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EUROPEAN LEGISLATION ON OTA IN FOOD AND FEED AND THE RISK OF ITS PRESENCE ON HUMAN AND ANIMAL HEALTH

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Abstract

Studies on ochratoxin A (OTA) showed that this mycotoxin is nephrotoxic, hepatotoxic, carcinogenic, immunesuppressive and teratogen.
Consumption of food contaminated with OTA affects the health of farm animals and their productivity leading to its presence in animal products.
Early identification and removal of feed and food chain products contaminated with OTA can be achieved by control strategies. This paperwork aims to present the impact on human and animal health, the probable risk of OTA residues in animal products and control strategies that apply in the feed industry.

Key words: *Ochratoxins , human toxicity, animals toxicity, control strategies.*

INTRODUCTION

Food safety and security, safe food procurement remain essential in most countries.
In the recent years intense national and international efforts have been made regarding food security. There are taken in consideration both microbiological and chemical risks.
World Health Organization (WHO) recognizes the chemical risks of food and feed contamination by mycotoxins (toxic metabolites of fungi), fishery products (toxins produced by algae) and edible plant species by plant toxins as an important source of food originated disease WHO, 2002a).
Mycotoxins have received the most attention in many parts of the world, as they are a major issue in food safety.
Many countries have adopted regulations regarding the mycotoxins in food and feed due to the severe effects that they can have on people and animals. The mycotoxin regulations have a major role in the protection of the human and animal health and economic interests of producers and traders.
Ochratoxin A is a fungus mycotoxin that aroused worldwide interest in terms of economic losses due to the effects on human health and animal productivity and the national and international trade.
The disease caused by the presence of this mycotoxin is called ochratoxicoza. The main target of this mycotoxin is the kidney.
Following epidemiological studies, it was demonstrate that OTA is involved in the pathogenesis of many forms of human renal disease, including kidney cancer (Marquardt and Frohlich, 1992; Ringot et al., 2006; Pfohl-Lenszzkowicz et al., 2007).
Due to high toxicity to human and animal body, ochratoxins are extensively studied in recent years.
The conclusions of these studies was that these toxins are nephrotoxic, immunotoxic, neurotoxic, myelotoxic and carcinogenic (Group 2B toxicity) according to the CIRC classification (CIRC, 1993).
The OTA elimination ways through kidney and in part by through may explain the degenerative changes in the epithelial cells of the kidney and liver. (Koynarski et al., 2007).
The genotoxicity and the oxidative way can be taken into account for the occurrence of nephrotoxic and carcinogenic effects as shown by research conducted in recent years. (Pfohl-Lenszzkowicz et al., 2007).

The attention paid to these mycotoxins is motivated by the many signs of food contamination: cereals, coffee, dried fruit, spices, chocolate, wine, cocoa (Bayman and Baker, 2006).
OTA can be found in animal tissues and products because mycotoxins can be transferred through the food chain.

OTA in feeds

OTA is a toxic by-product produced by species of Aspergillus and Penicillium.
The factors affecting ochratoxinogeneza are: temperature, humidity, water activity, degree of aeration, substrate biocoenosis. (Tabel 1)

Table 1. Growth conditions for ochratoxin production.

Growth conditions	Aspergillus ochraceus	Penicillium verrucosum
Optimum temperature for growth	24-37 C	20°C
Optimum temperature for ochratoxin production	31°C	20°C
Optimum growth	3-10	6.0 – 7.0
Minimum wather activity for ochratoxin production	0.8	0.86

OTA production is influenced by environmental conditions (temperature and water) as well as the seed's type and integrity. Aspergillus ochraceus grows better in oilseeds (soybeans, peanuts), Penicillium verrucosum grows better in cereal crops: wheat, corn. (Madhyastha et al., 1990).
The main source of ochratoxin in the vineyards of France, Spain and Italy A.carbonarius.
In cooler regions, OTA is produced by Penicillium and in warmer regions by Aspergillus (Pohland et al.,1992; Varga et al., 1996). Ochratoxin formes on acidic foods (Cuero et al., 1987).
Large amounts of ochratoxin occur during storage of agricultural products due to the humidity (18-24%) favoring mycotoxigen fungal growth (Shotwell et al., 1969; Zimmerli and Dick, 1996; Campbell et al., 2003).
The highest amounts were reported in Northern Europe and North America (World Health Organisation, 2002).
OTA formes mainly coffee during beans' storage.
Rapid drying of agricultural products after harvesting can reduce the production of OTA. (Levi et al., 1974; Urbano et al., 2001; Samson et al*.,* 2004; Frisvad et al., 2004).

OTA effects on animal health and production

After consumption of OTA contaminated food, major economic impact can be observed on monogastric animals, birds and pigs. The ruminants are more resistant to OTA contaminated food. The consumption of OTA contaminated food reduces the growth rate and thus lowers animal productivity.

Nephrotoxic action

The effects of consumption of OTA contaminated on animal health depend on dose, animal species and the amount ingested.
It is believed that pigs are the most sensible to OTA. (European Food Safety Authority-EFSA, 2006). Nephrotoxicity of ochratoxin is different from animal to animal. Consumption of food containing OTA in concentration of 1 to 3 mg/kg resulted in the appearance of nephropathy and kidney cancer in pigs and humans after the installation of the tubular degeneration. The immunosuppressive activity in 'natural killer' cells could explain tumour growth (Pfohl-Lenzkowicz et al., 1993; CIRC, 1993; Pfohl-Lenzkowicz et al., 2002).
The installed kidney necrosis can be explained as a result of increased lipid peroxidation demonstrated for OTA in vivo and in vitro. (Meki AR et al.,2001).
OTA inhibits the activity of many enzymes from the Krebs cycle, resulting in the decrease of the ATP production and the inhibition of the mitochondrial respiration. (Wei et al.,1985).
The ingestion of OTA produces polyuria and polydipsia. The increased blood urea and creatinine levels draw attention upon the renal impairment.
The consumption of food containing OTA in concentrations higher than 1 mg / kg leads to leukocytosis, increased neutrophils / lymphocytes ratio and decreased hemoglobin and erythrocytes levels. It was observed an impairment in the immune function associated with lymphocyte development and production of interleukins IL-2. (Harveyet al., 1992).

On necropsy there are found kidney discoloration and hypertrophy, atrophy and degeneration of proximal convoluted tubules, interstitial fibrosis and sometimes hyalinisation glomeruli. The impairment of the function renal was observed after consumption of OTA contaminated food in concentration of 200-4000g/kg. (Stoev et al., 2002).

On histology examination there were found proximal tubular lesions and interstitial fibrosis.

After consumption of OTA contaminated food in concentrations higher than those that cause nephrotoxicity, there are found embryotoxicity, immunotoxicity and teratogenicity. (Benforg et al., 2001).

On pigs, OTA ingestion lowers resistance to infection. (Stoev et al., 2000).

On poultry, it was observed a decrease of immunoglobulins levels and phagocytic capacity of monocytes, and decreased antibodies. (Thuvander et al., 1996). Many European countries have experienced episodes of porcine nephropathy. The pigs intoxicated with OTA showed biochemical lesions: glucosuria, proteinuria, enzimurie, reduced urine concentration, renal insufucienta. (Petkova et al., 1991; Pfohl et al., 2002).

The poultry are also affected by contamination with OTA. Chicken duck, turkeys are sensible to OTA. Ingestion of OTA contaminated food leads to decreased egg production, poor quality shelled eggs, decreased feed conversion, reduced weight gain, nephrotoxicity.

The administration of OTA contaminated food (2 mg/kg) on laying egg hens significantly decreases food consumption per day, egg and serum triglycerides production and increases liver weight. (Denli et al., 2008).

On chickens receiving OTA contaminated food (2 mg/kg) there were observed weight loss, diarrhea, excessive urine and kidney damage (Dwivedi et al., 1984). It was also observed a decrease a tocopherol concentration in the liver of chicken that consumes OTA contaminated food (2.5 mg/kg). (Hoehler et al., 1996).

On poultry, there were found the same biochemical and histological lesions as pigs.

Carcinogenic action

OTA administration on rodents causes kidney, breast, liver and testis tumors (IARC, 1993). Because of the genetic sensibility related to biotransformation, male rats are more sensible to OTA (Pfohl-Leszkowicz et al., 1998).

The administration of OTA contaminated food for 2 years in a group of female pigs has led to kidney cancer. This is due to metabolism and excretion ochratoxins relatively quickly with an RL50 (disposal) in pigs for several days. (Krogh and Role, 1992).

The OTA contamination of animal products

The contamination of animal products may occur after consumption OTA contaminated food or by direct contamination with fungi.

After the consumption of OTA contaminated food there is a rapid absorption of toxins into the bloodstream followed by relatively slow elimination through urine and feces. (Galtier, 1991; Mantle,2008).

A team of researchers found that after oral administration of a single dose of 500g/kg of OTA peak plasma concentration at 2 hours is about 30% of the OTA intake. (Vettorazzi et al., 2009).

The persistence of OTA in plasma is due to enterohepatic circulation and resorption in renal tubules. (Roth et al., 1988; Marquardt and Frohlich, 1992).

On pigs that were fed during the growth with OTA contaminated diet in concentration of 25 g/kg, residue in pork was up to 1 g/kg ((Malgutii et al., 2005).

On ruminants, OTA residue does not accumulate in a significant level, because this toxin is rapidly detoxified in the rumen in less toxic metabolites (Muller et a., 1998).

The porcine nephropathy, which originally appeared in Denmark, has been reported in many European countries. The main cause of this renal disease is the consumption of OTA contaminated food. (Stoev et al., 2002).

Macroscopic changes observed after examining the pig kidney is an indirect method of determining the level of OTA in carcass in Denmark (Jorgensen and Petersen,2002).

At an OTA level of 25g/kg in pig kidney the meat is checked to ensure that it does not exceed the value of 10 g/kg. After some studies, it was established that the OTA content in carcass is 40% in the case of pig kidney (Buchmann and Hald, 1985).

When the quantity of OTA varies between 10 and 25 g/kg in kidney, liver and pig kidney are rejected.

There was a correlation between OTA content in organs (kidney, liver, whole blood and plasma) and various forms of nephropathy after a study on two pigs in Sweden. (Rutqvist et al., 1978).

OTA concentration in the feed might be used to specify residues in pig tissues and organs. This can be done following correlations established between the consumption of foods with OTA and debris from kidney, liver, muscle and adipose tissue (Krogh et al., 1974; Krogh, 1976; Rutqvist et al., 1978).

After some studies, many researchers have tried to establish a relation between the average concentration of OTA in serum and concentration of OTA in pig's feed (Stoev, et al., 2002; Malgutti et al., 2005; Jarczyk et al., 2008**;** Aoudia et al., 2009), ar depicted in Figure 1.

Knowing that OTA content in blood serum reaches a plateau after 10-13 days, there have been experiments where exposure to OTA lasted at least 14 days. The equation in the figure below confirms the relation between OTA content in feed and its residues in blood serum (Hult et al., 1979; Aouila et al., 2009).

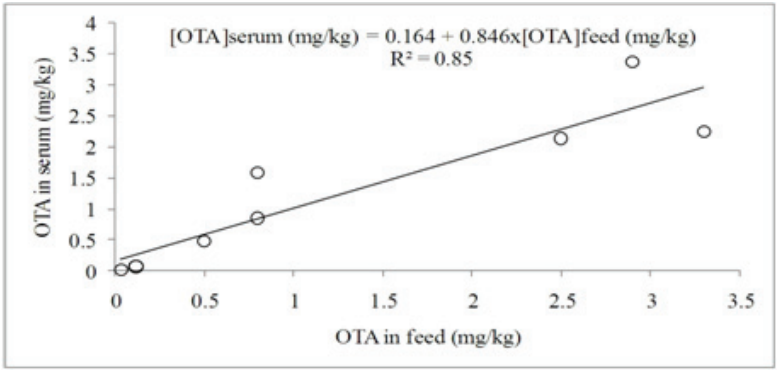

Figure 1. Relation between the concentration of OTA in the diet and its concentration in pig blood serum.

The consumption of OTA contaminated food led to accumulation of OTA residue in renal and hepatic tissues and growth of the organs which are involved in detoxification and elimination processes (Stoev, et al., 2002; Aoudia et al., 2009). A weight gain of these bodies was observed in experiments in which OTA contamination level exceeds the recommendation of 0.05 mg/kg indicated by the European Commission (Stoev, 2010).

There was detected OTA residues in chicken muscle and eggs (Marquardt and Frohlich, 1992).

At a consumption of OTA contaminated food in concentration of 2 mg/kg, there was an increase in the content of OTA in the liver (15.1 g/kg). (Denli et al.,2008). OTA did not exceed the limit of detection (0.05 micrograms/kg) in the analyzed eggs. After studies by several researchers concluded that intake of OTA by eating eggs is not a concern. (Tangni et al., 2008). On birds which were fed with an OTA contaminated diet in concentration of 10 mg/kg an contamination in concentration of 0.7-1.3 g/kg was observed (Neimiec et al., 1994).

OTA was not detected in Japanese quail eggs when administrated a diet of 1 mg OTA/kg (Piskorska and Juszkiewicz, 1990).

Toxic action of OTA on the human body

OTA is considered as a possible causative agent for two chronic diseases: Balkan endemic nephropathy (NEB) and chronic interstitial nephropathy (North Africa).

In Balkan region ochratoxicosis were confirmed in humans. The appearance of Balkan nephropathy was associated with nephrotoxic effect of OTA in humans.

In 1956, this disease was described, for the first time, in a study conducted on a group of 664 hospitalized patients for kidney diseases, in Bulgaria. The Balkan endemic nephropathy was diagnosed in Romania with a spreading area of five outbreaks in Oltenia and one in Banat. (Gluhovschi et al., 1994).

On patients in Bulgaria, suffering from the Balkan endemic nephropathy, there were

observed renal and bladder tumors similar to those obtained experimentally in rat kidney.
The link between exposure to ochratoxin and renal and bladder cancer incidence was found on patients in the region of Midi-Pyrenees. Several researchers argue OTA involvement in Balkan endemic nephropathy etiology (Petkova and Kernozemsky, 1988; Phofl et al., 1999; Abarca, 2001; Pfohl, 2002).
It was established that nephrotoxic Ochratoxin A is a mycotoxin, immunotoxic, myelotoxic and carcinogenic according to data from (CIRC, 1993).
The toxic action of ochratoxin occurs through several mechanisms: effects on lipid and carbohydrate metabolism on mitochondrial respiration and changes in the transcription and transduction.
Following epidemiological studies which demonstrate that OTA can cause in humans a higher incidence of renal tumors and nephropathy, the European Scientific Committee indicates for human alimentation a tolerable food consumption lower than 5 mg/kg/day.
After oral administration, OTA is present in the blood for 35 days. (Petzinger, and Weidenbach, 2002).
After some studies it was observed that renal tumors often appear on food consumption of 70 g/kg/day of OTA (Phofl et al,1993, Phofl et al,2007; Phofl,2009).

Legislation on OTA in food

Attempts to eliminate mycotoxins in animal nutrition and the food is impossible (Bennett et al., 2003).
Many countries and organizations have established levels of OTA in feed and food.
There were established regulations and guidelines establish maximum limits for mycotoxins that the: US Food and Drug Administration (FDA), Food and Agricultural Organization of the United Nations (FAO), European Union (EU), the Institute of Public Health of Japan.
The 1881 regulation issued in 2006 the European Commission has set maximum limits for mycotoxins (Regulation (EC) No 1881/2006). (Table 2) to basic products: cereals, cereal based products, dried fruits and wine, baby food, coffee.
Limits of different mycotoxins in feed, cereal and cereal products for animal feeding recommended guidelines for the maximum tolerable the Commission of the European Communities (Table. 2). (Recommendation 2006/576/EC)

Table 2. European Union Maximum level of ochratoxin permitted in foodstuff

Commodities	Maximum level (μg/kg)
Raw cereals	5.0
Cereal products	3.0
Infant based food	0.5
Dried vine fruit	10.0
Soluble coffee	10.0
Roasted coffee beans	5.0
Wine and grape juice	2.0

Table 3. Guidance values for OTA in feeding stuffs with a moisture content of 12%, as set in the Commission Recommendation 2006/576/EC

Products Intended for Animal Feed	Guidance Valuein mg/kg
Feed materials	0.25
Cereals and cereal products	
Complementary and complete feedingstuffs	
Complementary and complete feedingstuffs for pigs	0.05
Complementary and complete feedingstuffs for poultry	0.1

There were used several strategies to reduce the risk of ochratoxins appearance in food industry, as a result of the transfer or the food chain.
Public health issues are justified on the basis of demonstrated toxic effects caused by contamination with ochratoxin.
Mycotoxins can contaminate feed materials (cereals) before arriving in feed mills due to weather conditions.
It is necessary to develop the capacity determination of mycotoxins level in whole food chaine: plant-animal-animal originated food products. (Savu et al., 2004).
As a precaution, the quality control on each lot is in order. OTA is a hard to break mycotoxin when it appears in feed. Temperatures up to 250°C on a extensive time of a few minutes are demanded in order to destroy OTA compounds in foodstuffs. (Boudra, H.; Le Bars, P.; Le Bars, J).
If the OTA is considered to be a feed hazard, there can be used specific absorbents to block the mycotoxin in the digestive contents or microorganisms capable of transforming it into non-toxic metabolites. (Denli, 2008; Schatzmayr et al.2006).

Several studies indicate that antioxidants play a role in reducing the toxicity of OTA in several species. Abdel-Wahhap et al. and Ozcelik et al. concluded that melatonin shows a preventive effect of OTA-induced oxidative stress. (Özçelik et al.,2004; Abdel-Wahhab et al., 2005).
The use of a tocopherol in the diet decreased by 57% overall DNA adduct in the kidney caused by a single administration of OTA in mice and rats. (Grosse et al., 1997).
The ochratoxins' suppressive effect on egg production and the toxic effect of OTA in different organs lowered by adding a plant extract (artichokes) in laying eggs hens' diet (Stoev, 2010).
The official controls carried out in order to ensure the verification of compliance with feed and food under EC Regulation no. 882/2004 had a major role in ensuring food safety.
There were developed laws that establish sampling analyzing methods for the official control of feed and food:
• EC Regulation no. 401/2006 established sampling and analyzing methods for the official control of mycotoxins in food;
• EC Regulation no. 152/2009 established sampling and analyzing methods for the official control of feed.
Feed business operators have to be licensed in accordance with European Commission Regulation no. 183/2005 and to conduct a risk analysis and critical control points in HACCP implementation.
The application of HACCP system in all units that are parts of the the food chain is required.
Hazard analysis and critical control points (HACCP) is a scientific and systematic apparatus used to identify:
• Risks associated to a food product regarding food safety;
• Risk monitoring to ensure food innocuousness.
The legislation in food industry aims to reduce, eliminate and prevent a risk to human and animal health. The three components of risk analysis: risk assessment, risk management and risk communication lead to the establishment of efficient and accurate measures of health protection.
European Commission Regulation no. 178/2002 establishes safety requirements regarding feed and feed business operators' responsibilities. Food and feed traceability shall be established at all stages of production, processing and distribution.
European Food Safety Authority (EFSA) is endowed with a number of important tasks regarding: independent scientific advice on all aspects of food security, early warning systems and collaboration with national agencies, thus ensuring a high level of protection and consumer confidence.
EFSA is a decentralized organism of the European Community in food security and safety functions.
The Food and Veterinary Office Commission (FVO), as guardian of the Treaties of the European Community is responsible for ensuring that Community legislation on food safety, animal health, plant health and animal welfare is properly implemented and enforced. As a service of the Commission, The Food and Veterinary Office (FVO) plays an important role in this task.
The Food and Veterinary Office mission, through its audits, inspections and related activities contribute to:
• European Community development policy in food safety, animal health and welfare and plant health sectors;
• Development and implementation of effective control systems in food safety, animal health and welfare and plant health sectors.
The control strategies for OTA in food consist in: early identification and elimination of the contaminated products from the food chain.

MATERIALS 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 (450 nm), centrifuge, magnetic stirrer, paper filter, gradual pipette, 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 3).

Table 4. 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)

Table 5. The results of determinations made are shown in the table below

Matrices	Nr. Samples			OTA, (µg/ Kg)		
	2010	2011	2012	2010	2011	2012
Mixed fodder for pigs	3	3	10	Ned... 0.478	Ned.	Ned.
Corn beans	6	4	11	Ned.	Ned.	Ned.
Bran	3	4	9	Ned.	Ned.	Ned.
Ground grain	6	5	8	0.35.. 0.74	0.12.. 0.21	0.12.. 0.21
Pig kidney	-	-	6		-	Ned

Ned.-undetectable

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

RESULTS AND DISCUSSIONS

The strategies for reducing the risk of ochratoxins appearance in food as a result of transfer through the food chain consisted in quality control (test mycotoxicological) feed and animal products batches.
For that matter, between 2010 and 2012 there were analyzed feed samples (grains, pig feed, bran).
In 2012 there were analyzed 6 samples (matrix-kidney) for the determination of ochratoxin A.
The results of the performed determinations were interpreted according to the European legislation and are thus presented in the tables below.

CONCLUSIONS

This paperwork approaches about the problem of ochratoxin A in relation to toxicity and the mechanisms by which it exerts its toxicity to human and animal health and control strategies used in the feed industry.
The strategies used to reduce, eliminate or avoid the risk of ochratoxins are justified by the demonstrated toxic effects caused by contamination with ochratoxin.
Official controls performed to ensure the quality of feed and food under Regulation (EC) 882/2004, led to the conclusion that the EU legislation on food safety is observed.
The feed and animal products (pig kidney) samples that were collected and analyzed for the determination of ochratoxin A and compliance results show that they are not harmful to humans and animals

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