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Mycotoxins Co-contamination in Corn Grains

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Abstract

In this paper, we present the results of a research on 36 samples of maize. Mycotoxicological analyses showed that the most present mycotoxins were of the *Fusarium* mycotoxins group. During 2011, the maize samples were tested for the presence and content of total aflatoxins (AFB1, AFB2, AFM1, AFM2), deoxynivalenol (DON), zearalenone (ZON) and fumonisins (FUM). According to the results that we obtained through the research period i.e. vegetation year 2011, mycotoxicological profile for most maize samples was DON – FUM – ZON. DON mycotoxin was predominantly present in 2011. Precautionary measures are required especially with lower concentrations of mycotoxins when several mycotoxins are present simultaneously because this situation, according to conventional analytical methods, could be declared as quality safe or toxicologically negative finding.

Key words: mycotoxins, maize, mycotoxicological profile

Introduction

There is a lot of information published so far on the research of mycotoxins. However, due to their complexity, mycotoxins are still a subject of great interest of professional as well as of scientific community. There are many pitfalls and concerns about mycotoxins, and what makes them special and further complicates a perception and knowledge about mycotoxins, is the fact that one type of mold or species, can produce one or more mycotoxins, moreover one mycotoxin may be the product of few i.e. of a number of different mold species or genera.

It is not strange that mycotoxins have become a global problem. Starting from the first isolation of aflatoxin till today, a large number of toxins has been identified because endophytic molds, present on the plant, are able to synthesize toxic secondary metabolites of low molecular weight (Chu, 1992). High potential of toxigenic fungi to create mycotoxins in favorable agro-ecological conditions and the path of mycotoxins through the food chain with a cumulative effect, combined with the synergic relationships (Peraica et al., 1999), represent a major risk to the health of animals and humans. There is a reasonable assumption that in the body of an animal, that has been taking contaminated food, residues (mycotoxins and/or metabolites) could be found in various concentrations.

A chance for multiple mycotoxin contamination in food is relatively high. Numerous species of *Penicillium* (Pit, 2007) and *Fusaruium* (Bottalico, 1998) are known to be simultaneous producers of more than one mycotoxin.

However, it is believed that humans and animals are constantly exposed to simultaneous effects of several mycotoxins, mostly in low concentrations. Data on potential interactions occurring in a simultaneous introduction of several mycotoxins in the body of humans or animals are still incomplete (Milićević et al., 2014). The same authors quote that both humans and animals are constantly exposed to simultaneous effect of several mycotoxins, usually in low concentrations, and most probably with still unknown or unidentified mycotoxins.

In order to assess exposure to mycotoxins, it is necessary to apply reliable analytical methods, and take into account different levels of concentration in the samples, wherein errors are possible in every step. The problem begins with taking a sample, which is often not representative due to the dispersion of mycotoxins and thus it is a serious task (Lauren et al., 2006).

Distribution and concentration of mycotoxins and contamination of animal feed and raw materials vary, depending on climatic factors, harvest regimes and storage conditions. To create a mycotoxin profile, it is necessary to conduct a comprehensive and detailed research (with a greater number of samples) for the countries of different climate.

On the long term, it is difficult to forecast mycotoxins presense due to annual variations in prevalence of mycotoxins, but it is necessary to pay special attention to the simultaneous occurrence of various mycotoxins in the same sample (Knežević et al., 2014).

Materials and Methods

In this paper, we investigated the presence and concentration of the most important mycotoxins in maize samples intended for animal nutrition. The samples were collected successively in the autumn from October to December 2011. In the research, we examined a total of 36 samples of maize grains (*Zea mays*) intended for animal feed. The samples for mycotoxin testing were stored at -20 °C prior to testing for mycotoxins.

The research material for this work consisted of the samples of maize grains originating from 12 different locations, from feed mixers used in animal breeding farms in Banja Luka region. The collection of samples of maize grains consisted of 36 individual samples tested for the presence of aflatoxin (AF), deoxynivalenol (DON), zearalenone (ZON) and fumonisins (FUM) the maize samples being taken from 12 feed mixers in total, classified into two i.e. three groups.

To prove presence of mycotoxins we used instrumental methods that enable selective determination of mycotoxins, with a simultaneous low limits detection possibility i.e. possibility to determine very low concentrations of mycotoxins. For the purposes of this research and determination of mycotoxins a liquid chromatography (LC) method was used.

Results and Discussion

The average content of deoxynivalenol (DON) in maize samples was determined in 4 mixers of animal feed, and its content in maize was in average 2.37 ± 1.089 ppm, with a variation of 130.03% (Vk). The variation coefficient showed that the content of DON in maize samples considerably varied from one mixer to another.

Tab. 1. Contents of DON (ppm) in samples of maize from feed mixers of Group I
Садржај DON (ppm) у узорцима кукуруза из миксера сточне хране за групу I

Animal Feed Manufacturers *Произвођачи сточне хране*		$\bar{X} \pm s\bar{x}$	Vk
1.	Mixer II	0.24 ± 0.029	21.43
2.	Mixer III	0.47 ± 0.102	37.56
3.	Mixer IV	0.13 ± 0.019	26.02
4.	Mixer V	0.04 ± 0.009	42.33
Average / *Просјек*		0.22 ± 0.093	84.39

Data presented in Table 1 show that the content of DON in maize samples was determined in four mixers with average values of 0.04 to 0.47 ppm. The variation coefficient showed that the content of DON in maize samples between feed mixers varied 84.39% (Vk).

The presence of ZON mycotoxins in maize samples was found in 8 feed mixers. The average content of ZON in maize samples was 0.146 ± 0.096 ppm, with a variation of 197.74% (Vk). The variation coefficient showed that the content of ZON in maize samples between feed mixers varied highly.

Namely, in the Group I of feed mixers, ZON was detected in maize grains from four mixers (I, II, V and VI) with the content of 0.001 ppm. In Group II of feed mixers, ZON content ranged from 0.004 to 0.74 ppm (Table 2).

Tab. 2. Analysis of the content of ZON in feed mixers of Group II
Анализа садржаја ZON из миксера сточне хране за групу II

Animal Feed Manufacturers *Произвођачи сточне хране*		$\bar{X} \pm s\bar{x}$	Vk
1.	Mixer VII	0.004 ± 0.0009	39.22
2.	Mixer VIII	0.040 ± 0.012	51.34
3.	Mixer VIII	0.740 ± 0.084	19.67
4.	Mixer VIII	0.560 ± 0.130	40.18
Average / *Просјек*		0.327 ± 0.190	116.2

Analysis of the data in Table 2 shows that the average content of ZON in this group of fodder mixers was 0.327 ppm, with a very high variation of 116.2%.

In our research, mycotoxin zearalenone (ZON) was detected in very small amounts, although even low concentration of mycotoxins should not be neglected because in certain combinations there is a synergism in the interaction as documented in some cases. Low ZON contamination in grains indicates proper application of agro-technical measures, as quoted by authors from Croatia (Domijan et al., 2005).

Thus, a concentration of 0.2 to 1.0 ppm is toxic to rodents while animal feed with the content of ZON greater than 0.5 mg / kg is not recommended for animal feeding (D'Mello & Mecdonald, 1997). Comparing these quotes with our research results, in maize samples of 8 feed mixers, we found more than 0.5 ppm only in two mixers. The average content of ZON in maize samples from the mixing unit IX was 0.740 ppm, and in samples from the mixing unit X, the average for ZON was 0.560 ppm. In all other samples the average content of ZON was detected in traces.

The average content of FUM in maize samples from 6 animal feed mixers was 0.504 ± 0.163 ppm, with a variation of 85.44% (Vk). The variation coefficient showed that the content of FUM in maize samples between the observed feed mixers varied greatly.

In the first group of feed mixers, mycotoxin FUM was identified in three mixers and ranged from 0.01 to 0.88 ppm (Table 3), and the average FUM content was 0.486 ppm, with variation coefficient of 90.6%.

Tab. 3. Content of fumonisins (FUM) in maize samples from feed mixers of Group I
Садржај фумонизина FUM у узорцима кукуруза из миксера сточне хране за групу I

Animal Feed Manufacturers *Произвођачи сточне хране*		$\bar{X} \pm s\bar{x}$	*Vk*
1.	Mixer II	0.01 ± 0.003	43.69
2.	Mixer III	0.57 ± 0.089	27.21
3.	Mixer IV	0.88 ± 0.310	61.11
Average / *Просјек*		0.486 ± 0.254	90.6

In the Group II of feed mixers, FUM toxin was identified in three mixers ranging from 0.01 to 1.19 ppm (Table 4). The average content of FUM in maize samples in Group II of feed mixers was 0.543 ppm, with a variation coefficient of 110.1%.

Tab. 4. Contents of fumonisins (FUM) in maize samples from Group II of feed mixers
Садржај фумонизина (FUM) у узорцима кукуруза из миксера сточне хране за групу II

Animal Feed Manufacturers *Произвођачи сточне хране*		$\bar{X} \pm s\bar{x}$	*Vk*
1.	Mixer VII	1.19 ± 0.338	49.27
2.	Mixer VIII	0.43 ± 0.083	33.55
3.	Mixer X	0.01 ± 0.001	20.31
Average / *Просјек*		0.543 ± 0.345	110.1

Our research on maize samples showed that the total fumonisins (FB1 and FB2), were found in 6 feed mixers, but in a concentration that was lower than the dominant DON. Although the frequency and total concentration of FUM (0.504 ppm) was second to DON (2.370 ppm), its average value was more than 4 times lower. Only in two mixers (V and VI), FUMs were present in higher average values than DON.

Jakšić et al. (2011) in their research of the presence of mycotoxins in maize in Serbia, reported that the presence of FUM mycotoxins was 100%, ranging from 0.030 to 1.52 mg / kg in the samples from 2009. To recap, in the research done in Serbia, FUMs were dominantly present in the samples, and in our research FUMs were second to DON by its average content.

Fusarium mycotoxin contamination is common in co-contamination of maize, due to colonization of species of the genus *Fusarium*, which, in favorable conditions, synthesize mycotoxins from this group, being present on the plant in the field before harvest. Thus, Jakšić et al. (2011), in the reports from Serbia, quote that out of four analyzed mycotoxins (AF, OTA, ZON and FUM), the largest number of samples were contaminated with FUM.

Particular attention should be paid to fumonisin B1, due to its frequent simultaneous occurrence with other mycotoxins, because even at low concentrations, in relation to maximum allowed simultaneous concentration, this mycotoxin can have effect. However, the combined effects of mycotoxins in low concentrations have not been researched enough, as argued by Peraica and Rašić (2012).

In addition, it is evident from our work that DON is predominantly present and that ZON and FUM were detected to a lesser extent, as accompanying contaminants.

Conclusion

Our results of research on maize samples, regarding the presence of mycotoxins in 2011, showed a dominant presence of DON with the highest average concentration of 2.37 mg/kg, the presence of FUM with average concentration of 0.504 mg/kg, and the presence ZON with average concentration of 0.146 mg/kg.

Testing maize samples for aflatoxins showed that all samples were negative for the presence of these mycotoxins in 2011.

According to the results we obtained for the research period i.e. the vegetation year of 2011, mycotoxicological profile for maize had the following sequence: DON - FUM - ZON.

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Ко-контаминација микотоксинима код зрна кукуруза

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Сажетак

У раду је истражено 36 узорака кукуруза. Микотоксиколошке анализе показале су да је присуство микотоксина најчешће из групе фузаријумских токсина. Током 2011. године, узорци кукуруза испитани су на присуство и садржај укупних афлатоксина: (AFB1, AFB2, AFM1, AFM2), деоксиниваленол (DON), зеараленон (ZON) и фумонизини (FUM). Према резултатима које смо добили за испитивани период, вегетацијску 2011. годину, микотоксиколошки профил за већину узорака кукуруза је био DON – FUM – ZON. DON је доминантно присутан микотоксин у 2011. години. Посебне мјере опреза потребне су управо код нижих концентрација микотоксина и истовремено присутних више микотоксина, што би практично могло класичним аналитичким методама бити декларисано као токсиколошки безбједан квалитет или негативан налаз.

Кључне ријечи: микотоксини, кукуруз, микотоксиколошки профил

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