

## Zearalenone Contamination in Corn

Harčárová Michaela<sup>1</sup>, Čonková Eva<sup>2</sup>, Nad' Pavel<sup>1</sup>, Proškovcová Martina<sup>2</sup> and  
Zigo František<sup>1</sup>

<sup>1</sup> University of Veterinary Medicine and Pharmacy in Košice, Department of Animal Nutrition and Husbandry,  
Komenského 73, 04181 Košice, Slovak Republic

<sup>2</sup> University of Veterinary Medicine and Pharmacy in Košice, Department of Pharmacology and Toxicology,  
Komenského 73, 04181 Košice, Slovak Republic

### \*For Correspondence

#### Correspondence Author

Department of Animal Nutrition and Husbandry,  
University of Veterinary Medicine and Pharmacy in  
Košice, Slovakia  
[frantisek.zigo@uvlf.sk](mailto:frantisek.zigo@uvlf.sk)

**Keywords:** Cereals, Mycotoxin contamination,  
Zearalenone

**Abstract:** The most common problem in agricultural industry is the contamination of corn with zearalenone. Zearalenone may cause humans and animals intoxications. It is often a cause of the disruption the breeding process with consequent economic losses. The aim of this work was to determine the incidence and concentrations of zearalenone ( $\mu\text{g/kg}$ ) in corn samples using enzyme-linked immunosorbent assay (ELISA). Incidence of zearalenone in corn samples achieved 46.4 % (13 maize samples) with concentrations range 69.897 – 1531.617  $\mu\text{g/kg}$  and average value 399.578  $\mu\text{g/kg}$ . The detected levels of zearalenone did not exceed the maximum acceptable limits of 3,000  $\mu\text{g/kg}$ .

---

### I. INTRODUCTION

The economic importance of corn cultivation (*Zea mays*) is mainly related to the provision of human and animal nutrition. However, corn is also used for industrial purposes (in the starch, fat, distilling, chemical or milling industries and for the production of bioethanol or biofuel). Of the total world production, a substantial part of corn is mostly used for animal feed. Corn is a highly concentrated carbohydrate feed suitable for feeding all types of livestock and is the main component of compound feed for poultry and pigs. Intensive cultivation of corn also carries certain risks. Among other things, this is the contamination of corn with microscopic filamentous fungi, which can occur during plant growth in the field or during grain storage [1].

The most important field fungi are representatives of the genera *Fusarium* and *Alternaria* and the most common storage fungi are species of the genera *Penicillium* and *Aspergillus* [7]. *Fusarium* fungi have been found to colonize more than 50% of corn prior to harvest [10]. In addition to the degradation of corn due to reduced nutritional properties of the grain, fusaria are able to synthesize secondary metabolites, mycotoxins. Important *Fusarium* mycotoxins are trichothecenes (deoxynivalenol, nivalenol, T-2 toxin), zearalenone and fumonisins [6]. Zearalenone (ZEA), also known as F-2 toxin, is a secondary metabolic product of microscopic filamentous fungi of the genus *Fusarium*, mainly *Fusarium graminearum*, but also other species such as *Fusarium equiseti*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium nivale*, *Fusarium roseum*, *Fusarium sporotrichioides* and others [16].



Zearalenone is a non-steroidal mycoestrogen with a chemical structure similar to steroid hormone which ensures its ability to bind to the intracellular estrogen receptors of the uterus, hypothalamus and pituitary gland [2]. Zearalenone acts as an estradiol agonist and partial antagonist, inhibits follicle-stimulating hormone secretion and prevents ovarian follicle maturation in the preovulatory phase [8]. The estrogenic effects of zearalenone cause not only reproductive disorders in animals but also hyperestrogenic syndrome in humans [13]. Pigs are most susceptible to zearalenone intoxications compared to other animal species. Typical clinical signs of intoxication are edema and hyperemia of the vulva in sexually immature sows [5]. Persistent corpus luteum, anestrus and pseudogravidity may occur in sows if zearalenone is present in the feed in concentrations between 3,000 and 10,000 µg/kg. ZEA at concentrations above 30,000 µg/kg in feed can cause early embryonic death if given to the animal within 1 to 3 weeks after fertilization [11]. In addition to genital pathological effects, zearalenone can have hepatotoxic, hematotoxic, immunotoxic and genotoxic effects [17].

The aim of this work was to determine the incidence and levels of zearalenone (µg/kg) in corn samples using the enzyme-linked immunosorbent assay (ELISA).

## **II. Materials and Methods**

The incidence of zearalenone was detected in 28 samples of corn after harvest in 2018 (Tajba a. s., Čečejevoce, Slovak Republic). The analysis was performed using a commercial Veratox for zearalenone kit (Neogen Corporation, USA). The samples were prepared as follows: 25 ml 70% methanol was added to 5 g of the ground sample. The samples were shaken on an orbital shaker (Orbital Shaker - Biosan) for 3 minutes and filtered through Whatman 1 filter paper. After dilution with distilled water 1: 5, the samples were prepared for quantification using an ELISA kit. The resulting zearalenone concentrations (µg/kg) were read and evaluated using ELISA reader (Dynex Technologies, Inc., Virginia, USA).

## **III. Results and Discussion**

The occurrence of zearalenone in the analyzed corn samples is presented in Tab. 1. Out of a total 28 samples, zearalenone was confirmed in 13 samples, representing an incidence 46.4%. Zearalenone levels in examined samples ranged from 69.897 µg/kg to 1531.617 µg/kg and the average ZEA concentration was 399.578 µg/kg. The detected levels of ZEA did not exceed the maximum limits of 3,000 µg/kg.

Table 1. Incidence of zearalenone in corn samples (µg/kg)

Cereals	n/n*	I (%)	Concentrations of ZEA (µg/kg)	Average value (µg/kg)
Corn	28/13	46.4	69.897-1531.617	399.578

Note: n – total number of examined samples, n\* – number of samples with zearalenone, I – incidence of zearalenone, ZEA – zearalenone.

Corn is one of the cereals and they are a suitable substrate for the growth of microscopic filamentous fungi. The presence of micromycetes in cereals and environmental factors such as temperature and humidity are essential for mycotoxin synthesis [14]. Zearalenone is an important mycotoxin that poses a threat to human and animal health.

In the examined samples, zearalenone occurred in 13 samples with an incidence of 46.4%, in the concentration range of 69.897 - 1531.617 µg/kg. A higher percentage of corn contamination by zearalenone was confirmed in samples in Croatia, where the incidence of ZEA was up to 84%, but the concentration range (0.4 - 39 µg / kg) was lower than in the samples examined [3].

Similarly, Pleadin et al. (2012) found a higher incidence of zearalenone (88%) in corn samples from Croatia with concentrations range from 2 to 5,110 µg/kg [12]. The incidence of ZEA in samples from Switzerland was 79%, ranging from 16 to 1,260 µg/kg [4]. Lower contamination with zearalenone (8%) was recorded by Manova and Mladenova (2009) in samples from Bulgarian corn and Tabuc et al. (2011) in corn from Romania (32%) [9,15]. The maximum concentration of zearalenone in corn samples in Bulgaria reached up to 4050 µg/kg [9]. In monitoring the content of mycotoxins in feed, the Slovak Republic follows Directive 2002/32/EC on undesirable substances in feed and Commission Recommendation No. 576/2006/EC on limits for the content of mycotoxins



in feedingstuffs. The maximum level for zearalenone in corn by-products is 3,000 µg/kg. Zearalenone concentrations in the examined corn samples did not exceed the maximum limit.

#### IV. Conclusion

To ensure the production of quality agricultural crops, constant control is needed. This inspection begins at the time of crop growth in the field, during harvesting and also during storage. Regular monitoring of the incidence of microscopic filamentous fungi and their secondary metabolites can reduce the negative impact on livestock health. To eliminate the occurrence of microscopic fungi and mycotoxins various physical, chemical and biological methods are currently used. In addition to the use of decontamination procedures, good agricultural practice should be followed (efficient crop rotation, choice of suitable variety/hybrid, optimal sowing plan, adequate soil preparation and treatment, cultivation, collection, storage and crop transport).

#### V. Acknowledgements

This study was supported by the grant from the Culture and Education Agency of the Ministry of Education of the Slovak Republic for the project VEGA 1/0402/20 and KEGA 006UVLF-4/2020: *Implementation of new scientific knowledge in teaching and improving the practical training of students in breeding technology from subject Animal husbandry.*

#### References

- [1] S.A.O.Adeyeye, Fungal mycotoxins in foods: A review, *Cogent Food & Agriculture*, 2(1), 2016, 1-11.
- [2] M. Devreese, P. De Backer, S. Croubels, Overview of the most important mycotoxins for the pig and poultry husbandry, *Vlaams Diergeneeskundig Tijdschrift*, 8(4), 2013, 171-180.
- [3] A.M. Domijan, M. Peraica, Ž. Jurjević, D. Ivić, B. Cvjetković, Fumonisin B1, fumonisin B2, zearalenone and ochratoxin A contamination of maize in Croatia, *Food Additives and Contaminants*, 22, 2005, 677-680.
- [4] B. Dorn, H.R. Forrer, E. Jenny, F.E. Wettstein, T.D. Bucheli, S. Vogelgsang, *Fusarium* species complex and mycotoxins in grain maize from maize hybrid trials and from grower's fields, *Journal of Applied Microbiology*, 111, 2011, 693-706.
- [5] R.C. Gupta, *Reproductive and Developmental Toxicology* (USA: Elsevier Academic Press, 2011).
- [6] M.A. Haque, Y. Wang, Z. Shen, X. Li, M.K. He, C. Saleemi, Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review, *Microbial Pathogenesis*, 142, 2020, 104095.
- [7] I. Jedidi, C. Soldevilla, A. Lahouar, P. Marín, M.T. González-Jaén, S. Said, S., Mycoflora isolation and molecular characterization of *Aspergillus* and *Fusarium* species in Tunisian cereals, *Saudi Journal of Biological Sciences*, 25(5), 2018, 868-74.
- [8] H. Malekinejad, E.J. Schoevers, I.J. Daemen, C. Zijlstra, B. Colenbrander, J. Fink Gremmels, Exposure of oocytes to the *Fusarium* toxins zearalenone and deoxynivalenol causes aneuploidy and abnormal embryo development in pigs, *Biology of reproduction*, 77(5), 2007, 840-847.
- [9] R. Manova, R. Mladenova, Incidence of zearalenone and fumonisins in Bulgarian cereal production, *Food Control*, 20, 2009, 362-365.
- [10] S.A. Okoth, E. Siameto, Evaluation of selected soil fertility management interventions for suppression of *Fusarium* spp. in a maize and beans intercrop, *Tropical and Subtropical Agroecosystems*, 13(1), 2011, 73-80.
- [11] G.D. Osweiler, *Occurrence of mycotoxins in grains and feeds. Diseases of Swine* (USA: Blackwell Publishing, 2006).
- [12] J. Pleadin, M. Sokolović, N. Perši, M. Zadavec, V. Jaki, A. Vulić, Contamination of maize with deoxynivalenol and zearalenone in Croatia, *Food Control*, 28, 2012, 94-98.
- [13] M. Poór, S. Kunsági-Máté, N. Sali, T. Kőszegi, L. Szente, B. Peles-Lemli, Interactions of zearalenone with native and chemically modified cyclodextrins and their potential utilization, *Journal of Photochemistry and Photobiology B: Biology*, 151, 2015, 63-68.
- [14] M.C. Smith, Natural Co-Occurrence of Mycotoxins in Foods and Feeds and Their in vitro Combined Toxicological Effects, *Toxins*, 8(4), 2016, 94.
- [15] C. Tabuc, I. Taranu, L. Calin, Survey of mould and mycotoxin contamination of cereals in South-Eastern Romania in 2008–2010, *Archiva Zootechnica*, 14, 2011, 25-38.



- [16] G.L. Zhang, Y.L. Feng, J.L. Song, X.S. Zhou, Zearalenone: A Mycotoxin With Different Toxic Effect in Domestic and Laboratory Animals' Granulosa Cells, *Frontiers in Genetics*, 9, 2018, 667.
- [17] A. Zinedine, J.M. Soriano, J.C. Molto, J. Manes, Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin, *Food and Chemical Toxicology*, 45(1), 2007, 1-18.

