Occurrence of Zearalenone and Ochratoxin A in Wheat and Barley in Some Regions in Bulgaria

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Abstract

Feed grains are prone to fungal infections during growth, harvesting, and storage. Corns such as wheat and barley may be contaminated with mycotoxins leading to acute and chronic animal health exposure. The objective of this study was to obtain an information on the levels of ochratoxin A (OTA) and zearalenone (ZEA) in wheat (n=26) and barley (n=21) samples intended for feeding to farm animals. The samples were collected from different regions of Bulgaria in 2017 harvest and analyzed by using Enzyme-linked immunosorbent assay (ELISA). The results showed that 35% of wheat samples were contaminated with ZEA in the range of 51.65 μg kg^{-1}–126.54 μg kg^{-1} and in 29% barley samples the contamination was between 48.67–79.64 μg kg^{-1}. OTA pollution was registered in 19% of wheat samples in the range 4.86–7.32 μg kg^{-1} and in 29% barley samples it was between 4.46–11.70 μg kg^{-1} respectively. In all of the investigated samples the contamination with OTA and ZEA is bellow the limit set in the valid EC Recommendation 576/2006/EC.

Key words: barley, ELISA, mycotoxins, ochratoxin A, wheat, zearalenone,

Introduction

Mycotoxins are naturally secondary toxic metabolites produced by fungi such as *Aspergillus, Fusarium* and *Penicillium*. The most studied mycotoxins are aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, and fumonisin, due to their toxic nature and adverse effects on food quality and safety (Binder et al., 2007).

The favourable season for growth of fungus on feed ingredients are rainy season when optimum ambient temperature and humidity is available (Rai et al., 2011). From another side many foods and feeds can become contaminated with mycotoxins since they can form in commodities before harvest, during the time between harvesting and drying, and in storage. Due to their toxic effects, mycotoxins are considered as risky to the animals, and humans of contaminated feeds and foods (Omede, 2008). Adverse effects on animal health have been recognized in farmed animals such as poultry, swine, and cattle as consequence of the consumption of high levels of cereals (Charoenpornsook and Kavisarasai, 2006). Mycotoxins have been detected in various feed materials and are as considered as one of the most dangerous contaminants of animal feed. Commodities and products which are susceptible to mycotoxins contamination include corn, wheat, barley, rice, oats, nuts, milk, cheese, peanuts, and cottonseed.

Fusarium fungi are common contaminants of Bulgarian crop plants. They are the major fungus in wheat, and infection of wheat by this fungus usually occurs when there is cold, wet weather before harvest (Vrabcheva et al., 2002).

Several studies carried out in the wide range of countries, reported the high incidence of ZEA in cereals, and in animal feeding stuffs.

ZEA is a mycotoxin produced by the fungus's secondary metabolism, with estrogenic activity in mammals (Neish and Cohen, 1981). ZEA can caused cell deregulation and as well as may be attributed to an initial lesion in the cytosolic estrogen receptor which causes hormone control damage (Smith and Moss, 1985).

ZEA leads to a number of diseases in animals resulting in considerable losses of production, and a high rate of mortality (Valcheva and Valchev, 2007).

Typically for this mycotoxin is that it occurs during the growing period of seeds. It is called ''field mycotoxin''. It proliferates in mature grains, which were not sufficiently dried, owing to humidity, during harvest or storing period (Riley, 1998).

It exists in many cereal crops such as wheat, barley, oats, and sesame seeds (El-Desouky & Naguib, 2013).

OTA was isolated for first time from a commercial corn sample in 1969 (Shotwell et al., 1969). Only OTA, and rarely ochratoxin B, have been found to occur naturally in food and feed. OTA is produced by the next genera; Aspergillus ochraceus, Aspergillus niger and Aspergillus carbonarius, Penicillium verrucosum, and species of Penicillium, Petromyces, and Neopetromyces (Larsen et al., 2001).

Ochratoxigenic fungi originate in the soil or on decaying plant material and therefore are present in the field and on kernels prior to harvest, during which time OTA may be produced (Miller, 1995). However, OTA contamination is more often a result of grain harvested at high water content, improperly drying grain prior to storage, or storage under humid conditions (Kuruc et al., 2015).

Concerning this mycotoxin the cereal grains are considered as the main human dietary source of it.

Subsequently, OTA has been detected worldwide in animal feed and a variety of commodities and foods, including oats, wheat, rye, barley, corn, fruits, coffee, spices, fruit juice, wine, beer, poultry and milk (Abarca et al, 2001). Although, it has been suggested (Cicoňová et al., 2010) that pork products may also be a significant source of this toxin.

Humans are exposed to OTA either directly by eating foods contaminated with OTA or indirectly by consuming meat or milk from animals fed with OTA-tainted feed (Edwards et al., 2002). Toxicological studies have found OTA to be an immunosuppressant, with nephrotoxic, embryotoxic, teratogenic, neurotoxic, and genotoxic effects, and it has been classified as a 2B carcinogen by the International Agency for Research on Cancer (Khoury and Atoui, 2010).

Over the last years, the importance and application of immunoassays, especially ELISA has grown significantly. ELISA test kits became very popular due to their relatively low cost form and easy application. From anotehr side their results could be comparable with those obtained by other conventional methods (Feizy, 2014).

The study aims was to survey the occurrence of ZEA and OTA in wheat and barley obtained from different regions of Bulgaria by ELISA method.

Material and Methods

Wheat (26 samples) and barley (21 samples) were supplied by different regions of Bulgaria and namely Northeastern, Northwest, Northern central, Southeastearn, Southwestearn and South central. For the investigations the samples were prepared according to the instructions of the kit manufacturer R-Biopharm, Germany. Samples were grounded and 5 g of ground samples were mixed and processed using 70% methanol (Valerus, Bulgaria) as a solvent for extraction. Extraction was performed by shaking (3 minutes) and following dilution. 50 μL of the diluted solution was used for the analysis. The filtered samples were screened for ZEA and OTA by ELISA method. All reagents including standard solutions, conjugate solution, antibody, substrate/chromogen and stop solution must be bringing at room temperature before use. Microtitre plate with 48 wells was used. Wells are coated

with antibodies. Using the optical densities (OD) of the standard, the calibration curve is plotted against the concentrations of other standards, and the amount of mycotoxin in the sample is extrapolated from standard curve. The measurement was made photometrically at 450 nm. The absorbance was inversely proportional to the mycotoxin concentration in the sample. The values calculated for the standards were entered the Ridawin program, Computer Systems (ELx800 Universal Microplate Reader, BIOTEK® Instruments, Inc., USA).

Results and discussion

The total number of 47 samples of wheat and barley collected from different regions of Bulgaria were investigated for ZEA and OTA. Typically these areas are that they are characterized by different climatic conditions during growth, ripening and harvesting. The infections of grains usually occur when there is cold, wet weather before harvest. Such climatic conditions have been prevailing in the spring, and summer months of 2017, when heavy rainfall has been observed in all regions of the country. In the investigated wheat samples (35%) the quantity of zearalenone was between 51.65–126.54 μg kg^{-1} and in barley (29%) it was in the range 48.67–79.64 μg kg^{-1}.

Twenty-one samples of barley were investigated for ZEA. The results show that 19% (4 samples) were contaminated with ZEA at concentration in the range 40–70 μg kg^{-1}. Two samples had a concentration of ZEA in the range 70–100 μg kg^{-1}, and the greatest number of 71% (15 samples) was not contaminated. The determined by us results are lesser than the limit of ZEA referred in EC Recommendation 576/2006. Similar to our results are these of El-Desouky and Naguib (2013) where they have found ZEA mycotoxin in 4 out of the 15 analyzed samples of barley. Moreover, in all of other samples the results were within the permissible limit as recommended by the European Union. From another side Martins and Martins (2002) reported that the production of ZEA depends on the environmental conditions. Therefore, taking into account the similar climatic conditions in the neighboring Turkey the results of Bilal et al. (2014) are closed to ours. They have shown that feedstuffs and feeds were contaminated with varied levels of ZEA and these levels lesser than the tolerable limit. They considered that mycotoxin contamination and concentrations in feedstuffs and feeds can be vary according to regional climate, annual rainfall regime, harvesting methods, storing conditions etc.

The situation with detection of ZEA in wheat was a little different. The investigations were performed with 26 samples. The lowest detected concentration was 51.65 μg kg^{-1}, and the highest one was 126.54 μg kg^{-1}. It should be mentioned that this value is much lower to the recommended by EU limit. For another 5 samples in our study the concentration was varied between 40–70 μg kg^{-1} and 3 samples were contaminated with values upper 70 μg kg^{-1} but bellow 100 μg kg^{-1}. The remaining 17 wheat samples (65%) were not contaminated with ZEA. Concerning the results got by Manova and Mladenova (2009), who analyzed by high-performance liquid chromatography (HPLC) 54 wheat grain samples produced in Bulgaria, they have detected only one sample contaminated with ZEA. These results can be attributed to the fact that contamination of crops with ZEA largely depends on environmental conditions (Martins and Martins, 2002). For ex-

Table 1. Number of samples wheat and barley contaminated with ZEA

Crop	Zearalenone			
	40–70 μg kg^{-1}	70–100 μg kg^{-1}	≥ 100 μg kg^{-1}	Negative samples
Barley	4	2	N/A*	15
Wheat	5	3	1	17

**N/A – not applicable*

ample, the intense rains favoring fungal growth and mycotoxin production. In Table 2 are summarized the results connected with ZEA contamination obtained for the both feed grains.

The next mycotoxin for which wheat and barley samples were investigated was OTA. Concerning wheat samples analyzed for OTA in our study we found that (3 samples) 11.5% of 26 samples were contaminated with OTA more than 5 μg kg^{-1}. Two of wheat samples were contaminated with concentration lower than this value, and the remaining samples (81%) were not contaminated. Our results are little similar to these done by Elaridi et al. (2019). They have investigated wheat grains and wheat flour and found that wheat grains were not detected with OTA and only one wheat flour sample was contaminated with OTA level greater than the limit set by the European Commission. From another side, the results determined by us were lesser than those one determined by Zinedine et al. (2006) who reported a high percentage (38%) of contamination in wheat commercialized in Tunisia and Morocco, respectively. Unlike our results, Zebiri et al. (2018) also reported very high concentration of OTA contamination of Algerian wheat grains. They show that wheat grains were highly contaminated by OTA (69.23% and 92.85%, respectively), where 50% of positive samples exceeded the EU maximum limit for OTA. This is likely due to the climate conditions (high temperature and humidity), and inappropriate storage conditions, and the absence of the Good Manufacturing Practices during processing which promotes the development of storage fungi like *Aspergillus* species. It has to be mention that due to climatic conditions, *Aspergillus* species seem to be OTA producers. In addition, improper storage conditions can also lead to higher cereals contamination by mycotoxins (Petzinger and Weindenbach, 2002). The occurrence of OTA in cereal grains depends on the conditions of the grain at harvest, how carefully the grain is dried on the storage conditions (Eskola, 2002). Moreover, the corns in the mediterranean countries are often contaminated with OTA (Jedidi et al., 2016).

Table 2. Number of wheat and barley samples contaminated with OTA

Crop	Ochratoxin A	
	≤ 250 μg kg^{-1}	Negative samples
Barley	6	15
Wheat	5	21

Another set of twenty-one barley samples was also tested for availability of OTA. It should be mentioned that in all of the investigated samples, the measured concentrations were below those in the available EC Recommendation. Among 21 of tested samples 6 samples were contaminated with OTA and and 71% (15 samples) were negative.

Our values are lower than these one by Gumus et al. (2004). They reported that the OTA content in 26 of the 29 barley samples was between 0.53–12 μg kg^{-1}. The OTA content was determined to be below 3 μg kg^{-1} in 15% of the barley samples, 3–5 μg kg^{-1} in 31% and above 5 μg kg^{-1} in 54% of the barley samples. The OTA levels in 54% of positive barley samples was more than the permitted limit under Turkish food regulations (i.e., 5 μg kg^{-1} in unprocessed cereals). High-levels of OTA in barley samples can indicate that storage and/or processing conditions were not suitable. OTA formation can be prevented by storing the cereal under suitable conditions, aeration of storage, and keeping the moisture content of the barley below 13–14%. In addition, air conditions during the harvesting of barley, and the moisture in the air, can affect OTA formation.

Because the samples were delivered from the six regions of the country it has to be mention that for the analyzed wheat samples the highest contamination with ZEA and OTA was observed in the Northeastern region, ZEA was predominant in Southeastern and South central parts of Bulgaria while the OTA contamination was absent in Southeastern part and in South Central region it was around 10%. It is noteworthy that in the analyzed samples of wheat produced in the North Central, South western and North western regions no ZEA and OTA was found. In Figure 1 and Figure 2 can be seen the distribution of ZEA and OTA contaminated wheat and barley samples by these regions.

As shown in Figure 2, the content of OTA for barley produced in Northeastern region increased by factor of two than this one for ZEA.

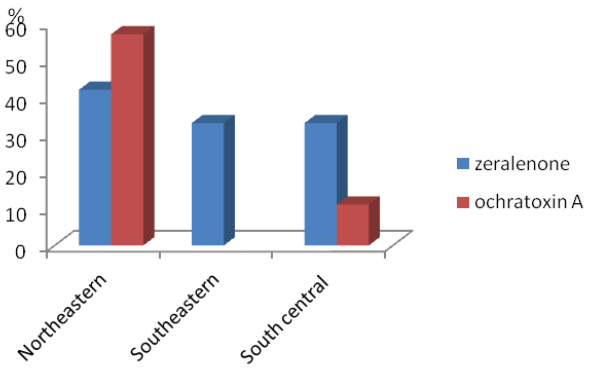

Fig. 1. ZEA and OTA occurrence in wheat and its distribution by regions during 2017

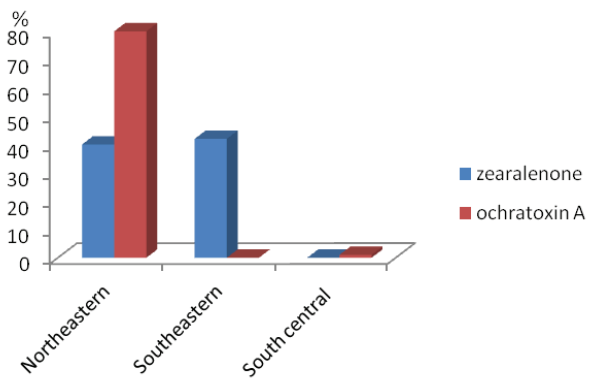

Fig. 2. ZEA and OTA occurrence in barley and its distribution by regions during 2017

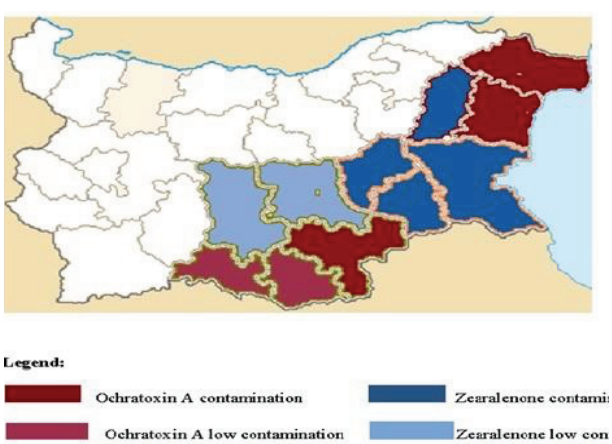

Fig. 3. Map depicting the location of ZEA and OTA contamination of wheat and barley grains in Bulgaria during 2017 year

The situation with wheat contamination in this region is almost the same. In Southeastern region again the ZEA contamination was predominant and in the South Central part the contamination of barley by the both mycotoxins is negligible. On Figure 3 it is interesting to see the local pollution on the territory of the country for the both examined crops.

Conclusion

The data obtained in this study showed that the feed grains were contaminated with different levels of ZEA and OTA, and that these levels are under tolerable limits set by EU regulation. The contamination of wheat and barley with the both mycotoxins was detected in Northeastern region. In Southeastern region, we have detected only ZEA contamination for the both kind of feed grains, and in South central part the contamination for barley samples was very scarce. In other parts of the country we did not register any pollution by the investigated mycotoxins. In addition, it has to be considered that mycotoxins contamination can be varying according to regional climate, harvesting, storing conditions, etc. Moreover, the monitoring programs have to be routinely implemented to ensure minimal contamination with mycotoxins of feed materials and this lead to better and safer feed and food.

References

Abarca, M. L., Accensi, F., Bragulat, M. R., & Cabañes, F. J. (2001). Current Importance of Ochratoxin A–Producing Aspergillus spp. *Journal of food protection*, *64*(6), 903-906.

Bilal, T., Aksakal, D. H., Sünnetci, S., Keser, O., & Eseceli, H. (2014). Detection of aflatoxin, zearalenone and deoxynivalenol in some feed and feedstuffs in Turkey. *Pakistan Veterinary Journal*, 34, 459-463.

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J., & Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal feed science and technology*, *137*(3-4), 265-282.

Charoenpornsook, K., & Kavisarasai, P. (2006). Mycotoxins in animal feedstuffs of Thailand. *Current Applied Science and Technology*, *6*(1), 25-28.

Cicoňová, P., Laciakova, A., & Mate, D. (2010). Prevention of ochratoxin A contamination of foodand ochratoxin A detoxification by microorganisms-a review. *Czech Journal of Food Sciences*, *28*(6), 465-474.

El-Desouky, T. A., & Naguib, K. (2013). Occurrence of zearalenone contamination in some cereals in Egypt. *Journal of Agroalimentary Processes and Technologies*, 19, 445-450.

Elaridi, J., Yamani, O., Al Matari, A., Dakroub, S., & Attieh, Z. (2019). Determination of ochratoxin A (OTA), ochratoxin B (OTB), T-2, and HT-2 toxins in wheat grains, wheat flour, and bread in Lebanon by LC-MS/MS. *Toxins*, *11*(8), 471.

Eskola, M. (2002). Study on trichothecenes, zearalenone and ochratoxin A in Finnish cereals: occurrence and analytical techniques. PhD Thesis. National Veterinary and Food Research Institute (EELA) and University of Helsinki.

Edwards, S. G., O'callaghan, J., & Dobson, A. D. (2002). PCR-based detection and quantification of mycotoxigenic fungi. *Mycological Research*, *106*(9), 1005-1025.

Feizy, J., Beheshti, H. R., Eftekhari, Z., & Zhiany, M. (2018). Survey of mycotoxins in wheat from Iran by HPLC using immunoaffinity column cleanup. *Journal of Chemical Health Risks*, *4*(1).

Gumus, T., Arici, M., & Demirci, M. (2004). A survey of barley, malt and beer contamination with ochratoxin A in Turkey. *Journal of the Institute of Brewing*, *110*(2), 146-149.

Jedidi, I., Cruz, A., González-Jaén, M. T., & Said, S. (2017). Aflatoxins and ochratoxin A and their Aspergillus causal species in Tunisian cereals. *Food Additives & Contaminants: Part B*, *10*(1), 51-58.

El Khoury, A., & Atoui, A. (2010). Ochratoxin A: general overview and actual molecular status. *Toxins*, *2*(4), 461-493.

Kuruc, J. A., Schwarz, P., & Wolf-Hall, C. (2015). Ochratoxin A in stored US barley and wheat. *Journal of food protection*, *78*(3), 597-601.

Larsen, T. O., Svendsen, A., & Smedsgaard, J. (2001). Biochemical characterization of ochratoxin A-producing strains of the genus Penicillium. *Applied and Environmental Microbiology*, *67*(8), 3630-3635.

Manova, R., & Mladenova, R. (2009). Incidence of zearalenone and fumonisins in Bulgarian cereal production. *Food control*, *20*(4), 362-365.

Martins, M. L., & Martins, H. M. (2002). Influence of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (Zea mays) by Fusarium graminearum. *Food Chemistry*, *79*(3), 315-318.

Miller, J. D. (1995). Fungi and mycotoxins in grain: implications for stored product research. *Journal of stored products research*, *31*(1), 1-16.

Neish, G. A., & Cohen, H. (1981). Vomitoxin and zearalenone production by Fusarium graminearum from winter wheat and barley in Ontario. *Canadian Journal of Plant Science*, *61*(4), 811-815.

Omede, A. A. (2008, June). Critical issues in poultry feed quality evaluation in Nigeria. In *Book of Abstracts and Congress Proceedings, XXIII World's Poultry Congress* (Vol. 64, No. supplement 2, p. 455).

Petzinger, E., & Weidenbach, A. (2002). Mycotoxins in the food chain: the role of ochratoxins. *Livestock production science*, *76*(3), 245-250.

Rai, R. B., Rahman, S., Dixit, H., Rai, S., Singh, B., Kumar, H., ... & Dhama, K. (2011). Analysis of feed ingredients for Afla and T-2 mycotoxins by ELISA in rural areas of Uttar Pradesh. *Ind J Vet Pathol*, *35*, 238-240.

Riley, R. T. (1998). Mechanistic interactions of mycotoxins: theoretical considerations. In: *Mycotoxins in Agriculture and Food Safety. Marcel Dekker, Inc., New York*, 227-253.

Shotwell, O. L., Hesseltine, C. W., & Goulden, M. L. (1969). Ochratoxin A: occurrence as natural contaminant of a corn sample. *Applied microbiology*, *17*(5), 765-766.

Smith, J. E., & Moss, M. O. (1985). *Mycotoxins. Formation, analysis and significance*. John Wiley & Sons Ltd..

Valcheva, A., & Valchev, G. (2007). The fusariotoxins zearalenon and deoxinivalenol as natural contaminators of some basic cereal components in the production of combined feed. *Bulgarian Journal of Agricultural Science*, *13*(1), 99-104.

Vrabcheva, T., Stroka, J., & Anklam, E. (2002). Occurrence of fumonisin B 1 in Bulgarian maize samples determined by ELISA and TLC methods using different clean up steps. *Mycotoxin research*, *18*(2), 46-56.

Zebiri, S., Mokrane, S., Verheecke-Vaessen, C., Choque, E., Reghioui, H., Sabaou, N., ... & Riba, A. (2018). Occurrence of ochratoxin A in Algerian wheat and its milling derivatives. *Toxin Reviews*. 37, 1-6.

Zinedine, A., Brera, C., Elakhdari, S., Catano, C., Debegnach, F., Angelini, S., ... & Miraglia, M. (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food control*, *17*(11), 868-874.

EU Commission. (2006). Commission recommendation (EC) No. 576/2006 of 17 August 2006 on the presence of deoxynivelenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. *Official Journal of the European Union*, *576*.