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ASSESS THE RISK OF MYCOTOXIN CONTAMINATION IN CEREALS AND MIXED FODDER

Maria Prisecaru, Ionuț Stoica, Tina Oana Cristea, Maria Călin, Adriana Bertha Deliu

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INTRODUCTION

Foods intended for human feeding and feedingstuffs that enter into animal feed, as well as the raw material from which are susceptible to alteration by moulds, some of which may produce toxic metabolites.

Mycotoxins, secondary metabolites excreted by moulds belonging mainly to the genera *Aspergillus*, *Penicillium* and *Fusarium*, are regarded as part of food contaminants, the most significant in terms of the impact on public health, food security and the economy of many countries. They are found on a wide variety of foods before, during and after harvest. Affect many agricultural products, namely grains, fruits, nuts, coffee beans, rice and oilseeds, which are very sensitive to contamination of substrates with the production of molds and mycotoxins. Mycotoxin contamination of the products is achieved when environmental conditions are met on the field for their appearance, as well as inadequate methods of harvesting, storage and processing when they are cumulated.

Through the diversity of their toxic effects and their synergistic features, the mycotoxins present a risk for the consumer of contaminated foods. So far, there have been identified over 400 mycotoxins, belonging to at least 21 classes different from the chemical point of view, and their number continues to grow.

The study aimed at assessing the degree of contamination with mycotoxins of cereals and mixed fodder during the period 2012-2014 in Bacău County on a total number of 1035 samples. We evaluated the presence of the following mycotoxins: *aflatoxin B1*, *ochratoxin A, zearalenone* and *deoxinivalenole*, regarded as among the most important, with significant risks for human and animal food safety.

MATERIAL AND METHODS

The methods utilized to analyze samples are validated and accredited.

For the determinations the immune-enzyme competitive ELISA method was used. The detection principle is photometry, because it is the most frequent enzyme for conjugate is peroxidase that can be determined in very small concentrations. Tetrametilbenzidina is used as the substrate and H_2O_2 as cosubstrat. These substances determine a blue coloration in the presence of peroxidase. Adding sulphuric acid can inhibit the reaction and changes the colour at the same time from blue to yellow.

Determinations were performed in the laboratory of the Sanitary Veterinary Department Bacău.

RESULTS AND DISCUSSIONS

1035 samples were analyzed, presented in table 1.

Table 1. The number and types of samples analyzed

The matrix type	2012	2013	2014
Combined feed for birds	114	302	181
Combined feed for pigs	67	94	24
Maize	15	51	67
Wheat	12	29	32
Barley	5	8	12
Oats	4	3	5
Soybean	1	2	3
Sunflower	1	2	1
TOTAL	**219**	**491**	**325**

Each laboratory and analyst establishes the limits under which emits the result in the analyses report. Thus, in determining the LOD and LOQ is 10 combined forage samples are analyzed as blank samples (table 2), the complete analytical procedure, analyzed beforehand and that do not contain aflatoxin B1 over LOD. For the verification method and analyst detection, a negative control, positive control and standards for each test and array must used. Control samples are prepared by contamination of a negative sample with the analyst that must be determined (fortified sample). This contamination must be carried out to the limit of detection.

The limit of detection (LOD) is the smallest limit of a compound that can be detected and identified.

The limit of quantification (LOQ) is the lowest concentration of a compound that may be

detected, identified and quantified. LOQ is the limit, up to which no one can appreciate exactly the amount of mycotoxins over LOQ can determine the exact quantity.

Table 2. Results of blank samples

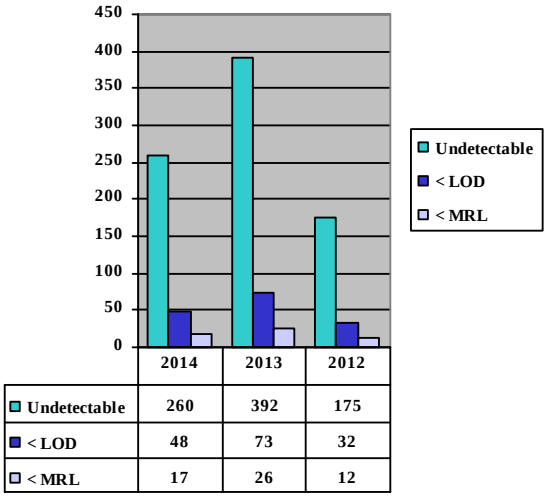
No. of sample	The result mg/kg
1	0.00097
2	0.00106
3	0.00055
4	0.00104
5	0.00085
6	0.00093
7	0.00105
8	0.001
9	0.0007
10	0.00071
Average	0,00089
Standard deviation	0,00018
Trust interval	0,00088619
	0,00088581
Uncertainty of measurement	0,00035
LOD	0,000532
LOQ	0,001063

If the result is < LOD must be reported as "undetectable".

If the result is > LOD, but LOQ < will be reported as LOQ.

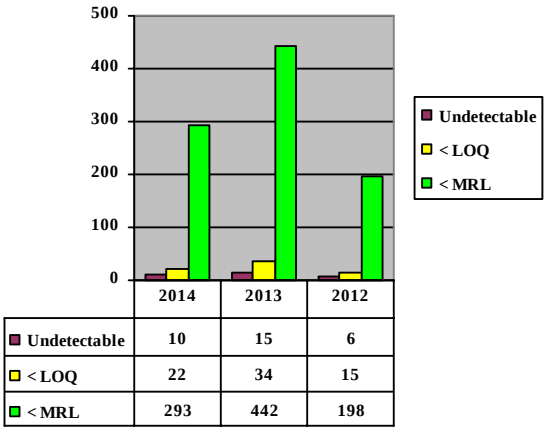
In case that the mycotoxin content of the sample is high, the sample should be diluted to a concentration that is within the calibration curve and the calculation shall take into account the dilution factor.

Below, in Figure 1, 2, 3 and 4 are presented the number of samples and mycotoxins contamination situation analyzed. Reporting was done to LOD and MRL standards (MRL = maximum residue limit).



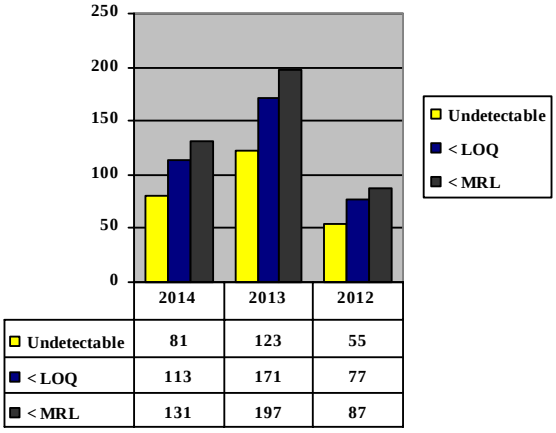
	2014	2013	2012
Undetectable	260	392	175
< LOD	48	73	32
< MRL	17	26	12

Figure 1. The degree of sample infestation with aflatoxin B1



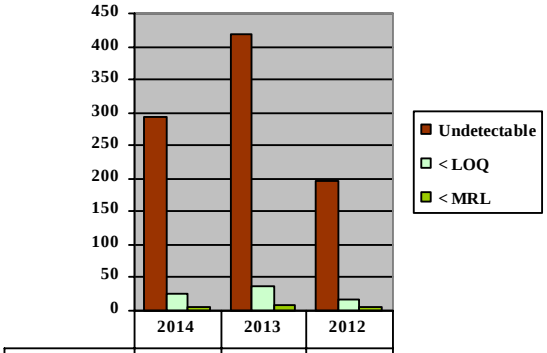
	2014	2013	2012
Undetectable	10	15	6
< LOQ	22	34	15
< MRL	293	442	198

Figure 2. The degree of sample infestation with ochratoxine



	2014	2013	2012
Undetectable	81	123	55
< LOQ	113	171	77
< MRL	131	197	87

Figure 3. The degree of sample infestation with zearalenone



	2014	2013	2012
Undetectable	293	418	197
< LOQ	26	38	17
< MRL	6	9	5

Figure 4. The degree of sample infestation with deoxynivalenol

According with the maximum permissible limits and the limits determined by the laboratory where the tests were carried out, the results obtained in the three years of study are the following:

- For **aflatoxin B1** (graph 1): 80% undetectable samples (827), 15% samples < LOQ (153) and the 5% sample in permitted limits (55).
- For **ochratoxin A** (graph 2): 3% undetectable samples (31), 7% samples < LOQ (71) and 90% sample in permitted limits (933).
- For **zearalenone** (graph 3): 25% undetectable samples (259), 35% samples < LOQ (361) and 40% sample in permitted limits (405).
- For **deoxynivalenol** (graph 4): 90% undetectable samples (908), 8% samples < LOQ (81) and 2% sample in permitted limits (20).

CONCLUSIONS

In no sample higher amounts of mycotoxin than maximum limit allowed were determined.

Toxicity of mycotoxins is quite large, very small amounts can affect the health of the body. The limits that mycotoxins became dangerous are of the order of micrograms or nanograms per kilogram of body per day, depending on the type of toxin.

Mycotoxins present a higher risk than food additives, contaminants and synthetic pesticides (there are 10,000 times more dangerous than pesticide residues).

The majority of mycotoxins, from which are included also the present ones, have carcinogenicity capacity, the target organ being the liver. The carcinogen mechanism is manifested through their ability to settle on the DNA, causing alterations in the form of mutations.

If used for animal feed stuffs are contaminated, then products like milk and meat will contain toxins, or biotransformation products. For example, cattle turn aflatoxin B1 in aflatoxin M1 which then is secreted into the milk. In the case of swine, the ochratoxin present in food accumulates in their flesh.

The higher chemical stability of the majority of mycotoxins, makes almost impossible the detoxification of mycotoxins contaminated products through physical or chemical methods.

ABSTRACT

The study aimed at assessing the degree of contamination with mycotoxins of cereals and mixed fodder during the period 2012-2014 in Bacău County on a total number of 1035 samples.

We evaluated the presence of the following mycotoxins: aflatoxin B1, ochratoxin A, zearalenone and deoxinivalenole, regarded as among the most important, with significant risks for human and animal food safety.

Determinations were performed in the laboratory of the Sanitary Veterinary Department Bacău and the immune-enzyme competitive ELISA method was used. In no sample higher amounts of mycotoxin than maximum limit allowed were determined.

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AUTHORS` ADDRESS

PRISECARU MARIA, STOICA IONUȚ - „Vasile Alecsandri" University of Bacau, Faculty of Science, Department of Biology and Environmental Protection, Marasesti Street, no. 157, Bacau, Romania, e-mail: prisecaru_maria@yahoo.com; ionut_stoica23@yahoo.com;

CRISTEA TINA OANA, MARIA CĂLIN - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, Bacau, code: 600388, e-mail: sclbac@legumebac.ro;

DELIU ADRIANA BERTHA - Sanitary Veterinary Directorate and Food Safety, e-mail: office-bacau@ansvsa.ro