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MANAGEMENT OF MYCOTOXINS CONTAMINATION OF FEED INPUTS ON THE AGRI-FOOD CHAIN

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Abstract

Mycotoxins are toxic compounds for animals and humans, naturally produced by different types of fungi. Exposure to mycotoxins through the consumption of contaminated food or feed leads, among others, to gastrointestinal and renal disorders to the point of immune deficiency and cancer. Most mycotoxins are chemically stable and persist in food processing. Given the implementation of the requirements of the European Green Deal, especially those related to organic products, and in the context of climate change, especially regarding temperature and humidity fluctuations, the increase of the presence of mycotoxins in food and feed is highly expected. However, the evaluation of the degree of mycotoxin contamination in different steps on the food chain, with fast and accurate methods, is still an issue for farmers, especially those producing inputs for feed. In the context of a rising organic products market, the frequency and severity of issues related to mycotoxins contaminations are expected to increase. The current work presents the most recent data on mycotoxins occurrence in the world and in Europe and review the mycotoxins monitoring regulations in Europe and Romania. In the same time, the paper analyses the diverse array of methods of analysis developed in recent years and propose a non-exhaustive list of authorized and accredited laboratories in Romania, where players of the agri-food chain can check the quality of the feed inputs and manage the quality of their products. Our analysis shows an increased interest of the Romanian producers on food and feed safety issues, which led to a diversification of services in mycotoxins analysis field in Romania.

Key words**:** *quality management, food safety, mycotoxins, ochratoxin A, cereals*

INTRODUCTION

Mycotoxins are secondary metabolites produced by different types of fungi, as *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, and *Alternaria* [7], and are toxic to animals and humans [10]. The term "mycotoxins" was introduced in 1961, after more than 100,000 turkeys died from a mysterious disease called "turkey X disease" in Great Britain [28, 33]. Studies showed that the cause of the disease was actually in the feed, which included peanuts contaminated with aflatoxins, secondary metabolites of *Aspergillus flavus* [28, 33]. Mycotoxins enter the food chain because of crop infection before or after harvest and are mainly found in cereals, dried fruits, nuts and fruits and vegetables [10], but also in molluscs, herbs and spices, feed materials [20]. Exposure to mycotoxins through consumption of contaminated food or from animals fed with contaminated feed, especially aflatoxins, ochratoxin A and toxins secreted by the genus *Fusarium*, (deoxynivalenols) leads to gastrointestinal and renal disorders up to immune deficiency and cancer in humans [10]. In animals, high concentrations of mycotoxins may lead to acute symptoms, while low levels have long term impact on animal performance, which include, but are not limited to reduced weight gain, limited

feed efficiency, decrease in egg production, low milk production, reproductive failure, etc. [7]. Mycotoxins in feed represent a particular risk, especially for poultry, as their feed is composed of different ingredients, primarily cereals (rice, wheat, barley, oats, rye, maize, sorghum, and millet), by-products of milling [27], that all are highly susceptible for mycotoxin contamination [26]. The mycotoxins mode of action is generally represented by breaking cell membranes, preventing or influencing the synthesis of DNA, RNA and proteins, both in humans and animals [34]. In the context of increasing the organic production, stimulated by the new European Green Deal, and the large fluctuations in the temperature and humidity regime favorized by the climate change (factors favoring the growth of fungi), an increase of mycotoxins incidence in food and feed is anticipated.

Currently, around 300-400 mycotoxins are known, but only 20-30 of them are supervised by the authorities, due to their increased toxicity [10]. According to other sources, a larger number of mycotoxins is proposed, between 20,000 and 300,000 mycotoxins [33]. According to the EC 2016/1319, special attention must be paid to cereals and cereal products used directly in the feed of animals in own households, because the use of uncontrolled feed in the daily ration should not lead to the exposure of animals to a very high level of mycotoxins [18]. It is also worth mentioning that most mycotoxins are chemically stable and survive household food processing [35, 38]. At global level it was created a committee of scientific experts jointly convened by the World Health organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), called Joint FAO/WHO Expert Committee on Food Additives (JECFA), responsible for assessing the health risk caused by natural toxins, including mycotoxins [38]. International standards and codes of practice for limiting exposure to mycotoxins in certain foods are established by the Codex Alimentarius Commission based on JECFA assessments. European Food Safety Authority (EFSA) pays special attention to the monitoring of mycotoxins in animal feed, financing projects focused on this topic [11], since ochratoxin A (OTA) was proven to be a strong nephrotoxic, hepatotoxic, and teratogenic compound [8]. Intake of feed contaminated with OTA affects animal health and productivity and may lead to the presence of OTA in animal products for human consumption. Control strategies for OTA in food products require immediate identification and removal of contaminated commodities from the food chain [8]. However, current analytical protocols may fail to identify contaminated products, especially in animal feed [5, 7]. OTA is produced by several species of *Aspergillus* and *Penicillium* and is a common mycotoxin that can contaminate food, forming during storage of agri-food products. Its most noticeable and notable effect is kidney damage, but the toxin can also have effects on fetal development and the immune system [38]. To minimize the risk of contamination with mycotoxins, it is important to remember that some molds can produce visible infections, but other can penetrate deep into food without being visible. There are general guidelines for storage, to avoid mold growth, such as efficient drying of goods and keeping them dry, proper storage in controlled, low humidity. However, to minimize the health risk caused by mycotoxins, people are advised to regularly inspect stored grains (especially maize, sorghum, wheat, rice), and avoid damage to the grains before and during drying and storage, as damaged grains are more prone to mold invasion and therefore mycotoxin contamination; to purchase grains as fresh as possible; to regularly check the storage conditions and the integrity of the storage spaces, to store for as short periods as possible and to ensure a diverse diet, which also reduces exposure to mycotoxins, but also improves nutrition [37]. Pre-harvest methods, as using resistant varieties, biological and/or chemical plant protection products and field and harvest management and post-harvest methods as drying, maintaining good storage conditions and chemical protection are also good ways of limiting mycotoxins production [7].

Still, all these measures do not grant themselves that food and feed is safe and mycotoxins free. Despite the efforts and good agricultural practices, it is estimated that today 25% of the world's grain production is contaminated with mycotoxins [35]. Such high pressure on the food safety side requires easy access to fast and sensitive analysis methods with low detection limit values.

As methods of reducing mycotoxins effects, various ways have been tested, including sorbent materials [7], addition of enzymes [27], addition of detoxifying microorganisms [33], etc., both in vitro and in vivo. Among the trials, activated chydrated sodium calcium aluminosilicate, cholestyramine esterified glucomannan, diatomaceous clays, bentonite are the binding agents used in animal feed to prevent the adverse effects of mycotoxins [7, 28]. In addition, biological control of the mycotoxin-producing fungi has been developed, based on antagonistic microorganisms [33]. Still, these treatments imply additional costs and should be correlated with the mycotoxins content.

The present paper reviews the most recent data on mycotoxins occurrence in the world and in Europe and review the mycotoxins monitoring regulations in Europe and Romania. At the same time, the mycotoxins methods of analysis are presented, including the laboratories in Romania that perform these analyses.

MATERIALS AND METHODS

To reach the paper objective, a comprehensive review of the available online and of offline bibliographical references (Reports, Statistics, research papers, books, textbooks etc.), using the following international databases: Web of Science - Core Collection, Scopus (Elsevier), Science Direct Freedom Collection (Elsevier), Oxford Journals, CAB Abstracts, Google Scholar, simple Google research. The key words used for databases research were "mycotoxin" "mycotoxin definition", "mycotoxins impact, food", "mycotoxins impact, feed", "sources of mycotoxin contamination", "mycotoxins monitoring", "mycotoxin analysis". The search aimed at emphasizing the main aspects related to the subject at the global level, and especially in Europe and Romania. The legal framework regarding mycotoxins management was provided by Food and Agriculture Organization of the United Nations (FAO), Europe Food Safety Authority (ESFA), European Commission regulations, recommendations, laboratory, World Health Organization, National Sanitary Veterinary and Food Safety Authority Bucharest, Romania and its laboratories operating under the Institute of Hygiene and Veterinary Public Health (IISPV). The content of this article reflects the authors' opinions based on their original approach of the topic, regarding the logical structure of the researched problems, the depth of the detailed information, and the conclusions drawn at the end of the study.

The assessment of the degree of contamination with mycotoxins of feed inputs on the agri-food chain was done within a research project destined to improve quality management of agri-food products.

RESULTS AND DISCUSSIONS

Occurrence of mycotoxins contamination of feed inputs on the agri-food chain in the world

As reported by EW Nutrition, one of the biggest companies in mycotoxins analysis, with global coverage, on out of more than 4,000 analyses (over 1,000 samples covering grain and by-products commonly used in animal feed worldwide), 95% of the samples were contaminated with at least one mycotoxin [21].

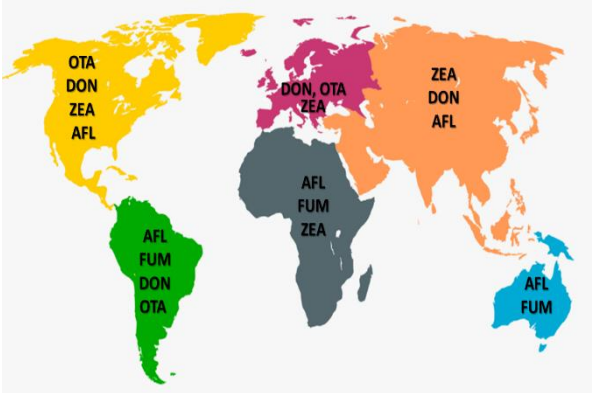

Fig. 1. Distribution of main mycotoxins by continent
Source: [35].

As a common finding of their assessment, most positive samples usually contained three or more mycotoxins, multiple contamination being the most usual [21].

The prevalence of mycotoxins varies by continent; in Europe, deoxynivalenol, ochratoxin A and zearalenone are the most common, while aflatoxins and fumonisins are most common in Australia (Figure 1).

Occurrence of mycotoxins contamination of feed inputs on the agri-food chain in Europe

With a solid set of legal acts implemented, EU strives to ensure that food is safe for its consumers, through one of the highest food safety standards in the world.

In 1979 an alert system was created to notify countries as fast as possible about food safety concerns.

Today, the Rapid Alert System for Food and Feed (RASFF) facilitate vital information exchanged in real time, making possible products recalling from the market before they could harm consumers [20].

According to the ACN Annual report 2021, mycotoxins were found in 450 food samples, in 2021, a number which is with 6% higher than in 2020 but with 23% lower than in 2019 [13]. The majority of positive samples – 399, were aflatoxins, of which 273 cases were reported in nuts [13]. As recurrent notifications, dried figs from Turkey where in 57 of cases.

The mycotoxin most frequently found in food was aflatoxin B1, but also ochratoxin A, with 47 positive samples, of which the majority were spices and dried figs [13]. For the current year, there were 414 alerts already issued including the word `toxin`, of the total 1,288 listed.

Out of the total alerts for 2022, 344 alerts where for aflatoxin, of the total of 1074 alerts for aflatoxin, 49 alerts were for ochratoxin A, of the total of 136 alerts, four alerts of lipophilic toxins, out of the total 11 listed, two alerts for azaspiracid toxins, two alerts for mycotoxins, in general, out of the five listed, one staphylococcal toxin out of the two alerts listed, one zearalenone alert out of the two listed, three alerts for fumonisins of the 10 alerts in total, and three patulin alerts out of the total eight listed [13].

As conclusion, in RASSF, mycotoxins represented in 2021 the third most notified hazard category, and three-quarters of the notifications were border rejections [13].

Mycotoxins monitoring and analysis in Europe

At EU level, the body coordinating the mycotoxin topic is the Directorate-General for Health and Food Safety [9].

The authority making the recommendations is the European Food Safety Authority (EFSA) [12]. The EU Reference laboratory (EURL) for Mycotoxins and Plant Toxins is the Wageningen Food Safety Research Institute (WFSR), specialized in (forensic) measurements, top level research and development of methods to detect substances in food and feed, for a safe and authentic food [37].

The legislation and regulatory acts in place guiding the mycotoxins' related activities are:

- Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs [14],
- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs [15],
- Commission Recommendation of 15 March 2012 on the monitoring of the presence of ergot alkaloids in feed and food [17],
- Commission Recommendation of 17 August 2006, on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding [16].

Therefore, the Commission for Food Safety Recommendations includes the following mycotoxins to be monitored:

-Aflatoxins
-Fusarium toxins
-Deoxynivalenol
-Fumonisins
-Nivalenol
-T-2 and HT-2 toxin
-Zearalenone
-Ochratoxin A
-Patulin (Figure 2)

Fig. 2. The mycotoxins that should be monitored as recommended by the EU Commission for Food Safety
Source: Own design based on [10].

In addition, following the EFSA opinions, the Standing Commission for Food safety also recommended the following mycotoxins to be monitored:
-Sterigmatocystin,
-Ergot alkaloids,
-Phomposins,
-Alternaria toxins
-Citrinin [19].
The Standing Committee on plants, animals, food and feed, the section of Toxicological Safety of the Food chain, held in Brussels several meetings in the period 2012-2014, and issued a "*Compilation of agreed monitoring recommendations as regards the presence of mycotoxins and plant toxins in food*", a document that includes valuable data on the methods, the limits of quantification and the foods /feeds to be targeted for monitoring the presence of tropane alkaloids, sterigmatocystin, deoxynivalenol, ergot alkaloids, phomopsins, citrinin, pyrrolizidine alkaloids, and Alternaria toxins [19, 31].

Mycotoxins monitoring and analysis in Romania

In Romania, the responsible authority for mycotoxins monitoring and analysis is the National Sanitary Veterinary and Food Safety Authority, Bucharest (ANSVSA), by the laboratories operating under the Institute of Hygiene and Veterinary Public Health (IISPV).
These laboratories, functioning as National Reference Laboratories (NRL), are:
- NRL for Mycotoxins and Plant Toxins in Food Products of Animal Origin and Animal Feed
- NRL for Mycotoxins in Food Products of Non-Animal Origin.

As regulatory acts, beside the EC regulations and recommendations, in Romania the following laws are in place:
- Order no. 63 of October 10, 2012 for the approval of the Veterinary Sanitary Norm that establishes the minimum standards regarding the protection of birds on the farm and during transport [3],
- Decision no. 1156 of December 23, 2013 for the approval of the sanitary-veterinary actions contained in the Program of actions for surveillance, prevention, control and eradication of animal diseases, of those transmissible from animals to humans, animal protection and environmental protection, identification and registration of cattle, pigs, sheep, goats and equids, of the actions provided for in the Food Safety Supervision and Control Program, as well as the related tariffs [4].

A special care related to animal feed is given by the law to the poultry feed, the maximum level of mycotoxins being the following:
a) deoxynivalenol: 8 mg/kg for cereals and cereal products, 12 mg/kg for corn products and 5 mg/kg for combined feed;
b) zearalenone: 2 mg/kg for cereals and cereal products and 3 mg/kg for corn products;
c) ochratoxin A: 0.25 mg/kg for cereals and cereal products and 0.1 mg/kg for combined feed;
d) fumonisin: 60 mg/kg for corn and corn products and 20 mg/kg for combined feed.
These values are the same for the breeding hens, chickens intended for meat production and turkeys intended for meat production [3].
In Romania, besides the central and local laboratories of the ANSVSA, there are also private laboratories that perform mycotoxins analysis, all of them being authorized both by the ANSVSA and accredited by the Romanian Accreditation Association (RENAR).
The entities listed in Table 1 are the first that appears on a simple Google search, using the key words "mycotoxin analysis".

Table 1. The Romanian authorized laboratories to perform mycotoxins analysis

Laboratory name	Region / City	Matrix	Mycotoxin
IBA București	Bucharest	Very diverse	DON, AFL, ZEA, OTA, AFLM1
ICA Research & Development	Bucharest	wheat, corn, spices, coffee, animal feed	DON, OTA, PA, T-2 / HT-2ZEA
APIS Laboratories	Moldova / Iași	milk, milk powder, animal feed, grains, grain products, corn-based foods and nuts	DON, ZEA, OTA, AFL, FUM
IQLAB Service SRL	Bucharest	Food and feed	AFLA, OTA, ZEA, DON
Eurolab laboratory services	International	Food and feed	MYC, AFL-M1, AFL-B1, DON, FUM, DAS, HT-2, OTA, PA, OTB, CTN, T-2, ZEA
SGS Romania S.A.	International	Not specified	Available on request
Synevovet	International	Diverse matrixes	AFLA, AFL-B1, AFL-M1, OTA, DON, ZEA, FUM, T-2
Primoris Bulgaria	Plovdiv, Bulgaria	animal feed, cereals and cereal products, coffee, dried fruits and nuts, milk, baby food, apple	AFL, DON, AFL-M1, FUM, T2, OTA, ZEA, ERG, PA, TA
ALS Laboratories	Ploiesti, Deva, Iasi	cereals, dried fruits, nuts, spices, cocoa	DON, AFL-M1, OTA, ZEA, PA, T-2
LABROM	Bacau, Alba Iulia	animal feed	AFL, OTA, DON

Aflatoxins (AFL)	**HT-2 toxin (HT-2)**
Aflatoxin B1 (AFL-B1)	Mycotoxins (MYC)
Aflatoxin M1 (AFL-M1)	Ochratoxina A (OTA)
Citrin (CTN)	Ochratoxina B (OTB)
Ergot alkaloids (ERG)	Patulin (PA)
Fumonizin (FUM)	Tropane alkaloids (TA)
Deoxynivalenol (DON)	T-2 toxin (T-2)
Diacetoxyscirpenol (DAS)	Zearalenon (ZEA)

Source: Own conception.

Mycotoxins analysis methods

Today, a high number of methods of analysis for mycotoxins are available, but researchers in food safety are continuously developing new methods, to obtain a faster and more reliable result. Among the developed methods, we name the thin layer chromatography (TLC) [30], gas chromatography - mass spectrometry (GC-MS) [6], liquid chromatography - tandem mass spectrometry (LC-MS/MS) [2; 24; 29], immunologic method - ELISA (Enzyme linked immunosorbent assay [22, 23], gold nanoparticle-based immunochromatographic assay. These quantitative methods, are based on different extraction and clean-up techniques, such as: liquid–liquid extraction (LLE), immunoaffinity columns (IAC), supercritical fluid extraction (SFE), solid phase extraction (SPE) [36], solid phase microextraction (SPME) [39], cation exchange resin [32], liquid-liquid microextraction in porous hollow fibers, and solid bar microextraction (SBME) - which using uses only a few milligrams of a sorbent wrapped in a hollow fiber micro-tube [1]. One of the most used methods for ochratoxin A analysis in feed inputs is the HPLC-FLR technique after purification on immuno-affinity columns due to its high sensitivity. Fluorimetric detector for HPLC remains the classical and stable method with a wide application area in mycotoxins analysis, where the influence of matrix is negligible.

In 2018 an LC–MS/MS method for the simultaneous determination of citrinin and ochratoxin A in a variety of feeds and foods was developed and validated, which shows the scientific community's concern for this challenge [25]. This method is also rather expensive, usually time-consuming and requiring deuterated internal standard, for these reasons, LC-MS/MS is more often used for the elucidation of OTA metabolites and for structural confirmation.

CONCLUSIONS

Mycotoxins are an increasing threat for food security, despite the efforts done to improve the agricultural practices and the post-harvesting practices. In the last years, special attention was given to the mycotoxin issues, many official recommendation and regulations being issued by the competent authorities, in the world, Europe and Romania. Despite the measures taken and awareness campaigns organized by different stakeholders, mycotoxins are still an issue of concern and the research on new methods for more sensitive and reliable results is ongoing. Diverse methods of analysis have been recently developed and many new authorized and accredited laboratories in Romania allow not to the players of the agri-food chain to check the quality of the feed inputs and manage the quality of their products. There is an increased interest of the Romanian producers on food and feed safety issues, which led to this diversification of analysis in Romania, including for mycotoxins that are not included in the EU or National regulations as mandatory.

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