

## AFLATOXINS IN FEED

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Received 13 December 2017; Accepted 25 March 2018

Published online: 16 April 2018

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### Abstract

*Background.* Mycotoxins are toxic secondary metabolites, which are synthesized from a large number of intermediates from the primary metabolism of saprophytic molds. Aflatoxins, due to their genotoxic and carcinogenic effects, are the most important group of mycotoxins from the aspect of their impact on human and animal health. Of all known aflatoxins, AFB1 is the most frequent, with the most harmful impact on human and animal health.

*Scope and Approach.* Due to their prevalence and toxicity, monitoring the presence of aflatoxins in the food chain is required. The scope of this paper is to provide information on the presence of aflatoxins in animal feed and in milk. This paper describes temperature increases in Europe that are contributing to the increased presence of aflatoxins in food, as well as aflatoxin prevention and protection measures.

*Key Findings and Conclusions.* During the last decade, serious contamination of corn with aflatoxins was recorded in southern Europe. In the summer of 2012, Serbia recorded high concentrations of aflatoxins in corn and milk. Based on climate change data, it is expected that aflatoxin contamination in corn will become more frequent.

**Key Words:** Aflatoxins, climate change, feed, preventive measures

## INTRODUCTION

Although a large number of different mycotoxins have been discovered, most attention is dedicated to those most commonly occurring as food and feed contaminants:

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aflatoxins, ochratoxin, zearalenone, fumonisins, trichothecenes, patulin (Sinovec et al., 2006).

Aflatoxins are the most important group of mycotoxins from the aspect of impact on human and animal health. The most important aflatoxins are aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, but also M<sub>1</sub> and M<sub>2</sub>, which are formed as metabolites of B<sub>1</sub> and B<sub>2</sub> (Milićević, 2016) and are contaminants of milk and milk products (Jakšić et al., 2017). Of all the above-mentioned aflatoxins, AFB<sub>1</sub> is the most frequent, toxic, and with the most expressed carcinogenic effect (Milićević, 2016).

Among many *Aspergillus* species, group B aflatoxins are most commonly synthesized by *A. flavus*, while group G aflatoxins are synthesized by *A. parasiticus* (Sinovec et al., 2006). These organisms are ubiquitous and can synthesize aflatoxins in corn, various types of cereals and cereal products, oilseeds, dry fruits, jams, spices, cocoa, dried meat products and cheese.

Aflatoxins cause aflatoxicosis, and the form and manifestation of the intoxication depends on the amount ingested, the frequency of the input, and genetic, physiological and external factors. The residues of mycotoxins which can be found in foods of animal origin are a special problem. The toxic effect of aflatoxins is expressed at the level of interaction with the genetic material. The aflatoxin molecule enters the cell and its nucleus, and then enters DNA base pairs, which slows the processes of transmitting DNA information, creating errors in DNA transcription. Their immunosuppressive and teratogenic effects were also determined (Sinovec et al., 2006).

Between 0.3 and 6.2% of AFB<sub>1</sub> ingested by cows is transformed into AFM<sub>1</sub>, and the highest amount of AFM<sub>1</sub> is present in milk between 6 and 24 h after cows consume contaminated feed. After that period, the amount of AFM<sub>1</sub> begins to decrease until complete elimination after 72 to 96 h, if the animal no longer ingests feed contaminated with AFB<sub>1</sub> (Kos, 2015).

### **Feed as source of aflatoxins**

The presence of mycotoxins in food is the result of the various physical, chemical and biological interaction and environmental factors on farm and during storage of food. The occurrence of *Aspergillus* and aflatoxin also largely depends on the geographical area, i.e. climatic conditions, the applied agrotechnical measures (Kos, 2015), damage to plants, and the presence of insects and rodents. These factors all greatly influence the development of mold and synthesis of toxins.

*Aspergillus* molds and the aflatoxins they produce are one of the most common corn contaminants. Most (80-90%) corn produced in Serbia is used as a component of animal feed, and the remainder is mainly used for human consumption and production of starch, so the contamination of corn with aflatoxin is of great concern. During 2012, Serbia experienced a drought as reported by the Republic Hydrometeorological Institute, which particularly affected agricultural crops. It was confirmed that, *A. flavus*

was present in all tested soybean, but was slightly less frequent on corn grain (95.3% was contaminated), barley (65.2%) and sunflower (57.1%), while the lowest frequency was on wheat grain (45.85%) (Lević et al., 2013).

In stock feeds, the highest concentrations of aflatoxins were found in haystacks, while lower levels were detected in feed mixtures, and the lowest values were recorded in silage (Stanivuk, 2011). In terms of production and storage, hay is the most common feed in Serbia, while silage is the safest feed, with regard to aflatoxin contamination, for dairy cows. In the same study, the amount of AFM<sub>1</sub> in milk samples was several times lower than the maximum allowed concentration (MAC) permitted by Serbian regulations (Stanivuk, 2011).

Analyzing 680 samples of corn in Serbia in the period 2012-2013, mean AFB<sub>1</sub> levels were 28.06 µg kg<sup>-1</sup>, 29.1 µg kg<sup>-1</sup> and 33.21 µg kg<sup>-1</sup> in the regions of Belgrade, Central Serbia and Vojvodina, respectively. Respectively in these three regions, 20.77%, 22.49% and 29.73% of corn contained AFB<sub>1</sub> at levels greater the MAC. The consequence of high concentrations of AFB<sub>1</sub> in corn was high concentrations of AFM<sub>1</sub> in milk, ranging by regions from 0.117 µg kg<sup>-1</sup> to 0.205 µg kg<sup>-1</sup> (Stefanović, 2014). Aflatoxins were not detected in corn from the four-year period from 2009-2011, but in 2012, 79% of 700 examined corn samples were contaminated with aflatoxins. This was explained by the extremely warm and dry weather conditions recorded in 2012. Aflatoxin was detected in 98.7% of 200 milk samples at concentrations of 0.01 to 1.20 µg kg<sup>-1</sup> (Kos, 2015).

In Serbia during 2013, 281 samples of complete feed mixtures for dairy cows were examined. Of the 281 feeds, 67 contained AFB<sub>1</sub> above the MAC. In 22% of corn, concentrations of AFB<sub>1</sub> were above the MAC, while AFB<sub>1</sub> was not found in sunflower meal, hay, corn silage, or turnip noodles (Spirić et al., 2013). Therefore, the contamination occurred only in corn and corn flour, which are the basic ingredients of the feed mixture for cows.

High frequencies of aflatoxin in corn were also recently published from Croatia, when 38% of 633 analyzed corn samples were contaminated with aflatoxins, the mean value being 81 µg kg<sup>-1</sup> (Pleadin et al., 2014). In the period 2014-2016 in Bosnia and Herzegovina, 272 samples of silage, corn or concentrated feeds were examined, and 2.57% had values above Bosnia's MAC (Dojčinović et al. 2017). In Serbia in the period 2012-2016, 50 samples of corn were examined for the presence of AFB<sub>1</sub> and significant temporal differences were found. The highest frequency of AFB<sub>1</sub> contamination was recorded during 2012, when 72% of corn contained AFB<sub>1</sub>. Lower frequencies (25%, 25%, 10%) were detected in 2013, 2015 and 2016, respectively (Kos et al., 2017). The authors emphasized weather conditions (dry and warm weather) as the main reason for these results (Kos et al., 2017).

In a study in the Netherlands in 2016, there was an increase in the presence of aflatoxin in corn and corn products compared to earlier years (Van der Fels-Klerx et al. 2018). More than 300 samples from Eastern Europe as well as from South and North America had a concentration of 6 µg kg<sup>-1</sup> or higher, of which 6 samples had

a concentration above the maximum limit under EU regulations. In the same period, there was a significant decrease in the concentrations of AFB<sub>1</sub> in sorghum, sunflower seed, maize gluten feed and soybean compared to previous years (Van der Fels-Klerx et al. 2018). Concerning aflatoxins in cereals, the frequency of positive cereals was highest in Southern Europe and South Asia (average frequencies of positive samples >30%), but in contrast, contamination of wheat and soybean by aflatoxins was much less common. However, in southern Europe, although 43% of wheat tested contained aflatoxins, the maximum value was only 6 µg kg<sup>-1</sup> (Guerre, 2016). Examination of AFB<sub>1</sub> in the period 2013-2015 in China showed the highest content of AFB<sub>1</sub> was in soybean, it was lower in corn and bran, and the lowest values were in wheat (Li et al., 2016). In the same research, in feed mixtures, the highest content of AFB<sub>1</sub> was in pig feed mixtures, while it was lower in feeding dough and in cattle feed mixtures.

### **Climate change in Europe**

Until a few years ago, aflatoxins were not recognized in Europe as a problem and aflatoxins were mainly found in products originating from countries with tropical weather conditions favorable for the growth of *Aspergillus* spp. Nevertheless, during the last decade, serious contamination of corn with aflatoxins in southern Europe has been recorded. This was particularly noticeable in 2003 and 2012 when contamination of corn with aflatoxin occurred in Italy and southern Europe, serving as a warning that the aflatoxin situation there is labile. It is evident that during recent years, there have been occurrences of climate change: increases of daily minimum temperatures, higher average temperatures and longer dry periods causing heat stress in plants, especially during the periods of corn flowering and corn silk tanning (Nešić et al., 2015).

Recently, a study modeled the possible contamination of AFB<sub>1</sub> in corn, depending on climatic conditions over the next hundred years in Europe (Batallini et al., 2016). The risk of AFB<sub>1</sub> contamination in three different climate scenarios is described: with the current average daily temperatures; with an average daily temperature increase of 2°C; and an average temperature increase of 5°C. For this purpose, the aflatoxin risk index (AFI) in corn was used, which predicts the growth of *A. flavus* and the production of aflatoxins in corn. AFIs range from 0 (no risk) to 200 (a high risk). Air temperature, relative humidity and rainfall data were used as parameters (Batallini et al., 2016).

Currently, the area with increased aflatoxin production in corn (AFI >0) is generally below 45° north latitude, due to the limiting conditions for the growth of *A. flavus* and the production of aflatoxins at higher latitudes. In the other two climate scenarios (+ 2°C, +5°C), the areas with the largest increase in AFB<sub>1</sub> are Eastern Europe, the Balkans and the Mediterranean region (Batallini et al., 2016). However, the highest AFI were noted for the +2°C scenario, under which the southern part of Europe is very likely to experience AFB<sub>1</sub> in corn. Because of this, in Southern Europe, production of corn, soybean and sunflower is likely to be reduced, as these crops will become more favorable for cultivation in northern areas. Corn production is expected to increase

by 30-50% in Northern Europe, and to significantly decrease in Southern Europe (Nešić et al., 2015). For European countries such as Romania, France, Hungary and northern Italy (which produced 60% of total EU corn in 2013), according to the +2°C scenario, the low to medium AFIs predict aflatoxin contamination will likely increase. According to the results, Serbia is located in an area with expected medium to high AFI increases. In the +5°C scenario, the areas in Europe which are exposed to aflatoxin contamination on corn would likely experience increased contamination.

Generally, contaminated crops will have a reduced economic value, and in that case, they would be diverted for other purposes, such as biofuel production. This could lead to the production of corn in the current corn-growing areas becoming uneconomical. In addition, the expansion of risk zones for aflatoxin could increase the chronic exposure of humans and animals to these mycotoxins (Batallini et al., 2016).

### Measures of prevention and protection

Since the main mode of mycotoxin intake is contaminated food/feed, the optimal solution is to prevent food/feed contamination by reducing plant mold infestation, rapid drying and proper storage of removed plants, or by using effective protective agents. In cases where mycotoxins are suspected or identified in nutrients and/or foods, their elimination can be carried out by mechanical separation, chemical extraction and decontamination or detoxification by using physical, chemical or biological methods.

In practice, the most common method of reducing or eliminating mycotoxins is the use of adsorbents. Adsorbents are substances that are not resorbed from the bowel and have the ability to bind certain chemicals and to prevent their resorption. The stability of the adsorbent-toxin bind in the wide pH range in the gastrointestinal tract is the most important criterion for assessing the efficacy of adsorbents. It should be noted that although *in vitro* tests show many mineral compounds are effective for adsorption of mycotoxins, *in vivo* analysis has shown that some adsorbents cannot protect animals against the mycotoxins. The most commonly used inorganic adsorbents are hydrated sodium calcium aluminosilicate, bentonite, clay and various aluminosilicates – zeolites. Bentonite, diatomite and zeolite adsorbed more than 95% of AFB<sub>1</sub> *in vitro* at different pHs (Bočarov-Stančić et al., 2011). In another study (Demir et al., 2018), the positive effects Clinoptilolite (CLP) zeolite (at 2%) on yield performance, some blood parameters and egg quality of laying hens were confirmed. The usage of CLP in the diet led to a significant decrease in feed consumption, a remarkable result for production economics. The possibilities of using organic adsorbents, especially modified mannann oligosaccharides isolated from the inner layer of the yeast cell wall, have been considered. Increased interest in the application of organic adsorbents came primarily from the need to overcome the problems associated with the inorganic adsorbents, which can adsorb important nutrients (Sinovec et al., 2006).

The use of chemicals to inactivate or remove mycotoxins is based on the use of acids, bases and other chemical compounds. Chemicals are efficient but they are unsafe due

their toxic residues. Also, individual agents can significantly degrade nutrition and produce a negative impact on palatability (Milićević, 2016).

Some bacteria and yeasts have proven detoxification ability against mycotoxins, utilizing their own enzymes to convert mycotoxins into less toxic metabolites, in very specific, efficient and environmentally friendly processes. Among many biotransforming strains which can transform mycotoxins, *Aspergillus*, *Stenotrophomonas*, *Pseudomonas*, *Mycobacterium*, and *Bacillus* have the capability to biotransform aflatoxins (Zhu et al., 2017). *Bacillus subtilis* from animals' guts and soil had the greatest ability to biotransform aflatoxin as well as inhibit the growth of pathogens (Gao et al., 2011).

## CONCLUSION

In many countries there is concern about the presence of AFB<sub>1</sub> in grain, products obtained from grain processing, foods of animal origin and industrial foods. Permanent screening and monitoring of foods and feeds for AFB<sub>1</sub> is becoming more common. Significant education on protection measures and prevention of mycotoxins is required for producers of raw materials and animal feed.

## Acknowledgements

This paper was supported by Ministry of Education, Science and Technological Development, Republic of Serbia, through the funding of Project No 31034

## Authors contributions

JD have made substantial contributions to conception and acquisition of data. Have been involved in drafting the manuscript and participated in technical details. MR have been involved in drafting the manuscript and revising it critically for important intellectual content. RS have been involved in drafting the manuscript and revising it critically. GS have been involved in drafting the manuscript. KM have been involved in drafting the manuscript in part measures of prevention and protection and helped in technical details. ŠD have been involved in drafting the manuscript and revising it critically. Have given final approval of the version to be published.

## Competing interests

The authors declare that they have no competing interests.

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## AFLATOKSINI U HRANI ZA ŽIVOTINJE

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### Kratak sadržaj

*Uvod.* Mikotoksini su toksični produkti sekundarnog metabolizma saprofitskih plesni, koji se sintetišu od velikog broja međuprodukata primarnog metabolizma. Aflatoksini zbog genotoksičnog i kancerogenog dejstva predstavljaju najznačajniju grupu mikotoksina sa aspekta uticaja na zdravlje ljudi i životinja. Od svih poznatih aflatoksina, AFB<sub>1</sub> je najfrekventniji sa najštetnijim uticajem na zdravlje ljudi i životinja.

*Cilj i pristup.* Zbog svoje zastupljenosti i toksičnosti aflatoksini zahtevaju praćenje njihovog prisustva u lancu hrane. Cilj ovog rada je da pruži informaciju o prisustvu aflatoksina u hrani za životinje i u mleku. U radu su opisane globalne temperaturne promene koje povećavaju prisustvo aflatoksina u hrani, kao i mere prevencije i zaštite

*Ključni nalazi i zaključak.* Tokom poslednje decenije, zabeleženo je ozbiljno zagađenje kukuruza sa aflatoksinima u južnoj Evropi. Republika Srbija je u leto 2012. godine zabeležila visoke koncentracije aflatoksina u kukuruзу i u mleku. Na osnovu podataka o klimatskim promenama, očekuje se da kontaminacije aflatoksinima u kukuruзу postanu učestalije.

**Ključne reči:** aflatoksini, klimatske promene, hrana za životinje, mere preventive