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Occurrence of mycotoxins in pastures: A systematic review

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REVIEW ARTICLE

Abstract

In this study, a systematic review is presented on the worldwide occurrence levels of mycotoxins in pastures reported in the scientific literature from January 1987 until December 2021. Trichothecenes and zearalenone were the most frequent mycotoxins found at high levels in pastures from countries in Europe, Oceania, and North America. Alternariol and Ergot alkaloids were also frequently detected, although at low levels. A few surveys were conducted in South American countries, and no information was available from the African and Asian continents, stressing the need for studies on the occurrence of mycotoxins in pastures from those regions, especially in tropical areas, where pastures are used as main sources for animal nutrition.

Keywords: pastures, animal feed; feed safety; mycotoxins; occurrence

Introduction

Mycotoxins are low-molecular, secondary metabolites produced by certain filamentous fungi species that develop on plant- and animal-derived foods, especially cereals (Cimbalo *et al.*, 2020). Foods or feeds contaminated with these compounds are potentially harmful to human and animal health (Gallo *et al.*, 2015). Metabolic functions of mycotoxins are still unclear, but it is believed that these toxic substances are involved in protection against parasites and predators, or inhibition of growth of environmental competitors (Yang *et al.*, 2020). Until the middle of the 20th century, few studies have been conducted on the harmful role of fungi in animal and human foods. Mycotoxin studies became popular in the 1960s due to the death of thousands of turkeys in England because of the so-called Turkey "X" disease, which led to the discovery and naming of the toxin produced by *Aspergillus flavus*, as aflatoxin (Vedovatto *et al.*, 2020). Since then, many other mycotoxins were discovered and, nowadays, around 400 secondary metabolites are classified as mycotoxins (Buszewska-Forajta, 2020).

Mycotoxins can occur in a variety of foodstuffs, such as cereals, legumes, fruits, and nuts (Bangar *et al.*, 2022). The fungi that produce these metabolites are ubiquitous and multiply by releasing spores that survive for long periods of time (Pereira *et al.*, 2019). In addition, a single fungus species may produce different mycotoxins. However, the presence of a fungus in foodstuffs does not necessarily indicate the presence of any mycotoxin because its production depends both on genetic makeup of the fungus and environmental factors (Cimbalo *et al.*, 2020). The main factors that favor the occurrence of toxigenic fungi and mycotoxins in food products include environmental humidity, temperature, and high water activity (Heshmati *et al.*, 2021).

Considering human and animal health perspectives, the most relevant mycotoxigenic fungi genera and their respective mycotoxins are: *Aspergillus*, which produces the aflatoxins (AFs), ochratoxin A (OTA), sterygmatocystin (STE), cyclopiazonic acid (CPA), and patulin (PAT); *Fusarium*, which produces fumonisins (FB), zearalenone (ZEN), trichothecenes type A (A-Trich) such as toxins

T-2 and HT-2, diacetoxyscirpenol (DAS), and type B (B-Trich) including deoxynivalenol (DON) and nivalenol (NIV); *Penicillium*, which produces OTA, CPA, citrinin (CIT), PAT, and rubratoxins (RT); *Alternaria*, which produces tenuazonic acid (TA) and alternariol (AOL); *Claviceps* and *Neotyphodium*, which produce Ergot alkaloids (EA) (Cimbalo *et al.*, 2020; Gallo *et al.*, 2015; Magnoli *et al.*, 2019; Pereira *et al.*, 2019). In particular, the AFs are considered among the most dangerous mycotoxins because of their carcinogenic effects and worldwide occurrence in food and feedstuffs (Heshmati *et al.*, 2021). These toxins are produced especially by *A. flavus* and A. *parasiticus* and rarely by *A. nomius*, and the main types produced by these fungi are AFB_1, AFB_2, AFG_1, and AFG_2 (Souza *et al.*, 2021).

Syndromes caused by ingestion of mycotoxins are called mycotoxicosis, which can be acute, subacute, or chronic, depending on the degree of exposure and clinical signs. Several production animals are susceptible to mycotoxin toxic effects, especially pigs (Cimbalo *et al.*, 2020), broiler chicks (Yang *et al.*, 2020), and fish (Fallah et al., 2014). The main health issues associated with the ingestion of mycotoxins include hepatotoxic, renal, neurological, estrogenic, immunosuppressive, carcinogenic, mutagenic, and teratogenic effects (Franco *et al.*, 2021). AFs have carcinogenic, mutagenic, teratogenic, and immunosuppressive effects (Heshmati *et al.*, 2021). AFB_1 is the most toxic metabolite among AFs, being classified as Group 1 human carcinogen by the International Agency for Research on Cancer (Mokhtarian *et al.*, 2020; Pires *et al.*, 2022; Souza *et al.*, 2021). In production animals, unspecific effects may be observed, especially at low doses, which are generally related to decreased performance that leads to economic losses. In some cases, under field conditions, mycotoxins are considered as invisible hazards for production animals, as there are no perceptible changes in animal health, although mycotoxin residues may be present in animal-derived food products, such as eggs, meat, milk, and cheese. (Adegbeye *et al.*, 2020; Buszewska-Forajta, 2020; Mohajeri *et al.*, 2013; Vedovatto *et al.*, 2020). Thus, the occurrence of mycotoxins in feedstuffs is a potential hazard for the animal industry and human health (Fallah *et al.*, 2014). Dairy products may contain aflatoxin M_1 (AFM_1), the hydroxylated metabolite of AFB_1 excreted in the milk of dairy animals that have consumed AFB_1-contaminated diets (Fallah *et al.*, 2015; Souza *et al.*, 2021). In addition, AFM_1 binds to casein and does not undergo significant modifications by heat treatments commonly applied during processing of dairy products (Gonçalves *et al.*, 2015). Therefore, the presence of toxin remains in the final dairy product, sometimes at higher levels, as observed in casein-concentrated products such as cheese and milk powder (Fallah *et al.*, 2015; Mohajeri *et al.*, 2013).

Animal fodder contaminated with mycotoxins is an entry route of xenobiotics such as mycotoxins in the human food chain. The Food and Agricultural Organization of the United Nations (FAO) indicated that about 25% of the crops in the world are contaminated with mycotoxins. Thus, the United States (U.S.) Food and Drug Administration (FDA), as well as the European Union (EU) and many countries around the world, have determined limits for mycotoxins in human foods and animal feed to prevent contamination or reduce the impact of mycotoxin intake. However, as it is not possible to completely eliminate fungi and their toxic metabolites from foodstuffs, more research and constant monitoring are necessary (Buszewska-Forajta, 2020; Cimbalo *et al.*, 2020; Magnoli *et al.*, 2019; Pereira *et al.*, 2019). Moreover, environmental changes, including the effects of global warming, have generated concern about the geographic spread and increase in the occurrence of crop pests and pathogens, making mycotoxins one of the most important risks to food and feed safety in the near future (Battilani *et al.*, 2016; Bebber *et al.*, 2013; Medina *et al.*, 2017; Moretti *et al.*, 2019).

It is generally accepted that ruminants are less susceptible to the toxic effects of mycotoxins. However, tolerance may vary according to the species, sex, and breed. There is little *in vivo* scientific evidence that unequivocally confirms mycotoxin effects on ruminant health and production. In relation to the meat production industry, while contemporary production systems are focused on genetic improvement for high productivity and the slaughter of younger animals, it is known that young, high-yielding animals have higher metabolic rate that make them more susceptible to mycotoxins, which may lead to greater accumulation of toxic metabolites in their products (Adegbeye *et al.*, 2020; Gallo *et al.*, 2015; Penagos-Tabares *et al.*, 2021; Rodrigues, 2014; Skládanka *et al.*, 2013; Vedovatto *et al.*, 2020).

Pastures are essential resources for animal feeding, as they reduce production costs. They also are the basis for meat and milk production in several countries, such as Argentina, Australia, Brazil, New Zealand, as well as parts of Europe and the United States. Beef cattle in some of these countries are almost all raised in pastures, and in dairy production, pastures are a significant part (or total) of the fodder provided to cattle. In general, there is less information on the occurrence of mycotoxins in pastures and conserved forage than in grains and cereals, which raises concern given the significant role of pastures in the beef and dairy industry (Dias-Filho, 2016; Gallo *et al.*, 2015; Gott *et al.*, 2017; Nichea *et al.*, 2015; Penagos-Tabares *et al.*, 2021; Reed and Moore, 2009; Štýbnarová *et al.*, 2016). As pastures are important sources of ruminant fodder for meat and milk production, the analysis of mycotoxin occurrence in pastures is essential from the point of view of feed safety and animal health. Therefore, the objective of this study was to conduct a systematic review of the literature published on the worldwide occurrence levels of mycotoxins found in pastures.

Search Strategy

A systematic literature search was conducted in PubMed, Science Direct, and Google Scholar (as the gray literature) databases using the following key terms: "mycotoxins" AND "occurrence" OR "contamination" AND "pastures" OR "grass" OR "forage." The search strategy was based on the PRISMA protocol (Moher *et al.*, 2015), as summarized in Figure 1. Data extraction and quality assessment of articles were based on Cochrane protocol (Higgins and Green, 2011). All relevant articles published from 1st January 1987 to 31st December 2021 that investigated the occurrence or levels of mycotoxins in pasture were retrieved and screened for eligibility. Besides the reference lists of articles, manual search was also performed to identify other suitable studies. During the primary screening, after excluding unsuitable articles due to irrelevant content, full texts of potentially eligible articles were downloaded. Then, downloaded articles were examined twice for eligibility. Inclusion criteria were: (1) Full-text article available, (2) Original research studies (not reviews), (3) Mycotoxin frequencies and levels described in positive samples, and (4) Accurate analytical methods mentioned. Articles that did not meet these criteria were excluded. Exclusion criteria included: (1) No original data (review, book, thesis, or workshop) articles, (2) Studies on other toxins or other related products, (3) Only analytical method development, or insufficient method description, or comparison of different analytical methods, and (4) Lack of specific data on the occurrence of mycotoxins in field pastures. After conducting the evaluation process, 82,462 articles were excluded. Finally, 13 articles fulfilled the inclusion criteria and were included in this review.

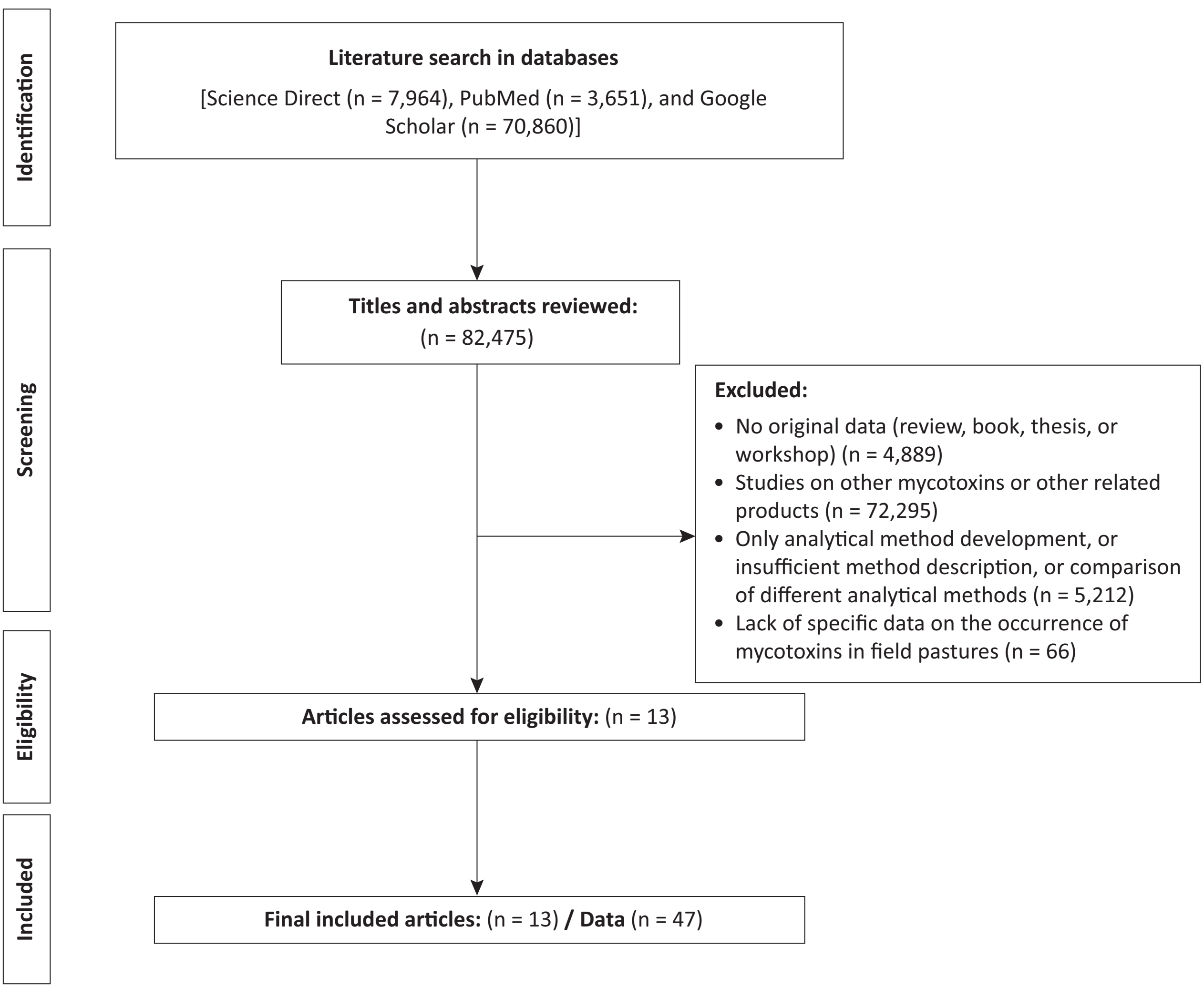

Figure 1. Flow chart describing the literature search, inclusion and exclusion criteria, and data collection based on the PRISMA guidelines (Moher *et al.*, 2015).

Main Findings

The available data on the occurrence of mycotoxins in pastures published in the scientific literature until December 2021 are presented in Table 1.

Oceania

One of the first studies on the occurrence of mycotoxins in pastures was carried out by Di Menna *et al.* (1987) in New Zealand, where the occurrence of ZEN was assessed in sheep pastures mainly made up by *Lolium perenne* and *Trifolium repens*, from January to April 1985, using high performance liquid chromatography (HPLC) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). ZEN was detected in 17% of samples in levels ranging from 400 to 4,000 µg/kg. Besides ZEN, the authors considered the hypothesis that other mycotoxins produced by the genus *Fusarium* could contaminate these pastures.

Reed *et al.* (2004) carried out a study in Southern Australia to assess the occurrence of ZEN in dairy and sheep pastures mainly made up by *Lolium perenne*, during the fall/winter of 1999 and 2000, analyzed by HPLC. ZEN was found in 93% of the samples collected in 1999 and in 74% of products in 2000, with maximum concentrations of 21,000 and 14,100 µg/kg, respectively. Later on, between 2005 and 2007, Reed and Moore (2009), assessed the occurrence of ZEN, DON, FB, and AF in pastures from Southeastern Australia containing predominantly *Lolium perenne* and *Trifolium subterraneum*. This study was divided into four trials, and trial 2 and 4 dealt specifically with the presence of mycotoxins in pastures. In trial 2, ZEN was detected in 36% of the samples at levels ranging from 180 to 4,950 µg/kg. In trial 4, ZEN and DON were found in 54 and 46% of the samples containing 60 to 3,060 and 129 to 682 µg/kg, respectively, while the incidence of fumonisin B_1 (FB_1) and AFB_1 comprised 23 and 15% of samples analyzed, with concentrations ranging from 158 to 998 and 14 to 16 µg/kg, respectively.

Europe

The first report dealing specifically with the presence of mycotoxins in pastures in Europe was provided by Skládanka *et al.* (2011) in the Czech Republic. The authors aimed at analyzing the occurrence of DON, ZEA, FB, and AF in pastures predominantly made up by *Lolium perenne, Festulolium pabulare, Festulolium braunii,* and combinations with *Festuca rubra* and *Poa pratensis* during 2008 and 2009, using enzyme-linked immunosorbent assay (ELISA). The most frequent mycotoxins were DON and ZEN, which were detected at levels of 12.34–71.43 and 3.7–173 µg/kg, respectively. FB and AF were absent or below the limits of detection of the analytical method. In another study, Burkin and Kononenko (2015) carried out a large survey in Western Russia to detect the presence of mycotoxins in grass and legume meadows between 2011 and 2014, using ELISA. In the Moscow oblast, AOL was the most prevalent mycotoxin, found in 96% of the samples at levels of 19–10,000 µg/kg, followed by STE in 64% of analyzed samples at levels of 8–200 µg/kg. The authors also reported CPA (60%; range: 115–2,455 µg/kg), EA (57%; range: 3–52,200 µg/kg), T-2 toxin (54%; range: 3–795 µg/kg), OTA (41%; range: 7–105 µg/kg), CIT (35%; range: 33–340 µg/kg), DAS (32%; range: 100–1,445 µg/kg, ZEN (21%; range: 25–5,700 µg/kg), and DON (19%; range: 78–930 µg/kg).

Orina *et al.* (2020), also in Russia, carried out a study to assess the presence of mycotoxins in legume pastures mainly made up by *Galega orientalis*, *Lathyrus pratensis*, *Medicago falcata*, *Medicago sativa*, *Melilotus albus, Melilotus officinalis, Trifolium hybridum*, *Trifolium* pratense, *Trifolium* Repens, *Vicia cracca*, *Vicia* sativae, and *Vicia sepio* in 2015, using ELISA. AOL was the most frequent mycotoxin, found in 100% of the samples at 20–1,549 µg/kg, followed by DAS (42%; range: 141–1,892 µg/kg), toxins T-2/HT-2 (41%; range: 4–27 µg/kg), and DON (32%; range: 100–631 µg/kg).

A study was performed in the mountains of the Czech Republic by Štýbnarová *et al.* (2016), to assess the occurrence of mycotoxins in dairy pastures mostly made up by *Nardus stricta*, *Dechampsia cespitosa*, *Avenella flexuosa*, *Bistorta officinalis*, *Calamagrostis villosa*, *Festuca supina*, *and Luzula sylvatica*, between June and September of 2014 and 2015, using ELISA. All samples were positive for *Fusarium* toxins, with mean levels of DON, T-2/H-T2 toxin, and ZEN equal to 667.5 µg/kg, 50.1 µg/kg, and 66.4 µg/kg, respectively. Penagos-Tabares *et al.* (2021) attempted to detect mycotoxins and other metabolites in Austrian dairy pastures mainly made up by *Lolium perenne, Dactylis glomerata, Poa pratensis, Festuca pratensis, Alopecurus pratensis, Phleum pretense, Trifolium pretense, Trifolium repens,* and *Medicago sativa,* correlating these findings with geoclimatic factors. Analyses were carried out between April and October 2019, using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Results showed 68 different fungal metabolites, and among relevant mycotoxins regarding animal production's health, the most frequent ones were NIV (83%; range: 38.1–574 µg/kg), AOL (61%; 1–23.7 µg/kg), ZEN (50%: range: 2.61–138 µg/kg), STE (44%; range: 1.03–7.34 µg/kg), EA (39%; range: 4.70–435 µg/kg), and DON (11%; range: 107–505 µg/kg). AF, FB, T-2, HT-2, and OTA were not detected. Metabolites belonging to the genus *Fusarium* were found in 100% of the samples, with B-Trich in 83% of them. Environmental

Table 1. Worldwide occurrence of mycotoxins in pastures reported from January 1987 until December 2021.

Country	Types of pastures	N	Types of mycotoxins	Positive samples		Concentration		LOD (µg/kg)	Analytical method	Reference
				n	%	Range (µg/kg)	Mean (µg/kg)			
Oceania:										
New Zealand	Predominantly *Lolium perenne* and *Trifolium repens*	60	ZEN	10	17	400–4,000	NR	100	HPLC GC-MS/MS	Di Menna *et al.* (1987)
Australia	Predominantly *L. perenne*	87	ZEN	72	83	0–21,000	1,380	NR	HPLC	Reed *et al.* (2004)
Australia	Predominantly *L. perenne* and *Trifolium subterraneum*	35 13 13 13	ZEN DON FB_1 AFB_1	15 6 3 2	43 46 23 15	60–4,950 129–682 158–998 14–16	1,028 268 621 15	32 50 NR 1	HPLC	Reed and Moore (2009)
Europe:										
Czech Republic	Predominantly *L. perenne, Festulolium pabulare, Festulolium braunii, Festuca rubra, Poa pratensis*	150	DON ZEN	NR	NR	12.34–71.43 3.7–173	42 61	NR	ELISA	Skládanka *et al.* (2011)
Czech Republic	Alpine meadows with predominance of *Nardus stricta, Dechampsia cespitosa, Avenella flexuosa, Bistorta officinalis, Calamagrostis villosa, Festuca supina,* and *Luzula sylvatica*	20	DON T-2/HT-2 ZEN	20 20 20	100 100 100	NR	667.5 50.1 66.4	NR	ELISA	Štýbnarová *et al.* (2016)
Russia	Meadow grass, Moscow Province (collected in 2014)	227	AOL STE CPA EA T-2 OTA CIT DAS ZEN DON	218 145 136 130 123 93 79 73 48 43	96 64 60 57 54 41 35 32 21 19	19–10,000 8–200 115–2,455 3–52,200 3–795 7–105 33–340 100–1,445 25–5,700 78–930	NR	NR	ELISA	Burkin and Kononenko (2015)
Russia	Predominantly *Galega orientalis*, *Lathyrus pratensis*, *Medicago falcata*, *Medicago sativa*, *Melilotus albus, Melilotus officinalis, Trifolium hybridum*, *Trifolium pratense*, *Trifolium repens*, *Vicia cracca*, *Vicia* sativae, and *Vicia sepio*	69	AOL DAS T-2/HT-2 DON	69 29 28 22	100 42 41 32	20–1,549 141–1,892 4–27 100–631	196 437 10 221	NR	ELISA	Orina *et al.* (2020)
Austria	Predominantly *L. perenne, Dactylis glomerata, Poa pratensis, Festuca pratensis, Alopecurus pratensis, Phleum pretense, Trifolium pretense, Trifolium repens,* and *Medicago sativa*	18	NIV AOL ZEN STE EA DON	15 11 9 8 7 2	83 61 50 44 39 11	38.1–574 1–23.7 2.61–138 1.03–7.34 4.70–435 107–505	170 6.41 29.6 2.94 163 306	NR	LC-MS/MS	Penagos-Tabares *et al.* (2021)
Americas:										
Argentina	Predominantly *Setaria geniculata, Cynodon plectostachyus, Cynodon dactylon, Panicum maximum, Paspalum notalum, Leersia hexandra,* and *Luziola peruviana*	29	ZEN	17	59	2–577.6	96,1	2	HPLC	Salvat *et al.* (2013)

(Continues)

Table 1 (continued).

Argentina	Natural pastures of unidentified grass, possibly *Leersia hexandra*, *Luziola periviana, Sorghastrum setosum, Spartina argentinensis, C. dactylon*	175	AOL ZEN STE T-2 HT-2	166 151 137 106 81	95 86 78 61 46	0.5–1,036 0.3–2,120 0.3–733 0.8–5,438 4–5,651	NR 62.95 NR NR NR	0.5 0.3 0.3 0.8 4.0	LC-MS/MS	Nichea *et al.* (2015)
United States	*C. dactylon*	157	ZEN T-2/HT-2 DON STE FUM	96 23 4 4 1	61.1 14.6 2.5 2.5 0.6	0–10,770 NR NR NR NR	664 NR NR NR NR	NR	LC-MS/MS	Gott *et al.* (2017)
United States	Pastures and conserved forage of *Schedonorus arundinacea*	40	B-Trich ZEN	23 11	57.5 27.5	NR	2,803 9,799	NR	HPLC LC-MS/MS	Gott *et al.* (2018)
United States	Pastures and conserved forage of *C. dactylon, Cynodon* spp., *Hemarthhria altíssima*	415	ZEN A-Trich B-Trich	249 69 40	60 16.6 9.6	NR	1,428 1,139 1,231	NR	LC-MS/MS	Gott *et al.* (2019)

N: number of samples analyzed; LOD: limit of detection; NR: not reported; ELISA: enzyme-linked immunosorbent assay; HPLC: high performance liquid chromatography; GC-MS/MS: gas chromatography coupled to tandem mass spectrometry; LC-MS/MS: liquid chromatography coupled to tandem mass spectrometry; AFB_1: aflatoxin B_1; AOL: alternariol; CPA: cyclopiazonic acid; CIT: citrinin; DAS: diacetoxyscirpenol; DON: deoxynivalenol; EA: Ergot alkaloids; FB_1: fumonisins B_1; FUM: fumonisins; HT-2: HT-2 toxin; NIV: nivalenol; OTA: ochratoxin A; STE: sterygmatocystin; T-2: T-2 toxin; ZEN: zearalenone; A-Trich: trichothecenes type A; B-Trich: trichothecenes type B.

temperature showed a positive linear correlation with the occurrence of mycotoxins. Temperatures higher than 15ºC led to an exponential increase in the metabolites produced by fungi of the genera *Alternaria* and *Fusarium.*

Americas

The first study carried out in America was conducted by Salvat *et al.* (2013) in the Province of Chaco, Argentina, to assess the occurrence of ZEN in natural and cultivated pastures from December 2011 and January 2012, using HPLC. ZEN was found in 59% of the samples, at levels of 2–577.6 μg/kg. Furthermore, Nichea *et al.* (2015) evaluated the occurrence of mycotoxins and other metabolites in natural grass pastures for beef cattle produced in the same Argentinean Province in 2011 and 2014, using LC-MS/MS. The authors detected 77 different metabolites, with 60 of them found in both years of analysis. Among mycotoxins relevant to animal production, AOL was the most prevalent compound, found in 94% of the samples at levels of 0.5–1,036 μg/kg, followed by ZEA (86%; range: 0.3–2,120 μg/kg), STE (78%; range: 0.3–733 μg/kg), T-2 toxin (61%; range: 0.8–5,438 μg/kg), and HT-2 toxin (46%; range: 4–5,651 μg/kg). AF, OTA, and CPA were not detected in any sample.

In the United States, Gott *et al.* (2017) carried out a study in Florida aiming at detecting mycotoxins in pastures of *Cynodon dactylon* L., using LC-MS/MS. ZEN was the most frequent toxin (61%; range: 27–1,936 μg/kg), followed by T-2 toxin (14.6%), DON (2.5%), STE (2.5%), and FB (0.6%). Subsequently, Gott *et al.* (2018) evaluated the occurrence of mycotoxins in pastures and conserved forage of *Schedonorus arundinacea* from the states of Kentucky and Georgia between August 2017 and January 2018, using HPLC and LC-MS/MS. EA were found in 100% of the samples, at a mean level of 410 μg/kg, followed by B-Trich (57.5%; mean level: 280.3 μg/kg) and ZEN (27.5%; mean level: 979.9 μg/kg). However, a larger study was also carried out by Gott *et al.* (2019) in the states of Florida, Texas, Alabama, Georgia, and Louisiana, to assess mycotoxins in pastures and conserved forage of *Cynodon dactylon, Cynodon* spp., *Hemarthhria altissima* between 2016 and 2019, using LC-MS/MS. The most frequent mycotoxin was ZEN (60%; mean level: 1,428 μg/kg), followed by Trich-A (16.6%; mean level: 1,139 μg/kg), and Trich-B (9.6%; mean level: 1,231 μg/kg).

Africa and Asia

No information on the occurrence levels of mycotoxins in pastures from the African and Asian continents was retrieved from the databases. However, a study conducted by Oluwafemi *et al.* (2014) in the city of Abeokuta, Nigeria, assessed the levels of AFM_1 in milk of dairy cows (n = 100) from herds raised in natural pastures. AFM_1 is the main metabolite produced after biotransformation of AFB_1 in the liver, being excreted in milk as a function of the ingested amount of AFB_1 (Gonçalves *et al.*, 2015). AFM_1 was found in 75% of the milk samples analyzed by Oluwafemi *et al.* (2014), at levels ranging from 0.009 to 0.456 μg/kg, hence indicating a significant contamination of the ingested pastures containing the parent compound (AFB_1).

Discussion

Data presented in the present study indicate that mycotoxins produced by the genus *Fusarium* were the most frequent ones in pastures, being reported in all articles evaluated. ZEN was the most prevalent mycotoxin, found in most of the studies, followed by trichothecenes. These results confirm, in part, the reports by Štýbnarová *et al.* (2016), Nichea *et al.* (2015), and Gott *et al.* (2017), in which fungi in the genus *Fusarium* are considered the most important mycotoxin producers in pastures, with DON, ZEN, AF, and FB as the most frequent toxins (Di Menna *et al.*, 1987; Gott *et al.*, 2017; Reed *et al.*, 2004; Reed and Moore, 2009; Salvat *et al.*, 2013; Štýbnarová *et al.*, 2016).

ZEN causes reproductive effects due to its estrogenic activity. It may cause problems of fertility and fetal development in ruminants raised in pastures. In addition, the consumption of ZEN and the excretion of its metabolites α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) in the urine of exposed animals may be confused with the use of grow promoters, substances that are banned in some countries of South America and the EU. Besides reproductive changes, the possible detection of α- and β-ZEL is one of the reasons why the occurrence of ZEN has been constantly studied in pastures (Di Menna *et al.*, 1987; Nichea *et al.*, 2015; Reed and Moore, 2009; Salvat *et al.*, 2013; Štýbnarová *et al.*, 2016). In this context, the levels of ZEN reported by Burkin and Kononenko (2015), Di Menna *et al.* (1987), Gott *et al.* (2017, 2018, 2019), Nichea *et al.* (2015), Reed *et al.* (2004), and Reed and Moore (2009) were higher than the lowest concentration that cause physiological effects (greater than 1,000 µg/kg), as determined by Reed and Moore (2009). Besides, together with the results by Salvat *et al.* (2013), these studies reported levels greater than the EU recommendations of 500 µg/kg for feed destined for calves, dairy cattle, sheep, and goats (European Commission, 2016).

Trichothecenes are important mycotoxins in terms of food safety and animal health worldwide due to their inhibitory effects on eucaryotic protein synthesis and mitochondrial function, changes in cell division and cell membranes, as well as potent immunosuppressing activity and digestive syndromes. Trich-B were the most prevalent mycotoxins found by Gott *et al.* (2018), Penagos-Tabares *et al.* (2021), Skládanka *et al.* (2011), and Štýbnarová *et al.* (2016), but at levels lower than the limits recommended for animal feed by the United States and EU. DON is the most prevalent Trich-B in ruminant feed all over the world. These animals have few acute effects, although chronic exposure can lead to reduced productivity due to gastrointestinal syndromes and increased vulnerability to other diseases (Burkin and Kononenko, 2015). Toxins T2 and HT2 (Trich-A mycotoxins) were found in most studies that analyzed the co-occurrence of mycotoxins in pastures. These mycotoxins are among the most toxic metabolites found in ruminant diets due to their effects on the upper digestive tract, pregnancy loss, and immunosuppressive activity. In one of the studies, levels observed were over 5,000 µg/kg, which is much greater than the limit recommended for animal feed in the EU (Burkin and Kononenko, 2015; Gott *et al.*, 2017, 2019; Nichea *et al.*, 2015; European Commission, 2013, 2016; FDA, 2016; Orina *et al.*, 2020; Štýbnarová *et al.*, 2016).

Regarding other *Fusarium* mycotoxins, FB was detected at low levels and frequencies in most studies. Few studies were carried out for the detection of AF, FUM, and OTA in pastures. Another important issue is the variability of methods employed in the detection of these toxins: ELISA, GC-MS/MS, HPLC, LC-MS/MS, which can yield results that may not be compared adequately. The occurrence of AOL was emphasized in the studies by Burkin and Kononenko (2015), who found it in 96% of the samples at levels ranging from 19 to 10,000, by Nichea *et al.* (2015) in 95% of the samples at levels of 0.5–1,036 µg/kg, by Orina *et al.* (2020) in 100% of samples ranging from 20 to 1,549 µg/kg, and by Penagos-Tabares *et al.* (2021) in 61% of samples containing 1–23.7 µg/kg. These are potentially toxic levels, since tolerance levels for this toxin have not been determined yet by the United States or EU. AOL is produced by the genus *Alternaria* and is generally related to the occurrence of digestive, muscular, and hemorrhagic syndromes in humans and poultry.

A was frequently detected in the studies by Burkin and Kononenko (2015), with 57% of the samples testing positive at levels of 3–52,200 µg/kg. Gott *et al.* (2018) and Penagos-Tabares *et al.* (2021) also reported high frequencies and concentrations of EA (100%, mean level: 410 µg/kg; 39%, mean level: 4.70–435 µg/kg, respectively). Despite the high occurrence in these studies, the levels observed were below the limits determined by the European Commission (2012). EA are produced by the genera *Claviceps* and *Neotyphodium*, and upon ingestion, they may cause infertility and reduced production indices, besides signs of ergotism, such as hyperthermia, gangrene, and seizures. There have been many reports on animal intoxication by EA, mainly in animals kept in pastures and fed conserved forage of *Lolium perene* and *Schedonorus arundinacea* (Canty *et al.*, 2014; Murty *et al.*, 2018)

Studies carried out by Baholet *et al.* (2019), Orina *et al.* (2020), and Skládanka *et al.* (2011), listed some factors that may interfere in mycotoxin production in pastures: (1) pasture management, as extensive management favors the occurrence of mycotoxins; (2) growth stage of the plant, with final stages favoring mycotoxin occurrence; (3) soil fertilization practices, as the use of biofertilizers may change levels and frequency of mycotoxin occurrence; (4) climate conditions, as mycotoxin production is generally related to stress caused by high temperatures and favorable humidity conditions. Moreover, the concerns presented by Battilani *et al.* (2016), Bebber *et al.* (2013), Medina *et al.* (2017), and Moretti *et al.* (2019) have pointed out towards a growing occurrence of mycotoxins due to global warming. In this context, the study by Penagos-Tabares *et al.* (2021) was one of the first to provide scientific evidence of the relationship between mycotoxin occurrence in pastures and geoclimatic factors, reinforcing the idea that global warming will increase the occurrence of mycotoxins in agricultural production, including in pastures, especially in Europe. Most of the studies evaluated, except for Gott *et al.* (2017, 2019), highlighted the occurrence of mycotoxins in pastures located in temperate climates. Therefore, studies on the occurrence of mycotoxins in tropical pastures are of fundamental importance, considering their regular use as nutrient sources in ruminant production systems of some countries, such as Brazil (Dias-Filho, 2016).

AFB_1 was detected in only one study by Reed and Moore (2009), who reported this mycotoxin in 15% of the pasture samples from Australia at levels of 14–15 μg/kg, which is close to the limit determined by the United States and EU for animal feed (European Commission, 2010; FDA, 2016). The occurrence of high levels of AFB_1 in feed of dairy cows is alarming, considering that 0.35–6.2% of the parent compound may be excreted into milk as AFM_1 (Souza *et al.*, 2021), and the fact that this metabolite also exhibits carcinogenic properties and toxic effects on the liver, kidneys, hematopoietic stem cells, and immune system (Daou *et al.*, 2022; Jafari *et al.*, 2021; Mokhtarian *et al.*, 2020). In addition, AFM_1 remains stable in milk and other dairy products even after conventional thermal processing performed in dairy plants (Pires *et al.*, 2022; Souza *et al.*, 2021), thus representing a remarkable danger to human health, especially to infants (0–12 months) (Daou *et al.*., 2022; Jafari *et al.*, 2021). Hence, the absence of data from African and Asian continents, and the few data reported in South American countries stress the need for studies on the occurrence of mycotoxins in pastures from those regions. In addition, the AFM_1 level (0.11 μg/kg) found by Oluwafemi *et al.* (2014) in the milk of Nigerian dairy cattle fed with natural pastures highlights the urgency of studies regarding the occurrence of AFB_1 in pastures, especially in African countries.

Concluding remarks

The most frequently found mycotoxins in pastures are metabolites produced by the *Fusarium* genus, such as ZEN and trichothecenes including DON, T2, and HT2 toxins. In 60% of the studies evaluated, ZEN was above the tolerance levels determined by the EU for animal feed. AOL and EA occurred most frequently in specific locations, such as Argentina, Austria, United States, and Russia, although at low or still unregulated levels. Temperature showed a linear correlation with mycotoxin production in Austrian pastures. Although these data show the general scenario of the occurrence of mycotoxins in pastures, this subject should be better studied in terms of co-occurrence and interaction, correlation with climate factors, different plant varieties, and in different regions. Further studies on the occurrence of mycotoxins in pastures from African, Asian, and South American regions are urgently needed.

Conflict of interest statement

None declared.

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