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DOI: 10.2478/fv-2023-0013

FOLIA VETERINARIA, 67, 2: 18—23, 2023

INCIDENCE OF TRICHOTHECENES DEOXYNIVALENOL AND T-2 TOXIN IN POULTRY FEED MIXTURES

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ABSTRACT

Trichothecenes are among the most important fusariotoxins. According to their chemical structure, they are divided into 4 groups (A–D). In terms of agriculture, trichothecenes of groups A and B are of greatest importance. In this study, the incidence of trichothecenes (deoxynivalenol and T-2 toxin) in mixed feed for broilers and turkeys were determined. Deoxynivalenol was detected in all analysed samples of feed mixture for broilers and turkeys (100 %) at an average concentration of 1.776 ppm; 0.675 ppm, respectively. T-2 toxin was present in 93.8 % of mixed feed for broilers at an average concentration of 36.625 ppb and in all of the tested samples (100 %) of turkey mixed feed (average level 25.899 ppb). The trichothecenes deoxynivalenol and T-2 toxin in feed samples for poultry did not exceed concentrations recommended by legislation.

Key words: ELISA; feed; mycotoxins; poultry; trichothecenes

INTRODUCTION

Trichothecenes represent a large group of secondary metabolites of microscopic filamentous fungi of the genus *Fusarium*. According to the characteristic functional chemical group (different substituents on C–3, 4, 7, 8 and 15), they are divided into four basic groups (A–D). The basis of the trichothecene molecule is a tricyclic sesquiterpene ring with a double bond between carbon C9, C10 and an epoxy ring in the position of carbon C12, C13, which is responsible for their toxicity. Trichothecenes are referred to as 12,13-epoxytrichothecenes [20]. The chemical structure of trichothecenes is variable and depends on the number and position of hydroxyl groups and also on the number and position of esterification groups. Trichothecenes of the genus *Fusarium* are relatively simple alcohols with short ester chains. The starting molecule in the biosynthesis of trichothecenes is trichodiene [13]. The occurrence of trichothecenes in grain is associated with cold and wet harvests and they are the most commonly found in wheat, barley and corn. In cereal grains are dominant trichothecenes of type A (T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol and verulol) and trichothecenes of type B (deoxynivalenol, acetylated forms of deoxynivalenol 3-ADON and 15-ADON, nivalenol and fusarenon-X) [9]. In terms of toxicity, trichothecenes are classified as gastrointestinal toxins, dermatotoxins, immunotoxins, hematotoxins and genotoxins [28]. Trichothecenes are resistant to inactivation during the processing of the grain. Therefore, the presence of trichothecenes in feed cannot be completely prevented and can be a cause of disruption of the productive

health of animals [8]. In poultry, trichothecene toxicosis is manifested acutely or chronically. The acute form of poisoning has a characteristic clinical picture, fast progress and is easily diagnosed. However, the chronic form manifests unspecific clinical symptoms and diagnosis is more difficult [26].

Deoxynivalenol – DON (Fig. 1) was first isolated in Japan in 1972 by Mooroko, as mentioned in the study by B r e i d e n b a c h et al. [2]. It is the most common worldwide distributed trichothecene [27]. Its producers are *F. graminearum, F. culmorum, F. sporotrichioides, F. poae* and *F. acuminatum* [25]. It occurs mainly in wheat, barley, corn, oats, rice, sorghum and millet. DON inhibits DNA and RNA synthesis at the level of ribosomes. It damages the expression of cytokines and increases the level of intracellular calcium, which leads to caspase activation and DNA and protein breaks with subsequent cell apoptosis [23]. In poultry, after feeding feed containing deoxynivalenol, a reduced weight of the egg itself, a deterioration in the quality of the eggshell, and residues in the egg was observed [21]. Progressive rickets was detected when broilers were fed trichothecene-containing feed for a long time [4].

T-2 toxin (Fig. 2) is produced by several species of *Fusarium* moulds, mainly *Fusarium sporotrichioides* and *Fusarium poae*. The occurrence of T-2 toxin has been recorded in cereals (corn, wheat, oats and barley), beans and soybeans [16].

T-2 toxin is readily metabolized to HT-2 toxin from which it differs structurally by the functional group at the C4 position. T-2/HT-2 toxins have been shown to produce numerous adverse effects on many animals, these two mycotoxins are frequently evaluated together [22]. T-2 toxin is a strong inhibitor of protein synthesis at the level of ribosomal RNA, disrupts DNA and RNA synthesis and induces immunosuppression [1, 12]. Trichothecene T-2 toxin is characterized by extreme skin and mucosal toxicity [15]. It is very toxic for poultry, in turkeys and chickens it causes necrosis on the beak, oral cavity, tongue and hard palate. Intoxication of poultry by T-2 toxin can lead to a reduction in weight gain, poor feathering, motor function impairment, and increased susceptibility to *E. coli* and *Salmonella* spp. [6, 14, 24].

The aim of this research was to determine concentrations of trichothecenes, deoxynivalenol and T-2 toxin in mixed feed for broilers and turkeys by immunoassay ELISA.

MATERIALS AND METHODS

Samples

A total of 20 samples of mixed feed for poultry were examined (16 samples were collected from mixed feed for broilers and 4 samples from mixed feed for turkeys)

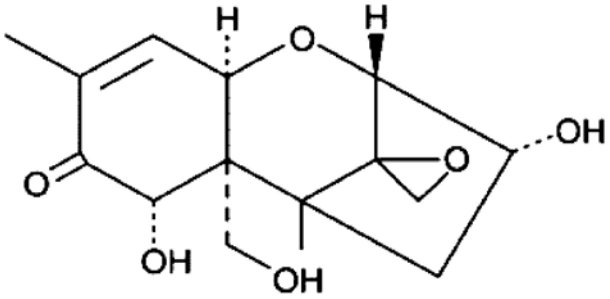


Fig. 1. Structural formula of deoxynivalenol

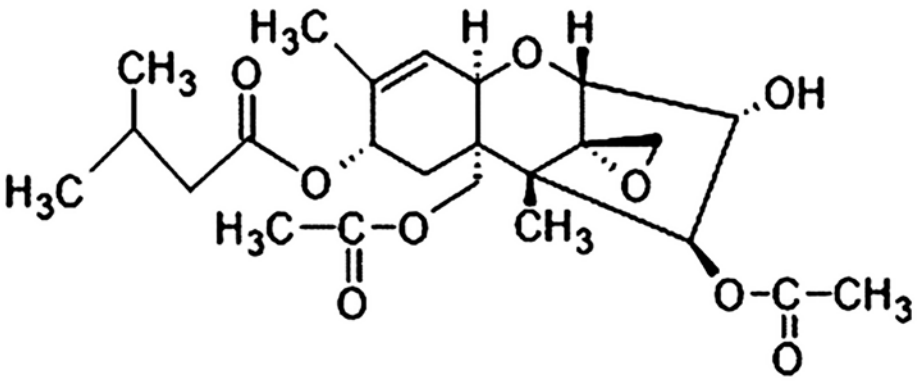


Fig. 2. Structural formula of T-2 toxin

(Tab. 1). The samples were obtained from commercial vendors in the form of pellets and were intended for different age categories of poultry. Mixed feed for broilers and turkeys was obtained from the producers from eastern Slovakia.

Determination of deoxynivalenol

The ELISA method was used to determine deoxynivalenol in samples of mixed feed for poultry. Analysis were performed using the Veratox 5/5 Quantitative DON Test (Neogen Corporation, Lansing, USA). Samples for the determination of deoxynivalenol were processed as follows: 50 g of each sample was ground and mixed with 250 ml of distilled water. The samples were mixed on a shaker for 3 minutes and then filtered through Whatman 1 filter paper (Cytiva, Kent, UK). The obtained filtrates from samples were diluted with distilled water (1 ml extract to 1 ml distilled water). Thus prepared samples were used in the ELISA analysis itself, which represents a direct competitive enzyme immunoassay. The principle of this method is the competition of unlabelled deoxynivalenol from samples and standards (standards with concentrations of deoxynivalenol 0, 0.25, 0.5, 1 and 2 ppm; $mg.kg^{-1}$) with enzyme labelled deoxynivalenol (conjugate) for antibody binding sites. After washing the samples, a substrate was added, which reacts with the conjugate to produce a blue colour. The more intense this colour reaction is, the less deoxynivalenol the sample contains. The resulting concentrations of deoxynivalenol (ppm) were determined spectrophotometrically at 650 nm using an ELISA reader (Dynex Technologies, Inc., Chantilly, USA).

Table 1. Number of analysed mixed feed for poultry

Mixed feed for broilers	Number of samples
BR1 (diet for fattening of broilers)	4
BR2 (diet for the growth of the broilers)	4
BR3 (final diet)	8
Mixed feed for turkeys	**Number of samples**
Morka Midi (diet for turkey from 9 to 12 weeks of age)	2
Morka Maxi (final diet)	2

Determination of T-2 toxin

The procedure for preparing samples for the determination of T-2 toxin was as follows: 25 ml of 70 % methanol was added to 5 g of the ground sample. The samples were shaken for 3 minutes on a shaker (Orbital Shaker Biosan) and filtered through a filter paper Whatman 1 (Cytiva, Kent, UK). After dilution with distilled water in a ratio of 1 : 1, they were prepared for quantitative determination using an ELISA kit. The procedure was performed according to manufacturer's protocol Veratox for T-2/HT-2 Quantitative Test (Neogen Corporation, Lansing, USA) The T-2 concentrations (ppb; $\mu g.kg^{-1}$) were determined at a wavelength of 650 nm by ELISA reader (Dynex Technologies, Inc., Chantilly, USA).

Statistical analysis

The statistical functions of the MS Excel software were used to evaluate the mean values and medians.

RESULTS

Table 2 shows the concentrations of trichothecenes deoxynivalenol and T-2 toxin in the mixed feed for poultry tested in this study. Deoxynivalenol in mixed feed for broilers was detected in all analysed samples (100 %) in a range of 0.435–1.829 ppm (average concentration 1.776 ppm). All tested samples of mixed feed for turkeys (100 %) were contaminated with deoxynivalenol in levels ranging from 0.283 to 1.067 ppm (average value 0.675 ppm). T-2 toxin

Table 2. Incidence [%] and concentrations of deoxynivalenol [$mg.kg^{-1}$] and T-2 toxin [$\mu g.kg^{-1}$] in poultry feed samples

Mixed feed	Parameter	DON	T-2 toxin
For broilers	n	16	16
	+/n	16/16 (100 %)	15/16 (93.8 %)
	Minimum	0.435	nd
	Maximum	1.829	42.285
	Average	1.776	36.625
	Median	1.179	17.419
For turkeys	n	4	4
	+/n	4/4 (100 %)	4/4 (100 %)
	Minimum	0.283	9.115
	Maximum	1.067	45.088
	Average	0.675	25.899
	Median	0.675	24.696

DON – deoxynivalenol; n – total number of samples; + – positive samples; nd – not detected

was present in 93.8 % of samples of mixed feed for broilers at an average concentration of 36.625 ppb and all the tested samples of mixed feed for turkey were contaminated (average value 25.899 ppb).

The recommended values of trichothecenes; deoxynivalenol and T-2 toxin were not exceeded in the tested samples.

DISCUSSION

Mycotoxin contamination of feed is a global agriculture problem [19]. Up to now, over 500 compounds have been identified as mycotoxins [17]. The most commonly studied mycotoxins with the greatest concern to human and animal health are aflatoxins, zearalenone, trichothecenes, patulin, ochratoxins, and fumonisins [11]. From the point of view of feed contamination, trichothecenes of groups A and B are of the greatest importance. Trichothecenes deoxynivalenol and T-2 toxin are predominantly found in cereals (wheat, maize), which are the basic part of feed mixtures for poultry. In the European Union, the maximum permitted content of deoxynivalenol in feed (EC Directive 2002/32/EC and EC Recommendations 2006/576/EC) is 5 $mg.kg^{-1}$ in supplementary and complete mixed feed and of T-2 toxin 250 $\mu g.kg^{-1}$ in mixed feed [5, 7, 29].

In our samples of poultry mixed feed, we recorded 100 % occurrence of deoxynivalenol in a range from 0.435 $mg.kg^{-1}$ to 1.829 $mg.kg^{-1}$. One hundred percent contamination of mixed feed for poultry with deoxynivalenol was also recorded by Cegielska-Radziejewska et al. [3]. However, DON was detected in the range of lower concentrations 0.003–0.099 $mg.kg^{-1}$ [3]. Greco et al. [10] reported that deoxynivalenol was found in 90 % of the analysed samples. According to Labuda et al., the mean DON content in positive samples (56 %) of mixed feed for poultry from Slovakia was 0.303 $mg.kg^{-1}$ [18]. In contrast, Magnoli et al. observed only 6 % deoxynivalenol contamination of poultry feeds in Argentina [19].

In our study, 93.8 % T-2 toxin contamination of mixed feed for broilers was confirmed. All tested samples of mixed feed for turkeys were contaminated by T-2 toxin. In the study of Labuda et al. [18], T-2 toxin was the most frequent mycotoxin detected. It was found in 90 % of the samples in relatively low concentrations ranging from 1 to 130 $\mu g.kg^{-1}$ (average value 13 $\mu g.kg^{-1}$). According to Greco et al. [10] T-2 toxin was detected in 38 samples out of 49 samples from Buenos Aires (78 %). However, in Poland, the presence of toxin T-2 was not detected in any of the total of 45 samples of feed mixture for poultry [3].

Deoxynivalenol and T-2 toxin in feed samples for poultry did not exceed maximum levels specified in the regulations and the concentrations detected were too small to cause any serious health problems to animals that consumed such feed.

CONCLUSIONS

The best protection against mycotoxins is the monitoring of their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product. Results indicate that the level of microbiological contamination in feeds for broiler chickens and turkeys produced in the Slovak Republic is within the requirements of the binding standards.

ACKNOWLEDGEMENT

This study was supported by the Scientific grant agency, VEGA project No. 1/0402/20 "Effect of additives in the nutrition of monogastric animals on production health, production parameters, products quality and environment."

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Received February 16, 2023
Accepted March 22, 2023