Monitoring of Mycotoxins in Feed for Dairy Cows and Swine from Muntenia Region, Romania

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Abstract

Mycotoxins have a strong negative impact on crops, animal health and human health. These secondary metabolites are toxic and can cause major economic losses and illnesses (Zain, 2011). This study aimed to evaluate the presence of the most prevalent mycotoxins, aflatoxin B1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEN), in feed intended for dairy cows and swine from farms located in the Region of Muntenia (Călăraşi, Giurgiu and Teleorman), Romania. 136 samples of fodder and forage intended for cattle and 37 samples for pigs were collected during 2017 - 2018. Some of the samples were analyzed within the national surveillance program and some on request. AFB1 has been extracted and purified in an immunoaffinity column specific for AFB1. Detection and quantification of toxic secondary metabolites were performed by high-performance liquid chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA). All four mycotoxins were detectable and quantifiable. The level of contamination was generally low. The maximum allowable limits for AFB1, OTA, DON and ZEN were not exceeded. The low level of contamination indicates no potential risks for animal and human health and suggests that the best practices in the management and storage of feed were applied in the studied area.

Keywords: dairy cows, feed, mycotoxins, monitoring, swine

Introduction

Mycotoxins are secondary metabolites of certain molds (fungi) that can adversely affect animal and human health because of their toxicity (Alshannaq and Yu, 2017; Fink-Gremmels and Van der Merwe, 2019). Their negative effects can be observed in all vertebrates, but a special interest has been given the species of animals that can provide food products for humans (cattle swine, sheep, goats, poultry, etc). Multiple studies conducted in the last decade involve the detection and

quantification of mycotoxins not only in animal origin products (Becker-Algeri *et al.*, 2016), but also in plant origin products (Barkai-Golan and Paster, 2008). From the last group, animal feed is apparently more prone to contamination with mycotoxins (Gruber-Dorninger *et al.*, 2018, Tola and Kibede, 2016). Feedstuffs for cattle and swine were analyzed worldwide in the last 5 years (Changwa *et al.*, 2018; Li *et al.*, 2014, Ma *et al.*, 2018). Monitoring primary production can prevent the contamination of future products and also of the food

chain, in general (Fink-Gremmels and Van der Merwe, 2019; Pinotti *et al.*, 2016; Schatzmayr and Streit, 2013). The importance of this step is linked to the economic losses that contaminated products can produce. Beside the economic losses that mycotoxins can cause, such as health problems or even death in humans, their occurrence implies significant economic losses also in areas like crop growth, animal production, food processing (animal or vegetable origin), veterinary care, national and international trades, research on the topic, etc (Bryden, 2012; Hussein and Brasel, 2001; Iheshiolor *et al.*, 2010; Zain, 2011).

Presently, over 300 mycotoxins have been identified and reported. This number includes some of the most dangerous mycotoxins: aflatoxins (AF), ochratoxins (OTA), fumonisins, patulins, zearalenones (ZEN), and trichothecenes including deoxynivalenol (DON) and T-2 toxin). These groups showed increased popularity on a global scale due to their high prevalence and toxicity (Marin *et al.* 2013).

AFB1 is a secondary metabolite of *Aspergillus flavus* and *Aspergillus parasiticus*. The exposure to this mycotoxin after contaminated feedstuff ingestion can lead to a very serious health issue, the hepatocellular carcinoma (HCC) (Hamid *et al.*, 2013; Magnussen and Parsi, 2013). Moreover, when AFB1 becomes metabolized in the organism of dairy cattle, another problem arises. AFB1 is converted to AFM1 and excreted in milk. Therefore, milk and milk products can also serve as an indirect source of aflatoxins (Iqbal *et al.*, 2015; Iqbal *et al.*, 2016). Lately, a great number of studies have been conducted on aflatoxins as a result of the concern for food safety and human health (Kumar *et al.*, 2017). The International Agency for Research on Cancer (IARC) described AFB1 and AFM1 as human carcinogens and placed them in Group 1 and Group 2B (IARC, 1993).

OTA is produced by several species of *Aspergillus* and *Penicillium* and it is nephrotoxic and nephrocarcinogenic (Bui-Klimke and Wu, 2015). OTA belongs to Group 2B, possibly carcinogenic to humans, based on demonstrated carcinogenicity in animal studies (IARC, 1993).

ZEN is a nonsteroidal estrogenic mycotoxin produced by several species of *Fusarium*. The toxin is responsible for oestrogenic effects and several studies refer to it a endocrine disruptor (Kowalska *et al.*, 2016; Metzler *et al.*, 2015).

DON is the most frequent trichothecene contaminant of agricultural crops throughout the world and it is produced by species such as *Fusarium graminearum*, *Fusarium cerealis* and *Fusarium culmorum*. DON does not constitute a significant threat to public health, but it can affect the immune system and cause temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever to both humans and animals (Sobrova *et al.*, 2010).

In 2018, Romania has ranked 3rd place in the European Union (EU) in grain harvest. Also, in the years before 2018 other records for maize were obtained. Being an important producer on the EU market, for products that can serve as either feed or food, requires careful monitoring of mycotoxins, especially in areas where the presence of AFB1, OTA, ZEN and DON were previously reported (e.g. West Romania, SE Romania, etc) (Alexa *et al.* 2013; Gagiuc *et al.*, 2018; Stanciu *et al.*, 2019; Tabuc *et al.*, 2011).

Materials and methods

Sample Collection

A total of 271 feed samples were taken from 3 counties located in Romania between January 2017 and December 2018. The counties of origin and the sample numbers are represented in Figure 1. The samples collected in 2017 were represented by fodder and forage for dairy cows and pigs (n=156). The fodder and forage material collected in 2018 was exclusively part of the feed for dairy cows (n=115). Various categories of samples were taken into study. The first category, cattle feedstuff, included combined fodder, grain legumes, grain cereals (barley, maize, wheat), soy grist, rapeseed grist, maize silage, corn, alfalfa hay, middlings, pickled fodder (maize silage/ alfalfa silage), sunflower grist. The second category, swine feedstuff, was composed of combined fodder, grain legumes, grain maize, grain cereals, soy grist and rapeseed grist.

Instrumental Analysis

In the process of selecting and implementing the appropriate method the following aspects were considered: compliance with international regulatory limits, facility and accessibility in active monitorization conditions (sample number and cost-analysis ratio).

The HPLC method performed on one part of the collected samples was described by Irakli *et al.*, 2017. The other part was analyzed using the

Figure 1. Studied Area during 2017-2018

ELISA method. The principle of the ELISA was described by Zheng *et al.*, 2006. The ELISA kits for the identification of AFB1, OTA, DON and ZEN were ordered and purchased from R-Biopharm (Darmstadt, Germany) and used according to the indications of the producer.

Results and discussions

The maximum residue limits (MRLs) for AFB1, OTA, DON, ZEN in feed are regulated by EC Directive 32/2002 and EC Commission Recommendation 576/2006. The MRLs were compared to the results obtained after performing Enzyme-Linked ImmunoSorbent Assay (ELISA) and high-performance liquid chromatography (HPLC) Analyses on Feed Samples.

Table 1 reveals contamination rate with different mycotoxins in feed samples collected between 2017-2018. Aflatoxin B1 (AFB1) was the mycotoxin with the highest incidence in dairy cow. Tabuc *et al.*, 2011 has mentioned that this toxin in particular must be continuously monitored in SE Romania. The results of ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEN) were generally low and followed the trend ZEN<DON<OTA<AFB1 in dairy cow feed from 2017 and 2018. It appears that in SE Romania the incidence of mycotoxins is different than in West Romania, where Alexa *et al.*, 2013 revealed that DON has recorded an incidence of 73.08% in the samples harvested in 2010.

Table 1. Mycotoxin Contamination Rate

Type of Feed	Period	AFB1	OTA	DON	ZEN	Aflatoxin Σ
Bovine Feed	2017	74.58%	10.17%	9.32%	5.93%	-
Bovine Feed	2018	63.72%	16.81%	10.62%	7.08%	1.77%
Swine Feed	2017	45.56%	2.7%	21.62%	5.41%	-

In this study, the incidence values of DON in dairy cow feed from 2017 were similar to the values of the same toxin, in the same samples from 2018 (Tab. 1). The incidence similarity between the years 2017 and 2018 was also observed in ZEN, although at lower levels. A slightly higher variability was observed in AFB1 and OTA in the consecutive years (marked with red circles in Figure 2).

The analysis of swine feed from 2017 showed that the most encountered mycotoxin was AFB1 with a rate of 45.56%, followed by DON (21.62%), ZEN (5.41%) and OTA (2.7%) (Tab. 1). The trend

AFB1>DON>ZEN>OTA is in accordance with the trend described by Gagi *et al.*, 2018.

Table 2 presents the distribution of mycotoxins in feed by county of origin. All of the samples collected from the 3 counties, regardless of the feed type, revealed strong positive results for AFB1. The highest prevalence of AFB1 was registered in Giurgiu county in 2018 (100% in dairy cow feed). Therefore, all different types of feed from Giurgiu were contaminated with AFB1 residues. OTA occurrence was mainly associated with Călărași county. In this location some of the feed samples coming from dairy cow feed revealed

Figure 2. Variability of Mycotoxin Contamination in Dairy Cow Feed

Table 2. Mycotoxin Distribution in Feed by County

Type of Feed	Period	County	AFB1	OTA	DON	ZEN	Aflatoxin Σ
Dairy Cow Feed	2017	Călărași	75.95%	13.92%	6.32%	3.8%	-
		Giurgiu	68.75%	-	18.75%	12.5%	-
		Teleorman	85.71%	14.29%	-	-	-
Dairy Cow Feed	2018	Călărași	63.64%	17.27%	10%	7.27%	1.82%
		Giurgiu	100%	-	-	-	-
		Teleorman	50%	-	50%	-	-
Swine Feed	2017	Călărași	74.92%	3.57%	17.86%	3.57%	-
		Giurgiu	50%	-	50%	-	-
		Teleorman	60%	-	20%	20%	-

OTA contamination between in 2017 (13.92%) and 2018 (17.27%). OTA was detected in a few samples of swine feed from 2017 (3.57%). In 2017 OTA was present in also in Teleorman (14.29%). DON and ZEN were present in 2017 in feed for dairy cows and swine collected from Călărași (6.32%, and 3.8% respectively 17.86% and 3.57%) and Giurgiu (18.75% and 12.5% respectively 50% and 0%). The following year, the same toxins were detected in dairy cow feed from Călărași (10%, respectively 7.27%).

In 2017, AFB1 had a 100% incidence in alfalfa hay and silage maize for dairy cows (Figure 3.). The

second product affected was cereal maize (90%), followed by grain cereals, cereals (wheat), grain maize, combined fodder (middlings), combined fodder, soy grist, rapeseed grist and sunflower grist. The highest level of contamination for AFB1 was 0.0033 ppm in cereals (grain maize) collected from Călărași, for OTA 0.0006 ppm in combined fodder and middlings from the same county, for ZEN <0.0001 ppm in various types of feed from Călărași and Giurgiu and for DON 0.089 ppm in barley grains from Călărași.

In 2018, AFB1 had a 100% incidence in grain cereal and sunflower grist for dairy cows

Fig 3. Comparative Contamination with AFB1 in Dairy Cow Feed

Figure 4. AFB1 Contamination Rate in Swine Feed from 2017

(Figure 3.). The incidence exceeded 50% in alfalfa hay, cereals (grain maize), silage maize and soy grist. However, the incidence of AFB1 dropped with 13.23% comparing to the previous year, while OTA, DON and ZEN increased. The highest incidence of DON (66.66%) and OTA (>50%) was recorded in cereals (wheat). The highest level of contamination for AFB1 was 0.016 ppm in cereals (maize), for OTA 0.0006 ppm in cereals (barley) and complementary feed, for ZEN 0.021 ppm in cereals (wheat) ppm and for DON 0.154 ppm in combined fodder, all coming from Călărași County. Stanciu *et al.*, 2019 associated DON and ZEA with wheat production from North Muntenia and linked their occurrence to the weather conditions.

In 2017, AFB1 was mainly found in grain maize for pigs. The other types of analyzed feed, such as combined fodder, grain cereal and soy grist had a similar incidence for AFB1 (Figure 4.). The highest level of contamination for AFB1 was 0.0007 ppm in combined fodder and grain maize, for OTA <0.0001 ppm in combined fodder, for ZEN <0.0002 ppm in grain legumes, all coming from Călărași. The highest level of contamination for DON was 0.520 ppm in combined fodder from Teleorman.

Conclusions

None of the samples exceeded the MRLs. However, residues of AFB1, OTA, DON and ZEN were quantifiable and therefore, concerns regarding the functionality of the quality management systems involved feedstuff production and storage conditions in the studied area exist. Due to its location (plain terrain), sudden climate changes are known to occur more often. The agro-climatic conditions could have most certainly contributed to the levels of mycotoxin contamination in the 3 counties (Călărași, Giurgiu and Teleorman) of SE Romania. The region should be continuously monitored especially nowadays when we are confronting global climate change. Special attention should be focused on the incidence of AFB1 and the level of contamination with DON in cereals for dairy cows and pigs (e.g. maize, barley, wheat), combined fodder, middlings and complementary fodder for dairy cows, and finally combined fodder and grain legumes for pigs.

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