Original scientific paper UDC 637.12:582.282.123.4

AFLATOXIN M1 IN MILK AND ASSESSING THE POSSIBILITY OF ITS OCCURRENCE IN MILK PRODUCTS

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Abstract

Aflatoxin M1 (AFM1) is a hepatocarcinogenic derivative of aflatoxin B1 excreted into the milk after ingestion of contaminated feed. The presence of AFM1 in milk and milk products is of huge concern for human health. In this paper, the results on long term assessment of AFM1 in milk produced in Serbia are presented. In the period 2013 to 2016, 427 milk samples were examined for AFM1. In 34.4 % of samples, the content of AFM1 was higher than 0.05 μg/kg. The article also offers a review of the fate of aflatoxin in milk products during the different operations in milk processing. The evaluation of the influence of processing on AFM1 stability can propose economic strategy for resolving cases of accidents due to AFM-1contamination of milk.

Key words: aflatoxin M1, milk, milk product

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AFLATOKSIN M1 U MLEKU I PROCENA MOGUĆNOSTI NJEGOVE POJAVE U PROIZVODIMA OD MLEKA

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Kratak sadržaj

Aflatoksin M1 (AFM1) je hepatokarcinogeni derivat aflatoksina B1 koji se izlučuje mlekom nakon uzimanja kontaminirane hrane. Prisustvo AFM1 u mleku i mlečnim proizvodima je od ogromnog značaja za ljudsko zdravlje. U ovom radu su prikazani rezultati dugoročnog ispitivanja AFM1 u mleku u Srbiji. U periodu od 2013. do 2016. godine, u 427 uzoraka mleka je ispitan sadržaj AFM1. U 34,4% uzoraka sadržaj AFM1 je bio veći od 0,05 μg/kg. U radu je takođe dat pregled istraživanja o sudbini aflatoksina u mlečnim proizvodima tokom različitih procesa tokom prerade kontaminiranog mleka. Utvrđivanje uticaja prerade mleka na stabilnost AFM1 može da obezbedi ekonomsku strategiju za rešavanje problema u slučaju kontaminacije mleka sa AFM1.

Ključne reči: aflatoksin M1, mleko, proizvodi od mleka

INTRODUCTION

Aflatoxins (AF) are the most important mycotoxins, and besides cereals, they can contaminate milk and milk products. International Agency for Research of Cancer evaluated aflatoxin M1 (AFM1) as proved carcinogen to humans, and considered it as belonging to Group 1 (IARC, 2002). Contamination of milk and milk products can occur by indirect contamination via contaminated feed or rarely by direct contamination, when molds grow on milk. There are different data about carry-over rate of aflatoxin B1 (AFB1) from feed to AFM1 in milk. According to Creppy (2002), approximately 0.3 – 6.2% of the total ingested AFB1 from feed is transformed into milk. Some investigations revealed different carry-over rate in different animals. For example, in cows, it ranges from 0.35% to 3%, while in sheep this rate is 0.08 to 0.33% (Bakirci, 2001).

AFM1 binds to the protein fraction of milk (casein (Brackett, 1982) and may be present in milk products produced from the contaminated milk in higher concentrations than in milk itself. Only combined action of heat and low pH is able to denature whey proteins to a point where they lost their AFM1-binding capacity (Barbiroli et al., 2007). Due to its semi polar character, AFM1 predominate in the nonfat fraction (Van Egmond and Paulish, 1986; Galvano et al., 1996; Prandini et al., 2009). AFM1 was found in pasteurized milk, UHT milk, powdered milk, infant milk formulas, yoghurt, feta cheese, white cheese, traditional cheese from Turkey, Iran and Brazil, ice-cream, butter (Campagnollo et al., 2016). Different factors can influence the amounts of free AF in milk and milk products: aflatoxin concentration, pH, heat processes, ionic strength, fermentation temperature, storage temperature, storage time, protein content, titratable acidity, strain utilized (Arab et al., 2012). Pasteurization processes, even those using UHT techniques, do not drastically affect AFM1 concentration because of its heat stability (Bakirci 2001; Galvano et al., 1996; Rama et al., 2015). The data from published studies showed variable findings regarding AFM1 reduction during different unit operations used in milk products processing (Camagnollo et al., 2016). There are opinions that these contraindications in aflatoxin stability studies are due to the differences in initial levels of contamination, the range of temperature and analytical methods for AF determination (Campagnollo et al., 2016).

The legislation for AFM1 in EU (EC 1881/2006) is very strict. Maximum level is 0.05 μg/kg. In the USA (FDA, 2011), maximal limit for AFM1 in milk is 0.5 μg/kg as well as in Asia (China) and South America (Brazil). In Serbia, legislation for AFM1 in milk was in accordance with the EU in 2011, when the new Regulation about maximum permissible residues in food and feed was adopted („Službeni glasnik RS" 28/2011). However, since then, maximum level of AFM1 has been changed several times (Table 1). Currently, there is valid modification of the Regulation under which the maximum acceptable amount of AFM1 in milk is 0.25 μg/kg. Except for milk, regulatory limits were established in some countries also for the presence of AFM1 in milk products (Table 2, Campagnollo, 2016).

Table 1. Maximum permissible limits of AFM1 in milk in Serbia

Maximum permissible concentration (µg/kg)	Reference	Period of validity
0.05	„Službeni glasnik RS" 28/2011	04.05.2011. - 01.03.2013.
0.5	„Službeni glasnik RS" 20/2013	01.03.2013. - 20.03.2014.
0.5	„Službeni glasnik RS" 29/2014	20.03.2014. - 01.07.2014.
0.05	„Službeni glasnik RS" 39/2014	01.07.2014. - 14.07.2014.
0.25	„Službeni glasnik RS" 72/2014	15.07.2014. - 31.12.2014.
0.05	„Službeni glasnik RS" 29/2014	01.01.2015. - 06.10.2015.
0.25	„Službeni glasnik RS" 84/2015	07.10.2015. - 05.04.2016.
0.25	„Službeni glasnik RS" 35/2016	06.04.2016. - 05.10.2016.
0.25	„Službeni glasnik RS" 81/2016	06.10.2016. - 05.03.2017.
0.25	„Službeni glasnik RS" 21/2017	05.03.2017. - 06.09.2017.

Table 2. Maximum permissible limits for AFM1 in milk and milk products in different countries (Campagnollo, 2016; Škrbić et al., 2015)

Country	Milk	Milk product
USA	0.50	
EU	0.050	
Iran	0.050	0.50 (milk powder)
		0.020 (butter and butter milk)
		0.250 (cheese)
Turkey	0.050	0.250 (cheese)
Brazil	0.50	5 (milk powder)
		2.5 (cheese)
Italy	0.050	0.250 (soft cheese)
		0.450 (hard cheese)
China	0.5	0.5 (milk powder)
Pakistan	0.05	0.050
Switzerland	0.050	0.250 (cheese)
Austria	0.050	0.250 (cheese)
France	0.050	0.250 (cheese)
The Netherlands	0.050	0.020 (butter and cheese)

The presence of AF is typical for the warm tropical areas, and therefore the occurrence of these toxins has not been characteristic for the climate in Serbia (Živkov-Baloš et al., 2008). Due to climatic changes, as well as the development of novel analytical methods for its determination, the detection of aflatoxins in Serbian corn became more frequent (Jakšić et al., 2015). Consequently, the problems about the presence of AFM1 in milk are more pronounced. After the incident in 2013, when high contamination of corn and milk was recorded, special attention was given to the monitoring of food safety in terms of the presence of aflatoxins. As obvious from the introduction paragraph, the legislation in Serbia regulates the presence of AFM1 in milk, but not in milk products. Only few papers have been published on the topic of monitoring AFM1 in milk products in Serbia, but there are no specific studies on the transfer of AFM1 from milk into traditional and most widely consumed products in Serbia. This paper provides a long-term assessment of AFM1 in milk produced in Serbia and implications for possible fate of AFM1 in milk during its processing.

MATERIAL AND METHODS

In a period from 2013 to 2016, in the laboratory of Scientific Veterinary Institute „Novi Sad“, 427 milk samples were analyzed for the content of AFM1. Milk samples were collected from milk collecting points or dairy plants, directly on the production line. Samples were collected directly from the production in dairies or sampled by an official of control.

The presence of AFM_1 was analyzed by enzyme-linked immunosorbent assay method, using Ridascreen® Aflatoxin M_1 (Art. No. R1121) test kit (R-Biopharm, Germany). The color intensity is measured photometrically at 450 nm (Multiskan FC, Thermo Scientific, China) and is inversely proportional to the mycotoxin concentration in the sample. Special software Rida® Soft Win (Art. No. Z9999, R-Biopharm, Germany) was used for the evaluation of enzyme immunoassays. According to the manufacturer´s description, the detection limit (DL) for AFM_1 was 0.005 μg/kg. Because of high toxin concentration and maximum permitted level of 0.5 μg/kg, in a part of examinations the samples were diluted, thus in that case DL was 0.05 μg/kg, while determination range encompassed concentrations from 0.05 to 0.80 μg/kg.

The analytical quality of the ELISA method was assured by determination of spiked samples as well as by participation in proficiency testing scheme (milk powder sample FAPAS 04224). Recovery for AFM_1 was 105%.

RESULTS

The results of AFM1 content in milk samples (2013–2016) are presented in Table 3.

Table 3. Contents of AFM_1 in milk samples in Serbia in 2013–2016

			No. of samples				
Year	Positive/ total no. of samples	Positive samples* (%)	< 0.05 (μg/kg)	0.05-0.25 (μg/kg)	0.26–0.50 (μg/kg)	0.51–0.80 (μg/kg)	> 0.80 (μg/kg)
2013	55/75	73.3	20	28	9	8	10
2014	26/66	39.4	40	22	3	1	/
2015	51/178	28.6	127	38	8	2	3
2016	15/108	13.9	93	12	1	1	1
Total	147/427	34.4	280	100	21	12	14

* above 0.05 μg/kg

Characteristic climatic conditions in Serbia during 2012 affected particularly corn production (Jakšić et al., 2015). Corn contamination with high levels of AF has led to consequent milk contamination with M_1. In 73.3% of samples, maximum EU level for AFM1 in milk (0.05 μg/kg) was exceeded. This result is in accordance with published results of Torovic (2015) where 75% of samples exceeded concentration of AFM1 of 0.05 μg/kg. Somewhat lower frequency of AFM1 contamination was recorded by Tomaševic et al. 2015, while some higher levels were reported by Kos et al., 2015. During the period 2013-2014, Tomašević et al. (2015) analyzed a total of 1,438 milk samples. AFM1 levels exceeded the EU maximum residue limit in 56.3% of raw milk and 32.6% of heat-treated milk samples. In the study conducted by Kos et al. (2015) during the first half of 2013, 176 samples of different types of milk were examined in Serbia, and 86.0% contained AFM1 greater than 0.05 μg/kg. After that, in the period 2014 to 2016, gradual decline in the percentage of contaminated samples was observed, ranging from 39.4% to 13.9%. Although corn gender 2014 and after was not significantly contaminated with AF (Nešić et al., 2015), occasional occurrence of samples with high concentrations of AFM1 was still evident, reaching levels of even over 0.8 μg/kg.

DISCUSSION

During the crisis with AF in 2013 and even later years, the producers and milk processors faced the problem what to do with contaminated milk. Although regulations on maximum levels of AFM1 do not include milk products, they still refer to the milk intended for processing. Besides that, questions were also asked about the possibility of processing such milk into the safe products. Results of AFM1 examination of milk products indicate that there is no available process which can completely destroy AFM1 (Table 4.). Contrasting data have been reported on the influence of milk products preparation. Numerous investigation showed that the increase of AFM1 levels in cheese is a function of cheese type, the type of unit operations and the amount of eliminated water during processing (Nilchian and Rahimi, 2012; Bakirci, 2001; Deveci 2007). According to Manetta et al. (2009), there is direct correlation between the AFM1 in milk and its level in the final product. Experimental data showed that compared to milk, AFM1 concentration increases in yogurt (sour milk) 2 times, in cheese with a long ripening period 4.5 times, while the concentration of AFM1 in whey decreases by 40% (Manetta et al., 2009). The study of Deveci and Szegin (2006) revealed that the total AFM1 contents were reduced by about 59-68% when original skimmed milk was spray-dried. Other authors have different conclusions, i.e., transformation of fluid milk into powder will result in great increase in AFM1 concentration (Campagnollo, 2016). During the production of cheese, AFM1 crosses into the cheese (because it is bound to casein) and whey (as it is soluble in water) (Tokar and Vengust, 2008). There was less AFM1 in cream and butter than in milk. In the soft cheese, the content of AFM1 was 2.5 to 3.3 times higher and in hard cheese 3.9-5.8 times higher than in milk from which the cheese is made (Yousef and Marth, 1989). According to Mohammadi et al. (2009), rennet temperature, press time, and saturated brine pH affected the amount of AFM1 in cheese production. The combination of pasteurization, the conversion of milk into feta cheese and at least 50 days of preservation in brine leads to a 50% reduction in initial concentration of AFM1 in milk (Motawee and McMahon, 2009). Studies about the stability of AFM1 in yoghurt during fermentation are controversial, similar as in case of cheese production. In some investigations, AFM1 proved resistant to thermal treatment and slightly acidic conditions in the production of cheese and yoghurt (Colak 2007 Oruc et al., 2006) and an increase of its concentration in yoghurt was observed (Bakirci 2001), while other studies recorded a decreased levels as compared to milk (Govaris et al., 2002). Possible reasons include different pH of yoghurts and fermentation conditions (Govaris et al., 2002).

Table 4. Selected studies about stability and fate of AFM1 during dairy processing

Result	Reference
The AFM1 content in ice-cream and in sherbet remained stable through 8 months of frozen storage.	Wiseman and Marth, 1983
No significant trends for short- and long-term stability of AFM1 in milk powders for 6 years at -20°C.	Josephs et al., 2005
Pasteurization at 63°C for 30 min caused <10% destruction of AFM1.	Motawee and McMahon, 2009
64.4% of AFM1 concentration from milk was found in cream. Mean AFM1 level of skim milk was 3% higher than those of milk.	Bakirci, 2001
Pasteurization and concentration on 30-33% dry mater reduced 35-40% of AFM1.	Deveci and Sezgin, 2006
Fermentation with different starters to pH 4.0 and 4.5 has impact on reducing AFM1 concentration by 25%.	Jasutiene et al., 2006
AFM1 remained at 42.87% and 34.73% in Turkish White and Kashar cheese samples, respectively. The change of AFM1 concentration during the white cheese ripening of 0-90 days was averagely 9.8%.	Colak, 2007
Loss of the initial amount of AFM1 in milk was estimated at about 13% and 22% by the end of the fermentation, and 16 and 34% by the end of storage for 4 weeks at 4°C, for yoghurts with pHs 4.6 and 4.0, respectively.	Govaris et al., 2002
During cheese making, the remaining AFM1 in milk was partitioned: 2/3 retained in the curd and 1/3 going into whey. 22-27% AFM1 reduced during storing feta cheese in brining solutions (8-12% w/w salt) during 10 days, 25-29 % after 60 days.	Motawee and McMahon, 2009

There are no studies on the effects of processing of contaminated milk on the concentration of AFM1 during manufacturing of traditional Serbian products, but there are few studies on the presence of AFM1 in milk products in Serbia. Fifty four samples of white and hard cheese were analyzed in Serbia in May-Jun, 2013. Seven samples (23%) exceed maximum acceptable level of

0.25 μg/kg (Škrbić et al., 2015). In addition to milk, Tomašević et al. (2015) analyzed milk products. Milk powders had the highest mean concentration of AFM1 (0.847±1.948 μg/kg) and were followed by hard (0.379±0.509 μg/kg) and white cheeses 0.146 ±0.170 μg/kg. However, based on these studies it is difficult to conclude about the degree of AF transfer into milk products. As can be seen, there is high standard deviation of AFM1 results in milk products, and there is also large difference between the concentration in the milk and milk products. Obviously, the lowest concentration of AFM1 was in yogurt as compared with other products which is in accordance with the presented studies from other countries.

Good nutrition in appropriate animal production system is essential to economically produce a healthy, high-quality product (Mirilović et al., 2015). It is necessary to act preventively in order to avoid milk contamination. Undoubtedly, it is very important to control the quality of feed and storage conditions in view of the presence of aflatoxin. Grains contaminated with AFB1 should not be fed to lactating animals to avoid contamination of milk.

CONCLUSSION

There is a factual risk of contamination of corn, milk and milk products with AFs in Serbia. In general, one can conclude that neither storage nor processing can fully eliminate AFM1 from milk. Further studies addressing the occurrence and stability of AFM1 in milk products should be carried out in order to evaluate the fate of AFM1 in traditional Serbian milk products. Avoiding economic losses due to processing of contaminated milk is possible if taking into consideration the following conclusions:

1. During milk processing, AFM1 passes from raw milk into cheese, yoghurt and whey.
2. The concentration of AFM1 in cheese depends on the production process and the process of cheese ripening and brining. Recent studies show that AFM1 concentrates in cheese in a large percentage - in contrast to older studies. This is perhaps influenced by the development of new and more accurate methods for determination of AFM1 in cheese. Variations in different studies about AFM1 content in cheese are partially due to the differences in analytical method used to quantify AFM1.
3. The content of AFM1 in yoghurt depends on the pH.
4. The temperature has little effect on reducing the concentration of AFM1, but studies on the presence of AFM1 in pasteurized and UHT milk have always revealed smaller number of contaminated samples of UHT milk.

5. AFM1 is concentrated in milk powder and remains stable in it for a long time.
6. Sour cream and butter have less AFM1, but there is little information on the percentage.
7. According to several studies, the storage of frozen milk and yoghurt results in the reduction of AFM1concentration of.
8. Milk pasteurization and cheese manufacturing process do not eliminate AFM1, so it is prudent to check the AFM1 incidence in cheese.

ACKNOWLEDGEMENT

This paper is published as part of the projects of the Ministry of Education, Science and Technological Development of Republic of Serbia No. 031071.

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Primljeno: 28.02.2017.
Odobreno: 08.06.2017.