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Review Article

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AFLATOXIN STANDARDS AND MAIZE TRADE

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Summary

Mycotoxin contamination is recognized as an unavoidable risk in agriculture production in both developed and developing countries. Health consequences of aflatoxin are much more serious problem in developing countries than in developed. The number of countries regulating aflatoxins has significantly increased over the years. In paper were discussed different limitations for aflatoxin in the world, as well as their relation to the global maize trade. Following the debate in Serbia, caused by various aflatoxin regulations in different years, we wanted to contribute to the establishment of the state policy that targets mentioned issue. One of main conclusions in paper is that nations with strong trade connections tend to have similar regulations on allowed level of aflatoxin within the maize. Also, it was concluded that incidental appearance of Aspergillus flavus in maize during 2012 in Serbia demonstrates the weakness of the control system, as well as weakness of the national legislation. Main recommendation is oriented to limitation of aflatoxin B_1 in animal feed, what is in same time the most effective measure for control of level of aflatoxin M_1 in milk. After completing this condition Serbia has to return back the allowed limit for aflatoxin in milk at level of 0,05 μg/kg.

Key words: *standards for aflatoxin, global maize trade, Serbia.*

JEL: *Q13, Q18, I15*

Introduction

Mycotoxins are secondary metabolites of molds that are produced by most of fungi of the genera *Aspergillus*, *Penicillium* i *Fusarium*. They are contaminating a wide range of crops before or after the harvest. According to FAO, more than 25% of the world's agricultural crops are contaminated with mycotoxins (Đorđević et al., 2009). Mycotoxin contamination

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is recognized as an unavoidable risk because the formation of fungal toxins depends of weather conditions, so effective prevention is impossible (Marković et al., 2010).

There are many important diseases in human population and at domestic animals caused by mycotoxins (known as mycotoxicoses). By consumption of food contaminated with mycotoxins, they enter in animal body, and later can be deposited in internal organs, muscle mass and other tissues, or it can be separated from milk or urine, as in case of ruminants. When aflatoxin B1 (AB1) enters with food into the body of dairy cattle, it metabolizes in liver and stands mainly in milk as aflatoxin M1 (AM1) and partly as type AB1. So after lactation residues of both factions can be ascertained in milk (Ožegović et al., 1995). The transfer of aflatoxin B1 from feed to milk depends on many factors, ranging from 0,3 to 6,2%. According to the IRAC classification from 2002, aflatoxin M1 is in the first group of carcinogens, but it is considered to have only 10% of carcinogenicity from its precursor aflatoxin B1 (Polovinski Horvatovic et al., 2009). AB1 is extremely thermo stable compound, which can be destroyed at temperatures between 140 and 160°C (temperatures that are not applied in food and processing industry). For example, during pasteurization or sterilization, their structure is not destroyed, so they can be found in milk or milk products (Škrinjar et al., 2005). It causes damage to the liver, kidneys, cardiovascular and neural system, expressing the carcinogenic, teratogenic, mutagenic and immunosuppressive effects (Shashidhar et al., 2005).

Aflatoxin is one of the causes of hepatocellular carcinoma (HCC), the most often and malignant primary tumor of the liver, which causes the death within 12 months from the onset of the symptoms in 93% of all cases (Ferlay et al., 2010). It is estimated that in global, aflatoxin is the main cause of HCC in at least 4,6% and maximally 28,2% cases (Liu et al., 2010).

According to geographical distribution, HCC is not distributed equally. It is typically highest in developing countries, especially sub-Saharan African and Asian countries. In these high-risk regions the primary cause of cancer is recognized in HBV (chronic hepatitis B virus) infection and then in food contaminated with aflatoxins. A particular problem in hepatocarcinogenesis is a synergistic interaction between aflatoxin B1 and HBV (Kew, 2003). Exposure to aflatoxin and HBV infection is more often in rural than in urban areas (Kew, 2010; Plymoth et al., 2008), so the appearance of HCC is more common in rural areas.

Geographic patterns of prevalence of HBV infections are presented in Table 1. By frequency of HBV infection Serbia is among countries with a medium level of endemism with prevalence of HBsAg + (infected with hepatitis B), or 2-7% (Lazarevic et al., 2007).

Table 1. Geographic patterns of prevalence of HBV infections

Element	Low	Intermediate	High
HBsAg positivity	0.2%-0.7%	2%-7%	8%-20%
Anti-HBs positivity	4%-6%	20%-55%	70%-90%
Childhood infection	Infrequent	Frequent	Very frequent
Neonatal infection	Infrequent	Infrequent	Frequent
Territories where was found	Australia, Western Europe. North America (South), South America	Eastern Europe, Japan, Middle East, ex-USSR, South America	China, Southeast Asia, tropical Africa, South America (Amazon Basin), Pacific Islands

Source: Maynard et al., 1989

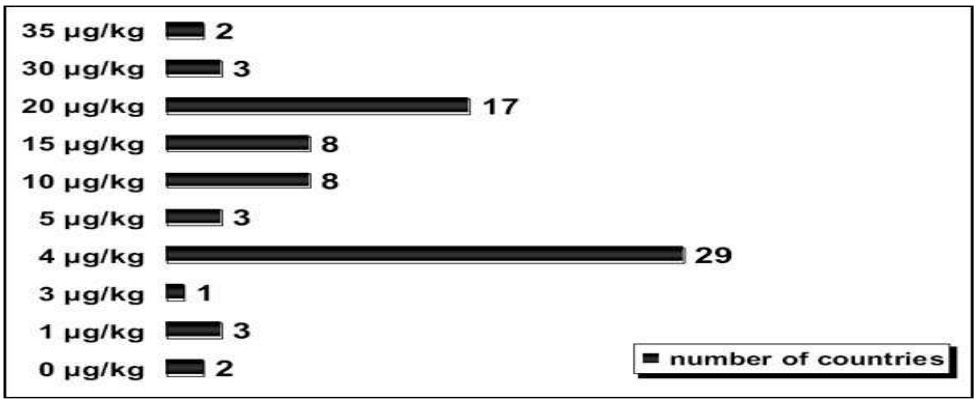
Health consequences of aflatoxin are much more serious problem in developing countries than in developed. In developing countries a lot of malnourished people are chronically exposed to high aflatoxin levels, primarily through the staple foods of maize and peanuts. These countries have a lack of resources, technology and infrastructure necessary for routine food monitoring and aflatoxin control, as well as optimal drying and storage practices. Unlike them, the developed countries have the resources and infrastructure and excellent control, thus in this countries aflatoxin contamination is reconsidered more as an economic as a health problem. Contaminated maize must be destroyed, or its price has to be reduced. Losses due to the presence of aflatoxin in maize, in the USA, are measured in hundreds of million of USD (Wu, 2004).

Global aflatoxin limitations

The number of countries that regulate aflatoxins has significantly increased over the years. The aflatoxin regulations are often detailed and specific for different foodstuffs, dairy products and feedstuffs. Most of these countries (76 countries in 2003) have regulations on total level of four predominant types of aflatoxin (B_1, B_2, G_1 and G_2), sometimes in combination with specific limit for aflatoxin B_1 (61 countries in 2003), (FAO, 2004).

Mostly used limit (Figure 1) is 4 µg/kg (applied in 29 countries), limit that was found in the harmonized regulations within the EU, the European Free Trade Association (EFTA) and candidate countries for accession to EU. Another frequently used limit is at 20 µg/kg and it is applied in 17 countries (half of them are in Latin America and several in Africa). Also, the United States, one among the first countries that established an aflatoxin action limit, follows the 20 µg/kg limit. The concentration of the sum of the aflatoxins B2, G1 and G2 is generally less than the concentration of pure aflatoxin B1 (Yabe, Nakajima, 2004).

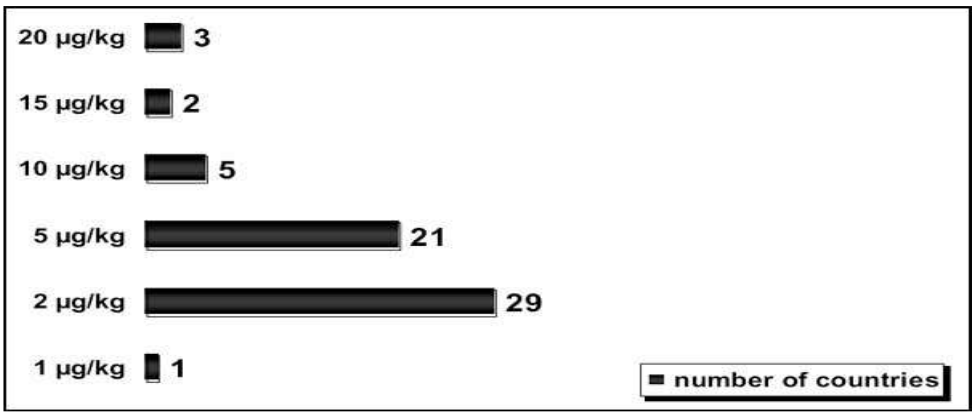
Figure 1. Worldwide limits for total aflatoxins in food



Source: FAO, 2004

The most often limit for aflatoxin B1 in food is 2 µg/kg and it is applied in 29 countries (Figure 2). Like before, limit is mostly established in the harmonized regulations within the EU, EFTA and candidate countries for EU accession. Other most important limit is at 5 µg/kg and it is followed by 21 countries (usually spread over Africa, Asia/Oceania, Latin America and Europe). The USA and Canada do not have a separate limit for aflatoxin B_1.

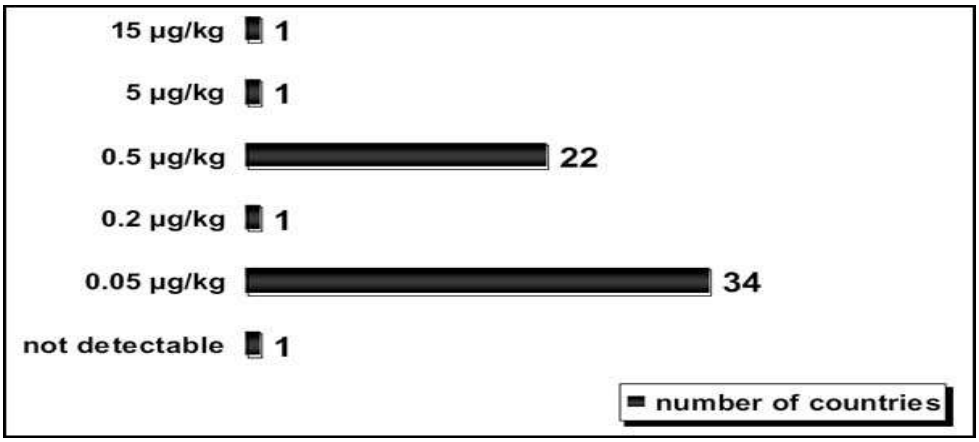
Figure 2. Worldwide limits for aflatoxin B_1 in food



Source: FAO, 2004

Regulations for aflatoxin M_1, by the end of 2003, existed in 60 countries (Figure 3). Again, as like in previous case, the EU, EFTA and candidate countries for EU accession have a highest share in the group of countries that established limit at 0,05 µg/kg, although some countries from Africa, Asia and Latin America also apply mentioned limit. Second mostly used limit is 0,5 µg/kg. This higher regulatory level is applied in the United States and several Asian countries, as well as it occurs most frequently in Latin America.

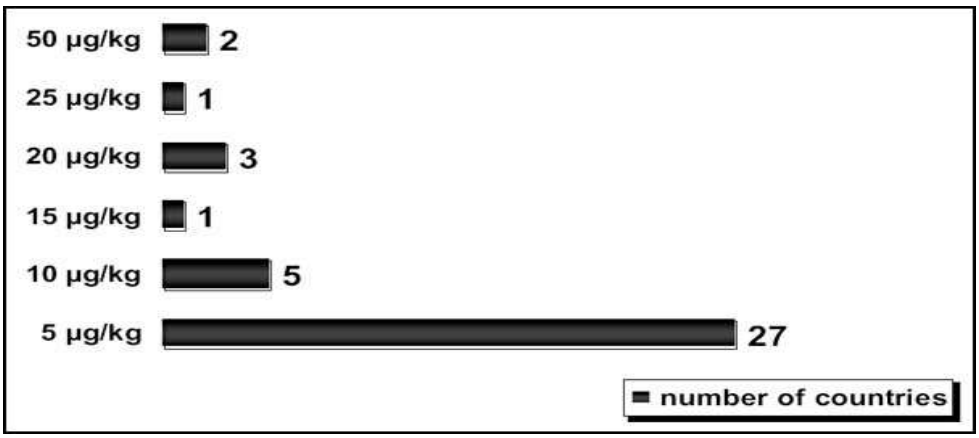
Figure 3. Worldwide limits for aflatoxin M_1 in milk



Source: FAO, 2004

Limitation of 5 µg/kg dominates within the distribution pattern for aflatoxin B_1 in feed for dairy cattle (Figure 4). Mentioned limit is applied by EU and EFTA countries. It is also followed in many candidate countries for EU accession and sporadically outside the Europe. Strict application will normally be effective to prevent that aflatoxin M_1 levels in milk remain below 0,05 µg/kg for dairy feed (where these countries have set their corresponding limit for aflatoxin M_1 in milk).

Figure 4. Worldwide limits for aflatoxin B_1 in feed for dairy cattle



Source: FAO, 2004

Global maize trade

As it can be seen in Table 2 (where were presented global top maize exporters and importers in previous decade), USA was the predominant exporter of maize in the world, exported more than half billion metric tons (MT). It has been exported four times more maze than second largest exporter, Argentina. Despite relatively small surface compared to

other leading exporters, Serbia also exported large quantities of maize (6.797.441 MT) during the previous decade. Japan is the largest maize importer in the world. Within the group of countries that have been also imported large quantities of maize over the last decade, precede Republic of Korea, Mexico, Egypt, Taiwan and Spain. Countries as are the USA, Canada, the Netherlands, Mexico and Germany were appeared in both observed groups (among top 20 maize exporters and importers).

Table 2. Top worldwide maize exporters and importers (based on volume of trade, period 2000–2009)

Rank	Top exporters	Total amount exported, 2000-2009 (MT)	Top importers	Total amount imported, 2000-2009 (MT)
1	USA	526,670,541	Japan	170,279,244
2	Argentina	123,527,253	Republic of Korea	90,841,881
3	France	71,269,591	Mexico	69,857,045
4	China	65,558,093	Egypt	51,446,403
5	Brazil	54,473,911	Thailand	47,282,122
6	Hungary	28,557,159	Spain	45,302,592
7	Canada	23,311,927	USA	33,978,967
8	Ukraine	19,568,172	Netherlands	28,629,716
9	South Africa	15,021,879	Malaysia	27,703,058
10	Paraguay	12,051,097	Iran	27,178,624
11	Mexico	11,923,079	Columbia	26,821,972
12	India	11,738,537	Canada	26,012,453
13	Germany	10,400,097	Algeria	20,230,143
14	Serbia	6,797,441	Italia	16,678,997
15	Thailand	5,366,268	Germany	16,548,899
16	Romania	4,859,320	Israel	15,658,213
17	Switzerland	3,620,319	Saudi Arabia	15,613,125
18	Netherlands	3,601,194	Portugal	14,245,589
19	Austria	3,394,665	Morocco	14,083,900
20	Bulgaria	2,962,606	UK	13,815,724

Source: Wu et al., 2012

As is shown in Table 3 many of the worldwide top maize exporters are also the countries that export maize to the largest number of countries. Like before, USA has the highest out-degree (i.e. the number of countries to which it has exported at least one consignment of maize within the period 2000–2009) exporting maize to the 181 different countries. The largest maize importers, as like Japan, Korea and Mexico (Table 2) are not included in Table 3, because they are importing large amounts of maize from a small number of countries. Within Europe, countries such as France, Germany, Italy, the Netherlands, Spain and UK have realized a significant volume of maize trade among themselves (Table 2 and 3).

Table 3. Nations with the highest level of maize export and import (number of countries with which they trade)

Rank	Exporting nations	Total number of nations to which they export	Importing nations	Total number of nations from which they import
1	USA	181	France	69
2	Argentina	150	Germany	66
3	South Africa	128	USA	66
4	France	122	Netherlands	62
5	Canada	108	Canada	58
6	Brazil	101	Italia	57
7	China	95	UK	56
8	Italia	94	Spain	53
9	Netherlands	86	Egypt	53
10	India	85	Switzerland	51
11	Australia	78	Turkey	47
12	Ukraine	72	Austria	46
13	Hungary	72	Saudi Arabia	46
14	Thailand	70	Russian Federation	44
15	Spain	70	South Africa	44
16	Germany	69	UAE	44
17	Turkey	69	Bulgaria	43
18	UAE	66	Israel	41
19	UK	63	Romania	40
20	Chile	60	Belgium	40

Source: Wu et al., 2012

The USA which has established a relatively high total standard/limit for aflatoxin, 20 µg/kg, primarily exports maize to other countries that also allow relatively large volume of aflatoxin in maize (Table 4). There are, however, several exceptions: several Latin American and Middle Eastern countries that have strict aflatoxin standards (Honduras, Cuba, Chile, Turkey, Tunisia and Syria) but import large amounts of maize from the USA. In general, aflatoxin regulations in two countries which trade with maize between each other do not differ more than 5 µg/kg. In fact, in the most of top 20 trade relationships, importing and exporting country have the same aflatoxin standard for maize (Wu et al., 2012).

Table 4. Top exporter - importer pairs and their total aflatoxin (AF) standards for maize in µg/kg (worldwide trade for period 2000–2009)

Rang	Exporter nation	AF standard	Importer nation	AF standard	Total quantity (MT)
1	USA	20	Japan	20	159,377,000
2	USA	20	Mexico	20	69,764,700
3	USA	20	Taiwan	15	44,212,000
4	USA	20	Korea	20	41,657,300
5	China	40	Korea	20	36,446,400
6	USA	20	Egypt	20	35,540,100
7	USA	20	Canada	15	25,933,000
8	USA	20	Colombia	20	21,726,900
9	Canada	15	USA	20	21,161,900
10	France	4	Spain	4	18,682,400
11	France	4	Netherlands	4	14,901,600
12	Brazil	30	Iran	30	12,588,000
13	Mexico	20	USA	20	10,947,000
14	Argentina	20	Chile	5	10,625,700
15	USA	20	Algeria	20	10,457,700
16	USA	20	Dominican Rep.	20	10,325,300
17	Argentina	20	Spain	4	10,311,600
18	Chile	40	Malaysia	35	10,119,800
19	France	4	UK	4	9,899,890
20	Argentina	20	Egypt	20	9,734,360

Source: Wu et al., 2012

Limits for aflatoxins and its appearance in food in Serbia

Until March 2013, maximum permissible concentrations of total aflatoxins (B1, B2, G1 and G2) in cereals and cereal-based products in Serbia were regulated by the Regulation on maximum residue levels of pesticides in food and feed (Official Gazette of RS, no. 25/2010 and 28/2011, Annex 5, paragraph 2.1). Mentioned Regulation is in accordance with the EU regulations (Commission regulation (EU) No. 165/2010 amending Regulation (EC) No. 1881/2006 that sets maximum level for certain contaminants in foodstuffs as regards aflatoxins). Allowed volume is in range from 0,1 to 5,0 µg/kg for aflatoxin B1 and 4,0 and 10,0 µg/kg for total aflatoxins (B1, B2, G1 and G2). Also, according to this Regulation maximal level of aflatoxin M1 in milk is 0,05 µg/kg. Regulation on the quality of the feed (Official Gazette of RS, no. 4/2010, Article 99) prescribes the maximum permissible concentrations of aflatoxin B1 in food and feed for animals, as a concentration of 0,01 to 0,05 mg/kg (10-50 µg/kg), as well as limit for aflatoxin B_1 in feed for dairy cattle at 10 µg/kg. Mentioned Regulation is not in line with the EU legislation, in fact the maximal allowable level of alfatoxin B1 in foods intended for feeding of dairy cows is twice higher than a level in the EU.

In 2012 in Serbia was observed incidental occurrence of *Aspergillus flavus* on maize. This happened due to extremely high temperatures that lasted from June to September and caused the drought, which has adversely affected the maize during the process of ripening. In such conditions, the development of *Aspergillus flavus* was optimal, and resulted in the emergence of aflatoxins in maize kernels (Škrinjar et al., 2013). There are different data

about frequency and occurrence of aflatoxin within the growing season. According to one study, in 78 samples of maize used for feeding animals, 44 contained aflatoxin (Kos et al., 2013). Also, 23,1% of infected samples contained toxins in concentrations in range from 1 to 10 μg/kg, 17,9 % in range from 10 to 50 μg/kg and 15,4% in range between 50 and 80 μg/kg. Matijevic (2013) reports that 12 maize samples that were tested for the presence of aflatoxins B1, B2, G1 and G2 were contaminated by these toxins at concentrations from 6,2 μg/kg to 145,8 μg/kg. Unfortunately, detailed monitoring has not been made, but tests made in some institutions suggested to increased occurrence of aflatoxin compering to previous period (Škrinjar et al., 2013).

Aspergillus flavus was isolated in samples of feed in 2005 and 2006 (Krnjaja et al., 2007). During the period 2007-2008, 90 milk samples are analyzed for the presence of aflatoxin M in milk. In that sum, 23 samples of raw milk were produced on the small individual farms and 67 samples were commercial milk bought in the local market. Within the 23 analyzed samples of raw milk (goat's, sheep's and cow's milk) from the small individual farms in 30,4% of samples was found the level of aflatoxin M which exceeds the allowable level by the EU legislation, but in any of the samples were not found higher concentration than allowed one by national legislation. Within the 67 analyzed samples of commercial milk (34 samples of pasteurized milk and 31 sample of UHT milk) in twenty (29,8%) samples were found certain amount of aflatoxin M, but in concentration that does not exceed EU or Serbian legislation (Polovinski Horvatović et al., 2009).

Different regulations in different years cause some confusion. After the occurrence of aflatoxin in 2012, the Serbia adopted a Amendments on Regulation on maximum residue levels of pesticides in food and feed (Official Gazette of RS, no. 20/13). Based on the Regulations, the maximum volume of aflatoxin M1 in raw milk, heat-treated milk and milk for production of dairy products is 0,5 μg/kg. In fact, the maximal llowable volume of aflatoxin is increased 10 times, and previous regulation that was in force until 2010 was returned into the power. This issue triggered a debate in Serbia. Leaving aside the debate, truth is that it will be impossible to expect the EU permitted level of aflatoxin M1 in milk if Regulation on the quality of the feed (Official Gazette of RS, no. 4/2010, Article 99) is not in line with the EU regulation. This is supported by work of Battacone et al., 2009. An experiment was carried out to investigate the transfer of aflatoxin M1 into the milk of dairy cows that were fed with nutrients contaminated with aflatoxin B1. Dairy cows were divided into four groups. One was the control group and it was fed with food that contains different levels of aflatoxin during the 14 days. Dairy cows that ate a feed with a maximum permitted level of aflatoxin B1 (5 μg/kg) gave the milk which contained 58% higher level of aflatoxin M1 (0,07929 μg/kg) than prescribed.

Discussion and conclusion

Standards for aflatoxin are very complex issue, as it is not just a health issue, but also a serious trade issue. Different countries have different limitations for aflatoxins. Countries and nations that share strong food trade relations tend to have similar regulations on allowable levels of aflatoxin in maize. The USA and EU are two different clusters of maize trade. There is no direct links between the USA and any EU country at the level of

1 million MTs of maize, traded in period from 2000 to 2009. Constant tension between the USA and the EU over genetically modified organisms (GMOs), (Papic Brankov et al., 2012) adoption of strict standards for aflatoxin by the EU can be seen through the prism of non-tariff barriers for import of genetically modified USA maize. In this way EU protect itself against rapid diffusion of GMOs which is impressive, but very uneven (Papic et al., 2008), as well as against the market monopolization by the multinational companies (Papic Brankov et al., 2010).

Most of the USA maize trade is done with Canada, Latin American countries and Middle Eastern countries. Within the EU cluster, France and Hungary are the main maize exporters, while Spain and the Netherlands are main importers which share the same aflatoxin limits. In between these two distinct clusters are the countries that export maize to multiple different parts of the world, as are Argentina, Brazil and China, which deliver maize to the Africa, Asia, the Americas, Europe and Middle East. Although Argentina and Brazil have relatively relaxed aflatoxin standards, they export some amount of maize to EU countries that have much stricter aflatoxin standards. However, all three mentioned countries trade more with countries that have relaxed standards, or do not have standards at all for aflatoxin in maize (Wu et al., 2012).

The 49th Joint FAO (Food and Agriculture Organization) and WHO (World Health Organization) Expert Committee on Food Additives' Meeting on Aflatoxin, assessed the effect of aflatoxin regulations on liver cancer depending on HBV prevalence (JECFA,1998; Henry et al., 1999). Their conclusion was: the effect of moving from an enforced aflatoxin standard of 20 µg/kg to 10 µg/kg, in one nation with HBV prevalence of 1% will reduce risk of 2 additional cancers per year per billion people. In the second country with HBV prevalence of 25% yielded a drop in the estimated population risk of 300 additional cancers per year per billion people. This means that in rich, food-importing countries, with low HBV prevalence, tightening of the aflatoxin standard would reduce cancer risk to amount so small to be detectable by epidemiological methods. Searching the database was showed that this is the only work on observed topic, and it does indicate the need for caution and further research.

By reconsideration of fact that Serbia is a country with intermediate risk, since prevalence of HBV is 2-7%, as well as the fact that Serbia is a big maize exporter, we believe that there is a need for strengthening of state control. Incidental occurrence of *Aspergillus flavus* on maize, in 2012, was shown the weakness of the used control system and legislation. HACCP should be applied to control the processes and not just the final products. Regulation on the quality of the feed must be in line with the EU regulation in order to obtain milk with a better quality. Consequently, limiting of aflatoxin B_1 in animal feeds is the most effective means for controlling of aflatoxin M_1 in milk. Authorized inspection must follow more timely notifications of the Rapid Alert System for Food and Feed (RASFF)[3]. The truth is that transfer of aflatoxin B1 from feed to milk ranging from

[3] RAFFS was established to provide food and feed control to authorities with effective tool for exchange of information about measures that was taken to response the serious risks detected in

0,3-6,2%, means that there is a small risk for causing of health problems, but risk still exists. Considering that just chronic exposure to aflatoxin can create serious health risks, we feel justified temporary returned level of allowed aflatoxin to 0,5 µg/kg in order to protect domestic production. At the same time, we believe that it is necessary, as soon as possible, to return the value of the standard at the level of 0,05 µg/kg.

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relation to food or feed. This exchange of information helps Member States to act faster and coordinated in process of response to a health threat caused by food or feed.

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STANDARDI ZA AFLATOKSIN I TRGOVINA KUKURUZOM

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Sažetak

Kontaminacija mikotoksinima se smatra neizbežnim rizikom u poljoprivrednoj proizvodnji i zemalja u razvoju i razvijenih zemalja. Zdravstvene posledice od prisustva aflatoksina su mnogo ozbiljniji problem u zemljama u razvoju nego u razvijenim. Broj zemalja koji zakonski regulišu nivo aflatoksina značajno je povećan poslednjih godina. U ovom radu smo razmatrali različite standarde za aflatoksin u svetu i njihovu ulogu u globalnoj trgovini kukuruzom. Prateci debatu u Srbiji izazvanu različitom zakonskom regulativom o aflatoksinu u različitim godinama, želeli smo da doprinesemo kreiranju državne politike o ovom pitanju. U radu se zaključuje da države sa jakim trgovinskim vezama slično regulišu dozvoljeni nivo aflatoksina u kukuruzu. Takođe, u radu se zaključuje da incidentna pojava Aspergillus flavus na kukuruzu u 2012. godini demonstrira slabosti sistema kontrole i zakona. Naša glavna preporuka je ograničavanje nivoa aflatoksina B1 u stočnoj hrani što je ujedno i najefikasnija mera kontrole nivoa aflatoksina M1 u mleku. Nakon ispunjavanja ovog uslova smatramo da Srbija treba da vrati dozvoljenu granicu aflatoksina u mleku na nivo od 0.05 μg/kg.

Ključne reči: *aflatoksin standardi, globalna trgovina kukuruzom, Srbija.*

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