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FUMONISIN B_1 IN MAIZE, WHEAT AND BARLEY GRAIN IN SERBIA

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Abstract: A wide variety of commodities in the world have been analyzed for fumonisins contamination. However, they have mostly been reported in maize and maize-based foods and feeds. Just a few scientific researches were conducted to obtain results on natural contamination of wheat and barley with these mycotoxins. This survey was conducted to evaluate fumonisin B₁ contamination in maize, wheat and barley grain in Serbia. A total of 203 maize, 180 wheat and 120 barley samples were obtained from different local warehouses between October 2007 and June 2009. Concentration of FB₁ were analysed with the Enzyme-Linked Immunosorbent Assay (ELISA). Positive results were found in 70.7%, 60.6% and 34.1%, in the maize, wheat and barley samples, respectively. FB ₁ concentration varied from 750 to 4900 µg kg⁻¹, and the mean levels recorded were: 1225.7 kg⁻¹ in maize; 852.7 μg kg⁻¹ in wheat; and 768.2 μg kg⁻¹ in barley. The mycotoxin contamination of cereals was affected by factors such as origin resistance, droughtstress, and insect damage and differed between the years of investigation The results obtained in this survey revealed that FB₁ is frequent contaminant of cereal grains in Serbia. Considering that these products are consumed in large amounts either directly or as components of foods and feeds, the levels of contamination reported herein indicate a potential threat to animal and public health.

Key words: fumonisin B₁, barley, maize, wheat

Introduction

Fumonisins are a group of 15 structurally related mycotoxins, and three of them (FB₁, FB₂ and FB₃) can cause serious health problems in animals such as equine leukoencephalomalacia (ELEM), pulmonary edema in swine and liver, or kidney cancer in rats or mice. Fumonisins are also associated with esophageal cancer in human (De Nijs et al., 1998).

Fumonisins are widely distributed as natural contaminants of cereals, especially maize, and maize-based food and feed. This group of mycotoxins, biosynthesised by species of the genus *Fusarium*, is interesting even for countries that lack conditions for their natural occurrence. For instance, in the Netherlands, it has been established that 61 out of 62 samples of maize imported for human consumption contained fumonisin B_1 (FB₁) ranging from 30 to 3,350 μ g kg⁻¹ (mean 640 μ g kg⁻¹). Eleven samples have contained more than 1,000 μ g kg⁻¹. Retail maize-based products in the UK have contained FB₁ in the range 1,000 to 8,000 μ g kg⁻¹ (*Candlish et al., 2000*). Higher concentrations of fumonisins (12-1,300 μ g kg⁻¹) were determined in Germany in maize-based products imported from Italy (*Usleber et al., 1994*). This points out to the fact that humans and animals all over the world are permanently exposed to fumonisin activities.

The frequency and concentrations of FB_1 in cereals, first of all, in maize grain, vary from a continent to a continent, form a country to a country. The natural incidence of FB_1 is more frequent in Europe (86%) than in other continents, but on the other hand, the maximum levels are lower than those in Africa, for instance (Bottalico, 1998). In Europe (Austria, Croatia, Germany, Hungary, Italy, Poland, Portugal, Romania, Spain and United Kingdom), FB_1 has been detected in 248 out of 719 samples of maize, or in 34.5% of samples (IARC, 2002). Concentrations of FB_1 have varied from 7 to 250,000 μ g kg $^{-1}$ in positive maize samples. Furthermore, these concentrations have amounted to 790, 6,100 and 9,818 ppb in maize-based products in Switzerland (Pittet et al., 1992), Italy (Doko and Visconti, 1994) and Germany (Bresch et al., 1998), respectively.

There are little data on the natural incidence of FB_1 in wheat grain, and particularly in barely grain, not only in Europe, but also in other regions world-wide. However, a high level of FB_1 in naturally contaminated wheat grains has been detected in Uruguay (*Piñeiro and Silva, 1997*), South Africa (*Mashinnini and Datton, 2006*) and Italy (*Castoria et al., 2005*).

The concentrations of FB₁ registered in the region of the western Balkan have been greater in maize grain in Croatia (Jurjević et al., 1998), smaller in Serbia (Abramović et al., 2005), while there is no data on the presence of this mycotoxin in BH and Montenegro. Generally, in Serbia, there are little data on cereal contamination with fumonisins, which is not consistent with widely distributed infection caused by producents of this mycotoxin on maize grain (Lević at al., 1997, 2009), wheat grain (Levć et al., 2004; Stanković et al., 2007) and barley grain (Lević et al., 2010). Besides, it has been determined that species of the genus Fusarium, isolated from cereal grain, are characterised with high potential of FB₁ biosynthesis (Stanković et al., 2007), as well as, that cereal grain contain high concentrations of this mycotoxin (Stanković et al., 2008, 2010). Consequently, this study encompasses comparative investigations of concentrations of FB₁ in grain of three cereals that are most often grown in Serbia.

Materials and Methods

A total of 203 maize, 180 winter wheat and 120 winter barley samples were obtained from 12 different locations in Serbia between October 2007 and June 2009. Grain samples of four wheat, seven maize and two barley varieties were collected after four to six months of storage in family barns. To obtained representative samples, primary large samples of approximately 10 kg were prepared by combining several samples collected from different parts of storage bags or containers. The primary samples were homogenised and quartered to obtain a 1 kg sample for laboratory analyses. All samples were stored in the dark at 4°C in paper aseptic bags and thoroughly ground for analyses. Samples were ground using a KnifetecTM 1095 sample mill and passed through a 20-mesh screen. After thorough sample mixing, subsamples of 20 g were prepared for further analyses.

All samples were analysed with the Enzyme-Linked Immunosorbent Assay (ELISA) using the Celer®Techna test kits. These kits were validated by the manufacturer for use in maize, wheat, barley and other commodities. Mycotoxins were extracted from a 20-g representative grounded sample by shaking it with 100 ml of 70% methanol and 4 g NaCl for 1 h at 180 rpm on a GFL shaker.

The extracted sample and enzyme-conjugated mycotoxin were mixed and added to the antibody-coated micro well. Mycotoxins in samples and control standards were allowed to compete with enzyme-conjugated mycotoxins for the antibody binding sites. The microwells were measured with a microplate reader with an absorbance filter of 450 nm. The optical densities (ODs) of the samples were compared to the ODs of standards and an interpretative result was obtained.

The incidence (I) of FB_1 per year was calculated by the equation: I (%) = [(Number of samples in which FB_1 occurred/Total number of samples) x 100.

Results and Discussion

Mycotoxin FB_1 was detected in grain of each three cereals collected in Serbia between October 2007 and June 2009. The largest variation of the incidence of FB_1 and the highest mean value of this mycotoxin were found in samples of maize (Table 1). It is interesting that there was about the same variation of 8.22, 7.78 and 8.49 from the mean value of FB_1 incidence for samples of maize, wheat and barley, respectively.

The incidence of FB_1 in maize grain varied from 60.7 to 80.1%, depending on the growing conditions over years (Table 1). In Canada, the presence of fumonisins has been established in all maize samples that had been collected from locations under conditions of drought and increased temperatures in the period from tasseling to the ear development (July-August) followed by conditions cooler and wetter weather than it was common during September and October

(Miller et al., 1995). According to the reports of the Republic Hydrometeorological Service of Serbia agrometeorological conditions in 2006 and 2007 were significantly different. In July 2006, the average daily temperatures were higher, with deficit rainfall, while in August, it was colder and more humid than the appropriate long-term average (1971-2000). In contrast, during July and August 2007 there was no precipitation and air temperature were very high. These conditions may explain an extremely low incidence of FB₁ in 2006 (60.7%) or extremely high in 2007 (80.1%). Furthermore, studies carried out by Logrieco et. al. (1993) showed that maize samples from dry and warm regions were mainly populated by the species F. verticillioides for which is known that, besides F. proliferatum, is the principal producent of fumonisins.

Table 1. Incidence (%) of fumonisin B_1 in grain of maize, winter wheat and barley collected in Serbia during the period 2006-2009

Grain	FB ₁ incidence (%) per year				Mean±SD
Sample	2006	2007	2008	2009	Mean±SD
Maize	60.7	80.1	75.1	71.9	72.0±8.22
Wheat			55.1	66.1	60.6±7.78
Barley			28.1	40.1	34.1±8.49

The values of standard deviation varied from 7.78 to 8.49% (Table 1). These values pointed out to small differences between variations of incidence of FB_1 in samples of wheat in relation to variations in samples of maize or barely.

Mycotoxin concentrations were higher in 2009 than in 2008 for both, winter wheat (55.1% in 2008 and 66.1% in 2009) and winter barley (40.0% in 2008 and 28.1% in 2009) (Table 1). This can be explained by differences in weather conditions between the two years. Fusarium head blight is strongly associated with moisture and the time of rainfall at the time of flowering (*JECFA*, 2001). According to reports of the Republic Hydrometeorogical Service of Serbia, the average monthly spring and summer temperatures and rainfall were higher in 2009 than 2008. In contrast, conditions in 2008 favoured the incidence of FB₁ in maize grains than in 2009.

Castella et al. (1999) found that the incidence of FB₁ was the highest in maize (87.3%) then in barley (72.4%,) and the least in wheat (47.7%). If these results are compared with results presented in this paper (Table 1) the tendency of the decrease in the intensity of FB₁ incidence is observed. Namely, the intensity of FB₁ incidence was on average lower by 15.3, 11.2 and 13.6% in grain of maize, wheat and barley, respectively. Naturally occurring FB₁ in samples of barley in Korea were found in 6% with an average of 16 μg kg⁻¹ (Park et al., 2002), which is significantly lower than our results, and especially than data presented by Castella et al. (1999).

Concentrations of FB_1 varied more among positive samples of wheat (750-4900 μ kg⁻¹) than among samples of maize (750-4300 μ kg⁻¹), and especially samples of barely (750-1225 μ kg⁻¹) (Table 2). However, according to the average concentrations of FB_1 the following relation was obtained: maize (1225.7 μ kg⁻¹) > wheat (852.7 μ kg⁻¹) > barley (768.2 μ kg⁻¹). *Gamanya and Sibanda (2001)* established a higher amount of FB_1 in maize grain (500-800 μ kg⁻¹) than in wheat grain (2.5-600 μ kg⁻¹), although these amounts in both cases were significantly lower than amounts obtained in our study. *Castella et al. (1999)* found that concentrations of FB_1 were the highest in maize (ranging from 200 to 24,900 μ kg⁻¹) then in barley (ranging from 200 to 11,600 μ kg⁻¹), and the least in wheat (ranging from 200 to 8,800 μ kg⁻¹). Our results differed from these results in relation to the lower concentrations of FB_1 in grain of maize, wheat and barely, and in relation to a lesser importance of FB_1 for barley grain than wheat grain.

Table 2. Sample size and concentration ($\mu g \ kg^{-1}$) of fumonisin B_1 in grain of maize, winter wheat and barley collected in Serbia from 2006-2009

Items	Grain sample			
Items	Maize	Wheat	Barley	
Sample size ^a	144/203	109/180	41/120	
Range (µg kg ⁻¹)	750-4300	750-4900	750-1225	
Mean ^b (μg kg ⁻¹)	1225.7	852.7	768.2	

^a Number of positive samples/Number of total samples

Fumonisins are widely distributed geographically, and their natural occurrence in maize has been reported in many areas of the world (Shepard et al., 1996). The maximum concentrations of FB₁ detected in maize grain in Serbia were lesser than concentrations of this mycotoxin established in certain European countries, for instance France (Le Bars and Le Bars, 1995), Croatia (Jurjević et al., 1998), Italy (Pietri et al., 1995), Hungary (Fazekas et al., 1998) and Slovakia (Šrobarova et al., 2000). On the other hand, concentrations of FB₁ determined in maize grain in Serbia were higher than concentrations of FB₁ registered in maize grain in Germany (Usleber and Martlbauer, 1998), Poland (Doko et al., 1995), Romania (Doko et al., 1995) and Sapain (Visconti et al., 1995).

Table 3. Distribution of fumonisin B_1 concentrarion in maize, winter wheat and barley grain samples collected from various regions in Serbia

ED (ug 1/g ⁻¹)	Total samples (%)			
$FB_1(\mu g kg^{-1})$	Maize	Wheat	Barley	
<100	28 .8	27 .1	31 .7	
100-500	35 .6	31 .1	31 .7	
500-1000	15 .3	16 .7	11 .7	
>1000	18 .6	15 .1	18.8	

^b Mean concentration in positive samples

Overall mycotoxin concentrations of samples are given in Table 3. A large number of samples (79.7%, 74.9% and 75.1% in maize, wheat and barley, respectively) contained FB₁ at levels lower than 1000 μ g kg⁻¹. The concentration levels of FB₁ in 18.6%, 15.1% and 18.8% of maize, wheat and barley samples, respectively, were higher than the maximum level in feed adopted by the EC.

Our results show that FB₁ is a significant contaminant not only of maize but also of wheat and barely grown under agroecological conditions of Serbia. A high level of FB₁ (over 6,000 µg kg⁻¹) in naturally-contaminated wheat grains was detected in Uruguay (*Piñeiro and Silva, 1997*). Naturally occurring FB₁ in samples of barley in Korea were found in an average of 16 µg kg⁻¹ (*Park et al., 2002*). On the other hand, it is considered that the natural occurrence of fumonisins in cereal grains, such as wheat and barley, is uncommon. Two hypotheses can explain this observation (*Marin et al., 1999*). First, *Fusarium Liseola* section is a more common contaminant of maize than of wheat and barley. Second, different nutritional components in barley and wheat could act as inhibitors of fumonisin biosynthesis or a component in maize may have the capacity to initiate the fumonisin development.

Preliminary data obtained by *Desjardins et al.* (2007) indicate a significant potential for fumonisin contamination of wheat in which *F. proliferatum* is present. These authors confirmed that *F. proliferatum* could cause wheat kernel black point disease. Our previous studies showed that the frequency of *F. proliferatum* in grain of wheat and barley were 13.3% (10/70) and 13.8% (4/29), respectively, with a maximum incidence up to 15% (*Lević et al., 2009*). Furthermore, the most frequently isolated species in 6-row barley were species from section *Liseola* (*F. proliferatum*, *F. subglutinans*, *F. verticillioides*) (*Lević et al., 2010*). These facts may explain the high concentrations of FB₁ identified in wheat grain, as well as, in barley grain in Serbia.

The contamination of maize, wheat and barely with FB_1 is especially important from the aspect of getting cereal-based products used in human nutrition. *Doko and Visconti (1994)* determined that products prepared from contaminated maize contained different levels of FB_1 . By processing maize contaminated with 5,310 μ g kg⁻¹ FB_1 , extruded maize with a higher concentration of this mycotoxin than the concentration of a raw material (6100 μ g kg⁻¹) is obtained. Also, grits, flour or polenta with a somewhat lower concentration of this mycotoxin (3760 μ g kg⁻¹) are obtained, while the least concentration of FB_1 was established in flakes (up to 60 μ g kg⁻¹), tortilla chips (up to 60 μ g kg⁻¹) and flaked maize (10 μ g kg⁻¹). Fumonisins were not recorded in distilled alcohol, but they remained up to 85% in residues of distillation products (*Bennett et al., 1996*). Utilisation of these residues in horse and pig feed can be harmful for these animals, because they are sensitive to relatively low levels of fumonisins.

Conclusion

Our studies showed that agroecological conditions in Serbia favoured the natural incidence of FB₁, not only in maize grain, but also in grain of wheat and barely. This fact, as well as, 15.1-18.8% of samples that had a higher maximum level of FB₁ than feed adopted by the EC, point out that it is necessary to take measures for lowering concentrations of this mycotoxin in grain of cereals important for agriculture in Serbia. Among the strategies for reducing risk of FB₁ contamination in cereals supplied to the market, development and deployment of *Fusarium* - resistant germplasm is a high priority. Breeding for increased grain yield and reduced mycotoxin levels is being practiced today in both commercial and public programmes, but the degree of resistance achievable may be limited due to complicated genetics and/or linkage to undesirable agronomic traits.

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Fumonizin B₁ u zrnu kukuruza, pšenice i ječma u Srbiji

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Rezime

Širok spektar proizvoda u svetu je analiziran na kontaminaciju fumonizinima. Međutim, prisustvo ovog mikotoksina uglavnom je zabeleženo u zrnu kukuruza i hrani na bazi kukuruza. Malo je istraživanja o prirodnoj kontaminaciji pšenice i ječma fumonizinima. Cilj ovog istraživanja bio je da se ispita kontaminacija fumonizinom B₁ zrna kukuruza, pšenice i ječma u Srbiji. Ukupan broj od 203 uzoraka zrna kukuruza, 180 uzoraka zrna pšenice i 120 uzoraka zrna ječma sakupljenih u različitim lokalitetima Srbije u periodu oktobar 2007. - jun 2009. godine. Koncentracija FB₁ u zrnu žita je analizirana ELISA testom. FB₁ je utvrđen u 72% uzoraka zrna kukuruza, 60,6% uzoraka zrna pšenice i 34,1% uzoraka zrna ječma. Koncentracija ovog mikotoksina je varirala od 750 do 4900 μg kg⁻¹, sa srednjim vrednostima: 1225,7 kg⁻¹ u kukuruzu; 852,7 μg kg⁻¹ u pšenici; i 768,2 μg kg⁻¹ u ječmu. Kontaminacija zrna sa FB₁ je varirala u zavisnosti od mnogih faktora, kao što je otpornost biljke, stres suše, povrede od insekata i

razlikovala se u ispitivanim godinama. Rezultati ovog istraživanja ukazali su da je FB₁ značajan kontaminant zrna žita u Srbiji. S obzirom da se ovi proizvodi koriste u velikim količinama, bilo kao hrana za ljude, bilo kao hrana za životinje, utvrđeni stepen kontaminacije ukazuje na visok potencijalni rizik po javno zdravlje.

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