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Determination of Zearalenone in Feed for Broilers and Turkeys Using the ELISA Assay

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Abstract: *The use of quality and safe feed is the basic element of prosperous livestock farming. Microscopic fungi can produce secondary metabolites - mycotoxins. The most common contaminant of cereals is zearalenone and its occurrence has been confirmed mainly in corn. In this study, a total of 24 poultry feeds (20 broiler feeds and 4 turkey feeds) consisting mainly of maize and wheat were investigated. Zearalenone was found in 18 broiler feed samples with an average concentration value of 163.937 µg.kg^{-1}. In turkey feed samples, zearalenone was present in 3 samples and the average concentration value was 18.896 µg.kg^{-1}. The determined values were in accordance with the valid EC Recommendation 2006/576/EC.*

I. INTRODUCTION

Feed safety is a fundamental element of efficient and economically beneficial livestock breeding. Through high-quality feed, among other things, the good health of animals, their efficiency and productivity is ensured. The basic components of feed for farm animals are cereals, which are primarily a source of carbohydrates in the form of starch, contain nitrogenous substances, fat, minerals and vitamins. For the production of feed for animals, cereals are grown mainly for the production of grains and provide the so-called concentrated feed. The most dietary important cereals are corn, wheat, barley, oats and rye [1]. The presence of corn and wheat is linked to the risk of the presence of foreign substances in feed, such as mycotoxins. Mycotoxins are toxic substances of natural origin with low molecular weight [2]. They arise as secondary metabolites of microscopic filamentous fungi of various genera. Agriculturally, the most important genera of micromycetes are *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. [3]. More than 400 mycotoxins are currently identified [4].

Zearalenone, which is produced by species such as *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium sporotrichioides* and others, is included among the most important mycotoxins worldwide. In terms of toxicity, it mainly affects the reproductive system, liver and kidneys, it is an important immunotoxin. Zearalenone and its metabolites show a structure similar to 17-β estradiol and are able to bind to estrogen receptors of reproductive organs, which has a negative effect on estrogen levels and may be the cause of disruption of the reproductive system of animals [5]. Poultry is relatively resistant to the toxic effects of zearalenone and it is related to the slower absorption of zearalenone in the organism and the faster elimination of its metabolites through the liver and the metabolic activity of intestinal microorganisms [6].

It has been reported that reproductive disorders in broilers and turkeys occur only at high concentrations of zearalenone of 400 $mg.kg^{-1}$ and lower concentrations (2 $mg.kg^{-1}$) may cause slow growth and achondroplasia [7]. Feeding with zearalenone-contaminated feeds for poultry may cause changes in biochemical parameters (decrease in the concentration of triacylglycerides and globulins, increase in AST and ALT) [5].
For simple, rapid and low cost detection, high sensitivity immunoassay methods were developed [8]. The most widely used immunoassay methods include enzyme-linked immunosorbent assay (ELISA), rapid immunochroma to graphic assays (ICA), immunochip, immunosensor and other [9-12].
The aim of this work was to determine the concentrations of zearalenone in feed samples for broilers and turkeys using quantitative ELISA analysis.

II. Materials and Methods

A total of 24 poultry feeds were examined. Twenty feeds were intended for the nutrition of broilers in different stages of fattening and 4 feeds were intended for turkeys. Feeds were obtained from sellers from eastern Slovakia. Feed for broilers and turkeys was mainly composed of cereals and other components (Tab 1).

Table 1 Type, quantity, composition and form of feed for broilers and turkeys

Feed/Number	Composition	Form
BR1/6	corn, wheat, soybean meal, dried blood monocalcium phosphate, salt, lysine, methionine, premix	peleted
BR2/6	corn, wheat, soybean meal, monocalcium phosphate, salt, lysine, methionine, premix	peleted
BR3/8	corn, wheat, soybean meal, monocalcium phosphate, salt, lysine, methionine, premix	peleted
Morka Midi/2	corn, wheat, soybean meal extracted, wheat mill residues, rapeseed meal, wheat bran, calcium carbonate, sunflower oil, monocalcium phosphate, sodium chloride, sodium bicarbonate	granulated
Morka Maxi/2	corn, wheat, soybean meal extracted, wheat mill residues, rapeseed meal, wheat bran, calcium carbonate, sunflower oil, monocalcium phosphate, sodium chloride and sodium bicarbonate	granulated

Abbreviations: BR1 – feed for fattening broilers up to 10 days of age, BR2 – feed for growth of broilers up to 30 days of age, BR3 – final diet, Turkey Midi – feed for turkeys from 9 to 12 weeks of age, Turkey Maxi – feed for turkeys from the 13th week of age until slaughter

The preparation of feed samples for the quantitative determination of zearalenone was carried out by following method: 25 ml of 70% methanol was added to 5 ml of ground sample, and then the samples were shaken for 3 minutes on a shaker (Orbital Shaker – Biosan) and filtered through filter paper Whatman 1. After dilution with distilled with water in a ratio of 1:5, were prepared for quantitative determination using ELISA analysis. The analyses themselves were performed using the Veratox for zearalenone kit (Neogen Corporation, Lansing, USA). The resulting concentrations of zearalenone (ppb; $\mu g.kg^{-1}$) were determined spectrophotometrically at 650 nm using an ELISA reader (Dynex Technologies, Inc., Chantilly, USA).

III. Results

The concentrations of the quantitative determination of zearalenone are shown in Tab 2 and Fig 1. Zearalenone was present in 18 broiler feed samples with an average concentration value of 163.937 $\mu g.kg^{-1}$. In turkey feed samples, the presence of zearalenone was confirmed in 3 samples and the average value of the concentration was 18.896 $\mu g.kg^{-1}$. Overall, the concentrations of zearalenone in feed for turkeys reached lower values than in feed for broilers (Fig 1). However, all determined values were in accordance with the valid EC

Recommendation 2006/576/EC and did not exceed the recommended values specified in this regulation (2000-3000 µg.kg^{-1}) [13].

Table 2 Determination of zearalenone (ppb) in feed mixtures for poultry

Parameters	Feed for broilers	Feed for turkeys
n	20	4
n*/%	18/90	3/75
Min.	nd	nd
Max.	372.438	56.205
x	163.937	18.896
Median	151.785	9.689

Abbreviations: n – total number of samples, n*/% – number and percentage of samples in which zearalenone was present, Min. – minimum concentration, nd – the value is lower than the range of the test kit, Max. – maximum concentration, x – the average value of concentrations

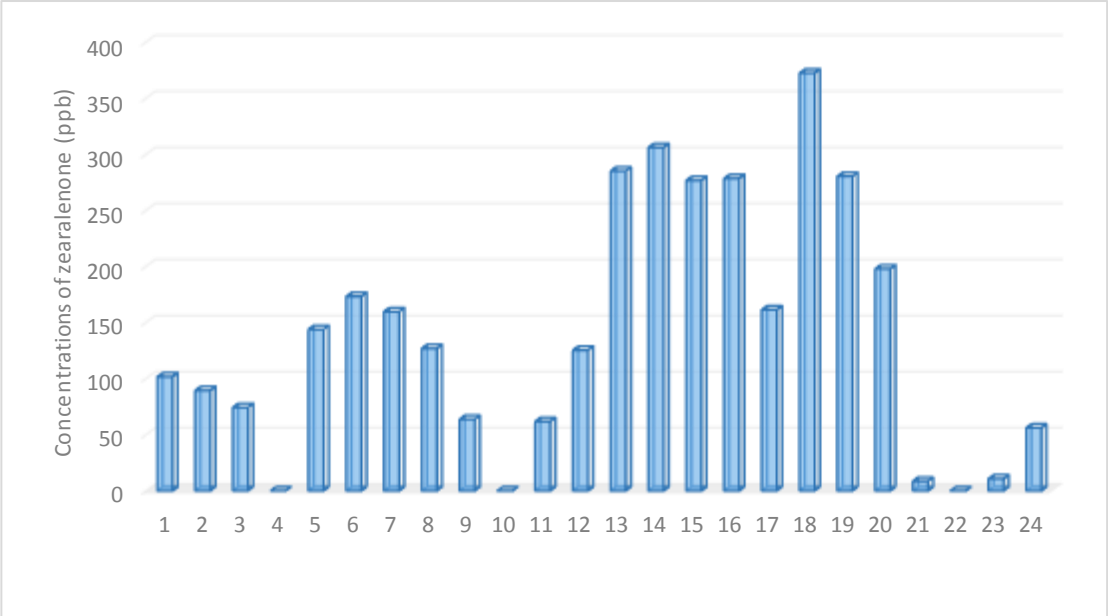

Figure 1: Concentrations of zearalenone (µg.kg^{-1}; ppb) in samples of feeds for broilers and turkeys
1-20 – feed samples for broilers, 21-24 – feed samples for turkeys

IV. Discussion

The cultivation of fodder crops, especially cereals, and their subsequent use for fodder purposes entails a risk associated with the presence of secondary metabolites of microscopic filamentous fungi - mycotoxins. Mycotoxin contamination of food and animal feed is a serious global problem [14]. Although there are many procedures and methods currently available to eliminate the growth of micromycetes and the degradation of mycotoxins, their occurrence cannot be completely prevented. Every year, under the influence of constantly changing weather and climatic conditions, a large amount of fodder is degraded by microscopic fungi and their secondary metabolites. The Food and Agriculture Organization of the United Nations (FAO) estimates that approximately 25% of the world's cereals are contaminated with mycotoxins [15].

Currently, the most agriculturally important genera of microscopic filamentous fungi include the genera *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. It is reported that in Europe, cereals are mainly

contaminated with mycotoxins of the genus *Fusarium*, mostly trichothecenes and zearalenone [16]. Zearalenone is often found in corn, even at very high concentrations [17]. Cereals are the major component of poultry feed, therefore monitoring the presence of mycotoxins is one of the essential elements of preventive measures. From a total of 24 poultry feed samples examined by us, zearalenone was present in 18 broiler feed samples and in 3 turkey feed samples, with an average concentration value of 163.937 $\mu g.kg^{-1}$; respectively 18.896 $\mu g.kg^{-1}$. Similar results were reported by Greco et al. (2014), who confirmed the occurrence of zearalenone in 44 samples out of a total of 49 samples (90%) and a median of 50 $\mu g.kg^{-1}$ [18].

Eighty-eight percent occurrence of zearalenone in poultry feed was recorded by Labuda et al. (2005), zearalenone was present in 44 samples out of a total of 50 with an average concentration value of 21 $\mu g.kg^{-1}$ [19]. In contrast, Magnoli et al. (2002) reported only 18% occurrence of zearalenone in 120 poultry feed samples [20]. In comparison, 16 samples out of a total 52 mixed-feed for laying hens were positive for zearalenone (30.7%) with concentrations ranged from 7.4 $\mu g.kg^{-1}$ to 61.4 $\mu g.kg^{-1}$ [21].

V. Conclusion

Continual monitoring of mycotoxin concentrations can partly prevent their negative impact on animal health and prevent economic losses in farms. For mycotoxins detection in agricultural products have been developed a number of assays, such as enzyme-linked immunosorbent assay (ELISA) and various chromatographic methods. Feeding mycotoxin-contaminated feed may not cause specific clinical symptoms in animals, therefore it is advisable to pay attention to the mycotoxic safety of the feed and observe the recommended levels specified in the legislation.

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