THE PRESENCE OF *OCHRATOXINS* IN FOODERS AND FOOD PRODUCTS AN THER IMPACT ON ANIMALS AND HUMAN HEALTH

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Abstract

Species of fungi: Aspergillus and Penicillium can produce and release, in certain circumstances of temperature, pH and humidity, secondary metabolites in fooders and food products type: Ochratoxins .
Toxicity of Ochratoxin depens on the type (A,B,C), source and their dose, duration of exposure . Up to the present time has been demonstrated nephrotoxic compounds, hepatotoxic and teratogenic effect. of OchratoxinA (OTA)
Due to the toxic effect of OTA the maximum level in fooder and food is subject to European legislation (Reg. CE N0 576/2006, Reg. CE 1881/2006)
The aim of the paper is to highlight the value of mycotoxins type OTA in feed and foods, as an possible risk on animals and human health. The working method used for teting was ELISA. Values obtained from determinations were performed according to the legislation.

Key words: *mycotoxins, OTA, animals health, human health*

INTRODUCTION

Mycotoxins, defined as "metabolites of fungi which, after ingestion inhalation or absorption through the skin, alter the ability of the body's reaction and cause illnesses or even death in animals" (Pitt JI,1996).
Once ingested they may lead to reduced performance and to change the metabolism of animals. Diseases that occur as a result of consumption of feed contaminated with mycotoxins are called micotoxicoze. They are negative: ingestion of feed; performance and changing metabolism of animals. The appearance of frequent and various forms of infecunditate is caused largely by the quality assurance of.

Contamination of agricultural products occur mainly during storage, being conditioned by the humidity and high temperatures. These factors favour the reproduction of fungi, leading to increased risk of mycotoxins. (Savu et al., 2004).

Due to the effects on human health and animal productivity and higher economic losses that occur inreaga world pay special attention to these mycotoxins.

Increasing control these mycotoxins contamination is due to numerous alerts on food: cereals, coffee, dried fruit(Bayman P, Baker J –2006) Species of fungi: *Aspergillus, Penicillium* generally develop after harvest and are called "mycotoxins of deposit". They can develop and release in feedingstuffs in certain circumstances secondary metabolites: Ochratoxin A (OTA)(Larsen et al.,2001; Pfohl-Leszkowicz et al., 2007).

These are:

In the genus *Penicillium:*

P. verrucosum

P. nordicum

In the genus *Aspergillus* :

- *A. ochraceus*
- *A.melleus*
- *A. auricomus*
- *A.ostianus*
- *A. petrakii,*
- *A.sclerotiorum*
- *A. sulfuroase*

In recent years the analyses of some food products and fodder demonstrated that, P. *viridicatum*, P.*griseofulvum* and possibly P. *solitum* also produced ochratoxins. From genus *Aspergillus*: *A niger* and A.*carbonarius* have been reported as ochratoxigenic fungi(Pitt, J.I.,1987; Abarca ML et al., 2001; National Library of Medicine, 2002).

Affects both animal health Ochratoxin and productive activity and can be match win in animal products such as meat, eggs, milk, presenting a potential risk for human health(Won-Bo Shim et al., 2004).

status of immune system, leading to reduced

In recent years due to its special form on the body human, animal and ochratoxinele are intensely studied. Depending on the degree and impact of health they may be divided into:

- Nephrotoxic
- Immunotoxic
- Neurotoxic
- Mielotoxice
- Carcinogens (IARC, 1993).

As a result of research carried out recently, you can pull up a stark signal of alarm that fungi pose for animal health and human health.

OTA- Toxicity in animals and in humans

OTA has cancerigena, genotoxica and mutagena to several species of animals and humans. Ochratoxin is primarily a toxin that affects the kidneys, but, if the concentration is high enough and injuries can occur in the liver(Rutqvist L. Et al. 1978; O' Brien E, Dietrich DR, 2005) .
By inhibiting the metabolism of glucose and insulin, OTA may cause the accumulation of glycogen in the liver.Neurotoxic effect was demonstrated in all mammalian species.
Main mechanisms by which manifests its toxicity mycotoxins are: stimulation of lipid peroxidation, apoptosis and inhibition of protein synthesis of DNA and RNA. In this respect immunotoxicity is the most important consequence of serious micotoxicozei (Bondy and Pestka, 2000).

Nefrotoxicitatea in pigs fed with feedingstuffs, at which the level of OTA has been comprised between 200-4000 g/kg, is manifested in the kidney, proximal tubular atrophy, fibrosis and glomerular sclerosis(Stefanovic, V.et al.,1991). Intoxicati pigs appear biochemical lesions: proteinuria, sugar, enzimurie, reducing the concentration of the urine. Later onset kidney failure (Petrova- BacharovaT et al., 1991; Pfohl-Leszkowicz et al.,2002)
Human epidemiological studies have shown that OTA can cause a higher incident of renal tumors, described for the first time in Bulgaria in the year 1956 (TanchevY,Dorossiev D -1956).
That is way the European Scientific Committee on Food indicates a lower tolerable intake, below 5 ng/kg /per day (Walker, R.; Larsen, J.C, 2005).
Carcinogeneza
In rodents, after ingesting food contaminated with OTA were detected in liver, kidney tumors, Mammary and testicular (IARC, 1993;Castegnaro, M. ,et al.,1998; Mantle, P.,et al.,2005)
Following administration of OTA in growing gilts over a period of 35 days found decreased phagocytic macrophages. Also the production of interleukin (IL) is compromised. (Harvey et al., 1992; Petzinger, E., Weidenbach, A. Mycotoxins,2002).
In the studies done have shown the effects of immunomodulation induced by ochratoxin, and the fact that OTA affects humoral immunity (antibody synthesis) in chickens, rats and mice (Surai, 2004).
Purified human lymphocytes after exposure to OTA has been a decrease in cellular capacity aspunde activation stimuli in vitro, is impaired production of IL-2 and IL-2 receptor by T cells activated. (Lea et al., 1989)The conclusion was that the toxin

led to immunosuppression by interfering with essential processes of cellular metabolism.
Inhibition of interferon production base has Locle after suppression of NK cell activity by OTA (Lea et al., 1989).
The man behind the studies conducted have found that kidney tumors often appear when food intake is greater than 70 g/kg per day of OTA(Pfohl-Leszkowicz, A et al. ,1993; Pfohl-Leszkowicz, A et al. 2007; Pfohl-Leszkowicz, A, 2009).

MATERIAL AND METHODS

We used the test kit with competitive enzyme immunoassay for the quantitative analysis of *Ochratoxin A (OTA)* in fodder and foods.
The determination is made based on working kit protocol used is based on the reaction of antigen - antibody. ELISA kit (Enzyme-linked immunosorbent assay-enzyme immunoassay, or EIA). After the sample preparation the test procedure, the measurement is made photometrical at 450 nm.
Reagents: - 1n HCl , 5 n HCl; CH_2Cl_2 ; 0,13M buffer ($NaHCO_3$) with pH=8,1
Equipment:
- microtiter plate spectrophotometer (450nm)
- centrifuge
- magnetic stirrer
- paper filter
- gradual pipettes
- micropipettes
- purification columns OTA

All reagents required for determinations had adequate quality according and the determinations were made using modern equipment from Sanitary- Veterinary and Food Safety Direction-laboratory of Brasov. This laboratory applies a GPL system and a quality system.
To avoid contamination of samples was taken into account the observance of rules, namely:
-when entering the laboratory, samples were pureed ;
-it was a laboratory sample is stored in the freezer representative until determination;
To obtain valid results has been considered subject to the following precautions:
-all reagents were brought to temperature 20-25 ° C and were mixed before use ;
-these steps were imposed by the kit work in compliance with time forced ;
-to work in the solvent extract preparation - 70% methanol (OTA) ;
-were observed using working volumes: 50, 100, 500 and 1000 µl-micropipets;
All kits must be certified according: detection limit (LOD), recovery rate, sample preparation and specificity (Table 1).

Table 1. Performance criteria for ELISA kit

Mycotoxin	Recovery %	LOD	Matrices
Ochratoxin A ***RidaScreen***	85	625 ppt*	Cereals, feed, food

*(ppb= ng/mL= µg/Kg ; ppt = ng/Kg)

RESULTS AND DISCUSSION

The present report refers to the determinations made in 2010-2012 on samples from farms, processing units, markets designated booth for animal feed and human food (cereals, wine, peanuts, etc).
The sample were representative of each batch separately and have respected the rules of collection.
The maximum level of *Ochratoxin A* in different types of products are indicate in following table 2:

Table 2. The maximum levels for OTA according to Europeans legislature

Mycotoxin type	Level	Products	Directive EU
Ochratoxin A	3,0 – 10,0 (ppb)	Cereals, peanuts , dry fruits, coffee	Regulation (EC) No 1881/2006
	2,0 (ppb)	Wine, juice of wine	
	0,50 (ppb)	Baby foods	
	0,05 – 0,25 (ppm)	Types of fodders	Regulation (EC) No 576/2006

Regarding the values of Ochratoxin A in the sample which we analysed are presented in the table below (Table nr.3).

Table 3. The values of OTA in same samples

Analysed sample	The variation of values for OTA (ppb)		
	2010	2011	2012
Cereals	0,00 – 3,200	0,00 - 2,84	0,22 – 1,64

Foods	0,00 – 0,120	0,00 - 0,300	0,00 – 4,28
Fodder	0,00 - 8,760	0,00- 0,54	0,12 – 0,24

Values obtained from determinations were performed according to the European legislation: Regulation (EC) No 576/2006 and Regulation (EC) No 1881/2006

CONCLUSIONS

The Ochratoxin A is on of the highly dangerous mycotoxins for human and animal health

The obtained results were reported to be tantamount to the laws regarding the levels of Ochratoxin A.

Since the values obtained from analyses did not exceed the maximum level permited by low , these samples are not harmful to humans and animals.

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