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Determination of Aflatoxins in Feed for Broilers

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Abstract: *The aim of this work was to quantitatively determine the concentrations of aflatoxins in samples of complete feed mixtures intended for fattening broiler chickens by ELISA analysis. From the total number of 17 samples, the presence of aflatoxins was confirmed in 4 cases (25.53%) with a maximum value of 19.342 µg/kg and an average concentration value of 6.654 µg/kg. The resulting concentrations are in accordance with the current legislation on undesirable substances in animal feed (2002/32 EC) and should not pose a risk of acute aflatoxicosis for broilers.*

Keywords: *broilers,ELISA assay, feed safety, mycotoxins*

I. INTRODUCTION

The first information about aflatoxins dates back to 1960 and is related to an outbreak of "Turkey X disease" on poultry farms in England. The toxin, which was isolated from the feed of affected individuals, was identified as the cause of death of over 100,000 turkeys that were fed a feed mixture containing peanut meal [1]. Aflatoxins are secondary metabolites (mycotoxins) that can be produced by various species of microscopic fungi of the genus *Aspergillus*, including *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. These micromycetes often contaminate agricultural crops during their growth in the field, during the harvesting, storage, transport and processing phases [2]. The ability of fungi to synthesize mycotoxins depends on various factors, such as geographical area, seasonal climatic conditions and temperature changes. One of the important factors for the development of mycotoxin contamination is the moisture content of feed [3,4]. Aflatoxins have been shown to have an affinity for oilseeds and nuts. The most commonly contaminated commodities include peanuts, corn and cottonseed, with corn and groundnuts being susceptible to pre-harvest contamination by aflatoxins. They can also be found in spices and foods of animal origin (milk and dairy products) [5].

All animal species are sensitive to the toxic effects of aflatoxins, with birds, fish, dogs and pigs being the most sensitive species, and young animals in general being the most sensitive. The presence of aflatoxins in poultry feed carries the risk of developing acute or chronic health complications [6]. The harmful effects of aflatoxins in poultry are non-specific and may be associated with changes in production parameters (reduced feed intake and feed conversion efficiency, low weight gain, reduced yield, reduced egg production) or with reproductive disorders. In broilers, reduced feed and water intake with subsequent weight loss has been described. Later, developmental depression may occur and affected individuals exhibit fluffy feathers, tremors, lameness, ataxia, paralysis of the legs, wing fluttering and even death. Pathological-anatomical findings in broilers include characteristic lesions on the enlarged liver, occasionally petechial haemorrhages, hypertrophic

gallbladder with dilated bile duct, and haemorrhagic catarrh of the digestive tract. The kidneys, liver, and spleen are hypertrophic, but the bursa of Fabricius and thymus are atrophied [6].

The aim of the work was to quantitatively determine aflatoxins in complete feed mixtures for broilers using immunoenzymatic analysis (ELISA) and compare the resulting concentrations with legal standards that limit the content of aflatoxins in poultry feed.

II. Materials and Methods

A total of 17 samples of complete feed mixtures for broilers were examined. The samples were obtained from different commercial vendors and were intended for different stages of broiler fattening (feed mixture of the first phase of broiler fattening - 3 pcs, feed mixture of the second phase of broiler fattening - 7 pcs, feed mixture of the third phase of broiler fattening - 7 pcs) (Tab. 1).

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

Feed/Number	Composition	Form
BR1/3	corn, wheat, soybean meal, dried blood monocalcium phosphate, salt, lysine, methionine, premix	peleted
BR2/7	corn, wheat, soybean meal, monocalcium phosphate, salt, lysine, methionine, premix	peleted
BR3/7	corn, wheat, soybean meal, monocalcium phosphate, salt, lysine, methionine, premix	peleted

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.

Representative samples (each 500 g) were stored in a dry and dark place before processing. Sample processing was carried out in the following way, 25 ml of 70% methanol was added to 5 g of ground and homogenized sample. Then a 3-minute shaking and extraction took place. The samples were filtered through Whatman filter paper No. 1 (Cytiva, Kent, UK) and the obtained filtrate was diluted with 1 ml of distilled water. The samples thus prepared were used for the quantitative determination of aflatoxins according to the RIDASCREEN FAST Aflatoxin, quantitative assay protocol (R-Biopharm AG, Darmstadt, Germany). The principle of the assay is an antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin antibodies. Standards (0 ppb; 1.7 ppb; 5 ppb, 15 ppb, 45 ppb), sample solutions, aflatoxin-enzyme conjugate and anti-aflatoxin antibodies were added to the wells. Free and enzyme-conjugated aflatoxin compete for the binding sites of the aflatoxin antibodies (competitive enzyme immunoassay). At the same time, the anti-aflatoxin antibodies are bound by the immobilized capture antibodies. Any unbound enzyme conjugate was then removed in a washing step using a washing solution. Substrate/chromogen solution was added to the wells and incubated for 5 minutes in the dark. The bound enzyme conjugate converts the chromogen to a blue product. Addition of stopping solution results in a colour change from blue to yellow. Measurement was performed photometrically at 450 nm, using an ELISA reader (Dynex Technologies, Inc., Chantilly, USA) where the absorbance is inversely proportional to the aflatoxin concentration in the sample.

III. Results

Table 2 shows the results of quantitative determination of aflatoxins (ppb; µg/kg) in 17 samples of complete feed mixtures for broilers. The incidence of aflatoxins was 23.53% and aflatoxins were present in 4 samples. The maximum concentration measured was 19.342 ppb and the mean concentration of alfatoxins was 6.654 ppb.

Table 2.Determination of aflatoxins (ppb) in feed mixtures for broilers

Parameters	Feed for broilers
Total number of samples	17
Number of samples with alfatoxins	4
Incidence (%)	23.53
Range of concentration (ppb)	nd-19.342
Average value of concentration (ppb)	6.654

Abbreviations: nd – not detected.

IV. Discussion

The Food and Agriculture Organization (FAO) has reported a 25% contamination of food and feed with mycotoxins worldwide [7]. However, a recent study has shown that up to 60-80% of crops worldwide are contaminated with mycotoxins, which significantly exceeds the figure reported by FAO [5,8]. According to Wu (2015), it is important to monitor aflatoxin concentrations in feed in poultry farms because they negatively affect health and serve as critical health indicators [9]. In addition to their carcinogenicity, their hepatotoxic, genotoxic, mutagenic, teratogenic, immunosuppressive, nephrotoxic and cytotoxic effects on the body are reported [10].

Currently, the immunoenzymatic method ELISAis considered a suitable and rapid method for detecting aflatoxins in feed and food, which is often used in research and various specialized laboratories. This technique is considered not only cheaper, but also easier to perform in practice compared to other analyses [11]. In the samples of complete feed mixtures for broilers examined by us using ELISA analysis, the presence of aflatoxins was confirmed in 4 samples out of a total of 17 samples, which represents an incidence of aflatoxins of 25.53%. The highest determined concentration of aflatoxins was 19.342 µg/kg and the average concentration value was 6.654 µg/kg. In the Czech Republic, they similarly investigated the determination of aflatoxins in complete feed mixtures for broilers and confirmed the presence of aflatoxins in 4 samples out of a total of 24 samples, which represents an incidence of 15.38% and the average concentration value was lower compared to our samples and represented a value of 0.104 µg/kg [12]. A higher incidence of aflatoxins was recorded in complete feed mixtures for broilers in samples in Pakistan. Out of a total of 30 samples, aflatoxins were present in 25 samples (83.33%) and the average concentration of aflatoxins determined in the aforementioned samples was as high as 20.63 µg/kg [13]. Sdogati et al., (2024) in their research monitored only the content of a specific type of aflatoxin - aflatoxin B_1 in poultry feed and the resulting incidence of aflatoxin B_1 was 8% with an average value concentration of 10.7 µg/kg [14]. According to Yunus et al. (2011), the concentration of aflatoxin B_1 necessary to reduce the performance of broilers would have to be 0.5 mg/kg (500 µg/kg) in feed [15]. Similar studies by other authors also agree with this statement (Dersjant et al., 2003; Devegowda et al., 2005)[16,17].

It should be noted that the aflatoxin concentrations determined by us are in accordance with the currently valid Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed, which sets a maximum level of aflatoxin in complete feed for poultry of 20 µg/kg [18].

V. Conclusion

Currently, the presence of secondary metabolites of microscopic filamentous fungi in feed and food cannot be completely prevented. They represent a hidden risk in human nutrition and in animal husbandry with a significant economic impact. In addition to generally known prevention procedures in the form of physical, chemical and biological methods, systematic monitoring of their occurrence is also an essential element of prophylaxis. In the samples examined by us, the concentrations of aflatoxins were in accordance with current legislation and should not pose a risk to animals of acute health complications in the form of aflatoxicosis.

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