

Aflatoxin M₁ in Serbia and the region, past and future

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MIROSLAVA POLOVINSKI HORVATOVIĆ¹, DRAGAN GLAMOČIĆ¹, IGOR JAJIĆ¹, SAŠA KRSTOVIĆ¹

¹ Faculty of Agriculture, Department of Animal Science, University of Novi Sad, Republic of Serbia

*Address for correspondence to: miroslavpolovinski@yahoo.com

Abstract

AFM₁ was in the center of public attention in Republic of Serbia and in the neighboring countries due to high increases in concentration during 2013. AFM₁ is metabolite of AF B₁. The huge outbreak in AFB₁ contamination, primarily of corn, during 2012 resulted in the outbreak of AFM₁ in milk and milk products. Countries of the region such as Croatia, Bosnia and Hercegovina, Slovenia, Hungary, Romania and Bulgaria had the similar problem. The end result was the sale drop of milk and milk products in the entire region. This review presents the data on the occurrence of AFM₁ in milk and milk products from Serbia and neighboring countries. Special attention is given to the contamination of AFB₁ in corn as the most probable source of contamination with AFB₁

Keywords: AFM₁, AFB₁, Serbia, milk

1. Introduction

Aflatoxins are mycotoxins, secondary metabolites of fungi, mostly produced by the genus *Aspergillus*. In the recent period, a wide public and scientific attention was given to AFs in Republic of Serbia and the region. The attention was attracted by huge problems caused by the increased level of those mycotoxins in feed, mainly in corn. As a consequence there was an increased level of AFM₁ in milk and milk products. Aflatoxins are highly toxic and mutagen and therefore are classified as I group human carcinogen (IARC 2012). AFM₁ is produced from AFB₁ as a result of contaminated feed consumption by dairy animals and its exertion in milk. Milk and milk products are very often consumed in Europe. According to the WHO estimation, the average per capita consumption in Europe regarding milk and milk products in total is 336.1 g/day (WHO 2003). Since Europe is a very heterogenic group of countries, the average milk and milk products consumption in Republic of Serbia is much lower (177.5 g/day) (Škrbić et al. 2014).

AFM₁ is the metabolite of AFB₁. The majority of this mycotoxin is excreted in urine and feces (Stubblefield et al. 1983) and some in milk. Concentration of AFM₁ in urine in humans is the indicator for human exposure to AFB₁ (Zhu et al. 1987). Carryover from feed to milk depends on numerous factors. The milk yield and days of lactation are the most important factors which influence carryover and it can be from 0.6 to 6% (Masoero et al. 2007) in some cases it can go up to 11.4% in very high yielding cows (Britzi et al. 2013). Milk processing in some milk products such as cheese, increases the amount of the AFM₁ since this mycotoxin is bound to casein (Campagnollo et al. 2016).

Any animal feed can be a source of contamination. In nutrition of dairy animals, the source of contamination is corn in most cases. However, the source of contamination can be silage or some byproduct incorporated in compound meal of dairy animals.

The aim of this work is to give the review on the current situation about AFB₁ contamination of feed intended for dairy animals and the occurrence of AFM₁ in milk and milk products in Serbia and the region.

2. Discussion

AFB₁

Legislation in EU countries for AFB₁ in animal feed, especially for dairy animals, is in function of preventing higher level of AFM₁ than allowed in milk by the EU regulation which is 50 ng/kg (EC 2006). Maximum permitted level for AFB₁ in grains intended for animal feed is up to 20 µg/kg, while for the dairy cattle feed maximum permitted level is 5 µg/kg (EC 2003). However, the estimation of maximum level of AFB₁ in feed which in milk will not cause the increases above the legal permit depends on the milk production. The study suggests that AFB₁ content in feed for high yielding Holstein cows with the average production of 45 kg/day in the early lactation should not exceed 1.4 µg/kg (Britzi et al. 2013). The corn is usually in the center of attention as the source of AFB₁ for dairy animals. However, silage or some byproduct as well can be contaminated and thus be the cause of the increases of AFM₁ in milk. In Serbia, allowed concentration of AFM₁ in milk was changed from 0.5 µg/kg to 0.05 µg/kg in 2011 (Serbian Regulation 2011). At the same time, the allowed concentration of AFB₁ in feed was kept on previous level which caused high problems in production in the 2013 year with AFs outbreak.

Table 1. RASFF notifications on AFB₁ in maize (2012-2014).

Country of origin	subject	reference
2012		
Aflatoxin B ₁ µg/kg - ppb		
Bulgaria	47.2; 55.5; 34.7; 35.2; 35.1; 31; 41.6; 32.5; 29.4; 29.1; 48.4	(RASFF 2012f)
Serbia	126.5; 161.1; 57.1; 80.8	(RASFF 2012c)
2013		
Romania	20.5; 39.8	(RASFF 2013h)
Bulgaria	96.1; 28.1; 42.9	(RASFF 2013g)
Romania and Bulgaria	20.1; 46.8	(RASFF 2013f)
Hungary	117.5; 102.5	(RASFF 2013e)
Bulgaria, Romania	22.4; 26.7	(RASFF 2013d)
Romania, Serbia, Bulgaria	1.9; 158.5	(RASFF 2013c)
Serbia, Romania, Bulgaria, Poland	37.1	(RASFF 2013b)
Serbia	204; 112; 38; 21	(RASFF 2013a)
Romania	57.6; 71.3	(RASFF 2013i)
Greece	29.4; 37.0; 35.5	(RASFF 2013n)
Bulgaria	34; 28.2; 43.8; 43; 37.3; 38.2	(RASFF 2013j)
Hungary	30.7; 11.5; 2.3; <1	(RASFF 2013m)
Hungary	75.2; 12.7; 20.1	(RASFF 2013l)
2014		
Croatia	84	(RASFF 2014a)
Romania	62.7	(RASFF 2014c)
Serbia	32	(RASFF 2014b)
Serbia	25	(RASFF 2014c)

Corn

Serbia is the country with the huge corn production. It is on the 8th place on the list of the world exporters of this crop with 2.964 million MT (“Corn Export Distribution Worldwide by Country, 2015 | Statistic” 2015). According to the report from 2015 the estimated corn production was 7.7 million MT with an average yield of almost 6.5 MT/HA (USDA 2015). Neighboring countries such as Hungary and Romania are also important producers of corn in Europe with the average of 1 million hectares cultivated with this grain (Yumpu.com 2016).

Europe was not traditionally related to the problem with contamination of crops with AFs. It was thought that imported food and feed from the countries with tropical and subtropical climate was the main source of the potential contamination. However, this perception has been changed by the recent developments. In Italy, as the result of climatic condition (drought and rainfalls combined with damage by insects), the corn from the region of Po valley was highly contaminated with AFB₁. AFM₁ was found in 6% of samples of milk at the beginning of 2003. By the end of the year, the number of samples with AFM₁ above regulatory limit increased to 7.8% (European Food Safety Authority (EFSA) 2004). Until 2012 RASFF reported no notifications concerning AFB₁ in maize from Serbia or the neighboring countries. However, only in 2013 there were thirteen RASFF notifications concerning AFB₁ in corn from Serbia, Romania, Hungary, Bulgaria and Greece, in the year before (2012) two from Bulgaria and Serbia and in the following year (2014) four from Serbia, Croatia and Romania. Moreover, in 2015 there was the notification concerning maize from Poland contaminated with AFB₁ up to 39.4 µg/kg (RASFF 2015).

It has become evident that the problem with AFs contamination can also be present in some parts of the Europe such as the region of Serbia and neighboring countries due to climatic changes. Some of the predictions have suggested that in Europe, in the years to come, in the case of climate scenario +2 °C AFB₁ will become food safety problem in maize (Battilani et al. 2016).

One of the possible problems which can occur during the outbreak of AFs contamination is sampling procedure. AFs contaminations have uneven distribution of fungi and their derivatives (mycotoxins) in the substrate. As can be seen from the table No 1, replicate test results are ranged from 21 to 204 µg/kg in one of the RAFF notification. Due to the problem concerning sampling procedure observed on corn from 2012 in Balkan outbreak Regulation (EC) No 152/2009 is replaced with the more accurate Regulation (EC) No 691/2013 (de Rijk et al. 2015).

Until 2013 when huge problems caused by AFB₁ in corn contaminated during 2012 occurred, the corn contaminated with this mycotoxin was only occasionally found in this region and at very low levels. In Romania, out of 56 samples of corn, wheat, oat and barley, AF was found in 30% of samples in concentration below 10 µg/kg (Tabuc et al., 2010). In the study from Croatia out of 300 samples taken from 20 largest feed producing companies, AFB₁ was found in 20% of samples in concentrations ranging from 1-10 µg/kg (Croatian Food Agency, 2012). In all three hundred samples of corn taken during 2009, 2010 and 2011 in Serbia, the concentration of AFB₁ was below 1 µg/kg (Kos, 2015). However, during 2012 in Serbia and the neighboring countries was a huge outbreak of AFs contamination, primarily in corn. Maize samples (n=633) from different regions of Croatia in 2013 showed high incidence of AFB₁. The mean value for this mycotoxin was 81 µg/kg, with the maximum found value of 2072 µg/kg (Pleadin et al. 2014). During that period in Serbia, in 700 samples of corn 149 (21%) samples contained AFB₁ below 1 µg/kg, while 171 samples (25%) contained AFB₁ above 50 µg/kg (Kos, 2015).

Byproducts

Sometimes the source of contamination of AFB₁ and consequently AFM₁ in milk can be the byproducts of food industry intended for animal diet. In Sweden, in the early 2006, elevated levels of AFM₁ were found in milk. Allowed concentration in milk in Sweden is the same as in the rest of EU and it is 50ng/kg. However, the Swedish Dairy Association set the action limit for starting the trace-back studies at 8 ng/kg (Nordkvist et al., 2009). 12 and 13 ng/kg AFM₁ were found in two samples of milk from one dairy. Trace-back study revealed contaminated milk samples up to 257 ng/kg in fresh milk taken from the producers. Feed and feed ingredients were analyzed and it was discovered that the source of contamination was a byproduct of food industry which was included in less than 10% (w/w) of compound cattle feed. Up to 56 µg/kg of AFB₁ was found in the ingredient (by-product of Basmati rice) (Nordkvist et al., 2009).

AFM₁

Legislation

Regulation for AFM₁ existed in 60 countries worldwide by the end of 2003 (FAO 2004). Milk and milk products are often on the menu of the average Europeans. Since legislation for AFM₁ in milk and milk products is 50 ng/kg in EU (EC 2006, 20) it is one of the lowest permitted levels of this mycotoxin in world. Besides EU countries, some other countries in Africa and Asia also have this permitted level. However, USA, Canada, Brazil and some other countries have legislation for AFM₁ which is ten times higher and it reaches 500 ng/kg. (FAO 2004).

In the past few years, Legislation regarding AFM₁ in Serbia has changed several times due to huge problems caused by this mycotoxin. As the result of aspiration of Serbia towards EU, the legislation was changed to 50 ng/kg since the previously allowed concentration was 500 ng/kg. In 2013 due to high contamination of corn with AFB₁, higher levels of AFM₁ were detected in the most milk and milk products from the region. Allowed concentration was again raised to 500 ng/kg until it was once again dropped to 50 ng/kg. From the 2015 to the April 2016 allowed concentration was 250 ng/kg.

Countries as Hungary, Croatia, Romania, Bulgaria and Greece are the members of the EU and thus the legislation is 50 ng/kg. Bosnia and Hercegovina, Macedonia, Albania and Serbia are not members and therefore legislation is somewhat different.

AFM₁ in the region

AFM₁ was in the center of public attention during 2013 due to the high level of presence of this mycotoxin in milk and milk products in the region. Consequently there was a rapid drop in milk sale. For example, in Romania there was a sale drop of 45% in only few weeks (USDA 2013). The farmers as primary producers experienced the most of the consequences. They were not able to place their production of milk, which resulted in the reduction in dairy cattle.

In the previous period, due to climatic conditions, it was thought that AFs rarely occurred in milk and milk products produced in Europe. On RASFF there was just one notification from 2007 concerning AFM₁ in milk from Hungary, while in 2012 and 2013 there were 6 notifications (Table no 2.).

The most extensive research on the occurrence of the AFM₁ in milk and milk products in the region was done in Serbia and Croatia which can be seen in Table no 3. Prior to the AFM₁ outbreak in 2013, only a few studies concerning this mycotoxin had been done in the region. Also, in some countries there was the evidence of the increases in AFB₁ levels in cereals, primarily in corn (Hungary, Romania, Bulgaria). However, there were no data concerning AFM₁ in milk and milk products from these countries. In some countries, increases in AFM₁ concentration were reported on RASFF. However, there were no studies on this mycotoxin in some of these countries.

Producers of crop and producers of dairy products were surprised and unprepared for the huge outbreak of the contamination of the AFB₁ in crops and consequently in milk and milk products which occurred in Serbia and region during 2012 and 2013. However, there were indications of this kind of possibility. Italy was one of the first European countries faced with unexpected outbreak of AF contamination back in 2003 due to the specific environmental condition and damage made by insects (European Food Safety Authority (EFSA) 2004). Mycotoxin monitoring data show the connection between unusual climatic conditions and high mycotoxin contamination (Battilani et al. 2016). Climate changes worldwide are inevitable. There are some predictions that in the case of +2 °C and +5 °C AFB₁ might become the problem in food safety in Europe, especially in case of +2 °C which is the most probable scenario of temperature increases expected in years to come (Battilani et al. 2016). AF contamination above the legal limit is to be expected. This will certainly lead to many problems and consequently changes in production technologies and even increases in costs of the food safety control. Moreover, some researchers have suggested that except temperature and water activity, the elevation of CO₂ level is one of the crucial factors, not for the growth of *Aspergillus flavus* but for the expression on genes responsible for the synthesis of aflatoxins (Medina et al., 2014). It is expected that the concentration of CO₂ in the next 10-25 year will be double to triple (from 350 to 700 or 900-1000 ppm) (Medina et al., 2014).

Table 2. AFM₁ in milk and milk products on the RASFF portal

Country of origin	Subject	reference
Hungary	0.121 µg/kg	(RASFF 2013k)
Hungary	0.073 µg/kg, 0.063 µg/kg	(RASFF 2012e)
Hungary	0.074 µg/kg	(RAFF 2012)
Hungary	0.095 µg/kg	(RASFF 2012d)
Hungary	0.072 µg/kg	(RASFF 2012b)
Slovenia	0.183 µg/kg	(RASFF 2012a)
Hungary	0.03 µg/kg; 0.45 µg/kg; 0.43 µg/kg	(RASFF 2007)

Table 3. AFM₁ in milk in Serbia and neighboring countries

Region	Maximum ng/kg	Number of samples	Reference
Bosnia and Hercegovina, Croatia	Raw milk 132.6 UHT 21.4	Raw milk (n=671) UHT (n=214)	(Bilandžić et al. 2016)
Croatia	Cow milk 162.3 Goat milk 40.8 Sheep milk 5.87 Donkey milk 10.4	Cow milk (n= 337) Goat milk (n= 32) Sheep milk (n= 19) Donkey milk (n= 14)	(Bilandžić et al. 2014)
Croatia	58.6	n= 61 samples of milk from small milking farms	(Bilandžić et al., 2010)
Croatia	37	n= 18	(Duraković et al. 2012)
Croatia	897.0		(Hengl et al., 2015)
Croatia	87.8	n= 60	(Markov et al. 2010)
Macedonia	408.1	n= 3635	(Dimitrieska-Stojković et al. 2016)
Romania	5% above 50	n= 1335	(USDA, 2013)
Romania	160	n= 22	(Macri et al., i 2014)
Serbia	14% samples above 500	n= 1438	(Tomašević et al. 2015)

Serbia	Cow milk 1200 Goat milk 240 Donkey milk 35 Breast milk 22 ng/kg Infant formula 22 ng/kg	Cow milk (n= 150) Goat milk (n=10) Donkey milk (n= 5) Breast milk (n=10) Infant formula (n=1)	(Kos et al. 2014)
Serbia	1440	n= 50	(Škrbić et al. 2014)
Serbia	7 samples above 50	n=90	(Polovinski Horvatić, et al., 2009)
Serbia	864	n=80	(Polovinski Horvatić et al. 2016)
Serbia (Kosovo and Metohija)	>50	Raw milk (n=826) UHT (n=69)	(Rama et al. 2016)

3. Conclusion

It is not questionable that the new outbreak of huge contamination with AFB₁ would happen in this region, it is only the matter of time when it will happen because of specific favorable environmental factors. Serbia and some of the countries in the region have other specific factors which can additionally increase susceptibility on the occurrence of AFM₁ in milk and milk products. Also, these factors can make problems solving more difficult. One of the issues is the fragmented agricultural production. Only 4% of producers of milk have the herd with 50-100 cows on one farm (Chamber of Commerce of Vojvodina, 2013). For example, average farm with the dairy cattle is much larger in Western Europe or in EU. Large agriculture producers have bigger economic power for the regular monitoring of food and feed in production. Also, larger producers have more technical possibility for production of feed such as silage. It remains to be seen, in time to come, what the extent of these problems will be like and their frequency of occurrence.

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