

Multiannual occurrence of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic

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Abstract

The aim of the study was to evaluate the individual and combined occurrence of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic in the years 2013–2017. The samples were analysed for the occurrence of aflatoxins (AF), deoxynivalenol (DON), zearalenone (ZEA), ochratoxin A (OTA), fumonisins (FUM), and T-2/H-2 toxins. The mycotoxin most commonly found in maize and small cereal grain samples was deoxynivalenol (94% and 53%, respectively). Deoxynivalenol was also the most frequent mycotoxin in all types of swine feed. The most frequent mycotoxin combinations in the total number of samples were DON + ZEA (56.63%), DON + OTA (11.61%) and DON + sum of T2/HT2 (9.76%). The maximum values set by the European Commission for mycotoxin levels in feed ingredients and complete feeds were exceeded in the case of AFB₁, deoxynivalenol, zearalenone, ochratoxin A and T-2/ H-2 toxins. Our findings support the need for further monitoring of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic.

Aflatoxin, deoxynivalenol, zearalenone, ochratoxin, fumonisins, T-2 toxin, maize, cereals, swine

Mycotoxins represent a serious problem in swine production because they can have a major effect on the health and productivity of pigs (Chaytor et al. 2011). Mycotoxins are secondary fungal metabolites that are toxic to both animals and humans. Approximately 400 mycotoxins have been identified to date, the majority being produced by species of the genera *Aspergillus*, *Penicillium* and *Fusarium* (Jard et al. 2011). The contamination of agricultural commodities and feed with mycotoxins can occur under favourable conditions during plant growth, storage and harvest. The growth of fungi is influenced mainly by temperature and moisture conditions (Binder et al. 2007).

Swine belong to the species that are often affected by mycotoxins. The most important mycotoxins affecting swine health are aflatoxin (AF), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEA) and fumonisins (FUM) (Chaytor et al. 2011). The contamination of swine feed can cause reduced animal productivity and immune suppression (Oswald and Comara 1998).

Various fungi can produce several mycotoxins simultaneously and commodities can also be contaminated by different fungal species. Therefore, the contamination of crops with

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more than one mycotoxin is extremely common (Streit et al. 2012). Interactions between mycotoxins can be antagonistic, additive or synergistic (Monbaliu et al. 2010).

The European Union has established regulations concerning the occurrence of mycotoxins in feed ingredients and complete feeds. Commission Directive 2002/32/EC sets maximum levels for AFB1. Commission Recommendation 2006/576/EC gives maximum levels for DON, ZEA, OTA and the sum of FUM B1 and B2.

Knowledge on the mycotoxin prevalence is required for the efficient prevention of mycotoxicosis in pigs. Therefore, we decided to conduct this study in order to evaluate the individual and combined occurrence of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic.

Materials and Methods

Samples of maize, small cereal grains and complete feeds for swine were collected from all over the Czech Republic in the years 2013–2017. They were delivered by feed producers, farmers and veterinarians. The samples were taken and prepared in compliance with the method described by the European Commission Regulation 152/2009. The samples were analysed for the occurrence of AF, DON, ZEA, OTA, FUM, and T-2 and H-2 toxins. The analyses were made according to the needs of feed producers, farmers and veterinarians. Since the samples were collected by private subjects we could not influence their choice of samples. Therefore the samples were not examined as random screening but as samples analysed based on previous suspect of contamination. Therefore each sample was analysed only for selected mycotoxins (as decided by private suppliers of samples). The individual samples were not analysed for the occurrence of all mycotoxins. The total numbers of analysed samples for particular mycotoxins are presented in Table 1. The performance of the analyses involved cooperation between four certified laboratories. The analyses were done at selected institutes (see below) by their high-performance liquid chromatography (HPLC) methods and the liquid chromatography with mass detection (LC-MS/MS) method or competitive direct enzyme-linked immunosorbent assay (ELISA) methods. Detailed procedures are subsequently listed for each workplace.

All samples were well homogenized by grinding before the analysis.

ELISA tests

Kits VERATOX by Neogen Corporation (Lansing, MI, USA) for DON, ZEA, T-2/HT-2 toxin and FUM were used for ELISA tests in the State Veterinary Institute in Jihlava.

Kits VERATOX by Neogen Corporation for T-2/HT-2 toxin and FUM were used for ELISA tests in the State Veterinary Institute in Olomouc.

Kits VERATOX by Neogen Corporation for FUM were used for ELISA tests in the State Veterinary Institute in Prague.

For determination of DON, 10 g of a well homogenized sample were extracted with 100 ml of distilled water on a high-speed homogenizer for about 2 min or on a shaker for about 5 min. The mixture was filtered through a microfibre glass filter paper. The filtrate was used for ELISA.

For determination of ZEA 5 g of a well homogenized sample was extracted with 25 ml of mixture of methanol + water (70 + 30) on a high-speed homogenizer for about 2 min or on a shaker for about 5 min. The mixture was filtered through a microfibre glass filter paper. This filtrate was diluted five times with distilled water (e.g. 1 ml of filtrate + 4 ml of distilled water), and the diluted filtrate was used for ELISA.

For determination of T-2/HT-2 toxin 5 g of a well homogenized sample was extracted with 25 ml of mixture of methanol + water (70 + 30) on a high-speed homogenizer for about 2 min or on a shaker for about 5 min. The mixture was filtered through a microfibre glass filter paper. The filtrate was diluted 1 + 1 with distilled water, and the diluted filtrate was used for ELISA.

For determination of FUM, 10 g of a well homogenized sample was extracted with 50 ml of mixture of methanol + water (70 + 30) on a high-speed homogenizer for about 2 min or on a shaker for about 5 min. The mixture was filtered through a microfibre glass filter paper. This filtrate was diluted with distilled water, 7 ml of distilled water were added to 1 ml of the filtrate, and the diluted filtrate was used for ELISA.

The limit of quantification is 100 µg/kg for DON, 25 µg/kg for ZEA, 25 µg/kg for T-2/HT-2 toxin, and 200 µg/kg for FUM; repeatability of this method is 10–15%, and recovery is 85–105%.

HPLC methods

The State Veterinary Institute in Jihlava

The liquid chromatograph Waters Alliance e2695 liquid chromatograph with detectors: 2998 PDA Detector and 2475 FLR Detector were used for HPLC analysis (Waters Corporation, Milford, MA, USA).

Aflatoxins

Aflatoxins AFB1, AFB2, AFG1, AFG2 were determined after purification of the sample on an AFLAPREP

Table 1. Occurrence of mycotoxins during the period of 2013–2017, summarized results from all 4 laboratories.

	Unit	AFB ₁	DON	ZEA	OTA	FUM	T-2/HT-2
Cereals	No. of samples	294	1436	732	442	174	424
	Mean \pm SD	0.38 \pm 0.70	506.67 \pm 1205.05	147.80 \pm 394.48	9.35 \pm 28.85	59.16 \pm 112.36	118.54 \pm 227.96
	Median	0.18	185.50	52.00	0.28	7.50	49.00
	Max	2.24	23350.00	2620.00	168.85	260.00	1325.00
	Prevalence	3.00	53.00	11.00	13.00	3.00	15.00
	Above limit	0.00	0.21	0.27	0.00	No limit	0.47
Maize	No. of samples	3321078	570159	175	224		
	Mean \pm SD	3.13 \pm 7.19	2767.82 \pm 3334.56	536.55 \pm 700.89	1.43 \pm 3.38	1314.28 \pm 2072.36	177.60 \pm 953.96
	Median	0.38	1613.00	310.00	0.13	360.00	50.00
	Max	30.16	29630.00	4920.00	14.50	9610.00	9610.00
	Prevalence	5.00	94.00	64.00	13.00	59.00	45.00
	Above limit	0.30	5.66	0.53	0.00	0.00	0.89
Creep feed	No. of samples	13	161	99	21	3	25
	Mean \pm SD	0.06	256.57 \pm 180.25	61.50 \pm 35.20	0.87 \pm 0.98	-	49.75 \pm 15.77
	Median	0.06	200.00	55.00	0.46	-	48.00
	Max	0.06	1190.00	160.00	3.10	0.00	70.00
	Prevalence	8.00	78.00	18.00	52.00	0.00	16.00
	Above limit	0.00	1.24	3.03	0.00	0.00	0.00
Starter feed	No. of samples	19	186	130	28	6	36
	Mean \pm SD	0.16 \pm 0.13	221.18 \pm 186.29	71.10 \pm 73.60	0.65 \pm 0.65	-	55.00 \pm 23.25
	Median	0.09	179.50	59.00	0.60	-	52.00
	Max	0.31	1579.00	342.00	2.38	0.00	85.00
	Prevalence	16.00	81	13.00	57.00	0.00	11.00
	Above limit	0.00	1.61	0.77	0.00	0.00	0.00

Table 1. Occurrence of mycotoxins during the period of 2013–2017, summarized results from all 4 laboratories.

	Unit	AFB ₁	DON	ZEA	OTA	FUM	T-2/HT-2
Grower feed	No. of samples	34	164	113	36	4	38
	Mean \pm SD	0.17 \pm 0.15	287.35 \pm 237.03	82.50 \pm 97.60	1.04 \pm 1.34	-	73.20 \pm 82.30
	Median	0.17	210.00	50.00	0.45	-	45.50
	Max	0.28	1272.00	495.00	5.24	0.00	302.00
	Prevalence	6.00	69.00	23.00	47.00	0.00	26.00
	Above limit	0.00	3.66	0.88	0.00	0.00	2.63
Grower – finisher feed	No. of samples	68	524	212	71	8	66
	Mean \pm SD	30.03 \pm 50.73	255.64 \pm 226.03	93.50 \pm 106.10	2.83 \pm 9.06	30.38 \pm 23.72	70.82 \pm 86.41
	Median	1.39	181.00	58.00	0.58	17.05	45.00
	Max	88.60	2445.00	477.00	54.50	57.77	383.00
	Prevalence	4.00	79.00	18.00	52.00	38.00	24.00
	Above limit	1.47	1.91	1.42	1.41	0.00	1.52
Gilts	No. of samples	11	140	99	14	4	23
	Mean \pm SD	-	299.16 \pm 620.54	56.10 \pm 21.60	0.60 \pm 0.35	-	28.00
	Median	-	180.00	56.00	0.58	-	28.00
	Max	0.00	893.00	100.00	1.25	0.00	28.00
	Prevalence	0.00	86.00	16.00	86.00	0.00	4.00
	Above limit	0.00	0.00	0.00	0.00	0.00	0.00
Gestating sow feed	No. of samples	24	342	274	30.00	4	64
	Mean \pm SD	-	342.87 \pm 936.76	72.20 \pm 43.90	2.61 \pm 5.60	-	63.04 \pm 40.59
	Median	-	200.00	60.00	0.56	-	49.00
	Max	0.00	13600.00	306.00	23.68	-	236.00
	Prevalence	0.00	82.00	30.00	70.00	0.00	41.00
	Above limit	0.00	2.92	0.36	0.00	0.00	0.00

Table 1. Occurrence of mycotoxins during the period of 2013–2017, summarized results from all 4 laboratories.

	Unit	AFB ₁	DON	ZEA	OTA	FUM	T-2/HT-2
Lactating sow feed	No. of samples	31	342	283	31	4	74
	Mean ± SD	0.07	255.51 ± 255.00	101.60 ± 70.60	0.69 ± 0.96	-	50.13 ± 18.35
	Median	0.07	178.00	80.00	0.40	-	46.00
	Max	0.07	2488.00	461.00	3.99	0.00	96.00
	Prevalence	3.00	80.00	49.00	68.00	0.00	20.00
	Above limit	0.00	2.92	1.77	0.00	0.00	0.00
Boar feed	No. of samples	14	83	83	8	0	17
	Mean ± SD	-	211.61 ± 170.32	79.60 ± 28.20	0.66 ± 0.44	-	47.00
	Median	-	170.00	70.00	0.50	-	47.00
	Max	0.00	1361.00	150.00	1.54	0.00	47.00
	Prevalence	0.00	71.00	20.00	88.00	0.00	6.00
	Above limit	0.00	1.20	0.00	0.00	0.00	0.00

No. of samples – number of examined samples; SD – standard deviation; Max – maximal mycotoxin concentration; Prevalence – percentage of positive samples; Above limit – percentage of samples with concentrations exceeding EU regulations; AFB₁ – aflatoxin; DON – deoxynivalenol; ZEA – zearealenol; OTA – ochratoxin; FUM – fumonisins; T-2/HT-2 – sum of mycotoxins

immunoaffinity column (R-Biopharm Rhone LTD, Darmstadt, Germany) by HPLC with post column derivatization using KOBRA CELL (R-Biopharm Rhone LTD) and fluorescence detection. The chromatographic analysis was performed on a Nova-Pak C18 column (250 mm × 4.6 mm, 4 µm) (Waters Corporation), mobile phase acetonitrile + methanol + redistilled water (1 + 1 + 3) with the addition of potassium bromide and 4 mol/l nitric acid solution, flow rate 1 ml/min, injection volume 20 µl, excitation wavelength 360 nm, emission wavelength 444 nm, column temperature 35 °C. The limit of quantification is 0.2–0.72 µg/kg; repeatability of this method is 10% and recovery 60–100%.

Ochratoxin A

OTA was determined after purification of the sample on a Neocolumn immunoaffinity column (Neogen Europe Ltd, Ayr, Scotland, UK) by HPLC with fluorescence detection. The chromatographic analysis was performed on a Nova-Pak C18 column (250 mm × 4.6 mm, 4 µm) (Waters Corporation), mobile phase acetonitrile + redistilled water + acetic acid (99 + 99 + 2), flow rate 0.8 ml/min, injection volume 20 µl, excitation wavelength 333 nm, emission wavelength 477 nm, column temperature 35 °C. The limit of quantification is 0.06 µg/kg; repeatability of this method is 7% and recovery 80–100%.

The State Veterinary Institute in Prague

Liquid chromatography with mass detection (LC-MS/MS) was used for the analysis of the AFB₁, AFB₂, AFG₁, AFG₂ and T-2 toxins. The samples were analysed after purification (NaCl + acetonitrile: methanol), extraction, shaking, filtration and dilution on column C 18 with a mobile phase 5 mM ammonium formate (NH₄FAc), 0.1% formic acid (FAc) in water and in methanol (MeOH), injection volume 60 µl. The limit of quantification is 0.05 µg/kg for aflatoxins and 10 µg/kg for the T-2 toxin, the repeatability of this method is 68%, and recovery 72–95%.

Liquid chromatography with fluorescence detection was used for the determination of OTA and ZEA. The samples were analysed after extraction with acetonitrile: redistilled water (60:40) for OTA, with acetonitrile: redistilled water (90:10) for ZEA on a Spherisorb column (250 × 4 mm, 5 µm) (Waters Corporation) with a mobile phase redistilled water (H₂O): acetonitrile

(ACN): acetic acid (CH_3COOH) (99: 99: 2), injection volume of 100 μl . The limit of quantification is 0.1 $\mu\text{g/kg}$ and 10 $\mu\text{g/kg}$ for OTA and ZEA, respectively. Repeatability is 7.5% for OTA, and 6.5% for ZEA. Recovery is 84% and 85% for OTA and ZEA, respectively.

Liquid chromatography with diode array detection was used for the determination of DON. The samples were analysed after extraction with redistilled water on a Spherisorb column (250 \times 4 mm, 5 μm) (Waters Corporation) (with a mobile phase $\text{MeOH:H}_2\text{O}$ (15:85), injection volume 50 μl . The limit of quantification is 100 $\mu\text{g/kg}$, repeatability of this method is 7%, and recovery 94%.

The State Veterinary Institute in Olomouc

Liquid chromatographs Agilent 1100 and Agilent 1290 (Agilent Technologies, Santa Clara, CA, USA), FLD detectors and DAD detectors were used for HPLC analysis.

Aflatoxins

AFB₁, AFB₂, AFG₁, AFG₂ were determined after purification of the sample on an AFLA test immunoaffinity column (VICAM, Waters Corporation) by HPLC with post column derivatization using KOBRA CELL (R-Biopharm Rhone LTD) and fluorescence detection. The chromatographic analysis was performed on a Nova-Pak C18 column (250 mm \times 4.6 mm, 4 μm) (Waters Corporation), mobile phase acetonitrile + methanol + redistilled water (1 + 1 + 3) with the addition of potassium bromide and 4 mol/l nitric acid solution, flow rate 1 ml/min, injection volume 100 μl , excitation wavelength 362 nm, emission wavelength 455 nm, column temperature 25 °C. The limit of quantification is 0.1 $\mu\text{g/kg}$, repeatability of this method is 10% and recovery 60–100%.

Ochratoxin A

OTA was determined after purification of the sample on a Neocolumn immunoaffinity column (Neogen Europe Ltd) by HPLC with fluorescence detection. The chromatographic analysis was performed on a SunFire C18 column (150 mm \times 3.0 mm, 3.5 μm , Waters Corporation), mobile phase $\text{ACN} + \text{H}_2\text{O} + \text{CH}_3\text{COOH}$ (99 + 99 + 2), flow rate 0.6 ml/min, injection volume 50 μl , excitation wavelength 333 nm, emission wavelength 477 nm, column temperature 30 °C. The limit of quantification is 0.2 $\mu\text{g/kg}$; repeatability of this method is 8% and recovery 80–110%.

Deoxynivalenol

DON was determined after purification of the sample on a DON test immunoaffinity column (VICAM, Waters Corporation) by HPLC with diode array detection. The chromatographic analysis was performed on an Ascentis Express C18 column (100 mm \times 4.6 mm, 2.7 μm , Sigma Aldrich, St. Louis, MO, USA), mobile phase $\text{ACN} + \text{H}_2\text{O}$ (10 + 90), flow rate of 0.6 ml/min, injection volume of 50 μl , wavelength of 218.2 nm and 360.1 nm, column temperature of 25 °C. The limit of quantification is 100 $\mu\text{g/kg}$; repeatability of this method is 10% and recovery 80–100%.

Zearalenone

ZEA was determined after purification of the sample on a ZEARALA test immunoaffinity column (VICAM, Waters Corporation) by HPLC with fluorescence detection. The chromatographic analysis was performed on Nova-Pak C18 column (250 mm \times 4.6 mm, 4 μm , Waters Corporation), mobile phase acetonitrile + redistilled water + methanol (46 + 46 + 8), flow rate of 1.0 ml/min, injection volume of 100 μl , excitation wavelength of 274 nm, emission wavelength of 440 nm, column temperature of 25 °C. The limit of quantification is 10.0 $\mu\text{g/kg}$; repeatability of this method is 7% and recovery 70–100%.

The Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic, National Reference Laboratory

The mycotoxins were determined by the multi-residue method of mycotoxin determination LC-MS/MS according to the Uniform Working Procedures – feed testing no. 10575.1

(<http://eagri.cz/public/web/ukzuz/portal/laboratore/dokumenty-a-publikace/jednotne-pracovni-postupy/metody-laboratoriho-zkouseni-krmiv.html>).

Results

Occurrence of aflatoxins

AFB₁ was detected in 5% of maize samples with a mean concentration of $3.13 \pm 7.19 \mu\text{g/kg}$ (max. 30.16 $\mu\text{g/kg}$). AFB₁ prevalence in small cereal grains was 3% with a mean concentration of $0.379 \pm 0.70 \mu\text{g/kg}$ (max. 2.24 $\mu\text{g/kg}$). In swine feed the highest prevalence was found in the starter feed (16%). The highest mean values of $30.03 \pm 50.73 \mu\text{g/kg}$ (max. 88.6 $\mu\text{g/kg}$) were found in grower-finisher feed. It was determined

that 0.30% of maize and 1.47% of grower-finisher feed samples were contaminated with AFB₁ at levels exceeding the maximum value set by the European Commission Directive (2002).

Occurrence of deoxynivalenol

Deoxynivalenol was detected in 94% of maize samples with a mean concentration of $2,767.82 \pm 3,334.56$ µg/kg (max. 29,630 µg/kg). DON prevalence in small cereal grains was 53% with a mean concentration of $506.67 \pm 1,205.05$ (max. 23,350 µg/kg). In swine feed the highest prevalence was found in the feed for gilts (86%). The highest mean values of 255.64 ± 226.03 µg/kg (max. 2,445 µg/kg) were found in the grower-finisher feed. It was determined that 5.66% of maize, 0.21% of small cereal grains, 1.24% of creep feed, 1.61% of starter feed, 3.66% of grower feed, 1.91% of grower-finisher feed, 2.92% of feed for gestating sows, 2.92% of feed for lactating sows and 1.20% of boar feed samples were contaminated with DON at levels exceeding the guidance value set by the European Commission (2006).

Occurrence of zearalenone

Zearalenone was detected in 64% of maize samples with a mean concentration of 536.55 ± 700.89 µg/kg (max. 4,920 µg/kg). Its prevalence in small cereal grains was 11% with a mean concentration of 147.80 ± 394.48 µg/kg (max. 2,620 µg/kg). In swine feed the highest prevalence was found in the feed for lactating sows (49%). The highest mean values of 93.50 ± 106.10 µg/kg (max. 477 µg/kg) were found in the grower-finisher feed. It was determined that 0.53% of maize, 0.27% of small cereal grains, 3.03% of creep feed, 0.77% of starter feed, 0.88% of grower feed, 1.42% of grower-finisher feed, 0.36% of feed for gestating sows and 1.77% of feed for lactating sows were contaminated with ZEA at levels exceeding the guidance value set by the European Commission (2006).

Occurrence of fumonisins

Fumonisin were detected in 59% of maize samples with a mean concentration of $1,314.28 \pm 2,072.36$ µg/kg (max. 9,610 µg/kg). FUM prevalence in small cereal grains was 3% with a mean concentration of 59.16 ± 112.36 µg/kg (max. 260 µg/kg). In swine feed positive samples were found only in the grower-finisher feed (38%) with mean values of 30.38 ± 23.72 µg/kg (max. 57.77 µg/kg).

Occurrence of ochratoxin A

Ochratoxin A was detected in 13% of maize samples with a mean concentration of 1.43 ± 3.38 µg/kg (max. 14.5 µg/kg). OTA prevalence in small cereal grains was 13% with a mean concentration of 9.35 ± 28.85 µg/kg (max. 168.85 µg/kg). In swine feed the highest prevalence was found in the boar feed (88%). The highest mean values of 2.80 ± 9.06 µg/kg (max. 54.50 µg/kg) were found in the grower-finisher feed. It was determined that 1.41% of grower-finisher feed samples were contaminated with OTA at levels exceeding the maximum value set by the European Commission (2006).

Occurrence of T-2 and H-2 toxins

These toxins were detected in 45% of maize samples with a mean concentration of 177.60 ± 953.96 µg/kg (max. 9,610 µg/kg). The prevalence of the toxins in small cereal grains was 15% with a mean concentration of 118.54 ± 227.96 µg/kg (max. 1,325 µg/kg). In swine feed the highest prevalence was found in the feed for gestating sows (41%). The highest mean values of 73.20 ± 82.30 µg/kg (max. 302 µg/kg) were found in the grower feed. It was determined that 0.89% of maize, 0.47% of small cereal grain, 2.63% of grower feed and 1.52% of grower-finisher feed samples were contaminated with T-2 and H-2 toxins at levels exceeding the maximum value set by the European Commission (2013).

Co-occurrence of mycotoxins

The most common combinations in maize samples were DON + ZEA (63.49%), DON + ZEA + sum of T2/HT2 (5.81%) and DON + sum of FUM (3.02%). The most important combinations in cereal grains samples were DON + ZEA (32.37%), DON + OTA (20.14%) and DON + sum of T2/HT2 (18.71%). The most frequent combinations in swine feed samples were DON + ZEA (57.67%), DON + OTA (19.06%) and DON + sum of T2/HT2 (10.40%). When counted from the total number of samples, the most frequent combinations were DON + ZEA (56.63%), DON + OTA (11.61%) and DON + sum of T2/HT2 (9.76%).

Discussion

Aflatoxins (B1, B2, G1, G2) are produced mainly by *Aspergillus flavus* and *A. parasiticus* primarily in regions with tropical and subtropical climates (Pleadin 2015). These mycotoxins can infect a wide range of crops such as corn, cotton, peanuts, seeds and tree nuts (Richard 2007). Aflatoxins can alter the structure and function of proteins. The main target organ for its toxicity is the liver (Agag 2004). Pigs are among the most susceptible species. Aflatoxicosis can result in reduced growth rate, liver damage and immunosuppression (Chaytor et al. 2011). Maximum values for AFB1 of 20 µg/kg for feed materials, 10 µg/kg for complementary and complete feed and 5 µg/kg for compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry animals have been established (European Commission Directive 2002).

We found a similar contamination of cereals (3%) and maize (5%) with AF as that found in a Polish study. Kosicki et al. (2016) reported that 5% of examined samples were contaminated with AF. In a study performed in Croatia, Pleadin et al. (2012) found a 13.3% prevalence of AFB₁ in feed for fattening pigs with a mean concentration of 1.10 ± 0.51 µg/kg (max. 1.53 µg/kg). In our study, we found a lower prevalence (4%), though higher mean and maximum values (mean 30.03 ± 50.73 µg/kg, max. 88.60 µg/kg), in feed for fattening pigs. In our study, 1.47% of grower-finisher feed samples were contaminated with AFB₁ at levels exceeding the maximum value set by the European Commission.

Deoxynivalenol is produced by *Fusarium culmorum* and *F. graminearum* primarily in cooler climates (Pleadin 2015). It is most prevalent in temperate zones of the world such as Europe and North America (Wu 2007). It affects cereal crops such as wheat, maize and barley (EFSA 2004a). Deoxynivalenol can enter into cells and inhibit DNA, RNA, and protein synthesis (Hussein and Brasel 2001). It alters the immune response (Oswald and Comara 1998) and intestinal functions (Kolf-Clauw and Oswald 2009). Pigs are the most sensitive animals to this toxin (Wu 2007). Guidance values of 8,000 µg/kg for DON in cereal feed materials, 12,000 µg/kg for maize feed materials, 5,000 µg/kg for complementary and complete feeds, and 900 µg/kg for complementary and complete feeds for swine have been established (European Commission 2006).

In our study, we found a high prevalence of DON in cereals (53%) and maize (94% of samples). Compared to our study, Kosicki et al. (2016) reported a higher prevalence of DON in cereal grain samples (98%) and a similar prevalence in maize (89%). In a Croatian study by Pleadin et al. (2012), the DON prevalence in feed for fattening pigs was 96.6% with a mean concentration of 817 ± 447 µg/kg (max. 1,860 µg/kg). A Portuguese survey conducted by Almeida et al. (2011) found that 16.6% of swine feed samples (fattening pigs) contained DON at a concentration of 223.2 ± 181.1 µg/kg (max. 864.0 µg/kg). In our study, we found significant contamination of grower-finisher feed with DON. This was manifested by high prevalence (79%) and mean values (255.64 ± 226.03 µg/kg, max. 2,445.00 µg/kg). In our study, 1.91% of grower-finisher feed samples were contaminated with DON at levels exceeding the maximum value set by the European Commission.

Zearalenone is produced by *Fusarium culmorum* and *F. graminearum* worldwide and can frequently co-exist with DON (Bottalico 1998). It infects corn, wheat, barley, sorghum and rye (EFSA 2004a). The toxin has oestrogenic effects because it can bind to oestrogen receptors instead of oestradiol-17B (Pleadin 2015). The pig is the species most affected by this toxin (Chaytor et al. 2011). Intoxication in pigs can cause premature puberty, embryonic death, smaller litter sizes, weak piglets and feminization of young boars (Richard 2007). The guidance values are 2,000 µg/kg for ZEA in cereal feed materials, 3,000 µg/kg for maize feed materials, 100 µg/kg for complementary and complete feeds for piglets and gilts, and 250 µg/kg for complementary and complete feeds for sows and fattening pigs (European Commission 2006).

In our study, we found a lower prevalence of ZEA in cereals (11%) compared to a Polish study (97%, Kosicki et al. 2016). In maize samples, our prevalence of ZEA (64%) was also lower to that found in the said study (92%). In a study conducted in Croatia by Pleadin et al. (2012), ZEA prevalence in the feed for fattening pigs was 93.3% with a mean concentration of 184 ± 21 µg/kg (max. 866 µg/kg). The maximum recommended level was exceeded in 17% of samples. According to a Portuguese study (Almeida et al. 2011), ZEA prevalence in the feed for fattening pigs was 24.9% with a mean concentration of 18.9 ± 17.9 µg/kg (max. 73.0 µg/kg). In the feed for sows, 29.9% samples were positive for ZEA (mean 19.4 ± 14.5 µg/kg, max. 57.7 µg/kg). In our study, we found a lower prevalence of ZEA in the grower-finisher feed (18%) than the above authors. Similar prevalence (30%) as in the Portuguese study was observed in the feed for sows. In our study, 1.42% of grower-finisher feed samples were contaminated with ZEA at levels exceeding the maximum value set by the European Commission.

Fumonisin is produced by *Fusarium proliferatum* and *F. verticillioides* worldwide (Richard 2007). There are three forms of the toxin – FB1, FB2, and FB3. The form FB1 is the most prevalent and harmful. The toxins mainly infect maize and its products (Pleadin 2015). Fumonisin toxicity can be explained by interference with enzyme N-acetyl transferase which is involved in sphingolipid metabolism (Hussein and Brasel 2001). Intoxication with this toxin causes pulmonary oedema in swine (Henry and Wyatt 1993). Guidance values of 60,000 µg/kg for FB1 and FB2 sum in maize feed materials and 5,000 µg/kg for complementary and complete feeds for pigs have been established (European Commission 2006).

We found a low prevalence of FUM in cereals (3%). This differs from the results of Kosicki et al. (2016) who detected no FUM in cereal samples. Our results (59%) are similar to those of Kosicki et al. (2016) who reported 58% prevalence in maize samples. Pleadin et al. (2012) found a 80% FUM prevalence in the feed for fattening pigs with a mean concentration of 405 ± 298 µg/kg (max. 1,043 µg/kg). In our study, we found lower prevalence (38%) and lower mean and maximum values (mean 30.38 ± 23.72 µg/kg, max. 57.77 µg/kg) in the feed for fattening pigs. According to Almeida et al. (2011) the FUM prevalence in the feed for sows was 8.7% with a mean concentration of 163.7 ± 127.8 µg/kg (max. 400.0 µg/kg). In our study, all samples of feed for sows were negative.

Ochratoxin A is produced by *Aspergillus ochraceus* and *Penicillium verrucosum* worldwide (Pleadin 2015). It contaminates mainly cereals, but can also be found in soybeans and peanuts (EFSA 2004b). The toxin primarily affects the kidneys causing nephropathy in pigs (Duarte et al. 2011). Guidance values of 250 µg/kg for OTA in cereal feed materials and 50 µg/kg for complementary and complete feeds for swine have been established (European Commission 2006).

We found the same prevalence of 13% in cereals and maize samples. In a study conducted in Poland by Kosicki et al. (2016), OTA was detected in 11% of maize samples and 28% of cereals. According to Pleadin et al. (2012), OTA prevalence in the feed for fattening pigs

was 26.6% with a mean concentration of 1.53 ± 0.42 µg/kg (max. 2.79 µg/kg). Almeida et al. (2011) found that OTA prevalence in feed for fattening pigs in Portugal was 7.6% with a mean concentration of 3.9 ± 1.2 µg/kg (max. 6.8 µg/kg). Compared to these authors, we found a higher prevalence of OTA in the feed for fattening pigs (52%) and 1.41% percent of the samples were contaminated with ochratoxin at levels exceeding the maximum value set by the European Commission.

The T-2 and H-2 toxins are produced by *Fusarium sporotrichoides* worldwide, but are most often found in northern parts of Europe such as Scandinavia and the UK (Edwards et al. 2009). They can infect maize, oats, barley, wheat, rice and soybeans (Pleadin 2015). The toxins inhibit protein and nucleic acid synthesis (Richard 2007). Swine is the species most sensitive to this toxin (Streit et al. 2012). Intoxication in pigs is manifested by a feed intake depression and a reduced weight gain (Rafai et al. 1995). Guidance values of the sum of the T-2 and HT-2 toxins of 2,000 µg/kg for oat milling products (husks), 500 µg/kg for other cereal products, and 250 µg/kg for compound feeds have been established (European Commission 2013).

In our study, we found a lower prevalence of the T-2 and H-2 toxins in cereals (15%) and maize (45%) compared to Kosicki et al. (2016). They detected the T-2 toxin in 78% of cereals and in 67% of maize samples. Pleadin et al. (2012) reported a 46.6% T-2 prevalence in the feed for fattening pigs with a mean concentration of 33.4 ± 13.5 µg/kg (max. 78.8 µg/kg). In our study, we found a lower prevalence of T-2 and HT-2 toxins (24%) in the feed for fattening pigs. In our study, 1.52% of grower-finisher feed samples were contaminated with T-2 and H-2 toxins at levels exceeding the maximum value set by the European Commission.

As documented by many studies, the co-occurrence of mycotoxins is an extremely common problem in the feed industry worldwide (Streit et al. 2012). For instance, Ma et al. (2018) found that 96.4% of complete feed samples were co-infected with AFB1, ZEA, and DON. In our study, the most frequent combinations in swine feed samples were DON + ZEA (57.67%), DON + OTA (19.06%), and DON + SUM-T2/HT2 (10.40%). The combination of DON + ZEA was also found to be the most frequent in the studies by Kosicki et al. (2016) and Zachariasova et al. (2014).

In general, the combined effects of mycotoxins can be additive or synergistic (Sun et al. 2014). Their implications for both animal and human health has not, however, been sufficiently investigated. The current safety legislation does not consider the toxicity of mycotoxin combinations. More studies are obviously necessary in order to produce objective data that could be considered when new safety standards are set in the future.

In general, mycotoxin concentration can be influenced by several factors that can occur under favourable conditions during plant growth, storage, and harvest. In our study, the samples were delivered by feed producers, farmers, and veterinarians according to their needs. Since they are private subjects we could not influence their choice of samples. Therefore, our study only presents the results of monitoring and makes it impossible to identify the particular reasons for higher mycotoxin concentrations.

It can be concluded based on our results that there is a need for further monitoring of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic.

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