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OCCURRENCE OF OCHRATOXIN IN COMPLETE FEED MIXTURES FOR MONOGASTRIC ANIMALS

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ABSTRACT

Ochratoxin A is a foreign substance of natural origin. It can be found in a wide range of commodities, including animal feed. Ochratoxin A is a dangerous contaminant, which can have a negative effect on the health and production of animals. In this study, the incidence of ochratoxin A in a complete feed for broilers (n = 25) and pigs (n = 6) was determined. Ochratoxin A was detected in one sample of pigs feed (16.67 %) and its concentration was 1.221 µg.kg^{-1}. This mycotoxin was not detected in the broiler feed samples. These results indicate that the feed samples collected were safe and do not pose a risk of acute mycotoxicosis in animals.

Key words: animals; feeds; mycotoxins; contamination

INTRODUCTION

Ochratoxins are produced in tropical and subtropical regions mainly by micromycetes of the genus *Aspergillus* (*A. ochraceus*, *A. westerdijkiae*, *A. niger* and others) and in colder regions mainly by microscopic filamentous fungi of the genus *Penicillium* (*P. verrucosum* and *P. nordicum*) [28]. Several types of ochratoxins occur naturally: ochratoxin A, ochratoxin B (dechlorinated ochratoxin), ochratoxin C (ethylated ochratoxin), ochratoxin D (4-hydroxy-ochratoxin), 10-hydroxyochratoxin, and ochratoxin α.

Ochratoxin A (OTA), which was first isolated from a culture of *Aspergillus ochraceus,* is the most common food and feed mycotoxin [37]. OTA production by *A. ochraceus* can occur at temperatures up to 37 °C, whereas OTA production by *P. verrucosum* occurs at ambient temperature of 4–31 °C [13]. OTA (Fig. 1) is a chemically stable compound in the form of a white crystalline substance with a melting point of 168–173 °C. It dissolves in chloroform, ethanol and methanol. From a chemical point of view and the method of biosynthesis, OTA belongs to the pentaketides derived from dihydrocoumarins linked to β-phenylalanine [34]. Ochratoxin A ($C_{20}H_{18}ClNO_6$) has a molecular weight of 403.81 g.mol^{-1} and its chemical name is *N*-{[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1*H*-isochromen-7-yl] carbonyl}-*L*-phenylalanine [9,17]. Under UV radiation, OTA shows intense green fluorescence in an acidic medium and blue fluorescence in an alkaline medium [27]. The International Agency for Research on Cancer (IARC) has classified this mycotoxin as a possible human carcinogen (group 2B). However, due to several evidence on the carcinogenicity of OTA, it is being considered in the future to re-evaluate its classification and include ochratoxin A in group 2A as a probable carcinogen for humans [23].

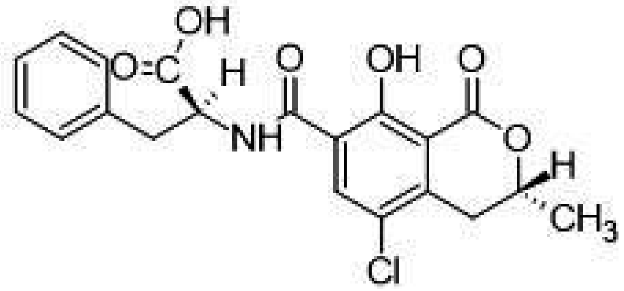

Fig. 1. Structural formula of ochratoxin A
Source: Own picture

Toxicity of ochratoxin A

Acute and chronic toxicity are associated with the ability of OTA to inhibit protein synthesis by competing with phenylalanine in reactions catalysed by phenylalanine-tRNA synthetase. In addition to inhibiting proteins, it increases lipid peroxidation, disrupts sugar metabolism, mitochondria functions and calcium metabolism [19]. As part of the toxicity of OTA, its nephrotoxic effects have been confirmed, but it can also have a hepatotoxic, neurotoxic, teratogenic and immunosuppressive effect. It is potentially nephrotoxic to all animal species and the most sensitive animals to the nephrotoxic effects of OTA are pigs [32]. It is reported that young animals are more sensitive to OTA due to the incomplete development of their immune system. The type and degree of toxicity varies depending also on the species of the animal. Polygastric animals are more resistant than monogastric to OTA toxicity, because in ruminants OTA is subjected to microbial degradation in the rumen [4,7]. Poultry are considered as more sensitive to ochratoxin A compared to mammals [40]. It is primarily nephrotoxic to poultry. In addition, ochratoxicosis in poultry may be manifested by reduced feed intake, low weight gain, reduced egg production, and egg quality [16, 21].

Occurrence of ochratoxin A

OTA has been found in a wide range of food and agricultural commodities, where its presence is mainly related to improper drying, and storage conditions [6]. Its occurrence has been confirmed in cereal grains (barley, maize, oats, rye, and wheat), silage, hay and compound feed [11]. As the presence of ochratoxin A in cereals cannot be completely avoided, guidelines for maximum levels of various mycotoxins in feed, cereals and cereal products intended for animal feed have been developed in EU (Table 1) [12].

Table 1. Reference values of ochratoxin A in products for animal feeding

Mycotoxin	Products for animal feeding	Reference value in μg.kg^{-1} (ppb) of feed in conversion to 12 % moisture content
Ochratoxin A	Feed ingredients	
	cereals and products of cereals	250
	Feed mixtures	
	for pigs	50
	for poultry	100
	for dogs and cats	10

Source: Commission Recommendation (2006/576/EC) [12]

The aim of this research was to determine concentrations of ochratoxin A in feed mixtures for broilers and pigs by enzyme-linked immunosorbent assay ELISA.

MATERIALS AND METHODS

Samples

A total of 31 samples of feed mixtures for monogastric animals were examined (25 samples were feed mixtures for broilers and 6 samples were feed mixtures for pigs). The feed samples were obtained from commercial sellers from eastern Slovakia and were in the form of pellets for broilers and in the form of meal for pigs. Feed mixtures were intended for different age categories of the above-mentioned monogastric animals (Tab. 2).

Table 2. Number of analysed feed mixtures for broilers and pigs

Feed mixtures for broilers	Number of samples	Form
BR1 (diet for fattening broilers; up to 10 days of age)	7	pelleted
BR2 (diet for the growth of the broilers; up to 30 days of age)	7	pelleted
BR3 (final diet)	11	pelleted
Feed mixtures for pigs	**Number of samples**	**Form**
Complete feed mixture (diet for growing pigs)	6	meal

Samples preparation and extraction

The compound feed samples were ground and mixed. Before extraction, an extraction solution (50 % methanol solution) was prepared by mixing 1 part of methanol with 1 part of distilled water for each sample tested. Ten grams of the ground sample were added to 40 ml of 50 % methanol/water and the samples were mixed vigorously for 5 minutes (Orbital Shaker Biosan). The extracts were filtered by pouring at least 5 ml of sample through a filter Whatman #1 (Cytiva, Kent, UK) and the filtrates were collected as a samples.

Procedure of ELISA analysis

The obtained filtrates from samples were used for ELISA analysis, which was performed according to the Veratox for Ochratoxin protocol (Neogen Corporation, Lansing, USA). The principle of this method is the competition of unlabelled ochratoxin from samples and standards (standards at concentrations of ochratoxin 0, 2, 5, 10 and 25 ppb; $\mu g.kg^{-1}$) with enzyme-labelled ochratoxin (conjugate) for antibody binding sites. After washing the samples, a substrate was added, which reacts with the conjugate to produce a blue colour. A higher concentration of OTA in the sample means a lower colour reaction. The resulting OTA concentrations (ppb) were determined from the calibration curve (Fig. 2) spectrophotometrically at 650 nm using an ELISA reader (Dynex Technologies, Inc., Chantilly, USA).

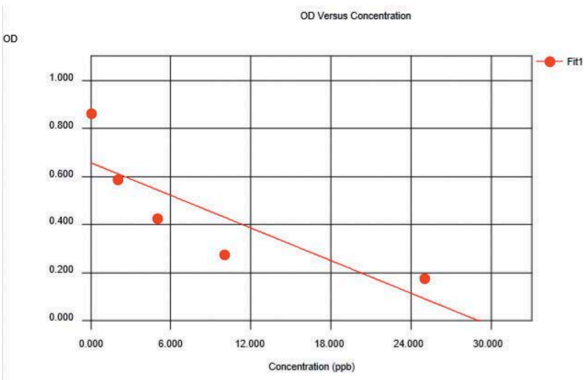

Fig. 2. Calibration curve; optical density (OD) versus concentration (ppb)

Statistical analysis

The statistical functions of the MS Excel software were used to evaluate the average values.

RESULTS

The results of the quantitative determination of ochratoxin A in feed mixtures for broilers and pigs are shown in Table 3. Ochratoxin A was detected at concentration 1.221 ppb in 1 sample of feed for pigs (16.67 %). In the other examined samples of feeds for broilers, ochratoxin A was not present.

Table 3. Occurrence (%) and concentrations of ochratoxin A (ppb) in samples of feeds for monogastric animals

Feed mixtures	Parameter	Ochratoxin A
for broilers	n	25
	+/n	nd/25
	Minimum	nd
	Maximum	nd
	Average	nd
for pigs	n	6
	+/n	1/6 (16.67 %)
	Minimum	nd
	Maximum	1.221
	Average	0.204

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

DISCUSSION

Quality nutrition is the basis of the productive health of animals. Feed represents a supply of energy and nutrients for animals [39]. The most important source of energy in feed is cereals, which are essential ingredients in compound feed for pigs and poultry. Cereals are a suitable substrate for the growth of microscopic filamentous fungi. Under suitable environmental conditions, fungi are able to synthesize secondary metabolites, mycotoxins. Mycotoxin contamination of feed limits the availability of nutrients and energy for livestock, which can result in suboptimal production, and mycotoxicosis in animals [22,39]. Pigs and poultry are important animals for livestock production and, due to their cereal-based diet, can be exposed to various mycotoxins [4]. Currently, 500 different mycotoxins are known and among the most important is ochratoxin A [3]. The accumulation of OTA at relevant levels in poultry meat and eggs occurs only when poultry are exposed to high levels of feed contamination. In pigs, OTA can accumulate in the kidneys and liver, and this may pose a risk to the human food chain [40].

The concentrations of OTA in animals feed varies from country to country and the highest OTA amounts have been reported in Northern Europe and North America

[38]. According to Binder et al. [5], OTA levels were detected in samples from Northern Europe at an average concentration of 8 μg.kg^{-1}, and maximum concentration of 10 μg.kg^{-1}. However, the higher concentrations were found in feed samples from Central Europe and average concentration was 231 μg.kg^{-1}, and maximum level of OTA was 530 μg.kg^{-1} [5].

In our samples of poultry feed mixtures, ochratoxin A was not present. According to Mikula et al. [20], OTA should not pose any significant threat to poultry in the Czech Republic [20]. In their study, OTA was present in 9 samples of feed for broilers (24.23 %) and the highest concentration was found to be 1.05 μg.kg^{-1}. This value is about 95 times lower than the guideline value (100 μg.kg^{-1}) established by the EU [12]. In their experiment, they also found that OTA occurs more in other poultry feeds (ducks, turkeys and quails), where the incidence of ochratoxin A was 64 % [20]. In the poultry feed samples from Spain, OTA was found in 42.9 % of samples in the concentration range from 0.42 to 0.58 μg.kg^{-1} [15]. A similar OTA incidence (38.5 %) was recorded in Jordan and poultry feed samples were containing an ochratoxin A with an average concentration of 2.90 μg.kg^{-1} [2]. Schiavone et al. confirmed the presence of OTA in all poultry feed samples with values ranging from 0.04 to 6.50 μg.kg^{-1} [31].

In our study, ochratoxin A was present in one sample (16.67 %) of complete feed for pigs and its concentration was 1.221 μg.kg^{-1}. Ochratoxin A was determined by Pozzo et al. in samples of pigs feed from northwest Italy and all feed samples were contaminated (100 %) with OTA, ranging from 0.22 to 38.4 μg.kg^{-1} [26]. Sdogati et al. (2024) confirmed the incidence of ochratoxin A (35 %) in pig feed samples from central Italy and one sample (0.6 %) contained OTA above the reference value according to EU (>50 μg.kg^{-1}) [29]. The analysis of compound feeds for pigs from Poland showed that 27 % was contaminated with ochratoxin A, and average concentration of OTA was 27.3 μg.kg^{-1} [24].

The occurrence of OTA is variable and its presence is related to the types of cultivated agricultural crops and climatic conditions [1]. The most relevant abiotic factors which influence of OTA production are water availability and temperature. According to Magan and Aldred, the moisture content of cereal grain should be lower than 14.5 % during storage to avoid higher OTA contamination [18]. Currently, other preventive measures for the elimination of mycotoxins are also known. These include physical (separation, cleaning, grinding, cooking, roasting, baking, etc.) [35], chemical (adding adsorbents, feed additives, inhibitors, antioxidants, etc.) [36], and biological methods (for example application of *Lactobacillus* spp., *Bacillus subtilis*, essential oils and other) [8, 10, 14, 25, 30, 33, 37].

CONCLUSIONS

Maintaining the productive health of animals also depends on quality nutrition. Foreign substances such as mycotoxins significantly disrupt animal health, and thus cause large economic losses. Regular monitoring of mycotoxin concentrations is a very important preventive measure. According to our results, we can assume that the samples that we have examined are safe and do not contain ochratoxin A, in critical concentrations, and meet the standards of the European Union.

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