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The risks associated with aflatoxins M1 occurrence in Lebanese dairy products

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ABSTRACT

Aflatoxins are potent carcinogens, teratogens and mutagens. When lactating ruminants are fed feedstuffs contaminated with aflatoxins B_1 (AFB1), this mycotoxin gets hydroxylated to aflatoxins M_1 (AFM1) 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 AFM1 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 AFM1 content using competitive ELISA (RIDASCREEN r-biopharm test kit). The exposure level of the Lebanese population to AFM1 was also assessed through food frequency questionnaires. Besides, factors affecting the level of AFM1, such as milk source, pasteurization, seasonal and processing effects, were studied. Results showed a significant ($p < 0.05$) difference in the AFM1 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 AFM1 above the EU limits (50 ng/L). Lebanese population daily exposure to AFM1 through consumption of dairy products was estimated to be 9.22 ng/L per person.

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_1 (AFB1), these toxins will be metabolized and excreted as aflatoxins M_1 (AFM1) in milk and this is the only route for transformation of AFB1 to AFM1 (Bakirci, 2001; Lopez, Ramos, Ramadan, & Bulacio, 2003). Bakirci (2001) and Creppy (2002) reported that 0.3–6.2% of AFB1 in ingested animal feed is transformed to AFM1 in excreted milk. AFM1 is detected in the milk 12–24 h after the first ingestion of AFB1 (Pittet, 1998); however, Battacone et al. (2003) reported that AFM1 is detected in sheep's milk within 6 h after consumption of contaminated feed. Researchers reported a higher incidence of AFM1 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).

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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 & McMahon, 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[1] 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

[1] 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.