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Ochratoxin Incidence in Poultry Feed Mixtures

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ABSTRACT— ***Ochratoxin is one of the most frequently occurring mycotoxins in food and feed. In humans and animals, it is the cause of health disorders, mycotoxicosis. Monitoring the content of ochratoxin in food and feed is an important part of preventive measures. This work was focused at determining the concentrations of ochratoxin in complete feed mixtures for broilers and turkeys by ELISA analysis. The presence of ochratoxin in concentrations lower than 2 ppb was confirmed in the examined samples. The ochratoxin content limit for supplementary and complete feed mixtures for poultry, which represents a value of 100 ppb in feed with a moisture content of 12%, was not exceeded in the samples.***
Keywords— mycotoxins, poultry, feed mixtures, ELISA analysis

1. INTRODUCTION

Ochratoxins represent a group of secondary metabolites of microscopic filamentous fungi, which are mainly synthesized by the genera *Asperillus* and *Penicillium* [1]. Currently, several types of ochratoxins occur naturally: ochratoxin A, ochratoxin B (dechlorinated ochratoxin), ochratoxin C (ethylated ochratoxin), ochratoxin D (4-hydroxyochratoxin), 10-hydroxyochratoxin and ochratoxin α. The most common food mycotoxin is considered to be ochratoxin A [2,3]. Its occurrence is mainly related to improper drying and storage conditions. It has been confirmed in a wide range of food and agricultural commodities [4]. The presence of ochratoxin A has been reported in cereals, legumes, oilseeds, dried fruits and other products [5]. Given that cereals are a basic component of feed mixtures for poultry, they represent a potential risk of developing health complications in animals through contaminated feed. When animals consume feed/feed mixtures spoiled by mycotoxins, the epithelial cells of the esophagus and gastrointestinal tract are the first to be negatively affected, leading to gastrointestinal dysfunction, gut microbial imbalance, malnutrition, diarrhea and ultimately to impaired animal production health [6]. In addition, ochratoxin A is nephrotoxic, hepatotoxic, teratogenic and immunotoxic in poultry [7]. Compared to mammals, poultry is more sensitive to the effects of ochratoxin A. Especially younger individuals are more sensitive due to an incompletely developed immune system [8].

In addition to disturbing the health of animals, the presence of residues in animal production products, which directly threaten the health of final consumers, is considered a risk factor. Residues of mycotoxins can occur in muscle, internal organs and eggs. The International Agency for Research on Cancer (IARC) classified ochratoxin A as a group 2B probable human carcinogen. However, in the future, it is being considered to reassess its classification and include ochratoxin A in group 2A as a probable carcinogen for humans [9].

Continuous monitoring of secondary metabolites of microscopic fungi in food and feed is considered a fundamental element of prevention of health complications in humans and animals. Therefore, the aim of this work was to determine the concentration of ochratoxin in complete feed mixtures for broilers and turkeys using immunoenzymatic ELISA analysis.

2. MATERIALS AND METHODS

2.1 The samples of feed mixtures

A total of 20 samples of feed mixtures for poultry were examined (16 samples were feed mixtures for broilers and 4 samples were feed mixtures for turkeys). The samples were obtained from different commercial sellers in the form of pellets and were intended for different stages of poultry fattening (feed mixture of the first stage of broiler fattening - 4 pcs, feed mixture of the second stage of broiler fattening - 4 pcs, feed mixture of the third stage of broiler fattening - 8 pcs and feed mixture for the first phase of fattening turkeys - 2 pcs and for the second phase of fattening turkeys - 2 pcs).

2.2 The ELISA analysis

The ELISA method was used for the *in vitro* determination of ochratoxin in samples of feed mixtures for poultry. Analyzes were performed using the Veratox kit - Veratox for ochratoxin (Neogen corporation, USA). Samples for ochratoxin determination were processed as follows: 10 g of each sample was ground and mixed with 40 ml of 50%

methanol. The samples were mixed on a shaker for 5 minutes and then filtered through filter paper Whatman 1. The obtained filtrates from the samples with a minimum volume of 5 ml were used in the ELISA analysis itself, which represents a direct competitive enzyme immunoassay. The principle of this method is the competition of free ochratoxin from samples and controls (standards with ochratoxin concentrations of 0, 2, 5, 10 and 25 ppb) with the ochratoxin-labeled enzyme (conjugate) for antibody binding sites. After washing the samples, a substrate was added, which reacts with the conjugate to produce a blue color. The more intense this color reaction is, the less ochratoxin the sample contains. The resulting concentrations of ochratoxin (ppb) were determined spectrophotometrically at 650 nm using an ELISA reader (Dynex Technologies, Inc., Virginia, USA).

3. RESULTS

The results of the quantitative determination of ochratoxin in feed mixtures for poultry are shown in table no. 1. From the total number of examined samples of poultry feed mixtures, the concentrations of ochratoxin were lower than the quantification range of the ELISA kit used, which was in the range from 2 to 25 ppb.

Table 1 Determination of ochratoxin (ppb) in feed mixtures for poultry

Sample ID	Location	(OD) Data	(OD) Mean	S.D.	C.V.	Dilution	(ppb) Conc.
T1	F1	1.045	1.045	*****	*****	1	-----
T2	G1	1.028	1.028	*****	*****	1	-----
T3	H1	1.020	1.020	*****	*****	1	-----
T4	A2	1.053	1.053	*****	*****	1	-----
T5	B2	1.021	1.021	*****	*****	1	-----
T6	C2	1.031	1.031	*****	*****	1	-----
T7	D2	1.021	1.021	*****	*****	1	-----
T8	E2	0.900	0.900	*****	*****	1	-----
T9	F2	0.947	0.947	*****	*****	1	-----
T10	G2	1.022	1.022	*****	*****	1	-----
T11	H2	0.947	0.947	*****	*****	1	-----
T12	A3	1.125	1.125	*****	*****	1	-----
T13	B3	1.197	1.197	*****	*****	1	-----
T14	C3	1.146	1.146	*****	*****	1	-----
T15	D3	0.917	0.917	*****	*****	1	-----
T16	E3	1.084	1.084	*****	*****	1	-----
T17	F3	1.139	1.139	*****	*****	1	-----
T18	G3	1.508	1.508	*****	*****	1	-----
T19	H3	1.133	1.133	*****	*****	1	-----
T20	A4	1.183	1.183	*****	*****	1	-----

Abbreviations: T1 – T16 - feed mixtures for broilers, T17 – T20 - feed mixtures for turkey, ----- - values lower than the range of ELISA analysis.

4. DISCUSSION

Potentially toxinogenic microscopic filamentous fungi are able to produce secondary metabolites - mycotoxins under certain environmental conditions. The most frequently occurring mycotoxins include, among others, ochratoxin. After aflatoxin B1, ochratoxin is the most important mycotoxin in terms of economic losses worldwide [10]. In addition to the direct negative effect of ochratoxin on the gastrointestinal tract of humans and animals, it is nephrotoxic, hetotoxic and immunotoxic [11]. Occurrence of ochratoxin has been confirmed in food (cereals, coffee, wine, dried fruits, nuts, spices) and in the form residues also in products of animal origin such as meat, organs and eggs [12]. An important element of prevention is the mycotoxic control of animal feed. It is the consumption of contaminated feed that can be the cause of damage to the health of animals, and through products of animal origin, the final consumer is also at risk.

In the samples of complete feed mixtures examined by us, ochratoxin was present in concentrations lower than 2 ppb. Similar ochratoxin values were determined in samples from Croatia by Domijan et al. [13]. Poultry feed samples contained ochratoxin in concentrations ranging from 0.42-6.19 ppb. Even lower concentrations of ochratoxin in the range of 0.04-6.5 ppb were found in poultry feed samples from Italy [14]. Similar results were reported in Spain, where ochratoxin concentrations in poultry feed averaged 1.53 ppb [15]. Using the ELISA method, ochratoxin concentrations were determined in samples of complete feed for broilers from Pakistan with resulting values in the range of 22-190 ppb [16]. The higher ochratoxin content in samples from Pakistan is probably related to the climatic zone that is favorable for the growth of microscopic fungi that are capable of producing mycotoxins [12]. Pakistan is one of the countries where there is no regulation and there are no legal standards to limit the content of mycotoxins in feed [17]. In Slovakia, the Commission Recommendation (2006/576/EC) currently applies, which deals with the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding, where the limiting value for supplementary and complete poultry feed with a moisture content of 12% is 100 ppb [18].

According to the achieved results of this work, it is clear that the poultry feed samples we examined meet the standards according to the current legislation in Slovakia.

5. CONCLUSION

Currently, emphasis is placed on the production of healthy food. The first prerequisite is the use of high-quality feed in livestock breeding. Monitoring the content of foreign substances that may occur in complete feed mixtures for animals is the first step in the prevention system. Foreign substances in feed also include secondary metabolites of microscopic filamentous fungi. Their occurrence cannot be completely prevented, but by regular monitoring and the use of elimination methods, we can reduce their concentrations to the values specified in the legal norms that are valid in Slovakia.

6. ACKNOWLEDGEMENT

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