

THE PRESENCE OF MYCOTOXINS (OTA AND ZEA) IN FEED FOR PIGS AND THEIR INFLUENCE ON REPRODUCTION

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Abstract

Today FAO estimates that about quarter of world cereal crops are contaminated with mycotoxins. Contamination of agricultural products can occur both before and after harvest, especially during storage, being conditioned by the humidity and high temperatures. These factors favor the growth of mold and increase the risk of mycotoxins. Species of fungi: *Aspergillus*, *Penicillium* and *Fusarium* can produce and release secondary metabolites in feed type: Ochratoxin A (OTA), Zearalenone (ZEA), impact on reproductive performance. Toxicity of mycotoxin depends on the source and their dose, duration of exposure and composition. Damaging effects of moldy feed management are felt especially in youth and female reproduction subject. If consumption of moldy feed, the effects recorded in breeding disorders manifested by abortions, complications of parturition, uterine involutions delayed, prolonged estrus after calving, followed by infecunditate endometritis and sterility. In this case the percentage of pregnant females is very low, even after the repeated treatments. This paper aims to address the presence of mycotoxins (OTA and ZEA) in feed intended for pigs, with their direct influence on reproduction. To minimize the impact of the presence of mycotoxins in pig feed, control measures are carried out to establish the quality of feed used. This mycotoxin has been evidenced by laboratory tests. The working method used was ELISA. Values obtained from determinations were performed according to the legislation.

Key words: mycotoxins, Ochratoxin, Zearalenone, feed, breeding swine.

INTRODUCTION

A recent definition posed mycotoxins as "fungal metabolites after inhalation ingestion or absorption through the skin, altering the responsiveness of the

body and cause illness or even death in animals (JI Pitt,1996).
 Species of fungi: *Aspergillus*, *Penicillium* and *Fusarium* can produce and release secondary metabolites in feed type: Ochratoxin A (OTA), zearalenone, impact on reproductive performance. (Table 1)

Table 1.Species of fungi that can generate OTA and ZEA in cereals

Fungi	Cereale/ matrice	Mycotoxins
<i>Aspergillus ochraceus</i> ; <i>Penicillium viridicatum</i> <i>Penicillium cyclopium</i>	Cereals	Ochratoxin A (OTA)
<i>Fuzarium culmorum</i> ; <i>Fuzarium graminearum</i> ; <i>Fuzarium sporotrichioides</i>	Cereals	Zearalenone

Regardless of their type, mycotoxins (aflatoxins, ochratoxin, zearalenone, fumonizine) cause serious damage to livestock. Food and Agriculture Organization (FAO) estimates that up to 25% of cereals crops are significantly contaminated with mycotoxins worldwide. Contamination of agricultural products can occur both before and after harvest, especially during storage, being conditioned by the humidity and high temperatures.

These factors favor the growth of mold and increase the risk of mycotoxins (Savu C. et al., 2004).

Following recent investigations, one can release a warning of the danger which poses for the health of animals. The occurrence of frequent and various forms of barrenness is caused largely by feed quality assurance.

Pigs are the most sensitive to the two mycotoxins: OTA and ZEA. High incidence of barrenness, profound disturbance of the sexual cycle (false estrus and repeated absence or irregularity of oestrus and vulvo vaginal swelling, vaginal and uterine prolapse are the main symptoms of moldy feed management. Reproduction disorders manifested by abortion complications at parturition, delayed uterine involutions, prolonged estrus after calving,

endometritis followed by infecunditate and sterility. in this case the percentage of pregnant females is very low, even after repeated treatments. Spores cross the gastrointestinal barrier through blood-and lymph, reach the gynecological organe, crossing the placenta (in de case of pregnant females). Roof covering the fetus and fetal multiply, causing abortion

Ochratoxin A (OTA) is considered a natural contaminant in cereal grains with affinity for plasma proteins that are fixed in 90%.(II' Icev YV, Perry,Rucher F et al,2002). OTA can delay the body's immune response,reducing cell-mediated immunity. As aflatoxins, it has carcinogenic action. In boars, OTA can reduce sperm motility and longevity.

Zearalenone is a non-steroidal estrogen mycotoxin produced by *Fusarium* spp it has been reported in many micotoxicoze at farm animals, especially in pigs.

Zearaleona is resistant to high temperatures and can be found in many cultures across the world contaminating grain, such as corn, oats, barley, wheat, rice and sorghum (Kuiper-Goodman et al., 1987, Tanaka et al., 1988).

Zearalenone is a known phytoestrogen that causes hormonal disorders in animals that ingest contaminated fodder, with the worst effects in pigs. Pigs are very sensitive to zearalenone, which produces a syndrome manifested by changes in estrogen and breast tissue of the vulva (congestion, increase in volume), abnormal lactation, infertility,abortion, birth of dead or viable products, vaginal and rectal prolapse .Absorbtion in pigs after a single oral dose of 10 mg / kg body weight was estimated at 80-85%. (Biehl et al., 1993) zearalenone and its metabolites were found in plasma of pigs less than 30 min after feeding began. (Kuiper-Goodman et al., 1987, Olsen et al.,1991, Biehl et al., 1993).

MATERIAL AND METHODS

To minimize the impact of the presence of mycotoxins in feed breeding pigs with direct influence on their determinations were carried out to establish the quality of feed used. This mycotoxins (OTA and ZEA) was evidenced by using ELISA method of working, a rapid quantitative method of screening. 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) contains:

- Microtiter plate consisting of 12 strips with 8 wells each, coated with antigen;
- Standards of different concentrations of mycotoxins (5 or 6) with standard Cuba Trace
- All reagents and buffers required (Anti-body - specifically of mycotoxin, Conjugate (with enzyme), Substrate Solution, Stop Solution , Washing buffer)

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, ZEA)
- were observed using working volumes: 50, 100, 500 and 1000 µl-micropipets

Upon completion of the determination and use of equipment contributed: centrifuge, shaker, stirrer ELISA, ELISA plate reader at 450nm.

All kits must be certified according: detection limit (LOD), recovery rate, sample preparation and specificity (Table 2).

Table 2. Performance criteria and standard solutions for same ELISA kit's

Mycotoxin	Recovery %	LOD	Matrices	Standards
Ochratoxin A <i>RidaScreen</i>	85	625 ppt	Cereals, feed	0.0; 25; 75; 225; 675; 2025 ppt
ZEA <i>BioScientific</i>	-	1,0 ppb	Cereals, feed	0.1; 0.25; 0.5; 1.5; 4.5 ppb

*(ppb= ng/mL= µg/Kg ; ppt = ng/Kg)

RESULTS AND DISCUSSION

This paper has proposed to address the presence of mycotoxins (OTA and ZEA) in feed intended for pigs in 2010-2011. Samples were representative sample for each lot and have to comply with harvesting. If consumption of moldy feed containing secondary metabolites such as: Ochratoxin A (OTA), zearalenone (ZEA) swine, especially youth and females may be affected, the impact on reproductive performance. Toxicity of mycotoxins depends on the source and their dose, duration of exposure and composition. Samples analyzed samples were represented by the following matrix: combined fodder for pigs, corn beans, bran, ground grain.

The results of determinations made are shown in the table below (Table 3).

Table 3. Determinative mycotoxins in swine feeds: 2010-2011

Matrices	Nr. Samples		OTA, (µg/ Kg)		ZEA, (µg/ Kg)	
	2010	2011	2010	2011	2010	2011
Mixed fodder for pigs	3	3	Ned...0,478	Ned.	Ned...16,82	Ned....28,5
corn beans	6	4	Ned.	Ned.	5,12...51,64	3,44...30,09
bran	3	4	Ned.	Ned.	Ned. ...35,10	1,12...28,14
ground grain	6	5	0,36..0,74	0,12..0,24	5,34...35,18	Ned. ..27,41

Ned.- nedetectabil

Values obtained from determinations were performed according to the legislation. Because toxic effects of mycotoxins, their highest level in feed for pigs is subject to COMMISSION RECOMMENDATION EC N0 567/2006 (Table 4)

Table 4. Maximum levels for ZEA and OTA in cereal for pigs feeding

Mycotoxin	Products intended for animal feed value	Value in mg/kg (ppm)
Zearalenone	Feed materials (*) — Cereals and cereal products with the	2

	exception of maize by-products — Maize by-products — Complementary and complete feedingstuffs for pigs	3 0,250
OTA	Feed materials (*) — Cereals and cereal products; Complementary and complete feedingstuffs for pigs	0,250 0,050

the previous year were much lower. This leads us to conclude that were rigorously respected veterinary rules on handling, transport and storage. Considering the results, it can be said that the fodder for feeding pigs were not any danger to their health and therefore did not affect reproductive performance default. Analyzing the results of determinations made, it can be seen that no sample (matrix) was not contaminated with OTA or ZEA values over the maximum allowed.

More than corn grain and bran samples analyzed both in 2010 and in 2011 did not contain OTA. The remaining samples were found only traces of OTA, obtained values were located below 1 µg/kg.(ppb)

In terms of values found in ZEA, the highest value obtained was recorded in 2010 maize grain (51.64 ppb) value less than about 60 times the maximum permitted.

All the same matrix to registering and in 2011 the highest value determined (30.09 ppb) approximately 100 times lower than the maximum allowed.It also notes that ZEA determinations made in 2011, compared to

CONCLUSIONS

Type mycotoxins: Ochratoxin A (OTA), Zearalenone (ZEA), can contaminate food agricultural products used in swine, reproductive performance impact;

Mycotoxins toxicity depends on the source and their dose, duration of exposure and composition;

The quality of feed used is determined by extensive laboratory occupying it an important quantitative determination of mycotoxin –working method ELISA, a quantitative screening method is based on the reaction antigen – antibody can contribute to obtaining valid results, because performance criteria of the kit used;

Due to the toxic effect of mycotoxins the maximum level in food for their pigs in subject to European legislation;

The results of determinations made on the matrices analyzed in 2010 and 2011 did not exceed the maximum allowed neither OTA and ZEA;

So it can be said that these fodder for feeding pigs were not any danger to their health and therefore did not affect reproductive performance default.

ACKNOWLEDGEMENTS :

This study is part of the doctoral dissertation included in the project: "doctoral Fellowships to support research activity in the field of agronomic and veterinary medicine" in the POSDRU/107/5/S/76888

REFERENCES

Abarca ML. Current importance of Ochratoxin A– producing *Aspergillus* spp. *Food Prot* 64, 2001.

Coman I., Mareş M. - *Micologie medicală aplicată*, Ed. Junimea, Iaşi, 75 - 270, 318 – 336, 2000
Coman I., Popescu. O - *Micotoxine şi micotxicoze*, Ed. Ceres, Bucureşti, 88 -126, 1985

Biehl et al, Biliary excretion and enterohepatic cycling of zearalenone in immature pigs. *Toxicol. Appl. Pharmacol.* 121, 152–159.

IARC - International agency for research on cancer) monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. Vol **56**, pp. 397-444. Lyon, France, 1993.

Il' Icev YV, Perry JL, Simon JD - Interaction of ochratoxin A with human serum albuminum. Preferential binding the dianion and the ph effects; *Phys Chem B* 2002;106:452-459

Il' Icev YV, Perry JL, Rucher F et al.- Interaction of ochratoxin A with human serum albuminum. Binding site localized by competitive interactions with the native protein and its recombinant fragments: *Chemico- Biological Interaction*

JECFA - Joint FAO/WHO Expert Committee on Food Additives, 32nd Meeting. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series 23. 1988

JECFA - Joint FAO/WHO Expert Committee on Food Additives, 53rd Report. Safety evaluation of food additives. WHO Food Additives , Series 44 , 2000

JEFCA- Evolution of certain mycotoxins (fifty sixth report of the Joint FAO /Who Expert Committee on Food Additives);WHO, Geneva;2002:906

Kuiper-Goodman, T.et al- Risk assessment of the mycotoxin zearalenone. *Regulatory Toxicology and Pharmacology*, **7**, 253-306, 1987

Kuiper-Goodman, T.et al.- Approaches to the risk assessment of fumonisins in corn-based foods in Canada. In L.S. Jackson, J.W. DeVries & L.B. Bullerman, eds. *Fumonisin in food*,p.369-393.NewYork,PlenumPress,1996

Kuiper, van der Saag, P.T., van der Burg, B. & Gustafsson, J.A. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinol*; **139**(10), 4252-4263,1998

Kuiper-Goodman T .- Food safety: mycotoxins and phycotoxins in perspective. In M. Miraglia, H.van Egmond, C. Brera & J. Gilbert, eds. *Mycotoxins and phycotoxins - developments in chemistry, toxicology and food safety*. Oxford, United Kingdom, International Union of Pure and Applied Chemistry (IUPAC), 1998

Olsen et al, Health evaluation of ochratoxin A in food products. *Nordiske Seminar- og Arbejds- rapporter*, Vol. **545**. Copenhagen, Nordic Council of Ministers,1991.

Pitt JJ- What are mycotoxins?; *Australian Mycotoxin Newsletter* 1996: 7(4):1.

Savu C. , Narcisa et all Georgescu - *Siguranta alimentelor* , Editura Semn E ,2004

Tanaka, T.et al, - Worldwide contamination of cereals by the *Fusarium* mycotoxins, nivalenol,deoxynivalenol, and zearalenone. 1. Survey of 19 countries. *J Agric Food Chem*, **36**, 979-983, 1988

Garon D, Richard E, Sage L, Bouchart V, Pottier D, and Lebailly P. Mycoflora and Multimycotoxin Detection in Corn Silage: Experimental Study.*J.Agric.Food Chem* 54,2006,p.3479-3484 .

**** COMMISSION RECOMMENDATION EC N0 567/2006 , On the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding, of 17 August 2006

**** Test kit, OTA - ELISA method, RIDASCREEN,Catalog # R1311

.**** Test kit, ZEA - ELISA method, BioScientific,Catalog # 1035