CORPUS PUBLISHERS

Corpus Journal of Dairy and Veterinary Science (CJDVS)

Volume 3 Issue 2, 2022

Article Information

Received date : March 16, 2022

Published date: March 28, 2022

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Keywords

Mycotoxins; Livestock Feed; *Aspergillus* spp; *Penicillium* spp; *Fusarium* spp; Dairy Cattle; Dairy Products; Aflatoxin M1; Mediterranean Sea

Review Article

Mycotoxin Contamination in Livestock Feed and Dairy Products, with Description of their Occurrence in Mediterranean Countries

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Abstract

Mycotoxins are produced by fungi that contaminate livestock feed in many parts of the World and can represent a challenge for human and animal health. The most frequent recovered fungi producing mycotoxins in dairy farms are represented by genera *Aspergillus, Fusarium*, and *Penicillium*. Cancerogenic properties were highlighted for mycotoxins, in particular for aflatoxins, ochratoxins, fumosin, highlighting the interest in recovery methods for these mycotoxins and focusing on possible mechanisms able to avoid fungal feed contamination and mycotoxins dispersal. Contamination by mycotoxins can thus spread from feed, damage cattle dairy and threaten human health. The countries bordering the Mediterranean Sea showed consolidated traditions in cattle livestock and several studies evidenced the concern of mycotoxins contamination in cattle farms and in dairy products, representing a serious challenge for human health. Moreover, climate change and temperature increase favour fungal production of mycotoxins, thus adding important concerns for human health. This concern assumes more serious aspects if considering that the Mediterranean basin is a hotspot in terms of climate change. This mini review describes the concern of mycotoxins contamination in dairy livestock feed and in dairy products, with a focus on countries bordering the Mediterranean basin.

Introduction

Mycotoxins are secondary metabolites of low molecular weight produced by several fungi, in particular by members of the genera *Aspergillus*, *Fusarium* and *Penicillium*. Mycotoxins present diverse chemical structures, evidencing different biological effects on animals and humans [1]. These secondary metabolites can develop in the field, contaminating maize, grain, barley, and rice then used as commodities of feed for livestock animal nutrition. Mycotoxins can also originate during ensiling, if environmental conditions favour the growth of fungi and, consequently, their synthesis [2]. Mycotoxins are present in approximately 25% of cereals consumed worldwide with higher mycotoxin contamination when climatic conditions are favourable, as in countries whose climate is characterised by high temperatures and high relative humidity [3]. Once entered into the food chain, mycotoxins can give rise to some concerns as mycotoxicosis onset in dairy cattle [4]. From animals, mycotoxins can thus pass in milk and dairy products and eventually reach humans. For this reason, mycotoxins can represent important contaminants of food, constituting a real and important problem for human health [5]. For the most investigated toxins, tolerable or provisional maximum tolerable daily intakes have been established [6].

Figure 1 describes the interaction between different compartments for the production and spread of mycotoxins. The latter can be produced in two different ways, directly into the field, with fungi affecting crops, or by fungi developing during ensilage procedures, depending on adapt conditions in terms of temperature and water concentration. Mycotoxins can thus reach dairy cattle, entering the animal body and, eventually, cause damages to animal health, or be transferred to milk and dairy products and thus mycotoxins can reach humans, representing great health concern. This mini review provides a description of the mycotoxins produced by various filamentous fungi and their diffusion in feed and dairy products. A survey on the spread of mycotoxins in the countries of the Mediterranean basin is also reported.

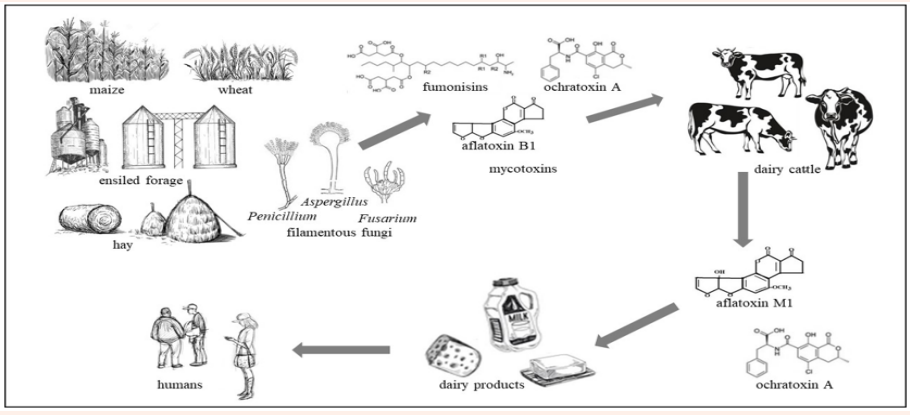

Figure 1: Mycotoxins development in livestock feed and transmission to cattle dairy and, through the milk and dairy products, to humans.

Mycotoxins Features

Mycotoxins are natural molecules that represent a heterogeneous group characterized by the common capability to induce toxicity, disease and death upon exposure, in humans and in animals [7]. They are produced by filamentous fungi that are characterized by high adaptability and enzymatic efficiency, a high versatility in using many different organic compounds for their metabolism, and the capability to resist broad ranges of temperature, pH, and humidity. Due to these features, filamentous fungi are widespread in the environment and are able to grow on crops, both in the field or in storage conditions [2]. There are many mycotoxins produced by fungi, with the concern of mycotoxins contamination of in green forage, hay, or silage (ensiling material) and thus feed, being one of the most dangerous aspects for human and for animal health [8]. Filamentous fungi are often detected in situ in the fields of production and they can grow in forages and filamentous fungi can be detected in silage or in hay [9,10]. Several fungal genera as *Fusarium* and *Alternaria*, typically contaminate forage before harvest and are defined field-fungi. The common contaminant *Aspergillus* is both present in the pre-harvest and in the post-harvest phases of forage treatment [11].

Mycotoxins are secondary metabolites produced by fungi and usually develop in sites with a low water activity (a_w) and which exhibit characteristics unsuitable for bacterial growth [12]. Production of mycotoxins by fungi depends on environmental conditions, such as weather situations. For instance, a high water activity and a temperature around 28 °C – 30 °C favour fungal growth associated with mycotoxins production [13-15]. During ensiling, the main part of fungi succumbs [16,17]. Nevertheless, members of several fungal species are able to resist ensiling, as it occurs in strains of the species *Aspergillus fumigatus*, *Penicillium roqueforti*, *P. paneum*, *Fusarium oxysporum*, *Monascus ruber*, as they are able to tolerate high levels of organic acids present during ensilage, and high levels of carbon dioxide (CO_2), along with low availability of oxygen (O_2) [18,19]. It can happen that few concentrations of oxygen can enter into silage portions during storage or feed-out, and some oxygen can be allowed to reach ensilage mass permitting fungal growth and mycotoxins production. In high-quality ensilage, the presence of lactic acid bacteria avoids the growth of fungi, although a little rise in oxygen concentrations can permit fungal growth as in *P. roqueforti* and *P. paneum*. Anyhow, in case of evaporation of organic acids as acetic and lactic acids, eventually along with CO_2 evaporation, oxygen (O_2) concentration increases, allowing growth of cereal associated filamentous fungi [11,20]. Although there are more than 100,000 known fungal species, the majority of mycotoxins are produced by fungal strains belonging to a few species, mostly from the genera *Aspergillus*, *Fusarium* and *Penicillium* [21]. The discovery of mycotoxins can be traced back to the 1960s, when a veterinary disease occurred in England, on a farm close to London, with an amount of 100,000 turkey poults dying because of an unknown disease, defined as "mysterious turkey X disease" [21]. The turkey X disease was then associated with groundnut contamination by secondary metabolites, representing a toxin, produced by members of the fungal species *Aspergillus flavus*, thus called 'aflatoxin' [21].

Being secondary metabolites, mycotoxins have no influence on fungal growth and its further development [22]. Mycotoxins can thus have a defensive role against insects, other microorganisms, animals and humans. From a chemical point of view, all the identified mycotoxins range from molecules with four carbon atoms up to compounds with complex structures [3]. Some mycotoxins are toxic to humans and animals and can represent a real public health concern [23]. There are currently about 300 mycotoxins that differ from each other in their fungal origin and the biological effects they cause, with few mycotoxins having important health effects [24]. In comparison to monogastric species, ruminant animals are generally considered to be less susceptible to the adverse effects caused by contamination of feeds with mycotoxins. This is based on the assumption that the rumen microbial flora degrades and inactivate mycotoxins, thus protecting the animal. A number of mycotoxins, however, resist microbial rumen degradation, causing distinct clinical signs of intoxication. Moreover, due to their complexity of feed, dairy cattle may be exposed to a varying number of mycotoxins originating from different materials [25]. In order to prevent cancer risks from exposure to mycotoxins, the International Agency for Research on Cancer (IARC) in Lyon, France, has performed the carcinogenic hazard assessment of some mycotoxins in humans, on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies [26,27]. Mycotoxins are low-molecular-weight natural compounds evidencing a broad chemical diversity, within common the capacity to have toxic effects in humans and other animals, causing diseases and death [21]. The most important and well investigated mycotoxins include aflatoxins, fumonisins, ochratoxins, zearalenone and trichothecenes [21].

Mycotoxins in Livestock Feed

Among mycotoxins, aflatoxins belong to the category of difuranocoumarins and can be produced by fungi in presence of warm and humid environmental conditions. Aflatoxins are produced for the main part by fungal strains belonging to the species included in the genus *Aspergillus* [28]. *Aspergillus flavus*, *A. nomius*, and *A. parasiticus* are three species whose strains are able to produce aflatoxins commonly found in food and feeds. The species *A. flavus* and *A. parasiticus* are found worldwide in the soil and in the air [28], preferring to grow at temperatures between 22 °C and 35 °C and at values of water activity (a_w) in a range from 0.95 to 0.98 [29]. Aflatoxins are the best known among all mycotoxins, due to the impact they have on human and animal health. Among more than 20 known aflatoxins, four main types were characterized in deep, based on the fluorescence aflatoxins emit upon UV light exposure, aflatoxins B1 and B2 display a blue fluorescence, whereas aflatoxins G1 and G2 emit on the green.

According to the classification by the International Agency for Research on Cancer, aflatoxin B1 was recognized as carcinogenic in 1987 and assigned to category 1A [30]. The four most studied aflatoxins can be classified based on the toxicity range of B1 > G1 > B2 > G2 [31]. Aflatoxin B1 is considered to be the most potent carcinogenic toxin known in mammals [32]. No tolerable daily intake for humans (tolerable daily intake, TDI) are contemplated for aflatoxin B1 [33]. Worldwide, the risk of exposure to aflatoxins in contaminated foods exists for more than 4.5 billion people. The EU legal limit for aflatoxin B1 (Figure 2) in processed cereal foods is 0.02 μg kg^{-1} [34]. Aflatoxins production involve pre-harvest and post-harvest factors that are strictly related to the production of these mycotoxins. It is thus important to consider that pre-harvest weather conditions associated with periods of drought and heat stress during flowering and fruit growth can represent the main factors responsible for the onset of infection with aflatoxins produced by fungal strains of the species *A. flavus* and *A. parasiticus* in maize [35]. Other stress factors in plants, such as inadequate nutrition, insect nutrition from growing fruits, weed competition, overgrowth of plants, and plant diseases, can favour fungal infection and the production of aflatoxins [35]. After harvest, aflatoxins can develop at higher concentrations if stored in the presence of humidity and at a temperature that favours their production by filamentous fungi. Aflatoxins biosynthesis is inhibited with a_w lower than 0.8334. Maintaining the temperature in the storage system below 15 °C regulate a_w at a value of 0.934, allowing minimal production of mycotoxins [36].

Aflatoxins are very harmful as they can induce carcinogenicity, mutagenicity, teratogenicity and can exert an immunosuppressive effect. Moreover, aflatoxins can cause aflatoxicosis both in animals and humans [32]. Acute aflatoxicosis symptoms in humans include vomiting, abdominal pain, pulmonary oedema, coma, convulsions and death. Chronic aflatoxicosis can cause cancer, inhibition of the immune system, liver damage [32]. In areas of the world with a high incidence of liver cancer as in southeast Asia and sub-Saharan Africa, important risk factors are considered chronic hepatitis C infection and exposure to aflatoxins, since they can even act synergistically [37]. In India in 1974, the most serious outbreak of human hepatitis occurred, with 108 patients dying after consuming maize with high contamination by aflatoxins at levels of 0.25 - 15 mg kg^{-1} [38]. The largest and most serious case of acute aflatoxin poisoning in humans was recorded in April 2004 in Kenya, with 125 dead people after eating infected maize, presenting aflatoxin levels on a range from 5 to 20 mg kg^{-1} [39]. Again in Kenya, in 2005, an epidemic event based on aflatoxins occurred causing 16 deaths [40]. In children, symptoms such as encephalopathy and visceral degeneration were assigned to Reye's syndrome, which is strictly linked to aflatoxin toxicity [29,41]. Ochratoxins, chemically are a weak organic acid, composed by a part of dihydro isocoumarin joined to 1-phenylalanine by a hydrogen bond (Figure 2) and can be present in three forms (A, B and C). Ochratoxins are produced by fungi of the genera *Aspergillus* and *Penicillium* [21,42]. The major species implicated in ochratoxin A production includes *Aspergillus ochraceus*, *A. carbonarius*, *A. melleus*, *A. sclerotiorum*, *A. sulphureus* [43]. Ochratoxin A causing cancer was reported for kidneys as targets [44]. Ochratoxin A evidenced genotoxicity and formation of ochratoxin A-DNA adducts), suggesting a role of this mycotoxin in oxidative stress and the identification of epigenetic factors involved in ochratoxin carcinogenesis [45].

Fumonisins result from being synthesized by the condensation of alanine into an acetate-derived precursor (Figure 2). Fumosinins are produced by *Fusarium* spp. fungal strains [46]. Fumonisins (B1 and B2) are cancer-promoting metabolites originated from strains of the species *Fusarium proliferatum* and *F. verticillioides* that have a long-chain hydrocarbon unit (similar to that of sphingosine and sphinganine) which plays a role in their toxicity. Fumonisin B1 is the most toxic and has been shown

to promote tumours in rats and cause equine leukoencephalomalacia and porcine pulmonary oedema. The naturally co-occurring aminopentol isomers (formed by base hydrolysis of the ester-linked tricarballylic acid of fumonisin B1) have been suggested to exert toxic effects due to their structural analogy to sphingoid bases [47]. Some correlation studies have suggested a link between the consumption of maize with a high incidence of *F. verticillioides* and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of South Africa [48] (Figure 2). A large family of mycotoxins is represented by trichothecenes, which are produced by members of different fungal genera such as *Fusarium*, *Trichoderma* or others. From the chemical point of view, trichothecenes all comprehend a 12, 13-epoxytrichotechene skeleton and an olefinic bond joining different side chains. A further chemical distinction allows trichothecenes to be distinguished between macrocyclic and non-macrocyclic. Well, known trichothecenes include deoxynilvalenol and T2 toxin (Figure 2) [21,42]. The trichothecene mycotoxins contain an epoxide at the C12,13 positions, which is responsible for their toxicological activity. At the cellular level, the main toxic effect of trichothecene mycotoxins appears to be a primary inhibition of protein synthesis. They affect actively dividing cells such as those covering the internal gastrointestinal tract, the skin, lymphoid and erythroid cells. The toxic action of trichothecene results in extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effect on the digestive tract and decreased bone marrow and immune function [49]. The trichothecene mycotoxins occur worldwide in grains and other commodities. Toxin production is greatest with high humidity and temperatures of 6 °C – 24 °C. The natural occurrence of trichothecene has been detected in corn, wheat, barley, and rice. Examples of type A trichothecene include T-2 toxin and HT-2 toxin. Deoxynivalenol is a common naturally occurring type B trichothecene [49] (Figure 2).

Biosynthesis of zearalenone occurs through a polyketide pathway [21]. Zearalenone is a mycotoxin produced by *F. graminearum* and other *Fusarium* spp. fungi using corn, wheat, barley, oats and sorghum as substrates. It is a non-steroidal compound that exhibits estrogen-like activity in certain farm animals, including cattle. Zearalenone is a phenolic resorcylic acid lactone (Figure 2) with potent estrogenic properties [49]. Zearalenone is a phytoestrogen compound known as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid l-lactone. It is a metabolite associated with several *Fusarium* species (i.e. *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*), with *F. graminearum* which is the species most responsible for the onset of estrogenic effects. Alcohol metabolites of zearalenone (i.e. α-zearalenol and β-zearalenol) are also estrogenic inducers [9,50].

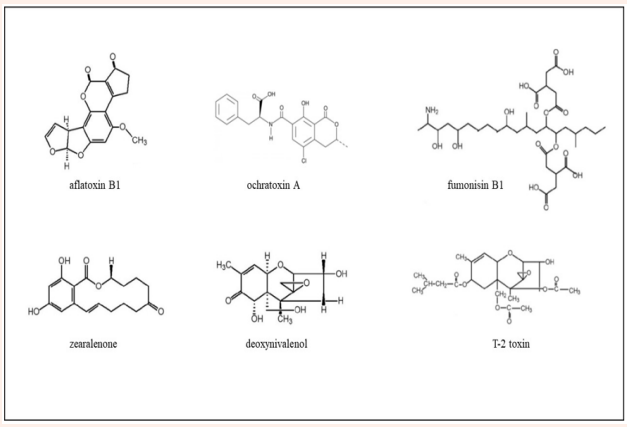

Figure 2: Formulae of frequently recovered mycotoxins in livestock feed.

Mycotoxins Contamination in Milk and Dairy Products

The first case of milk contamination by mycotoxins was registered in the 1960s, when the first case of an aflatoxin M1, which is an aflatoxin B1 metabolite produced in the animal rumen and secreted in milk, was detected [12,51].

Once ingested, aflatoxins B1 and aflatoxin B2 can be transformed by the animal organisms, by P-450 liver enzyme as in the case of aflatoxin B1, obtaining the hydroxylated form of the molecule, the aflatoxin M1 (Figure 3), that is a potentially carcinogenic compound, included in the category 2B [30]. Because of the presence of the hydroxyl group, aflatoxins M1 are very soluble in water, with the consequent excretion through substrates containing water, such as urine, bile, faeces, and milk [52]. Different maximum upper limits are set worldwide for aflatoxin M1 in milk or milk products, with Codex Alimentarius and the EU setting the limit to 0.05 μg kg^{-1} for aflatoxin M1, whereas the US and some Latin American countries set it to 0.5 μg kg^{-1} [53]. In milk and in dairy products, other mycotoxins, more than aflatoxin M1, have been also identified, including ochratoxin A [54]; zearalenone [55]; fumonisins [56]; T-2 toxin and deoxynivalenol [57,58]. Milk is a source of nutrients, particularly useful and widely used in children, so its safety must be carefully checked. Milk from cattle is the main type of milk used for human consumption, accounting for 83% of world milk production [59].

Moreover, the presence of aflatoxin B1 was recovered in heat-treated milk samples and in pasteurized and UHT milk, with average values of contamination equal to 1.476 μg L^{-1} and 0.690 μg L^{-1}, respectively. Because aflatoxin B1 is more toxic than aflatoxin M1 (Figure 3) [3], the presence of aflatoxin B1 in milk should also be examined. Aflatoxin M2 obtained from hydroxylation of aflatoxin B2, has also been detected in powdered milk samples and in UHT milk samples commercialized in Brazil, with values >0.08 μg kg^{-1} and >0.009 μg kg^{-1}, respectively [3].

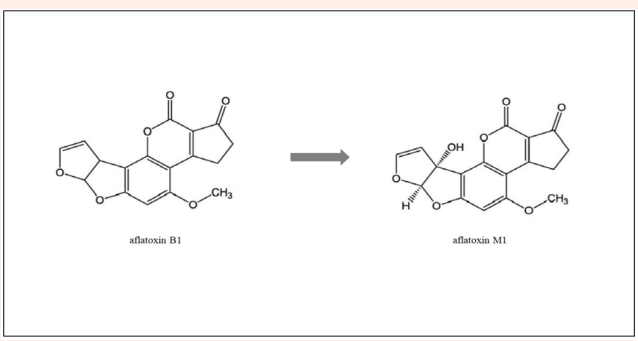

Figure 3: Aflatoxin B1 hydroxylation to aflatoxin M1 by cytochrome P450 in cattle dairy liver.

Mycotoxins in the Mediterranean Sea Bordering Countries

Mycotoxin contamination in feed grain and animal feed sourced in Europe and the Mediterranean Region evidenced the highest values for fumonisin, with mean value equal to 754 μg kg^{-1}; followed by deoxynivalenol recovered at a mean value of 304 μg kg^{-1}; zearalenone 174 μg kg^{-1}; aflatoxin B1 67 μg kg^{-1}; T-2 toxin 30 and ochratoxin A 6 μg kg^{-1} [60]. As an example, Morocco is a country in the North of Africa bordering the Mediterranean Sea, with a climate characterized by high humidity and a high temperature which favour the growth of fungi [32]. Mycotoxin contamination and climate change are correlated, and the effects in the Mediterranean area can be considered as climate change can affect mycotoxin production by fungi. Mycotoxins emerge as a particular concern since their prevalence and concentrations in food and feed may vary due to climatic conditions, as in the case of production of aflatoxins, the most toxic mycotoxins [61].

Mycotoxins as fusaproliferin, beauvericin and enniatins are mostly present in Northern Africa and the Mediterranean area, with their presence related to climatic conditions [62]. Algeria, Morocco and Tunisia are countries of North Africa disposed front to the Mediterranean Sea, presenting a characteristic climate with high temperatures and high relative humidity, offering environmental conditions favourable to fungal growth and mycotoxins production. The presence of toxigenic fungi and related mycotoxins as aflatoxins, ochratoxin A, and emerging *Fusarium* mycotoxins (enniatins, beauvericin and fusaproliferin) are significant and with high contamination levels in the three North African countries. Prevention of mycotoxin production in feeds is present in particular in Morocco and must be diffused further in Algeria and Tunisia and in the whole region of North Africa (Table 1) [63]. In Southern European Countries, mycotoxins were detected in feed materials, such as aflatoxins, zearalenone, type B trichothecenes (deoxynivalenol and acetyldeoxynivalenol), type A trichothecenes (T-2 toxin and HT-2 toxin), fumonisins and ochratoxin A [64]. The highest incidences were detected for type B trichothecenes and fumonisins with a high frequency (Table 1) [64]. Fungal strains of the genus *Fusarium* are of great concern in the Mediterranean area, particularly for cereals contamination and they are able to produce trichothecenes, zearalenone and fumonisins. Trichothecenes can cause diseases in animals, including haemorrhagic syndromes of internal organs. Zearalenone is about the most diffused mycotoxin originating from members of the genus Fusarium and several times it

is revealed at high concentrations as it happened in maize. Mycotoxin zearalenone is responsible for toxicoses in animals and can induce estrogenic diseases with reproductive disorders [30].

Table 1: Mycotoxins in feed and feed commodities in countries bordering the Mediterranean Basin.

Country	Substrata	Mycotoxins	Concentrations µg kg^{-1}	Fungi	Reference
Algeria	barley, maize, rice, wheat	T-2 toxin, citrinin, beauvericin, deoxynivalenol, fumonisins (B1 + B2), enniatins B and B1, zearalenone			[65]
Croatia	maize	aflatoxin B1			[66]
Egypt	maize	aflatoxin B1, fumonisin B1, ochratoxins A		*Aspergillus*, *Penicillium*, *Fusarium*	[67]
France	maize	aflatoxin B1		*Aspergillus* section *Flavi*	[68]
Greece/ Cyprus	feed	fumonisins, deoxynilalenol/ acetyldeoxynilvalenol, zearalenone	1,371, 268, 13 mean values, respectively		[64]
Israel	corn silage	fusaric acid, fumonisins, beauvericin, monilifomin, equisetin, zearalenone and enniatins		*Fusarium* spp.	[69]
Italy	feed	fumonisins, deoxynilalenol/ acetyldeoxynilvalenol, zearalenone	1,840, 752, 408 mean values, respectively		[64]
Italy	hay			*Cladosporium cladosporioides*, *Alternaria alternata*, *Rhizopus stolonifer*	[8]
Portugal	feed	fumonisins, deoxynilalenol/ acetyldeoxynilvalenol, T-2 toxin/HT-2 toxin, zearalenone	631, 191, 14, 10 mean values, respectively		[64]
Spain	feed	deoxynilalenol/ acetyldeoxynilvalenol, fumonisins, zearalenone	293, 33, 19 mean values, respectively		[64]
Spain	silage	fumonisins, aflatoxins		*Penicillium*, *Fusarium*, *Geotrichum*, *Monascus*	[4]
Turkey	feed	aflatoxin B1, deoxynivalenol, ochratoxin A, T-2 toxin, zearalenone	116.86, 500, 15.85, 29.30, 4.96 maximum value, respectively		[70]
Turkey	feed	aflatoxin B1, ochratoxins A, fumonisins (B1 + B2)	0.04, 0.344, 1.208 mean value respectively		[71]

North African populations are exposed to the risk of mycotoxins due to the consumption of contaminated food. These countries are surrounded by the Mediterranean Sea and have a climate characterized by high humidity and temperature, which probably favours fungal growth. During the last decades, many studies have reported the occurrence of different mycotoxins in food commodities in North African countries [72]. Contamination by mycotoxins in dairy products was detected in countries bordering the Mediterranean basin, some examples are reported in Table 2.

Table 2: Mycotoxins in milk and dairy products in countries bordering the Mediterranean Basin.

Country	Substrata	Mycotoxins	Concentrations µg kg^{-1}/µg L^{-1}	Method	Reference
Croatia	raw milk	aflatoxin M1	0.006-0.027	ELISA	[73]
France	raw milk	ochratoxin A	0.005-0.0066	HPLC	[74]
France	milk	aflatoxin M1			[12]
Greece	feta cheese	aflatoxin M1		ELISA	[75]
Italy	raw milk, fresh whole, high quality, organic	fumonisin B1	0.32, 0.38, 0.43, 0.38, respectively	LC–MS/ MS	[56]
Italy	milk infant formula	aflatoxin M1	0.012-0.015	HPLC	[76]
Italy	raw milk	aflatoxin M1	0.0072 and 0.022	ELISA/ HPLC-FD	[5]
Lebanon	raw milk	aflatoxin M1	0.0026-0.126	ELISA	[77]
Libya	cheese	aflatoxin M1	0.11-0.52	HPLC	[78]
Morocco	pasteurized milk	aflatoxin M1	0.001-0.117		[79]
Morocco	UHT milk; powder milk	aflatoxin M1	0.01476±0.01021, 0.0255±0.01206, respectively	HPLC	[80]
Portugal	UHT milk, pasteurized milk	aflatoxin M1	0.007-0.07	ELISA	[81]
Spain	UHT milk	aflatoxin M1	0.002-0.014	ELISA	[82]
Turkey	fluid milk, white cheese	aflatoxin M1	0.001-0.030, 0.024-0.452, respectively	ELISA	[83]

Conclusion

Mycotoxins represent an important challenge threatening human and animal health and damaging crops and livestock, thus constituting a concern also from the economic point of view. They diffuse in the environment, in particular in those presenting high humidity and high temperatures. Mycotoxins originated from filamentous fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium* can have consequences on cattle dairy health and by chemical affinity can enter in milk and in dairy products, eventually reaching humans. The presence of mycotoxins is a quite common event, going to increase in a near future due to climate change. As future research and insights for next investigations, it should be of extremely importance focus on method, preferably by biological approaches (i.e. yeasts and other microorganisms competing with filamentous fungi), to diminish mycotoxins production in livestock feed, thus avoiding their spread in milk and dairy products.

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