# Survey of moulds and mycotoxin contamination of cereals in South-Eastern Romania in 2008-2010

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SUMMARY

Fungal mycoflora and mycotoxin contamination were determined in 86 samples (21 maize, 21 wheat, 11 barley, 4 oats, 1 rye, 12 soya, 6 sunflower, 4 colza, 3 rice, 3 triticale), coming from the south–eastern part of Romania during the 2008 to 2010 period.

The most frequent fungal contaminants belonged to the *Aspergillus* and *Fusarium* genera, maize was the most contaminated cereal. The main toxinogenic species identified were *A. flavus*, *A. fumigatus, F. graminearum*, *F. culmorum* in all cereals

Aflatoxin B1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUMO), contents were analyzed by ELISA. More than 90 % of the samples were found to be contaminated by at least one toxin. The most frequent mycotoxin was the deoxynivalenol (71.60%). Around 40% of samples were contaminated with AFB1 and FB. Ochratoxine A and zearalenone were found in 16% and 32% of samples respectively. These results demonstrated that cereals produced in Romania present a particular pattern of fungal mycoflora and mycotoxin contamination since DON, ZEA and FUMO as well as AFB1 and OTA were observed.

Keywords: fungal contamination survey, mycotoxin contamination survey, feedstuff ingredients, Romania

## INTRODUCTION

Fungal development in alimentary substrates can lead to different detrimental effects: alterations of aspect and technological properties, modifications of nutritive value, development of mycosis and allergy agents, and production of mycotoxins (Bennett and Klich, 2003). In Europe, due to the temperate climate, *Fusarium* fungi are usually found as major contaminants of cereals (Domijan and al., 2005; Krysinska-Traczyk et al., 2007). Their development may lead to contamination of raw material with zearalenone (Cervero and al. 2007; Mankeviciene and al., 2007; Shollenberger et al., 2006), fumonisins (Arino et al., 2007; Domijan and al., 2005; Engelhardt et al., 2006;

Fandohan et al., 2005; Pietri et al., 2004) and deoxynivalenol (Mankeviciene et al., 2007; Pietri et al., 2004; Shollenberger et al., 2006). The development of *Penicillium* species, such as *P. verrucosum* may lead to Ochratoxin A contamination (Domijan et al., 2005; Krysinska-Traczyk et al., 2007). More rarely in Europe, the development of *Aspergillus* species such as *A. flavus* and *A. parasiticus*, can lead to the presence of aflatoxin B1, especially after warm summers (Giorni and al., 2007; Martins et al., 2007; Pietri et al., 2004).

Mycotoxins are usually considered as relevant for public health (Milicevic et al., 2010), some of them being considered as carcinogenic. Thus, aflatoxin B1 (AB1) is considered the most potent natural carcinogen, responsible for the appearance of humans and animals hepatocarcinoma. AFB1 is included in group 1 human carcinogen molecules by IRCC (Autrup et al., 1991; Vainio and al., 1992). Ochratoxin A (OTA) is nephrotoxic causing impairment in humans and animals tubulonecrosis (Pohland et al*.,* 1992; Marquardt and Frohlich, 1992; Abouzied et al., 2002; Vrabceva et al., 2000; Vrabceva et al., 2004). OTA is included in group 2B carcinogens molecules to animals and possibly carcinogenic to humans. (Vainio and al., 1992).

Fumonisins toxicity is characterized by the appearance of clinical signs very different depending on the species: to cause equine leukoencephalomalacia (liquefaction cerebral white matter) and histopathological abnormalities in the liver and kidneys, (Bailly et al., 1996) and pulmonary oedema in pig (Osweiler and al., 1992; Ross and al., 1992). In humans fumonisin B1 (FB1) is suspected of being involved in cancer of the oesophagus (Luo et al., 1990; Yoshizawa et al., 1994). FB1 is included in group 2B carcinogens molecules to animals and possibly carcinogenic to humans. Zearalenone (ZEN) induce hyper-oestrogenism in animals (Rainey et al., 1991), characterized by oedema vaginal (Kaliamurthy et al., 1997), ovarian atrophy, enlarged mammary glands, reduced testosterone levels and sperm quality, the appearance of sterility" (Etienne and Dourmad, 1994).

The toxic effects of deoxynivalenol (DON) are associated with gastrointestinal disorders. Intoxications characterized by refusing food poisoning, nausea, vomiting, were observed in pigs (Pollman et al., 1985; Trenholm et al., 1984; Harvey et al., 1989), lambs (Harvey et al., 1986), chickens (Hamilton et al., 1985; Huff et al., 1981; Huff et al., 1986; Kubena et al., 1985; Kubena et al., 1989; Swamy et al., 2002), ducks (Boston et al., 1996) and turkeys (Hamilton et al., 1985; Morris et al., 1999). All species are sensitive to the action of deozynivalenol, but toxicity is different from one species to another.

Within this context, efforts to assess human exposure in European Union (E.U.) have been undertaken within SCOOP projects (Scientific Cooperation on Questions relating to Foods), (Milicevic, 2010, Schothorst, van Egmond, 2004). They aimed to evaluate food and feeds' mycotoxins contamination and the

intake of mycotoxins by EU inhabitants (Gareis et al., 2004; Miraglia and Brera, 2002). Moreover the optimization of fungi and mycotoxin detection, facilitate the monitoring of these contaminants in foods and feeds in Europe and around the world. For example, extensive survey data on FBs in maize indicate that a high proportion of maize is contaminated with this toxin (WHO-IPCS. 2000; Placinta et al., 1999; Murphy et al., 1996). The level of FB contamination varies between different regions of each continent according to the climatic conditions. In most samples, FB1 has been the most prevalent toxin, followed by FB2 and FB3. In countries of Africa, North and South America, Asia, and Europe, FB1 has been reported at levels ranging from 0.01 to 155 mg/kg, FB2 occurring also from 0.01 to 22.96 mg/kg (Taranu et al., 2008). Other example showed that deoxynivalenol is the most prevalent trichothecene in crop production in Europe and North America (Schothorst, van Egmond, 2004, CAST, 2003).

Romania was not included in such surveys and only few data are available concerning mycotoxin contamination of cereals produced in this new E.U. member state (Curtui et al., 1998). Moreover due to its continental climate with cold winter and hot and dry summers, fungal mycoflora and mycotoxin contamination of cereals produced in may differ from that reported in other European countries. A first survey study on fungal mycoflora (*Aspergillus, Fusarium*, *Penicillium)* and mycotoxin (ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisins) contamination of cereals produced in the South East part of Romania during the 2002 to 2004 years (110 cereal samples (54 corn samples, 35 wheat samples and 21 barley samples) showed that cereals produced in Romania present a particular pattern of fungal mycoflora and mycotoxin contamination (Tabuc et al., 2009). Indeed, DON and ZEA appear important contaminant as reported for cereals produced also in North and Central part of Europe. Also, AFB1 in maize often overtakes EU regulation in human food, as observed after exceptional dry and hot summer in some countries of south Europe. This last point specially highlights that the carcinogenic mycotoxin AFB1 has still to be monitored in Europe, since local or temporary climatic conditions can lead to contamination of food and feeds.

The aim of the present study was to continue the Romanian survey by analysing fungal mycoflora and mycotoxin contamination of cereals produced in the South East part of Romania during the 2008 to 2010 years.

## MATERIAL AND METHODS

### *Solvents and reagents*

All solvents and reagents were purchased from Merck (Germany) and were of analytical grade.

### *Samples*

A total of 86 samples (21 maize, 21 wheat, 11 barley, 4 oats, 1 rye, 12 soya, 6 sunflower, 4 colza, 3 rice, 3 triticale), coming from South Eastern part of Romania were analysed for fungal and mycotoxin content before being used in the manufacture of feed. Although no statistical sampling plan was done, this study was made on all cereal samples received by INCDBNA during 2008-2010 from different merchants; that is why the number of samples varied from one year to another according to the following schedule: 42 samples in 2008; 32 in 2009 and 12 in 2010. They were analysed at reception, in the INCDBNA's laboratory.

### *Fungal count and identification*

Twenty grams of sample were dispersed in 180 ml of 0.05% Tween 80 solution using a warring blender mixer. 1 ml of each decimal dilution was plated on both malt agar medium (2% agar, 2% malt, 50 ppm chloramphenicol) (*32*) and malt salt agar medium (malt agar + 6% NaCl) (Rabie and al., 1997). Typical fungal colonies were counted after 3, 5 and 7 days of culture at 25 and 31°C and results were expressed as Colony Forming Unit (CFU)/g of sample. After isolation and planting out on Czapek medium or Potato Dextrose Agar (Oxoid, Dardilly, France), *Aspergillus*, *Penicillium and Fusarium* were then identified to species level according to Rapper and Fennell (1965), Pitt (1988) and Nelson and al. (1983).

### *Mycotoxin quantification*

300 g of samples were finely grounded into powder. A 20g sub-sample was then extracted by mechanical agitation for 3 min in a warring blender using 100 ml of 70% methanol for aflatoxin B1, zearalenone and fumonisins, 50% methanol for ochratoxin A, and de-ionized water for deoxynivalenol. Extract was filtered on Whatman n°1 filter.

The concentration of the monitored mycotoxins (ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisins) was determined using immunoenzymatic ELISA kits (Veratox, Neogen, MI, 48912, USA/Canada) as recommended by the manufacturer (Abouzied and al., 1996; Dorner and al., 1993). The optical density was read within 20 min on a microplate reader (TECAN SUNRISE, Austria) at 650 nm. Limits of detection (LOD) and quantification (LOQ) were respectively 1 µg/kg for ochratoxin A, 0.5 µg/kg for

aflatoxin B1, 0.1 mg/kg for deoxynivalenol, 10 µg/kg for zearalenone, 50 mg/kg for fumonisins.

## RESULTS

### *Fungal population of cereal samples*

The analysis of fungal contamination revealed that maize was the most contaminated sample with a mean fungal count of 355 $\times 10^3$ CFU/g followed by wheat and oats that presented respective mean fungal counts of 222.6 and 206 $\times$ $10^3$ CFU/g.

Analysis of fungal mycoflora at the genus level revealed only weak differences depending on the nature of the cereals. *Aspergillus* was the predominant genus, found in 80.95, 71.87 and 91.66% of 2008, 2009 and 2010 years respectively. *Fusarium* and *Penicillium* species were also frequently found (Table 1).

Table 1. Fungal contamination (% of samples)

| | 2008 (n = 42) | 2009 (n = 32) | 2010 (n =12) |
|---|---|---|---|
| *Aspergillus sp.* | 80.95 | 71.87 | 91.66 |
| *A. niger* | 54.70 | 59.40 | 75.00 |
| *A. flavus* | 42.80 | 31.20 | 66.60 |
| *A. versicolor* | 38.00 | 31.20 | 58.30 |
| *A. ochraceus* | 16.60 | 25.00 | 16.60 |
| Other | 19.00 | 43.70 | 33.30 |
| *Fusarium sp.* | 54.76 | 37.51 | 33.33 |
| *F. graminearum* | 47.60 | 18.70 | 33.30 |
| *F. culmorum* | 33.30 | 28.10 | 16.60 |
| *F. verticillioides* | 7.10 | 0.00 | 16.60 |
| Other | 26.20 | 21.80 | 16.60 |
| *Penicillium sp.* | 28.57 | 31.25 | 48.00 |
| *P. citrinum* | 14.20 | 12.50 | 16.60 |
| *P. purpurogenum* | 4.76 | 9.30 | 16.60 |
| *P. brevicompactum* | 0.00 | 15.60 | 8.30 |
| Other | 9.50 | 6.20 | 25.00 |
| *Mucor* | 14.28 | 15.62 | 25.00 |
| *Rhizopus* | 7.14 | 15.62 | 25.00 |
| *Cladosporium* | 9.52 | 6.25 | 8.33 |
| *Absidia* | 2.38 | 6.25 | 0.00 |

Because mycotoxins contamination depends on the fungal species developing on the substrate, identification of *Aspergillus*, *Fusarium* and *Penicillium* strains was done to the species level. Results are reported in Table 1. Among *Aspergillus* species, *A. niger* was the most frequently found in all

tested cereals, followed by *A. flavus*, *A. versicolor* and *A. ochraceus*. Other identified *Aspergillus* species were *A. candidus*, *A. glaucus*, *A. restrictus and A. terreus*.

The *Fusarium* species found in cereal samples mainly belonged to *F. graminearum* and *F. culmorum* species. In maize, *F. verticillioides* was also found as a contaminant of 7.1 and 16.6% of samples in 2008 and 2010 years respectively.

*Penicillium* isolates mainly belonged to *P. citrinum, P. purpurogenum* and *P. brevicompactum,* species. As observed for *Aspergillus* and *Fusarium*, only mild differences could be noted between cereals.

Many of these fungal species are known to be potentially toxigenic. Therefore, mycotoxin content of samples was analysed.

*Mycotoxin contamination of cereal samples*

Cereal samples were analysed for aflatoxin B1 (AFB1), ochratoxin A (OTA) deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FB) contamination. Results are reported in Table 2.

Table 2. Mycotoxins contamination of cereal samples in 2008-2010

| | 2008 (n=42) | 2009 (n=32) | 2010 (n=12) |
|---|---|---|---|
| Aflatoxin B1 | | | |
| Mean (± SE) | 1.71 (± 8.3) | 0.59 (± 0.94) | 4.11 (± 12.3) |
| Range of contamination (µg/g) | 0 – 51.7 | 0 – 2.3 | 0 – 42.6 |
| Numbers of samples > 5 µg/kg* | 2 (4.76%) | 0 | 1 (8.33%) |
| Ochratoxin A | | | |
| Mean (± SE) | 1.92 (± 12.5) | 1.86 (± 5.2) | 1.44 (± 4.12) |
| Range of contamination (µg/g) | 0 – 81 | 0 – 25.8 | 0 – 14.4 |
| Numbers of samples > 5 µg/kg * | 1 (2.38%) | 1 (3.12%) | 1 (8.33%) |
| Deoxynivalenol | | | |
| Mean (± SE) | 165.43 (± 370.62) | 61.73 (± 145.26) | 56.92 (± 42.73) |
| Range of contamination (µg/g) | 0 – 2248.2 | 0 – 632.7 | 0 – 158.9 |
| Numbers of samples > 1750 µg/kg * | 1 (2.38%) | 0 | 0 |
| Zearalenone | | | |
| Mean (± SE) | 8 (± 1.84) | 91.87 (± 109.92) | 48.47 (± 194) |
| Range of contamination (µg/g) | 0 – 336 | 0 – 282.4 | 0 – 496.1 |
| Numbers of samples > 100 µg/kg * | 1 (2.38%) | 12 (37.5%) | 1 (8.33%) |
| Fumonisins | | | |
| Mean (± SE) | 61.10 (±142.53) | 53.74 (±189.42) | 36.17 (± 80.32) |
| Range of contamination (µg/g) | 0 - 784.5 | 0 – 1008.1 | 0 – 260.2 |
| Numbers of samples > 100 µg/kg * | 6 (14.28%) | 4 (12.5%) | 2 (16.66%) |

*EU regulation

AFB1 contamination was observed in 38 % of maize samples. Mean level of contamination was about 3.2 µg/kg, the highest contamination levels observed being about 42.6 µg/kg. 4.76% exceeded EU regulation for that

contaminant. For wheat, barley, oats, rye, soya, sunflower, colza, rice and triticale, AFB1 contamination appeared very rare, no sample out of 81 analysed exceeded the regulatory EU limit of 5 µg/kg (FAO, 2004), the level of AFB1 was between 0.1 and 2.3 µg/kg.

OTA contamination was observed in 13.5 % of samples analysed, only 3 samples out of 81 analysed exceeded the regulatory EU limit of 5 µg/kg (FAO, 2004),). The level of OTA was between 0.8 and 81 µg/kg. Ochratoxin A was not observed in barley, oats, rye and rice samples analysed.

DON was the most frequent mycotoxin of analysed samples since more than 70% of samples were found to be contaminated. Only one samples of maize exceeded 1750 µg/kg that is EU regulation for DON (EU regulation, 2005), 2248.2 µg DON/kg (maize). The level of DON was between 5.4 and 880 µg/kg.

ZEA was observed in 32 % of samples analysed, but 53.85 % of samples contaminated exceeded 100 µg/kg that corresponds to EU regulation for ZEA (EU regulation ,2005), the level of zearalenone was between 1.6 and 496.1 µg/kg. ZEA was not observed in oats, rye and rice samples analysed.

FUMO was observed in 40.74 % of samples analysed, 47.61 % of wheat samples and 57.14 % of maize samples were found to be contaminated with fumonisins. 14.81 % of samples analysed exceeded 100 µg/kg that corresponds to EU regulation for FUMO (EU regulation, 2005), with highest level 1008.1 µg FUMO/kg (soya).

## DISCUSSION

Romania is located in the South East part of Europe, a continental climate characterized by dry and cold winter and hot summer prevails in this country. This climate may influence fungal species able to develop on cultures grown in this country, and both fungal and mycotoxins contamination of cereals may differ from those reported in other European countries.

We showed that *Aspergillus* fungi were very frequent contaminants of maize, wheat and barley. This is in agreement with other study done in countries where climate during spring and summer may be comparable such as Italy or Spain (Giorni et al., 2007; Medina et al., 2006). *Aspergillus* identification at the species level revealed that *Aspergillus niger* was the most frequent contaminant of cereals. This species is a very frequent fungal contaminant found worldwide on various substrates such as cereals, but also grapes, or coffee bean (Battilani et al., 2006; Leong and al., 2007; Magnoli et al., 2007). Although this species is not considered as an important mycotoxin producer, it has been shown to produce ochratoxin A, usually at low level (Hajjaji et al., 2006).

*Aspergillus ochraceus*, the most important producer of ochratoxin A (Pardo and al., 2006; Mateo and al., 2011), was isolated in the analysed samples, 16.6%, 25% and 16.6% in 2008, 2009 and 2010 respectively. OTA was found in 13.5 % of samples analysed, the mean level was 15.26 µg/kg. Only 3 samples out of 81 analysed exceeded the regulatory EU limit of 5 µg/kg. Ochratoxin A was not observed in barley, oats, rye and rice samples analysed.

*Aspergillus flavus* known to produce the carcinogenic mycotoxin AFB1 (Moreno and Suarez Fernandez, 1986), were also frequent contaminants in Romania. Two sample of maize and one sample of soya exceeded EU regulation for AFB1 (16.2, 42.6 and 51.7 µg AFB1/kg respectively). Although the presence of AFB1 in maize produced in Europe is surprising, this is not the first report of such a contamination. Indeed, recent surveys in Italy demonstrated the presence of AFB1 contamination of foods and feeds, (Giorni and al., 2007; Pietri and al., 2004).

*Fusarium* species were also frequently isolated from Romanian samples, as reported in other European countries. *F. graminearum* and *F. culmorum* were present on the samples analysed and more than 70% of samples were found contaminated with DON or ZEA. These results are in agreement with those reported in other regions of Romania (Curtui et al., 1998; Tabuc et al., 2009). They are also in agreement with results observed in countries located in north part of Europe such as the North of France, Germany, Norway, Belgium, Poland or Netherlands (Isebaert et al., 2005; Krysinska-Traczyk et al,. 2007; Shollenberger et al,. 2006). Among the tested samples, maize appeared the most contaminated with DON, in agreement with results from SCOOP task (Gareis aetnd al., 2003). Levels of contamination by deoxynivalenol were moderated, only one sample of maize exceeded EU regulation 1750 µg/kg) (EU regulation , 2005).

Zearalenone is also an important contaminant since 53.85 % of analysed samples exceeded the content of 100 µg/kg that is EU regulation value for this toxin (EU regulation, 2005).Once again these contamination levels appear higher than those usually reported in other European countries (Gareis and al., 2003; Mankeviciene et al., 2007).

*F. verticilloides* was identified in maize samples. The frequency of contamination of maize grains with this fungal species is in agreement with occurrence observed in near countries, such as Croatia (Domijan et al., 2005). Indeed, *Fusarium verticillioides* prevalence was less than 10% in maize samples, whereas results coming from Spain, Argentina or Benin report a *Fusarium verticillioides* prevalence higher than 60% (Arino et al., 2007; Fandohan et al., 2005; Ono et al., 1999.). Fumonisins were found in 40.74 % analysed samples. The highest level was of 1008.1 µg FUMO/kg (soya).

Taken together, these results demonstrate that cereals produced in Romania present a particular pattern of fungal mycoflora and mycotoxin

contamination. Indeed, DON and ZEA appear important contaminants as well as AFB1, This last point specially highlights that AFB1 has still to be monitored in that country.

## REFERENCES

Abouzied, M.M., S.D. Askegard, C.B. Bird, and B.M. Miller. 1996. Fumonisin Veratox. A new rapid quantitative ELISA for determination of fumonisin in food and feed. Adv. Exp. Med. Biol. 392: 135-144.

Abouzied M.M., Horvath A.D., Podlesny P.M., Regina N.P., Metodiev V.D., Kamenova-Tozeva R.M., Niagolova N.D., Stein A.D., Petropoulos E.A., Ganev V.S., (2002), Ochratoxin A concentrations in food and feed from a region with Balkan Endemic Nephropathy, Food Addit Contam, 19 (8), 755-764

Arino, A., T. Juan, G. Estopanan, and J.F. Gonzalez-Cabo. 2007. Natural occurrence of Fusarium species, fumonisin production by toxigenic strains and concentration of fumonisins B1, and B2 in conventional and organic maize grown in Spain. J. Food Prot. 70: 151-156.

Autrup, J.L., Schmidt, J., Seremet, T., Autrup, H., (1991), Determination of exposure to aflatoxins among Danish workers in animal-feed production through the analysis of aflatoxin B1 adducts to serum albumin. Scand. J. Work Environ. Health, 17, 436-440

Battilani, P., C. Barbano, S. Marin, V. Sanchis, Z. Kozakiewicz, and N. Magan. 2006. Mapping of Aspergillus section Nigri in southern Europe and Israel based on geostatistical analysis. Int. J. Food Microbiol. 111 : S72-S82

Bailly J.D, Raymond I., Le Bars P., Guyomard Y., Abadie J., Le Bars J., Guerre P., Delverdier M., Burgat V., (1996), Leucoencéphalomalacie des équidés: cas rapportés au CNITV, Rev. Méd. Vét., 147, 787-796.

Bennett, J.W., and M. Klich. 2003. Mycotoxins. Clin. Microbiol. Rev. 16: 497-516.

Boston S., Wobeser G., Gillespie M. 1996. Consumption of deoxynivalenol contaminated wheat by mallard ducks under experimental conditions, J. Wildlife Diseases, 32 (1), 17 - 22.

Council for Agricultural Science and Technology. Mycotoxins: risks in plant, animal and human. Potential economic costs of mycotoxins in United States. Ames (IA): CAST Task Force Report No. 139. 2003;138:136–42

Cervero, M.C., M.A. Castillo, R. Montes, and E. Hernandez. 2007. Determination of trichothecenes, zearalenone and zearalenols in commercially available corn-based foods in Spain. Rev. Iberoam. Micol. 24: 52-55.

Curtui, V, E. Usleber, R. Dietrich, J. Lepschy, and E. Martlbauer. 1998. A survey on the occurrence of mycotoxins in wheat and maize from western Romania. Mycopathologia 143: 97-103.

Domijan, A.M., M. Peraica, B. Cvjetkovic, S. Turcin, Z. Jurjevic, and D. Ivic. 2005. Mould contamination and co-occurrence of mycotoxins in maize grain in Croatia. Acta Pharm. 55: 349-356.

Domijan, A.M., M. Peraica, Z. Jurjevic, D. Ivic, and B. Cvjetkovic. 2005. Fumonisin B1, fumonisin B2, zearalenone and ochratoxinA contamination of maize in Croatia. Food Addit. Contam. 22: 677-680.

Dorner, J.W., P.D. Blankenship, and R.J. Cole. 1993. Performance of two immunochemical assays in the analysis of peanuts for aflatoxin at 37 filed laboratories. J. Assoc. Off. Anal. Chem. Int. 76: 637-643.

Engelhardt, G., J. Barthel, and D. Sparrer. 2006. Fusarium mycotoxins and ochratoxin A in cereals and cereal product: results from the Bavarian Health and Food Safety Authority in 2004. Mol. Nutr. Food Res. 50: 401-405.

Etienne M., Dourmad J.Y., (1994), Effects of zearalenone or glucosinolates in the diet on reproduction in sows: A review, Livestock Production Sci., 40, 99-113.

European Union. 2005. Commission regulation 856/2005 amending Regulation (EC) No 466/2001 as regards Fusarium toxins. Off. J. E. U. 143: 3-8.

Fandohan, P., B. Gnonionfin, K. Hell, W.F. Marasas, M.J. Wingfield. 2005. Natural occurrence of Fusarium and subsequent fumonisin contamination in preharvest and stored maize in Benin. Int. J. Food Microbiol. 99, 173-183.

F.A.O., worldwide regulations for mycotoxins in food and feed in 2003. FAO food and nutrition papers, Rome, Italy, 81, 2004.

Gareis, M., R. Schothorst, A. Vidnes, C. Bergsten, and B. Paulsen. 2003. Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states. Report of SCOOP task 3.2.10. Available at http://ec.europa.eu/food/fs/scoop/task3210.pdf

Giorni, P., N. Magan, A. Pietri, T. Bertuzzi, and P. Battilani. 2007. Studies on Aspergillus section flavi isolated from maize in northern Italy. Int. J. Food Microbiol. 113: 330-338.

Hajjaji, A., M. El Otmani, D. Bouya, A. Bouseta, F. Mathieu, S. Collin, and A. Lebrihi. 2006. Occurrence of mycotoxins (ochratoxin A, deoxynivalenol) and toxigenic fungi in Moroccan wheat grains: impact of ecological factors on the growth and ochratoxin A production. Mol. Nutr. Food Res. 50: 494-499.

Hamilton R.M., Trenholm H.R., Thompson B.K., Greenhalgh R., 1985. The tollerance of white Leghorn and broiler chicks and turkey poults to diets contained deoxynivalenol (vomitoxin) contaminated wheat, Poult. Sci., 64 (2), 273 - 286

Harvey RB, Kubena LF, Corrier DE, Witzel DA, Phillips TD, Heidelbaugh ND., 1986. Effects of deoxynivalenol in a wheat ration fed to growing lambs, Am J Vet Res., 47 (7), 1630-1632

Harvey RB, Kubena LF, Huff WE, Corrier DE, Clark DE, Phillips TD., 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs, Am J Vet Res., 50 (4), 602-607

Huff W.E., Doerr J.A., Hamilton P.B., Vesonder R.F., 1981. Acute toxicity of vomitoxin (deoxynivalenol) in broiler chickens, Poult. Sci., 60 (7), 1412 - 1414.

Huff WE, Kubena LF, Harvey RB, Hagler WM Jr, Swanson SP, Phillips TD, Creger CR., 1986. Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens, Poult Sci., 65 (7), 1291-1298

Isebaert, S., G. Haesaert, R. Devreese, P. Maene, F. Fremaut, and G. Vlaemynck. 2005. Fusarium spp and Fusarium mycotoxins in maize: a problem for Flanders? Commun. Agric. Appl. Biol. Sci. 70: 129-136.

Kaliamurthy J., Geraldine P., Thoams P.A., (1997), Effect of zeralenone on food consumption, growth rate, organ weight and serum testosterone level in male rats, J.Environ. Biol., 18, 115-120.

Krysinska-Traczyk, E., J. Perkowski, and J. Dutkiewicz. 2007. Levels of fungi and mycotoxins in the samples of grain and grain dust collected from five various cereal crops in eastern Poland, Ann. Agric. Environ. Med. 14: 159-167.

Kubena L.F., Swanson S.P., Harvey R.B., Fletcher O.J., Rowe L.D., Phillips T. D., 1985. Effects of feeding deoxynivalenol (vomitoxin)-contaminated wheat to growing chicks, Poult. Sci., 64, 1649-1655

Kubena LF, Huff WE, Harvey RB, Phillips TD, Rottinghaus GE., 1989. Individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks, Poult Sci., 68 (5), 622-626

Leong, S.L., L.T. Hien, T.V. An, N.T. Trang, A.D. Hocking, and E.S. Scott. 2007. Ochratoxin A producing Aspergilli in Vietnamese green coffee bean. Lett. Appl. Microbiol. 45: 301-306.

Luo Y., Yoshizawa T., Katayama T., 1990, Comparative study on the natural occurrence of Fusarium mycotoxins (trichothecenes and zearalenone) in corn and wheat from high- and low-risk areas for human esophageal cancer in China, Appl. Environ. Microbiol., 56 (12), 3723-3726

Magnoli, C.E., A.L. Astoreca, S.M. Chiacchiera, and A.M. Dalcero. 2007. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. Mycopathologia, 163: 249-260.

Mankeviciene, A., B. Butkute, Z. Dabkevicius, and S. Suproniene. 2007. Fusarium mycotoxins in lithanian cereals from the 2004-2005 harvests. Ann. Agric. Environ. Med. 14: 103-107.

Marquardt R.R., Frohlich A.A., (1992), A review of recent advances in understanding ochratoxicosis, J. Anim. Sci., 70, 3968-3988

Martins, H.M., M.M. Mendes Guerra, and F.M. d'Almeida Bernardo. 2007. Occurrence of Aflatoxin B1 in dairy cow feeds over 10 years in Portugal (1995-2004). Rev. Iberom. Micol. 24: 69-71.

Mateo, E.M., Gil-Serna, J., Patiño,. B, Jiménez, M. 2011. Aflatoxins and ochratoxin A in stored barley grain in Spain and impact of PCR-based strategies to assess the occurrence of aflatoxigenic and ochratoxigenic Aspergillus spp. Int. J. Food Microbiol. 149(2):118-126

Medina, A., F.M. Valle-Algarra, R. Mateo, J.V. Gimeno-Adelntado, F. Mateo, and M. Jimenez. 2006. Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of Alternaria, Aspergillus and Fusarium. Int. J. Food Microbiol. 108: 196-203.

Milićević, D. R., M. Škrinjar and T. Baltić. 2010. Real and Perceived Risks for Mycotoxin Contamination in Foods and Feeds: Challenges for Food Safety Control

Miraglia, M., and C. Brera. 2002. Assessment of dietary intake of ochratoxin A by the population of EU member states. Report of SCOOP task 3.2.7. Available at ec.europa.eu/food/fs/scoop/3.2.7_en.pdf

Moreno Romo, M.A., and G. Suarez Fernandez. 1986. Aflatoxin producing potential of Aspergillus flavus strains isolated from Spanish poultry feeds. Mycopathologia 95: 129-132.

Morris C.M., Li Y.C., Ledoux R.D., Bermudez A.G., Rottinghaus G.E., 1999. The individual and combineted effects of feeding moniliformin supplied by Fusarium fujikuroi culture material and deoxynivalenol in young turkey poults, Poult. Sci., 78 (8), 1110 – 1115

Murphy, P.A., Hendrich, S., Hopmans, E.C., Hauck, C C, Lu, Z., Buseman, G. and Munkvold, G. 1996. Effect of processing on fumonisin content of corn. Adv. Exp. Med. Biol., 392, 323-334

Nelson P.E., Toussoun T.A. and Marassas, W.F.O. 1983. Fusarium species: an illustrated manual for identification. Penn. State Univ. Press., PA, USA.

Ono, E.Y., Y. Sugiura, M. Homechin, M. Kamogae, E. Vizzoni, Y. Ueno, and E.Y. Hirooka. 1999. Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the state of Parama, Brazil. Mycopathologia 147 : 139-148.

Osweiler G.D., Ross P.F., Wilson T.M., Nelson P.E., Witte S.T., Carson T.L., Rice L.G., Nelson H.A., 1992, Characterization cf an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings, J. Vet. Diagn. Invest., 4, 53-59

Pardo, E., S. Marin, A.J. Ramos, and V. Sanchis. 2006. Ecophysiology of ochratoxigenic Aspergillus ochraceus and Penicillium verrucosum isolates.

Predictive models for fungal spoilage prevention: a review. Food Addit. Contam. 23: 398-410.

Pietri, A., T. Bertuzzi, L. Pallaroni, and G. Piva. 2004. Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in northern Italy. Food Addit. Contam. 21: 479-487.

Pitt, J.L. 1988. Laboratory guide to common Penicillium species. Academic Press, London,

D'Mello, J.P.F., Placinta, C.M. and Mac Donald, A.M.C. 1999. Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity, Anim. Feed Sci. Technol., 80, 183-205.

Pohland A.E., Nesheim S., Friedman L., 1992, Ochratoxin A, a review, Pure, Appl. Chem., 64, 1029-1046

Pollmann D.S., Koch B.A., Seitz L.M., Mohr H.E., Kennedy G.A., 1985. Deoxynivalenol contaminated wheat in swine diets, J.Anim. Sci., 60 (1), 239-247

Rabie, C.J., A. Lübben, G.J. Marais, and H. Jansen van Vuuren. 1997. Enumeration of fungi in barley. Int. J. Food Microbiol. 35: 117-127.

Rapper, K.B., and D.I. Fennel. 1965. The genus Aspergillus, Williams & Wilkins, Baltimore, Maryland.

Rainey M.R., Tubbs R.C., Hardin D.K., Cox N.M., 1991, Clinical manifestations of prepubertal exposure to zearalenone In: gilts, Agri-practice, 12, 35-41.

Ross P.F., Rice L.G., Osweiler G.D., Nelson P.E., Richard J.L., Wilson T.M., 1992, A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by Fusarium isolates, Mycopathologia, 117, 109-114.

Shollenberger, M., H.M. Muller, M. Rufle, S. Suchy, S. Plank, and W. Drochner. 2006. Natural occurrence of 16 Fusarium toxins in grains and feedstuffs of plant origin from Germany. Mycopathologia 161: 43-52.

Schothorst RC, van Egmond HP. 2004. Report from SCOOP task 3.2.10 "Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states". Subtask: trichothecenes. Toxicol Lett. 2004; 153:133–43.

Swamy H.V.L.N., Smith T K., Cotter P.F., Boermans H.J., Sefton A.E., 2002. Efffects of feeding blends, of grains naturally contaminated with Fusarium mycotoxins on production and metabolism in broilers, Poult. Sci., 81 (7), 966 - 975

Tabuc C., Marin D., Guerre P., Sesan T., Bailly J.D., 2009, Molds and mycotoxin content of cereals in southeastern Romania, J. Food. Prot., 72, 662-665

Taranu I., D. E. Marin, S. Bouhet and I. P. Oswald. 2008. Effect of fumonisin on the pig. Mycotoxins in Farm Animals, Transworld Research Network publisher, ISBN: 978-81-7895-312-0, Trivandrum-695 023, KERALA.

Trenholm H.L., Hamilton R.M.G., Friend D.W., Thompson B.K., Hartin K.E., 1984. Feeding trials with vomitoxin (deoxynivalenol)-contaminated wheat: effects on swine, poultry and dairy cattle, J. Am. Vet. Med. Ass., 185 (5), 527-531

Vainio H., Magee P., McGregor D., McMichael A., 1992, Mechanisms of Carcinogens in Risk Identification, IARC Scientific Publications No 116, IARC, Lyon.

Vrabcheva T., Usleber E., Dietrich R., Märtlbauer E., 2000, Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy, J Agric Food Chem., 48 (6), 2483-2488

Vrabcheva T., Petkova-Bocharova T., Grosso F., Nikolov I., Chernozemsky I.N., Castegnaro M;, Dragacci S., 2004, Analysis of ochratoxin A in foods consumed by inhabitants from an area with Balkan endemic nephropathy: a 1 month follow-up study, J Agric Food Chem., 52 (8), 2404-2410

WHO-IPCS. 2000, Environ. Health Criteria 219. WHO, (Eds), Geneva, 219.

Yoshizawa T., Yamashita A., Luo Y., 1994, Fumonisin occurnece in corn from high and low areas from esophageal cancer in China, Applied Envriron. Microbiol., 60, 1626-1629.