

Occurrence of mycotoxins in complete poultry feeds in the Czech Republic – Multiannual survey (2013–2018)

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Abstract: To assess the contamination and potential health risks for poultry, we investigated concentrations of selected mycotoxins in complete feeds sampled within the period of 2013–2018 in the Czech Republic. Broiler feeds, feeds for laying hens, chickens and/or other poultry species were investigated by ELISA methods or chromatography. Our results demonstrated that unlike other mycotoxins, (such as aflatoxins, ochratoxin A or zearalenone), deoxynivalenol (DON) may represent a potential threat. The prevalence of this mycotoxin in all the feed categories was very high, always reaching at least 81.29%. Moreover, in 14 of the 139 analysed broiler feed samples (i.e., in 10.81% of samples), DON was detected in concentrations exceeding the recent guidance value of 5 000 µg/kg established by EU legislation. Since previous studies demonstrated that the long-term feeding of such a highly contaminated diet could cause stress in the animals and could lead to their growth suppression, good agricultural practices and the further systematic monitoring of Czech poultry feeds are still highly needed.

Keywords: broilers; deoxynivalenol; feed contamination; filamentous fungi; *Fusarium*; mycotoxin

The contamination of agricultural commodities, feed ingredients or complete feeds by various filamentous fungi (moulds) represents a potential threat, since under favourable field or storage conditions,

they may easily grow and produce toxic secondary metabolites – mycotoxins (Bryden 2012). Among several other genera, *Aspergillus* sp. and *Fusarium* sp. have been considered the most important mycotoxin

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sources. While *Aspergillus* sp. is well known due to its aflatoxin (AFLA) or ochratoxin (OTA) synthesis, the latter genus is responsible for feed contamination by trichothecenes [e.g., deoxynivalenol (DON) and the T-2/HT-2 toxin], fumonisins (FUM) or zearalenone (ZEA) (D’Mello et al. 1999; Bryden 2012). A previous comprehensive survey of feeds and the raw materials of the feeds focused on Europe and Asia revealed the presence of detectable amounts of at least one mycotoxin in 72% of the samples. Moreover, the co-contamination with two or more mycotoxins was found in 38% of the samples (Streit et al. 2013a).

The mycotoxin presence in feeds is highly unwanted, due to their potential harmful effects on the health and productivity of the animals (Kipper et al. 2020). Depending on the compound which the animals are exposed to, numerous possible toxicity targets exist. While AFLA are hepatotoxins and potential carcinogens (Rawal et al. 2010), the exposure to OTA may lead to kidney damage (Heussner and Bingle 2015). On the other hand, DON targets the epithelial cells of the gastrointestinal tract and also affects immunity (Awad et al. 2013; Ghareeb et al. 2015; Aguzey et al. 2019) and fumonisins, well-known disruptors of sphingolipid metabolism, may act as an etiologic agent in diseases such as equine leukoencephalomalacia or porcine pulmonary oedema (Grenier et al. 2017). Unlike other mycotoxins, ZEA acts as a natural mycoestrogen, thus inducing a reproductive dysfunction (D’Mello et al. 1999; Chang et al. 2017). The frequent co-contamination by multiple mycotoxins implies that any possible synergistic or additive interactions should always be taken into account (Abidin et al. 2011; Streit et al. 2013b; Murusegan et al. 2015). As there are physiological differences among animal species, their sensitivity towards mycotoxins varies and certain species-specific effects appear (Bertero et al. 2018).

Poultry is the fastest growing agricultural subsector that is driven by growing populations, rising incomes and urbanisation (Narrod et al. 2007; Mottet and Tempio 2017). The growth is most significant in rapidly developing countries such as Brazil or China (Narrod et al. 2007). According to the Food and Agriculture Organization of the United Nations (FAO), the continuous increase in the world production of poultry meat is expected to increase from the 132 067 kt (kilotonnes) being produced in 2020 to an estimated 145 711 kt for 2029 (OECD/FAO 2020).

The mycotoxin contamination of a feed has an increasing global economic impact on the poultry production (Antonissen et al. 2014). The exposure does not necessarily have to cause any mortality, the main adverse effect of mycotoxins on poultry rather represents a reduced food intake that can lead to a decrease in the growth performance due to the low nutrient uptake and metabolism (Abd El-Hack et al. 2018; Wang and Hogan 2019). Moreover, it was demonstrated that broiler chickens exposed to certain *Fusarium* mycotoxins (DON, fumonisins) were predisposed for the development of necrotic enteritis (Antonissen et al. 2014; Antonissen et al. 2015).

The aim of our study was to investigate, for the first time, the prevalence and concentrations of the selected mycotoxins in poultry feeds from the Czech Republic. The obtained results were evaluated considering the guidance values/limits set up in the relevant EU legislation and discussed from the viewpoint of possible adverse effects on the poultry’s health and productivity.

MATERIAL AND METHODS

Complete poultry feed samples were obtained within the period from 2013 to 2018 from various private subjects (either feed producers, farmers or veterinarians) who delivered them to the laboratories specified in Table 1 approved by the competent authority of the Czech Republic. The studied feeds involved various broiler starters, growers and finishers, complete feeds for laying hens or chickens as well as other poultry species (such as turkeys, ducks or quails). Although the compliance with the relevant EU legislation, i.e., regulation No. 152/2009, laying down the methods of sampling and analysis for the official control of feed (European Commission 2009) was ensured, it should be emphasised that the sampling was not always random, and was rather focused on the possible contamination by mycotoxins. The presence of AFLA, OTA, DON, T-2/HT-2 toxin, FUM and ZEA in the feeds was investigated; however, as decided by the sample suppliers, each sample was only analysed for the selected mycotoxins and not for all of them. The analyses were carried out at either the State Veterinary Institutes in Jihlava, Olomouc or Prague or at the Central Institute for Supervising and Testing in Agriculture (the national reference laboratory in Brno) by the methods de-

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Table 1. Mycotoxins in the poultry feeds from the Czech Republic – A description and the characteristics of the analytical methods

1. State Veterinary Institute in Jihlava		
Mycotoxin	Method	LOD/LOQ (µg/kg)
AFLA ^c	HPLC-FLD	0.64 ^b
DON	ELISA*	100 ^a
FUM	HPLC-FLD	200 ^b
OTA	HPLC-FLD	0.06 ^b
T-2/HT-2	ELISA*	50 ^a
ZEA	ELISA*	50 ^a
2. State Veterinary Institute in Olomouc		
Mycotoxin	Method	LOD/LOQ (µg/kg)
AFLA ^c	ELISA* (HPLC-FLD)	1.0 ^a (0.1 ^b)
DON	ELISA* (HPLC-DAD)	100 ^a (80 ^b)
FUM	ELISA*	50 ^a
OTA	HPLC-FLD	0.2 ^b
T-2/HT-2	ELISA*	25 ^a
ZEA	ELISA* (HPLC-FLD)	25 ^a (10 ^b)
3. State Veterinary Institute in Prague		
Mycotoxin	Method	LOD/LOQ (µg/kg)
AFLA ^c	LC-MS/MS	0.05 ^b
DON	LC-DAD	100 ^b
FUM	ELISA*	200 ^a
OTA	LC-FLD	0.1 ^b
ZEA	LC-FLD	10 ^b
4. Central Institute for Supervising and Testing in Agriculture (Brno)		
Mycotoxin	Method	LOD/LOQ (µg/kg)
AFLA ^d	LC-MS/MS	1 ^b
DON	LC-MS/MS	50 ^b
FUM ^e	LC-MS/MS	10 ^b (20 ^b)
OTA	LC-MS/MS	5 ^b
ZEA	LC-MS/MS	20 ^b

AFLA = aflatoxin; DON = deoxynivalenol; ELISA = enzyme-linked immunosorbent assay; FUM = fumonisins; HPLC-DAD = high performance liquid chromatography coupled with diode array detection; HPLC-FLD = high performance liquid chromatography coupled with fluorescence detection; LC-MS/MS = liquid chromatography coupled with tandem mass spectrometry; LOD = limit of detection; LOQ = limit of quantification; OTA = ochratoxin; ZEA = zearalenone
^aLOD; ^bLOQ; ^cAFLA = ΣAFB1 + AFB2 + AFG1 + AFG2;
^dAFLA = AFB1; ^eLOQ_{fum. B1} = 10 µg/kg, LOQ_{fum. B2} = 20 g/kg;
 *VERATOX kit (Neogen Corp.)

scribed in Table 1. The sampling and analytical procedures used for the determination of the mycotoxin concentrations in the poultry feeds were the same as was previously described in detail in Svoboda et al. (2019).

RESULTS AND DISCUSSION

A brief summary of the results of the mycotoxin analyses in the feed samples from the Czech Republic is reported in Table 2. Although poultry, especially turkeys, are extremely sensitive towards AFLA (Rawal et al. 2010), the concentrations of these toxins detected in our study were generally very low, regardless of the feed category. The highest detected concentration (0.174 µg/kg) was still far below the EU legislation limits (either 20 µg/kg for broilers, laying hens and other poultry species or 5 µg/kg for chickens) (European Parliament and the Council 2002). The AFLA prevalence ranged from the 5.88% detected in the feeds for laying hens to the 20.00% found in the feeds for other poultry species. This is much lower than the prevalence reported outside the Europe. While 82.2% of the broiler finishers in Kuwait were contaminated by detectable concentrations of these mycotoxins (Beg et al. 2006), the prevalence of aflatoxin B₁ (AFB₁) in Thailand was as high as 93% (Kongkapan et al. 2016). Relatively higher AFLA concentrations and its prevalence compared to our results were also observed in Cameroon, Nigeria, Brazil, and Argentina (Kana et al. 2013; Akinmusire et al. 2019; Rossi et al. 2013 and Dalcerro et al. 1998, respectively).

Similar to AFLA, our results suggest that the feed contamination by OTA should not represent any significant threat for the exposed poultry in the Czech Republic. The highest concentration detected in our study was 6.31 µg/kg, which was about 15 times lower than the current legislation limit, or rather the guidance value (100 µg/kg) established by the EU (European Commission 2006). Moreover, when detected, the OTA concentrations in the feeds were usually below 1.00 µg/kg. A varying prevalence in the feed contamination by OTA within the four feed categories tested was observed with a minimum of 18.18% found in complete chicken feeds and a maximum of 61.54% detected in the feeds for other poultry species such as ducks, turkeys or quails. In fact, both the OTA con-

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Table 2. Mycotoxin concentrations detected in the feed samples from the Czech Republic

Mycotoxin	Feed category	<i>n</i>	Positives	Prevalence (%)	Range (µg/kg)	Mean ± SD* (µg/kg)	Median* (µg/kg)	Limit† (µg/kg)	Limit† exceeded samples/(%)
AFLA	broilers	26	4	15.38	< LOQ–0.174	0.104 ± 0.046 7	0.083	20 ^a	0/0
	laying hens	34	2	5.88	< LOQ–0.052	0.052 ± 0.000 7	0.052	20 ^a	0/0
	chickens	10	1	10.00	< LOQ–0.065	0.065	0.065	5 ^a	0/0
	others	15	3	20.00	< LOQ–0.158	0.119 ± 0.035 2	0.119	20 ^a	0/0
DON	broilers	139	113	81.29	< LOD–11 995	1 564 ± 2 591.9	437	5 000 ^b	14/10.07
	laying hens	169	144	85.21	< LOD–2 135	504 ± 451.8	320	5 000 ^b	0/0
	chickens	36	33	91.67	< LOD–3310	711 ± 720.6	369	5 000 ^b	0/0
	others	52	49	94.23	< LOD–1970	497 ± 496.7	330	5 000 ^b	0/0
FUM	broilers	1	0	0.00	< LOD	–	–	20 000 ^b	0/0
	laying hens	7	6	85.71	< LOD–136	59 ± 50.2	41	20 000 ^b	0/0
	chickens	2	1	50.00	< LOD–36	36	36	20 000 ^b	0/0
	others	1	0	0.00	< LOD	–	–	20 000 ^b	0/0
OTA	broilers	37	9	24.32	< LOQ–1.05	0.380 ± 0.279 6	0.320	100 ^b	0/0
	laying hens	35	15	42.86	< LOQ–6.31	0.867 ± 1.571 3	0.410	100 ^b	0/0
	chickens	11	2	18.18	< LOQ–0.94	0.569 ± 0.522 0	0.569	100 ^b	0/0
	others	13	8	61.54	< LOQ–0.55	0.319 ± 0.137 8	0.290	100 ^b	0/0
T-2/HT-2	broilers	54	19	35.19	< LOD–66	40 ± 12.5	36	250 ^c	0/0
	laying hens	70	11	15.71	< LOD–194	67 ± 53.3	48	250 ^c	0/0
	chickens	13	2	15.38	< LOD–66	58 ± 12.0	58	250 ^c	0/0
	others	26	10	38.46	< LOD–160	72 ± 37.8	69	250 ^c	0/0
ZEA	broilers	74	44	59.46	< LOD–2 255	343 ± 450.2	119	no limit	–
	laying hens	126	44	34.92	< LOD–460	108 ± 108.8	70	no limit	–
	chickens	26	13	50.00	< LOD–500	125 ± 155.2	53	no limit	–
	others	32	16	50.00	< LOD–420	122 ± 118.3	75	no limit	–

AFLA = aflatoxin; DON = deoxynivalenol; FUM = fumonisins; LOD = limit of detection; LOQ = limit of quantification; OTA = ochratoxin; ZEA = zearalenone

*Mean/median calculated from the positive samples only; †Either the maximum content or guidance value; LOD (for the ELISA method)/LOQ (for the chromatographic methods); ^aEuropean Parliament and the Council (2002); ^bEuropean Commission (2006); ^cEuropean Commission (2013). Feeds signed as “others” represented the ones intended for feeding ducks, turkeys or quails

centrations and prevalence found in our study were quite similar to those reported from Brazil (Bordini et al. 2016), while a much higher contamination was found in Kuwait (Beg et al. 2006). The ochratoxicosis of broiler chickens was previously demonstrated by Elaroussi et al. (2006). The OTA toxicity involved a reduced feed consumption and conversion, a reduced body weight in the exposed individuals, anaemia and a decrease in the white blood cell count. Nevertheless, it should be noted that throughout the experiment, the chickens were fed for 5 weeks with a diet contaminated by either 400

or 800 µg/kg of OTA (Elaroussi et al. 2006). Chicken co-exposure by OTA and another mycotoxin penicillic acid were investigated by other authors and although the toxic effects already appeared in the lowest OTA concentration tested (130 µg/kg) (Stoev et al. 2004), considering the OTA concentrations detected in the Czech feeds, the poultry still seem to be safe.

The occurrence of *Fusarium* mycotoxins, especially DON, showed a different pattern than AFLA or OTA. With almost 400 feed samples tested within the period of 2013–2018, DON rep-

resented the most commonly analysed mycotoxin in the Czech Republic. Moreover, the detection frequency (i.e., prevalence) of this mycotoxin in all the feed categories was very high always reaching 81.29% at least (as in the case of broiler feeds). A maximum observed mycotoxin concentration was 11 995 µg/kg, thus exceeding the EU legislation guidance value of 5 000 µg/kg (European Commission 2006). In total, excessive concentrations of DON were found in 14 broiler feeds. It should be noted that they were all sampled in 2015. *Fusarium* sp. represents a typical member of the so-called field fungi that can cause plant disease and pre-harvest mycotoxin contaminations (Kanora and Maes 2009). It is well described that climatic factors, such as humidity and temperature, have a significant influence on both the fungal growth and mycotoxin production (Kanora and Maes 2009; Bryden 2012; Murusegan et al. 2015).

Since the data provided by the Czech Hydrometeorological Institute (CHMI) suggested that the summer seasons of 2013 and 2014 were the coldest ones with the highest total rainfall throughout the whole investigation period (CHMI 2020), we assume that the raw materials for the preparation of the analysed complete feeds in 2015 might have been contaminated by DON more than ever. DON, as a member of the trichothecene family, is the most commonly found mycotoxin in Europe, or rather, in temperate climate zones in general (Binder et al. 2007; Cegielska-Radziejewska et al. 2013). Although its concentrations were low, this mycotoxin was detected in all 45 feeds for the broiler chickens analysed in Poland (Cegielska-Radziejewska 2013). In Slovakia, on the other hand, the DON prevalence in the feeds reached 56%, while the maximum concentration was as high as 1 230 µg/kg (Labuda et al. 2005). Despite the maximum DON concentration that was referred to here was approximately ten times higher, it should be noted that the feeds in our study were sampled and analysed as suspicious of being infected by fungi. Broiler chickens that were fed for 15 days by a diet contaminated with 4.6 mg/kg of DON previously showed a corticosterone stress response (Antonissen et al. 2017). The consumption of a feed containing an even higher DON concentration by broiler chickens led to their growth suppression which might be the result of a partial feed refusal and an alteration in the intestinal mucosa structures (Wang and Hogan 2019).

Considering these findings, the prolonged consumption of the most contaminated Czech feeds would probably cause adverse health effects in the poultry. On the other hand, it should be emphasised that the medians of the DON concentrations in the feeds of all four target poultry categories ranged from 320 µg/kg to 437 µg/kg. Moreover, these values were calculated exclusively from positive samples.

The T-2 toxin and its deacetylated form, the HT-2 toxin, represent the other trichothecenes investigated in our study. Similar to DON, both mycotoxins are produced by *Fusarium* sp. and often infect cereals, especially under cool and moist conditions (Nathanail et al. 2015). Neither concentrations detected in our study nor the percentage of the positive samples were high. While the highest T-2/HT-2 toxin concentration of 194 µg/kg was found in the feed sample for laying hens, the maximum prevalence (38.46%) was revealed in the feeds for other poultry species. As the guidance value recommended by the EU Commission is 250 µg/kg, the contamination of the Czech feeds by these mycotoxins themselves should not pose a risk for the poultry, unless one regards their possible synergistic interactions with other contaminants. Slightly lower concentrations of these toxins were reported from Slovakia, nevertheless the percentage of the detection was higher in Slovakia than in the Czech Republic (Labuda et al. 2005). A very high contamination by these toxins was previously found in several barley and wheat samples analysed in Canada (Shi et al. 2019). The experimental exposure through a diet contaminated by the T-2 toxin (in the concentrations ranging from 200 µg/kg to 600 µg/kg) affected the lipid peroxidation and antioxidant status in different poultry species. Geese represented the most sensitive species that was followed by ducks and chickens (Mezes et al. 1999).

Zearalenone (ZEA), another *Fusarium*-produced mycotoxin, was the second most commonly analysed compound in our study. In total, 258 feed samples were investigated with the prevalence ranging from 34.92% (complete feeds for laying hens) to 59.46% (broiler feeds). From the four feed categories studied, these were the broiler feeds that were the most significantly contaminated. While the maximum ZEA concentration detected was 2 255 µg/kg, the median concentration calculated from the positive samples of this category was 119 µg/kg. We are not aware of any restrictions or

EU legislative limits set up directly for complete poultry feeds; however, the ZEA guidance values for feed materials such as cereals, cereal products or maize by-products were recommended (European Commission 2006). This is perhaps due to the fact that poultry respond to the presence of ZEN in the feed only at very high dietary concentrations and can generally be considered as resistant (EFSA CONTAM Panel 2017). The low oral bioavailability and rapid elimination of the mycotoxin in the poultry species may be the main reasons of this characteristic (Devreese et al. 2015).

Fumonisin (FUM) represented the remaining group of mycotoxins in our interest; however, only 11 feed samples were analysed. Although the FUM concentrations were very low (a maximum of 136 µg/kg was detected in one sample of the feed for laying hens) or even below the LOD (LOQ) – limit of detection (limit of quantification), a convincing interpretation of these results is impossible as the number of samples was extremely limited. Previous studies conducted outside Europe suggested the common contamination of poultry feeds by FUM with a concentration maxima in the order of thousands of µg/kg and almost 100% prevalence (Beg et al. 2006; Rossi et al. 2013; Kim et al. 2014).

Taken together, within the period of 2013–2018, DON represented the most important mycotoxin contaminant of the Czech complete poultry feeds. Although the concentrations of other the mycotoxins were always below the limits (guidance values) established by EU legislation, the DON levels detected in 14 broiler feed samples investigated during 2015 exceeded 5 000 µg/kg. Previous studies demonstrated that the long-term feeding of broilers with such a highly contaminated diet may contribute to their stress, partial feed refusal or even growth suppression. On the other hand, it should also be mentioned here once more that the feeds analysed in our study were sampled as suspicious of being infected by toxigenic fungi, therefore, not that alarming results could probably be obtained by using a random sampling strategy. These findings emphasise the need for the further systematic monitoring of mycotoxins in poultry feeds in the Czech Republic.

Conflict of interest

The authors declare no conflict of interest.

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