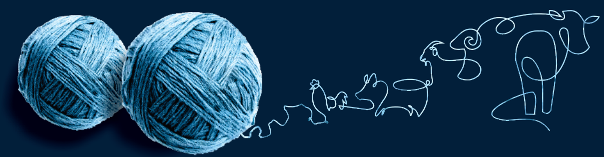
12th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION



PROCEEDINGS

9 -11 October 2019, Belgrade, Serbia

Institute for Animal Husbandry
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Proceedings of the 12th International Symposium
Modern Trends in Livestock Production
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THE EFFECT OF CLIMATE CONDITIONS ON AFLATOXIN CONTAMINATION OF CEREAL GRAINS AND FEEDS

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Review paper

Abstract: Production and consumption of healthy food occur as the necessity of the modern life habits. The reduced risk of diseases improves the quality of life. Feed safety is a priority trend in all process of animal production. However, toxigenic fungal species and their secondary metabolites (mycotoxins) are the common feed contaminants. The aflatoxins and the producing *Aspergillus* species section *Flavi* in cereal grains and feeds has emerged as a serious concern with potential health hazards in humans and animals. This paper aimed to give an overview on of the effect of climatic factors on *Aspergillus* and aflatoxin contamination of cereals and feeds in agro-ecological conditions of Serbia and other European countries. Since the reduction of health risks and diseases in the livestock as consequences of aflatoxins presence in feed have become the priority tasks in feed safety control strategy thus the aim of this review has also been to recommend some of the measures for preventing their adverse effect.

Key words: cereal grains, feeds, *Aspergillus* spp., aflatoxins, climate factors

Introduction

The high-quality feed is a significant component of a successful animal rearing. Cereal grains (maize, wheat, barley, and ray) are the main components of feed for farm animals. However, infection of grains by toxigenic fungi is inevitable occurred in the field and during storage. Fungal development can reduce the nutritional quality of grains and provoke the production of their secondary metabolites (mycotoxins) (*Krnjaja et al., 2012*). Mycotoxins are toxic compounds and can cause health disorders in humans and animals (*Zain, 2011*). The one mycotoxin can be produced by several fungi, and that a fungus can produce several mycotoxins (*Smith et al., 2016*).

The most common toxigenic fungal species belong to the genera *Alternaria, Aspergillus, Fusarium* and *Penicillium*. *Alternaria* and *Fusarium* are classified as field fungi, while *Aspergillus* and *Penicillium* species are consider as storage fungi, based on their different moisture requirements *(Logrieco et al., 2003)*. Field fungi require higher moisture conditions (20–21%) while the lower requirements of moisture (13–18%) is necessary for the developement of storage fungi (*Santin, 2005*). The interactions of host crop, genotype, soil type, stress, and biological factors encourage the preharvest fungal development. The complex interactions of environmental factors (moisture, temperature, oxygen, and carbon dioxide), substrate, and biotic factors (insects and microorganisms) are favorable for postharvest fungal contamination (*Miller 1995; Santin, 2005*).

According to the reports of The Food and Agriculture Organization of the United Nations, approximately 25% of the cereals produced in the world are contaminated by mycotoxins (*Reddy et al., 2010*). Mycotoxin contamination of cereal grains represents a potentially high risk for the health and production of farm animals (pigs, poultry and cattle). Mycotoxins are introduced into animal and human organisms most often through contaminated feeds and foods. Health disorders are most prominent in high-productive farm animals, given the significantly higher consumption of fodder. Factors such as the type and amount of mycotoxins in feed, duration of feeding with contaminated feed by the organism, species, breed, category, age, housing (rearing) of animals, etc. may also affect the degree of animal health disorders (*Krnjaja et al., 2009*).

Aflatoxins, ochratoxins, fumonisins, zearalenone, deoxynivalenol, T-2 toxin, other trichothecenes, and ergot alkaloids are the most commonly detected mycotoxins in cereal grains *(Binder, 2007)*. These mycotoxins cause significant economic losses in livestock production worldwide (*Zain, 2011*). Favorable environmental factors (high humidity and temperature) can increase the occurrence of mycotoxins in the crops. In particular, the occurrence of the aflatoxin is highly depended by the weather conditions. The global climate heating can cause that aflatoxins become a serious problem in grain production (*Mannaa and Kim, 2017*).

Considering that feed safety is a priority trend in all process of livestock production, the main aim of this review paper is to present the data on the effect of climatic factors on the natural occurrence of aflatoxins in cereal grains and feeds under agro-ecological conditions in Serbia (Europe). In addition, the aims have also been to indicate on potential hazards and harmful effects of these contaminants in the animal production, and to recommend some of the preventive measures for the reduction of these contaminants in cereals and feeds.

Aflatoxins

Aflatoxins are difuranocoumarin derivatives produced by the *Aspergillus* species of section *Flavi* (*Monge et al., 2013*). Four major aflatoxins founded in feedstuffs are aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2), while two metabolic products, M_1 (AFM_1), and M_2 (AFM_2) has been detected as milk contaminants. *A. flavus* and *A. parasiticus* primarily produce AFB_1 and B_2, while *A. parasiticus* produce AFG_1 and G_2 (*Ajani et al., 2014*). AFM_1 and M_2 are hydroxylated metabolites of AFB_1 and AFB_2, respectively, which are produced in the liver of humans and animals fed with contaminated foods/feeds and are secreted in the milk of mammals. Among the mentioned aflatoxins, AFB_1 is the highest toxic and most potent (*Luongo et al., 2014*). According to the International Agency for Research on Cancer AFB_1 has been classified as the human carcinogen, Class 1 (*IARC, 1993*).

Aflatoxin occurrence in different types of feeds is a major risk for animal health, and causes high losses in livestock production. These toxins have been occurred all over the world in feedstuffs due to international trade in these commodities that contributes to their worldwide dispersal. They can also be found in animal-derived food namely meat, eggs, milk, and milk products if animals were fed with contaminated feed (*Smith et al., 2016*).

$AFB_1$was a first mycotoxin isolated from the feed and caused the death of 100.000 turkeys in 1960 in England (*Bennet and Klich, 2003*). Aflatoxins have been determined worldwide in different types of animal feeds, and their quantity varies depending on numerous factors. There are great differences in the levels of livestock feed contamination in particular years. In favourable years, such as warm and dry seasons, the fungi may accumulate aflatoxins before grain harvest. Also, as *Aspergillus* spp. tolerates low water activity, grain commodities under storage conditions can subsequently be contaminated (*Bryden, 2012*).

The poultry, pigs, and cattle are the most sensitive farm animals to aflatoxins, while sheep and rabbits are the least sensitive. Younger, gravid animals and males are more sensitive than mature ones, non-gravid, and females, respectively *(Krnjaja et al., 2009)*. Clinical signs and symptoms of aflatoxicosis in animals are vomiting, feed refusal, abdominal pain, pulmonary edema, gastrointestinal dysfunctions, fatty infiltration and necrosis of the liver, reproductivity disorders, anemia and jaundice (*Talebi et al., 2011; Ajani et al., 2014*). Aflatoxins may induce hepatotoxic, carcinogenic, teratogenic, and immunosuppressive effects on humans and animals (*Smith et al., 2016*).

From that there are the risks by mycotoxin contamination, many countries have regulations for maximum permissible levels of mycotoxins in foods and feeds. According to Serbian regulation (*Službeni Glasnik RS 22/2018*), it adopted maximum permissible levels of total aflatoxins, aflatoxin B_1 (AFB_1) and aflatoxin M_1 (AFM_1) in different agricultural commodities and limits of AFB_1 in feedstuffs according to adequate regulations. These legislation limits are complied with EU regulations (*EC, 2010*), except for AFM_1. According to the mentioned Serbian and EU regulations, in all cereals and cereal products including also maize and rice, the maximum permissible level of AFB_1 and sum of aflatoxins (B_1, B_2, G_1, and G_2) have been set in 2–5 μg kg^{-1} and 4–10 μg kg^{-1}, respectively.

According to the EU regulation (*EC, 2010*), the maximum level of AFM_1 is 0.05 μg kg^{-1}. However, in Serbia, the regulation limit of AFM_1 in milk is 0.25 μg kg^{-1} (*Službeni Glasnik RS, 90/2018).* This limit has been reviewed every six months, since 2013 year, and has been determined based on available data from official laboratories on contamination of milk and feedingstuffs (*Jakšić et al., 2018*).

The European community set the maximum permissible level for AFB_1 to 20 μg kg^{-1} for all feed materials (*EC, 2003*). According to the Serbian Regulation on the quality of animal feeds (*Službeni Glasnik RS, 27/2014*), the maximum level of AFB_1 for all feed materials is 30 μg kg^{-1}.

Natural occurrence of aflatoxins in cereal grains and feeds in European countries under climate change conditions

Contamination of cereal grains by *Aspergillus* spp. and higher occurrence of aflatoxins has been rare under climatic conditions of many European countries Austria, Belgium, Germany, Hungary, Poland, Romania, UK, etc. (*Lević et al., 2013*). In support of that, by mycotoxins analyzing of cereal grains samples collected during 2005–2009 in five countries from Southern Europe (Greece, Cyprus, Spain, Italy, and Portugal) *Griessler et al. (2010)* obtained the average aflatoxins levels which were not above EU regulation limits. However, in Northern Italy during 2003 it has been reported the intensive occurrence of *A. flavus* and aflatoxin presence in the maize crops (*Moretti et al., 2004*). The other one, in Northern Italy, it has noted the maize contamination by aflatoxins due to drought conditions in 2012–2013 favorable to *A. flavus* infection (*Perrone et al., 2014*). Likewise, aflatoxin contamination of maize was seven times higher in 2012 than in 2011 due to differences in weather conditions (*Accinelli et al., 2014*). Furthermore, in southeastern Romania, *Tabuc et al. (2009)* reported that 20% of maize samples collected during 2002–2004 had AFB_1 level above the adopted EU maximum limit (5 μg kg^{-1}) (*EC, 2010*). Similar to that, in Hungary, *Borbély et al. (2010)* have determined 4.8% cereal and feed samples with AFB_1 levels above the EU limit. Then, in Serbia, the epidemic incidence of *A. flavus* and the increased aflatoxins levels in the maize grain crops during 2012 were determined. These intensive occurrences of *Aspergillus* spp. and aflatoxins can be explained with specific environmental and climatic conditions. Very high temperatures from the anthesis to maturity stages, then, the dry summers and the low seasonal rainfalls reported in 2012 in Serbia contributed to increase the development of *Aspergillus* spp. and aflatoxins levels in the maize crops (*Kos et al., 2012, 2014, 2017; Lević et al., 2013; Krnjaja et al., 2013; Obradović et al., 2018*). By analyzing maize kernel samples during 2012–2016, *Kos et al. (2018)* detected aflatoxins in 72.3%, 24.7%, 36.7%, and 5% of the maize samples in 2012 (range 1–111.2 μg kg^{-1}), 2013 (range 1.2–65.2 μg kg^{-1}), 2015 (range 1.1–76.2 μg kg^{-1}), and 2016 (range 1.3–6.9 μg kg^{-1}), respectively, while during the wet year of 2014, aflatoxins were not detected. *Obradović et al. (2018)* established AFB_1 level above a regulation limit in 16.7% of 90 investigated maize kernel samples in 2013 originating from production plots in Zemun Polje (suburb of Belgrade). However, *Krnjaja et al. (2015, 2016, 2018)* were not established the mean levels of aflatoxins above the regulation limits in post-harvest maize samples originating from different FAO maturity groups and different districts in Serbia in years 2013, 2014 and 2016. By analyzing the samples of different types of cereal grains (wheat, barley, oats, maize, and ray) after the harvest in 2012 from the main cereals growing area in Serbia, *Kos et al. (2014)* established aflatoxins only in the maize samples. Then, in Spain, the presence of aflatoxins under international regulation limits in stored barley grains collected from 2008–2010 has reported by *Mateo et al. (2011).* Similar that, in Poland, by analyzed mycotoxins in different genotypes of wheat, *Rachoń et al. (2016)* detected aflatoxins in trace amounts.

According to the results of *Krnjaja et al. (2019)*, it has been reported that in 14.29% of chicken feed samples collected from different farms in Serbia during 2016, AFB_1 level was above the regulation limit. Similarly, in Republic of Srpska (Bosnia and Herzegovina), *Dojčinović et al. (2017)* have reported that AFB_1 levels were above the regulation limit ($>$ 20 μg kg^{-1}) in one of 101, four of 85 and two of 86 tested samples of concentrated feed from 2014, 2015 and 2016, respectively.

Based on listed results, cereal grains and feed safety of fungal and aflatoxin contamination have been strongly influenced by climate conditions. The temperature, humidity, and precipitation are major climatic factors which directly affect fungal growth and infection. *Aspergillus* species have been occurred in tropical and subtropical regions in which droughts and high temperatures exist. Those factors also increase aflatoxin contamination of grain crops in the field. Until recently, *Aspergillus* species have not been often isolated in Europe, but in some years after the 2000s which are connecting with hot and dry summers the occurrence of *A. flavus* and aflatoxin contamination of maize crops greatly increase (*Van der Fels-Klerx et al., 2016*). *A. flavus* can cause pre- and post-harvest contamination of cereal grains. Although, the soil is the native habitat of this species, many types of organic substrates can be invaded by this fungus. Since aflatoxins are synthesize under the high moisture and temperature conditions, *A. flavus* produces them commonly in warmer, tropical and subtropical climates. So, in the European countries, the moderate climate should not be the risk of aflatoxins occurrence in cereals and other agricultural products. However, the effects of global climate heating started to influence, that aflatoxins become more prevalent in countries with the moderate climate (*Baranyi et al., 2013*). Serbia with continental to moderate continental climate has also been under the risk of the effect of climate changes which may contribute to increased aflatoxins occurrence in cereals. In the future, it is predicted that AFB_1 will become inevitable in production of maize in Eastern Europe, the Balkan Peninsula, and the Mediterranean regions (*Milicevic et al., 2019*).

Additionally, *Battilani et al. (2016)* reported the prediction of aflatoxin contamination in maize and wheat crops under a +2°C and +5°C climate change scenario where AFB_1 will become a feed safety issue in maize in Europe, especially in the +2°C scenario which will be expected scenario in the upcoming years.

Mitigation of aflatoxin cereal grains and feeds contamination

The control strategy of mycotoxin contamination in cereal grains and feeds consist of prevention of fungal development and mycotoxins production and detoxification of existing mycotoxins.

The preventive pre- and post-harvest measures, such as the healthy seeds, early sowing, harvest in full maturity, good storage conditions can be used to reduce fungal and aflatoxin contamination of cereal grains. Regular and correct ventilation and aeration of storage facilities may also prevents the increase in fungal contamination. Generally, only the dry grains with the moisture content of approx. 13% should be stored (*Krnjaja et al., 2009*).

Physical adsorption, chemical, and biological detoxification methods are used for adsorption and degradation of toxic into non-toxic compounds. In practice, zeolite and clay minerals are used as adsorbents of aflatoxins. They are from a group of alumosilicate minerals. Also, some of the biological adsorbents, such as modified glucomannan (isolated from cell walls of *Saccharomyces cerevisiae* yeast) are applied for inactivation of aflatoxins *(Devegowda and Murthy, 2005)*. Individual or combinations of organic acid (propionic, sorbic, acetic acids, etc.), salts of organic acid (calcium propionate and potassium sorbate) and copper sulphate are some of the chemicals which can inhibit fungal growth and proliferation (*Akande et al., 2006*). Lactic acid bacteria (LAB) are used in silage feeds. Probably, organic acid by LAB inhibits fungal growth, or there is the competition for nutrients in the substrate between fungus and LAB. Additionally, growing in the culture medium the bacteria can adapt faster than fungi. Then, binding AFB_1 and AFM_1 by LAB influenced by specified strains, matrix, temperature, pH, etc. The binding is a slightly reversible phenomenon. However, this biocontrol method could be a promising measure against fungal growth and aflatoxins in feeds (*Ahlberg et al., 2015*).

Conclusion

It has been predicted that aflatoxins will continue to be of increasing importance in cereal grains and feeds. Therefore, there is a need for detailed information on the effect of climate conditions on the aflatoxin production by *Aspergillus* spp. Determination of climate factors will be the crucial task to control strategy and risk assessment of aflatoxin contamination. Monitoring of weather conditions in certain regions and constant control of aflatoxins level in cereals and feeds are necessary for health risk assessment of humans and animals.

For successful livestock production, it is necessary to ensure both healthy and high-quality fresh components that are included in the feed mixtures, and ready-made mixtures without contaminants that may cause adverse effects in the production chain. Given the importance of the aflatoxins as a hepatotoxic and carcinogenic agent that causes aflatoxicosis in animals, the best solution to control the formation of this mycotoxin is to prevent the growth of fungi on maize as the main component of animal feed and also on other susceptible commodities. Pre-harvest and post-harvest measures should be raised to cereal grains safety by aflatoxin contamination.

Uticaj klimatskih uslova na kontaminaciju žita i hrane za životinje aflatoksinima

Vesna Krnjaja, Slavica Stanković, Ana Obradović, Tanja Petrović, Violeta Mandić, Zorica Bijelić, Marko Jauković

Rezime

Proizvodnja i potrošnja zdrave hrane je potreba savremenog načina života. Bezbedna hrana za životinje je prioritet u svim procesima stočarske proizvodnje. Međutim, toksigene vrste gljiva i njihovi sekundarni metaboliti (mikotoksini) su najčešći kontaminenti hrane za životinje. Aflatoksini i produkujuće *Aspergillus* vrste iz sekcije *Flavi* u žitima i hrani za životinje pojavljuju se kao ozbiljan problem sa potencijalnim opasnostima po zdravlje ljudi i životinja. Cilj ovog preglednog rada je da se razmotri negativan uticaj klimatskih faktora na infekciju/kontaminaciju zrna žita vrstama roda *Aspergillus* i aflatoksinima, kao i hrane za životinje u agroekološkim uslovima Srbije i drugih evropskih zemalja. S obzirom da smanjenje rizika koji ugrožavaju zdravlje i smanjenje bolesti u stočarstvu, kao posledica pojave aflatoksina u hrani za životinje, postaje prioritetan zadatak u strategiji kontrole bezbednosti hrane za životinje, cilj ovoga rada bio je, takodje, da se preporuče neke od mera.

Ključne reči: žita, hrana za životinje, *Aspergillus* spp., aflatoksini, klimatski faktori

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