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A survey of seasonal variations of aflatoxin M1 in raw milk in Polog and Pelagonia region

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Abstract

In this paper the incidence of contamination of aflatoxin M1 (AFM1) in raw milk samples collected in Polog and Pelagonia region was investigated. Aflatoxin M1 (AFM1) analysis was carried out by using the competitive enzyme-linked immunosorbent assay (ELISA) technique. For that purpose raw milk samples were collected from the bulk tank in the period of three year. The highest incidence of samples exceeding the maximum residual limit (MRL) were observed during winter in both regions (0.135μg.kg^{-1}; 1.003μg.kg^{-1} respectively). Considering seasonal variability, this study showed that AFM1 incidence and levels in raw milk samples obtained in winter were significantly higher ($p< 0.05$) than those obtained in summer also there is a statistical significant difference between the samples obtained in spring than those obtained in summer ($p< 0.05$).

Key words: aflatoxin M1, raw milk, ELISA

Introduction

Fungal toxins are important contaminants that can be transferred to the milk. Aflatoxins are metabolic by products produced mainly by molds Aspergillus flavus and Aspergillus parasiticus (Tomasevic et al., 2015). There are four types of aflatoxins (B-1, B-2, G-1, G-2). AFM1 is a metabolite of AFB1 that could be found in milk when dairy cows are fed with contaminated feed (Jovaisiene, et al., 2014). According to Giovati et al. (2015), contamination of milk may be mitigated either directly or indirectly, directly by decreasing the AFM1 content in contaminated milk, or indirectly, decreasing AFB1 contamination in the feed of dairy animals. On the other hand contamination may occur either pre- or post-harvest and is more frequent in areas with a hot and humid climate (Giovati et al., 2015). The extent of transfer from feed to milk (carry-over) is influenced by various nutritional and physiological factors, including feeding regimens, rate of ingestion, rate of digestion, health of the animal, hepatic biotransformation capacity and actual milk production. About 0.3-6.2% of AFB1 consumed by dairy cattle is metabolized to AFM1 and ejected into the milk and the toxin can be detected in milk 12 - 24h after consuming AFB1 (Fink-Gremmels, 2008). This can cause a serious health problems, that's why AFM1 has been classified by the International Agency for Research on Cancer (IARC) as a group 2B toxin.

The maximum residue levels (MRL) of AMF1 in milk are set up in many countries and can vary significantly and these limits are not universal to all countries. The European Commission Regulation 1881/2006 sets a maximum limit of 0.05µg.kg^{-1} (raw milk, heat treated milk and milk for the manufacture of milk based products) and 0.025µg.kg^{-1} for adults and for infants, respectively (European Commission, 2006[a], 2006[b]). On the other hand, the USA has set an upper limit of 0.5µg.kg^{-1}, which is 10 times higher than the standards set by the EC.

In the Republic of Macedonia MRL of AMF1 are set according to the European Commission Regulation (Official Gazette 102/2013), but there are countries like Pakistan where Pakistan Standard and Quality Control Authority has set the maximum limit for aflatoxins in milk as 10µg.kg^{-1}, and no further limits have been set for AFM1 of milk products and other mycotoxins in milk and milk products (Aslam & Wynn, 2015). The situation is Serbia is different they set different limits of the period from 2011 till 2014 and now they are harmonize with EU regulations (Serbian Regulation, 2014).

The aim of this paper is to determine the impact of the season on the quality and safety of raw milk followed by the aflatoxin M1.

Material and Methods

This study was carried out to evaluate the quality and safety of raw cow milk in the dairy plant at Polog and Pelagonia region. The results were obtained during three years of examination on aflatoxin M1 in raw milk. A total of 360 samples, were analyzed during the period, including all seasons. The milk was processed in consumption milk, yoghurt, cheese and sour cream. For the research, 200mL of milk were collected in sterile plastic cups. The milk temperature was kept < 6°C at all time. Before collection, the milk was stirred by the agitator of the tank for 5min. Milk samples were taken to the laboratory immediately after collection and stored at -18°C until laboratory analysis.

Determination of AFM1 was done by Enzyme Linked Immunosorbent Assay (ELISA) method using standard validated commercial kit RIDASCREEN Aflatoxin M1 test kit (R-Biopharm, Darmstadt, Germany), with a limit of detection (LOD) of 5ng.L^{-1} for milk. The ELISA test procedure for the detection of AFM1 in raw milk was performed according to the manufacturer's instructions. All standards, controls, and samples were performed in duplicate on the 96-well plate, coated with anti-AFM1 antibodies. The Metertech Model 6+ Miniphotometer (Metertech Inc. Version 2.03, Taiwan) was used to measure the optical density at 450nm.

The obtained results reached were statistically processed in the usual variation and statistical methods in Microsoft Office Excel. The arithmetic mean value, mean ± standard deviation (SD), concentration range (min-max) were calculated and with a t test the statistical significance of the differences between the seasons was determined at the level of $p<0.05$ and the results were shown in tables.

Results and Discussion

Milk and dairy products are good sources of high-quality protein (Thorning et al., 2016). Consumption of aflatoxin-contaminated foods can potentially pose serious long-term health problems, especially in infants and in 7–12-year old children, one of the major groups of milk consumers, who are relatively sensitive to the effects of AFM1 (Ghiasian et al., 2007).

AFM1 occurrence and levels in raw milk samples are shown by seasons. The mean levels determined during four seasons were in the ranges (μg.kg^{-1}): winter 0.004±1.003; spring 0.003-0.04; summer 0.004-0.075; autumn 0.002-0.072 (Table 1). The highest total AFM1 mean concentration was (1.003μg.kg^{-1}) were measured in winter (December).

In 320 out of 360 samples (88.89%), the AFM1 level was under detectable limit (<0.001μg.kg^{-1}), 5 samples (1.39%) had levels ranging from 0.001-0.050μg. kg^{-1}and 35 samples (9,72%) exceeded the limit of 0.50 μg.kg^{-1} for AFM1 in milk (Table 2).

Considering seasonal variability, this study showed that AFM1 incidence and levels in raw milk samples obtained in winter were significantly higher ($p< 0.05$) than those obtained in summer also there is a statistical significant between the samples obtained in spring that those obtained in summer ($p< 0.05$) (Table 1). Also there is a statistical significance between the samples from both groups during the winter period ($p<0.05$) (Table 3). Compared with other studies from Macedonia Dimitrieska-Stojkovic et al. (2016) reported the significant increasing of the AFM1 concentration during the period August-November in 2013, exceeded the MRL values. During spring, there were no elevated AFM1 levels. In summer five sample were found with elevated concentration, this is linked with poor and inadequate storage conditions of feed for dairy cows (Bilandzic et al., 2015) also in this period of the year a large quantity of fresh animal feed, such as pasture and green fodder, are available for lactating animals (Tomasevic et al., 2015).

These findings are consistent with previous studies indicating seasonal trend in AFM1 contamination with higher occurrence and levels in cold seasons (Tomasevic et al., 2015; Bilandzic et al., 2015; Guo et al., 2016). Fallah et al. (2015) reported 3.5 times higher levels of AFM1 in winter compared to summer season. The increased level of AFM1 in cold seasons has been explained by the fact that due to using silage feedstuff during wintertime and having the ideal fungal development condition and consequently the concentration of AFB1 exceeded the standard level. Aflatoxin B1, is considered the only silage-associated mycotoxin of potential concern for the safety of milk and dairy products, due to its high carry-over rate into the milk as aflatoxin M1 and the high toxicity of this toxin (Driehuis, 2013).

Conclusions

The obtained results in this study showed increased AFM1 concentration in milk. Moreover, there is a seasonal trend, with a higher occurrence and levels of AFM1 in winter than in summer and this situation can lead to serious health problem of consumers. Furthermore, it can be concluded that elevated concentrations were found in 35 samples during the winter, summer, and autumn months, which was a result of using contaminated feed for dairy farms. The results further support the conclusion that continuous inspection and control of AFM1 in milk and dairy products, together with the control of AFB1 in raw material and supplementary feedstuffs for dairy cattle is necessary. Additionally, it has to include implementation of good agricultural practice in pre-harvest and post-harvest management of feedstuff for lactating cows.

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Table 1: The occurrence of AFM1 in raw cow milk during seasons at both regions (N=360)

Season	*Concentration (µg/kg)*	
	Range, µg.kg^{-1}	**Mean ± SD**
Winter	0.004-1.003	0.0664±0.0301^{a}
Spring	0.003-0.040	0.0063±0.0021^{b}
Summer	0.004-0.075	0.0146±0.0173^{a,c}
Autumn	0.002-0.072	0.0103±0.0138

*The difference in the values with different superscripts are statistically significant at the level of a:b; b:c $p<0.05$

Table 2: Results of analyzed cow raw milk for the presence of AFM1 in Polog and Pelagonia region (N=360)

Cow milk	**< 0.001 µg.kg^{-1}**	**0.001 - 0.05 µg.kg^{-1}**	**> 0.05 µg.kg^{-1}**
Samples (N=360)	320	5	35
Percent (%)	88.89	1.39	9,72

Table 3: The occurrence of AFM1 in raw cow milk during seasons at Polog and Pelagonia region (N=360)

Season	*Concentration (µg/kg)*	
	Polog region- range, µg.kg^{-1}	**Pelagonia region- range, µg.kg^{-1}**
Winter	0.004-0.135^{a}	0.030-1.003^{b}
Spring	0.003-0.009	0.009-0.04
Summer	0.004-0.075	0.008-0.064
Autumn	0.002-0.066	0.004-0.072

*The difference in the values with different superscripts are statistically significant at the level of a:b $p<0.05$