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FUSARIOTOXINS CONTAMINATION IN CEREALS FOR FEED PRODUCTION IN THE PERIOD 2018–2019

Nadezhda Sertova, Maya Ignatova

Agricultural Academy, Institute of Animal Science, Kostinbrod, Bulgaria
E-mail: sertova@hotmail.com

ABSTRACT

The occurrence of zearalenone and deoxinivalenol in feed materials and its concentrations were presented in this survey for 2018–2019 crops harvest. A total number of 177 representative samples of wheat (n=70), barley (n=65) and maize (n=42) collected from differerent regions of Bulgaria were analyzed by Enzyme–Linked Immunosorbent Assay method. The highest zearalenone concentration of 168 µg/kg was detected in maize samples followed by barley of 156.78 µg/kg and 143 µg/kg in wheat samples for 2019 crop. The results obtained in this survey revealed that mycotoxins produced by Fusarium spp. are frequent contaminants of feed grains in Bulgaria. The highest concentration of deoxinivalenol detected was 2.86 mg/kg in maize, 2.29 mg/kg in barley, and 0.782 mg/kg in wheat samples, 2019 crop. The reported pollution levels are lower than those referenced in the European regulation. Despite the low levels, the results have shown the need for more comprehensive research on these two mycotoxins in feed materials.

Key words: mycotoxins, wheat, barley, maize, ELISA.

Introduction

Mycotoxins are secondary metabolites produced by a wide variety of fungal species during their growth in the field and in storage. In highly contaminated feed, they cause intoxication, but their cumulative, immunosuppressive and immunotoxic effects proved to be more important (Dospatliev and Palagacheva, 2009; Surai et al., 2010).

The widespread mycotoxins produced by *Fusarium* spp. are fumonisins, zearalenone (ZEA) and trichothecenes (deoxynivalenol, nivalenol and T–2 toxin). Suitable substrates for their accumulation are crops such as maize, wheat, barley, rye, rice and oats (Goyarts et al., 2007).

ZEA is a mycotoxin which has the ability to show strong estrogenic activity (Zinedine *et al.*, 2007) and leads to a number of diseases in animals sometimes resulting in a high rate of mortality (Valcheva and Valchev, 2007). It can be produced during plant vegetation and during longer storage of grains if they are not treated properly **(**De Boevre *et al*., 2012; Zhang, 2016). This toxin was detected in feed and silage maize, as well as in food products (Sirot *et al*., 2013; Bai *et al.*, 2018).

Another mycotoxin produced by mold fungi belonging to the *Fusarium* family is deoxinivalenol (DON). It is trichothecene mycotoxin and is one of the most frequently found in cereal grains (Bando et al., 2007). DON is known as vomitoxin because it causes vomiting when the pigs consume contaminated feed. It causes adverse symptoms such as feed refusal, reduced weight, diarrhea, vomiting (Moazami and Jinao, 2009), and neurotoxic disturbances (Ndossi et al., 2012).

In Bulgaria the mycotoxins produced by *Fussarium* spp. are the major fungus in wheat and infection of wheat by this fungus usually occurs when there is cold, wet weather before harvest (Vrabcheva, 2004).

The aim of the present study was to evaluate the natural occurrence of mycotoxins from *Fussarium* spp. immediately after harvest and their distribution in cereals from different parts of Bulgaria in the period 2018–2019.

Materials and methods

177 representative corn samples freshly harvested from different regions of Bulgaria were analyzed. Wheat (70 samples), barley (65 samples) and maize (42) were ground and 5 g of ground samples were mixed and processed using 70% methanol as solvent for extraction. The filtered samples were screened for ZEA and DON by Enzyme–Linked Immunosorbent Assay (ELISA) method. For the investigations, the samples were prepared according to instructions by the kit manufacturer (R–Biopharm). The kits were validated by the manufacturer.

Before use, all reagents including standard solutions, conjugate solution, antibody, substrate/chromogen and stop solution were adjusted to room temperature. Microtitre plate with 48 wells was used. Wells were coated with antibodies.

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

Results and Discussion

The investigations carried out for the period 2018–2019 gave us the possibility to detect the mycotoxicological status of the evaluated cereals with respect to fusariotoxins ZEA and DON. In Table 1 contamination with zearalenone is shown, and its quantity does not vary widely.

Table 1: ZEA contamination of feed crops

Crop	Number of samples	Number of positive samples	% of positive samples	Range (min-max) µg/kg	Average (µg/kg) ±SD
Wheat					
2018	38	15	39	55,30–79,65	69±7,6
2019	32	9	28	50,92–143	126.88±28
Barley					
2018	35	12	34	57,14–82,00	68.27±9,31
2019	30	6	20	76,18–156,78	135±29
Maize					
2018	20	8	40	56–126,5	91.8±13.8
2019	22	5	23	72–168	133±37

The data showed the presence of ZEA in all samples for the study period. Similar percentages for positive samples show wheat 39% and maize 40% for 2018 harvest. Lower percent positive samples were shown in maize 23% and even lower in barley 20% for 2019 crop.

Highest concentrations of 168 µg/kg of ZEA in maize and 156.78 µg/kg in barley were proved for 2019 crop. The lowest concentrations in the range 55.30–79.65 were detected in wheat samples, followed by barley samples for 2018. Following the results related to the frequency of ZEA, it could be mentioned that it decreased significantly in 2019 harvest.

Maize, wheat and barley were the most favorable substrates for ZEA accumulation and namely the higher incidence was registered with maize samples followed by wheat and barley samples.

The quantity of ZEA for all studied cereals was lower for 2019 compared to 2018 crop. It could be connected with climatic conditions. The rainy summer might promote the formation of fusariotoxins. It is known, they are field mycotoxins and are formed during growth, maturation and harvesting of the grain.

On the other hand, the proven average concentrations of ZEA for 2018 crop under study were considerably lower than 2019, which was not in correlation with the established high percent of damage by moulds of genus Fusarium registered in our investigations.

It has to be mentioned that the registered values by us were below the reference values set out in EC Recommendation 576/2006.

The results found are in accordance with the previous ones obtained by Manova and Mladenova (2009) that in the incidence of ZEA in wheat, barley and maize in Bulgaria all obtained ZEA value were bellow those ones recommended in the valid EC Regulation 1881/2006. Nevertheless, the occurrence of ZEA on wheat and barley in both studies intended for feeding stuffs and for foodstuffs was below the set up maximum levels as referred in EC legislation.

Bilal et al. (2014) have also shown that feedstuffs and feeds available in Turkey were contaminated with varied levels of ZEA and that these levels were lesser than the tolerable limit.

On the other hand, our results are close to those reported by Galbenu et al, (2011) in neighboring Romania. In their investigations performed on wheat, barley and maize by ELISA method they found high occurrences of ZEA, where 100% in barley, 10% in wheat and 33% in maize but the values were lower than these mentioned in EC legislation. It should be considered that mycotoxins contamination and concentrations in feeds can vary according to regional climate, harvesting, storage conditions and etc.

The frequency of ZEA contamination in the samples evaluated by us was found mainly in the North part of Bulgaria, especially Northwestern Region where the contamination was higher compared to the South part, followed by North Central Region because of the weather conditions, mainly summer rainfall, which is typical weather for this part of the country. It should be mentioned that the samples of wheat and barley originating in the Northwestern region were contaminated with a high concentration of ZEA, followed by samples collected from the North Central Region (Fig. 1).

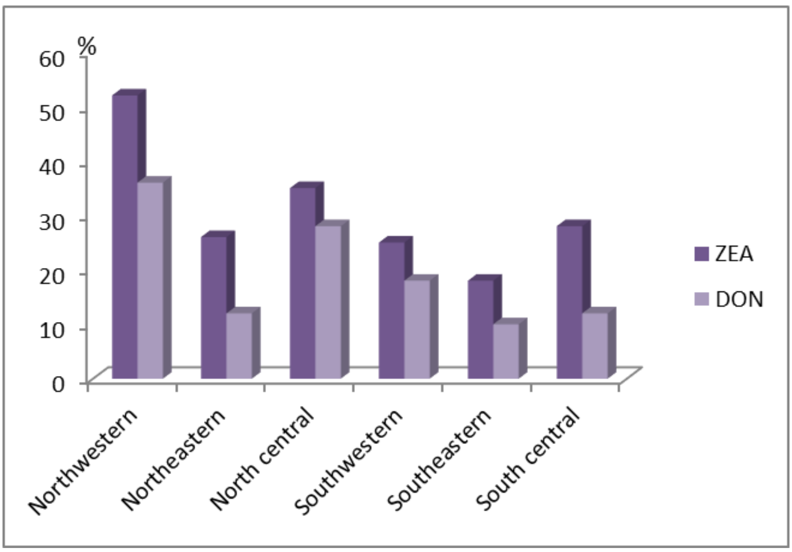

Figure 1: ZEA and DON occurrence in feed materials and its distribution by regions during 2018–2019

The situation with maize is slightly different, where samples originating in the south of the country show higher contamination than those in the north.

The ZEA is frequently found as a natural contaminant of cereals together with DON (Valcheva, 2000). The main producer of ZEA and DON is *Fusarium graminearum* (Molto et al., 1997).

This was the reason why samples analyzed for ZEA were examined for the presence of DON.

In Table 2 are shown the results of determination of DON contamination of the evaluated grains.

Table 2: DON contamination of feed crops

Crop	Number of samples	Number of positive samples	% of positive samples	Range (min-max) mg/kg	Average (mg/kg)
Wheat					
2018	38	8	21	0.452–0.782	0.673±0.11
2019	32	N/D	N/D	N/D	N/D
Barley					
2018	35	6	17	0.271–0.356	0.314±0.04
2019	30	3	10	0.544–2.29	1.56±0.91
Maize					
2018	20	7	35	0.307– 0.776	0.540±0.19
2019	22	5	23	0.287–2.86	2.05±1.02

Highest percent of positive samples for the studied period was found in maize, followed by wheat and barley samples. DON was not detected in wheat samples in 2019 crop. For all evaluated samples the high percent contamination was observed in 2018 harvest. The hot and humid environmental conditions might explain the high incidence levels of DON in the studied samples. The samples mostly originate from the North part of the country.

The high percent positive samples for maize did not correlate with average concentrations for 2018 crop. The highest concentration of 2.86 mg/kg in maize samples had been proven for 2019 crop followed by barley 2.29 mg/kg.

The lowest value of contamination was detected in barley samples in South part of the country and namely 0.271 mg/kg, followed by maize and wheat samples in 2018 crop. The presence of DON was lower compared to ZEA presence in the studied feed grains, Fig. 1. The most infected with DON were maize samples originating from Southwestern Region. It should be noted that the average concentrations with ZEA and DON for 2018 crop were lower than those in 2019, which was not in correlation with the established high percent.

In terms of averages, our results for wheat samples were close to those of Bryla et al. (2016) from Poland. He documented that 100% of the wheat samples were positive, with DON at average of 770.7 μg/kg.

On the other hand, our result at average value of 0.540 mg/kg determined in maize is closer to the value found by Iqbal et al. (2020). They detected the lowest average level of cornbread to be 0.619 mg/kg.

Martos et al. (2010) from Canada, investigated 15 samples of corn for the occurrence of DON, and 14 samples were found to be positive, at a mean level of 1513.5 μg/kg (ranged from 574 to 4865 μg/kg).

The change in mycotoxin levels in cereals may be due to the year of harvest (Kuiper–Goodman, 1999). However, the spread of fungi and the production of mycotoxins in cereals may occur as a result of both climatic and improper storage conditions (Iqbal et al., 2010).

Serbia is located in the moderate continental climate belt, where the most frequently isolated fungi contaminating cereals, feedstuffs, vegetables and fruits are from *Fusarium, Penicillium* and *Aspergillus* genera (Lević et al., 2004).

In this connection it is interesting to note that Igor Jalic et al. (2008) found that the concentration range of DON in maize was 0.042−2.460 mg/kg, at average value 0.536 mg/kg. This average value is very close to our one of 0.540 mg/kg found in 2018 maize crop.

The detected values of DON contamination were bellow those referred in EC Recommendation 576/2006.

Conclusion

The contamination of ZEA and DON as natural contaminants in wheat, barley, and maize samples was observed to be lower than the permissible allowed limits of the EU. It was found that ZEA prevailed in all representative samples while DON contamination was not observed in 2019 wheat samples. Samples of maize originating from North part of the country were more contaminated with ZEA compared to DON. High incedince of ZEA contamination was observed in the Northwestern part. The factors causing a high incidence of ZEA in cereals might be the use of varieties which were sensitive to fungal attack, old traditional farming practices and no–till farming. However, it is recommended to continuously monitor the seeds in order to protect them against the risk of mycotoxins contamination.

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