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Review Article

AFLATOXIN M_1 IN MILK: OCCURRENCE, TOXICITY AND MITIGATION

PATEL S.N.*, PARNERKAR S. AND PATEL D.C.
Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Rajpur (Nava), Himmatnagar, 383010, India
*Corresponding Author: Email - patelsweta3268@gmail.com

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Abstract: Aflatoxins are fungal metabolites which are produced by Aspergillus species. Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM2) are commonly found in nature. Among all, AFB_1 represents the highest degree of toxicity, followed by AFM_1, AFG_1, AFB_2 and AFG_2, Hence, AFB_1 is listed as a group I carcinogen by the International Agency for Research on Cancer. Aflatoxin residue might occur in dairy milk and other dairy products of animal that ingested aflatoxin contaminated feedstuff. Aflatoxin M_1 is a hydroxylated metabolite of AFB_1 which is excreted in milk from the mammary glands of both humans and lactating animals. Milk and dairy products, contaminated with AFM_1 are considered to pose certain risks for human health. AFM_1 can cause serious human disease, especially primary liver cancer, DNA damage and acute toxicity and carcinogenicity compared to the parent molecule AFB_1; therefore, it is now classified by the International Agency for research on cancer as a group 1 human carcinogen. A number of research investigations have been conducted to study the occurrence of AFM_1 and determination of AFM_1 in milk. Various physical, chemical and biological methods have been studied to detoxify AFB_1 and AFM_1 from milk and milk products. Now a days, essential to focus on bring up to date the current global status of AFM_1 contamination of milk and milk products destined for human consumption and the effects of processing and reduction methods on the elimination of aflatoxins from milk and milk products.

Keywords: *Aflatoxin B_1, Aflatoxin M_1, Occurrence, Toxicity, Mitigation*

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Introduction

Milk and milk products are commonly consumed by people of all age groups, especially children as milk are nutritionally balanced and provide all essential amino acids. Hence, the quality of milk needs to be good for human consumption. Humans and animals are more subjected to "biological hazard" from natural toxicants that occur in food and feed. Aflatoxins (AFs) are fungal metabolites which are produced by Aspergillus species, mainly by *A. flavus* and *A. parasiticus, A. ochraceoroseus, A. nomius, A. fumigitus, A. pseudotamari* [86]. Aflatoxins are the most studied group of mycotoxins [51], and they were initially isolated and identified as the cause of the Turkey 'X' disease in 1960, upon investigation it was discovered that the Turkeys had been fed Brazilian peanut meal containing the *Aspergillus flavus* [10]. The major types of AFs are: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2). AFB1 represents the highest degree of toxicity; followed by AFM1, AFG1, AFB2 and AFG2 [48]. AFB1 is considered as a most potent hepatocarcinogen, teratogen and mutagen [59], hence, listed as a group I carcinogen by the International Agency for Research on Cancer [54]. According to the Food and Agriculture Organization (FAO), up to 25% of the world's food crops are significantly contaminated with mycotoxins [40]. It is approximated, 4.5 billion people living in developing countries are chronically exposed to uncontrolled number of aflatoxins [115]. After the discovery of aflatoxins, researchers suggested that its residue might occur in milk and other animal products from animal that have been ingested contaminated feedstuff [107]. Aflatoxin M1 is a metabolic product of AFB1, which eliminated in milk via mammary glands of both humans and lactating animals [11, 39]. The mycotoxin contamination of milk and dairy products can occur by indirect contamination when lactating animals ingest AFB1 contaminated feed which will pass to the milk as AFM1, and also by direct contamination, when molds can grow in milk (very unlikely) or on dairy products as intentional additives or accidental contamination [94].

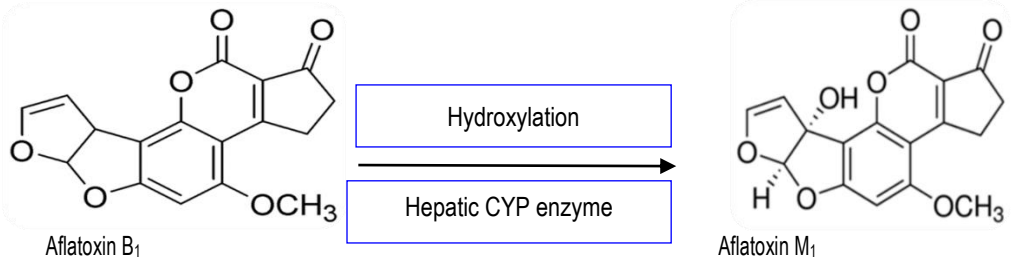
Therefore, milk and dairy products are particularly susceptible to contamination by AFM1 and are considered to pose certain risks for human health. Subsequently, milk has the greatest demonstrated potential for the introduction of aflatoxin residues in the human diet [44]. AFM1 can cause serious human disease, especially primary liver cancer, DNA damage and acute toxicity and carcinogenicity compared to the parent molecule AFB1; hence it is now classified by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen [54]. AFM1 exposure to children especially infants, is of particular concern, as they have potentially higher vulnerability and sensitivity than adults and their capacity for biotransformation of carcinogens is slower than adults [72]. A number of researches conducted on the occurrence of AFM1 in milk but currently need to focus on the development of an efficient and sensitive method for determination of AFM1 in milk and milk products. Various physical, chemical and biological methods are used for the inactivation and mitigation of AFB1 and AFM1 from milk and milk products [80]. So, there should aims to bring up to date the current global status of AFM1 contamination of liquid milk destined for human consumption and the effects of processing and reduction methods on the elimination of aflatoxins from milk and milk products.

Sources of Aflatoxins in Milk

The presence of mycotoxins in milk and milk products results mainly due to indirect contamination and occasionally by direct contamination.

1. Indirect contamination

The dairy animals consuming feeds or fodder contaminated with AFB1 excrete AFM1 in their milk [83]. *A. flavus* and *A. parasiticus* are ubiquitous fungi, have affinity for oily seeds as a growth source *e.g*., peanut meal, maize and cottonseed meal. These funguses colonize in the plants or crops in field of tropical and subtropical climate area but they can also colonize products in post-harvest processing and storage.



Aflatoxin B_1 Aflatoxin M_1

Fig-1 Conversion of AFB1 to AFM1

Aflatoxin production occurs with temperature between 20°C to 30°C and this higher limit is also the optimal one for the aflatoxin production in the feeds of dairy animals. *A. parasiticus* prefers a soil environment and is more common on peanuts while *A. flavus* is adapted to an aerial environment and more found on dry fruit, cotton and corn. Aflatoxin production strongly correlate to moisture content of feeds and environmental condition particularly the temperature and water stress. Cropping system play an important role in aflatoxin production *e.g.,* monoculture and the employment of hybrids that unsuitable for the cultivation area, with low resistance to insect attack are favorable factors for aflatoxin production. The Factors that accentuate water stress, like unsuitable seeding density and timing, sandy soil, inadequate pest control and excessive nitrogenous fertilization, increase aflatoxin accumulation in the plants. Harvesting time and conditions of drying and storage can play an equally important role in aflatoxin production. A drying process is more important to control fungal activity during storage, feed should be preserved with moisture of 13% or less. It is fundamental to maintain a low and uniform grain moisture level because irregular moisture of the mass, accentuated with mixture of different lots, favors fungal development in the mass [16].

2. Direct contamination

Direct contamination of dairy products with mycotoxins results due to fungal growth used for fermentation or unintentional fungal growth. Moulds that are intentionally grown on cheese are cheese starter cultures, such as Penicillium species on French Roquefort and Camembert cheeses; under certain conditions (contamination of starter cultures with toxigenic strains or environmental contamination) these fungi are able to produce mycotoxins. Another possible contamination of dairy products is the accidental occurrence of mould on products, although good manufacturing practices will often prevent dairy products from getting contaminated [106].

Metabolism of AFB1 to AFM1

Aflatoxin M1 is the 4-hydroxy derivative of aflatoxin B1 which has a relative molecular mass of 328 Da and has the molecular formula $C_{17}H_{12}O_7$ [112]. AFB1 and its metabolites (AFM1, AFQ1, and AFP1) are excreted through the faeces, urine, and in the case of lactating mothers, also in breast milk after consumption of aflatoxin contaminated food [61]. Kiessling *et al*., (1984) stated that after ingestion, contaminated feed goes to the rumen of animals, the rumen microflora and microfauna provided the first line of defense to ruminating animals by converting mycotoxins such as aflatoxins, ochratoxin, trichothecenes, into their fewer toxic metabolites aflatoxicol, ochratoxin-alpha, and de-epoxy-trichothecenes, respectively [67]. Further studies on many other mycotoxins were carried out and it showed that not all mycotoxins are subject to enzymatic cleavage by rumen micro flora [17, 62]. The rumen protozoal population has highest capacity to detoxify the ingested mycotoxins but this may vary in different classes of mycotoxins and contribution of bacteria. The very less amount of AFB1 degraded in rumen and converted into secondary metabolite aflatoxicol [41]. AFB1 that escapes rumen degradation is converted into aflatoxin M1 (AFM1) by hepatic metabolism [69], which is excreted with milk at a transfer rate that varies from 1% to 6% [29]. The pure form of AFB1 is not mutagenic and its biotransformation in mammalian tissues is accomplished by microsomal cytochrome P450 monooxygenases and their subfamilies which are found at different concentrations in the most tissues of animals with abundance in the liver [28]. Basically, there is four pathways which describe the possible metabolism of AFB1 include, O-dealkylation to AFP1, ketoreduction to aflatoxicol, epoxidation to AFB1-8, 9-epoxide, and hydroxylation to AFM1, AFP1, AFQ1 or AFB2a [51].

Detoxification of AFB1-8, 9-epoxide and AFM1 in mammalian tissues is carried out via conjugation by glutathione and catalyzed by glutathione -S- transferase. AFB1-8, 9- epoxide is further hydrolyzed to a dihydrodiol [71, 75]. The Bovine hepatocytes metabolize AFB1 to AFM1 predominately, but there were measurable amounts of AFB1 epoxide, AFB1 dihydrodiol, and AFB1-GSH conjugates also present [68]. AFB1 epoxide exist as two stereoisomeric form which are endo- and exo-epoxides, respectively and exo-epoxide being the DNA-reactive form. AFM1 further activated to form an AFM1-8, 9-epoxide which binds to DNA and excreted into urine in form of AFM1-N7-guanine [30]. Biomarkers of AFB1 exposure include urinary aflatoxin metabolites, such as AFB1-N7-guanine and AFM1, serum AF-albumin and AFM1 in milk [108,114].

AFM1 is the primary aflatoxin metabolite in animals and human milk comprising 95% of the total amount of aflatoxins excreted in milk [61]. AFM1 excreted in to milk of lactating dairy cows is approximately equal to 1-3% of the dietary concentration of AFB1 [106]. It has been estimated that 0.09– 0.43% of dietary intake is excreted in human milk as AFM1 [120]. AFM1 is normally detected in milk within 12 h of administration of AFB1 contaminated feed to the animals [26]. A continuous daily exposure to constant levels of AFB1 results in increase of AFM1 excretion in milk for several days before achieving a steady-state, when an equilibrium between intake and excretion is established, and AFM1 excretion declines as contaminated feed is withdrawn, reaching an undetectable level after 4–5 days [47].

According to Pettersson (1998), AFB1 and AFM1 has a strong correlation, and he proposed the equation ($r^2 = 0.915$) to estimate the transfer of AFM1 in milk: [AFM1 (ng/kg milk) = 10.95 + 0.787 × (µg AFB1 intake per day)]. This equation indicates that the animals must ingest less than 50 and 25 µg AFB1 per day to comply with the European regulatory levels of contamination in milk set at 0.05 and 0.025 µg/kg of milk for adults and infants, respectively. Thus, cows must ingest less than 10 and 5 kg of feed contaminated at the maximum authorised level (5 µg AFB1/kg feed for dairy cattle) to maintain a safe level of AFM1 in milk [82]. Many studies were conducted to find the carryover of AFM1 in milk of cows ingested naturally as well as artificially AFB1 contaminated diet. Chopra *et al*., (1999) found that AFM1 excretion as % of AFB1 intake varied between 0.04- 0.6% and after complete withdrawal of AFB1 contaminated ration, AFM1 excretion in milk dropped to a negligible level (<0.01 µg/1) within 4-5 days [19]. Garg *et al*., (2004) stated that AFB1 excretion in milk as AFM1 in buffaloes (0.95 to 2.27%) was significantly lower as compared to cows (2.06 to 4.65%) [46].

Factors Affecting Carryover of AFB1 to AFM1

The extent of carry-over of AFB1 to AFM1 in dairy cows is influenced by numerous nutritional and endogenous host factors, including breed, health of the animal, hepatic biotransformation capacity, lactation stage and actual milk production [111]. The excretion of AFM1 in milk may vary between individual animals, from day to day, and from one milking to the next. The rate of AFB1 carry-over as AFM1 in milk of dairy cows was established to range from 0.3% to 6.2% [110]. The major factors that affect the AFM1 concentration in milk are animal type, stage of lactation, milk yield and season [61].

I. Animal type: The excreted amount of AFM1 in milk of dairy cow was 1-3% of ingested AFB1 [106], for ewes it ranges from 0.60 to 0.72% (with a maximum of 2.7%), and for goats 2.5 to 2.7%, respectively [95]. In Mares, the calculated mean for carryover of AFM1 with daily milk yield of 3kg was 0.04 - 0.05 %, which is around 10 times lower than that in dairy cattle suggesting a better ability of mares to degrade AFB1 [15].

Table-1 *Legislation for AFM1 levels in milk worldwide [43]*

Country	Foodstuffs	AFM_1 max. level
Austria, Belgium, Bosnia, Cyprus, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Poland, Portugal, Spain, Sweden, Turkey, United Kingdom, Saudi Arabia, United Arab Emirates, Kuwait, Oman, Qatar	Raw milk, heat-treated milk and milk for the manufacturer of milk-based products	0.050 µg/kg
Belarus, Bulgaria, Chile, Hungary, Iran, Israel, Malta, Morocco, Switzerland, Turkey	Milk	0.05 µg/kg
Argentina, Brazil, Paraguay, Uruguay	Fluid milk, Powder milk	0.5 µg/L 5 µg/kg
China [76]	Milk and milk products	0.5 µg/kg
Mexico	Pasteurized, ultra-pasteurized, sterilized and dehydrated milk, milk products	0.5 µg/L
Syria [40]	Liquid milk Dried milk (not used in baby food)	0.2 µg/kg 0.05 µg/kg
Indonesia [40]	Milk	5 µg/kg
Armenia, Barbados, Croatia, South Korea, Latvia, Peru, Romania, Russian Federation, Serbia, Singapore, Taiwan, Ukraine, Venezuela, Vietnam [40]	Milk	0.5 µg/kg
Codex Alimentarius, USA, India [57], South Africa, Kenya	Milk	0.5 µg/kg

Table-2 *Maximum limits for AFM1 in milk products*

Country	Milk (µg/kg)	Dairy products (µg/kg)	Reference
USA	0.05	0.50 (milk products)	[105]
EU	0.050	0.050 (milk products)	[36]
Iran	0.050	0.50(milk powder) 0.020 (butter& butter milk) 0.250 (cheese)	[60]
Turkey	0.050	0.250 (cheese)	[21]
Brazil	0.50	5 (milk powder) 2.5 (cheese)	[8]
Italy	0.050	0.250 (soft cheese) 0.450 (hard cheese)	[7]
China	0.5	0.5 (milk products)	[76]
Pakistan	0.05	0.05 (milk products)	[58]
Switzerland	0.050	0.250 (cheese)	[22,38]
Netherland	0.050	0.020 (butter & cheese)	[22,38]

Table-3 *Occurrence of AFM1 in animal milk samples in Europe*

Country	Collection year	Milk type	Total samples	No. and % of positive samples	C.min –C.max	Method of analysis	Reference
Austria	1999	Raw milk	20	0	< 10 ng/kg	-	[29]
France	1999	-	234	0	< 30 ng/kg	-	[29]
Germany	1999 2000	Raw milk Raw milk	6537 3618	211 (3.2%) 4 (0.1%)	< 10 - 50 ng/kg < 10 - 50 ng/kg	-	[29]
Greece	2000 -2001 2010	Raw milk Raw milk	51 196	36 (70.6%) 91 (46.5%)	> 50 ng/L 10 ng/L	TLC ELISA	[91] [103]
Italy		UTH milk Raw milk	161 12	125 (78%) 50%	<1-23.5 ng/l <3-10 ng /l	ELISA ELISA	[45] [93]
Netherland	1999	-	30	5 (16.7%)	10-50ng/kg	-	[29]
Spain	2000	Raw milk	92	5 (5.4%)	14-24.9 ng/l	ELISA	[109]
UK	2001	-	100	3 (3%)	10-50ng/kg	-	[29]

(C. min= Concentration minimum, C. max = Concentration maximum, ELISA= enzyme- linked immunosorbent assay, TLC= thin liquid chromatography)

II. Stage of lactation

AFM1 can be found in milk within 12–24 h after the first ingestion of AFB1 [15], and increased as soon as the first milking after animal ingestion with a pattern of increment up between 7th and 12th days of AFB1 ingestion [74]. The carry-over of AFM1 in the milk was higher in the early stage and it declined to in the late lactation stage in the dairy cows [110].

III. Milk yield

Milk yield is one of the major factors affecting the total excretion of AFM1. The high yielders have high concentration of AFM1 In their milk as compared to low yielders [74]. High yielding dairy cows with a production of up to 40 liters of milk per day, showed a carry-over percentage as high as 6.2 % [110].

IV. Season

The mean contamination level of AFM1 in autumn and winter was significantly higher than those of spring and summer [64], due to the fact that grass, pasture, weed, and rough feeds were found more commonly in spring and summer than in winter [18] and in winter, cows fed with greater number of concentrates or compound feed which content high amount of AFB1 [102].

Toxicity of AFM1

AFM1 can cause acute and chronic both type of toxicity, by direct ingestion of contaminated milk or dairy products and AFB1 metabolism in the liver [78]. Recent reports highlighted the occurrence of AFM1 in plants, produced by Aspergillus spp. through a different biosynthetic pattern not involving AFB1, or possibly by insect pest's metabolism from AFB1 [37, 99]. In humans, exposure of AFM1 occurs mainly through consumption of milk [117]. Acute hepatotoxicity of AFM1 was initially observed in ducklings fed with AFM1 contaminated milk [4]. After that, studies in different animal species confirmed the hepatotoxicity of AFM1 and its carcinogenic effect, although lower by about one order of magnitude as compared to AFB1 [117].

There is evidence found for *in-vivo* carcinogenic effect of AFM1 and AFB1 in rat models and *in-vitro* in murine and human liver microsomes [47]. The limited ability to metabolize AFM1 into the DNA-reactive epoxide may thus account for the reduced extent of DNA damage and pre-neoplastic lesions as compared to AFB1. The mutagenic effects of AFM1 found during *in-vitro* studies on Salmonella typhimurium strains [116], a similar genotoxic effect observed in Drosophila melanogaster with AFB1 in *in-vivo* studies, which show the possible adverse effects in mammalian cells *in vivo* [96].

Table-4 *Occurrence of AFM1 in animal milk samples in Africa*

Country	Collection year	Milk type	Total samples	No. and % of positive samples	C.min –C.max	Method of analysis	Reference
Egypt	1999-2000 2010	- Powder cow milk	15 125	3(20%) 54 (43.2%)	5000-8000 ng/l 0.3-21.8 ng/l	LC-FLD ELISA	[33] [34]
Libya	2002	Raw milk	49	35 (71.4%)	30-2680 ng/l	ELISA	[31]
Nigeria	2006	Raw milk	22	3 (13.6%)	2040-4000ng/l	TLC	[12]
Kenya	2006	Pasturized & UHT milk	613	473 (77.2%)	5-780 ng/kg	ELISA	[65]
Sudan	2009	Raw milk	44	42 (95.5%)	220-6900 ng/l	LC-FLD	[35]

Table-5 *Occurrence of AFM1 in animal milk samples in Asia*

Country	Collection year	Milk type	Total sample	No. and % of positive samples	C.min –C.max	Method of analysis	Reference
China	2006-2007 2012	UHT milk Raw & powder cow milk	233 50	112 (48%) -	21.49-95.73 ng/kg 42.9-237.4 ng/kg	ELISA LC-MS/MS	[122] [50]
India	- -	Pasteurized Raw buffalo milk	12 216	4 (32) -	164 ng/kg >500-48000ng/l	ELISA ELISA	[88] [101]
Iran	2001 2007-2008	Raw milk Raw milk UHT milk	111 75 210	85 (76.6%) 58 (78.7%) 116(5.2%)	15-280 ng/l 60.1 ng/l 8-249 ng/l	TLC ELISA ELISA	[64] [86] [86]
Japan	2001-2001 2004	UHT milk Raw milk	208 299	207 (99.5%) -	1-29 ng/kg 5-11 ng/l	LC- FLD	[77] [100]
Pakistan	2005 2012	Raw milk Raw milk	168 104	167 (99.4%) 39 (37.5%)	10-700 ng/l 4-890 ng/l	LC- FLD LC- FLD	[52] [58]
Turkey	2010	Raw milk	36	22 (61.1%)	1-10ng/kg	ELISA	[3]

Table-6 *Occurrence of AFM1 in animal milk samples in America*

Country	Collection year	Milk type	Total sample	No. and % of positive samples	C.min-C.max	Method of analysis	Reference
Argentina	1999 2007	Raw milk Raw milk	56 94	6 (10.7%) 60 (64%)	12-30 ng/l 10-70 ng/l	ELISA LC-MS/MS	[72] [5]
Brazil	1992 2004-2005 -	Pasteurized milk Pasteurized milk Pasteurized cow milk	52 12 47	4 (7.7%) 7 (58.3%) 39 (83%)	70-370 ng/l <10-200 ng/l 9-437 ng/l	LC-FLD LC-FLD LC-FLD	[24] [79] [55]
Colombia	2005	Pasteurized milk	121	96 (79.3%)	10.6- 288.9 ng/l	LC-FLD	[27]
Mexico	-	Raw milk	40	32 (80%)	6-65 ng/l	ELISA	[90]

Table-7 *Occurrence of AFM1 in human milk*

Country	No. of sample	No. of positive sample	Range (µg/l)	Reference
Turkey	50 (Samsun and neighbor provinces) 61 (Istanbul) 75 (Ankara) 73 (Eastern Turkey)	33 (66%) 8(13.1%) 75 18 (24.6%)	0.038-0.0943 0.0051-0.0069 60.90-299.9 0.001-0.006	[2] [66] [49] [13]
UAE	140	92%	-	[1]
Zimbabwe	54	6(11%)	>0.05	[113]
France	42	none	-	[113]
Kuwait	12	5	0.0883-0.0152	[23]
Brazil	100	2	0.03-0.08	[56]
Australia	73	11 (15%)	0.028-1.031	[32]
Thailand	11	5	0.039- 1.736	[32]
Iran	160(Tehran) 182(Tabriz- Iran)	157 (98.1%) 11%	0.0003-0.0267 0.00510.008	[92] [73]
Egypt	388	36%	0.0103-0.022	[63]

Initially, AFM1 was categorized as group 2B human carcinogen by IARC [53], Further research and studies allow to reclassify AFM1 as a group 1 human carcinogen [54]. AFB1 must be converted into its reactive epoxide to bind protein and exert acute toxic effects, but this process does not seem crucial to the cytotoxicity of AFM1. AFM1 direct cytotoxicity was revealed in cultured human intestinal enterocytes- Caco-2 and cytotoxicity results with intracellular reactive oxygen species (ROS) generation [121]. AFM1 may cross the placental barrier from the pregnant mother and thus affect the foetus by exposing the newborn to aflatoxin risk. The presence of aflatoxins in human cord sera found at the birth and in serum obtained immediately after birth from mother [25], reveals the transfer of aflatoxin by the feto-placental route, which may be of biological importance. The presence of AFM1 in milk and milk products, is worldwide concern for humans as even small amounts of AFM1 is important for consumers, especially children's, who are most susceptible to the adverse effects of aflatoxins [44].

The young children who weaned on cow's milk at an early age; consumption of milk contaminated with AFM1 may reduce the development of their immune competence and making them more susceptible to other diseases [47].

Determination of AFM1 in MILK

It is essential to control the sources of contamination using rapid, sensitive, reliable and cost-effective methods. A number determination methods of AFM1 have been developed which can be classified as two main groups: chromatographic methods and immunochemical methods [61]. A liquid chromatographic technique, namely Thin Liquid Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) predominantly used for aflatoxin analysis as they are of low molecular weight, possess significant UV absorption and fluorescence properties [64, 95]. Various Immunochemical methods are used for rapid screening of aflatoxins, such as, enzyme-linked immunosorbent assay (ELISA), immunoaffinity column assays (ICA), sequential injection immunoassay (SIIA) and radioimmunoassay (RIA). However, ELISA based techniques are mainly used for rapid mycotoxins screening [104], but there are disadvantages of false-positive results and unacceptable quantification accuracy, therefore confirmatory analysis is required for aflatoxin determination [89]. There are many rapid screening ELISA test kits available commercially for qualitative confirmation of aflatoxin in various food and milk samples.

Effect of Processing of Milk on AFM1 Concentration

Milk, as a liquid, is a highly perishable product that rapidly loses its quality and spoils if not to be treated. Since, milk is processed in numerous ways, the effects of storage and processing on stability and distribution of AFM1 are of great concern. AFM1 is relatively stable in raw and processed milk and milk products as AFM1 resistant to thermal inactivation during milk processing like pasteurization and autoclaving [84]. Many experiments were carried out to study the effects of various heat treatment on stability and distribution of AFM1 in processed milk. Choudhary *et al*., (1998) revealed that, sterilization of milk at 121°C for 15 minutes degrade 12.21% of AFM1, whereas boiling decreased 14.5 % AFM1 in milk respectively [20]. Pasteurization of milk can cause decrease in the level of AFM1 at the rate of 7.62% [14]. AFM1 also found in ultra-heat treatment (UHT) milk samples [97]. However, depending up on the conditions employed to heat the milk like temperature and time of heating can decrease 12-35% AFM1 content of the milk [83].

Mitigation of AFM1 from Milk

To minimize risks associated with unavoidable exposure to aflatoxins, regulation and monitoring measures must be supported by in-field (pre-harvest) and storage (post-harvest) interventions which may be applied to minimize aflatoxin contamination. AFM1 is excreted in milk of dairy animals following ingestion and metabolism of AFB1 contaminated feed. Contamination of milk may, thus, be reduced either directly, decreasing AFM1 content of contaminated milk, or indirectly, decreasing AFB1 contamination in feed of dairy animals [47].

Direct methods for mitigation AFM1 in milk

Various chemical, biological, radiation and Toxin adsorbents methods are used for direct reduction or mitigation of aflatoxin in milk .Use of toxin adsorbents is one of the main methods to reduce AFM1 in milk. Adsorbents like bentonite, vermiculite, hydrated sodium calcium aluminosilicate (HSCAS) and active carbon are known for absorbing various aflaoxins [118]. For the chemical treatment peroxide, benzoyl peroxide and sulphite are used to eliminate AFM1 from milk [6, 9]. The ultraviolet radiation to the milk for 20 minutes effective for reduction the AFM1 up to 65-67% in milk, however ultraviolet radiation with peroxide treatment is more effective for reduction of AFM1 in milk [119]. Use of bacteria (*Flavobacterium aurantiacum*) and probiotics such as lactic acid bacteria (LAB) and Saccharomyces spp. are effective for elimination of AFM1 from milk as they have affinity to bind with aflatoxins [47].

Indirect Methods for Mitigation of AFM1 into Milk

Milk contaminated with aflatoxins is produced next to use of contaminated feedstuff. Therefore, reduction in AFM1 in milk indirectly via control of livestock feed hygiene is possible. To achieve the aim, it is essential to apply hygiene principles and health considerations on farming and production of crops, livestock feed factories and storage of livestock feed. Use of aflatoxin binders such as dietary clay minerals (alumino silicates, bentonite, montmorillonite, Zeolite *etc.*), activated carbon / activated charcoal, complex carbohydrates (cellular wall of yeasts and bacteria, glucomannan) in feed is very effective way to mitigate AFM1 from milk via bind AFB1 with binders and reduce the absorption of AFB1 in animal body [118]. Sodium bentonite (1%) and activated charcoal (1%) in goat feed can significantly reduce the excretion rate of AFM1 by 66% and 75% and also reduce carry over percentage of AFM1 in goat milk by 65% and 76%, respectively [87]. Many of researchers concluded that, Sodium bentonite is effective aflatoxin binder for dairy animals as it significantly eliminates the AFM1 in milk of dairy cows [26, 81]. The hydrated sodium calcium aluminoilicates (HSCAS) are highly selective and effective aflatoxin binders for dairy animals as they have high binding affinity and capacity with aflatoxins which may reduce absorption of AFB1 in animal body, preventing toxin distribution and metabolism thus reducing the carryover in milk. Kutz *et al*., (2009), included two different HSCAS (marketed as Solis and Novasil plus) in dairy cow's diet, suggested that, inclusion of HSCAS at 0.5% in cow's diet significantly reduce the AFM1 excretion in milk 36% and 35%, respectively [70]. Montmorillonite, a clay mycotoxin adsorbent added at the rate of 1% dairy cow's diet, significantly reduce the excretion if AFM1 in cow milk [85]. Use of HSCAS at 2% and 4% in goat's diet, results in significant decrease the AFM1 excretion in goat milk [98]. The yeast cell wall extracts (Saccharomyces cerevisiae) are effectively used as aflatoxin binders by many researchers and suggested that the yeast cell wall extracts are good microbial entero-adsorbents for dairy animals. Firmin *et al*., (2011) concluded that, use of yeast cell wall extracts (Saccharomyces cerevisiae) in dairy ewes reduce the excretion and carryover of AFM1 in ewe's milk [42]. Use of microbial binders for aflatoxin is a promising strategy to reduce chronic low-level exposure to AFM1 in milk by effective and specific natural binders which may also deliver benefits as probiotics. However, essential to conduct further researches for mitigation of AFM1 in liquid milk by developing effective decontamination processes to eliminate AFB1 from animal feed.

Conclusion

Aim of this review was to discuss the sources, metabolism, carryover, determination, international legislation, occurrence and mitigation of AFM1 in liquid milk. There is wide variation between the levels of AFM1 in liquid milk in worldwide may be due to different climate condition, geographical area, feeding practices and level of contamination. Therefore, to obtain a low level of AFM1 contamination in liquid milk, milk products and animal feed should be evaluated and controlled continuously by developing simple, rapid, economic and accurate method for aflatoxin determination. Presence of AFM1 in human milk is of great concern as it can cause serious health hazard to mother, foetus and infant as well. To prevent a public health hazard, regular periodic survey of milk is must. Moreover, scientific evidence is also required for the harmful effects resulting from chronic exposure to low levels of AFM1. Good agricultural practices, different heat treatment and dietary interventions by aflatoxin binders, can reduce but not complete eliminate the contamination of milk with AFM1. These decontamination methods can be implemented as a part of prevention and control of food safety and quality assurance to reduce the public health hazards, however, further research should be conduct for development of effective decontamination processes.

Application of Research: For the development of effective methods for determination and elimination of AFM1 form milk and milk products and prevention of health hazards related to aflatoxins.

Research Category: Veterinary Science and Animal Husbandry

Abbreviations: AFB1- Aflatoxin B1, AFB2 -Aflatoxin B2, AFG1- Aflatoxin G1, AFG2- Aflatoxin G2, AFM1- Aflatoxin M1, AFM2- aflatoxin M2, , AFP1- Aflatoxin P1, AFQ1- Aflatoxin Q1, AFB2a – Aflatoxin 2a, AFs- Aflatoxins, HSCAS- hydrated sodium calcium aluminosilicate, TLC- Thin Liquid Chromatography, HPLC- High Performance Liquid Chromatography, C. min- Concentration minimum, C. max - Concentration maximum, ELISA- Enzyme- Linked Immunosorbent Assay, LC-FLD- Liquid Chromatography Fluorescence Detector, LC MS/MS= Liquid Chromatography with tandem Mass Spectrometry, ICA- Immunoaffinity Column Assays, SIIA- Sequential Injection Immunoassay, RIA- Radioimmunoassay

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****Principal Investigator or Chairperson of research: Dr S N Patel**
University: Kamdhenu University, Rajpur (Nava), Himmatnagar, 383010, India
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