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DETERMINATION OF INCREASED LEVELS OF AFLATOXINS IN FEEDSTUFFS AND RAW MILK IN REPUBLIC OF MACEDONIA APPLYING SCREENING AND CONFIRMATORY METHODS

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Abstract

The frequency of occurrence of AFM1 in commercially available milk and dairy products during the Western Balkan Countries outbreak in 2013, led to an increased concern about the establishment of measures to control Aflatoxin B_1 contamination in feed, as well as Aflatoxin M1 in milk samples. In the light of these concerns, a comprehensive surveillance program for AFM1 in raw milk was established in Macedonia. The Aflatoxin M_1 contamination in 105 samples (2.9 %) was above the maximum allowed limit. The investigation of the feed sampled as follow-up revealed increased AFB1 presence with 13.4 % noncompliance. The sampling season is likely highly influencing the AFM1 content in raw milk samples.

Key words (bold): aflatoxin B_1, aflatoxin M_1, feed, milk, contamination, seasonal variations

1. INTRODUCTION

Since the beginning of the 21st century there are a numerous reports and modeling simulations by competent expert bodies, United Nations Institutions and published articles showing that climate changes affect significantly the agricultural production, diminishing thereby food safety as well as treating public health [1]. Drought stresses reduces the crop plants resistance and extreme precipitations and heat waves increases the possibility of growth of plant pathogenic moulds, producing the toxic metabolites – mycotoxins, both prior to and post harvest [2]. Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic fungal metabolites from Aspergillus found in foods and feeds. The attention to the presence of aflatoxins in feed is very important because of the possible contamination of the milk produced by animals fed with aflatoxin-contaminated feed. Aflatoxin M1 (AFM1), the hydroxylated metabolite of Aflatoxin B1 (AFB1), may be found in milk and milk products obtained from livestock that have ingested contaminated feed. Generally, it is deemed that approximately 1–6% AFB1 present in animal feed appears as AFM1 in milk, depending from animal to animal, season of milking and many other factors [3]. At the end of 2012 and beginning of 2013 the risk of mycotoxins was brought to public attention in Balkans and Central European countries following the European Commission Rapid Alert System for Food and Feed, where ten alerts for the presence of increased amounts of AFB1 in maize panel, have been reported [4]. Until 2013, the reported positive Aflatoxin M_1 samples from the Balkans region were only sporadic, with a negligible MRL exceeding [5-7]. Recently a few published papers have stressed the regional aflatoxin problem, mainly arising from the long-lasting drought in 2012 [8-10].

This paper presents the preliminary results from the survey on the presence of AFB1 in feedstuffs and AFM1 in raw milk from Republic of Macedonia, conducted during the 2013.

2. Materials and methods

2.1. Sample collection

This paper presents some of the results from the survey on AFB1 in feedstuffs and AFM1 in raw milk from Republic of Macedonia, conducted during the 2013. In total 117 samples of feedstuffs and 3099 samples of raw milk were tested for the presence of AFB1 and AFM1, respectively. The samples were collected within the survey of the Food and veterinary agency of Republic of Macedonia.

2.2. Methods of testing

The milk samples were tested applying the immunochemical screening method (ELISA kit – TECNA, Trieste - Italy). The positive samples exceeding the maximum residue level (MRL) of 0.050 μg/kg were confirmed with high performance liquid chromatography (HPLC) with fluorescence detection (FD) on Alliance system consisted of a 2695 separation module and 2475 multi-wavelenght fluorescence detector (Waters, Milford, MA, USA) [11]. Feed samples were tested for aflatoxin B_1 applying the HPLC-FD method on Perkin Elmer Series 200 pump and LC 240 fluorescence detector (Norwalk, Connecticut, USA) [12]. Testing methods were validated and confirmed to be sensitive, selective, accurate and precise according to the requirements of Commission Regulation 401/2006/EC [13].

2.2. Statistical analysis

Statistical calculations for the between month and between season variations of AFM1 concentration were performed applying the IBM SPSS Statistics for Windows, version 2.2. (IBM Corp., Chicago, USA). To evaluate differences in the means between two series of samples t-test for independent samples has been applied. The differences between the values were considered significant at probability level (P) ≤0.05.

3. Results and discussion

3.1. Aflatoxin B_1 testing

During the survey period, a total of 117 dairy cow feed samples were tested for AFB1 presence applying the validated HPLC-FD method. The results from detection of the aflatoxin B_1 are presented at Figure 1. 29.9 % of the total feed samples were found containing AFB1 over the detection limit of the applied method. Among them, in 11 samples (9.4 %) the established MRL values have been exceeded [14].

Predominantly, the non-compliances have been revealed at complementary dairy cows feed samples (22.6 %) with obtained concentration range from 0.005 μg/kg up to 51.4 μg/kg. Regarding the maize panel samples, the AFB1 prevalence was 17.2 %, with four detected MRL exceeding. The concentration range was from 0.005 up to 75.0 μg/kg. Furthermore, the mean level of AFB1 in complementary feed samples was significantly higher (P<0.05) than that of maize panel samples. The AFB1 contamination in raw feed (silage) was negligible.

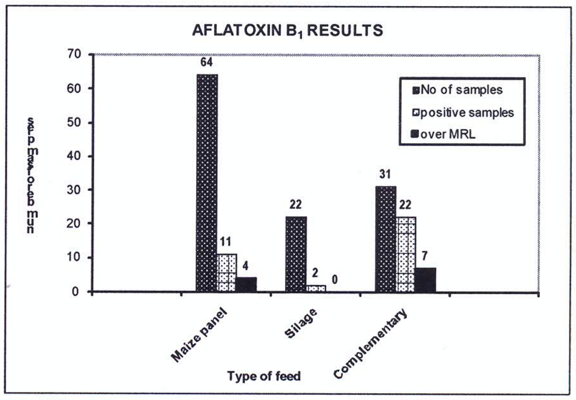

Fig. 1 Aflatoxin B1 results in feed

3.2. Aflatoxin M1 testing

For better comprehension of the AFM1 distribution during the whole year, the monthly occurrence and concentrations in the surveillance period from February 2013 until January 2014 are presented on Figure 2. After the initial incidence of 5.4 % and 2.9 % in February and March, respectively, slight decrease in the number of non-compliant samples was observed in the period from April to July, when the EU MRL exceeding [14] dropped to less than 2 %. The long lasting drought during the second half of 2013, and thus the lack of fresh feed caused significant increasing of the AFM1 concentrations during the period August-November. Therefore, the MRL has been exceeded in 3.7 %, 5.5 %, 6.7 % and 4.6 % of the tested samples for August, September, October and November, respectively. During this period, the AFM1 contamination levels were determined in the range from 6.6 to 408.4 ng/kg. The highest positive mean (34.8 ng/kg) and the highest level of AFM1 (408.4 ng/kg) were measured in October, with MRL exceeding in 6.7 % of the samples tested.

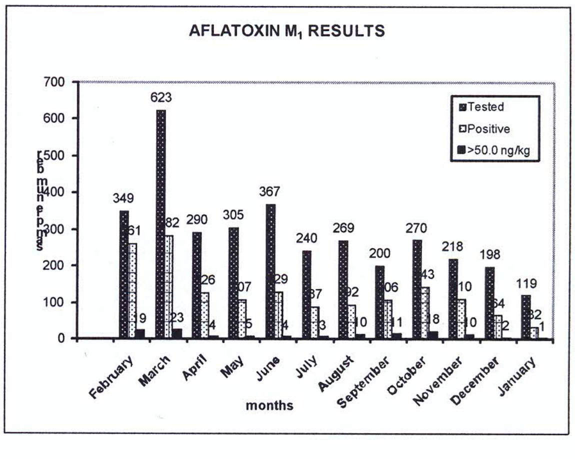

Fig. 2 Aflatoxin M1 results by months

Nevertheless, in this study, AFM1 was detected in 42.4 % of the samples which is considered highly critical for AFM1 risk assessment, as trends in contamination can be timely identified and may act as alert to milk producers before exceeding the maximum tolerable level. The significant decreasing of the positivity during December and January, 32.3 % and 26.9 %, was likely due to the stricter official controls, increased farmer awareness, and more intensive self controls in the dairy industry. Moreover, it is important to stress that in this period, the non-compliancy was significantly reduced to less than 1 %.

Elevated AFM1 concentrations were detected in two reports for cow's milk from Serbia [9], where concentrations ranged from 0.01 to 1.2 μg/kg in 98.7 % of milk samples, and 0.01 to 1.44 μg/kg with 76 % positivity [10]. In the investigations conducted in Croatia during 2013, the determined concentration ranged from LOD up to 1135 ng/kg and 183.5 ng/kg, for raw and UHT milk, respectively [8]. These investigations were fully in line with the assumption that the whole region of Western Balkan countries, including Macedonia, was highly affected by the usage of contaminated feed with AFB1. The comparison among these countries is possible, apart from the different analytical procedures applied, due to the very similar climatic conditions, storage conditions, the sampling period and very likely same origin and similar AFB1 contamination of feed.

However, other studies mainly performed in Mediterranean or Asian countries, show enhanced occurrence of AFM1 in milk and dairy products, whereas in Sardinia the incidence was 31.5% [15], 16.6% in Italy [16], 100% in Iran [17], mainly attributed to the favorable climatic conditions for AFB1 production and the way of feed storage. According to the presented reports, the occurrence of Aflatoxin M1 is a global problem in regions affected by dry climate, or with seasons of long drought periods that favor the mould development. It can be concluded that the elevated Aflatoxin B1 levels in feed is a consequence of climatic and technical conditions.

Regarding the fact that dairy products are the most potent source of aflatoxins, countries with week system of feed control should increase the frequency of feed and milk samples, and should increase the level of breeder's education about the harmful effects of aflatoxins to feedings.

3.3. Seasonal variations of AFM1 in raw milk

The sampling season is likely highly influencing the AFM1 content in raw milk samples. The present study revealed that 4.2 % of raw milk samples from the autumn-winter season exceeded as compared to only 1.8 % of spring-summer samples regarding the EU MRL value as showed in Table 1. The significant difference in the mean values has been confirmed by a suitable statistical test at confidence level $P \leq 0.05$.

Many previously reported incidences indicate that AFM1 contamination in raw milk is season-dependant [8, 18, 19] which is very probably due to the seasonal variation in the type and quality of feed given to dairy cows. Recently published study from Brazil [20] reported significantly lower AFM1 concentrations in raw milk during the rainy season, than in the drought periods. This confirms the influence of microclimate variability on the AFM1 contamination.

Table 1. Between season variations of AFM1 concentration

Testing period	Tested (*n*)	Positive[a] *n* (%)[b]	>50.0 ng/kg *n* (%)	Average ± SD ng/kg
Spring-summer	1271	474 (37.3)	26 (2.0)	10.1±16.5
Autumn – Winter	1828	1091 (59.7)	84 (4.6)	16.8±23.1

[a]over LOD of the ELISA method
[b]the number in parenthesis is incidence expressed in %

Some authors [21] suggest that the biggest risk with respect to mycotoxins from climate change will be found in developed countries with temperate climates (e.g. parts of Europe and the United States of America, etc.). The climate of these regions will become warmer reaching temperatures of 33 °C, close to the optimal for aflatoxin production. This may be the case with the crops, especially so if recognised aflatoxin-susceptible plants (e.g. maize) are grown increasingly to exploit the new conditions.

4. CONCLUSION

The present study is reporting the results from the survey investigating the presence of AFM1 in raw milk samples collected from dairy production facilities in Macedonia, in the period February 2013 - January 2014, as well as Aflatoxin B_1 presence in lactating cows feed. The contamination in 105 samples (2.9 %) was above the maximum allowed limit. The investigation of the feed sampled as follow-up revealed increased AFB1 presence with 13.4 % noncompliance. In summary, our results indicate that AFB1 in imported corn for dairy cattle is a major contributor to AFM1 content in raw milk. To ensure the safety of milk for human health it is extremely important to avoid providing feed contaminated with AFB1 to cows. Hence, regular monitoring of not only the AFM1 level in milk, but also the AFB1 level in feed, will be required to protect the public, especially infants and young children, against AFM1 toxicity. Future study could possibly focus on risk assessment of AFM1 in milk and dairy products.

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