THE IMPORTANCE OF MONITORING OF AFLATOXINS AT THE DAIRY FARMS

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Aflatoxins (AFs) are one of the most important contaminants of food and feed, regarding its toxicity and carcinogenic properties. They belong to the Group 1 of human carcinogens clasified by the International Agency for Research on Cancer (IARC). From a European perspective, imported feed such as peanut cake, palm kernel, copra and corn gluten meal (depending of origin) is considered to be the most common source of exposure (**Streit et al.,** 2012). Aflatoxins are metabolic products of *Aspergillus flavus* that mostly occures in areas with tropical and subtropical climates, but under certain conditions (high temperature, drought and insect damage), AFs can appear in other climate zone. *Aspergillus* produces four major aflatoxins: B1, B2, G1 and G2, while aflatoxin B1 (AFB1) is the most toxic in the group and the toxicity is in the order of B1 > G1 > B2 > G2 (**Abass et al.,** 2004). In the 2003, the EFSA report included findings from Italy reporting the detection of AFB1 in maize originating from the Po valley. It occured after a growth period characterized by high temperatures, drought and insect damage. As a result, regional milk samples collected after this particular harvest were found to be contaminated with aflatoxin M1 (AFM1) concentration exceeding EU limitations (**EFSA J**., 2004) . AFM1 is a major metabolite of AFB1, which is formed when animals ingest feed contaminated with AFB1. These metabolites are not destroyed during the pasteurization and heating process. The amount of AFM1 which is found in milk depends on several factors, such as animal breed, lactation period, mammary infections etc. AFM1 can be detected in milk 12-24 h after the AFB1 ingestion, reaching a high level after a few days. When AFB1 intake is stopped, the AFM1 concentration in milk decreases to an undetectable level after 72 h (**Sibanda et al.**, 1999; **Gunsen and Buyukyoruk**, 2002; **Sarimehmetoglu et al**., 2004). Maximum tolerated levels of mycotoxins in animal feed have been established in many countries.

Allowed limits of mycotoxins in feed on territory of European Union are regulated by regulation of European Union (**EC 32**/2002**, EC 100**/2003**, EC 576**/2006). Guidelines for establishing these limits are based on epidemiological data and extrapolations from animal models, taking into account the inherent uncertainties associated with both types of analysis. Estimations of an appropriate safe dose are usually stated as a tolerable daily intake (**Kuiper-Goodman**, 1998; **Kuiper-Goodman**, 1994; **Smith et al**., 1995). Countries, members of European Union have harmonized their regulations and other countries, like Serbia, have their own regulations. Allowed limits of mycotoxins in animal feed in Serbia are determined by regulations of Serbia (**Pravilnik o kvalitetu hrane za životinje, Sl. Glasnik 4**/2010). In Serbia, monitoring of mycotoxins is not obligatory in present. The aim of our work was screening of mycotoxins in animal feed from the region of Vojvodina. Permanent screening is needed on all levels of production and storage, as well as the use of known methods to reduce mould contamination or toxin content in feedstuffs.

In the year 2012, there was occurence of AFs in corn in Serbia and the region, causing a major problem in the storage, transport and feeding of animals. After Rapid System Alert in October 2012 when high level of AFs were found in corn exported from Serbia to Italy, Ministry of Agriculture of Republic Serbia ordered systematic monitoring of corn storage systems. It was found that about 30 % of corn samples were contaminated by AFs in the concentrations above level of 50 μg/kg according to Serbian regulation (**Pravilnik o kvalitetu hrane za životinje, Sl. Glasnik 4/2010**). Since the greatest influence AFs had on the dairy cows, causing occurence of AFM1 in the milk as a consequence, milk samples collected from market had a significant level of AFM1, exceeding Serbian and EU permitted level (0.05 μg/kg). The Provincial Ministry for agriculture, forestry and water management organized and supported monitoring of dairy farms regarding to presence of AFs in the raw materials (corn), corn sylage and mixed feed as well as presence of AFM1 in milk.

MATERIAL AND METHODS

Representative samples (1-2 kg per sample) from the total of 14 dairy farms in Vojvodina were collected during February 2013. The collected samples included 35 samples of feedstuffs (corn, corn silage and mixed feed) as well as 14 samples of raw milk. All samples were taken according to proper sam-

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pling procedure (**Guidance Document**, 2010). Samples were examined in accredited Laboratory of Institute of Food Technology during February 2013. The amounts of total aflatoxins (AFS – B1+B2+G1+G2), aflatoxin B1 (AFB1) and aflatoxin M1 (AFM1) were determined. Screening method for analysis was done using Neogen Veratox® testing kits with limits of detection of 0.250 mg/kg for total aflatoxins. For analysis of AFM1 it was used I 'screen AFLAM1 test kit (Tecna S. r. I., Trieste, Italy), with detection limit 0.25 µg/kg for aflatoxin M1 as well as with limit of 0.04 mg/kg for AFB1.

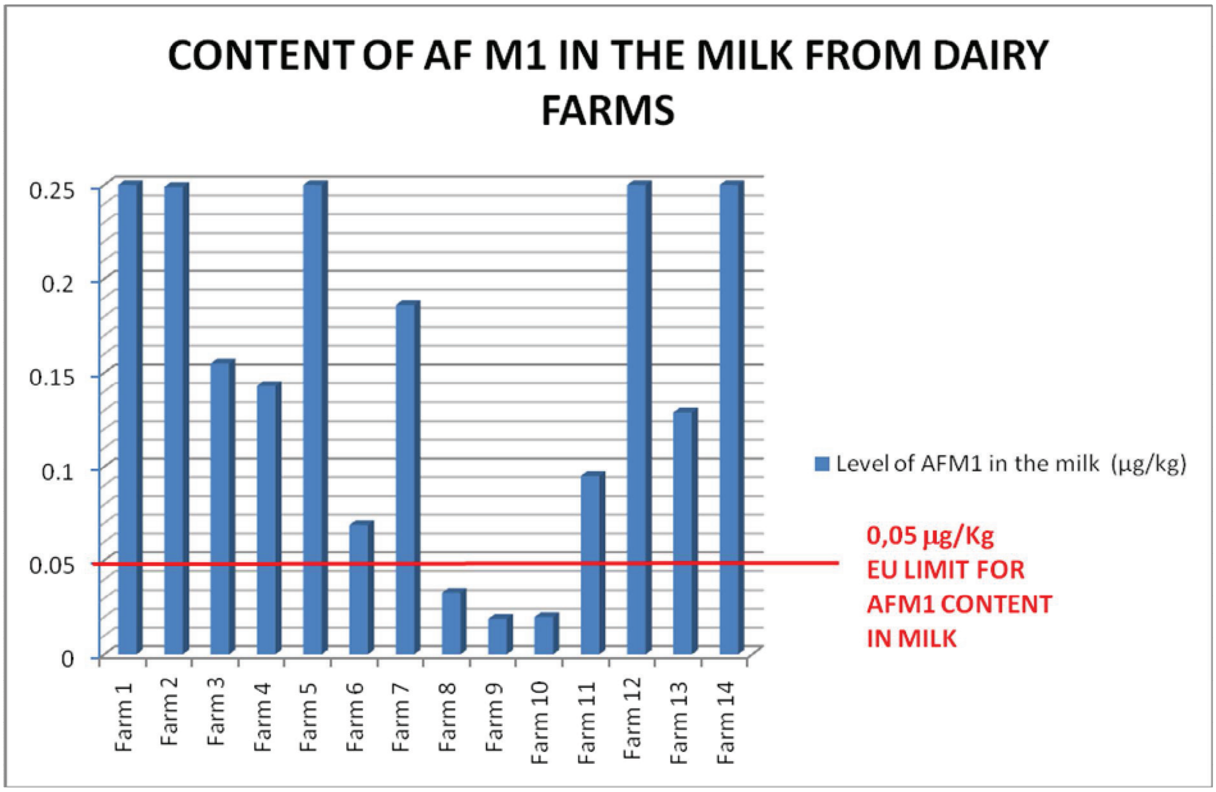
The test itself is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA). Free mycotoxins in the samples and controls are allowed to compete with enzyme-labeled mycotoxins (conjugates) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color. The test is read in a microwell reader (Thermolabsystem, Thermo, Finland) to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of mycotoxin.

RESULTS AND DISCUSSION

Fourteen samples of corn silage, ten samples of corn and eleven samples of mixed feed (total 35 samples) were analyzed for total aflatoxins and AFB1 level. Results of analyses are presented in the table 1.

Results showed that there was less contamination on the farms (Farm 4, 7, 10 and 12) that used complete feed mixes from industrial production then in other that used their own feed. In the half of analysed corn samples (50%) total AFs level exceeded permitted level according to regulation (0,050 mg/kg) and in the other 50% of corn samples level of total AFs was below limit. In the 14,3 % samples of corn silage, total AFs were above limit. The level of AFB1 is not regulated by Serbian low but according to EU regulation (EC 32/2002) 17.1% samples were contaminated in the higher level then permitted (0.020 mg/kg). Obtained results showed that level of AFB1 in 5 samples was out of range of used testing kit (> 0.040 mg/kg). Summarizing total samples contamination it can be concluded that 22.9% of samples could not be used in feeding of dairy cattle according to total AFs level as well as 34.2% samples can not be used for feeding of dairy cattle regarding to AFB1 level (according to EU regulation). Results of analyses of raw milk taken from the farms are shown on the graph 1.

Regarding the level of AFM1 in the raw milk, in only 3 samples level was below permitted value. In other samples it was found from 2 to 5 times more AFM1. Feedstuffs from farms number 8, 9 and 10 had acceptable levels of total AFs



Graph 1. **Results of AFM1 level in milk samples taken from dairy farms**

Table 1. **Results of analyzed samples taken from dairy farms**

		AFLATOXINS B1+B2+G1+G2 (mg/kg)	AFLATOXIN B1 (mg/kg)
Farm 1	Corn silage	0.023	0.001
	Corn	0.071	>0.040*
	Mixed feed	0.040	>0.040*
Farm 2	Corn silage	0.078	>0.040*
	Mixed feed	0.034	0.010
Farm 3	Mixed feed	0.042	0.002
	Corn	0.087	0.021
Farm 4	Corn	0,005	0.004
	Mixed feed	0,005	0.001
	Corn silage	0.018	0.010
Farm5	Corn	0.063	0.011
	Mixed feed	0.025	0.010
	Corn silage	0.031	0.020
Farm 6	Corn	0.008	0.005
	Mixed feed	0,005	0.002
	Corn silage	0.011	0.002
Farm 7	Corn	0,005	0.001
	Mixed feed	0,005	0.003
	Corn silage	0.065	>0.040*
Farm 8	Corn silage	0.011	0.002
	Corn silage	0.018	0.003
Farm 9	Corn silage	0,005	0.002
	Corn	0.039	0.020
Farm 10	Mixed feed	0.005	0.001
	Corn silage	0.026	0.020
Farm 11	Corn	0.058	>0.040*
	Corn silage	0.005	0.001
Farm 12	Mixed feed	0.005	0.003
	Corn silage	0.028	0.001
Farm 13	Corn	0.040	0.008
	Mixed feed	0.019	0.019
	Corn silage	0.005	0.002
Farm 14	Corn	0.054	0.040
	Mixed feed	0.015	0.012
	Corn silage	0.019	0.015

*results are out of range of testing kit

and AFB1. Also, it is noticed that in some farms (number 4, 6 and 12) even level of AFs were acceptable, presence of AFM1 was evident. It can be explained by previous consummation of feed, since we did not have information about AFs content in those feedstuffs. AFM1 can be detected in milk after 12–24 h of the first ingestion of AFB1. The occurrence of AFM1 in milk reaches maximum within two days after the intake of the contaminated feed. It disappears within 4–5 days after the withdrawal of the contaminated source. The amount of AFM1 has direct relationship with concentration of AFB1 in feed consumed by the animals (**Hussain et al.**, 2008).

CONCLUSIONS

The detected total AF contents in the dairy cow feed samples were below the limit of 0.050 mg/kg in more than 75% of samples. But, consenquently higher level of AFB1 in the feeds (according to EU regulation) contributed to the higher level of AFM1 in the raw milk, so detected AFM1 contents were higher than the legal limit in Serbia of 0.05 µg/kg. This fact showed that harmonization between Serbian and EU regulative is necessary. Missing of AFB1 level regulation in feed materials is a gap which has to be taken in consideration especially in feeding of dairy cattle since AFs have great and immediate influence on the raw milk. Permanent monitoring of feed materials is obligatory.

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Key words: *dairy farms, corn, silage, aflatoxins, milk, aflatoxin M1*

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