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AFLATOXINS OCCURRENCE AND LEVELS IN MAIZE FROM A ROMANIAN FEED MILL

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Abstract

The present study provides a two years overview of quantitative determination of total aflatoxin in maize from a Romanian feed mill. The aim of the study was to monitor the occurrence and levels of total aflatoxins in maize samples analyzed during the 2020 and 2021; maize used as raw material in the compound feed production was prelevated for analyzes both in the raw material reception stage and from the unit's stock. To determine the aflatoxins concentration of maize from the reception stage, 1034 analysis were carried out in 2020 and 1191 analyzes in 2021. In order to assess aflatoxins occurrence and levels for deposited maize, were prelevated samples from the stock of the studied unit (48 samples in 2020 and 43 in 2021. Results showed that in 2020, from 1034 samples, 77.3% (n = 800) were positive, while in 2021, from 1191 samples, 57.9% were positive. For the determination of the total aflatoxin content of maize from the units stock, the incidence of positive samples was slightly higher in 2021 (65.1%), compared to year 2020 (56.2%). It can be concluded that it is important for feed mills to establish adequate control measures for mycotoxin contamination.

Key words*: aflatoxins, feed mill, feed and food safety, maize.*

INTRODUCTION

Due to the frequent contamination of food and feed by different contaminants, food and feed safety is an major issue (Lorenzo et al., 2018; Marin et al., 2022).

Mycotoxins, a group of toxic compounds produced by spore-forming fungi, could contaminate a wide range of food products both pre- and post-harvest (Zain, 2011).

Feed and food contaminated products pose high risks to animal health and human metabolic conditions, ranging from acute symptoms of severe disease to long-term effects (Anukul et al., 2013; Pinotti et al., 2016; Matei et al., 2023).

Due to incidents of mycotoxin poisoning (Probst et al., 2007; Galarza-Seber et al., 2016), most countries have regulatory levels for the presence of mycotoxins in certain staple food or feed, and therefore testing for those specific regulated mycotoxins is required (Smit et al., 2004); to verify food safety and protect consumer health, specific and selective analytical techniques adapted are used (Pleadin et al., 2019).

Maize, one of the most important cereals produced for human and animal consumption in the European Union, is primarily grown for grain and forage; over 80% of maize grain is utilized for feed (Palumbo et al., 2020).

Aflatoxins are a class of carcinogenic mycotoxins produced by *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, and are commonly identified in staple foods such as corn, rice, peanuts, dried fruits and spices, and dairy products (Reddy et al., 2010; Ismail et al., 2018; Rushing & Selim, 2018). *Aspergillus* fungi contaminate cereal crops and may produce aflatoxins in different harvest stages (Sserumaga et al., 2020). Corn kernels can contain aflatoxins up to 400,000 ppb, therefore sampling is very important in analyzing contamination levels (Richard, 2015).

All primary conversions of aflatoxin B_1 lead to the production of hydroxyl metabolites. The most significant resultant toxin, in terms of toxicity, is aflatoxin M_1, famously dubbed the "milk toxin" due to its excretion in the milk of farm animals fed on aflatoxin B_1 (Prandini et al., 2009). According to studies, aflatoxin B_1 is genotoxic, induces genetic mutations and chromosomal changes (IARC, 2002).

During the 2000s, the European Union revamped its food safety policy, aligning with the integrative concept of "from farm to fork", ensuring a high standard of food product safety across all stages of the production chain (Lapusneanu et al., 2021; Matei & Pop, 2022); even feed mills, like food units must to have auto control programs for contaminants (Lapusneanu et al., 2020; Reg. EU No 183/2005).

The present study provides a two years overview of quantitative determination of total aflatoxin in maize, as a raw material for compound feed, in a Romanian feed mill.

Our study aimed to monitor the occurrence of total aflatoxins in maize samples collected during 2020 and 2021 from a Romanian feed mill. Maize used as raw material in compound feed production underwent analysis both upon arrival at the raw material reception stage and from the unit's stock. Investigating aflatoxin concentrations at the feed mill level was necessary to identify aflatoxin levels in maize. Therefore, information regarding aflatoxin levels in the feed mill will be crucial for ensuring feed safety.

MATERIALS AND METHODS

To ascertain the total aflatoxin content in maize content from the feed mill reception stage, 1034 analyzes were carried out in 2020, respectively 1191 analyzes in 2021; to determine the total aflatoxin of maize from the feed mill stock, 48 analyzes were carried out in 2020, respectively 43 analyzes in 2021.

To quantify total aflatoxins, we employed the laboratory kits Ridascreen®Fast Aflatoxin. Initially, 5 g of ground sample was weighed into a suitable container, to which 25 ml of methanol (70%) was added. After shaking for three minutes, the extract was filtered through filter paper. The resulting filtrate was diluted with 1 ml of distilled water, and 50 µl of this solution was used per well in the test. A sufficient number of wells were inserted into the microwell holder for all standards and samples, with their positions recorded accordingly. Next, 50 µl of standard or prepared sample was pipetted into separate wells, using a new pipette tip for each. To each well, 50 µl of enzyme conjugate and 50 µl of anti-aflatoxin antibody solution were added, followed by manual shaking of the plate and incubation for 10 min at room temperature (20- 25°C). Subsequently, the liquid was emptied from the wells into a sink, and the microwell holder was tapped upside down onto a clean filter towel to remove any remaining liquid. The wells were then filled with washing buffer (250 µl per well) using a wash bottle or multichannel pipette, and the liquid was emptied again to ensure thorough washing. This washing step was repeated twice more. Following this, 100 µl of substrate/chromogen was added to each well, and the plate was gently mixed before incubating for 5 min at room temperature in the dark. Finally, 100 µl of stop solution was added to each well, gently mixed by manual shaking, and the absorbance was measured at 450 nm. Readings were taken within 10 minutes after the addition of the stop solution (R-biopharm Ridascreen®Fast Aflatoxin).

The data obtained from the analyses underwent statistical processing and interpretation. The minimum and maximum values were determined, and position and variation estimators were calculated. Specifically, the arithmetic mean ($\bar{x}$) and the standard deviation (s) were computed for samples that yielded positive results.

The results obtained underwent comparison with the values regulated by European legislation. Interpretation of these results led to the formulation of conclusions regarding feed safety.

RESULTS AND DISCUSSIONS

The results of the analyzes were interpreted and compared with reference to the European Union legislation (Table 1) regarding the maximum permitted limits of mycotoxins in the raw materials and compound feed.

Table 1. Maximum permissible limits for aflatoxin B_1 in feed (Reg. EU No 574/2011)

Animal feed	Maximum content (ppb)
Feed materials	20
Complete feed, except:	10
- compound feed foryoung poultry	5
- compound feed for poultry (except young animals)	20

To determine the mycotoxins concentration of maize, analyzes were carried out to determine the total aflatoxin content of the samples from the reception stage and from the stock of the feed mill, during the years 2020 and 2021 (Table 2).

To assess the total aflatoxin content of maize from the reception stage, 1034 analyzes were carried out in 2020, and 1191 analyzes in 2021. Depending on the maize quantities received by the unit, the number of samples analyzed monthly ranged from 23 (in April) to 203 (in May) in 2020, and from 13 (in August) to 189 (in February) in 2021.

Incidence studies revealed that in 2020, out of 1034 samples analyzed, 77.3% (n = 800) tested positive. Conversely, in 2021, out of 1191 samples analyzed, 57.9% tested positive.

In both 2020 and 2021, there's a noticeable peak in aflatoxin contamination during the summer months, particularly in July. This peak is quite pronounced, with percentages reaching as high as 84.7% in 2020 and dropping slightly to 40% in 2021. While the summer months consistently show higher levels of aflatoxin contamination, there are also fluctuations throughout the rest of the year. For instance, there's a notable decrease in contamination percentages during September and October in both 2020 and 2021 (Figure 1). This could be due to factors such as changes in climate conditions, harvesting practices, or crop rotation patterns.

The average values established for each month in which analyzes were carried out varied from 1.1 ppb to 2.6 ppb in 2020, respectively from 0.6 ppb to 3.9 ppb in 2021. The maximum values identified in the reception stage were 70.4 ppb for the analyzes carried out in 2020, respectively 62.1 ppb for the analyzes carried out in 2021.

From all the analyzes carried out in 2020, for one sample (0.09% of the total) a total aflatoxin concentration above the maximum limit allowed by legislation was found; from all the analyzes carried out in 2021, for nine samples (0.7% of the total) the concentration in total aflatoxin was above the maximum allowed limit. Since the identification of exceeding the maximum allowed limit of total aflatoxin content in maize samples occurred at the reception of raw materials, the respective batches were rejected based on this parameter

In order to assess aflatoxins occurrence and levels for deposited maize, were prelevated samples from the stock of the studied unit (48 samples in 2020 and 43 in 2021). For the determination of the total aflatoxin content of maize from the stock (Table 3), the incidence of positive samples was slightly higher in 2021 (65.1%) than in 2020 (56.2%). On the other hand, the average value established was slightly lower in 2020 (0.9 ppb) compared to 2021 (1.4 ppb).

In Lăpuşneanu et al. (2023) study, they investigated the microbiological contamination of raw materials and compound feed, noting the presence of the *Aspergillus* genus in over 50% of analyzed maize samples. The total fungal count ranged from 4.0×10^2 to 4.9×10^3 cfu/g. Such contamination can contribute to the production of aflatoxins. A survey conducted in 2019 in Romania focused on the prevalence of aflatoxins in maize. In total, 95 maize samples were collected, revealing that 88 samples (92.63%) were contaminated. Among these, only one sample exceeded the limit, with a concentration of 77.59 µg/kg (Smeu et al., 2020).

Table 2. Results of total aflatoxin assessment for maize from reception stage (ppb)

Specification		2020 Year						2021 Year					
		n	Positive (%)	x̄	s	Min.	Max.	n	Positive (%)	x̄	s	Min.	Max.
Maize reception stage	Jan.	105	79	2	0	2	2	117	56.1	1.7	3.9	0.1	34.8
	Feb.	136	75.7	2	0.5	1.7	7.5	189	66.1	1.1	0.8	0.1	4
	Mar.	88	78.4	2	0.3	2	4.5	128	59.3	1	0.8	0.1	3.3
	Apr.	23	65.2	2	0	2	2	86	54.6	0.9	0.6	0.1	2.9
	May	203	82.2	2	0.2	1	4.3	79	59.4	0.9	0.6	0.1	2.7
	June	111	76.5	1.8	0.5	0.1	4.5	143	57.3	0.8	0.6	0.1	4
	July	46	84.7	1.5	1.4	0.1	5.2	30	40	0.8	0.4	0.2	1.9
	Aug.	0	-	-	-	-	-	13	61.6	0.6	0.5	0.1	1.3
	Sept.	105	56.1	2.6	8.9	0.3	70.4	80	62.5	1.5	4.4	0.1	30.1
	Oct.	71	61.9	1.3	1	0.01	4.1	80	37.5	3.9	11.7	0.1	62.1
	Nov.	58	87.9	1.5	1	0.1	3.8	129	59.5	2	6	0.1	36.9
	Dec.	88	86.3	1.1	0.7	0.12	2.9	117	51.2	3	9.6	0.03	60.7

n - number of samples analysed; x̄ - mean; s - standard deviation; Min. - minimum value identified; Max. - maximum value identified.

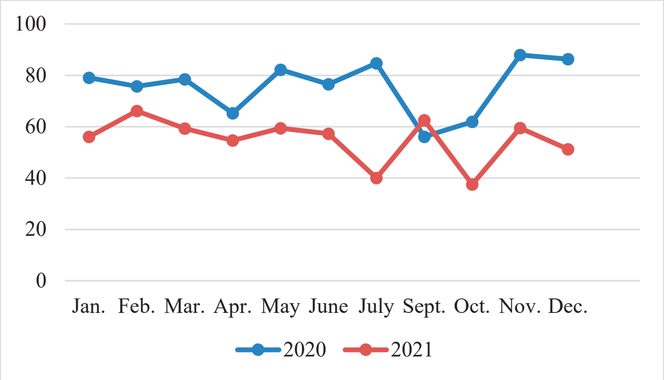

Figure 1. Overview of total aflatoxin positive maize samples depending on seasonality (%)

Table 3. Results of total aflatoxin assessment for maize from stock (ppb)

2020 Year						2021 Year					
n	Positive (%)	x̄	s	Min.	Max.	n	Positive (%)	x̄	s	Min.	Max.
48	56.2	1.4	0.7	0.14	2.5	43	65.1	0.9	0.5	0.09	2.32

CONCLUSIONS

The present study underscores maize as a potential source of aflatoxin exposure, both in maize itself and in compound feed. Our outcomes revealed that only a small fraction of the analyzed samples contained unsafe levels of aflatoxins. However, the percentage of total aflatoxins exceeding the threshold set by European regulations was 0.09% in 2020 (maximum 70.5 ppb) and 0.7% in 2021 (maximum 62.1 ppb).

It is important for feed mills to establish adequate control measures for mycotoxin contamination of the raw materials, in order to guarantee feed and food safety; considering that in our study, exceeding the limits of the allowed content in mycotoxins were identified at the reception, the maize batches were rejected and did not enter in production.

Understanding the seasonality of aflatoxin contamination is crucial for implementing effective mitigation strategies and ensuring food safety throughout the year. By identifying peak contamination seasons and addressing the underlying factors contributing to contamination, stakeholders can work towards minimizing the risk of aflatoxin exposure and protecting public health.

The application of every measure capable of preventing feed and food contamination, along with the potential adverse effects of toxins on human and animal health, as well as economic losses, is paramount for ensuring feed and food safety. Therefore, further research is imperative for feed mills to investigate aflatoxin and other mycotoxin contamination in maize.

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