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Long-term monitoring of deoxynivalenol in Serbian maize: A 2010–2023 study

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A B S T R A C T

The trichothecenes are a diverse group of mycotoxins primarily produced by molds from the *Fusarium* genus, and can occur both in the field and in storage units. In this study, a total of 576 samples were processed over a 14-year period, from 2010 to 2023. The number of samples in which some concentration of deoxynivalenol (DON) was detected was 288. The average value during the observed period (2010–2023) ranged from 0.176 in 2020 to 2.420 mg/kg in 2014. The highest concentration was recorded in 2014 at 9.498 mg/kg, while other maximum values in different years reached up to 3.822 mg/kg. The occurrence of DON varies from year to year, primarily depending on climatic conditions. The number of positive samples ranged from 5% in 2018 to 100% in 2014. However, contamination of maize with Fusarium can happen not only in the field but also during storage. Therefore, continuous monitoring is necessary. Our findings suggest that the presence of DON may affect animal production; this impact may have been overlooked due to other pressing issues.

Keywords: contamination, fusarium toxins, mycotoxins, regulatory compliance

И З В О Д

Трихотецени су разнолика група микотоксина које првенствено производе плесни из рода *Fusarium*, а могу се јавити и на пољу и у складишним условима. У овој студији, укупно 576 узорака је обрађено током периода од 14 година, од 2010. до 2023. године. Број узорака у којима је детектована нека концентрација деоксиниваленола (ДОН) био је 288. Просечна вредност током посматраног периода (2010–2023) кретала се од 0,176 у 2020. до 2,42 mg/kg у 2014. години. Највећа концентрација је забележена 2014. године и износила је 9,498 mg/kg, док су остале максималне вредности у различитим годинама достизале и до 3,822 mg/kg. Појава DON-а варира из године у годину, првенствено у зависности од климатских услова. Број позитивних узорака кретао се од 5% у 2018. до 100% у 2014. години. Међутим, контаминација кукуруза фузаријумом може се догодити не само на пољу већ и током складиштења. Стога је неопходно континуирано праћење. Наши налази указују на то да присуство ДОН-а може утицати на сточарску производњу; овај утицај је можда био превиђен због других хитнијих проблема.

Кључне речи: контаминација усева, фусаријум токсини, микотоксини, законска регулатива

1. Introduction

The trichothecenes are a diverse group of mycotoxins primarily produced by molds from the Fusarium genus (Eriksen and Pettersson, 2004). Deoxynivalenol (DON) is a mycotoxin that is commonly found around the world (Eriksen and Pettersson 2004). The contamination of cereals with DON can occur both in the field and in storage units. DON is the least acutely toxic fusarium toxin (IARC, 1993); however, at the same time, it is an indicator of the occurrence of the other Fusarium toxins (Holanda and Kim, 2021). Its occurrence can be found in many cereals, but it is particularly significant in maize, as maize is the most commonly used energy source for animals. DON is in group 3 by the International Agency for Research on Cancer (IARC, 1993), which means unclassifiable as to carcinogenicity in humans; therefore, there is no evidence at present that it causes cancer in humans. While DON is not carcinogenic, its presence can lead to various issues in humans and animals. The DON impacts animal production by affecting the immune system, feed intake, and overall production parameters (Eriksen and Pettersson, 2004; Tiemann et al., 2008). Among the animal species used in animal production, it seems that pigs are the most susceptible to the effects of DON (Eriksen and Pettersson, 2004).

The agricultural land in Vojvodina covers an area of 1,983,154 ha, which is 38.29% of the total agricultural land in the Republic of Serbia (Novković et al., 2022). Vojvodina has the most intensive crop

production in Serbia due to its geographic and climatic conditions, characterized by very flat land and a branched river network. In recent years, maize has been harvested on approximately 900,000 hectares in Vojvodina, with average annual production ranging from 4.6 to 7.8 million metric tons, depending on climatic conditions and rainfall (USAD, 2023). The average need for domestic animal production is about 4.4 million MT, meaning a significant amount of maize is available for export. The Republic of Serbia is one of the top ten maize exporters in Europe (USAD, 2023).

Numerous countries have enacted legislation regarding DON in food and feed due to its economic impact on animal production and human health. In Serbia, the maximum levels of undesirable substances have been recently updated. The legal limit for maize intended for animal feed is 12 mg/kg, while the allowed concentration for the complete feed mixture intended for pig nutrition is 0.9 mg/kg (Sl. Glasnik RS, 4/2010, 113/2012, and 27/2014). As for human consumption, according to EU and Serbian regulations, the maximum residue limit (MRL) for DON in maize is 750 µg/kg (Sl. glasnik RS, 73/2024 and 90/2024). Polygastric animals are generally insensitive, while non-ruminants, particularly pigs, are very responsive to the presence of DON in their food. The legislation in Serbia concerning the permissible concentration of DON for human consumption is aligned with European Union regulations.

In 2012, a significant outbreak of aflatoxin B1 occurred in the Balkans (de Rijk et al., 2015), greatly impacting all aspects of agricultural production in the region (Jajić et al., 2019; Kos et al., 2018). As a result, the focus on mycotoxin research shifted predominantly to aflatoxin B1, while DON received relatively less attention. Maize is one of the primary crops, and pig breeding is the most common form of livestock production in Vojvodina. Compared to other livestock sectors in Serbia, it has successfully avoided a huge decline and maintains relatively strong numbers. When climatic conditions are favorable for the growth of molds from the genus Fusarium, maize is not the only grain that can contribute to the presence of DON in the diets of pigs and other animals (Jakšić et al., 2012). The presence of DON in larger quantities can significantly impact pig production. Despite a steady decline in recent years, Serbia still has approximately 3 million pigs each year (Statistical Yearbook of the Republic of Serbia, 2021). It is crucial to ensure the quality of grain, particularly maize, to maintain animal production and potentially enhance it.

When DON is present in animal production, limited options remain. Mycotoxin deactivators with varying effects can be utilized (Weaver et al., 2014; Gallo et al. 2020). Research on mycotoxin degradation shows varying degrees of success (Jajić et al., 2016; Krstović et al., 2021). However, all of that additionally requires time, an economic component, and ultimately increases production costs.

This study aimed to investigate the prevalence of DON in maize from 2010 to 2023, while keeping in mind its effects on animal production, most specifically pig production.

2. Materials and methods

2.1. Samples

Maize samples were randomly collected from Serbian producers in accordance with Commission Regulation (EC) No 401/2006. A total of 576 samples were obtained from various locations across Serbia after each harvest over 14 years (2010–2023). Approximately 300 g of each sample was prepared by grinding in a laboratory mill using a 0.5 mm sieve, followed by thorough homogenization. The homogenized samples were packed in plastic bags and stored at –20 °C until analysis. Before analysis, samples were brought to room temperature.

2.2. Analyses

DON determination was performed using a 1260 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) and a Hypersil ODS column (150 × 4.6 mm i.d., 5 µm particle size). A total of 12.5 g of homogenized sample was extracted with 50 ml of acetonitrile:water (84:16, v/v), and the resulting extracts were cleaned using Mycosep™ 225 multifunctional cleanup columns (Romer Labs Inc., Union, MO, USA). A 3 mL aliquot of the cleaned extract was evaporated to dryness at 60 °C under a gentle stream of nitrogen, and the residue was reconstituted in 300 µL of mobile phase. Chromatographic conditions for DON were applied according to the method described by Abramović et al. (2005). Quality control procedures included the use of blank samples, spiked samples, and control standards in each analytical run. The method was validated in-house using a maize certified reference material (BRM 3024, Romer Labs, Austria), with a certified DON concentration of 901 ± 110 µg/kg. The trueness of the method for DON was 92.3%. The limit of quantification (LOQ), established based on the signal-to-noise ratio and confirmed experimentally through spiking of blank samples, was 150 µg/kg. Linearity of the method was demonstrated with a regression coefficient (R^2) greater than 0.999.

3. Results and Discussion

The results of the occurrence of DON in 1167 samples during fourteen years of research are presented in Table 1. The occurrence of DON in maize varied significantly from year to year. The highest incidence of DON was in 2014. During this year, all samples were positive and contained some amount of DON. The average value during the observed period (2010–2023) ranged from 0.176 in 2020 to 2.420 in 2014. The highest concentration was recorded in 2014 at 9.498 mg/kg, while other maximum values in different years reached up to 3.822 mg/kg. From Table 1, it is clear that the number of positive samples varies from year to year, likely due to the climatic conditions during each year. The number of positive samples ranged from 5% in 2018 to 100% in 2014.

For DON to increase in prevalence within cereals, conditions must be favorable for the growth of fungi from the Fusarium genus. Its occurrence is more common in the temperate regions of Europe. However, the prevalence of DON can vary depending on climatic and agronomic conditions (EFSA, 2013). It is not

uncommon to observe a high incidence of DON depending on yearly climatic conditions in Serbia (Jajić et al., 2008; Jajić et al., 2014; Kos et al., 2017).

Table 1.
The Occurrence of DON in maize from 2010 to 2023 in the Republic of Serbia

Year	2023	2022	2021	2020	2019	2018	2017	2016	2015	2014	2013
N	18	41	44	9	34	56	5	66	163	74	15
N of positive samples	13	9	8	2	8	3	3	54	63	74	14
N of positive samples (%)	72	22	18	22	24	5	60	82	39	100	93
Average	0.59	1.03	1.06	0.17	0.79	0.37	0.54	0.59	0.567	2.42	0.44
SD	0.29	1.09	0.67	0.01	0.70	0.10	0.13	0.46	0.62	1.63	0.59
CV	0.48	1.06	0.63	0.08	0.89	0.27	0.24	0.77	1.13	0.67	1.33
Median	0.44	0.75	0.85	0.18	0.57	0.33	0.55	0.41	0.30	2.10	0.26
Min	0.27	0.28	0.34	0.17	0.19	0.30	0.40	0.09	0.06	0.37	0.12
Max	1.05	3.82	2.02	0.19	2.38	0.48	0.66	2.27	2.63	9.50	2.43

Our findings are consistent with those reported in previous studies. In the research conducted by Kos et al. 2017, the occurrence of DON in maize was highest in 2014, during the three years from 2013 to 2015. The concentration range in that year varied from 0.260 to 9.050 mg/kg, with an average of 3.063 ± 1.264 mg/kg.

Croatia, the neighboring country of Serbia, has similar climatic conditions and agricultural practices. A study by Kovač et al., 2022 revealed that Fusarium toxins are the most prevalent contaminants in cereals in Croatia. Among the cereals examined— maize, wheat, barley, rye and oats —deoxynivalenol (DON) was identified as the most frequently occurring mycotoxin. Furthermore, the highest level of contamination was observed in corn. There was a significant difference in the occurrence of DON in cereals between 2016 (72.5% of samples) and 2017 (32.6%). Authors of this study concluded that climatic conditions, humidity, and temperature significantly influence the colonization of mycotoxigenic fungi and the production of mycotoxins.

A study (Pleadin et al., 2017) conducted in Bosnia and Herzegovina (BiH) analyzed 257 samples of unprocessed cereals, including maize, wheat, and barley, over a three-year period from 2013 to 2015. The most commonly detected mycotoxin in these samples was DON, with the highest prevalence found in maize. Notably, 2014 recorded the highest incidence of DON in maize.

In 2014, it seems that the entire region experienced significant contamination of cereals with DON primarily due to climatic conditions. Maize was the most affected crop and exhibited high levels of contamination. Our findings support this observation and suggest that this contamination is likely to have a substantial impact on animal production.

4. Conclusions

The comparative analysis of wheat samples C, PU I, PU II and PU III revealed remarkable differences in morphological, chemical and techno-functional properties relevant for applications in the food industry. PU I had a high starch content, indicating good milling and flour performance. PU II exhibited favourable properties, including a balanced starch and protein content, strong gluten potential and good water and oil absorption, making it suitable for baked goods and dough-based products. PU III, with lower starch and higher lipid content, may be more suitable for formulations requiring improved mouthfeel and lipid binding capacity. Sample C, which served as a control, showed constant but moderate values for all parameters. The results underline the value of initial multiplied seeds not only in crop production but also in supporting specific functional requirements in cereal-based food systems. These results indicate that selected wheat varieties, in particular PU II and PU I, have promising properties for targeted food applications and could serve as valuable candidates for further breeding or industrial-scale use.

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