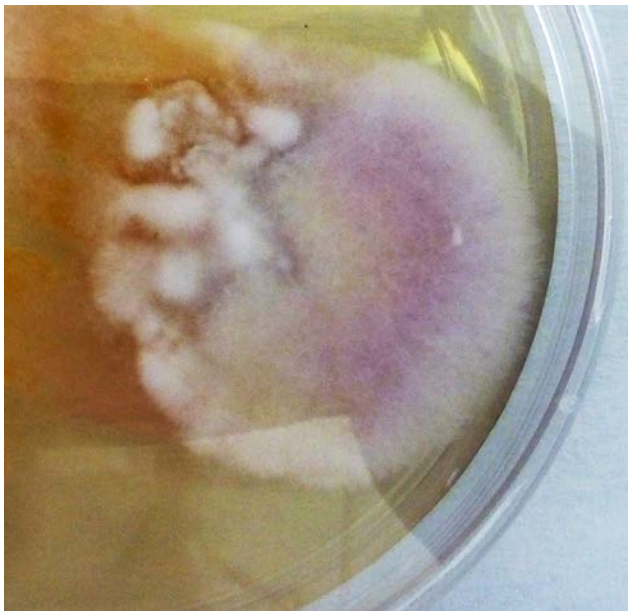

Fig. 2. *Fusarium* spp. on potato dextrose agar plate

health disorders and mortality in animals [23, 37]. Mycotoxins exhibit toxic actions that are characterized by their carcinogenic, mutagenic, teratogenic and estrogenic properties. [12, 16, 26]. A secondary contamination of human consumers by residues in eggs, meat, or milk has a significant importance [37]. The data of the Food and Agriculture Organization reveal that about 25 % of foodstuffs produced worldwide are contaminated with mycotoxins [17]. More than 350 mycotoxins in nature have been identified so far. Aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol and others) zearalenone and fumonisins are important in agriculture [38]. Deoxynivalenol is one of the most frequent contaminant in cereals in Europe, Asia and the Mediterranean [3]. It has been shown that deoxynivalenol and fumonisins decrease the height of intestinal villi and the surface of the intestinal mucosa [4]. Deoxynivalenol reduces intestinal cell proliferation and the protective function of the intestinal mucosa. Fumonisins and deoxynivalenol increase the invasion of *Salmonella* spp. and *E. coli* and they are also predisposing factors of necrotic enteritis. In infections caused by coccidia, deoxynivalenol and fumonisins increase the amount of lesions in the colon and the number of oocysts in the faeces [1, 40]. Zearalenone, the world's most pervasive mycotoxin, contaminate about 32 % of the cereals and their products [2]. It occurs mainly in maize, oats, rye, rice, sorghum and wheat [11]. Zearalenone has the structure of non-steroidal acid resorcyclic lactone, similar to the steroid hormones, which increases its ability to bind to oestrogen receptors. It acts as an agonist, and partial antagonist of estradiol. It inhibits the secretion of follicle stimulating hormone (FSH) and inhibits the maturation of ovarian follicles in the pre-ovulation phase [35]. Pigs are particularly sensitive compared to other animal species. In their liver the biotransformation of zearalenone and the synthesis of α-zearalenol takes place, which is more toxic than zearalenone alone [33]. The feeding of zearalenone containing feed is closely related to the occurrence of changes in the genital apparatus in females, such as ovarian atrophy, decrease in the number of litters and reduced birth weight of piglets. In males, atrophy of testes has been observed [21].

The complete compound feed used for livestock fattening is a mixture of feed cereals and vitamin-mineral supplements. The presence of cereals in these mixtures involves the risk of the occurrence of microscopic filamentous fungi and mycotoxins.

The purpose of our study was to determine the mycological contamination and to detect the presence of mycotoxins deoxynivalenol and zearalenone in the feed cereals (wheat, barley, corn and oats).

MATERIALS AND METHODS

Samples

Fifty-six samples of feed cereals were used for this analysis. Thirty-two samples were of wheat (*Triticum aestivum*), 16 samples of corn (*Zea mays* spp. *Mays*), 6 samples of barley (*Hordeum sativum*) and 2 samples of oats (*Avena sativa*). The samples were obtained from Tajba a. s. (Čečejovce, Slovak Republic) after harvesting in 2016 and 2017.

Cultivation and microscopy

Endogenous contamination

The endogenous fungal contamination was determined by the direct cultivation of the grains on the following agar media: PDA (potato dextrose agar), DRBC (agar containing dichloran, chloramphenicol and bengal rose), DPCA (dichloran chloramophenicol peptone agar) and SDA (Sabouraud dextrose agar) (HiMedia, Čaderský-Envitek, spol. s r.o., Brno, Czech Republic). The cultivation was carried out for 7 days at 25 ± 1 °C. To determine the species, the identification based on macroscopic and microscopic characteristics according to Burgess et al. [6], Samson et al. [42] and Leslie and Summerell [32] were used:

Macroscopic features were determined by:

rate of colonies growth, the shape of the colonies, edge of the colonies, surface of the colonies, colour of the colonies, creation and elimination of pigments and formation of exudates on the colonies surface.

Microscopic characters were determened by:

the presence of unborn spores, their shape and size, the method of forming and arranging spores, type of vegetative structure, its shape and layout, the presence of sex structures and spores, the presence or absence of sclerosis, sporodiachia and chlamydospore.

Calculation of parameters:

The isolation frequency (Fr) and relative density (RD) of genera were calculated according to Gonzalez et al. [19] as follows:

$$Fr\ (\%) = (ns/N) \times 100$$

ns — number of samples with a genus

N — total number of samples

$$RD\ (\%) = (ni/Ni) \times 100$$

ni — number of isolates of a genus

Ni — total number of fungi isolates

Mycotoxins analysis

The preparation and extraction procedures of samples were made according to the manufacturer's protocol (Veratox for deoxynivalenol, Veratox for zearalenone; Neogene Corporation). Cereal samples for the determination of deoxynivalenol were processed as follows: 10 grams of each sample was ground and mixed with 100 ml of distilled water. The mixing of the samples on a shaker (Orbital Shaker-Biosan) for 3 minutes was followed by filtration through Whatman 1 filter paper. The filtrates were dissolved with distilled water in a ratio of 1 : 2. The sample preparation for the determination of zearalenone was carried out as follows: the cereal samples were milled, followed by adding 25 ml of 70 % methanol (MIKROCHEM, Ltd., Pezinok, Slovak Republic) to 5 g of the sample. The samples were then mixed on a shaker for 3 minutes. After mixing, they were filtered through Whatman 1 filter paper and diluted with distilled water in a ratio of 1 : 5. The diluted samples were prepared for a quantitative assay, which was performed by ELISA and evaluated by an ELISA reader (Dynex Technologies).

RESULTS

Table 1 shows the occurrence of the mould's genus and their relative density in the feed cereals. In the analysed samples the Fr (%) of *Alternaria* (67.8 %) was the highest frequency recorded. The *Fusarium* genus (44.6 %) was identified in 25 samples. The *Penicillium* genus (39.2 %) was found in 22 samples. *Mucor* (30.3 %) was diagnosed in 17 samples, *Rhizopus* (28.5 %) in 16 samples and *Cladosporium* in 12 samples (21.4 %). The genus *Scopulariopsis* was detected in 5 samples (8.9 %) and in 1 sample (1.7 %) the genus *Aspergillus* was found. The relative density RD (%) of the genus *Alternaria* was 27.9 %, and of the genus *Fusarium* 18.3 %. The RD of other genera of moulds reached values from 0.1 % to 16.1 % (Tab. 1).

Tables 2 and 3 show the incidence and concentration of DON and ZEA in the samples. Deoxynivalenol was found in 24 samples (42.8 %). The highest concentration of deoxynivalenol was recorded in the samples of corn

Table 1. Fungal genera present in feed cereals samples

Genus	Number of isolates	Fr [%]	RD [%]
Penicillium	22	39.2	16.1
Cladosporium	12	21.4	8.8
Rhizopus	16	28.5	11.7
Scopulariopsis	5	8.9	3.6
Mucor	17	30.3	12.5
Alternaria	38	67.8	27.9
Fusarium	25	44.6	18.3
Aspergillus	1	1.7	0.1

Table 2. The presence of deoxynivalenol in feed cereals samples

Feed cereals	n/n*	I [%]	Concentration [ppm]
Wheat	32/8	25.0	0.021—0.596
Corn	16/14	87.5	0.183—1.925
Barley	6/2	33.3	0.019—0.052
Oats	2/nd	nd	nd

n—number of investigated samples; n*—number of positive samples; I— incidence; nd—not detected

Table 3. The presence of zearalenone in feed cereals samples

Feed cereals	n/n*	I [%]	Concentration [ppm]
Wheat	32/nd	nd	nd
Corn	16/14	87.5	2.043—1457,652
Barley	6/nd	nd	nd
Oats	2/1	50.0	367,363

n —number of investigated samples; n*—number of positive samples; I – incidence; nd—not detected

(1.925 ppm). None of the samples exceeded the regulatory limits of deoxynivalenol contamination (5 mg); valid in the EU. Fifteen samples (26.7 %) were zearalenone positive, with the highest detected level in the samples of corn (1457.652 ppb). In the samples of wheat and barley zearalenone was not present.

DISCUSSION

The contamination of animal feed by microscopic filamentous fungi a nd their secondary metabolites (mycotoxins) is one of the major threats to human and animal health [7]. In our study eight mould genera were recovered, three of them may produce mycotoxins [22]. The most commonly detected genera of fungi included: *Alternaria* (67.8 %), *Fusarium* (44.6 %), *Penicillium* (39.2 %), *Mucor* (30.3 %), *Rhizopus* (28.5 %), *Cladosporium* (21.4 %), *Scopulariopsis* (8.9 %) and *Aspergillus* (1.7 %). Almost the same results were presented in a study by Dancea et. al. [14]; the micromycete species with the highest frequency in samples from Transylvania (Romania) were: *Fusarium*, *Aspergillus*, *Alternaria*, *Penicillium* and *Rhizopus*. Tančinova et. al. [43] analyzed the fungal contamination of stored wheat from Slovakia and the most frequent genera were: *Aspergillus*, *Penicillium* and *Cladosporium* (90 % positive samples), followed by *Alternaria* (81 %) and *Fusarium* (54 %). The most predominant microscopic fungi in Pakistan were found to be the *Aspergillus* species, followed by *Penicillium*, *Fusarium* and *Alternaria* [41]. According to Labuda and Tančinova [29] *Fusarium*, *Aspergillus*, *Mucor* and *Rhizopus* were typical fungi contaminating feed mixtures. The most frequently found genera of moulds in cereal samples from Poland and eastern Slovakia were *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus* [9, 10].

According to our study, DON was present in 24 samples (42.8 %). In positive samples of wheat, the incidence of deoxynivalenol was 25.0 % with a range of 0.021—0.596 ppm. The occurrence of DON in corn was 87.5 % and the concentrations were 0.183—1.925 ppm and in barley was detected DON in 2 samples (33.3 %) with concentrations of 0.019—0.052 ppm. Deoxynivalenol was not present in samples of oats. Similar results of wheat contamination with deoxynivalenol have been reported by several studies. Fifty five wheat samples were examined in Serbia and the resulting incidence of deoxynivalenol was 34.5 % with a maximum DON concentration of 0.420 ppm [24]. The incidence of deoxynivalenol in Norway was 29.4 % and the maximum mycotoxin level was 0.890 ppm [44]. However, in the Czech Republic, the deoxynivalenol content was up to 100 % with a maximum level of 2,265 ppm [20]. The occurrence 100 % of DON was also reported in Lithuania and the maximum deoxynivalenol concentration was 2,230 ppm [34]. In the corn samples of Argentina DON

was detected in 19.0 % with a maximum of 0.834 ppm [5]. The study in Spain showed 26.8 % DON contamination of orn. The concentrations ranged from 0.026 to 0.131 ppm [8]. K r y s i ń s k a – T r a c z y k [28] has reported 75.0 % deoxynivalenol contamination of maize samples in Poland. Although this study did not record the occurrence of DON in samples of oats, other studies of Poland, Lithuania and Norway confirm the incidence of deoxynivalenol in oats [31, 34, 39]. In our study, zearalenone was detected in 15 samples (26.7 %) of feed cereals. Fourteen samples of aize and 1 sample of oats contained ZEA. In samples of wheat and barley zearalenone was not present. The concentrations in corn ranged from 2 to 1457 ppb and in oats the maximum level achieved was 367,363 ppb. Other authors detected ZEA only in 3 out of 300 samples [13]. However, J a r a m i l l o [25] found zearalenone in most of the samples and levels of zearalenone ranged between 100 and 7000 ppb. In Croatia the presence of zearalenone reached 83.6 % in samples of the corn with a maximum level of 2.54 ppb [15]. The occurrence of ZEA (15 %) was confirmed also in Morocco in corn [46]. However, in a study from Argentina, the presence of zearalenone in samples of corn was not reported [5]. The occurrence of ZEA in oats was recorded in Lithuania in 57.1 % of the samples and in Poland in 19.2 % of the samples [34, 39]. These results point to the fact that the presence and concentration of deoxynivalenol and zearalenone in cereals is related to the climate conditions of the environment in which the crops are grown.

CONCLUSIONS

Mycotoxins are secondary metabolites produced by filamentous microscopic fungi toxic to humans, animals and plants. Their ingestion, inhalation or dermal absorption may cause different diseases and even death. Mycotoxins cause undesirable contamination of feed. We confirmed the presence of microscopic filamentous fungi in feed cereals and also the presence of their secondary metabolites (deoxynivalenol and zearalenone). Therefore, it is very important to regularly examine and monitor the maximum limits of mycotoxins in food and feed. The complete elimination of moulds and mycotoxins is not possible, but the addition of adsorbents, antioxidants and other biologically active substances may reduce the incidence.

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