Mycotoxins in Bovine Milk and Dairy Products: A Review

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Abstract: This paper presents a literature review of the occurrence of several mycotoxins in bovine milk and dairy products, because it is the main type of milk produced and marketed worldwide. Mycotoxins are produced by different genera of filamentous fungi and present serious health hazards such as carcinogenicity and mutagenicity. Under favorable growth conditions, toxigenic fungi produce mycotoxins which contaminate the lactating cow's feedstuff. During metabolism, these mycotoxins undergo biotransformation and are secreted in milk. Data show that there is a seasonal trend in the levels of mycotoxins in milk, with these being higher in the cold months probably due to the prolonged storage required for the cattle feeds providing favorable conditions for fungal growth. Good agricultural and storage practices are therefore of fundamental importance in the control of toxigenic species and mycotoxins. Although aflatoxins (especially aflatoxin M_1) are the mycotoxins of greater incidence in milk and dairy products, this review shows that other mycotoxins, such as fumonisin, ochratoxin A, trichothecenes, zearalenone, T-2 toxin, and deoxynivalenol, can also be found in these products. Given that milk is widely consumed and is a source of nutrients, especially in childhood, a thorough investigation of the occurrence of mycotoxins as well the adoption of measures to minimize their contamination of milk is essential.

Keywords: cow's milk, occurrence, toxicity

Introduction

Mycotoxins are a group of highly toxic compounds produced by fungi or yeast, which commonly develop in places with low water availability and unsuitable for bacterial growth (Jouany 2001). The preparation and storage methods of animal feedstuffs, such as grains and silage, can propitiate mycotoxin contamination (Bryden 2012). When animals ingest contaminated foodstuffs, mycotoxins are metabolized, biotransformed, and transferred to animal products, such as milk or meat, thus becoming a risk to human health (Bruerton 2001).

The concern for mycotoxin contamination in dairy products began in the 1960s, with the 1st reported cases of contamination by aflatoxin M_1 (AFM_1), which is an aflatoxin B_1 (AFB_1) metabolite produced in the animal rumen and secreted in milk. During the 1960s, the intake of feed contaminated with AFB_1 was relatively high, milk production was low, and analytical methods were poorly developed. Studies have indicated that values between 0.3% and 6.2% of AFB_1 in animal feed is metabolized, biotransformed, and secreted in milk in the form of AFM_1, although the rate of biotransformation varies between animals and other factors, including nutritional and physiological, such as diet, rate ingestion, digestion rate, animal health, biotransformation capacity liver, and dairy animal production (Creepy 2002; Unusan 2006; Iqbal and others 2013, Duarte and others 2013).

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Other mycotoxins, such as ochratoxin (OTA; Breitholtz-Emanuelsson and others 1993; Boudra and others 2007; Pattono and others 2013), zearalenone (ZEN; Yiannikouris and Jouany 2002; Coffey and others 2009; Huang and others 2014), fumonisins (Gazzotti and others 2009), T-2 toxin and deoxynivalenol (DON; Swanson and Corley 1989; Sorensen and Elbæk 2005), have also been identified in milk samples and dairy products, although these have not been as extensively studied as AFM_1, and despite the amount of these mycotoxins and its conjugated derivatives in milk being small, the shortage of reports is of concern since milk is a source of nutrients, particularly in children.

Cow milk is the main milk type used for human consumption corresponding to 83% of world milk production, followed by buffalo milk with 13%, goat milk with 2%, sheep milk with 1% and camel milk provide 0.3%. The remaining share is produced by other dairy species such as equines and yaks (FAO 2015).

Thus, other than the most commonly found mycotoxin, AFM_1, there is a wide range of milk and dairy product contaminants; therefore, this study reviews the contamination and incidence of various mycotoxins in bovine milk and dairy products intended for human consumption.

Mycotoxin Contamination of Milk

Milk quality, in relation to toxic contaminants, is directly related to the type and quality of animal feed following the metabolism of mycotoxins and their subsequent excretion in milk (Jobim and others 2001). The level of excretion of mycotoxins in milk is generally low and is affected by the molecular weight and lipophilicity of a given mycotoxin. The transport rate is also influenced by the pH gradient between the blood plasma and milk, which changes according to the animal's health status (Yiannikouris and Jouany 2002; Kalac 2011). Mycotoxin absorption by the mammary gland can occur through intercellular filtration, passive diffusion across the cell membrane, or active transport (Jouany 2001; Yiannikouris and Jouany 2002), it depends on the mycotoxin, their ionic state, among others.

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When the animal feed is supplemented with silage, the main mycotoxin contaminants are DON and ZEA. Contamination contents can be reduced by the rumen microbiota in healthy animals, thus reducing the risk of milk contamination (Kalac 2011). The rumen fluid, containing protozoal microbiota and rumen bacteria is considered the first defense system against mycotoxins such as ZEA, OTA, T-2 toxin, and diacetoxyscirpenol (DAS), but is ineffective for AFB_1, fumonisin, and patulin (Kiessling and others 1984; Obremski and others 2009; Prandini and others 2009). Nevertheless, the rumen barrier can be altered by animal diseases, changes in the diet or high mycotoxin contamination in the animal feed. An example is that different feed destiny to dairy cows with high genetic potential for milk production could induce a lowering of rumen pH which, in its turn, may also influence the rate of OTA degradation (Pattono and others 2011).

Legislation

Many countries have regulations to control AFB_1 levels in foodstuff and to establish the maximum permissible levels of AFM_1 in milk and dairy products in order to reduce disease risks (Rastogi and others 2004), as described in Table 1.

Mycotoxin Occurrence

Aflatoxins in milk

Aflatoxins are polycyclic structures belonging to the furanocoumarin class of compounds, which are hepatotoxic, carcinogenic, and immunosuppressive fungal metabolites (Williams and others 2004) mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Prandini and others 2009). When lactating mammals, such as cows, are fed with AFB_1-contaminated feed, the ingested mycotoxin is metabolized in the liver by cytochrome P450 enzyme into several metabolites (Figure 1). Through hydroxylation of the tertiary carbon of the di-furanocoumarin ring, AFB_1 is converted to its major metabolite, AFM_1 (Faletto and others 1988). Due to the hydroxyl group, these compounds are very soluble in water, allowing their rapid excretion through urine, bile, feces, and milk (Van Egmond 1983).

AFM_1 is thermally resistant and is not completely inactivated by pasteurization, sterilization, or other milk treatment processes (Assem and others 2011). Thus, because milk is a primary nutrient of significant human consumption, especially by children, control of the incidence of AFM_1 in this food is of paramount importance.

Although AFM_1 is the most frequent aflatoxin in milk (Coffey and others 2009), the concept that AFB_1 is completely converted to AFM_1 is refuted by some studies. Carvajal and others (2003) reported the presence of AFB_1 (0 to 0.4 μg/L) in heat-treated milk samples and Scaglioni and others (2014) detected in pasteurized and UHT milk, with averaging contamination of 1.476 and 0.690 μg/L, respectively. Because AFB_1 is more toxic than AFM_1 (Zain 2011), the presence of AFB_1 in milk should also be examined. Aflatoxin M_2 (AFM_2), another AFB_1 metabolite, has also been investigated in milk, although a previous study did not detect it (Garrido and others 2003), Sartori and others (2015) detected, respectively, in 17 (24%) of the powdered milk samples (>0.08 μg/kg) and in 3 (18%) of the UHT milk samples (>0.009 μg/kg) commercialized in Brazil; Lee and Lee (2015) also detected AFM_2 in UHT and pasteurized milk.

Factors of influence in AFM_1 milk contamination

AFM_1 contamination in milk and dairy products shows variations according to geographical region, season, environmental conditions, level of development, farming systems diversity, green forage availability, and consumption of feed concentrates, among others (Tajkarimi and others 2008; Dashti and others 2009; Ghazani 2009; Pei and others 2009; Bilandzic and others 2010; Heshmati and Milani 2010; Rahimi and others 2010; Xiong and others 2013). It has been shown that milk produced during the warm seasons is less contaminated by AFM_1 than that produced in the cold seasons (Ghiasian and others 2007; Peng and Chen 2009). According to Dasthi and others (2009), the higher contamination incidence in the cooler months is due to the prolonged storage of cattle feed, which provides favorable conditions for fungal growth. Furthermore, environmental factors, such as temperature and humidity, agricultural products used in feed and seasonal effects in the country of origin can all affect feed AFB_1 contamination levels (Dasthi and others 2009).

Xiong and others (2013) evaluated raw milk contamination during different seasons and found that the occurrence of AFM_1 in milk was significantly higher during winter (0.123 μg/L; $P < 0.05$) compared to the remaining seasons, that there was no significant difference (spring 0.029 μg/L, summer 0.032 μg/L, and autumn 0.032 μg/L; $P > 0.05$). In Croatia, Bilandzic and others (2010) also verified the concentration distribution of AFM_1 in milk during the different seasons and found that the concentration was statistically higher between January and April (0.036 to 0.059 μg/L), corresponding to winter and spring, than between June and September (0.012 to 0.015 μg/L), corresponding to summer and autumn. According to the authors, fresh feed, such as pasture, grass, weeds, and raw feed, is available during the spring and summer. However, during the cold months, dried or concentrated preparations are frequently used. Furthermore, in the cold seasons, animals are often fed with dry hay which, in inappropriate storage conditions, can lead to the appearance of aflatoxigenic fungi and aflatoxins (Kamkar 2005; Tajkarimi and others 2008; Heshmati and Milani 2010).

Through monitoring of AFM_1 levels in milk, Veldman (1992) demonstrated that the AFB_1 conversion rate can be up to 6.2%, with means of 1.8%. Because manufacturing processes do not affect the concentration of AFM_1 mainly due to its thermal stability, the most effective method to control AFM_1 concentration in milk is by reducing AFB_1 contamination of raw materials and cattle feed through the application of Good Agricultural and Storage Practices (Diaz and Espitia 2006; Nuryono and others 2009; Prandini and others 2009; Bilandzic and others 2010; Elzupir and Elhussein 2010; Rahimi and others 2010).

Han and others (2013) observed that, although all analyzed feed samples destined for cattle were within the AFB_1 levels allowed by the European Commission (EC; 5 μg/kg), the AFM_1 content in 3 out of 200 milk samples was above the maximum established levels by the EC (0.050 μg/L). Battacone and others (2009) also reported that there is no guarantee that the AFM_1 concentration in milk will always be lower than the EC legal limit when levels of AFB_1 in animal feed are within the limits.

In a study conducted in Portugal, higher levels of AFM_1 were detected in milk samples produced in the Azorean islands than in continental samples (Duarte and others 2013). According to the authors, this suggests the existence of local factors related to feed storage time and conditions that deserve more attention and may influence analysis. In the Azores, supplementation with conserved forages, mainly corn silage and grass, is needed given the cattle energy requirements and the shortage of seasonal pasture during the summer at low altitudes and during winter at high altitudes (Rego 2010). It is considered that milk production from fresh

Table 1–International limits for AFM_1 in milk and dairy products for human consumption.

Country	Raw milk (μg/kg)	Dairy derivatives (μg/kg)
EUROPEAN UNION	0.05	0.05
Germany	0.05	–
Belgium	0.05	–
Italy	–	0.01 (foods for children)
Sweden	–	0.05 (fluid milk foods)
Uruguay	0.5	0.5
Barbados	0.05	–
Cyprus	0.5	0.5
Mauritius	10	10
Áustria	–	–
	0.05; 0,01(infant pasteurized milk)	0.02 (butter); 0.25 (cheese); 0.40 (powder milk)
France	0.05; 0,03 (for children <3 years old)	0.05; 0.03 (for children <3 years old) (powder milk)
Switzerland	0.05	0.025 (whey and its products); 0.25 (cheese); 0.02 (butter); 0.10 (powder milk)
Bulgaria	0.50	0.1 (powder milk); 0 (powder milk and infant foods); 0.02 (infant foods)
Romania	0	–
Czech Republic	0.50	5; 0.1 (infant foods)
EUA	–	0.50
Brazil	*–	0.50 (fluid milk); 5.0 (powder milk); **2.5g/kg (cheese)
Argentin	0.05	0.50; 0.05 (powder milk)
Honduras	0.05	0.25 (cheese); 0.02 (infant foods)
Nigeria	1	–
Egypt	0	0
Turkey	0.05	0.25 (cheese)
MERCOSUL	0.5	5.0 (powder milk)

Source: Adapted from Kaniou-Grigoriadou and others (2005).
[a]There is no legislation for raw milk in Brazil.
[b]Limit set by Brazil (2011).

pasture cattle presents a low risk of aflatoxin incidence (Fink-Gremmels 2008a; Motawee and others 2009) and that the risk is higher in milk from cattle fed concentrated feed (Duarte and others 2013).

Still, some studies emphasize that carry-over of AFB_1 is higher in cow's milk (0.03%) which sheep´s and goat´s milk, probably due extensive variability in the expression and catalytic activity of hepatic enzyme families (for example, cytochrome P450 and glutathione-transferase) involved in the biotransformation and detoxification of AFB1 among this species (Battacone and others 2003; Diaz and others 2004; Hassain and Kassaify 2014).

Occurrence of AFM_1 in milk

The occurrence of AFM_1 has been reported in various locations worldwide (Table 2), with many of the studies raising concerns about AFM_1 contamination levels being higher than the maximum level set by European Union (EU) legislation (0.05 μg/kg; EC 2001). Nevertheless, AFM_1 levels are often lower than the maximum limits (0.5 μg/kg for fluid milk) established by national authorities, including the Institute of Standards and Industrial Research of Iran (ISIRI 2002), the Turkish Food Codex (TFC), the National Health Surveillance Agency in Brazil – ANVISA (Brasil 2011), and the Food and Drug Administration in the U.S.A. (FDA 1996), among others.

Collected data from studies conducted in recent years, evidence the incidence of AFM_1 in milk samples and milk products is relatively lower in European countries (Portugal, Turkia, Italy, and Croatia), independent of the sample type. In contrast, in studies in Asiatic countries like China, Thailand, and Taiwan were observed frequency of occurrence of mycotoxins in up to 100% of samples.

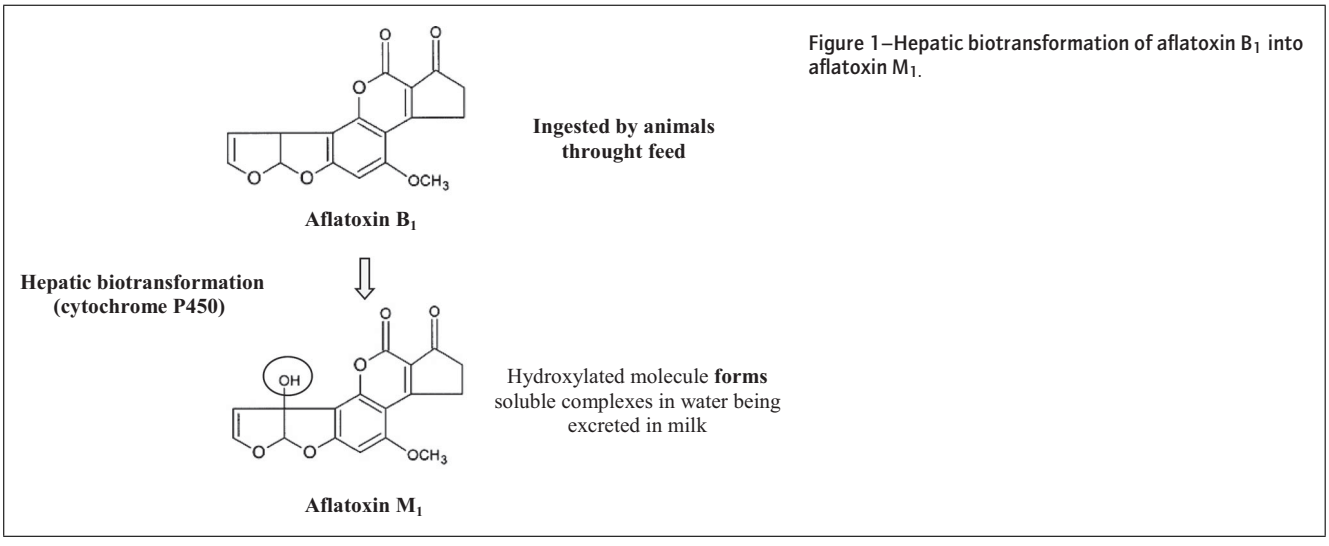


Figure 1–Hepatic biotransformation of aflatoxin B_1 into aflatoxin M_1.

Table 2–Occurrence of AFM_1 contamination in bovine milk.

Country	Sample	Frequency (%)	Min–Max (μg/L)	Reference
Serbia	Pasteurized milk	(35/36) 97.2	0.06–1.2	Kos and others (2014)
	UHT milk	(69/70) 98.5	0.02–0.41	
	Organic milk	(6/6) 100	0.01–0.08	
	Raw milk	(40/40) 100	0.08–1.2	
	Raw milk	(382/678) 56,3	0.282–0.358	Tomasevic and others (2015)
	Heat treated milk	(143/438) 32,6	0.09–0.145	
Lebanon	Raw milk	(28/38) 73.6	0.0026– 0.126	Assem and others (2011)
	Pasteurized milk	(17/25) 68.0	0.0033–0.084	
	Powder milk	(5/14) 35.7	0.0092–0.016	
Spain	UHT milk	(68/72) 94.4	0.002–0.014	Cano-Sancho and others (2010)
Brazil	Pasteurized milk	(26/30) 87	0.009–0.437	
	UHT milk	(13/17) 76	0.008–0.215	
	Milk with additives	(13/17) 76	0.009–0.061	Iha and others (2013)
	Powder milk	(12/12) 100	0.02–0.76	
	Infant formula	(0/7) 0	–	
	Fluid milk	(26/65) 40	0.009–0.069	Jager and others (2013)
	Powder milk	(2/4) 50	0.5–0.81	
	UHT milk	(23/75) 30.7	1.0–4.1	Oliveira and others (2013)
	UHT milk	(133/152) 87.5	0.002–0.121	Silva and others (2015)
South corea	Raw milk	(48/100) 48	0.002–0.08	Lee and others (2009)
Portugal	UHT and pasteurized milk	(11/40) 27.5	0.007–0.07	Duarte and others (2013)
Italy	Infant formula	(2/185) 1,1	0.012–0.015	Meucci and others (2010)
Thailand	Raw milk	(240/240) 100	0.05–0.101	Ruangwises and Ruangwises (2010)
Turkey	Fluid milk	(43/50) 86	0.001–0.030	Ertas and others (2011)
	UHT milk	(75/129) 58.1	0–0.544	Unusan (2006)
	UHT milk	(67/100) 67	0.01–0.63	Tekinsen and Eken (2008)
Morocco	Pasteurized milk	(54/61) 88.8	0.001–0.117	Zinedine and others (2007a)
Índia	Infant formula	(17/18) 94	0.143–0.77	Rastogi and others (2004)
Taiwan	Pasteurized milk	(44/48) 90.9	0.002–0.083	Rastogi and others (2004)
Iran	Raw milk	(85/111) 76.6	0.015–0.28	Kamkar (2006)
	Pasteurized milk	(83/116) 71.5	0.006–0.528	Fallah (2010)
	UHT milk	(68/109) 62.3	0.006–0.516	
China	Raw milk	(12/12) 100	0.16–0.5	Pei and others (2009)
	Raw milk	(45/200) 32.5	0.005–0.06	Han and others (2013)
	UHT milk	(84/153) 54.9	0.006–0.16	Zheng and others (2013)
	Pasteurized milk	(25/26) 96.2	0.023–0.154	
Sudan	Raw milk	(42/44) 95.5	0.22–6.9	Elzupir and Elhussein (2010)
Pakistan	Raw milk	(76/107) 71	0.004–0.845	Iqbal and Asi (2013)
Croatia	Raw milk	(72/3543) 2	0.006–0.027	Bilandzic and others (2015)

In Brazil, due to its large extent, differences in climate and production of milk and dairy products, the results of frequency vary as the percentage of occurrence of the mycotoxin (30.7% to 100%) and about the levels of contamination found (0.0018 to 4.1 μg/L).

In a study carried out by Fallah (2010), in samples collected in Iran, when considering the AFM_1 limits established in milk by the FDA and ISIRI (0.5 μg/kg), only 1.7% of pasteurized milk samples and 2.7% of ultra-high temperature (UHT) milk samples were above the stipulated levels. However, according to the limits set by the EC (0.05 μg/kg), 27% and 17% of pasteurized and UHT milk samples, respectively, were above the acceptable limits. In Syria, 80% and 60% of pasteurized milk samples exceeded the limits set by the EC and FDA, respectively (Ghanem and Orfi 2009). In studies carried in Turkey, 47% and 3.2% of the UHT milk samples had levels above the limits allowed by EC and FDA, respectively (Unusan 2006), and 31% of UHT milk samples showed levels above the EC regulation (Tekinsen and Eken 2008). Furthermore, 64% (Çelik and others 2005) and 47% of UHT milk samples (Unusan 2006) exceeded 0.05 μg/kg. In Iran, 78% (Oveisi and others 2007), 80% (Kamkar and others 2008), and 18% (Alborzi and others 2006) of milk samples were detected to have AFM_1 concentrations greater than 0.05 μg/kg and Gholampour and others (2008) examined 111 cartons of pasteurized and UHT milk produced during the winter and found that 100% of the samples were contaminated with levels above the EC limits. In Sudan, 83.3% of raw milk samples showed substantially higher levels than the maximum allowed by the FDA (0.5 μg/kg), whereas 100% of the samples exceeded the upper limit set by the EC (Elzupir and Elhussein 2010).

In Brazil, Pereira and others (2005) detected AFM_1 in 38% of pasteurized milk samples with an average level of 0.059 μg/L, but the concentrations obtained were within the maximum limits established by Brazilian regulations (0.5 μg/L). However, recent results obtained by Oliveira and others (2013) are quite alarming, since 31% of UHT milk samples showed very high levels of AFM_1, ranging from 1.0 to 4.1 μg/L, which are equivalent to twice or more of the maximum tolerable limit by Brazilian regulations. Other studies conducted in Brazil detected the presence of AFM_1 in 95% (0.01 to 0.2 μg/L; Shundo and others 2009), 40% (0.009 to 0.069 μg/L; Jager and others 2013), 53% (mean level of 0.074 μg/L; Pereira and others 2005), and 24% (mean level 0.680 μg/L; Sassahara and others 2005) of milk samples collected in São Paulo, Pirassununga, Lavras, and northern Paraná, respectively.

Occurrence of AFM_1 in dairy products

The variations in AFM_1 concentration in cheese and other dairy derivatives observed in different studies (Table 3) may be explained by the different levels of milk contamination, cheese

Table 3–Occurrence of AFM_1 contamination in dairy products.

Country	Sample	Frequency (%)	Min–Max (μg/kg)	Reference
Kuwait	White cheese	(32/40) 80	0.024–0.452	Dashti and others (2009)
Turkey	Cheese	(68/72) 94.4	0.012–0.378	
	Yoghurt	(28/50) 56	0.0025–0.078	Ertas and others (2011)
	Dairy dessert	(26/50) 52	0.0015–0.08	
	Butter	(92/92) 100	0.01–7.0	Tekinsen and Uçar (2008)
	Cream cheese	(99/100) 99	0–4.1	
	Yoghurt	(70/80) 87.5	0.01–0.475	Atasever and others (2011)
Iran	White cheese	(93/116) 80.1	0.052–0.745	Fallah and others (2009)
	Cream cheese	(68/94) 72.3	0.058–0.785	
	Lighvan cheese	(49/75) 65.3	0.03–0.313	Fallah and others (2011)
	Cheese	(47/88) 53.4	0.082–1.254	Rahimi and others (2009)
	White cheese	(30/50) 60	0.041–0.374	Tavakoli and others (2012)
	Feta cheese	(66/80) 82.5	0.15–2.41	Kamkar (2006)
Libya	Cheese	(15/20) 75	0.11–0.52	Elgerbi and others (2004)
Brazil	Cheese	(3/10) 30	0.091–0.3	Jager and others (2013)
	Yoghurt	–	–	
Greece	Feta cheese	(0/54) 0	–	Kaniou-Grigoriadou and others (2005)
Pakistan	White cheese	(93/119) 78	0.004–0.595	
	Cream cheese	(89/150) 59	0.004–0.456	Iqbal and Asi (2013)
	Butter	(33/74) 45	0.004–0.413	
	Yoghurt	(59/96) 61	0.004–0.616	
Serbia	Milk products	(122/322) 37,8	0.268–0.952	Tomasevic and others (2015)

manufacturing practices, types of cheese, cheese ripening conditions, and the analytical methods employed (Sarimehmetoglu and others 2004; Fallah and others 2011). Considering the preferred affinity of AFM_1 for the casein fraction, a high concentration of this toxin in the curd may occur during the cheese manufacturing process (Sengun and others 2008), resulting in a 3-fold higher AFM_1 concentration in many soft cheeses and a 5-fold higher concentration in hard cheeses compared to the milk from which they were produced (Ardic and others 2009). The occurrence of AFM_1 in samples of dairy products such as cheese, yogurts, and butters is considerably high in several regions of the world. As shown in Table 3, except for samples in Greece, other studies showed occurrence frequency of the mycotoxin up to 100%, with contamination reaching 7.0 μg/L (butter sample originated in Turkey).

Tavakoli and others (2012) observed a seasonal variation in cheese contamination, where the samples collected in winter were significantly ($P < 0.03$) higher than those collected during the summer.

Iha and others (2013) evaluated the stability of AFM_1 in dairy products and observed that there was a 3.2% decrease in AFM_1 concentration after 28 d in the Minas Frescal cheese, the authors attributed this decrease to possible analytical variations. These results are similar to those in the study by Oruc and others (2006), in which AFM_1 was seen to remain stable in 2 types of cheese (Kashar cheese for a period of 60 d and canned white cheese for 90 d) during the storage and maturation periods.

Govaris and others (2002), studying AFM_1 stability in artificially contaminated yogurt during 4 wk of storage at 4 °C and at 2 different pH values (4.0 and 4.6), observed a 6% concentration decrease in yogurt, which are conflicting results to those obtained by Iha and others (2013). The results showed that, at pH 4.6, AFM_1 levels did not change significantly ($P > 0.01$); however, yogurt at pH 4.0 showed a significant decrease in AFM_1 concentration ($P < 0.01$) after the 3rd and 4th wk of storage at both concentration levels (0.05 and 0.1 μg/L), probably associated with the low pH. Govaris and others (2002) further reported that during yogurt fermentation, AFM_1 levels decreased significantly from the initial values in milk and attributed this to different factors such as low pH, formation of organic acids, the presence of *Lactobacillus* sp.

Studies have shown that lactic acid bacteria inhibit the biosynthesis of aflatoxins, and can even remove various mycotoxins either through the accumulation of fermentation products (lactic acid, diacetyl, acetaldehyde, acetic acid, hydrogen peroxide, bacteriocins, or hydrogen) reaching inhibitory levels in certain foods and beverages (Onilude and others 2005; Bovo and others 2012), or through their ability to bind mycotoxins (Dalié and others 2010). *Saccharomyces cerevisiae*, the yeast widely used in food fermentation, has been studied as a potential agent for mycotoxin decontamination in food (Shetty and Jespersen 2006).

Fumonisin

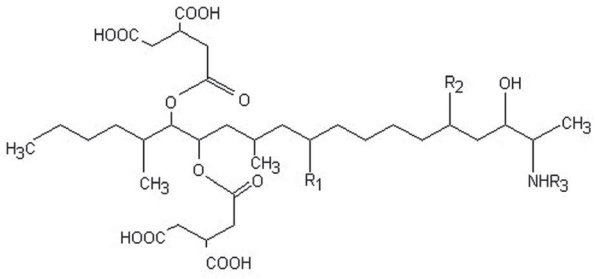
Fumonisins were discovered in 1988 through their isolation in cultures of *Fusarium verticillioides* (Gelderblom and others 1988), being also produced by *F. proliferatum* and other *Fusarium* species (Glenn 2007) like *F. fujikuroi* (Proctor and others 2004), *F. globosum* (Marasas and others 2004), *F. oxysporum* (Proctor and others 2004), and *F. nygamai* (Leslie and others 2005). Animal and human health problems related to these mycotoxins are almost exclusively associated with the consumption of contaminated maize or its derivatives (Bolger and others 2001; Wang and Merril 2004; Abbas and others 2006). Eighteen different types of fumonisins have been isolated and identified all differing in their hydroxyl groups (Figure 2). The most toxigenic and predominant molecular form, produced by *Fusarium moniliforme*, is fumonisin B_1 (FB_1; Seo and Lee 1999), which, together with fumonisin B_2 (FB_2), is responsible for about 70% of all fumonisin found in nature and food (Seo and others 2001; Niderkon and others 2009). FB_1 is classified by the Intl. Agency for Research on Cancer as a Group 2B possible carcinogen to humans.

Ruminants appear to be tolerant to FB_1 because this mycotoxin is barely metabolized by rumen microbiota of the liver (Caloni and others 2000; Spotti and others 2001a). However, the transfer of these contaminants to milk has been previously confirmed (Maragos and Richard 1994; Hammer and others 1996; Spotti and

others 2001b), although some studies found no FB_1 contamination in milk (Scott and others 1994; Richard and others 1996). Values of 0.05% have been reported for the secretion of FB_1 in milk, the animals were administered 3 mg of toxin per kg of feed (Hammer and others 1996).

The incidence of fumonisins in milk samples has not been extensively studied. It must be noted, however, that in all the latter cases the limits of quantitation of the methods used were considered high (between 1 and 50 μg/kg). Gazzotti and others (2009) developed a method to determine and quantify FB_1 in milk samples by means of liquid chromatography-mass spectrometry; of the 10 samples analyzed, 8 were contaminated above the limits of quantification (0.26 μg/kg).

Because FB_1 and FB_2 in milk samples are stable to heat treatments such as pasteurization (62 °C/30 min) and storage at 4 °C for 11 d (Maragos and Richard 1994), the incidence of these contaminants is a major issue for human health. Therefore, establishing methodologies for their detection and quantification and the subsequent regulation of the minimum levels allowed are of paramount importance.



Fumonisina	R1	R2	R3
B1	OH	OH	H
B2	H	OH	H
B3	OH	H	H
B4	H	H	H
A1	OH	OH	CH3CO
A2	H	OH	CH3CO

Source: Diaz and Boermans (1994).

Figure 2–Molecular formula of fumonisin.

Zearalenone

Zearalenone (ZEN) is a lactone mycotoxin which can be produced by various *Fusarium* species, *Fusarium graminearum,* and *Fusarium culmorum* are its major producers (Zinedine and others 2007). The reduction of ZEA to its metabolites (Figure 3) primarily occurs in the liver, but it also can be converted by rumen protozoa (Malekinejad and others 2006).

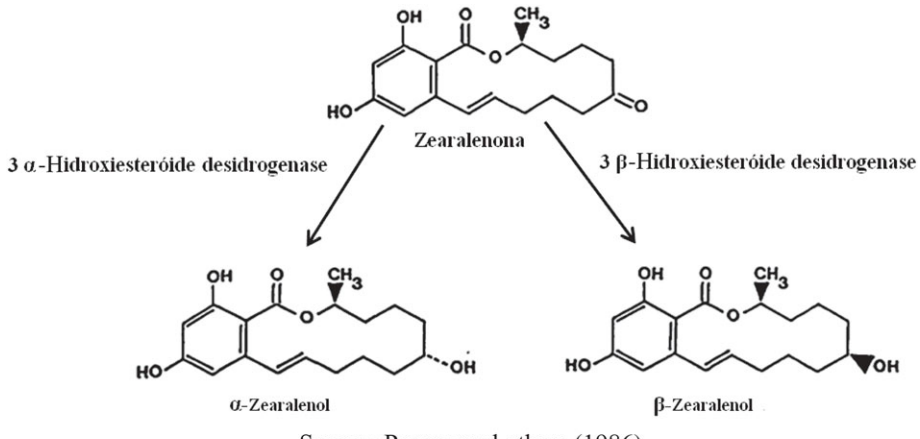
Unlike the aflatoxins, ZEN is not considered carcinogenic. Nevertheless, this mycotoxin has estrogenic effects, with the estrogenic activity of α-ZEA being 3 to 4 times greater than the parent compound ZEN (Minervini and others 2005).

Although ZEN is not 1 of the higher mycotoxins of occurrence in milk and its derivatives, some studies have reported ZEN contamination. In a study conducted in Italy, Meucci and others (2011) detected ZEN in 9% (maximum 0.76 μg/L), α- ZEN in 26% (maximum 12.91 μg/L), and β- ZEN in 28% (maximum of 73.24 μg/L) of milk-based infant formula samples studied, and the authors obtained limits of detection (LOD) of 0.02, 0.02, and 0.2 μg/L for ZEN, α- ZEN, and – ZEN, respectively. Furthermore, there were significant variations in the ZEN metabolite levels of the infant formula brands evaluated. According to the authors, this could be attributed to different manufacturing practices and probably the variation in the quality of the raw materials used. In Argentina, Signorini and others (2012) estimated that the concentration of ZEN was 0.125 μg/L in raw milk. In China, Huang and others (2014) observed the occurrence of ZEN in samples of raw milk, pasteurized milk, and powdered milk, and despite the LOD is approximately 10 times less than that obtained by Meucci, contaminated samples percentages were similar at 23.3%, 16.7%, and 25%, respectively.

Ochratoxin

Ocratoxin (OTA) is a mycotoxin synthesized by a variety of different species of fungi, mostly from the genera *Aspergillus (A. ochraceus, A. melleus, A. sulphureus, Aspergillus section Nigri, A. carbonarius, A. awamori)* and *Penicillium (P. verrucosum, P. crysogenum, and P. nordicum*; Zheng and others 2005; Bayman and Baker 2006; Magan 2006; Rodríguez 2011). This toxin has been shown to be nephrotoxic, hepatotoxic, teratogenic, and immunotoxic. The Intl. Agency for Research on Cancer classified OTA in 2B group (possibly carcinogenic agent; Intl. Agency for Research on 1993).

According to Nogueira and Oliveira (2006), the main route of contamination of OTA is the gastrointestinal tract, with the



Source: Pompa and others (1986)

Figure 3–Chemical structure of zearalenone and its metabolites.

mycotoxin being slowly absorbed. This occurs in most species, there are a 1st and rapid absorption in the stomach due to its acid characteristics, followed by a slow absorption, intestinal level. According to Völkel and others (2011), only a small percentage of OTA remains in the blood stream. Nevertheless, due to OTA being degraded by microorganisms in the cattle rumen, it has been suggested that its rate of excretion in milk is minimal (Fink-Gremmels 2008b).

In a study of OTA detection and quantification in Italian milk samples, Pattono and others (2011) analyzed 63 milk samples from organic farms and 20 nonorganic, commercially available milk samples. The limit of quantification (LOQ) of the method was 0.05 μg/L and the results showed that only 3 organic samples were contaminated with OTA in amounts ranging from 0.07 to 0.11 μg/L. None of the nonorganic samples resulted positive for OTA. In a survey in France, Boudra and others (2007) evaluated 132 samples of bovine milk from farms in the northwest of the country in order to determine the presence of AFM_1 and OTA. Only 3 samples were found to contain OTA above the LOQ of the method (0.005 μg/L). Breitholtz-Emanuelsson and others (1993) found that from the 36 milk samples evaluated, 5 (14%) were positive for OTA (LOQ 0.005 μg/L). In a study of mycotoxins in animal feed, Galtier (1991) calculated that a cow fed with an oral dose of 1 g of OTA/d would result in 100 μg/kg of OTA in milk, corresponding to a transfer ratio of 0.01%. Another study in Turin, Italy, examined OTA levels in colonial cheeses made with raw milk. Of thesee 32 cheese samples evaluated, 6 were positive for the presence of OTA with values between 18.4 and 146.0 μg/kg in the interior part and between 1.0 and 262.2 μg/kg in the cheese hull. The presence of mycotoxins on the surface of the cheese can be explained due to the environmental conditions of the ripening chamber (for example, temperature and water activity) during cheese maturation, which can be favorable for the metabolic activity of some mycotoxin-producing strains (Pattono and others 2013).

Trichothecenes

Trichothecenes are produced by several genera of fungi, including *Fusarium, Stachybotrys, Myrothecium, Trichothecium, Trichoderma, Cephalosporium, Cylindrocarpon, Verticimonosporium,* and *Phomopsis* (Scott 1989). Are mycotoxins which may be present in most cereals during harvest and storage. The fusaric acid, which is often present in cereals, increases the toxicity of trichothecenes through a synergistic mechanism (Yiannikouris and Jouany 2002). Despite the large number of these compounds found in nature, only a few have been detected by natural contamination in foods, for example, DON, nivalenol, T-2 toxin, HT-2, and diacetoxyscirpenol toxin (Radová and others 1998; Kotal and others 1999; Garda and others 2004). In general, milk is not considered a significant source of trichothecene toxins; however, high levels of contamination by feed and silage mycotoxins together with animal physiological imbalances can result in significant levels being present in milk. Recent studies have revealed that animals ingesting feed contaminated by type A or B trichothecenes (DON or T-2 toxin) showed mycotoxin biotransformation and excretion in fluids such as milk, thus classifying these as a risk to human health (Danicke and Brezina 2013).

Deoxynivalenol

DON, also known as vomitoxin, is mainly produced by *Fusarium graminearium* and, in some regions, by *Fusarium culmorum* (Richard 2000). The main product of the microbial metabolism of mycotoxin in the animal body is di-epoxy-DON (Figure 4), a less toxic metabolite that may be eliminated in both urine and stool and secretions such as milk (Maul and others 2012; Shephard and others 2013). In a study, Seeling and others (2006a) studied the "carry-over" (ratio of DON concentration or its metabolites in milk to DON concentration in animal feed) and found values between 0.0001 and 0.0002 for DON, and 0.0004 and 0.0024 for di-epoxy-DON, concluding that, in healthy cows, the mycotoxin is detected in blood and milk mainly as de-epoxy-DON.

Studies of DON metabolism and excretion in milk samples have been performed since the 1980s (Côté and others 1986; Charmley and others 1993). Nowadays, other metabolites arising from DON feed contamination have been studied in milk (Seeling and others 2006b; Danicke and Brezina 2013); however, there are few reports of the natural occurrence of these toxic compounds in milk samples for human consumption (Signorini and others 2012).

Despite the amount of DON and its conjugated derivatives in milk be very small, some authors evaluated the incidence of these mycotoxins in this food. Thus, Rubert and others (2012) developed and validated a methodology capable of extracting and simultaneously quantify and identify 21 different mycotoxins from foods intended for infants, including fluid milk. Among the mycotoxins studied, the authors evaluated the natural incidence of DON, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol in 3 types of baby food, but none of the milk samples were naturally contaminated by DON nor its metabolites.

The high incidence of DON in cereals for animal feed (Soleimanyand others 2012) reinforces the need for studies to evaluate the impact of this mycotoxin and its degradation products in milk samples for human consumption. Furthermore, laws to regulate the level of these contaminants in milk and its derivatives should be implemented.

T-2 toxin

The T-2 toxin is a mycotoxin produced mainly by fungi of the genus *Fusarium sporotrichioides* (Cast 2003) and belongs to the group of nonmacrocyclic trichothecenes.

T-2 milk contamination through contaminated feed has been proven by several studies (Robison and others 1979; Yoshizawa and others 1981). These studies have also predicted the metabolic fate of this mycotoxin in the animal body and the possible compounds formed during food digestion (Yoshizawa and others 1981). Nevertheless, there are no reports in the literature describing the natural occurrence of this mycotoxin and its metabolites in milk samples intended for human consumption.

Conclusions

Given the extensive occurrence of different types of mycotoxins not only in milk but also in other dairy products, as well as concern regarding their animal and human toxicity and the fact that milk is a source of nutrients of particular importance for infants, it is essential to adopt measures to minimize food contamination by such mycotoxins. Thus, special care should be taken with lactating cow's feedstuff and in increasing the awareness of Good Agricultural and Storage Practices.

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