# Aflatoxins – recent items about influence on vegetable – animal – food relationship

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*Abstract:*: - Mycotoxins are considered to be the most significant food contaminants from the point of view of world public health, food security and even economy influence. They are found in various food stuffs, before and after harvest time, such example: a lot of agricultural products: cereals, fruits, nuts, coffee beans, rice and oil plants, that all represent very sensitive substrates for moulds contamination and aflatoxin Throughout their diverse toxic effects and also their sinergetic properties, micotoxins represent a highest risk for contaminated food consumers, by their capacity to modify biological structures, also on animals and human population. This paper proposes correlation on relevant aspects about aflatoxin incidence in feed stuffs, with emergence on animal tissue or animal products, and forward, in food products derived from them.

*Key-Words:* - micotoxin, feed stuffs, Elisa, method validation

## 1 Introduction

The oldest document about mould intoxication is about 55 B.C., in Denmark and after that time, also Hippocrate and Cesar of Rome, have mentioned some aspects about negative effects consumption of flour stored in humidity conditions.

The precise moment on micotoxins presence and influence is dated 1962, England, when have died a hudge number (over 100 thousands) of turkeys and other domestic poultry [1, 2].

In 1987, World Health Organization classified Group 1 of aflatoxin as carcinogen, with a great impact on liver health and DNA destroyer agents, M1 aflatoxin being considered the most aggresive. The incidence of M1 aflatoxin in milk could be explained by molded feed (B1) consumed by animals [3, 4, 5].

Short-time ingestion of large quantities of aflatoxins can lead to acute poisoning, which can manifest through bleeding, acute liver failure and even death. Lethal dose depends on several factors, eg the amount of aflatoxin ingested, age, health and nutritional status of the individual. Eating a small amount, but for a long period of time may cause chronic poisoning. Effects and symptoms are often difficult to identify, both because of low intensity but mostly because of their nonspecific character.

This case was the beginning process for isolation and identification of the first micotoxin. The contamination was related with aflatoxins, subsequent feed consumption, feeds that were infected by *Aspergillus flavus* [6, 7, 8]

*Aspergillus flavus* doesn't contaminate cereals in the field, but after the storage,its needs for humidity being very low.

The aflatoxin quantity depends on *Aspergillus* strain. Their toxic and theratogen effects have conducted to liver dysfunction, they are carcinogenic [9, 10].

## 2. Problem Formulation

Elisa competitive method is used especially for molecular compounds with small molecular mass. For this kind of structures, that have not antigenic properties, that means, they doesn't produce antibody, we have to proceed competitive Elisa method.

In this method, an exact quantity of enzymatic conjugate is in challenge with sample or standard, for a limited, fixed antibody quantity. This antibody is binded with a solid phase-the places from the strip-that is why the name of the method:"enzyme-linked-immuno sorbent assay" or ELISA.

The principle of detection it is photometry, peroxidase enzyme could be detected in least quantities.
The steps for sample conditioning are:
- the sample is grinded till powder; 3 grams of sample is extracted with 9 $cm^3$ methanol. After homogenization, at room temperature, for several minutes, the sample is centrifuged at 2000 r.p.m. or it can be filtered.
An aliquot part of 50µl from supernatant (centrifuged or filtered) is then diluted with 150 µl buffer solution.. For Elisa test we have used 50 µl
The kit board consists of 12 stripes with 8 places, coated with rabbit antibody and anti IgG mouse. In the first step, are added specific antibody (mouse anti aflatoxine), aflatoxine marqued with enzyme conjugated and AFM1 standards and also the samples to be analysed. Specific antibody were binded by rabbit antibody strike-bound, and, in the same time, free aflatoxin(unbinded), from standards or samples and marqued aflatoxin are in competition for binding situs of specific antibody, that is immunoenzyme competitive test.
After one hour, the reagents (marqued with enzyme) unbinded, were washed. The enzyme quantity that is binded was revealed by adding chromogen substrate (tetramethylbenzidine, TMB). This will turn chromogen in a coloured one. The reaction was stopped by adding 100 µL $H_2SO_4$ 1M solution. The colour intensity was measured by spectrophotometry, at 450 nm, with ELISA reader system, taking account that the aflatoxine M1 concentration is in inverse ratio with sample colour intensity. In the same time was established a calibrating curve with standard solutions of M1 aflatoxine: 5(S1); 10(S2); 25(S3); 50(S4); 100(S5) şi 200(S6) ng/L.
The samples were prepared in compliance with technical informations of R-Biopharm AG, Ridascreen kit for AFM1.
Milk samples were centrifugated for fat carrying-off for 10 min / 3500 g and after centrifugation, the Kit contain the following:

- microtitration board (96 places), 12 stripes with 8 places, coated with mouse antibody anti-IgG;
- 6 aflatoxine M1 flasks (1,3 mL) with concentration 0 ng/L (zero standard), 5 ng/L , 10 ng/L , 25 ng/L , 50 ng/L , 100 ng/L, 200 ng/L;
- 1 conjugate solution flask (aflatoxine marqued with peroxidase) manifold drying antibody anti- M1 (manifold drying 1,3 mL);
- chromogen substrate solution flask (tetramethylbenzidine), (10 mL);
- buffer solution flask pH 7, (20 mL);
- stopping solution flask, $H_2SO_4$ 1N ( 14 mL);
- washing buffer solution flask( 30 mL).

With kits indicated for ELISA method could be effectuated 96 determinations, including standards.
Reagents that are used, out of kit:

- methanol
- n-heptane
- diclormethan
- buffer solution, pH 7.2: (0.55 g $NaH_2PO_4$ x $H_2O$ + 2.85 g $Na_2HPO_4$ x 2 $H_2O$ + 9 g NaCl în 1000 ml distilled water)
- Equipment assembly line Reader 230 S and incubator SHAKER 50 X BIOMERIEUX
- Centrifuge EBA-20
- Micropipete de 20-200 µl şi respectiv 200-1000 µl
- Pasteur pipette
- Graduate pipette
- Gloves

## 3 Problem Solution

We have done an experiment with different feeds over three years: 2009, 2010, 2011, also raw materials and finished products.
For each of micotoxins: B1, Ochratoxin, Zearalenone, DON we have established **LOD**-the smallest concentration for a component, that can be detected and identified with specified method and equipment, and also LOQ - the smallest concentration for a component that can be detected, identified and quantified with a reasoning statistical uncertainty.
If the result is < LOD, that must be reported as " undetectable".
If it is> LOD and < LOQ it have to declare < LOQ mg/kg and if the value is > LOQ, we must declare the exact quantity and compare with maximum residue limit(MRL)
The results obtained are as following

- Aflatoxin B1: 80% samples undetectable , 15% samples < LOQ and 5% samples in the range of admitted limits.
- Ochratoxin A: 3% undetectable , 7% < LOQ şi 90% samples in the range of admitted limits.
- Zearalenone: 25% undetectable, 35% < LOQ şi 40% samples in the range of admitted limits.;
- Deoxinevalelol: 90% undetectables , 8% samples < LOQ and 2% in the range of admitted limits.

We have to reveal that doesn't exist samples over limits.

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Maximum admited limits are:

- aflatoxine B1 -0.005mg/kg and 0.02 mg/kg
- ochratoxine A - 0.05mg/kg and 0.25 mg/kg
- deoxinivalenol - 0.9mg/kg and 12 mg/kg
- zearalenone - 0.1mg/kg and 3 mg/kg

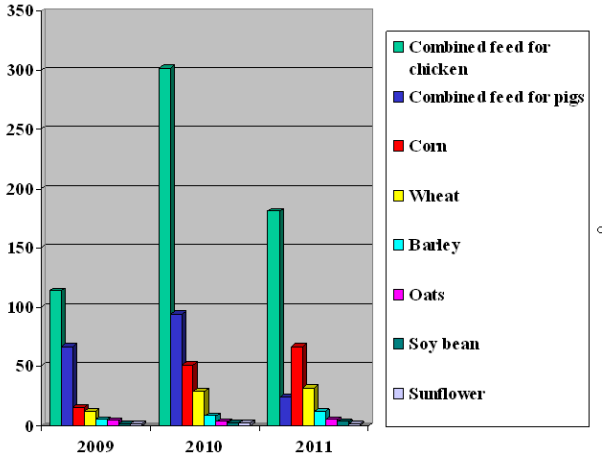



**Fig. 1. Results evolution in 2009-2011 year**

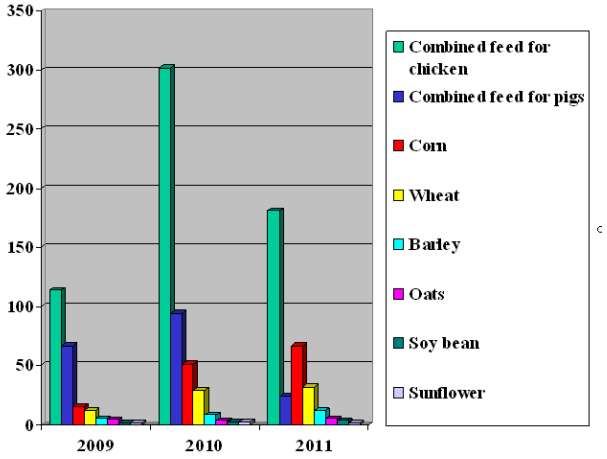
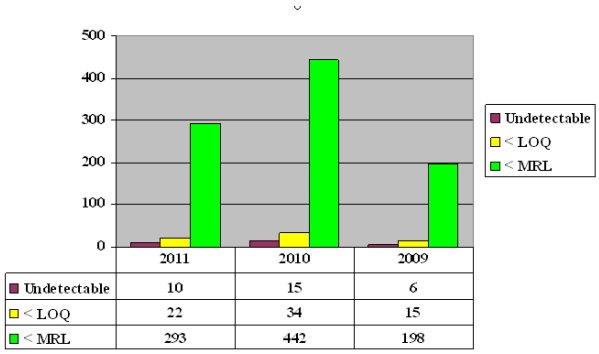



**Fig.2. Annual micotoxin evolution**




| | 2011 | 2010 | 2009 |
|---|---|---|---|
| Undetectable | 10 | 15 | 6 |
| < LOQ | 22 | 34 | 15 |
| < MRL | 293 | 442 | 198 |

**Fig.3. B1 Contamination level**

LOQ- Limit value for quantification
MRL- Maximum residual limit

# 4 Conclusion

This is an ever-growing research field.

- The toxin cannot be destroyed by physical means (high temperature sterilization, refrigeration, dehydration, desiccation, lyophilization, irradiation or metabolism). Thus an animal that ate infected corn toxin can forward it to the man who consumes its meat.
- Experts believe that the most effective method of avoiding the consequences of mycotoxin infections is preventing fungal infestation of crops. Food safety measure provide that foodstuff with a risk of Mycotoxin contamination must be supervised.
- Depending on the type of toxin, mycotoxins can be allowed as a microgram or nanogram per kilogram body weight. If the body gets more than that, mycotoxins attack the genetic material, causing cancer.
- Health changs does not occur in all animal population, this means that mycotoxins are not infectious;
- Mycotoxins have the property to produce antibodies.
- The main target of aflatoxins is the liver, the kidney is ochratoxins, zearalenone, genital syndrome estrogen and deoxynivalenol which has the lowest toxicity associated with acute gastrointestinal syndrome.
- A U.S. study shows that mycotoxins are 10,000 times more dangerous than pesticide residues.

Our country has implemented the most important regulations in many fields of activity, since 2005, especially in those domains with great impact in our trade collaboration with UE members or non-UE members.

Monitoring of mycotoxins content in foods is a constant concern of the authorities, and mainly for food processors in this industry.

Feed products are susceptible to contamination with aflatoxins, reasons why preventive measures should be taken, such as: ensuring optimal storage, monitoring of mycotoxin levels, risk assessment in accordance with the requirements of applicable law and achieve traceability to prevent aflatoxins contamination.

It is also necessary to develop procedures and guidelines of good management practices to prevent, reduce and / or eliminate contamination with aflatoxin B1, Ochratoxin, Zearalenone and DON.

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