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**Deepak Soni**
M.V.Sc., Department of Veterinary, Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

**Sneh Lata Chauhan**
Scientist, Department of Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

**Corresponding Author:**
**Deepak Soni**
M.V.Sc., Department of Veterinary, Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

# A review of aflatoxins in milk: Occurrence, toxicity and detection

**Deepak Soni and Sneh Lata Chauhan**

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**Abstract**
Aflatoxins are a major threat owing to their high heat tolerance and ability to cause serious health implications. The most prevalent form of aflatoxins found in feed and feed products is aflatoxin B1 (AFB1). AFM1 and AFM2 are the hydroxylated metabolic by products of AFB1 and AFB2, respectively. Dairy products contain aflatoxin M1 because it attaches to milk proteins, particularly casein. Aflatoxin contamination of milk and similar products is a problem for public health. AFM1 is cytotoxic and can also result in DNA damage, mutation, and chromosomal changes. AFB1 is one of the most potent known hepato-carcinogens. A number of monitoring techniques have been employed to gather data and find aflatoxins. These range from simple tests like the ELISA and HPLC to more recent proprietary methods like the CLIA (chemiluminescence enzyme immunoassay method) or the use of an immunochromatographic approach. The difficult requirements to develop a novel analytical method are the method's sensitivity and repeatability.

**Keywords:** Aflatoxins, Milk, AFB1, AFM1

## Introduction
Aflatoxins can occur in a wide range of vital foods, including maize, nuts, rice, spices, corn and dried fruit. Aflatoxins are a major threat owing to their high heat tolerance and ability to cause serious health implications. Aflatoxins are carcinogenic, hepatotoxic, genotoxic agents causing hepatitis, chronic liver failure and impaired immune system (Bailey *et al.*, 1994; Williams *et al.*, 2004) [4, 53].

Based on their physical, chemical, and biological characteristics, aflatoxins can be categorized into a number of classes. When exposed to UV radiation, aflatoxin group B emits blue light, whereas aflatoxin group G emits yellow-green light. According to Wacoo *et al.* (2014) [50], these are further categorized as B1, B2, G1, and G2 in that order.

The most prevalent form of aflatoxins found in feed and feed products is aflatoxin B1 (AFB1). The hydroxylated metabolic byproducts of AFB1 and AFB2 are AFM1 and AFM2, respectively. Mammals who ingest AFB1 release AFM1 in their milk (Henry *et al.*, 2011) [18].

AFM1 is present in milk and related products because it interacts to the casein protein (Prandini *et al.*, 2009) [36]. AFM1's tolerated daily intake was determined by Kuiper-Goodman (1990) to be 0.2 ng/kg b.w., and the potential for harm from AFM 1 caused the WHO-International Agency for Research on Cancer (IARC) to reclassify it from group 2 to group 1 (IARC, 2012) [21]. When evaluating the cancer risk, infants are more vulnerable because milk is a necessary component of a child's diet. They consume a lot of milk in their diet. Additionally, young animals have been found to be more vulnerable to aflatoxins than adults. Therefore, the presence of aflatoxins in milk and dairy products is considered to be disagreeable. Contamination of milk and dairy products with aflatoxins occurs in two manners. Aflatoxin-producing fungus can cause post-production contamination of milk and milk related products or transfer of toxins to milk from animals.

The European Union (EU) and the Codex Alimentarius Commission (CAC), among other international regulatory authorities, set the maximum permitted limit (MPL) of AFM1 in milk at 0.05 ng/mL. However, the MPL of AFM1 in milk was set at 0.5 ng/mL by the Food Safety and Standards Authority of India (FSSAI, 2011) [12]. Public health is endangered by the presence of aflatoxin metabolites in milk and its related products. Due to trade restrictions put

in place by the importing nations, AFM1 contamination of milk may potentially have an impact on the export of everyday necessities. The study describes the many techniques used to find aflatoxin in milk and milk-related products in India and other nations since aflatoxin in milk is crucial for both consumer safety and public health.

**Table 1:** Occurrence of AFM1 in milk and dairy products

| S.N. | Method | Country | Samples | Number Of +ve samples | Reference |
|---|---|---|---|---|---|
| 1 | HPLC -fluorescence detection | Brazil | 111 samples | 29 (17 UHT, 12 pasteurized) samples exceeded the limit of 0.05, ηg/mL of EU | Garrido *et al.* (2003) [13] |
| 2 | Enzyme immunoassay | Argentina | 77 samples | 18 samples were found to positive with ranging 0.010–0.030 ηg/mL | López *et al.* (2003) |
| 3 | ELISA and HPLC | Spain | 92 samples of raw cow milk | 3 with the mean concentration found using ELISA was 0.02 0.005 ηg/mL, while by HPLC it was 0.017 0.003 ηg/mL | Velasco *et al.* (2003) [49] |
| 4 | Thin layer chromatographic method | Giza Governorate | 90 milk samples | 20% of milk samples contained form 0.378 to 0.342 ηg/mL of AFM1 | Nassib *et al.* (2005) [34] |
| 5 | ELISA | Iran | 328 branded milk products and liquid milk samples | 96.3% samples were found to positive with ranging between 0.001–0.113 ηg/mL | Oveisi *et al.* (2007) [33] |
| 6 | ELISA | Syrian | 126 samples | 80% of samples were found to positive with ranging from >0.020 to 0.765 ηg/mL | Ghanem and Orfi (2009) [14] |
| 7 | Immunoaffinity column chromatography and HPLC - fluorescence detection | South Korea | 100 raw milk samples | 48 samples contained AFM1 but were below the Korean regulations (0.5 ηg/mL) for AFM1 in milk | Lee *et al.* (2009) [29] |
| 8 | HPLC -fluorescence detection | Pakistan | 169 milk samples 55 (buffalo), 40 (cow), 30 (goat), 24 (sheep), and 20 (camel) | 15.8% and 20% of positive samples of buffalo and cow, respectively and none of sheep, goat and camel milk exceeded EU MPL (0.05 ηg/mL) | Hussain *et al.* (2010) [20] |
| 9 | ELISA | Pakistan | 232 milk samples | 177 (76.3% of the total) milk samples were positive with average concentration was 0.252 ηg/mL | Sadia *et al.* (2012) [40] |
| 10 | HPLC with fluorescence detector | Karnataka and Tamilnadu, India | 45 samples | 38% of the plain UHT milk samples tested positive for AFM (1) and had concentrations more than 0.5 g/kg. | Siddappa *et al.* (2012) [45] |
| 11 | HPLC with fluorescence detector | China | 31 milk samples | 8 samples but value did not exceeded Chinese legal regulation (0.5 ηg/mL) | Wang *et al.* (2012) [51] |
| 12 | HPLC -fluorescence detection | Brazil | 83 samples of different heat treated milk products | AFM1 was found in 83% of the milk samples (>3 ng/kg), with values in fluid milk ranging from 8 to 437 ng/kg and powdered milk from 20 to 760 ng/kg. | Iha *et al.* (2013) [22] |
| 13 | HPLC -fluorescence detection | Turkey | 176 samples of raw milk | 53 (30.1%) 0.033-1.50 ηg/mL in different seasons | Golge (2014) [15] |
| 14 | ELISA | Pakistan | 84 milk samples | 81 milk samples (96.43%) were found positive with AFM1 ranging from 0.01–0.76 ηg/mL | Jawaid *et al.* (2015) [23] |
| 15 | HPLC-FL | China using HPLC-FL | 560 milk samples | 14.1% of raw cow milk samples with concentrations between 0.01 and 0.144 g/mL were positive. | Fallah *et al.* (2016) [10] |

**Toxicity**

Aflatoxins causes acute and chronic toxicity. Symptoms lke nausea, vomiting, abdominal pain, and convulsions can be seen in acute toxicity, whereas immunotoxicity, hepatotoxicity, and teratogenicity can be seen as complications of chronic toxicity. One of the main causes of hepatocellular cancer in poor nations is aflatoxin. Since AFB1 is one of the most potent known hepato-carcinogens, long-term exposure to even very small amounts of aflatoxins in the diet raises safety concerns for people. AFM1 can also result in DNA damage, chromosomal abnormalities, and gene mutations, among other genetic disorders. However, compared to AFB1, AFM1 is less mutagenic and genotoxic.

**Exposure**

AFM1 may manifest in internal organs and tissues, including the liver and kidney, as well as in milk and associated products if an animal consumes food infected with AFB1. Geographically varying sources of aflatoxin contamination in animal feedstuffs are possible. The most significant aflatoxins are found in groundnut, cottonseed, and maize meal, which are present in many feeds. Aflatoxin contamination of agricultural products is a global issue, not just in developing nations where climatic and technological factors promote aflatoxin development.

**Testing methods**

Data collection has used a variety of screening techniques, and results are often verified using the HPLC method. These can be as simple as an ELISA test or as sophisticated as using an immune chromatographic approach or a CLIA (chemiluminescence enzyme immunoassay method) test. Data have been published in the literature and are also available for various monitoring for aflatoxins (Trombley *et al.* 2011, Bailey *et al.* 2012, Abhijith *et al.* 2013) [48, 5, 1].

**Electrochemical biosensors**

According to Mascini (2001; Mascini *et al.* 2001) [31], the target analyte's binding to the corresponding element immobilized on an appropriate support medium constitutes the detection principle. The biosensors can be categorised into four classes based on how the signal is transduced: electrochemical, optical, electric, and piezomagnetic. Electrochemical and optical biosensors are most frequently utilised in the detection of aflatoxins (Mottram *et al.* 2002) [33].

**ELISA**

One of the most popular techniques for identifying and evaluating the quantity of aflatoxins is this one. Due to its sensitivity, selectivity, and versatility as a tool, immunoassay has become very popular (Catala and Puchades 2008) [6].

ELISA tests have been used in recent years for detecting AFM1 from infant formula (Er *et al.* 2014) [7], raw and imported powdered milk (Guan *et al.* 2011; Ali *et al.* 2014) [16, 2] and other dairy products (Sarımehmetoglu *et al.* 2004; Kaniou-Grigoriadou *et al.* 2005; Yaroglu *et al.* 2005; Gurbay *et al.* 2006; Ardic *et al.* 2009; Fallah 2010b; Kazemi Darsanaki *et al.* 2013; Santini *et al.* 2013) [43, 26, 54, 17, 3, 9, 27, 41]. This assay's 245 ng/L detection limit was reported by

Sassahara *et al.* in 2005 [44]. The ELISA method does have some limitations, such as a lengthy incubation period and wash and mix phases.

**Chromatography**

The most often used technique for analyzing mycotoxins is chromatography. The most frequently employed chromatography methods are Gas Chromatography (Rodrguez-Carrasco *et al.* 2012, 2014) [37, 38], High Pressure Liquid Chromatography (Soleimany *et al.* 2011; Rubert *et al.* 2012; Michel *et al.* 2013; Kong *et al.* 2014) [46, 39, 32, 28], and LC (Frenich *et al.* 2011; Soleimany *et al.* 2012; Warth *et al.* 2012) [11, 47, 52]. Among these, High Pressure Liquid Chromatography and LC are the most frequently used method Although this method offers good accuracy, it also necessitates trained personnel, thorough sample pretreatment, and expensive equipment (Sapsford *et al.* 2006) [42].

**Liquid Chromatography**

HPLC is most commonly used technique for detecting aflatoxins in food products. This approach offers a nice balance of sensitivity, dynamic range, and adaptability. Liquid chromatography detection is frequently carried out using the HPLC-fluorescence, HPLC-UV, and derivatization techniques. Use of a fluorescence detector turned into mostly implemented for AFM1 evaluation due to its greater sensitivity and greater selectivity technique. Aflatoxin detection wavelengths can range from 360–365 nm and 274–334 nm.

**High-performance thin-layer chromatography (HPTLC)**

International laboratories frequently use thin-layer chromatography for the qualitative assessment and quality control of food products. Between TLC and HPTLC, the stationary phase has different dimensions. Their main benefit was that they were inexpensive, but because they do not offer a sufficient quantification limit (LC), they are not widely utilized in laboratories. For the identification of AFM1 in milk, screening techniques based on this TLC approach are available (Kamkar 2005, 2006; Fallah, 2010a) [24, 25, 8].

**Spectrometry**

The MS method is employed for affirmation because it produces spectra with distinctive fragmentation patterns.

AFM1 and other mycotoxins including Ochratoxin A, zearalenone, and o-zearalenone were all simultaneously determined in a recent work by Huang *et al.* (2014) [19] employing high pressure liquid chromatography in conjunction with a mass spectrometer and ions. The recovery was between 87.0 and 109%, and the limits of quantification (LOQ) for doses of 0.025, 0.1, and 0.5 ug/kg were between 0.003 and 0.015 ug/kg. The suggested approach is suitable for determining AFM1, Ochratoxin A, and other metabolites like zearalenone and a-zearalenone simultaneously, and it can be used to analyze mycotoxins in milk.

**Conclusion**

Aflatoxin poisoning will continue to be a problem for global public health, but there are many different obstacles that each nation and region must overcome. The problem has become more complicated as a result of global trade, climatic changes, and various regulatory frameworks. AFM1 is frequently found in dairy products and milk from animals. However, more research is required to provide specific scientific data on the risk to human health from subchronic exposure over a lengthy period of time. The sensitivity and reliability of AFM1 analysis methods present a considerable challenge in developing new analytical techniques. These should ensure that a large batch's testing sample is typical of the entire batch and that the analysis is precise and sensitive enough to be applied on the largest imaginable scale.

**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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