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Exposure assessment to mycotoxins in gluten-free diet for celiac patients

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ABSTRACT

Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium*, which attack crops in the field, and grow on foods also during storage under favorable conditions of temperature and humidity. Foods mainly contributing to the intake of mycotoxins with diet are cereals, maize being the most risky commodity due to the potential co-occurrence of more than one mycotoxin, this can be of particular concern especially for vulnerable group of population such as celiac patients that show increased maize-based products consumption. In this study the exposure of celiac patients to fumonisins (FBs) and zearalenone (ZON) has been assessed. The higher exposures, for all the matrices and for both the selected mycotoxins, were for children age group. The lower and upper bound exposure ranged between 348–582 ng/kg bw/day for FBs and 22–83 ng/kg bw/day for ZON; these values result well below the TDI for the selected mycotoxins, representing the 17–29% and 9–33% of the TDI set for FBs and ZON, respectively. Even considering the worst scenario the exposure values reported for children were lower, namely 1385 ng/kg bw/day for FBs and 237 ng/kg bw/day for ZON, than the corresponding toxicological thresholds.

1. Introduction

Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi of genera *Aspergillus*, *Penicillium* and *Fusarium*, which, under favorable conditions of temperature and humidity, attack crops in the field and grow on food commodities also during storage. Consequently, consumers can be exposed to mycotoxins either directly by ingesting contaminated foods or indirectly by consuming animal origin products derived from animals exposed to mycotoxins by feed. Thus, the metabolism of ingested mycotoxins can result in their accumulation in different organs and tissues, potentially affecting human health since mycotoxins may enter into the whole human food chain through a wide spectrum of foodstuffs such as cereals, meat, milk, wine, beer, dried fruits and spices.

Mycotoxins are regularly implicated in toxic syndromes in animals and humans (Smith et al., 1995; Berry, 1998). The toxic properties of mycotoxins associated with animals and human include genotoxicity, carcinogenicity, teratogenicity, mutagenicity, nephrotoxicity and immunotoxicity. In particular, the specific immunotoxic action leads to depression of the lymphocytes activity, suppression of antibody production and damage to the functionality of macrophages and neutrophyles (Milićević et al., 2010).

The most relevant mycotoxins found in foods are aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (type A: T-2 and HT-2 toxin, and type B: deoxynivalenol), zearalenone (ZON) and fumonisins B_1 (FB_1) and B_2 (FB_2). Mycotoxins are estimated to affect as much as 25% of the world's crops each year (Lawlor and Lynch, 2005). Foods that mainly contribute to the intake of mycotoxins with diet are cereals, accounting for 50%, followed by alcoholic beverages, dried fruits, cocoa and coffee (SCOOP Task, 2002, 2006). Among cereals, maize is the most risky commodity and the potential co-occurrence of more than one mycotoxin can be of particular concern especially for vulnerable groups of population that result high consumers of maize-based products due to regional diet habits or to specific pathologies like celiac disease.

Many countries have adopted regulations to limit the mycotoxin exposure. According to the annual report of the Rapid Alert System for Food and Feed (RASFF), in 2012 mycotoxins were the main hazard in border rejection notification in the European Union (RASFF, 2012).

A full review by Marín et al. (2013) reported several dietary exposure studies to mycotoxins.

The estimate of population exposure to mycotoxins is traditionally based on food consumption patterns and mycotoxin

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contamination levels. However, for estimates of individual exposure such approaches are less useful, particularly in light of heterogeneity in contamination and variations in food processing and cooking (Wild and Gong, 2010). The use of biomarkers in risk assessment studies provides different information related to the biological responses to the intake of a mycotoxin (biomarkers of effect), the quantitative detection of the parent toxin and its metabolites (biomarkers of exposure) or the indication of the variabilities of susceptibility of an organism to the effects of an exposure to a toxic compound such as mycotoxins (biomarkers of susceptibility).

Basically, the biomarker measurement is an additional approach with clear advantages in reducing uncertainties in risk assessment. Conversely, the not yet ascertained metabolic pathways for some mycotoxins, the unknown bioavailability of the parent compound, the lack of availability of commercial reference standards of metabolites constitute real disadvantages in using this approach. In addition, epidemiological studies, bioinformatics and advanced statistical methods are needed to substantiate the findings. Anyway, biomarkers will not replace traditional approaches used in risk assessment, but should be considered as an additional approach.

Recently the estimation of dietary intake has been also focused on vulnerable groups and particular attention was devoted to children, vegetarian people and sub-groups of population, warning about possible higher exposure due to unfavorable body weight/intake ratio, dietary habits or pathologies (Cano-Sancho et al., 2012; Dall'Asta et al., 2012; Ostrý and Ruprich, 1998).

In this study, the exposure of celiac patients to the main maize contaminating mycotoxins was evaluated; based on the occurrence data obtained, exposure calculations were conducted for FBs and ZON. This is the first study that assess the celiac patients exposure to ZON, while few works, focused on the exposure assessment to fumonisins of celiac patients, are present in literature (Cano-Sancho et al., 2012; Dall'Asta et al., 2012; Ostrý and Ruprich, 1998). The present study was performed in the framework of an ongoing three years project in cooperation with, and supported by the Italian Celiac Association.

2. Materials and methods

2.1. Sample

Gluten-free (GF) products were purchased from the Italian market. All products were collected from retail shops and pharmacies specialized in dietetic foods. Almost all products were foods specifically formulated for coeliacs of the Italian National Register of gluten-free products. In addition, also simple maize flour samples were collected. A total amount of 376 gluten-free samples were purchased, namely 133 GF pasta of different shapes, 109 GF savory snacks including crackers, nachos and breadstick, 87 GF bread and 47 GF flour to be used in recipes (polenta, pizza, cakes, breadcrumbs). Most of the collected samples were maize-based products. All the information about the sample composition was obtained from the labels. Samples were randomly selected collecting as many as possible leader and minor brands available on the market.

2.2. Determination of mycotoxins

All the collected samples were analyzed for the determination of aflatoxin B_1 (AFB_1), ochratoxin A, fumonisins B_1 e B_2 (FB_1, FB_2), deoxynivalenol (DON), zearalenone and toxins T-2 and HT-2. The selected mycotoxins were simultaneously determined according to Brera et al. method (submitted for publication). Briefly, the ground samples were extracted with AcCN:MeOH:H_2O (20:20:60, v:v:v), the extract was then centrifuged, filtered and injected in the UPLC–ESI MS/MS system without any further purification step.

The method was in-house validated on GF pasta and GF bread matrices. The method performances, including precision and accuracy, were evaluated by analyzing 6 replicates for two spiked levels. The in-house validation results were compliant with the criteria reported in the European Commission Regulation 401/2006 (EC, 2006a).

2.3. Exposure assessment

The exposure was calculated by a deterministic approach combining the mycotoxin contamination value with the food consumption divided by the body weight (EFSA, 2011a; WHO, 2009). The exposure assessment calculations were performed according to four age categories: children (3–9.9), teenagers (10–17.9), adults (18–64.5) and elderly ($\geqslant$65); with the exception of children age group, also the gender was evaluated. For each category lower and upper bound exposures were assessed. Exposure calculations at the 95th percentile (P95) of consumption were also performed with the aim of evaluating a worst-case scenario.

2.3.1. Consumption data

In literature, few consumption data expressly assessed for celiac patient exist (Dall'Asta et al., 2012; Gibert et al., 2006), but they do not really fit with the purpose of this work; therefore the Authors preferred to refer to the food consumption survey published by the Italian Institute for Nutrition (INRAN) (Leclercq et al., 2009), which is highly representative of the Italian population in terms of geographical area considered and of the number of households involved in the survey (3323 respondents involved). The basic assumption of this choice is that the celiac patient has the same diet habits of the population involved in the survey, with the substitution of the gluten-based foods with GF products specifically formulated for celiac patients (pasta, bread, etc.). This assumption is also supported by the consumption data reported, for Italian celiac patients, by Gibert et al. (2006), which are in line with the assumed substitution.

2.3.2. Concentration data

The concentration data used for exposure calculation were derived from the analyses conducted within this study. As regards the management of left-censored data, a substitution method was applied (EFSA, 2010; GEMS/Food-EURO, 1995) generating lower and upper bound contamination values by substituting with zero or LOQ (Limit Of Quantification) value the results reported to be below the LOQ. As a consequence lower bound and upper bound exposures were assessed.

3. Results and discussion

3.1. Mycotoxin occurrence

The results obtained from the analysis of the collected samples, are reported in Table 1. The mean contamination reported in Table 1 is calculated attributing LOQ/2 value to all the samples reported to be lower than LOQ for all considered mycotoxins. As reported in Table 1, none of the analyzed samples was contaminated by AFB_1, despite it often occurs in maize and maize-based foods. Only few samples were found positive for OTA, DON and T-2+HT-2 content. As regards the OTA contamination, some of the positive samples exceeded the maximum level set by the European legislation (EC, 2006b). However, the positive samples ranged between the 2% and 6% of the analyzed matrices and the mean value reported was well below the legal limit set for OTA. Also for DON contamination, one snack sample was found to contain about the double of the reported legal limit, but similarly to the OTA situation, the mean value for the snack samples was well below the legal limit. As regards the FBs content, the 29% of the analyzed samples resulted contaminated, the presence of FBs was detected in all the selected food categories. However, the number of positive samples was below the occurrence reported in other studies (Dall'Asta et al., 2009, 2012; Lo Magro et al., 2011) where the percentage of contaminated samples ranged between 54% and 88%. Zearalenone contamination was found in all the investigated food categories with the exception of GF pasta. The positive samples represented the 11% of the overall collected samples.

3.2. Exposure assessment

Due to the poor amount of positive samples for AFB_1, OTA, DON and T-2 and HT-2 contamination, the exposure assessment was performed only for FBs and ZON. The FBs exposure calculation was made for all food categories (i.e. pasta, bread, flour and snack), while for ZON, pasta was not included since none of the analyzed samples resulted contaminated. The exposure assessment for FBs and ZON is reported in Tables 2 and 3, respectively. To stress the