Bull Environ Contam Toxicol (2011) 86:294–297
DOI 10.1007/s00128-011-0219-x

Determination of T-2 and HT-2 Toxins in Commodities and Feed in Croatia

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Received: 4 October 2010 / Accepted: 8 February 2011 / Published online: 18 February 2011

Abstract Data on the occurrence of T-2 and HT-2 toxins in commodities and feed in Croatia and in Europe are very limited. In the present study, 50 feed and commodity samples tested for the presence of these mycotoxins yielded highest concentrations in cattle feed (67.68 ng/g) and oat samples (32.94 ng/g). In the future, screening should include greater numbers of feed and commodity samples to shed more light on the presence of T-2 and HT-2 toxins.

Keywords T-2 HT-2 · Feed · Commodities

A variety of fungi from the genus *Fusarium* produce a number of different mycotoxins, which are secondary metabolites of fungi capable of producing acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic and estrogenic effects on animals (Van Egmond 2004). *Fusarium* mycotoxins are divided into several classes: trichothecenes, fumonisins and zearalenone (European Commission, Scientific Committee on Food 2002). Trichothecenes form a group of more than a hundred compounds, which all contain a 12,13-epoxytrichothene skeleton (Bennett and Klich 2003; Milanez et al. 2006; Boermansn and Leung 2007).

T-2 and HT-2 toxins are type A trichothecene mycotoxins produced by *Fusarium (F.) poae, F. sporotrichioides, F. kyushuense* and *F. langsethiae* (Glenn 2007). The major producer of T-2 and HT-2 toxins is *F. sporotrichioides*, which grows at −2–35°C and in water activities above 0.88 (Richard 2007; Creppy 2002). Therefore, *F. sporotrichioides* is prevalent in North America, South America, Asia, Africa and Europe (Richard 2007). Due to the broad prevalence of this fungus, many different crops including corn, oat, barley, wheat, rice and soya beans can be infected with T-2 and HT-2 toxins.T-2 toxin is a very potent cytotoxic and immunosuppressive toxin, which can cause acute intoxication and chronic diseases in both humans and animals (Ueno et al. 1983; Peraica et al. 1999; Zhou et al. 2008). The symptoms of acute intoxication are nausea, vomiting, abdominal pain, diarrhea, bloody stools and weight loss. In animals, symptoms also include decreased production of milk or eggs, increased incidence of cracked eggs and oral lesions in poultry (Morgavi and Riley 2007). The major effect of T-2 toxin is inhibition of protein synthesis, which leads to secondary disruption of DNA and RNA synthesis (Richard 2007; Creppy 2002; Bennett and Klich 2003). The immune system is also a target of T-2 toxin, and the effect includes changes in leukocyte count, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection and blastogenic response to lectins (Creppy 2002). HT-2 toxin is a metabolite of T-2 toxin and is formed in microbial transformation via deacetylation reaction (Zhou et al. 2008). This reaction is performed by several intestinal microorganisms in different animals (Young et al. 2007; Fuchs et al. 2002). The high toxicity of T-2 and HT-2 has led to defining a tolerable daily intake (TDI) value of 0.06 μg/kg bw/day for humans.

Data on the occurrence of these mycotoxins in Europe are very limited (Commission Recommendation 2006). Considering the high toxicity of both T-2 and HT-2 toxins, there is the need to collect more data on their occurrence and to set up legal limits for these mycotoxins (Meister 2008; Scientific Commitee on Food 2002). In Croatia, Sokolović and Šimpraga have reported a survey on trichothecene mycotoxins performed by use of thin layer

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chromatography during the 1998–2004 period, however, without records on HT-2 toxin (Sokolović and Šimpraga 2006).The aim of this work was to perform a screening for T-2 and HT-2 toxins in commodities and feed during a 1 year period using enzyme-linked immunosorbent assay (ELISA) as a sensitive screening method for quantitative determination.

Materials and Methods

A Ridascreen® T-2/HT-2 Toxin kit for ELISA was provided by R-Biopharm (Darmstadt, Germany). Each kit contains a microtiter plate with 96 wells coated with capture antibodies; standard solutions (0, 0.25, 0.75, 1.5, 3.0 and 9.0 ng/mL); peroxidase conjugated T-2 toxin; anti-T-2/HT-2 antibody; substrate/chromogen solution; stop solution and washing buffer (salt). All other chemicals used in the analysis were of analytical grade. ELISA was performed by ChemWell (Awareness Technology, Inc., USA).

All samples, both commodities and feed, were collected within the scope of the National Control Program at farms in different regions of Croatia. A total of 50 samples, 25 commodity samples and 25 feed samples, were analyzed. Commodities included wheat, corn, oat, barley and soya samples, while feed included pig, poultry and cattle feed.

Samples were ground on an analytical mill (Cylotec 1093, Tecator). After grinding, 5 g of sample was extracted with 25 mL of methanol/distilled water (70/30; v/v) solution. The extraction was performed within 10 min on a magnetic stirrer, followed by extract filtering through a filter paper (Whatman, Black Ribbon). The filtrate obtained was diluted at 1:2 ratio with distilled water. Diluted filtrate was applied onto ELISA kit wells. When calculating the concentration of T-2/HT-2 toxins in samples, the results obtained from the calibration curve were multiplied by the corresponding dilution factor.

Competitive ELISA was performed as described in the manufacturer's instructions. All standards and samples were analyzed in duplicate. Fifty µL of diluted enzyme conjugate and 50 µL of diluted anti-T-2/HT-2 toxin antibody were added to 50 µL of standards and prepared samples. Microwell holder was mixed gently and incubated for 1 h at room temperature. After incubation, the wells were washed 3 times with 250 µL of washing buffer. Then, 100 µL of substrate/chromogen solution was added to each well and incubated for 15 min at room temperature. The reaction was stopped by adding 100 µL of stop solution and absorbance was measured at 450 nm.

Statistical data analysis was performed by use of the Statistica Ver. 6.1. software (StatSoftInc. 1984–2003, USA).

Results and Discussion

The high toxicity of T-2 and HT-2 toxins, and monitoring of these toxins in other EU countries have resulted in launching the National Control Program for these mycotoxins in Croatia. In the present study, 25 feed samples collected from different feed factories in Croatia were analyzed by ELISA method. A typical ELISA calibration curve of absorbance (%) versus concentration (ng/mL), obtained by measuring absorbance at 450 nm, is presented in Fig. 1.

T-2/HT-2 were detected in 19 of 25 feed samples. The mean (±SD) and maximum concentrations of T-2/HT-2 in feed samples are presented in Table 1.

The concentrations of T-2/HT-2 in feed samples ranged from 10.47 ± 4.38 to 25.79 ± 29.29 ng/g. The highest concentration of 67.68 ng/g was detected in cattle feed and lowest concentration of 6.09 ng/g in poultry feed. Other authors report on T-2 toxin concentration in poultry feed of 130 ± 30 ng/g (Lincy et al. 2008) and 13 ± 22 ng/g (Labuda et al. 2005). In Croatia, Sokolović and Šimpraga (2006) have reported on T-2 toxin concentrations of 100–500 ng/g in undefined feed samples. All results obtained in our study were below the maximum allowed level (500 ng/g), set in the Croatian legislative until 2010 (Official Gazzete of the Republic of Croatia 2007). As the results were below the maximum allowed level, there was no need to use a confirmatory method. Our results were below the maximum allowed levels regulated in other countries. In Canada, the maximum allowed level is 100 ng/g HT-2 toxin in cattle and poultry feed; in Israel, 100 ng/g T-2 toxin in all feed, and in Russia in cereals, wheat flour and bran (Milanez et al. 2006), whereas

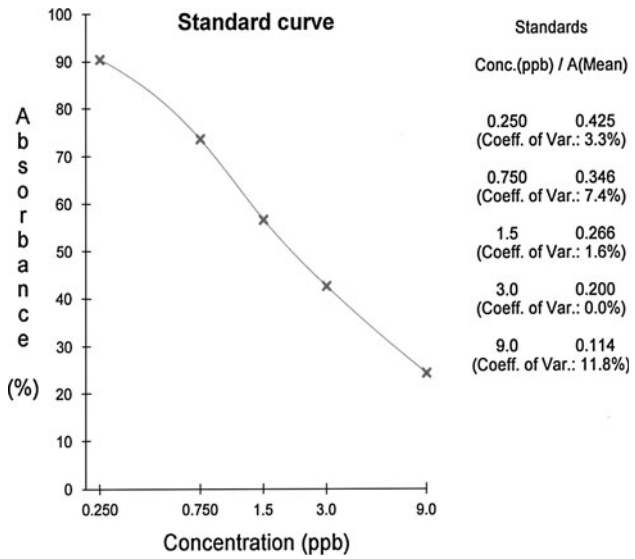

Fig. 1 Typical ELISA calibration curve for T-2/HT-2 determination

Table 1 Mean (± SD) and maximum T-2/HT-2 concentrations (ng/g) determined in feed samples

Feed type	n (Number of samples)	Maximum (ng/g)	Mean ± SD (ng/g)
Pigs feed	16[a]	29.98	11.91 ± 10.11
Cattle feed	4	67.68	25.79 ± 29.29
Poultry feed	5	16.04	10.47 ± 4.38

[a] in 6 samples T-2/HT-2 were not detected

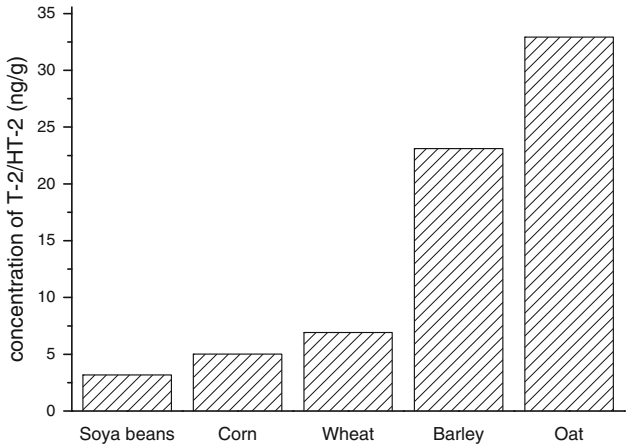

Fig. 2 Maximum concentrations (ng/g) of T-2/HT-2 toxins determined in commodities

Slovenia and Ukraine have even higher maximum allowed levels of 1,000 and 250 ng/g, respectively (Wu 2007). The results obtained in our study are below the range reported previously in Croatia (Sokolović and Šimpraga 2006).

Maximum concentrations (ng/g) for different types of commodities, ranking from lowest to highest, are presented in Fig. 2.

The concentrations of T-2/HT-2 toxins in commodities ranged from 2.61 to 32.94 ng/g. The highest concentration (32.94 ng/g) was determined in oat sample from a feed factory in the north of Croatia. In their study, Binder et al. (2007) determined the presence of T-2 toxin in 26 oat samples from Europe and Mediterranean region, and report on the mean and maximum concentration of 124 ng/g and 814 ng/g, respectively. Schollenberger et al. (2005) report on T-2 and HT-2 concentrations in oat samples marketed in Germany of 6–11 and 5–23 ng/g, respectively. Mankevičiene et al. (2007) report on T-2 toxin concentration in oat samples from Lithuania of 17.2–121.5 ng/g in 2004 and 10.8–41.8 in 2005. In Norway, Langseth and Rundberget (1999) found the mean HT-2 and T-2 concentrations in oat samples of 115 and 60 ng/g, respectively. Besides oat, barley is grain that is often contaminated with T-2 and HT-2 toxins. Binder et al. report on a T-2 toxin level of 921 ng/g in barley, which is even higher than in oat samples. Sokolović and Šimpraga (2006) report on the levels of T-2 toxin ranging from 100 to 700 ng/g in different types of commodities in Croatia. Langseth and Rundberget (1999) have reported lower concentrations of T-2 and HT-2 toxins. In their study, the highest T-2 and HT-2 concentrations recorded in barley samples were 85 and 73 ng/g, respectively.

In our study, the maximum concentration found in barley samples was 23.11 ng/g. In other commodities, the maximum T-2/HT-2 concentrations were 6.91 ng/g in wheat, 5.02 ng/g in corn, and 3.17 ng/g in soya beans.

In conclusion, the concentration of T-2/HT-2 toxins in all study samples was below 500 ng/g as the maximum allowed level in the Republic of Croatia until 2010. In the future, screening should include greater numbers of feed and commodity samples, oat in particular, to shed more light on the presence of T-2 and HT-2 toxins. Information on the composition of feed samples will be highly useful to establish the possible association between the presence of T-2 and HT-2 toxins and feed ingredients. In addition, there is a need to develop a more sensitive method for T-2/HT-2 toxin determination and confirmation.

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