OCCURRENCE OF AFLATOXIN M1 IN ALBANIAN RAW MILK DETECTED BY ELISA TECHNIQUE

Fatmira SHEHU

Department of Veterinary Public Health , Faculty of Veterinary Medicine, Agricultural University of Tirana, s_fatmira@yahoo.it

Enkela ZANI

Food Safety and Veterinary Institut, Tirana, Albania, enkela.zani@yahoo.com

Suzana KOLA

Food Safety and Veterinary Institut, Tirana, Albania, kolasuzana@hotmail.com

Ermira MARKU

Food Safety and Veterinary Institut, Tirana, Albania, elmiramarku@hotmail.com

Bizena BIJO

Department of Veterinary Public Health , Faculty of Veterinary Medicine, Agricultural University of Tirana , bizena.bijo@yahoo.com

ABSTRACT

AFM1 (Aflatoxin M1) is the major mycotoxin frequently found in milk and dairy products. Tolerance limits for aflatoxins in dairy products have been established internationally. According to the requirements of EU Regulation (EC) No.401 / 2006 the maximum residue level (EU MRL) of AFM1 is 50 ng/kg for milk and dairy products. The purpose of this study was to detect the occurrence and determinate the presence of AFM1 milk In Albania, there are scarce published data about AFM1 level in dairy products and none of the consumer exposure to this toxin. A total of 180 milk samples were collected in January-December 2016, from 13 small farms of Myzeqeja region in Albania. Samples were analyzed for the presence of AFM1 content using competitive ELISA (RIDASCREEN R-biopharm test kit). The competitive enzyme immunoassay- Ridascreen method highlighted the contamination with AFM1 in 98/180 milk samples. Beside to the high number of samples we detected huge level of concentration. AFM1 levels oscillated from 3.25 - 131 ng / L, where one of the most important factor affected was considered the season of the year. Results demonstrated a significant ($p < 0.05$) difference of the AFM1 content in milk samples between autumn and spring seasons. Milk samples collected in February and March presented a high positivity of and the highest AFM1 level (131 ng / L).

Key words: raw milk, aflatoxin M_1, ELISA, public health

Introduction

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on both humans and animals. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer, and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products (Castegnaro M et al 1998)

Approximately 18 aflatoxins have been identified. Among them, aflatoxin B1 (AFB1) is a highly toxic, mutagenic, teratogenic and carcinogenic compound that causes DNA damage, gene mutation, chromosomal anomalies and cell transformation, and therefore has been classified as a class I human

carcinogen (carcinogenic) (IARC, 1993, 2002). About 0.3–6.2% of AFB1 in animal feed is transformed to AFM1 in milk (Whitlow, L. W., et al 2002). Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country (Creppy E. E 2002).

Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with aflatoxin B1. 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 could 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 (Decastelli, L., et al 2007). Many countries have established regulations to control the levels of aflatoxin B1 in feeds and to have maximum permissible levels of aflatoxin M1 in milk to reduce this hazard (Bilandzic et al 2010).

Considering that milk and milk derivatives are consumed daily and, moreover, that they are of primary importance in the diet of children, most countries have set up maximum admissible levels of AFB1 in feed (European Commission, EC, 2003a) and of AFM1 in milk, which vary from the 50 ng/kg established by the EU, to the 500 ng/kg established by US FDA (EC, 2003b, U.S. Food and Drug Administration, FDA, 2011). More restrictive MRLs have been implemented by the EU for the presence of AFM1 in baby food (EC, 2004)

Climatic conditions regarding temperature and moisture in tropical and subtropical regions favour the growth of the toxigenic Aspergillus (Picinin et al., 2013). Also, long periods of high temperatures andlong-lastingdrought insummerin other climatic regions, e.g. the northern temperature zone, may also favour the development of mycotoxins (Bilandzic N., et al., 2010, Decastelli et al., 2007). Respectively, variations of AFM1 levels have been determined during different seasons, with higher concentrations in winter than in summer (Iqbalet S.Z et al 2013, Tajkarimi et al., 2008).

Several methods for aflatoxin M1 determination have been developed, including high performance liquid chromatography associated with fluorescence or mass spectrometric detection. Immunochemical methods have also been described and are employed as screening methods in routine analysis, mainly because of their simplicity and rapidity (Gallo, P.,et al 2006, Gilbert, J., et al 2002, Magliulo, M., et al 2005, Muscarella, M., et al 2007)

Some studies have been done about aflatoxin M1 contamination in raw milk in the world and their results have presented exceeded concentrations regarding the European Community and Codex Alimentarius regulatory limit (Codex Alimentarius 2001, Creppy E. E 2002, Montagna, M.T., et al 2008). The aim of this study was to investigate the presence of AFM1 in raw milk samples produced in Myzeqeja region in Albania by ELISA method.

Materials and Methods

Preparation of samples

A total of 180 milk samples were collected in January-December 2016, from 13 small farms of the Myzeqeja region of Albania. All Samples defatted through cooling Centrifuge for 10 min, 3500g at 4°C. The upper fat layer was removed. 100 µl (per well) of this solution was used in the test (R-Biopharm GmbH, Darmstadt, Germany 2015,)

ELISA test procedure

The quantitative analysis of AFM1 in raw milk samples was performed competitive enzyme immunoassay based on antigen-antibody reaction (ELISA RIDASCREEN AFM1, R-Biopharm). The procedure as described by R-biopharm GmbH (R-Biopharm GmbH, Darmstadt, Germany 2015).Before starting the test, the reagents were brought up to room temperature. After centrifugation, the upper creamy layer was completely removed by aspirating through a Pasteur pipette and from the lower phase (defatted phase), 100 µL was directly used per well in the test.

The AFM1 standards and test samples (100 µl per well) in duplicate were added to the wells of a micro-titer plate pre-coated with antibodies for AFM_1 and incubated at room temperature in dark for 60 min. After the washing step, 100µl of peroxidase conjugate was added to the wells and plate was incubated again for 60 min at room temperature in dark. After the three washing step, the unbound conjugate was removed during washing. Subsequently, 50 µl each substrate (urea peroxide) and chromogen (tetramethyl-benzidine) were added to the wells and incubated for 30 min in dark. Finally, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with ELISA plate reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations.

Evaluation of AFM1

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standards) and multiplied by 100 (percentage maximum absorbance). Therefore, the zero standard is thus made equal to 100%, and the absorbance values are quoted in percentages.

Results and Discussion

A total of 180 raw milk samples was analyzed with competitive ELISA. Analytical results showed that the incidence of AFM1 contamination in raw milk samples was high. 54,4% of samples were contaminated with AFM1, the toxin concentration was higher than the maximum tolerance limit (50 ppt) accepted by European Union and Codex Alimentarius Commission. The occurrence of AFM1 was shown in Table 1.

Table 1. Occurrence of AFM1 in raw milk samples from Myzeqeja region-Albania

AFM_1 levels ng/l	Sample No.	(%)	Range
Not detected	82	-	-
< 10	7	3,9	3.5- 9.7
11-25	17	9,4	11.2- 21.5
26-50	20	11.1	27.5-48
≥ 50	54	30	51.2- 131
Total samples	180	54.4	2.1- 131

The AFM1 contamination levels, as seen, are shown at a large number of samples. Also, they had high levels of concentration. The aflatoxin M1 contamination levels were between 3.25 - 131 ng / L. Besides, factor affecting the level of AFM1, such as seasonal effect, is studied. Results showed a significant ($p < 0.05$) difference in the AFM1 content in milk samples between fall and spring seasons. Milk samples collected in February and March presented a high positivity and the highest AFM1 level (131 ng / L).

Conclusion

This study shows the importance of continuous aflatoxin level monitoring in animal feed and the necessary implementation of strict regulations for mycotoxins in Albania. The results indicated that the contamination of the dairy products in such a level could be a serious public health problem at the moment. According to these values, it should be expected Albanian population is exposed to a significant risk from aflatoxin M1 including average and high consumers. Therefore, it is important to set the effective control of raw milk and dairy products in accordance with the defined maximum residue levels set by the European Union (EU).

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