ASSESSMENT OF AFLATOXIN M1 IN RAW MILK IN THE MARAMURES PROVINCE OF ROMANIA

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REZUMAT. Un sondaj cu privire la apariția aflatoxinei M1 a fost efectuat în județul Maramures (România) pe un total de 120 de eșantioane de lapte crud provenit de la 5 ferme, pentru 12 luni (ianuarie-decembrie 2010). Probele au fost analizate prin cromatografie în strat subțire (TLC). Datele au fost analizate statistic prin aplicarea ANOVA. Aproximativ 3,33% din probe au fost contaminate cu aflatoxina M1 depășindu-se limita Uniunii Europene (0.05 micrograme kg-1). Incidența . aflatoxinei M1 a fost mult mai mare în sezonul ploios octombrie (33,3%), decât în timpul verii

Cuvinte cheie: lapte crud,cromatografie, aflatoxina

ABSTRACT. A survey on the occurrence of aflatoxin M1 was carried out in the province of Maramures (Romania) on a total of 120 samples of raw milk from 5 farms for 12 months (January-December 2010). Samples were analyzed by thin layer chromatography (TLC). The data were analyzed statistically by applying ANOVA. About 3.33 % of the samples were contaminated with aflatoxin M1 and exceeded the European Union limit (0.05 µg Kg^{-1}). The incidence of aflatoxin M1 was much higher in rainy season October (33.3%), than during summer.

Key words: raw milk, chromatography, aflatoxin

1. INTRODUCTION

Milk is a major nutrient for children, and cereals are an important source of nutrition in their diet and are among the first solid foods eaten. The presence of chemical contaminants in the human diet, and especially in the diet of vulnerable populations such as infants, is of great concern. (Alvito, Sizoo, Almeida, & van Egmond, 2010)

Mycotoxins are secondary metabolites produced by various fungi (Schmidt-Heydt & Geisen, 2007). Aflatoxins are fungal metabolites produced by three species of Aspergillus, namely *A. flavus, A. parasiticus* and *A. nomius*. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. One of the mycotoxins, aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 (AFB1) and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs (Prandini, et al., 2009). About 1-2% of AFB1 in animal feed is transformed to AFM1 in milk; it may vary from animal to animal, rom day to day and from milking to the next. 12-24 h after the first AFB1 ingestion, the toxin can be detected in the milk (Ebrahim 2010). The occurrence of aflatoxin M 1 in milk is transitory in nature and reaches maximum within two days after the intake of the contaminated commodity (Hussain & Anwar, 2008).

Aflatoxins represent a serious risk for animal and human health, especially for children, who are the major milk consumers (Rosi, et al., 2007). The hepatotoxic, genotoxic, carcinogenic, teratogenic, immunosuppressive and antinutritional effects of aflatoxins are well documented (Wangikar, Dwivedi, Sinha, Sharma, & Telang, 2005; Williams, et al., 2004). Aflatoxins are considered to be human liver carcinogens, AFB1 being the most potent. AFM1 has a potency approximately one order of magnitude lower than that of AFB1 (Tajkarimi, et al., 2008)

The presence of AFM1 in milk and dairy products can be a potential threat to the health of consumers (Manetta, et al., 2009). Exposure to AFM1 through milk products is a serious problem for public health. Several countries have established regulatory limits for AFM1 in raw milk and milk products, which vary from country to country (Ruangwises & Ruangwises, 2010). At present, aflatoxin presence in feed, milk

and dairy products can be systematically controlled in Europe and other developed countries (Yaroglu, Oruc, & Tayar, 2005).

Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another (Stoloff, van Egmond, & Park, 1991). The European Commission proposes a maximum permissible level of 0.05 μg Kg^{-1} AFM1 in milk and milk products (EC, 2006) . In our country, legal limits for AFM1 in milk are 0.05 μg Kg^{-1}.

In Maramures province of Romania, there is little data in the literature on the occurrence of AFM1 in raw milk. Since they are mycotoxins hazardous to human health, the aim of this survey was therefore to determine concentrations of AFM1 in raw milk produced in some farms of the Maramures province of Romania by thin layer chromatography (TLC).

2. MATERIALS AND METHODS

Sampling. A total of 120 samples of raw milk were collected from five farms in the county of Maramures, Figure 1 for a period of 12 months (January-December 2007), each farm were taken every two samples each month. The raw milk were (kept on the ice during the transportation and were analyzed immediately upon arrival at the laboratory.

Determination of AFM1. Milk samples were analyzed for the presence of AFM1 by thin layer chromatography (TLC), method described in AOAC Official Methods of Analysis (1998)

Fig. 1 *Map indicating sampling locations*

Statistical analysis. For the interpretation of the analytical results obtained, we analyzed the statistical signification of the differences between the averages studied using the F-test (Fisher) test at all the levels of ($P<0.05$). Probabilistic statistical determinations were made with the algorithm of determination ANOVA Single Factor using the Microsoft EXCEL program.

Table 1 *Occurrence of AFM1 in raw milk samples*

Monthly Interval	Farm Number	Analyzed (n)	No. of positive above EU limit n(%)*	AFM1 concentration (μg Kg^{-1})		
				Minimum	Maximum	$\bar{x} \pm Sr$**
January - March	1	6	0	0.015	0.024	0.018 ± 0.22
	2	6	0	0.009	0.018	0.013 ± 0.11
	3	6	0	< LOD***	0.031	0.012 ± 0.47
	4	6	1(16.6)	0.027	0.067	0.038 ± 0.31
	5	6	0	0.004	0.038	0.022 ± 0.30
April - June	1	6	0	< LOD***	0.024	0.014 ± 0.37
	2	6	0	0.025	0.029	0.024 ± 0.56
	3	6	0	0.002	0.037	0.017 ± 0.34
	4	6	0	0.027	0.048	0.031 ± 0.35
	5	6	0	0.014	0.020	0.019 ± 0.37
June-September	1	6	0	< LOD***	0.014	0.007 ± 0.18
	2	6	0	0.004	0.034	0.027 ± 0.35
	3	6	0	0.016	0.025	0.019 ± 0.38
	4	6	0	0.021	0.041	0.039 ± 0.35
	5	6	0	0.014	0.032	0.017 ± 0.53
October-December	1	6	0	0.015	0.020	0.016 ± 0.37
	2	6	1(16.6)	0.023	0.058	0.033 ± 0.32
	3	6	0	< LOD***	0.015	0.013 ± 0.37
	4	6	2(33.3)	0.037	0.087	0.040 ± 0.37
	5	6	0	0.007	0.042	0.037 ± 0.13

* < EU limits for AFM1 (0.05 μg Kg^{-1})
** Standard deviation, calculated from results generated under repeatability conditions.
***LOD Limit of detection μg Kg^{-1}

3. RESULTS AND DISCUSSION

Table 1 summarizes the number of samples analyzed and the number of samples found to contain detectable levels of AFM1 contamination in dairy farms. From 120 samples, 4(3.32%) contained AFM1 in concentrations ranging from 0.05 to 0.057 μg Kg^{-1}

The number of positive samples for AFM1 in raw milk form farm no.4 were 3(2.5%) during

February, October and November (rainy season.) At the farm no.1, 2 and respective 5 shows that the absence AFM1 contamination, which indicated implementation of HACCP systems, while the farm no.2 system is ongoing implementation

It was also observed that during the winter months, contamination levels in the samples from the farms were higher than they were during the summer months in the year 2010. This could be explained by the prolonged storage required for feed, therefore AFM1 contamination of milk is the result of cows feeding on material containing aflatoxin AFB1. The concentration of this mycotoxin in animal feedstuffs is influenced by the type, the time and method of harvesting and temperature and relative humidity of storage facilities (Tajkarimi, et al., 2007) , AFM1 concentrations in milk samples were significantly higher in the colder seasons (Rahimi, Shakerian, Jafariyan, Ebrahimi, & Riahi, 2009) One reason for this is that milking animals are fed with compound feeds in winter that are prone to aflatoxin B1 contamination (Abolfazl, 2005; Galvano, Galofaro, & Galvano, 1996; Hussain & Anwar, 2008) (Abolfazl, 2005)

4. CONCLUSION

In conclusion, raw milk have to be controlled continuously for presence of AFM1 contamination. It is also extremely important to maintain low levels of AFM1 in the feeds of dairy animals. Efficient control of AFM1 in milk requires efficient and easily performed analytical methods, allowing low quantification which increases the percentage of positive samples.(Luzia and Myrna 2006). Therefore, animal feeds should be checked regularly for aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled.

Implementing a food control system, such as the HACCP system, in the food industries is suggested as an efficient means for limiting aflatoxin contamination in Maramures.

The obtained data in our research are the first results of investigations on the occurrence of mycotoxins in raw milk from province of Romania

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