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Occurence of aflatoxin M1 in Northern Africa: A short review

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Abstract

Moldy feed toxicosis was recognized as a serious livestock problem in the 1950's. Mycotoxins are toxic metabolites synthesized by some naturally occurring fungi under suitable physical, chemical and biological factors. The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins. The aflatoxins constitute a group of fungal metabolites that have varied toxic properties. Among 17 aflatoxins isolated and identified, only 4 of them are well known and studied extensively ; B1, B2, G1 and G2. Aflatoxin B1 ingested by lactating animals is biotransformed in the liver to a monohydroxylated metabolite, aflatoxin M1 (AFM1), which is a toxic molecule excreted in the milk. Higher analytical costs of AFM1 are one of the reported reasons for unavailability of surveillance data in the majority of developing countries. In this review, occurrence of AFM1 in milk and milk products in northern Africa, as well as its metabolisme, properties and analytical methods are summarised.

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Introduction

The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins (Rice and Ross, 1994). In 1960, during the investigations in the United Kingdom of moldy feed toxicosis which was called Turkey "x" disease, *Aspergillus* species were identified as the organisms responsible for the elaboration of the toxin in feed (Klich *et al.*, 2000; Dhanasekaran *et al.*, 2011). Since then, many mycotoxins have been discovered, the last major group being, in 1988, fumonisins (Yiannikouris and Jouany, 2002; Zinedine and Idrissi, 2007).

Aflatoxins (AFs) are a group of mycotoxins that are of greatest significance for food and feedstuffs safety (Josephs *et al.*, 2005).

They may be present in many raw and processed food commodities including cereals, milk and milk products (Aidoo *et al.*, 2011).

Among aflatoxins, aflatoxin B1 (AFB1) is the most commonly found in food (Reid *et al.*, 2016). Aflatoxin B1 ingested by lactating animals is biotransformed to a monohydroxylated metabolite, aflatoxin M1 (AFM1), which is subsequently excreted in the milk (Firmin *et al.*, 2011). This latter is a very important food source for humans due to its chemical composition and nutritive properties (Kalla *et al.*, 2015).

Toxic and carcinogenic effects of AFM1 have also been extensively demonstrated (Riley and Pestka, 2005). Despite the toxicity of aflatoxins, control systems are not always in place in all countries, especially in developing countries where AFM1 is a real public health threat.

Writing this short review article on aflatoxin M1 is motivated by the importance of this toxin as a milk contaminant with very serious risks on human health. Furthermore, choosing to study its occurrence in Northern Africa, through the rare studies made in this region, is a first attempt to evaluate the importance of this toxin in this area.

The aim of this short review is to present some of the major properties of aflatoxin M1; the different analytical techniques used to detect AFM1 in milk and milk products and also the different decontamination strategies used. This article presents as well a review of studies conducted in six countries of Northern Africa: Algeria; Morocco; Tunisia; Sudan; Egypt and Libya in order to evaluate the occurrence of this toxin in this part of the world.

Mycotoxins

Mycotoxins are toxic metabolites synthesized by some naturally occurring fungi under suitable physical, chemical and biological factors (Yiannikouris and Jouany, 2002 ; Agag, 2004 ; Galtier *et al.*, 2005 ; Repussard *et al.*, 2013 ; Zbib *et al.*, 2014). Until recently, only three species (*Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*) have been widely recognized as producers of aflatoxin (Klich *et al.*, 2000; Mohammadi *et al.*, 2009).

Many authors have showen that seasonal effect influences concentration of aflatoxin M1 in milk. They have reported higher concentration of AFM1 in cold seasons as compared to hot seasons (Bilandzic *et al.*, 2010; Fallah, 2010), the reason being in winter time, milking animals are mostly fed with compound feeds and thus concentration of aflatoxin B1 increases which in turn enhances AFM1 concentration in milk.

Although 17 aflatoxins have been isolated (WHO, 1979), only 4 of them are well known and studied extensively from toxicological point of view. Being intensely fluorescent in ultraviolet light these four aflatoxins are designated by the letters B1, B2, G1 and G2 representing their blue and green fluorescence in UV light (Devero, 1999). Other significant members of the aflatoxin family are M1 and M2 (Dhanasekaran *et al.*, 2011), found in milk of animals previously exposed to B1 and B2. Of all the known aflatoxins, aflatoxin B 1 (AFB1) is the most acutely toxic to various species (Oyedele *et al.*, 2017). It is highly toxic, in terms of both acute and chronic toxicity (Sweeney and Dobson, 1998; Moss, 2002).

Two pathways of the dietary exposure have been identified, the first one is direct ingestion of aflatoxins (mainly B1) in contaminated foods of plant origin such as maize and nuts and their products, the second pathway is ingestion of aflatoxins carried over from feed into milk and milk products including cheese and powdered milk, where they appear mainly as aflatoxin M1 (WHO, 1979; Agag, 2004).

Aflatoxin M1 metabolism

Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1 forming in liver by means of cytochrome P450- associated enzymes (Ardic, 2008). It may be found in milk or dairy products obtained from livestock that have ingested contaminated feed with AFB1. There is a linear relationship between the amount of AFM1 in milk and AFB1 in feed consumed by animals (Ardic, 2008).

After absorption, the highest concentration of AFB1 is found in the liver (Mintzlaff *et al.*, 1974). Once there, aflatoxin B1 is metabolized by microsomal enzymes to different metabolites through hydroxylation, hydration, demethylation and epoxidation. The hydroxylation of AFB1 at C4 produces, AFM1 (Patterson and Roberts, 1970; Busby and Wogan, 1985), which is the principal hydroxylated aflatoxin metabolite present in the milk of dairy cows fed a diet contaminated with aflatoxin B1 (Groopman *et al.*, 1985; Bhatnagar *et al.*, 2002) (Fig. 1).

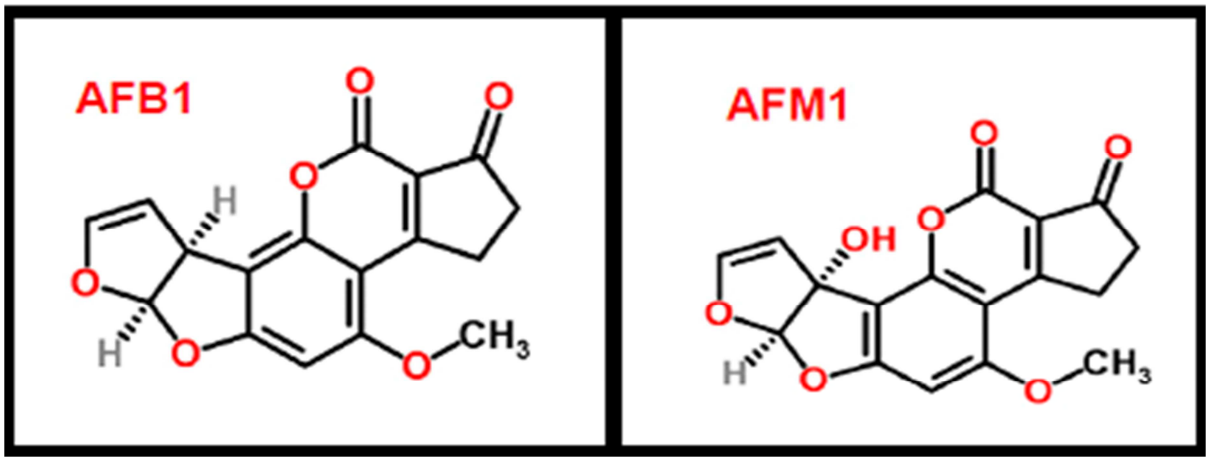

Fig. 1. Chemical structure of Aflatoxin B1 and Aflatoxin M1 (Alshannaq and Yu, 2017).

AFM1 is usually considered to be a detoxification product of AFB1 (Neal *et al.*, 1998). It could be detected in milk 12-24 h after the first AFB1 ingestion, reaching a high level after a few days. When the intake of AFB1 is finished, the AFM1 concentrations in milk decreases to an undetectable level after 72 h (Mohammadi, 2011).

Aflatoxin M1 toxicity

Aflatoxins constitute a group of fungal metabolites that have varied toxic and carcinogenic properties, depending on dose and duration of exposure (Agag, 2004). They have been implicated as potential factors in the increased incidence of human gastrointestinal and hepatic neoplasms in Africa, The Philippines and China (CAST, 1989). Indeed, Aflatoxins have damaging effects on human and animal liver tissue, which can lead to liver cancer or even death (Dhanasekaran *et al.*, 2011). Among aflatoxins, aflatoxin B1 (AFB1) is the most common contaminant of food, and is also the most toxic and carcinogenic. AFB1 can induce immune toxicity in various animal species (Peng *et al.*, 2017).

When animals consume feed that is fungal (aflatoxin B1) infected, they will excrete aflatoxin M1 (AFM1) in their milk. This aflatowin should not be present in the milk as per World Health Organization Standard. When such milk is consumed it will act like a cumulative poison and it will damage the liver and cause cirrhosis (Suliman and Abdalla, 2013).

Aflatoxin M1 (AFM1) in milk and milk products is considered to pose certain hygienic risks for human health.

It is considered to be a potential carcinogen for animals and humans (Cavaliere *et al*., 2006). Lafont *et al.* (1989) observed a high genotoxic activity of AFM1, although it was lower than that of AFB1. The International Agency for Research on Cancer (IARC, 2002) has classified both AFB1 and AFM1 as agents belonging to Group 1 (highly poisonous toxic substances), carcinogenic for humans.

Decontamination strategies in milk and milk products

There is currently no known procedure for destroying aflatoxin M in milk without destroyin the milk. For all practical purposes, aflatoxin M is stable in raw milk and processed milk products, and is unaffected by pasteurization or milk processing (Stoloff, 1980; Gallot *et al*., 2000; Josephs, *et al*., 2005).

Global review of the literature indicates the existence of methods of partial decontamination of AFM1, however; evidence based studies do not suggest that any single strategy as a coherent and complete solution to the issue (Ismail *et al*., 2015).

Early experiments demonstrated good reductions with bentonite (Applebaum and Marth, 1982). More recently, the ability of saponite-rich bentonite to reduce AFM1 contamination in milk was investigated. The detoxification capacity of the bentonites used was efficient, bringing contamination of milk below the European standard limits for AFM1 (50 ng/kg). Bentonite residues retained in milk (0.4%) were of no concern for human health (Carraro *et al*., 2014).

Inclusion of enterosorbents in the diet of dairy animals may reduce absorption of AFB1 in the animal body, preventing further steps of toxin distribution and metabolism, thus reducing carry-over in milk (Giovati *et al*., 2015). Significant reductions of the concentration of AFM1 in milk were observed when clay enterosorbents were included in the diet of lactating dairy cattle and goats fed with feed contaminated with AFB1 (Phillips *et al*., 2008).

Nowdays, some interventions exploit microorganisms (Ehrlich *et al*., 2011; Ismail *et al*., 2015), purified microbial enzymes (Gonçalves *et al*., 2017), dietary clay minerals, and specific antibodies induced by vaccination to reduce directly or indirectly AFM1 contamination of milk (Giovati *et al*., 2015).

Analytical methods

The frequent detection of AFM1 in commercial milk and dairy products, the high consumption of these products, especially in infants and the carcinogenicity of AFM1 led to an increased public awareness and therefore to the establishment of measures to control AFM1 contamination of food and feedstuffs (Josephs, *et al*., 2005 ; Ehrlich *et al*., 2011).

Currently the limits of AFM1 are highly variable, depending upon the degree of development and economic standing of the countries. A maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg (Codex Alimentarius Commissions, 2001).

Analytical techniques in use for detection of AFM1 in milk and milk products include : Chromatographic techniques (Rice and Ross, 1994 ; Reiter *et al*., 2009 ; Orata, 2012 ; Laura *et al*., 2016 ; Shephard, 2016), immunochemical methods (Jiang *et al*., 2013 ; Vdovenko *et al*., 2014 ; Yao *et al*., 2015 ; Kos *et al*., 2016), rapid methods (Zheng *et al*., 2006 ; Maragos and Busman, 2010),other research methods have potential utility for the analysis of mycotoxins. They include : infrared spectroscopy (De Girolamo *et al*., 2009 ; Pérez *et al*., 2017 ; Sieger *et al*., 2017), capillary electrophoresis (Maragos and Appell, 2007 ; Shephard, 2008), electronic nose (Olsson *et al*., 2002 ; Campagnoli *et al*., 2009), biosensors (Bram Van Der *et al*., 2003 ; Logrieco *et al*., 2005), mlecular imprinting polymers (Visconti *et al*., 2005 ; Appell and Mueller, 2016), fluorescence polarization (Chun *et al*., 2009 ; Sheng *et al*., 2014).

Among chromatographic techniques, HPLC is considered the reference method for AFM1 analysis (Beltran *et al*., 2011; Diniz Andrade *et al*., 2013).

Recent research has focused on the development of structure switching signaling aptamer assay, transducing the aptamer-target recognition event into an easily detectable signal (Istamboulié *et al*., 2016).

Sharma *et al.*, (2016) demonstrated the development of structures witching aptamer assay for determination of aflatoxin M1 (AFM1) employing the quenching-dequenching mechanism.

The application of these techniques to achieve higher levels of accuracy in analytical results appears to require various degrees of enhanced sensitivity and safety besides rapidity and cost effectiveness. Higher analytical costs of AFM1 are one of the reported reasons for unavailability of surveillance data in the majority of developing countries (Trucksess, 2001).

Studies on AFM1 in Northern Africa

Algeria

In the very first study curried out by Redouane-Salah *et al.* (2015) on milk consumed in Algeria, AFM1 was detected in 5 out of 47 samples (11 %) at levels ranging from 9 to 103 ng/L, with one sample exceeding the limit of 50 ng/L set by European regulations. Traces of AFM1 (less than 8 ng/L) were also found in 11 other samples. The incidence of AFM1 contamination was higher in imported powdered milk (29 %) than in raw milk (5 %). Although the concentration of AFM1 in contaminated samples was low. Authors concluded that the relatively considerable prevalence found in this exploratory study justifies more detailed and continuous monitoring to reduce consumers' exposure to AFM1.

Morocco

Zinedine *et al.*, (2007), using immunoaffinity columns and liquid chromatography coupled to fluorescence detection of aflatoxin M1 (AFM1) in pasteurized milk produced in Morocco, reported that 88.8% of the samples were contaminated with AFM1 ; 7.4% being above the maximum level of 0.05 µg/L set by the European regulations for AFM1. An important incidence of AFM1 in milk was observed (77.7% - 100%), with a mean value of 0.0186 µg/L of AFM1. In their study, Zinedine *et al.*, (2007) estimated that daily intake of AFM1 was 3.26 ng/person/day.

ElMarnissi *et al.* (2012) reported that AFM1 was detected in 13 out of 48 samples of raw milk (27%) at concentrations ranged between 10 and 100 ng/l. Within the positive samples, four (8% of the total) were above the European legislation limit of 50 ng/L. Authors revealed a variation of contamination in milk samples collected in autumn compared to those collected in other seasons suggesting a link between feeding practices and AFM1 contamination of milk.

Tunisia

In this country, only a small number of studies on aflatoxin M1 in milk from lactating dairy cows are available. In the study conducted by Abbès *et al.* (2012), 112 raw cow milk samples were collected from farms and local markets. Milk samples were analysed for aflatoxin M1 (AFM1), using enzyme-linked immunoabsorbent assay (ELISA) technique. Authors found that recovery was 81%–92% for AFM1, while the limit of detection (LOD) was 0.01 mg/l for AFM1.

Results revealed the presence of AFM1 in 60.7% of the cow raw milk samples examined (median 13.62±1.4 µg/l). Contaminated levels were higher than the EU limit of 0.05 µg/L. It was concluded that more precaution should be taken on hygiene controls in order to prevent fungal contamination (Abbès *et al.*, 2012).

Sudan

Using high-performance liquid chromatography (HPLC) with fluorescence detection, presence of AFM1 in dairy cattle milk was investigated by Elzupir and Elhussein (2010). A total of 44 bulk dairy cattle milk samples were collected and analyzed. The rate of contamination was 95.45% (42/44), with contamination level ranging between 0.22 and 6.90 µg L^{-1} and average concentration of 2.07 µg L^{-1}.

More recently, a study was carried out by Suliman and Abdalla (2013) to detect aflatoxin M1 in dairy cattle milk in Khartoum State - Sudan. It included 143 raw milk samples. The technique used for detection of AFM1 was (ELISA). Suliman and Abdalla (2013) found that all examined milk samples were contaminated with AFM1. 141 (98.6%) of the samples had AFM1 greater than the European maximum tolerance limit (50ng/L). Authors concluded that AFM1 levels in milk appear to be a serious public health problem in this country.

Egypt

In Egypt, a first study was carried out by Salem (2002), to investigate the natural occurrence of total aflatoxins in feedstuffs and aflatoxin M1, in raw milk of dairy farms in Assiut province. A total 85 raw milk samples were collected and analysed with ELISA technique. Aflatoxin M1 was found in 50 (58.8%) of the investigated milk samples. The concentrations of aflatoxin M1 ranged from 15 ng/L. Results obtained revealed that aflatoxin M1 levels in analyzed milk samples did not reach the maximum tolerated limit of EU countries (50 ng AFM1/L milk). 16/50 positive samples exceeded the Swiss limits, which are the most restrictive in the world (10 ng/L) (Salem, 2002).

In another study, Motawee *et al.* (2009) analysed milk from buffalo, cow, goat and camel species collected in Ismailia province. Most milks (80%, 74%, 66% and 52% of the camel, goat, cow and buffalo milks, respectively) contained levels of contamination below the European Union maximum of AFM1<50 ng/L and all milk samples were <500 ng/L (Motawee *et al.*, 2009). 500 ng/L being the level for AFM1 in fluid milk in the United States (FDA, 2005).

In another recent study conducted by Ghareeb *et al.* (2013), a total of 48 raw milk samples were collected from various dairy farms in the Qena region and investigated for the presence of AFM1. Additionally, 30 dry powdered milk samples were also purchased from supermarkets in the same region. Results showed that the occurrence of AFM1 was 97.92 % (47/48 positive samples) and the mean level of AFM1 was 62.81±32.10 ng/L. The level of AFM1 in 53.19 % of raw milk samples was higher (79.85 ± 17.30 ng/L) than the maximum tolerance limit (50 ng/L) established by European Union (EU). Concerning powdered milk, only 60.0% (18/30 samples) were positive for AFM1 with mean level of 1.81±1.02 ng/Kg. According to Egyptian standards, the amount of AFM1 in the positive samples (47 from 48 samples, 97.92 %) goes beyond the tolerated levels, suggesting that the contamination of raw milk is very high (Ghareeb *et al.*, 2013).

Libya

In Libya, 49 samples of raw cow milk and 20 samples of fresh white soft cheese were collected in the north-west region of Libya, and analysed using HPLC technique for the presence of aflatoxin M1 (AFM1) by Elgerbi *et al.* (2004). Results showed that 71.4% (35/49) of milk samples had AFM1 levels between 0.03 and 3.13 ng ml^{-1}, and 75.0% (15/20) of white soft cheese samples showed the presence of AFM1 in concentrations between 0.11 and 0.52 ng g^{-1} of cheese. In this study concentrations of AFM1 were lower in cheese products than in raw milk samples (Elgerbi *et al.*, 2004).

Furthermore, in a preliminary study conducted by Elgerbi (2005), results showed that 66.7% of 27 human breast milk samples were contaminated with AFM1 ranging from 0.015 to 0.343 pg·mL^{-1}. Indeed AFM1 is also present in the milk of human nursing mothers consuming foodstuffs containing the toxin (Neal *et al.*, 1998).

Conclusion

Aflatoxin M1 (AFM1) in milk and milk products is considered to be a potential carcinogen for animals and humans. Although several surveys were curried out to monitor the presence of AFM1 in milk and dairy products all over the world, there is scarce information from countries of Northern Africa. In the fiew studies conducted in this part of the world, a high prevalence of AFM1 in milk and milk products was revealed. This situation should draw attention of researchers studying this aflatoxin.

The high prevalence of AFM1 contaminating milk and milk products poses a potential risk for consumer health. Hence the need for strict regulations for mycotoxins levels, not only in human foods but also in animal feeds with special focus on occurrence of AFB1 in the feed offered to dairy cows and AFM1 in milk and milk products.

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