Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 116, 75—80, 2009

UDC 637.12:582.281/.282(497.143)
DOI:10.2298/ZMSPN0916075P

Miroslava S. Polovinski-Horvatovic[1], *Verica B. Juric*[1], *Dragan Glamocic*[1]

[1] Agriculture Faculty, Trg Dositeja Obradovica No. 8, 21000 Novi Sad, Serbia

THE FREQUENCY OF OCCURRENCE OF AFLATOXIN M_1 IN MILK ON THE TERRITORY OF VOJVODINA

ABSTRACT: Aflatoxin is one of the most common mycotoxins which can be found in milk. It represents a natural metabolite of aflatoxin B_1 that occurs as a result of animal metabolism and the body's attempt to detoxificate it. It is excreted in milk, feces and urine of animals that consumed contaminated feed with aflatoxin B_1. The carry-over from feed to milk depends on many factors, ranging from 0.3 to 6.2%. Aflatoxin M_1 is in the first group of carcinogens according to the IRAC classification from 2002, but it is considered to have only 10% of carcinogenicity from its precursor aflatoxin B_1. Legislation in member countries of European Union for this mycotoxin in milk intended for people is 0.05 μg/l, while the rest of the countries that also have legislation for this mycotoxin allow the concentration that is ten times higher, and that is 0.5 μg/l.

In this paper, we have tried to provide on insight into the quality of milk, food often consumed by children, from the standpoint of mycotoxicology, and to compare the obtained data with data available from literature, from countries in the region that have similar climatic and agricultural conditions. From a total of 65 samples of processed milk, aflatoxin M_1 was found in 18 samples and none of the samples exceeded the level of 0.05 μg/l, which is allowed by the legislation of the European Union.

KEY WORDS: aflatoxin M_1, milk

INTRODUCTION

Mycotoxins are large group of compounds, secondary metabolites of fungi, in which aflatoxins are one of the most frequent and most toxic. The first time aflatoxins were heard about was in 1961, when Blount described Turkey "X" disease (B l o u n t, 1961), which is now known to be caused by aflatoxin B_1 and most likely cyclopiazonic acids. Since that time, a golden era studies of mycotoxins as possible contaminants of food for people and animals begins. Mycotoxins rarely act acutely as toxins, and are usually found in small quantities in food for people or animals. Since their action chronic in character, they are often called the silent killers.

Aflatoxins usually generate fungi from the genus *Aspergillus*, most often *A. flavus* and *A. parasiticus*. Aflatoxin M_1 is 4-hydroxy derivative of aflatoxin

B_1, which occurs in the body of animals during metabolism in the liver. Cytochrome responsible for this biotransformation is the CYP450, which is accompanied with two dominantly present isoforms, CYP3A4 and CYP1A2. Both isoforms of enzyme catalyse biotransformation of aflatoxin B_1 in high reactive form of exo-aflatoxin B_1 8,9-epoxide (G u e n g e r i c h, 1998), and later in aflatoxin M_1. Aflatoxin M_1, according to the classification of IRAC (IRAC, 1993), was first classified in the second group of carcinogens (2B), but in the year 2002, the same organization classified it in the first group of carcinogens (IRAC, 2002). All aflatoxins, as well as aflatoxin M_1, are very stable compounds during processing and do not reduce significantly. Even more, during the production of cheese and other milk products it comes to the multiple increase of the amount of aflatoxin M_1 (depending on the type of cheese coefficient the increase ranges for soft cheeses from 2.5 to 3.3, and from 3.9 to 5.8 for hard cheeses). Average consumption of milk and milk products in the European Union per day is about 340 g per person, while the average consumption of milk is 290 g (H e n r y S. H. et al., 2004).

It is believed that the rate of samples with aflatoxin B_1, and the aflatoxin M_1 in Europe, due to climate conditions (for fungi of the genus *Aspergillus*, the most appropriate regions with subtropical and tropical climate) are relatively poorly represented, and that aflatoxin B_1, found in food for people and animals, comes mainly from food imported in the European countries from the subtropical and tropical regions. However, recent data from the EFSA (European Food Safety Authority) publications (EFSA, 2004) raised serious doubts. In the area of North Italy, due to unfavourable climate factors (rotation of drought and rainy interval) and insect damage caused contamination of corn with significant concentration of aflatoxin B_1, which resulted in increased aflatoxin M_1 in milk in the amount which exceeds allowed level of 6%, and that percentage increased to 7.3% at the end of the same year. The usual practice in the countries of the European Union is monitoring of milk and milk products for the presence of aflatoxins M_1. Legislation of this toxin in the European Union is one of the most restrained legal regulations in the world and is 0.05 mg/l (Commission Regulation (EC) N. 466/2001), while other countries that also have legislation for this mycotoxin (including Serbia) allow the value that is ten times higher and amounts 0.5 μg/l.

MATERIALS AND METHODS

All 65 samples were taken from September to December 2008. Method used to determine aflatoxin M_1 combined clean up process with immunoaffinity columns and TLC determination (G r o s s o et al., 2004; S h u n d o L. and S a b i n o M., 2006). The milk samples were centrifuged for 15 min and the upper fat layer was discarded. The skimmed milk (100ml) was passed throught an immnuoaffinity column (AFLAPREPM$_1$ R-Biopham, Rhone Ltd). Column was washed with distillated water (40 ml). Bound aflatoxin M_1 in the immunoaffinity column was released by the elution with 2.5 ml acetonitrile--methanol (3:2; v/v) and 2.5 ml methanol, the elute was evaporated to dryness

using rotary evaporator. Concentrate of AFM_1 was resuspended in 1 ml acetonitrile and evaporated again. The last concentrate was resuspended in 200 ml toluene acetonitrile (9:1; v/v).

In accordance with the TLC procedure, concentrate samples from 50 and 100 ml were used and TLC plate samples were applied (TLC aluminum sheets. 20x20 cm, Silica gel 60). The plates were developed in chlorophorm: acetone:isopropanol (87:10:3; v/v). After the plate had dried, it was read under long wave (366nm) light. The concentration of AFM_1 was determined by taking into account the spots of samples and spots of standards.

RESULTS

A total of 65 samples of commercial milk brought into the local markets from different manufacturers (a dairy), and with different content of milk fat (fat content of milk varied from 0.9% to more than 3.2%, depending on the characteristics of the producer) were analysed. From a total of 65 analysed samples, 34 samples were pasteurized milk, while the 31 samples of milk were long-term UHT milk. In eighteen milk samples, different concentrations of aflatoxin M_1, were found while in other samples content was less than the limit of detection (0.01 mg/l). None of the samples exceeded the allowed concentration of 0.5 μg/l, but also a single sample is not exceed allowed level by the legislation of European Union (0.05 μg/l).

Tab. 1 — Overview of the analyzed samples of pasteurized and UHT milk

Type of milk	< 0,01 μg/l	od 0,0125—0,05 μg/l	> 0,05 μg/l	Σ
pasteurized	27	7	0	34
UHT	20	11	0	31
Summary	47	18	0	65
Percent	72.31	29.69	0	100

As seen in Table 1, from a total of 65 samples of analyzed commercial milk, in 18 samples (269,69%) certain amount of aflatoxin M_1 was detected (ranging from 0.01 mg/l to 0.03 mg/l). The mean value for aflatoxin M_1 was 0.014 mg/l.

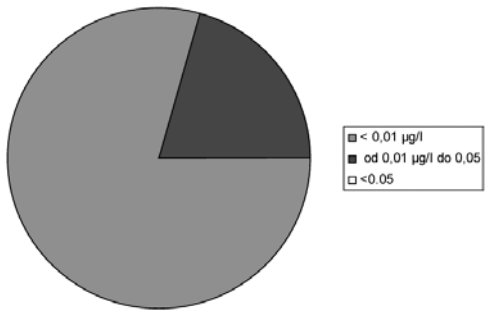

Fig. 1 — Graphically presented results

DISCUSSION

There are a lot of accessible data on the content of aflatoxin M_1 in milk and milk products from the countries of the European Union, because there are long-term monitoring on the presence of this mycotoxin, however, there is little or no data about this problem on the territory of Serbia or even beyond, in the territory of former Yugoslavia. From 11,831 processed samples from the territory of the European Union, 280 were from individual small farms, the concentration of aflatoxin M_1 in all the samples greater than 0.05 μg/l was detected in only 0.06% of samples, which indicates that the incidence of this mycotoxin are extremely low (EFSA, 2004). Countries in the region that are not members of the EU, which implemented some kind of monitoring, have higher values of concentration aflatoxin M_1 in milk and milk products then the member countries. In the study taken in Albania on samples of milk from individual farms, 42% of samples had lower value then 0.05 μg/l, while 58% of samples had higher concentrations of aflatoxin M1. Maximum concentration found was 0.85 μg/l (Panariti, 2001). The study made in Turkey in the UHT milk showed that 47% of samples exceeded the allowed limit of the EU. The average value found was 0.108 μg/l (Unusan, 2006). We must also take into account the data taken from the EU before introduction the regulation that sets 0.05 μg/l as a limitin 2003. Data from work Dragacci & Frémy, 1993 in which results of 15-year observation was summarized, regarding milk quality in France over period 1978—1992, there were two periods of high contamination with aflatoxin M_1 in milk, the first was 1978—1984 and another in the winter 1988—1991 year. Very contaminated samples, were found, some of reached 5 mg/kg aflatoxin in M1 (in the period 1978—1983). In the north of Italy in the period of 2003 surprisingly high concentration of aflatoxin B_1 in food for feeding milk cows were found resulted in higher concentration of aflatoxin M_1 in milk (EFSA, 2004). As a result of the situation in the country, strategy of surveillance and control was erected which is already near timeframe and so far has given towards the good results reduction of the percentage of contaminated samples of food and milk (Decastelli et al., 2006). Also, in the analysis of the results from this work, it should be noted that the samples analyzed were commercially available milk products, which means that this milk was produced from merging milk from a large number of producers, thus the contribution of the singular manufactures to the aflatoxin burden of milk products was masked. It is not surprising to obtain such results, since our previous work showed that 28% of milk samples collected from individual forms, exceeded the maximum concentration allowed for aflatoxin M_1 (Polovinski et al., 2008).

CONCLUSION

Although the results presented in this paper are encouraging, from the standpoint of mycotoxicological quality of milk in our market, it is certainly of general interest to introduce system of regular monitoring in order to always

have available data. The variation in the content of aflatoxin B_1 and M_1 are large and depend on the year and even within one year there may be significant variations depending on many factors. As a country which is clearly aimed at joining, establishing systems of monitoring to achieve better food and feed quality is a significant determinant.

ACKNOWLEDGE

This work is supported by means of the Ministry of Science, the Republic of Serbia.

REFERENCES

Blount, W. P. (1961): *Turkey "X" disease*, J Br Turk Fed; 9:52—4.

Commission Regulation (EC) N. 466/2001 (2001): Commission Regulation (EC) N. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.

Dragacci, S. & Frémy, J. M. (1993): *Contamination of milk with aflatoxin M_1*, Sci. Aliment., 13, 711—722.

EFSA (2004): *Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Aflatoxin B_1 as undesirable substance in animal feed*, The EFSA Journal 39, 1—27.

Grosso, F., Fremy, J. M., Bevis, S. and Dragacci, S. (2004): *Joint IDF--IUPAC-IAEA(FAO) interlaboratory validation for determining aflatoxin M_1 in milk by using immunoaffinity clean-up before thin-layer chromatography*, Food Additive, and Contaminant, 21, 348—357.

Guengerich, F. P., Johnson, W. W., Shimada, T., Ueng, Y. F., Yamazaki, H., Langouet, S (1998): *Activation and detoxication of aflatoxin B_1*, Mutat. Res. 402:121—8.

Henry, S. H., Whitaker, T., Rabbani, I., Bowers, J., Park, D., Price, W., Bosch, F. X., Pennington, J., Verger, P., Yoshizawa, T., Egmond van H., Jonker, M. A., Coker, R. (2004): *Aflatoxin M_1* (http//www.irmmi.jrc.be).

IARC (1993): *Monograph on the Evaluation of Carcinogenic Risk to Humans. Some Naturally Occurring Substances: Food Items and Constituent Heterocyclic Aromatic Amines and Mycotoxins* (Lyon: IARC).

IARC (2002): *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. Summary of data reported and evaluation*, IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Vol. 82. International Agency for Research on Cancer, Lyon, France.

Panariti, E. (2001): *Seasonal variations of aflatoxin M_1, in the farm milk in Albania*, Arh Hig Rada Toksikol, (2000), 52: pp. 37—41.

Polovinski, M., Juric, V., Glamocic, D. (2008): *Sadržaj aflatoksina M_1 u mleku na području Srbije*, Savremena poljoprivreda. In press.

Shundo, L., Sabino, M. (2006): *Aflatoxin M_1 in milk by immunoaffinity column cleanup with TLC/HPLC determination*, Brazilian Journal of Microbiology, 37, 164—167.

Unusan, N. (2006): *Occurrence of aflatoxin M_1 in UHT milk in Turkey*, 44(11): 1897—900.

УЧЕСТАЛОСТ ПОЈАВЉИВАЊА АФЛАТОКСИНА M_1 У КОНЗУМНОМ МЛЕКУ НА ТЕРИТОРИЈИ ВОЈВОДИНЕ

Мирослава С. Половински-Хорватовић[1], Верица Б. Јурић[1], Драган Гламочић[1]

[1] Пољопривредни факултет, Департмант за сточарство, Трг Доситеја Обрадовића бр. 8, 21000 Нови Сад, Србија

Резиме

Афлатоксин M_1 је један од најчешћих микотоксина који може да се јави у млеку. Представља природни метаболит афлатоксина B_1 који настаје као последица метаболизма животиње и покушаја организма да детоксикује афлатоксин B_1. Излучује се путем млека, фецеса и урина животиња које су конзумирале храну контаминирану афлатоксином B_1, проценат преласка зависи од многобројних фактора и креће се од 0.3 до 6.2%. Афлатоксин M_1 се убраја у прву групу карциногена према IARC класификацији из 2002. године, али се сматра да поседује само 10% од канцерогености свог прекусора афлатоксина B_1. Законска регулатива у земљама чланицама Европске уније за овај микотоксин у конзумном млеку намењеном за исхрану људи износи 0.05 μg/l, док у осталом делу земаља које поседују законску регулативу за овај токсин (у које спада и Србија) дозвољена концентрација је десет пута већа и износи 0.5 μg/l.

У овом раду смо покушали да дамо увид у стање квалитета млека, као животне намирнице коју чешће конзумирају деца, са становишта микотоксикологије, и упоређивањем добијених података са подацима приступачним из литературе, из земаља у окружењу са сличним климатским и пољопривреним условима. Од укупно 65 обрађених узорака млека, у 18 узорка је пронађен афлатоксин M_1, и ни у једном узорку није премашивао дозвољену количину по законској регулативи Европске Уније од 0.05 μg/l.