Multitoxin Contamination

Subjects: Public, Environmental & Occupational Health

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Mycotoxins, as natural products of molds, are often unavoidable contaminants of food and feed, to which the increasingly evident climate changes contribute a large part. The consequences are more or less severe and range from economic losses to worrying health problems to a fatal outcome. One of the best preventive approaches is regular monitoring of food and feed for the presence of mycotoxins. However, even under conditions of frequent, comprehensive, and conscientious controls, the desired protection goal may not be achieved. In fact, it often happens that, despite favorable analytical results that do not indicate high mycotoxin contamination, symptoms of their presence occur in practice. The most common reasons for this are the simultaneous presence of several different mycotoxins whose individual content does not exceed the detectable or prescribed values and/or the alteration of the form of the mycotoxin, which renders it impossible to be analytically determined using routine methods. When such contaminated foods enter a living organism, toxic effects occur.

multitoxin food feed

1. Introduction

Mycotoxins are already-known natural products of low molecular weight that are synthesized as secondary metabolites of filamentous fungi, usually *Fusarium*, *Penicillium*, and *Aspergillus*, and are often found as contaminants in food and feed [1]. They represent a toxic and chemically heterogeneous group, classified together for their ability to damage human and animal health (mycotoxicosis) and even cause fatal outcomes. They were named in 1962 after an unexpected animal health crisis near London, England, in which about 100,000 turkeys died from the mysterious X disease, which was linked to peanut meal contaminated with aflatoxins, secondary metabolites of *Aspergillus flavus*. This motivated numerous studies and expanded the number of mycotoxins discovered. In the period from 1960 to 1975, a large number of scientists engaged in well-funded research into new mycotoxins, which is why these years are also known as the mycotoxin rush. Today, there are about 400 different compounds bearing this name, and among them, there are a dozen that attract special attention because they pose a threat to human and animal health [2].

Mycotoxins can be synthesized by fungi directly in the field, already on the plants during the growing season (if they remain in the agricultural products at the time of their collection), or produced during storage or technological processes. Their presence is related to the fungal genus or species, the agronomic practices, the type of crop, and the harvesting, handling, and storage conditions. However, they are considered one of the foodborne risks that correlate with climatic changes. In this context, it is important to keep in mind that the two most important factors affecting the life cycle of all microorganisms, including mycotoxic molds, are water availability and temperature. In general, the amount of toxins synthesized depends on several factors: physical factors such as the temperature, the relative humidity, the moisture of the matrix, the water activity (aw), and the degree of mechanical damage to the grains; chemical factors such as the oxygen and carbon dioxide content, the composition of the substrate, and the presence of pesticides and fungicides; and biological factors such as the plant variety affected, the presence of stressors, the influence of insects, and the spore load [3]. Anything that can influence these factors can directly or indirectly change the mycotoxicological situation.

Based on these facts, weather extremes have been found to influence the occurrence of mycotoxins in food and feed. For example, years with regional weather conditions described as extreme (high temperatures, lack of precipitation, and pronounced drought) are prone to the occurrence of aflatoxins (AFs) in crops. This was reported in 2021, when the highest recorded prevalence of AFs in corn samples was 84% in Serbia and 40% in Croatia. Earlier reports for corn originating from the 2018–2020 cropping period are much more favorable, showing contamination in 10% of Serbian and 20% of Croatian samples. [4]. The problem of the presence of AFs in cereals from Serbia was the cause of the elevated levels of aflatoxin M1 in milk and dairy products a few years earlier [5], while the risk assessment in Croatia for the period 2016–2022 indicated that there is cause for concern in autumn and winter, especially for consumers of large quantities of milk [6]. The occurrence and contamination level of Fusarium mycotoxins in the maize samples analyzed in the study from the same region for the period 2018–2022 varied from year to year and might be related to climatic conditions. FUMs were identified as the most prevalent contaminants in maize in Serbia and Croatia. When analyzing the ten-year results from 2012 to 2022, the highest levels of DON and ZEN were found in maize samples from 2014. This could be due to the extreme rainfall observed in both countries in that year [7].

Such findings are not surprising, given the report of the Intergovernmental Panel on Climate Change (IPCC) [8], which states that the effects of global warming have already been observed and that this has already affected ecosystems and some of the services they provide. Climate-related risks to health, livelihoods, food security, water supply, human security, and economic growth are projected to increase with 1.5 °C of global warming and to be worse with a 2 °C increase. The IPCC further summarizes that there is a high probability of extreme heat in most populated regions and an increase in average temperatures in most terrestrial and marine regions, while it predicts a medium probability of heavy precipitation in several regions and the likelihood of droughts and precipitation deficits in others. In the face of these predictions, the safety of food (and feed) will be compromised in a number of ways [9], and only one of these is infestation with toxic molds and contamination with their secondary metabolites—mycotoxins.

Various preventive measures and control strategies, as well as treatments, can be tried [10][11][12], although the absolute elimination of these natural substances is not possible. In this struggle, the monitoring of food and feed and the timely determination of the nature and quantity of these contaminants [13] have a very important place in controlling the harmful effects on living organisms caused by their consumption. However, even on this path, despite modern techniques and growing knowledge on the subject, there are some obstacles.

2. Multitoxin Contamination

Mycotoxins are a huge global food safety concern, especially in light of recent estimates that they contaminate 60–80% of the food produced worldwide [14] and that this group consists of hundreds of identified species. Among them, aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), deoxynivalenol (DON), patulin (PAT), nivalenol (NIV), the T-2 toxin (T-2), the HT-2 toxin (HT-2), and zearalenone (ZEA) and its derivatives are of greatest concern as they are common and frequently detected in nature [15]. Therefore, many countries have set individual limits for various foods intended for human and animal consumption [16]. Current legislation refers to the assessment of risks posed by individual mycotoxins and, initially, by the metabolites of mycotoxins together with the parent compound. However, they do not take into account the multiple dynamics and potential interactions between co-occurring groups of mycotoxins [17].

Adding to all of the above the increasingly frequent extreme climatic events, to which fungi and their secondary metabolism are highly susceptible, the real mycotoxicological picture is often far more complex than typical patterns and goes far beyond one-sided viewpoints. Since a single fungal species can produce various mycotoxins and fungi of different genera may infect the same crops, the co-occurrence of several mycotoxins and simultaneous exposure in the total diet are even more likely. Such exposure to a combination of mycotoxins can cause the same effects or lead to a combination of different side effects. As stated by van den Brand et al. [18], when assessing the combined risk of co-occurring mycotoxins, this should be taken into account.

A significant analysis of the literature data on the presence of mycotoxins in foods derived from cereals in Europe and their natural co-occurrence was provided by Palumbo et al. [19]. The database for the presence of mycotoxins in cereals included twelve crop groups: corn, wheat, barley, oat, rye, sorghum, triticale, buckwheat, spelt, rye, millet, and soy. Mycotoxins were commonly noted in wheat and maize, with the highest occurrence of FUMs, DON, AFs, and ZEA. The highest content of FUMs (B1 + B2) was reported in corn (both for feed and food) and was above legal maximum levels (MLs). Similar results were found for DON in food, whose maximum concentrations in corn, wheat, oats, and barley exceeded the MLs. The co-occurrence of two or more mycotoxins was reported in 54.9% of the total records. Co-contamination of DON is usually observed with FUMs in corn and ZEA in wheat, while the combination of DON + NIV and DON + T2/HT2 is often reported in barley and oats. Some publications, analyzed in a Portuguese study [20], reported the co-occurrence of mycotoxins in 75% of foods derived from cereals. A similar percentage for the three mycotoxins ZEA, DON, and NIV was noted in 74% of wheat samples from Brazil sampled in 2009 and 12% originating in 2010, with precipitation during flowering or harvest periods explaining the difference between these results [21]. In Korea, co-occurrence of mycotoxins with glucoside conjugates has been reported with a maximum of 49% [22]. Another Korean study [23] investigated the levels of 13 mycotoxins in five types of commercial cereals (brown rice, maize, millet, sorghum, and mixed grains) and showed that *Fusarium* mycotoxins (FUMs, DON, NIV, and ZEA) were more frequently and simultaneously detected in all cereals and with higher mean concentrations than other toxins, like ochratoxin A and aflatoxins. Joshi et al. [24] examined multi-mycotoxin contamination in Nepalese maize, and they found out that all the samples contained at least a couple of mycotoxins, while more of them were found in 87% of the examined samples. The most frequently encountered binary, ternary, and quaternary contaminants were DON + AFs, AFs + FUMs + DON, and AFs + FUMs + ZEA + DON, respectively. An Algerian survey on 120 grain samples from markets (corn, wheat, barley, and rice) was performed to evaluate the presence of 15 mycotoxins. The test results showed that 65% of the samples (78 of them) were contaminated with some of the mycotoxins, while 50% contained between three and nine different types, with deoxynivalenol, the T-2 toxin, beauvericin, and citrinin being the most commonly found [25].

As reviewed by Tolosa et al. [26], animal feed is particularly vulnerable to contamination by multiple mycotoxins as it is a mixture of several raw ingredients, so it already becomes the rule, not the exception, while the transfer of mycotoxins from animal feed to food of animal origin is often demonstrated. According to Gruber-Dorninger et al. [27], out of 74,821 samples of feed and feed raw materials collected from 100 countries from 2008 to 2017, 64% proved positive for at least two mycotoxins. The most frequently observed mycotoxin mixtures were combinations of DON, ZEA, and FUMs, as well as FUMs and AF B_1. According to their extensive global survey, they concluded that (co-)contamination of animal feed with mycotoxins is common, regionally defined, and partially driven by climate and weather.

Considering the results of multi-mycotoxin monitoring studies in Europe, a large percentage of feed has been found to contain more than one mycotoxin. Belgium's Monbaliu et al. [28] reported that 75% of samples (sow feed, wheat, and maize) were contaminated with more than one type of mycotoxin, while type B trichothecenes and FUMs occurred most often. Up to 90% of the 1384 samples of raw materials and products for animal feed (complete feed and components like small grain, corn, and corn silage) from Poland, analyzed by Kosicki et al. [29], showed to contain DON and ZEA. They commonly found the combination of DON, T-2, and HT-2; ZEA, T-2, and HT-2; DON, T-2, HT-2, and ZEA; and DON and FUMs in maize. According to Romanian authors Stanciu et al. [30], 42% of the 116 cereal samples tested contained between two and five mycotoxins, with enniatin B being the most prevalent, followed by DON and ZEA. Castaldo et al. [31] dealt with the presence of 28 mycotoxins in 89 pet food samples from Italy. They reported mycotoxin contamination in 99.9% of the samples, with all the positive samples showing co-occurrence of mycotoxins and the simultaneous presence of up to 16 mycotoxins per sample.

Finally, an increasingly topical issue is the occurrence of so-called "emerging mycotoxins". This group consists of currently non-regulated mycotoxins produced by *Fusarium* spp., which include beauvericin (BEA), enniatins (ENNs), and fusaproliferin (FUS). Permitted levels have not been set for these mycotoxins as there are not enough data regarding their toxicity, occurrence, and contamination levels [32]. However, beauvericin (BEA) and enniatins (ENNs) are found in cereals to a large extent, although the pattern of contamination varies between crops and the contents strive to be higher in cold climates [26]. Their co-occurrence was observed in 47% of the samples, as stated by Tolosa et al. [33]. Also, the co-contamination of 87% of maize silage samples with ENNs, BEA, and other *Fusarium* mycotoxins, such as DON and NIV, was reported by Reisinger et al. [34], which Krizova et al. reviewed later [35].

Regular monitoring of different mycotoxins and the collection of data on their presence and co-occurrence, with details of the location of the crops, as well as the availability of this information, should be a priority. By adding knowledge about weather forecasting and plant phenology, it is possible to build predictive models to estimate mycotoxin risk levels with great confidence [36]. This would be highly beneficial for different stakeholders: farmers; the feed and food industry, including collectors, millers, and processors; food safety authorities; and above all, final consumers [3]. **Table 1** summarizes information on the presence of multiple mycotoxins in different feed and food samples, their incidence, and data sources.

Table 1. Presence of multiple mycotoxins—literature summary.

Co-Occurring Toxins	Matrix	Incidence of Multiple Contamination [%]	Source
FUMs, DON, ZEA, NIV, T-2/HT-2	maize, wheat, barley, oats, etc.	54.9	[19]
OTA, ZEA, DON	cereals	75	[20]
ZEA, DON, NIV	wheat	74 and 12	[21]
DON, DON3G NIV, NIV3G	market foods	49	[22]
FUMs, DON, NIV, ZEA	commercial cereals	3 for quadruple 22 for triple 30 for double	[23]
DON + AFs AFs + FUMs + DON AFs + FUMs + ZEA + DON	maize	100 for double 87 for triple and more	[24]
T-2, citrinin, BEA, DON	market cereals	50 for triple and more	[25]
DON, ZEA, FUMs o rFUMs + AFB1	feed and feed materials	64	[27]
FUMs with type B trichothecenes	feed and feed materials	75	[28]
DON, ZEA, T-2/HT-2, FUMs	feed	up to 90	[29]
ENN B, DON, ZEA	cereals	42	[30]
Up to 16 analytes	pet food	99	[31]
BEA + ENNs	feed and feed materials	47	[33]
More than 5 *Fusarium* mycotoxins	maize silage	87	[34]

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