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# Presence of *Fusarium* mycotoxins in feedstuffs and cow milk sampled from Croatian farms during 2015

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*Jelka Pleadin[1], Ana Vulić[1], Manuela Zadravec[2]*, Tina Lešić[1], Miroslav Benić[3], Vesna Jaki Tkalec[4], Nada Vahčić[5]*

[1]Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska Cesta 143, 10000 Zagreb, Croatia
[2]Croatian Veterinary Institute, Laboratory for Microbiology of Feed, Savska Cesta 143, 10000 Zagreb, Croatia
[3]Croatian Veterinary Institute, Laboratory for Mastitis and Raw Milk Quality, Savska Cesta 143, 10000 Zagreb, Croatia
[4]Croatian Veterinary Institute, Veterinary Centre Križevci, Ivana Z. Dijankovečkog 10, 48260 Križevci, Croatia
[5]University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

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**Abstract**

Mycotoxins may contaminate food of animal origin due to the carry-over effect and represent a potential risk to human health. The problem of *Fusarium* mycotoxin contamination becomes an issue especially during rainy years characterised by substantial temperature changes. The aim of this study was to investigate into the level of *Fusarium* mycotoxins zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FUM) in maize silage (n=21), concentrated dairy cattle feeds (n=56) and cow milk samples (n=105), taken during 2015 from households located in four Croatian regions. The presence of mycotoxins was determined using validated ELISA methods. A high level of feedstuffs' contamination was evidenced, especially with ZEN, with values higher than recommended observed in 9.5 % of maize silage samples. Fourteen point three percent (14.3 %) of milk samples were DON positive, with the toxin concentrations ranging from 5.4 to 67.3 μg/L. ZEN was determined in 94.3 % of milk samples, ranging from 0.3 to 88.6 μg/L. FUM were not detected in any of the analysed milk samples. Given the tolerable daily intakes (TDIs) defined for these mycotoxins, human health risks arising from the consumption of cow milk can generally be considered low, even in times characterised by weather conditions that facilitate the production of *Fusarium* mycotoxins in cereals subsequently used as dairy cattle feed. The exception represents particular milk samples in which high ZEN concentrations were found.

*Key words*: *Fusarium* mycotoxins, cow milk, dairy cattle, feedstuffs, Croatian farms

## Introduction

As natural and unavoidable contaminants of important agricultural commodities, mycotoxins have continued to severely impact animal and human health (Coffey et al., 2009). Mycotoxins of the *Fusarium* species have traditionally been associated with cereal contamination (Glenn, 2007) that may occur before harvest (in the field) and/or after it (in warehouses and silos). The level of contamination also depends on the storage methods and conditions, and varies across geographical areas and climatic

*Corresponding author/Dopisni autor: E-mail: zadravec@veinst.hr

regions, influenced by the formation of moulds, moisture level, temperature, aeration, the presence of insects and mechanical damage to the cereals stored (Placinta et al., 1999; Pleadin et al., 2015). Studies have shown that the problem of *Fusarium* mycotoxin contamination emerges in particular during rainy years characterised by substantial temperature changes (Pleadin et al., 2012a; Pleadin et al., 2012b).

Residues of mycotoxins may be present in eggs, milk, meat and offal due to the carry-over effect and represent a potential risk to humans (Yiannikouris and Jouany, 2002; Cavret and Lecoeur, 2006; Fink-Gremmels, 2008). In ruminants, the rumen flora can convert a number of mycotoxins into metabolites of lower or no health risk. The rumen of healthy animals is thus an important barrier, which can be impaired due to various ruminant diseases. In comparison to monogastric animals such as pigs, ruminants are generally more resistant to adverse effects of mycotoxins, since the microorganisms in the rumen have the ability to degrade these mycotoxins into less toxic compounds (Keese et al., 2008). In cows, the presence of zearalenone or the *Fusarium* species producing this mycotoxin has been associated with infertility, reduced milk production and hyperestrogenism (Warth et al., 2013). Dairy cows are considerably more tolerant to deoxynivalenol, as exemplified by the lack of any adverse impact on feed intake and milk production (D'Mello et al., 1999).

The excretion of mycotoxins through milk is generally low and is affected by molecular weight and lipophilicity of a mycotoxin. The transport rate is also influenced by the pH gradient between blood plasma and milk, which changes according to cow's health (Coffey et al., 2009; Kalač, 2011). Mycotoxin analysis of biological samples enables not only the evaluation of exposure to these contaminants, but also the assessment of consumer risk arising on the grounds of contaminated foodstuffs of animal origin (Dänicke and Winkler, 2015). At the same time, it is known that cow milk is an important component of diet of humans of all ages, since it provides important nutrients. Children are especially frequent consumers of milk as one of the principal foodstuffs taken during the first years of life.

Given that data on natural occurrence of *Fusarium* mycotoxins in cow milk are scarce, the aim of this study was to determine the levels of the most representative *Fusarium* mycotoxins deoxinivalenol (DON), zearalenone (ZEN) and fumonisins (FUM) in cow milk taken from farms seated in four Croatian regions. During the same period, maize silage and concentrated dairy cattle feedstuffs were sampled from different farms and analysed for the presence of the abovementioned mycotoxins. Furthermore, the aim of this study was to compare the mycotoxin levels established in milk with the Tolerable Daily Intake (TDI) values defined for these mycotoxins, as well as to evaluate human health risks arising from the consumption of cow milk potentially contaminated with *Fusarium* mycotoxins.

## Materials and methods

### *Sampling and sample preparation*

Samples of dairy cattle feedstuffs, of which 21 samples of maize silage and 50 samples of concentrated cattle feeds were retrieved throughout 2015 from dairy farms situated in the northern (Varaždin, Međimurje, Koprivnica-Križevci and Krapina-Zagorje County), central (Zagreb, Sisak-Moslavina and Bjelovar-Bilogora County), eastern (Slavonia, Baranja and Srijem County) and western (Istria and Gorski Kotar County) part of Croatia. Maize silage and cereals subsequently used for feed production were of the genus 2014 (used on farms during 2015). Sampling and preparation of the samples were performed in full line with the ISO 6497:2002 and ISO 6498:1998 standards, respectively. All samples of feedstuff were thoroughly ground in an analytical mill (Cylotec 1093, Tecator, Sweden) to achieve a particle size of 1.0 mm and were stored at 4 °C prior to analysis.

During 2015, a total of 105 cow milk samples were sampled from households also seated in the northern, central, eastern and western part of Croatia. Sampling of milk was performed in full line with the ISO 707:2008. All milk samples were fresh, not pre-treated, and were divided into groups based on the sampling region.

### *Extraction of mycotoxins*

*Feedstuffs:* After grinding, five grams of each feedstuff sample were extracted using 25 mL of distilled water so as to be analysed for DON presence,

or using 25 mL of methanol/water (70/30) solution so as to be analysed for ZEN and FUM presence. The extraction was performed by vigorous 3-minute shaking on a shaker, following which the extracts were filtered through a filter paper (Whatman, Black Ribbon, GE Healthcare, Buckinghamshire, United Kingdom). The supernatants obtained with all three analytes were appropriately diluted according to the ELISA kit manufacturer's instructions, and used for the determination of mycotoxin concentrations via the ELISA immunoassay.

*Milk:* Samples were centrifuged (3,000 g, 15 min) at 4 °C. The upper cream layers were removed. 20 $\mu$L of glucoronidase/arylsulphatase Helix pomatia (Art No. 4114, Merck) were added to 1 mL of a sample, and incubated for 3 h at 37 °C. To 0.9 mL of hydrolyzed and defatted milk, 0.1 mL of methanol (to the effect of ZEN and FUM presence detection) or water (to the effect of DON presence detection) was added. The resulting solution (50 $\mu$L) was used for analyses by the ELISA methods.

*Determination of mycotoxins using the ELISA*

The determination of mycotoxins concentration was performed using competitive RIDASCREEN® ELISA kits: DON (Art. No. R5906), Zearalenon (Art. No. R1401) and Fumonisin (determination of fumonisin B1, B2 and B3) (Art. No. R3401). Analytical steps were performed completely according to the test procedures declared by the kits' manufacturer (R-Biopharm, Darmstadt, Germany). The ELISA kits contained a micro-titre plate with 96 wells coated with antibodies, standard solutions containing different concentrations of mycotoxins, an enzyme conjugate, an anti-antibody, a substrate, a chromogen solution (urea peroxide/tetramethylbenzidine), a stop solution, and washing and dilution buffers. Standards employed with the validation of analytical methods were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used for analyses were of an analytical grade.

ELISA tests were performed using a ChemWell auto-analyzer (Awareness Technology Inc. 2910, USA), where the absorbance thereby being measured at 450 nm. In order to determine mycotoxin concentrations in milk samples, a standard curve illustrative of skim milk was plotted for each mycotoxin analysed. When establishing final mycotoxin concentrations in a given sample, the dilution factor and the mean recovery rate determined for each mycotoxin were taken into account.

*Validation of the ELISA method*

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the average of ten toxin-negative industrially produced milk samples (first analysed for the presence of *Fusarium* mycotoxins and then used for validation as a blank material) plus three-fold standard deviation (LOD = mean ± 3SD) and six-fold standard deviation (LOQ = mean ± 6SD), respectively. For each mycotoxin, the recovery rate was determined at two different levels (10 and 50 $\mu$g/L for DON and ZEA; 50 and 100 $\mu$g/L for FUM) by virtue of fortifying toxin-negative milk samples with standard working solution of the analysed mycotoxins (200 $\mu$g/L), followed by the analysis of three replicates at each "spiking" level.

*Estimation of mycotoxins intake*

The estimation of mycotoxins intake through the milk consumption pathways was made based on the data on average milk consumption published by the FAOSTAT (2014), link to published TDI values (EC 856/2005) and mean mycotoxins concentration determined in this study. According to FAOSTAT the average milk consumption in Croatia is 322 g/capita/day and for the sake of calculations, the average body weight was estimated at 70 kg (EFSA, 2012). From these data amount of mycotoxin in a daily portion of milk was calculated and related to the given TDI values.

*Statistical analysis*

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY, IBM, 2013). In order to determine the statistical significance of the differences between samples coming from different Croatian regions, the One-way ANOVA and the Tamhane's T2 *post-hoc* test were used. Statistical significance of data was estimated at the level of $p<0.05$.

## Results and discussion

Some investigations have been conducted to assess the carry-over of *Fusarium* mycotoxins into edible tissues, eggs and milk (Flores-Flores et al., 2015). Published data show a limited deposition of these mycotoxins in meat and other edible tissues, and a low transmission rate into milk and eggs. It is known that mycotoxins are never completely removed from the feed-food chain, or from milk via pasteurisation, since the majority of them are heat-stable.

Earlier studies have concluded that, given that measurable *Fusarium* mycotoxin levels imply vast consumption, milk does not normally pose as a human health hazard arising from contaminated feeds lactating dairy cows are fed on (Prelusky et al., 1990; Dänicke and Winkler, 2015). However, some authors have emphasised the necessity for further investigations into the levels of *Fusarium* mycotoxins in milk (Coffey et al., 2009; Flores-Flores et al., 2015), especially in heavy rainfall periods characterised by significant temperature changes that favour the formation of these mycotoxins in cereals, consequently causing the contamination of dairy cow feeds.

In this study, the presence of mycotoxins in feedstuffs and milk was determined using the ELISA methods. The results of validation of the methods used for cow milk analyses are shown in Table 1. The validation data pertaining to the methods used for the determination of these mycotoxins in feedstuffs (cereals) were published earlier (Pleadin et al., 2013).

LOD and LOQ values were the lowest for ZEA and the highest for FUM. Validation of the employed methodology resulted in the mean recovery rates of 80.4 % for DON, 91.0 % for ZEA and 79.0 % for FUM, and in the coefficients of variation (CV) ranging from 5.8 to 11.4 %. Based on the obtained validation results and the validation criterion given under the Commission Decision 2002/657/EC (EC, 2002), the applied quantitative ELISA methods can be considered suitable for the determination of the investigated mycotoxins in milk. However, since the method employed in the determination of FUM shows high LOD and LOQ values, it can't be considered sensitive enough to be used for the determination of lower FUM levels in milk samples.

It is known that mycotoxin levels in maize silage and concentrated feeds are correlated with mycotoxin concentrations in milk (Signorini et al., 2012). In this study, dairy cows were fed on feedstuffs produced from cereals of the genus 2014, i.e. the cereals harvested in the year known for its heavy rainfall periods. Concentrations of *Fusarium* mycotoxins in feedstuffs sampled from Croatian dairy cow farms during 2015 are shown in Table 2. The obtained results are also displayed according to the sampling region (Table 3), although milk producers do not produce feedstuffs themselves but rather resort to centralised feed production taking place in feed mill plants from which the feed gets to be distributed across Croatia.

In total, DON was detected in 77 %, ZEN in 66 %, and FUM in 80 % of maize silage and concentrated feed samples. DON and ZEA concentrations higher than recommended for dairy cattle feedstuffs under the Commission Recommendations 2006/576/EC (EC, 2006) were determined in 4.2 % and 9.2 % of samples, respectively. Although FUM concentrations were not higher than recommended in any of the samples, a significant level of contami-

Table 1. Results of validation of the ELISA method employed in the determination of mycotoxins in milk

| Mycotoxin | LOD (μg/L) | LOQ (μg/L) | Spiked level[a] (μg/kg) | Recovery (%) | CV (%) |
|---|---|---|---|---|---|
| DON | 4.0 | 5.4 | 10 | 78.5 | 5.8 |
| | | | 50 | 82.3 | 6.1 |
| ZEA | 0.8 | 1.2 | 10 | 87.6 | 4.3 |
| | | | 50 | 94.3 | 10.1 |
| FUM | 20.1 | 25.3 | 50 | 77.3 | 11.4 |
| | | | 100 | 80.6 | 9.3 |

DON - deoxynivalenol; ZEN - zearalenone; FUM - fumonisins; LOD - limit of detection; LOQ - limit of quantification;
[a]Industrial dairy milk was first analysed for mycotoxins under study and then utilised as a blank (mycotoxin-negative) material used for spiking during the recovery determination

nation was observed, particularly in some maize silage samples (the maximal value of 6,300 μg/kg). Generally, a high level of feedstuffs' contamination was observed, especially with ZEN, whose concentrations were higher than recommended in 9.5 % of maize silage and 8.9 % of concentrated feed samples. Mostly higher level of feedstuffs contamination was observed in Central and Eastern in comparison to Western and Northern part of Croatia, although not significantly different ($p>0.05$).

Given that high mycotoxin concentrations are usually associated with climatic conditions, in particular humidity and temperature as the factors most critical for mould formation and, thus, also mycotoxin production (Pleadin et al., 2013), high contamination of feedstuffs observed in this study could be explained by weather conditions evidenced during the period of cereal growth and harvesting. Official weather reports for 2014 show that in the period of cereal growth and harvesting (May-September), the

Table 2. *Fusarium* mycotoxins in maize silage and concentrated dairy cattle feed sampled from Croatian farms during 2015

| Mycotoxin | Material | GV (μg/kg) | % over GV[a] | % of positives[b] | Mean ± SD (μg/kg) | Range of positives (μg/kg) |
|---|---|---|---|---|---|---|
| DON | Silage | 10,000 | 4.8 | 81 | 3,879±4,893 | 38.3-13,407 |
| | Feed | 5,000 | 3.6 | 72 | 2,147±2,245 | 24.1-10,120 |
| ZEN | Silage | 3,000 | 9.5 | 74 | 2,084±2,723 | 9.2-11,424 |
| | Feed | 500 | 8.9 | 58 | 526±562 | 5.7-2,298 |
| FUM | Silage | 60,000 | 0 | 88 | 849±1,125 | 49.3-6,300 |
| | Feed | 50,000 | 0 | 71 | 855±865 | 33.1-1,854 |

DON - deoxynivalenol; ZEN - zearalenone; FUM - fumonisins; GV - guidance value for feedstuffs given under the European Commission Recommendations 2006/576/EC;
[a]Samples in which mycotoxin concentrations were higher than the guidance value stated under the European Commission Recommendations 2006/576/EC;
[b]Samples in which mycotoxin concentrations were higher than the LOQ value;

Table 3. *Fusarium* mycotoxins in feedstuffs sampled in different Croatian regions during 2015

| Mycotoxin | Region | No of samples | % of positives[a] | Mean ±SD (μg/kg) | Range of positives (μg/kg) |
|---|---|---|---|---|---|
| DON | Central | 18 | 82 | 3,112±3,561 | 24.1-9,256 |
| | Eastern | 25 | 84 | 4,293±4,591 | 27.3-13,407 |
| | Western | 10 | 68 | 2,022±2,897 | 46.2-9,302 |
| | Northern | 24 | 72 | 2,625±3,225 | 26.3-11,402 |
| | In total | 77 | 77 | 3,013±3,569 | 24.1-13,407 |
| ZEN | Central | 18 | 75 | 1,516±2,011 | 8.2-9,431 |
| | Eastern | 25 | 71 | 1,214±1,731 | 10.1-11,424 |
| | Western | 10 | 56 | 1,112±1,093 | 5.7-425 |
| | Northern | 24 | 62 | 1,377±1,735 | 7.1-4,546 |
| | In total | 77 | 66 | 1,305±1,643 | 5.7-11,424 |
| FUM | Central | 18 | 85 | 925±1,231 | 33.1-5,612 |
| | Eastern | 25 | 82 | 1,138±1,278 | 52.4-6,300 |
| | Western | 10 | 72 | 631±614 | 33.8-2,147 |
| | Northern | 24 | 79 | 715±856 | 40.7-4,793 |
| | In total | 77 | 80 | 852±995 | 33.1-6,300 |

DON - deoxynivalenol; ZEN - zearalenone; FUM - fumonisins;
[a]Samples in which mycotoxin concentrations were higher than the LOQ value

investigated parts of Croatia were warm (75-91 %) to very warm (91-98 %) (MHS, 2014). As for the humidity, the year 2014 was highly (91-98 %) to extremely humid (>98 %). The obtained results, in terms of higher mean concentrations of *Fusarium* mycotoxins in maize silage and final feed products sampled in 2015 (genus 2014), could also be linked to the weather conditions witnessed in 2014 during the cereal growth and harvesting period. In that period high to very high temperatures and extreme humidity was observed, which could facilitate a significant mould growth and consequently also the production of these mycotoxins.

Table 4 shows concentrations of *Fusarium* mycotoxins in milk samples determined in different sampling regions an also expressed as the total value. FUM concentrations are not shown, since this mycotoxin was not detected in any of the milk samples analysed, meaning that FUM concentrations were actually lower than the ELISA method's LOQ (25.3 μg/L).

14.3 % of samples were determined to be DON positive, with the toxin concentrations ranging from 5.4 to 67.3 μg/L and the maximal concentration being determined in the Northern region. The maximal mean concentration was determined in the Central region (28.8±30.6 μg/L), while the maximal mean concentration determined in the Northern region was substantially lower (9.1±6.3 μg/L). There was no statistically significant difference ($p>0.05$) between the investigated regions. Studies have shown that in the rumen DON gets to be swiftly bio-transformed into deepoxy-deoxynivalenol (DOM-1), while its non-metabolized portion gets to be excreted into milk in an extremely low quantity (1-3 μg/L) (Côté et al., 1986; Keese et al., 2008). Galtier (1998) determined that DON transfer into cow milk is small (0.22 %). After a single intravenous dose of 4,000 μg DON/kg body weight administered to ewes, Prelusky et al. (1987) determined 61 μg/L of DON and 1,220 μg/L of DOM-1 in their milk. After the application of DON doses of 16,500 to 18,860 μg DON/kg b.w., the maximal DON concentration in milk was 17 μg/L, while that of DOM-1 equalled to 205 μg/L. Charmley et al. (1993) concluded that diets containing DON in concentrations of up to 6 mg/kg did not reduce cow feed intake and that DON and deepoxydeoxynivalenol were not transferred into milk. Higher DON concentrations observed in this study in some milk samples can be linked to an extremely high contamination evidenced in some of the maize silage and feed samples. Observed results can also be associated with possible liver dysfunction that impaired cow metabolism and resulted in the lesser conversion of DON into its metabolite DOM-1. In that case, the ELISA method used within the frame of this study is not specific enough to be able to determine it, as proclaimed also by the kit manufacturer.

Table 4. *Fusarium* mycotoxins in cow milk sampled in different Croatian regions during 2015

| Mycotoxin | Region | No of positives | % of positives[a] | Mean ±SD (μg/L) | Median (μg/L) | Range of positives (μg/L) |
|---|---|---|---|---|---|---|
| DON | Central (n = 29) | 5 | 17.2 | 28.8±30.6 | 8.1 | 5.6-67.3 |
| | Eastern (n = 34) | 4 | 11.8 | 27.2±24.8 | 25.6 | 5.7-52.0 |
| | Western (n = 14) | 3 | 21.4 | 19.4±10.9 | 22.7 | 7.2-28.3 |
| | Northern (n = 28) | 3 | 10.7 | 9.1±6.3 | 5.5 | 5.4-16.4 |
| | In total (n = 105) | 15 | 14.3 | 21.1±18.2 | 8.1 | 5.4-67.3 |
| ZEN | Central (n = 29) | 27 | 93.1 | 8.3[b]±22.3 | 1.3 | 0.3-88.6 |
| | Eastern (n = 34) | 33 | 97.1 | 3.8[b]±11.1 | 1.6 | 0.5-60.3 |
| | Western (n = 14) | 13 | 92.9 | 2.0[b]±2.5 | 1.5 | 1.2-14.0 |
| | Northern (n = 28) | 26 | 92.9 | 7.8[a] ±12.0 | 3.6 | 1.3-48.0 |
| | In total (n = 105) | 99 | 94.3 | 5.5±30.5 | 1.6 | 0.3-88.6 |

n - the number of analysed samples; DON - deoxynivalenol; ZEN - zearalenone;
[a]Samples in which mycotoxin concentrations were higher than the limit of quantification (LOQ);
Results tagged with different letters ([a,b]) are statistically significantly different ($p<0.05$)

The mean representation of ZEN positive samples obtained in this study was 94.3 %, the toxin concentrations thereby spanning from 0.3 to 88.6 μg/L. The maximal mean value of 8.3±22.3 μg/L was determined in the central Croatia, while the lowest mean value of 2.0±2.5 μg/L was found in the samples coming from the Western region. Statistical analysis showed a statistically significant difference ($p<0.05$) in ZEN concentrations between the Croatian regions under study, the Northern region thereby being pinpointed as significantly different from the others. According to the 2004 EFSA opinion statement, ZEN has a limited tissue deposition and a low transmission rate into milk (EFSA, 2004). In accordance with the aforementioned, low ZEN levels in cattle and sheep liver, meat, milk and cheese have been reported. However, it was also observed that ZEN transfer into milk varies in its carry- over rates (Coffey et al., 2009). In the UK, ZEN was detected in 3 % of milk samples at levels ranging from 1.2 to 5.5 μg/L (EC, 2003). The maximal obtained concentration was 12.5 μg/L (El-Hoshy, 1999). Usleber et al. (1992) concluded that ZEN contamination is low even after high oral ZEN doses. But in contrast to these results, Mirocha et al (1981) found a high level of ZEN and its metabolites in concentration of 1,359 μg/L seven days after the administration of 200 mg ZEN daily, given to dairy cattle.

Mirocha et al (1981) estimated the transfer rate of ZEN and its metabolites into milk to be 0.05 %. Yiannikouris and Jouany (2002) reported on ZEN transfer rates of 0.06 %, 0.016 % or 0.008 %, dependent on the toxin intake. Other studies have claimed these rates to be 0.00625 % and 1.924 % (Galtier, 1998). Winkler et al. (2015) estimated the rate of transfer of ZEN and its metabolites into milk to be 0.008 %. In view of the above, it can be concluded that human exposure to ZEN coming from milk is not to be considered a health risk, but studies have also pointed out that the toxicity of ZEN metabolites should be taken into consideration, for example that of α-zearalenol, whose oestrogenic potential is three-fold higher than that of ZEN (Mirocha et al., 1981). Since *in vitro* studies have evidenced that the main representative of fumonisines, fumonisin B1 (FB1), was poorly metabolized in the rumen (Caloni et al., 2000), it was concluded that FB1 could reach milk (Flores-Flores et al., 2015). In the study by Richard et al. (1996), after the implementation of dietary equivalent of FUM of approximately 75 mg/kg and the average of 3 mg FB1/kg b.w./day, methods having a sensitivity of 5 μg/L failed to detect FUM in any of the milk samples. Also, a transmission study on four cows dosed with pure FB1 either orally or using an intravenous injection, showed no detectable residues of FUM in milk (Scott et al., 1994). Maragos and Richard (1994) reported FB1 presence in only 1 out of 155 analyzed samples found in the concentration of 1.29 μg/L, whereas in the study by Gazzotti et al. (2009) FB1 was found in 8 out of 10 analysed milk samples in the maximal concentration of 0.43 μg/L. Bottom-line, it has been concluded that in dairy cows the appearance of FUM in milk, or their carry-over from feed to milk, is not significant and does not represent a hazard or food safety concern when it comes to humans (Richard et al., 1996; EFSA, 2005).

However, in the recently published review, Flores-Flores et al. (2015) pointed out that, given that only a few studies investigated the possibility of carry-over of FUM from feed to milk and the obtained results were contradictory, more investigations of FUM contamination of milk on a large number of samples were needed. In the present study, FUM was not detected in any of the milk samples. Although a low FUM presence in milk samples analysed within this study cannot be dismissed, the LOQ of 25.3 μg/L established for the ELISA method employed within this study frame renders the detection of low FUM levels in milk impossible. Therefore, our further research shall seek for a more sensitive confirmatory technique capable of determining lower concentrations of this analyte in milk.

The mean and the maximum concentrations determined for DON and ZEA (no positives for FUM) were compared against the TDI values established for these mycotoxins, taking into account the available data on milk consumption across the Croatian population. The results descriptive of the estimated *Fusarium* mycotoxin intake through the milk consumption path are shown in Table 5.

When it comes to the mean and the maximal concentration determined in milk, the calculations showed the DON intake (expressed as the percentage

of the TDI) to be 9.7 % and 31.0 %, respectively. The ZEN intake, expressed as the percentage of the TDI calculated based on the mean ZEN concentration determined within this frame was 12.9 %, but when the calculation used the maximal concentration determined in milk as the rationale, the obtained value was 203.6 %. Given that FUM were not detected in any of the milk samples, but taking also into account that FUM concentrations higher than 25.3 μg/L (i.e. the LOQ of the ELISA assay) were not possible to detect by the used method, we insofar presume that milk is not a significant source of these mycotoxins and, thus, does not pose a threat to human health.

Since milk represents only one component of human diet and in view of the fact that *Fusarium* mycotoxins can be present in a number of food groups, for instance cereals, their total intake (i.e. the percentage of the TDI actually entering the body) could be higher than estimated herein. Also, when it comes to specific population such as children, who consume higher amounts of milk a day, mycotoxin intakes could also be higher than here stated. In light of the foregoing, it is evident that the prevention of contamination of feedstuffs and foodstuffs with *Fusarium* mycotoxins is of a great importance for the protection of public health. To prevent their presence, the identification of key critical control points, which include production, processing and storage of food and feed, is essential. Measures taken to the above effect require the application of effective techniques capable of reducing the mycotoxin presence in food and feed or of decontaminating the latter. Systematic control over *Fusarium* mycotoxins using modern analytical methods should be implemented in order to prevent contamination of the entire food/feed chain from farm to table.

## Conclusions

Levels of *Fusarium* mycotoxins in dairy cattle feedstuffs higher than recommended were evidenced for ZEN and DON and linked to extremely rainy conditions during the period of cereal growth and harvesting seasons. Given the TDIs defined for these mycotoxins, human health risks arising from the consumption of cow milk can be considered low, except for samples in which the maximal ZEN concentration was observed. Further studies are needed to determine a carry- over effect from commodities and dairy cattle feed products to cow milk, especially when it comes to DON and ZEA. Analytical method used for the determination of FUM should be of a higher sensitivity, so as to be able to detect lower concentrations of these contaminants in milk.

Table 5. Estimation of *Fusarium* mycotoxin intake through the milk consumption path, expressed as the percentage of the Tolerable Daily Intake (TDI)

| Mycotoxin | TDI[a] (μg/kg bw/day) | Region | Mean concentration (μg/L) | % of TDI | Maximal concentration (μg/L) | % of TDI |
|---|---|---|---|---|---|---|
| DON | 1 | Central | 28.8 | 13.3 | 67.3 | 31.0 |
| | | Eastern | 27.2 | 12.6 | 52.0 | 23.9 |
| | | Western | 19.4 | 8.9 | 28.3 | 13.1 |
| | | Northern | 9.1 | 4.1 | 16.4 | 7.6 |
| | | In total | 21.1 | 9.7 | 67.3 | 31.0 |
| ZEN | 0.2 | Central | 8.3 | 19.3 | 88.6 | 203.6 |
| | | Eastern | 3.8 | 8.6 | 60.3 | 138.6 |
| | | Western | 2.0 | 4.6 | 14.0 | 32.1 |
| | | Northern | 7.8 | 17.9 | 48.0 | 110.7 |
| | | In total | 5.5 | 12.9 | 88.6 | 203.6 |

DON - deoxynivalenol; ZEN - zearalenone;
[a]Tolerable Daily Intakes published in EC 856/2005

## *Prisutnost mikotoksina roda Fusarium u krmivima i kravljem mlijeku uzorkovanim sa hrvatskih farmi tijekom 2015. godine*

**Sažetak**

Mikotoksini mogu kontaminirati hranu životinjskog podrijetla putem *carry-over* efekta i predstavljaju potencijalni rizik za ljudsko zdravlje. Problem kontaminacije mikotoksinima iz roda *Fusarium* izražen je naročito tijekom kišovitih godina, koje ujedno karakteriziraju i značajne promjene temperature. Cilj ovog istraživanja bio je ispitati razinu fuzarijskih mikotoksina zearalenona (ZEN), deoksinivalenola (DON) i fumonizina (FUM) u kukuruznoj silaži (n=21), koncentriranoj hrani za mliječne krave (n=56) i uzorcima kravljeg mlijeka (n=105), uzorkovanih tijekom 2015. godine sa obiteljskih poljoprivrednih gospodarstava iz četiri hrvatske regije. Prisutnost mikotoksina određena je primjenom ELISA validiranih metoda. Utvrđena je visoka razina kontaminacije stočne hrane posebno za ZEN, s vrijednostima većim od preporučenih u 9,5 % uzoraka kukuruzne silaže. Na DON je bilo pozitivno 14,3 % uzoraka mlijeka, s koncentracijom u rasponu od 5,4 do 67,3 $\mu$g/L. ZEN je određen u 94,3 % uzoraka mlijeka, u rasponu od 0,3 do 88,6 $\mu$g/L. FUM nije određen u niti jednom od analiziranih uzoraka mlijeka. S obzirom na prihvatljiv dnevni unos (TDI) definiran za ove mikotoksine, zdravstveni rizik za ljude koji proizlazi iz konzumacije kravljeg mlijeka općenito se može smatrati niskim, čak i tijekom razdoblja koja karakteriziraju vremenski uvjeti pogodni za proizvodnju fuzarijskih mikotoksina u žitaricama, a koje se nadalje koriste kao hrana za muzne krave. Izuzetak predstavljaju pojedini uzorci mlijeka u kojima su određene visoke koncentracije ZEN.

*Ključne riječi*: mikotoksini roda *Fusarium*, kravlje mlijeko, mliječna goveda, krmiva, hrvatske farme

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