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# AFLATOXIN PRESENCE IN SERBIAN POULTRY FEED DURING 2013-2014 PERIOD*

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***Summary:*** *The aim of this study was to compare the presence and the content of aflatoxin in poultry feed samples from two years (2013-2014). A total of 349 samples of complete feeding mixtures for turkeys, broiler chicks and laying hens were investigated. In 203 samples from 2013 year (10 samples of feed for turkeys, 145 samples of feed for broilers and 48 samples of feed for laying hens), overall aflatoxin content was ranged from 2 to 87 µg/kg, with the average value of 19 µg/kg. On the other hand, in 146 samples from 2014 year (14 samples of feed for turkeys, 78 samples of feed for broilers and 54 samples of feed for laying hens), overall aflatoxin content was notably lower (12 µg/kg) and ranged from 5 to 35 µg/kg. The obtained aflatoxin occurrence may lead to a conclusion that the high aflatoxin content during 2013 was an exception in comparison with 2014 and the studies from previous years. However, these results were not surprising taking into account a high degree of corn infestation by A. flavus molds during 2012, which came as a result of favorable agricultural conditions for the development of these molds.*

***Key words:*** *aflatoxin, poultry feed, ELISA, Serbia.*

## INTRODUCTION

Secondary fungal metabolites, as a natural food and feed contaminants, became an important factor in terms of food safety and public health. Mycotoxin production can occur during plant growth, maturity, harvesting, storage, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) (Bernardo, 2004; Lanyasunya et al., 2005). The Food and Agriculture Organization (FAO) estimated that 25% of the world's crops are affected by mycotoxins, of which the most notorious are aflatoxins (WHO, 1999). Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2) are secondary metabolic products of some *Aspergillus* spp.: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, and belong to

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a group of potent carcinogenic and teratogenic compounds (Kurtzman et al., 1987; Martins, 1989). The most potent known hepatocarcinogenic substance is aflatoxin B1 (AFB1); recently, after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011).

Many authors investigated aflatoxin toxicity and presence of its residues in poultry (Ghosh et al., 1990; Quezada et al., 2000; Oguz et al., 2002; Bintvihok and Kositcharoenkul, 2006; Iqbal et al., 2014). The highest concentrations of aflatoxins are usually found in liver and kidneys. This is in accordance with generally accepted fact that these organs play key role in metabolism of xenobiotics (Iqbal et al., 2014). Chickens that consume aflatoxin-contaminated feed have increased levels of hepatic enzymes in their blood, likely due to necrosis of liver tissue (Oguz et al., 2002). Gosh et al. (1990) found that AFB1 decreased the values of T-lymphocyte counts in broilers. These conditions lower immune response and make chickens highly susceptible to various bacteria and fungal infections. Chronic exposure of chickens to aflatoxins is often related to the loss of weight and has impact on reproductive capabilities (Quezada et al., 2000; Bintvihok and Kositcharoenkul, 2006). These problems may cause great economic losses in poultry industry. Aflatoxins (AFs) also show carcinogenic, teratogenic and mutagenic properties. The concentrations of aflatoxin residues in muscles depend on the concentrations of aflatoxins in feed as well as on the age of the chicken. The metabolism of aflatoxins and their elimination from tissues is more rapid in older chickens. After 15 days of feeding chickens with meals without of aflatoxin, the concentration of aflatoxins in meat decreases below the limit of detection (Hussain et al., 2010). Aflatoxin residues in poultry meat represent a great concern for human health, since poultry meat is very common food commodity (Herzallah, 2009).

To reduce the risk of exposure, many countries have regulated the levels of aflatoxin in feed. Currently, the legal limits of aflatoxin in feedstuffs are highly variable from the European Union (EU) Countries to other countries. The EU has a limit of 20 µg/kg for complete feedstuffs for pigs and poultry (except young animals) and 10 µg/kg for other complete feedstuffs (Commission Directive, 100/2003). In Serbia, proposed maximum permissible levels of aflatoxins in complete and complementary feedstuffs for pigs and poultry (except young animals) is 20 µg/kg and in complete and complementary feedstuffs for calves, lambs, kids, piglets, chicks, turkey poult, ducklings is 5 µg/kg (Službeni glasnik RS, 27/2014).

Aflatoxin was monitored by the United States Food and Drug Administration (FDA), and a level of 20 µg/kg has been set as the limit of aflatoxin content for corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals (FDA, 2000). The mentioned regulation also establishes maximum level of aflatoxin in cottonseed meal intended for beef, cattle, swine, or poultry (300 µg/kg) and corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry (100 µg/kg) ( FDA, 2000).

The purpose of this study is to give the information on the occurrence of aflatoxin in complete feeding mixtures for poultry during two years period (2013-2014).

## MATERIAL AND METHODS

*Samples* of poultry feed were collected randomly from different farms in Serbia during 2013 and 2014 year. These samples (349) included complete feeding mixtures for turkeys, broiler chicks and laying hens. Immediately after sampling, 200 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. Then, sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20 ºC until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

*Extraction.* Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

*Analysis.* The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities on the basis of which aflatoxin content was calculated, were read using the reader of microtitration plates with a 630 nm filter (BioTec Instruments, USA).

*Quality control***.** In order to ensure quality of obtained results, method for determination of aflatoxin was validated. Validation parameters of the method were estimated according to European Commission (2006). Limit of detection (LOD) for aflatoxin in feed samples was 0.45 µg/kg while limit of quantification (LOQ) was 1.37 µg/kg. Average recovery value, based on analysis of certified reference material TR-A100 (Trilogy, USA) was 106.2% which is within acceptable limits according to Commission Regulation 401/2006. Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable" according to Commission Regulation 401/2006. Therefore, the method was suitable for the determination of aflatoxin in animal feed.

## RESULTS AND DISCUSSION

The occurrence of aflatoxin was investigated in 349 samples of complete feeding mixtures for turkeys, broilers and laying hens, and the results are presented in table 1. By analyzing 203 samples from 2013 year (10 samples of turkey feed , 145 samples of broiler chicken feed and 48 samples of laying hen feed ), overall aflatoxin content was ranged from 2 to 87 µg/kg, with the average value of 19 µg/kg. In complete feed for turkeys and broilers, average aflatoxin content (18 µg/kg) was somewhat lower than in complete feed for laying hens (21 µg/kg). On the other hand, in 146 samples from 2014 year (14 samples of turkey feed , 78 samples of broiler feed and 54 samples of laying hen feed ), overall aflatoxin content was notably lower than in 2013 (average value 12 µg/kg) and ranged from 5 to 35 µg/kg.

Overall aflatoxin incidence was higher in samples from 2013 year (54.2%) than in samples from 2014 (24.0%). This is not a surprise because the cereal grains used for preparation of feeding mixtures in 2013 originated from 2012 harvest when the aflatoxin presence and content were found to be surprisingly high (Kos et al. 2013; Jajić et al. 2013b). Namely, Jajić et al. (2013b) reported about aflatoxin contamination in 63.6% of the analyzed maize samples, with concentration range of 5-367 µg/kg. Somewhat higher contamination (68.5%) was reported by Kos et al. (2013), with a concentration range of 1.01-86.1 µg aflatoxin/kg. All this has contributed to the aflatoxin contamination of compound feed in 2013 (Jajić et al. 2013a).

As can be seen in table 1, very high rate of positive samples (80%) was established in samples of feeding mixtures for turkeys originating from 2013, but this probably does not represent the actual situation because rather small number of samples (10) was analyzed. On the other hand, on the basis of more representable number of samples (48) of feeding mixtures for laying hens originating from 2013, the incidence of aflatoxin was also very high 81.3%. In the most representable group of 145 samples (feeding mixtures for broiler chicks) originating from the same year, the presence of aflatoxin was found in 43.4% of analyzed samples.

In 2014, aflatoxin presence was found to be quite lower than in 2013. In feeding mixtures for broilers, the presence of aflatoxin was 15.4% but in feeding mixtures for laying hens and turkeys it was quite higher (31.5% and 42.9%, respectively).

***Table 1.*** Aflatoxin occurrence in poultry feed samples in the year 2013. and 2014.

| | 2013. | | | | 2014. | | | |
|---|---|---|---|---|---|---|---|---|
| | Turkeys | Broilers | Laying hens | Total | Turkeys | Broilers | Laying hens | Total |
| No of samples | 10 | 145 | 48 | 203 | 14 | 78 | 54 | 146 |
| Average (µg/kg) | 18 | 18 | 21 | 19 | 10 | 13 | 11 | 12 |
| Coefficient of variation (%) | 15 | 18 | 19 | 18 | 6 | 8 | 7 | 7 |
| MIN (µg/kg) | 7 | 2 | 5 | 2 | 5 | 6 | 5 | 5 |
| MAX (µg/kg) | 48 | 78 | 87 | 87 | 22 | 35 | 32 | 35 |
| No of positive samples (%) | 8 (80.0) | 63 (43.4) | 39 (81.3) | 110 (54.2) | 6 (42.9) | 12 (15.4) | 17 (31.5) | 35 (24.0) |
| No of samples above regulation (%) | 2 (20.0) | 53 (36.6) | 8 (16.7) | 63 (31.0) | 1 (7.1) | 12 (15.4) | 2 (3.7) | 15 (10.3) |

Regarding legislative in our country (Službeni glasnik RS, 27/2014) and the European Union (Commission Directive, 100/2003), contamination levels in 2013 were above the maximum limit in 2 (20.0%) samples of feeding mixtures for turkeys, 53 (36.6%) samples of feeding mixtures for broilers and in 8 (16.7%) samples of feeding mixtures for laying hens. As was expected, much lower number of samples from 2014 contained aflatoxin levels above the maximum permissible limit. Namely, this was in 1 (7.1%) sample of feeding mixtures for turkeys, 12 (15.4%) samples of feeding mixtures for broilers and in 2 (3.7%) samples of feeding mixtures for laying hens.

As for our neighboring countries, aflatoxin contamination was monitored in Romania (Braicu et al., 2008; Tabuc et al., 2010; Macri et al., 2014) and Croatia (Šegvić Klarić et al., 2009; Pleadin et al., 2014). In 22 feedstuff samples, collected between January and August 2013 in Romanian province Transylvania, Macri et al. (2014) found that 100% of the samples were positive for aflatoxin B1, with the concentration range of 1.13–92.92 µg/kg. Samples were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA). Tabuc et al. (2010) analyzed 56 cereal samples (corn, wheat, barley and oats). They determinated the level of fungal contamination and aflatoxin content; *Aspergillus* species were present in over 80% of the samples, and aflatoxin B1 has been identified in almost 30% of the samples, mainly in corn with the low concentrations <10 µg/kg. Braicu et al. (2008) investigated forty-three samples of different cereals (wheat, maize, rye and Triticale). It was found that 58.1% of the samples were contaminated with various concentrations of different mycotoxins: aflatoxin B1 (1.6–5.7 µg/kg), aflatoxin B2 (0.89–4 µg/kg), aflatoxin G1 (1.2–5.76 µg/kg), aflatoxin G2 (0.96–3.4 µg/kg) and 4.3–30 µg/kg ochratoxin A. The concentration of total aflatoxin ranged from 11.2 to 10.8 µg/kg. Among different cereals, the highest number of contaminated samples was found to be in the wheat samples (62.5%). Pleadin et al. (2014) analyzed 633 maize samples from 2013 using ELISA technique as the screening method and High Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) as the confirmatory method. They reported the mean value for AFB1 of 81 µg/kg, and the maximal concentration of 2072 µg/kg. The authors gave information about validation method for both techniques that were used. Šegvić Klarić et al. (2009) analyzed 37 samples of cereals and feed randomly collected in 2007 from households of an endemic nephropathy area in Croatia. The incidence of aflatoxins was 24.3 % with the average value of 4.6 µg/kg.

Based on everything stated above, it can be said that the high aflatoxin content during 2013 was an exception in comparison with 2014 and the studies from previous years. These results can be explained by the uncommonly high frequency and incidence of *A. flavus* infestation of maize grain in 2012 (Lević et al., 2013). Lević et al. (2013) described that this infestation was caused by extremely stressful agrometeorological conditions, high temperatures and drought over the period from flowering to waxy maturity of maize in the same year.

## CONCLUSION

Based on the results presented in this paper it can be concluded that the presence of aflatoxin and aflatoxin content during 2013 were rather high as the consequence of the outbreak of *A. flavus* in 2012. It was an exception in comparison with the results from 2014 and the studies from previous years when environmental conditions were unfavorable for *A. flavus* growth and toxin production. Namely, the average aflatoxin content in 2013 was 19 µg/kg with the obtained maximum value of 87 µg/kg. The overall presence was 54.2% with 31.0% of samples above the maximum limit permitted by Serbian and EU regulations. In the opposite, samples from 2014 showed significantly lower average values of the same mycotoxin (12 µg/kg), maximum value (35 µg/kg), overall presence (24.0%) and most importantly, percentage of samples with aflatoxin concentration above the maximum limit permitted by Serbian and EU regulations (10.3%).

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## PRISUSTVO AFLATOKSINA U SMEŠAMA ZA ISHRANU ŽIVINE U SRBIJI U PERIODU 2013-2014 GODINE

*Igor JAJIĆ, Vojislava BURSIĆ, Sandra JAKŠIĆ, Gorica VUKOVIĆ, Saša KRSTOVIĆ*

**Izvod:** Cilj istraživanja je bio da se uporedi prisustvo i sadržaj aflatoksina u uzorcima smeša za ishranu živine koje potiču iz dve različite godine (2013-2014). Ispitano je ukupno 349 uzoraka potpunih smeša za ishranu za ćurki, brojlera i koka nosilja. Ispitivanjem 203 uzoraka iz 2013. godine (10 uzoraka hrane za ćurke, 145 uzoraka hrane za brojlere i 48 uzoraka hrane za koke nosilje) utvrđen je ukupni sadržaj aflatoksina u opsegu od 2 do 87 µg/kg, sa prosečnom vrednošću od 19 µg/kg. S druge strane, analiziranjem 146 uzoraka iz 2014. godine (14 uzoraka hrane za ćurke, 78 uzoraka hrane za brojlere i 54 uzoraka hrane za koke nosilje), utvrđen je znatno niži sadržaj aflatoksina (12 µg/kg) koji se kretao u koncentracionom opsegu od 5 do 35 µg/kg. Dobijeni rezultati prisutnosti aflatoksina mogu navesti na zaključak da je visok sadržaj aflatoksina tokom 2013. predstavljao izuzetak u odnosu na 2014. ali i na rezultate istraživanja iz prethodnih godina. Ovi rezultati ipak ne predstavljaju naročito iznenađenje uzimajući u obzir pojavu visokog stepena infestacije kukuruza plesnima *A. flavus* tokom 2012. godine, nastalu kao posledica povoljnih agroekoloških uslova za razvoj ovih plesni.

**Ključne reči:** aflatoksin, hrana za živinu, ELISA, Srbija.

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