



# The risks associated with aflatoxins M1 occurrence in Lebanese dairy products



Hussein F. Hassan<sup>a</sup>, Zeina Kassaify<sup>b,\*</sup>

<sup>a</sup> Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Beirut, Lebanon

<sup>b</sup> Department of Nutrition & Food Sciences, Faculty of Agricultural & Food Sciences, American University of Beirut, Beirut, Lebanon

## ARTICLE INFO

### Article history:

Received 21 February 2013

Received in revised form

23 July 2013

Accepted 20 August 2013

### Keywords:

Mycotoxins

AFM1

ELISA

Milk

Cheese

Lebanon

## ABSTRACT

Aflatoxins are potent carcinogens, teratogens and mutagens. When lactating ruminants are fed feedstuffs contaminated with aflatoxins B<sub>1</sub> (AFB<sub>1</sub>), this mycotoxin gets hydroxylated to aflatoxins M<sub>1</sub> (AFM<sub>1</sub>) and appears in excreted milk. Tolerance limits for aflatoxins in dairy products have been established internationally. In Lebanon, there are scarce published data on the level of AFM<sub>1</sub> in dairy products and none on the consumer exposure to this toxin. A total of 524 samples of milk and dairy products characterized by their distinct processing methods have been collected from the Bekaa region of Lebanon. Samples were analyzed for their AFM<sub>1</sub> content using competitive ELISA (RIDASCREEN r-biopharm test kit). The exposure level of the Lebanese population to AFM<sub>1</sub> was also assessed through food frequency questionnaires. Besides, factors affecting the level of AFM<sub>1</sub>, such as milk source, pasteurization, seasonal and processing effects, were studied. Results showed a significant ( $p < 0.05$ ) difference in the AFM<sub>1</sub> content in dairy products between fall ( $25.16 \pm 1.97$  ng/L) and spring ( $40.28 \pm 1.97$  ng/L) seasons. Significant ( $p < 0.05$ ) difference existed as well between milk sources (sheep:  $2.72 \pm 0.09$  ng/L, goat:  $5.70 \pm 0.15$  ng/L, cow:  $22.18 \pm 5.8$  ng/L) and between the different assessed dairy products. No significant ( $p > 0.05$ ) difference was found between raw ( $10.74 \pm 2.01$  ng/L) and pasteurized milk samples ( $9.65 \pm 2.01$  ng/L). 21% of dairy products tested were contaminated with AFM<sub>1</sub> above the EU limits (50 ng/L). Lebanese population daily exposure to AFM<sub>1</sub> through consumption of dairy products was estimated to be 9.22 ng/L per person.

© 2013 Elsevier Ltd. All rights reserved.

## 1. Introduction

Aflatoxins are extremely toxic mycotoxins produced by the metabolism of common fungi *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. Under favorable conditions of temperature and humidity, they can be produced during any stage of production including harvesting, storage, transport, and processing. Aflatoxins are found in various plant products including peanuts, copra and soya or in cereals such as maize, rice and wheat. They are both acutely and chronically toxic for animals and humans. Aflatoxins are immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (Kocabas & Sekerel, 2003; Piva, Galvano, Pietri, & Piva, 1995; Peraica, Radic, Lucic, & Pavlovic, 1999). Worldwide, there has been major attention

towards the presence of organic contaminants. Aflatoxins are a class of compounds of great interest, since they can reach the consumer's diet through animal feed, and hence through the animals' final food products (Gallo, Salzillo, & Rossini, 2006; Hussein & Brasel, 2001; Khayoon, Saad, Lee, & Salleh, 2012).

When animals eat foodstuffs containing aflatoxins B<sub>1</sub> (AFB<sub>1</sub>), these toxins will be metabolized and excreted as aflatoxins M<sub>1</sub> (AFM<sub>1</sub>) in milk and this is the only route for transformation of AFB<sub>1</sub> to AFM<sub>1</sub> (Bakirci, 2001; Lopez, Ramos, Ramadan, & Bulacio, 2003). Bakirci (2001) and Creppy (2002) reported that 0.3–6.2% of AFB<sub>1</sub> in ingested animal feed is transformed to AFM<sub>1</sub> in excreted milk. AFM<sub>1</sub> is detected in the milk 12–24 h after the first ingestion of AFB<sub>1</sub> (Pittet, 1998); however, Battacone et al. (2003) reported that AFM<sub>1</sub> is detected in sheep's milk within 6 h after consumption of contaminated feed. Researchers reported a higher incidence of AFM<sub>1</sub> contamination during cold seasons when livestock are fed with animal feed compared to hot seasons when animals consume grass (Bachner, Martlbauer, & Terblan, 1998; Decastelli, Manco, & Sezian, 2006; Garrido, Iha, Santos Ortolani, & Duarte Favaro, 2003; Lopez et al., 2003).

\* Corresponding author. Tel.: +961 1 350000x4456, +961 3 290165; fax: +961 1 744460.

E-mail addresses: [zk18@aub.edu.lb](mailto:zk18@aub.edu.lb), [zeina.kassaify@aub.edu.lb](mailto:zeina.kassaify@aub.edu.lb) (Z. Kassaify).

**Table 1**  
Number of dairy products during the months of September, October, March and April.

Month	Dairy product	# Of samples
September	Raw Milk – Cow	15
	Pasteurized Milk – Cow	15
	Akkawi (cow)	16
	Halloum (cow)	16
	Karishe (cow)	17
	Shanklish (cow)	16
	Yogurt (cow)	17
	Kashta (cow)	15
October	Raw Milk – Cow	15
	Pasteurized Milk – Cow	15
	Akkawi (cow)	16
	Halloum (cow)	16
	Karishe (cow)	17
	Shanklish (cow)	16
	Yogurt (cow)	17
	Kashta (cow)	15
March	Raw Milk – Cow	15
	Pasteurized Milk – Cow	15
	Akkawi (cow)	16
	Halloum (cow)	16
	Karishe (cow)	17
	Shanklish (cow)	16
	Yogurt (cow)	17
	Kashta (cow)	15
April	Raw Milk – Cow	15
	Pasteurized Milk – Cow	15
	Raw Milk – Goat	4
	Pasteurized Milk – Goat	4
	Raw Milk – Sheep	4
	Pasteurized Milk – Sheep	4
	Akkawi (cow)	16
	Halloum (cow)	16
	Karishe (cow)	17
	Shanklish (cow)	16
	Yogurt (cow)	17
Kashta (cow)	15	

Milk and dairy products are major sources of nutrients for humans, especially children; however, they showed a potential to introduce health hazards, such as AFM1, from foods of animal origin into the human diet (Akkaya, Birdane, Oguz, & Cemek, 2006). When cheese is made from AFM1 contaminated milk, the toxin can be carried over into curd (Colak, 2007; Deveci, 2007; Kamkar, Karim, Aliabadi, & Khaksar, 2008; Manetta et al., 2009; Motawee & McMahan, 2009). Due to the insolubility of the AFM1 in butter and its absorbability in curd during production of milk products, the level of the AFM1 shows differences according to the properties of the dairy product components, extraction technique, processing method, type and degree of milk contamination and differences in milk quality (Blanco et al., 1988). For instance, it was found that AFM1 concentration ranges from 2.5 to 3.3 and 3.9–5.8 times higher in soft and hard cheeses, respectively, compared to milk (Yousef & Marth, 1989). AFM1 is resistant to thermal inactivation, pasteurization, autoclaving, cold storage, freezing, fermentation, concentration and drying (Deshpande, 2002; Gelosa & Buzzetti, 1994; Galvano, Galofaro, & Galvano, 1996; Park, 2002).

The European Community and Codex Alimentarius established a maximum level of AFM1 in liquid milk of 50 ng/L (Codex Alimentarius Commissions, 2001). On the other hand, according to US regulations, the limit of AFM1 in milk should not be higher than 500 ng/L (Stoloff, Park, & Van Egmond, 1991). The tolerance limits for aflatoxins in milk are established by competent national authorities. Regulatory limits throughout the world are influenced by considering each country's conditions, and may vary from one country to another (Van Egmond, 1989; Stoloff et al., 1991).

Routine laboratory analysis for AFM1 is performed by screening methods, such as Enzyme-Linked Immunosorbent Assay (ELISA) and lateral flow strips, and by confirmatory methods, such as High-performance liquid chromatography (HPLC) with fluorescence detection (Gallo et al., 2006). Monitoring programs are currently the main strategy to diminish exposure risk to aflatoxins for both animals and humans (Lopez et al., 2003). Mycotoxin detoxification processes of human food are still not efficient in terms of food safety, nutritional elements retention as well as cost (Piva et al., 1995).

The consumption of milk and dairy products is widespread in Lebanon; however, there are scarce surveys done on AFM1 content in these products (ElKhoury, Atoui, & Yaghi, 2011; Elkak, El Atat, & Abbas, 2011; Elkak, El Atat, Habib, & Abbas, 2012) and no existing data on the exposure of the Lebanese population to this toxin. The aim of this study was to estimate the risk, assess the consumer exposure and determine the prevalence of AFM1 contamination in milk and selected dairy products.

## 2. Materials and methods

### 2.1. Samples

A total of 524 samples of milk from different sources (sheep, goat and cow) and various kinds of processed dairy products made from cow milk (kashta, karishe, shanklish, yoghurt, akkawi, and halloum) have been collected from 15 small or medium farms and manufacturers in the Bekaa<sup>1</sup> region of Lebanon. Detailed information about the number, types, sources and periods of collection of the samples are summarized in Table 1. Samples were transported on ice in an ice box to the laboratory where they were kept in the refrigerator at 4 °C and analyzed for their AFM1 content within 24 h. To study the effect of seasonal change and pasteurization on the AFM1 content, 120 samples of cow's raw and pasteurized milk were randomly collected during September and October, as well as during March and April. During the same period, 388 samples distributed between kashta, karishe, shanklish, yoghurt, akkawi and halloum cheeses were collected to evaluate the processing effects on the levels of AFM1 contamination in dairy products. In order to evaluate the relationship between AFM1 and milk source, 4 raw sheep milk, 4 pasteurized sheep milk, 4 raw goat milk and 4 pasteurized goat milk samples were randomly collected during last week of April.

### 2.2. Testing procedure

The Determination of AFM1 has been based on an Enzyme-Linked Immunoassay (ELISA) using the RIDASCREEN test kit (R-biopharm, Germany, Product No: R1101). This method is quick, reliable and cost effective for the estimation of AFM1 and has been included in the official collection of test procedures by the German Federal Board of Health. This test kit is sufficient for 96 determinations (including the calibration curve). The basis of the test is the antigen–antibody reaction. The mean lower detection limit of RIDASCREEN® AFM1 test is 5 ng/L for milk and 50 ng/L for cheese (Anonymous, 1999).

Milk samples were centrifuged 10 min/3500 rpm/10 °C. After centrifugation, the upper cream layer was completely removed using a Pasteur pipette, the remaining skimmed milk was used directly in the test (100 µL per well). A representative cheese

<sup>1</sup> Bekaa region is located in Eastern Lebanon where ~75% of Lebanese dairy factories are located as per the statistics of the Ministry of Agriculture in Lebanon.

sample was macerated coarsely (Halloum, Akkawi and Shanklish) and thoroughly mixed.

Dichloromethane (40 ml) was added to the triturated cheese sample (2 g) in a screw-top glass vial and shaken for 15 min. The suspension was filtered and 10 ml of the filtrate dried at 60 °C under nitrogen. The oily residue was redissolved in 0.5 ml methanol, 0.5 ml PBS buffer and 1 ml heptane, and then mixed thoroughly and centrifuged at 2700 g for 15 min. The upper heptane layer was completely collected. Aliquot of the lower methanolic-aqueous phase was poured off carefully using a Pasteur pipette. 100 µL of this aliquot was brought up to a 10% methanol concentration by addition of 400 µL of buffer 1 and 100 µL was used per well in the test (Anonymous, 1999; Sarmehmetoglu, Kuplulu, & Cleik, 2004).

Sample solutions of 100 µL were added to the wells to occupy the binding sites proportionately then mixed gently and incubated for 60 min at room temperature (20–25 °C) in the dark. Then, the liquid was poured out of the wells and the wells were filled with 250 µL washing buffer and poured out the liquid again. This washing step was repeated twice. In the next stage, 100 µL of enzyme conjugate were added to occupy the remaining free binding sites and in washing step 250 µL of washing buffer washed any unbound enzyme conjugates. Then, 50 µL of enzyme substrate and 50 µL of chromogen were added to the wells and incubated for 30 min at room temperature in the dark. Bond enzyme conjugate converted the chromogen into a blue product, and then 100 µL of stop solution was added to each well, which leads to yellow discoloration of chromogen. The measurement of AFM1 was done photometrically at 450 nm against air blank within 60 min (Anonymous, 1999).

The mean values of the absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100. The zero standard was thus made equal to 100% and the absorbance values were quoted in percentages. The values calculated for the standards were entered in a system of coordinates on semi logarithmic graph paper against the AFM1 concentration in ng/L. The AFM1 concentration in ng/L corresponding to the extinction of each sample can be read from the calibration curve. In order to obtain the AFM1 concentration in ng/L, the concentration read from the calibration curve was multiplied by the corresponding dilution factor (1 for milk and 10 for cheese) (Anonymous, 1999).

### 2.3. Level of Lebanese population exposure to AFM1

A random sample of 200 individuals of different educational background, living area and age group was selected to participate in the study during the period of March and April. A AA food frequency questionnaire was prepared to obtain information about their socioeconomic status as well as the consumption rate/trend of some highly consumed dairy products including fresh milk, halloum, akkawi, karishe, shanklish, yogurt and kashta. The questionnaire was prepared in Arabic and validated. The mean daily intake of each dairy product concluded from the questionnaire was multiplied by the average respective AFM1 content reported in our study, then the values were summed up to reveal the level of exposure of the Lebanese population to AFM1.

### 2.4. Statistical analysis

Statistical analysis was conducted using Statistical Package for the Social Science (SPSS version 14) (SPSS version 8.0 for Microsoft Windows; SPSS, Chicago, IL). The extract of each dairy product in this study was tested in duplicate in the r-biopharm testing kit. Results were expressed as mean values ± standard error. Analysis of

**Table 2**

AFM1 (ng/L) in raw and pasteurized milk samples from different sources during April.

Milk source	Milk type	AFM1 (ng/L) mean ± SE <sup>a</sup>
Sheep <sup>x</sup>	Raw <sup>b</sup>	3.33 ± 0.09
	Pasteurized <sup>b</sup>	2.10 ± 0.09
	Total	2.72 ± 0.09
Goat <sup>x</sup>	Raw <sup>b</sup>	6.85 ± 0.15
	Pasteurized <sup>b</sup>	4.55 ± 0.15
	Total	5.70 ± 0.15
Cow <sup>y</sup>	Raw <sup>b</sup>	22.05 ± 5.80
	Pasteurized <sup>c</sup>	22.30 ± 5.80
	Total	22.18 ± 5.80

<sup>xy</sup>Milk sources followed by different superscripts are significantly different at  $P < 0.05$ .

<sup>a</sup> Standard error.

<sup>b</sup>  $N = 4$ .

<sup>c</sup>  $N = 15$ .

variance (ANOVA) was conducted to determine differences in AFM1 content among milk sources, dairy products and seasons. ANOVA was also used in the calculation of the Lebanese population exposure level to AFM1. The randomized complete block design (RCBD) was used in all experimental designs. A probability of  $<0.05$  was considered significant. In case of significant difference, Tuckey and Duncan's were used in the analysis.

## 3. Results and discussion

AFM1 content in raw and pasteurized milk from three sources (sheep, goat and cow) is presented in Table 2. The total mean of AFM1 content in the raw milk samples from the three sources was  $10.74 \pm 2.01$  ng/L, compared to  $9.65 \pm 2.01$  ng/L in the pasteurized milk samples. There was no significant ( $p > 0.05$ ) difference between raw and pasteurized milk samples. This conforms to the findings of previous studies which reported no effect of pasteurization on the AFM1 levels in milk due to its heat stability (Galvano et al., 1996; Gelosa & Buzzetti, 1994). AFM1 levels in sheep's milk (raw and pasteurized) samples reached  $2.72 \pm 0.09$  ng/L, compared to  $5.70 \pm 0.15$  (SE) ng/L for goat's milk (raw and pasteurized), and  $22.18 \pm 5.8$  ng/L for cow's milk (raw and pasteurized). There was no significant ( $p > 0.05$ ) difference in AFM1 content between sheep and goat's milk; whereas, there was a significant ( $p < 0.05$ ) difference between sheep and cow's milk, and between goat and cow's milk. This observed difference may be due to the fact that sheep and goats in Bekaa area tend to consume grass in April; whereas, cows consume concentrated feed during that period of year. Since animal feed is contaminated with AFB1 at higher levels than grass (Bachner et al., 1998; Blanco et al., 1988; Decastelli et al., 2006; Garrido et al., 2003; Lopez et al., 2003), the mean AFM1 in cow's milk was found ~ five folds more than the mean AFM1 in sheep and goat's milk. In addition, Battacone et al. (2003) reported that the carry-over ratio (AFM1 excreted in milk/AFB1 ingested) is lower in sheep than in cow, which explains the lower AFM1 levels in sheep's milk.

Table 3 shows the AFM1 content in different dairy products collected during September, October, March and April. Overall mean AFM1 content was 25.6, 24.8, 41.5 and 31.1 ng/L respectively. The highest contamination level was observed in akkawi, followed by shanklish, kashta, halloum, karishe and yogurt. Percentages of samples of various dairy products contaminated with AFM1 during the months of September, October, March and April are presented in Table 4. During the fall season, 224 (88.2%) of overall dairy products samples had an AFM1 contamination level below the LOD of the kit (5 ng/L for milk and 50 ng/L for cheese); whereas, 30 (11.8%) of the samples have exceeded EU limits ( $>50$  ng/L). The total mean of AFM1

**Table 3**  
AFM1 content in different dairy products during months of September, October, March and April.

Month	Dairy product	AFM1 (ng/kg) mean ± SE <sup>a</sup>
September <sup>x</sup>	Cow's Raw Milk <sup>b</sup>	13.89 ± 5.80 ng/L
	Cow's Pasteurized Milk <sup>b</sup>	11.88 ± 5.80 ng/L
	Akkawi <sup>c</sup> a	40.50 ± 5.62 ng/kg
	Halloum <sup>c</sup> b	25.03 ± 5.62 ng/kg
	Karische <sup>d</sup> c	14.25 ± 5.45 ng/kg
	Shanklish <sup>c</sup> d	38.69 ± 5.62 ng/kg
	Yogurt <sup>d</sup> a	21.27 ± 5.45 ng/kg
	Ashta <sup>b</sup> r	38.87 ± 5.80 ng/kg
	Total <sup>e</sup>	25.55 ± 1.97 ng/kg
	October <sup>y</sup>	Cow's Raw Milk <sup>b</sup>
Cow's Pasteurized Milk <sup>b</sup>		17.51 ± 5.80 ng/L
Akkawi <sup>c</sup> a		32.50 ± 5.62 ng/kg
Halloum <sup>c</sup> b		31.28 ± 5.62 ng/kg
Karische <sup>d</sup> c		17.53 ± 5.45 ng/kg
Shanklish <sup>c</sup> d		33.09 ± 5.62 ng/kg
Yogurt <sup>d</sup> c		19.35 ± 5.45 ng/kg
Ashta <sup>c</sup> d		29.23 ± 5.80 ng/kg
Total <sup>e</sup>		24.81 ± 1.97 ng/kg
March <sup>z</sup>		Cow's Raw Milk <sup>b</sup>
	Cow's Pasteurized Milk <sup>b</sup>	26.33 ± 5.80 ng/L
	Akkawi <sup>c</sup> a	80.07 ± 5.62 ng/kg
	Halloum <sup>c</sup> b	53.63 ± 5.62 ng/kg
	Karische <sup>d</sup> c	45.11 ± 5.45 ng/kg
	Shanklish <sup>c</sup> d	61.56 ± 5.62 ng/kg
	Yogurt <sup>d</sup> a	32.64 ± 5.45 ng/kg
	Ashta <sup>b</sup> f	60.78 ± 5.80 ng/kg
	Total <sup>e</sup>	49.47 ± 1.97 ng/kg
	April <sup>t</sup>	Cow's Raw Milk <sup>b</sup>
Cow's Pasteurized Milk <sup>b</sup>		22.30 ± 5.80 ng/L
Akkawi <sup>c</sup> a		57.16 ± 5.62 ng/kg
Halloum <sup>c</sup> b		31.65 ± 5.62 ng/kg
Karische <sup>d</sup> c		28.82 ± 5.45 ng/kg
Shanklish <sup>c</sup> d		41.77 ± 5.62 ng/kg
Yogurt <sup>d</sup> a		24.94 ± 5.45 ng/kg
Ashta <sup>b</sup> f		20.02 ± 5.80 ng/kg
Total <sup>e</sup>		31.09 ± 1.97 ng/kg
Total		Cow's Raw Milk <sup>b</sup>
	Cow's Pasteurized Milk <sup>b</sup>	19.51 ± 2.90 ng/L
	Akkawi <sup>c</sup> a	52.56 ± 2.81 ng/kg
	Halloum <sup>c</sup> b	35.40 ± 2.81 ng/kg
	Karische <sup>d</sup> c	26.43 ± 2.72 ng/kg
	Shanklish <sup>c</sup> d	43.78 ± 2.81 ng/kg
	Yogurt <sup>d</sup> e	24.55 ± 2.72 ng/kg
	Ashta <sup>b</sup> f	37.23 ± 2.90 ng/kg
	Total <sup>e</sup>	32.77 ± 1.97 ng/kg

<sup>xyz</sup>All months followed by different superscripts are significantly different at  $p < 0.05$ .

All dairy products within the same month followed by different online letters are significantly different at  $p < 0.05$ .

<sup>t</sup>N = 60.

<sup>a</sup> Standard error.

<sup>b</sup> N = 15.

<sup>c</sup> N = 16.

<sup>d</sup> N = 17.

<sup>e</sup> N = 127.

**Table 4**  
% Of AFM1 contamination levels exceeding EU limits and % contamination in different dairy products during different months.

	% Contaminated <sup>a</sup>					% >EU limits				
	Sep	Oct	Mar	Apr	Average	Sep	Oct	Mar	Apr	Average
Akkawi	75	75	12.5	31	48	19	19	75	44	39
Halloum	75	75	44	31	56	13	19	44	50	31
Karische	100	88	71	76	84	0	6	18	12	9
Shanklish	50	81	31	63	56	50	19	63	31	41
Yogurt	65	94	59	71	72	18	6	24	6	14
Kashta	87	93	33	87	75	13	7	67	7	24

<sup>a</sup> % contaminated in the range 0–50 ng/L.

contamination in dairy products was 25.16 ± 1.97 ng/L. On the other hand, during the spring season, 195 (72.2%) of overall dairy products samples have shown a contamination level below the LOD; whereas, 75 (27.8%) of the samples have exceeded the EU limits (>50 ng/L). The total mean was 40.28 ± 1.97 ng/L. Therefore, there was an increase of ~38% in levels of AFM1 contamination in the spring compared to fall season in the dairy products. According to the records of the Lebanese Agricultural Research Institute (LARI) in Tal Amara, Bekaa, average relative humidity during the fall season is around 55.2% compared to 63.0% during the spring season. Due to the fact that mold growth and relative humidity levels are directly proportional (Yiannikouris & Jouany, 2002), higher AFB1 levels were expected in the animal feed during spring; thus, more AFM1 has been excreted into the milk and detected in derived dairy products. According to LARI, during the fall season, average temperature is 18.9 °C versus 10.5 °C during spring; therefore, AFM1 contamination was higher during colder season. This is mainly due to the fact that, in Bekaa, animals graze more during the warmer fall season. This conforms to the findings from previous studies (Bachner et al., 1998; Blanco et al., 1988; Decastelli et al., 2006; Garrido et al., 2003; Lopez et al., 2003).

During the months of September, October, March and April, 419 (80.0%) of overall dairy products samples tested showed a contamination level below the LOD; whereas, 105 (20.0%) of the samples were higher than the European Union limits (>50 ng/L). The level of contamination of the cheese samples with AFM1 levels exceeding EU limits was high for the akkawi (39%), halloum (31%) and shanklish (41%) products. AFM1 gets associated with the casein during cheese production making cheese the strongest source of aflatoxins among dairy products (Brackett & Marth, 1982; Galvano et al., 1996; Lopez et al., 2003). Elkak, El Atat, Habib, and Abbas (2012) reported that 68% of different types of cheeses manufactured in Lebanon are contaminated with AFM1. In this study, the contamination level was 92%. This is may be due to the fact that the types of cheeses assessed in Elkak's study were different than the ones in this study and they have used a different type of analysis kit.

With respect to milk, in Brazil, Garrido et al. (2003) reported an incidence of AFM1 contamination of 73% in pasteurized milk with 15% exceeding 50 ng/L. In Italy, Galvano et al. (2001) found out that 78% of milk had AFM1; however, none of them exceeded the EU limits. In Portugal, the incidence of AFM1 in raw milk and UHT milk was found to be 81% and 84%, respectively (Martins, 2004). In Iran, 78% of milk samples exceeded the EU limits (Oveisi, Jannat, Sadeghi, Hajimahmoodi, & Nikzad, 2007). In Syria, 80% of milk samples were contaminated with AFM1 and 53% contained levels beyond the EU regulations (Ghanem & Orfi, 2009). ElKhoury et al. (2011) reported that AFM1 was detected in 41% of tested Lebanese milk samples with 17% exceeding the EU limits. Elkak et al. (2011) reported that in Lebanon, the AFM1 content in 6% of locally produced cow and goat milk exceed the EU limit. Our study showed that 3% of the pasteurized milk samples exceeded the EU limits; thus, the level of AFM1 contamination in Lebanese milk beyond the EU limit is relatively lower to that observed in other places around the world. This can be attributed to the low AFB1 contamination of animal feed.

With respect to yogurt, Galvano et al. (1996) reported the incidence of AFM1 contamination in 80% of tested yogurt samples in Italy. In Portugal, 4% of yogurt samples contained AFM1 (Martins, 2004). In Brazil, Sylos, Rodriguez-Amaya, and Carvalho (1996) did not detect the presence of AFM1 in the tested yogurts. Kim et al. (2000) reported that 50% of yogurt in South Korea is contaminated with AFM1. ElKhoury et al. (2011) found that 32% of the Lebanese yogurt had AFM1 with 4% exceeding the EU limit. In our study, 72% of the yogurt samples were found to have AFM1 (Table 4)

with 13% of them beyond the EU limit (Table 4); thus, the level of AFM1 contamination in the Lebanese yogurt is comparable to other places in the world.

In this study, AFM1 levels in soft cheeses (akkawi, kashta and karishe) were 2 times higher compared to the AFM1 levels in the milk; whereas, AFM1 levels for hard cheeses (halloum and shanklish) were 2.05 times higher, which is equal to the one found by Battacone et al. (2005). However, Yousef and Marth (1989) found that the factor was 2.5–3.3 for soft cheeses and 3.9–5.8 for hard cheeses.

The mean intake for fresh milk among the tested sample of the Lebanese population calculated from the food frequency questionnaire was found to be  $43.83 \pm 6.39$  mL/day, for yogurt  $125.43 \pm 13.85$  mL/day, for halloum  $81.00 \pm 6.57$  g/day, akkawi  $33.22 \pm 2.74$  g/day, for shanklish  $3.67 \pm 0.88$  g/day, for karishe  $2.84 \pm 0.71$  g/day and for kashta  $10.22 \pm 1.17$  g/day. The daily AFM1 exposure, in relation to the consumption of the various dairy products, was 9.22 ng per kg of dairy product consumed per person. The calculated mean exposure to AFM1 per day through pasteurized milk is 0.9 ng/L. This calculated value conforms to the findings of Creppy (2002) who calculated the intake of AFM1 from milk 0.7 ng/person per day for the Middle Eastern diet. According to Creppy (2002), an AFM1 intake of 0.11 ng/kg body weight per day results in 1.5–9.4 additional cancer cases/year per  $1 \times 10^6$ . In Lebanon, AFM1 exposure through consumption of dairy products was calculated to be 0.14 ng/kg body weight per day; therefore, AFM1 is a public health issue that should be monitored by the authorities and dairy producers. The most effective method of controlling AFM1 in dairy products is to reduce the contamination of animal feed with AFB1 by applying preventive measures in terms of temperature and humidity control that reduce fungal growth.

#### 4. Concluding remarks

Dairy products represent a major constituent of the human diet. AFM1 contamination of dairy products is an issue of public health concern. In our study, cow milk showed the highest AFM1 content among different milk sources. No significant ( $p < 0.5$ ) effect of pasteurization on the AFM1 in three sources of milk was observed. Our study showed that 21% of dairy products tested, mainly akkawi, halloum and shanklish, are contaminated with AFM1 above EU limits. Further studies must be routinely performed to ensure the safety of local dairy products. Legislations and monitoring programs from governmental agencies must be set to minimize the exposure of Lebanese population to this toxin.

#### References

- Akkaya, L., Birdane, Y. O., Oguz, H., & Cemek, M. (2006). Occurrence of aflatoxin M1 in yogurt samples from Afyonkarahisar, Turkey. *The Bulletin of the Veterinary Institute in Pulawy*, 50, 517–519.
- Anonymous. (1999). *Enzyme immunoassay for the quantitative analysis of aflatoxin M1*. Art. no. R 1101. Darmstadt, Germany: R-Biopharm GmbH.
- Bachner, U., Martlbauer, E., & Terblan, G. (1998). Detecting aflatoxin M1 in milk from selected parts of Bavaria by using ELISA. German Federal Republic. *On Dairy Science Abstract*, 52, 901.
- Bakirci, I. (2001). A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. *Food Control*, 12, 47–51.
- Battacone, G., Nudda, A., Cannas, A., Borlino, A. C., Bomboi, G., & Pulina, G. (2003). Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B1. *Journal of Dairy Science*, 86, 2667–2675.
- Battacone, G., Nudda, A., Palomba, M., Pascale, M., Nicolussi, P., & Pulina, G. (2005). Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. *Journal of Dairy Science*, 88, 3063–3069.
- Blanco, J. L., Dominguez, L., Gomez-Lucia, E., Garayzabal, J. F. F., Garcia, J. A., & Suarez, G. (1988). Presence of aflatoxin M1 in commercial UHT treated milk. *Applied Environmental Microbiology*, 56, 1622–1623.
- Brckett, R. E., & Marth, E. H. (1982). Association of aflatoxin M1 with casein. *Z Lebensm Unters Forsch*, 174, 439–441.
- Codex Alimentarius Commissions. (2001). *Comments submitted on the draft maximum level for aflatoxin M1 in milk*. Hauge, The Netherlands: Codex Committee on Food Additives and Contaminants, 33rd sessions.
- Colak, H. (2007). Determination of aflatoxin M1 levels in Turkish white and Kasha cheeses made of experimentally contaminated raw milk. *Journal of Food and Drug Analysis*, 15, 163–168.
- Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*, 127, 19–28.
- Decastelli, L., Manco, A., & Sezian, A. (2006). Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005. *Food Control*, 18, 1263–1266.
- Deshpande, S. S. (2002). Fungal toxins. In S. S. Deshpande (Ed.), *Handbook of food toxicology* (pp. 387–456). New York: Marcel Dekker.
- Deveci, O. (2007). Changes in the concentration of aflatoxin M1 during manufacture and storage of White Pickled cheese. *Food Control*, 18, 1103–1107.
- van Egmond, H. P. (1989). Aflatoxin M1: occurrence, toxicity, regulation. In H. P. van Egmond (Ed.), *Mycotoxins in dairy products* (pp. 11–55). London: Elsevier Applied Science.
- Elkak, A., El Atat, O., & Abbas, M. (2011). A survey on the occurrence of aflatoxin M1 in raw and processed milk samples marketed in Lebanon. *Food Control*, 22, 1856–1858.
- Elkak, A., El Atat, O., Habib, J., & Abbas, M. (2012). Occurrence of aflatoxin M1 in cheese processed and marketed in Lebanon. *Food Control*, 25, 140–143.
- ElKhoury, A., Atoui, A., & Yaghi, J. (2011). Analysis of aflatoxin M1 in milk and yoghurt and AFM1 reduction by lactic acid bacteria used in Lebanese industry. *Food Control*, 10, 1695–1699.
- Gallo, P., Salzillo, A., & Rossini, C. (2006). Aflatoxin M1 determination in milk: method validation and contamination levels in samples from Southern Italy. *Italian Journal of Food Science*, 18, 251–259.
- Galvano, F., Galofaro, V., & Galvano, G. (1996). Occurrence and stability of aflatoxin M1 in milk and milk products: a worldwide review. *Journal of Food Protection*, 59, 1076–1090.
- Galvano, F., Galofaro, V., Ritieni, A., Bognanno, M., De Angelis, A., & Galvano, G. (2001). Survey of the occurrence of aflatoxin M1 in dairy products marketed in Italy: second year of observation. *Food Additives and Contaminants*, 18, 644–646.
- Garrido, N. S., Iha, M. H., Santos Ortolani, M. R., & Duarte Favaro, R. M. (2003). Occurrence of aflatoxins M1 and M2 in milk commercialized in Ribeirao Preto-SP, Brazil. *Food Additives and Contaminants*, 20, 70–73.
- Geloso, M., & Buzzetti, Y. (1994). Effetto del trattamento termico del latte sul contenuto di aflatoxina M1. *Rivista di Scienza dell'Alimentazione*, 23, 115–117.
- Ghanem, I., & Orfi, M. (2009). Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market. *Food Control*, 20, 603–605.
- Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism and impact of mycotoxins on humans and animals. *Toxicology*, 167, 101–134.
- Kamkar, A., Karim, G., Aliabadi, F. S., & Khaksar, R. (2008). Fate of aflatoxin M1 in Iranian white cheese processing. *Food and Chemical Toxicology*, 46, 2236–2238.
- Khayoon, W. S., Saad, B., Lee, T. P., & Salleh, B. (2012). High performance liquid chromatographic determination of aflatoxins in chilli, peanut and rice using silica based monolithic column. *Food Chemistry*, 133, 489–496.
- Kim, E. K., Shon, D. H., Ryu, D., Park, J. W., Hwang, H. J., & Kim, Y. B. (2000). Occurrence of aflatoxin M1 in Korean dairy products determined by ELISA and HPLC. *Food Additives and Contaminants*, 17, 59–64.
- Kocabas, C. N., & Sekerel, B. E. (2003). Does systemic exposure to aflatoxin B1 cause allergic sensitization? *Allergy*, 58, 363.
- Lopez, C. E., Ramos, L. L., Ramadan, S. S., & Bulacio, L. C. (2003). Presence of aflatoxin M1 in milk for human consumption in Argentina. *Food Control*, 14, 31–34.
- Manetta, A. C., Giammarco, M., Di Giuseppe, L., Fusaro, L., Gramenzi, A., Formigoni, A., et al. (2009). Distribution of aflatoxin M1 during Grana Padano cheese production from naturally contaminated milk. *Food Chemistry*, 113, 595–599.
- Martins, M. (2004). Aflatoxin M1 in yogurts in Portugal. *International Journal of Food Microbiology*, 91, 315–317.
- Motawee, M. M., & McMahon, D. J. (2009). Fate of aflatoxin M1 during manufacture and storage of feta Cheese. *Toxicology and Chemical Food Safety*, 74, 42–45.
- Oveisi, M. R., Jannat, B., Sadeghi, N., Hajimahmoodi, M., & Nikzad, A. (2007). Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. *Food Control*, 18, 1216–1218.
- Park, D. L. (2002). Effect of processing on aflatoxin. *Advances in Experimental Medicine and Biology*, 504, 173–179.
- Peraica, M., Radic, B., Lucic, A., & Pavlovic, M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77, 754–766.
- Pittet, A. (1998). Natural occurrence of mycotoxins in foods and feeds—An updated review. *Revue de Médecine Veterinaire*, 149, 479–492.
- Piva, G., Galvano, F., Pietri, A., & Piva, A. (1995). Detoxification methods of aflatoxins – a review. *Nutrition Research*, 15, 767–776.
- Sarmehmetoglu, B., Kuplulu, O., & Cleik, T. (2004). Detection of aflatoxin M1 in cheese samples by ELISA. *Food Control*, 15, 45–49.
- Stoloff, L., Park, D. L., & Van Egmond, H. P. (1991). Rationales for the establishments of limits and regulations for mycotoxins. *Food Additives and Contaminants*, 8, 213–221.
- Sylos, C. M., Rodriguez-Amaya, D. B., & Carvalho, P. R. N. (1996). Occurrence of aflatoxin M1 in milk and dairy products commercialized in Campinas, Brazil. *Food Additives and Contaminants*, 13, 169–172.
- Yiannikouris, A., & Jouany, J. P. (2002). Mycotoxins in feeds and their fate in animals: a review. *Animal Research*, 51, 81–99.
- Yousef, A. E., & Marth, E. H. (1989). Stability and degradation of aflatoxin M1. In H. P. Van Egmond (Ed.), *Mycotoxins in dairy products* (pp. 127–161). London: Elsevier.