Biomonitoring of zearalenone and its main metabolites in urines of Bangladeshi adults

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ABSTRACT

The *Fusarium* toxin zearalenone (ZEN) is of concern due to its pronounced estrogenic effects in mammalian species. ZEN contaminates various cereal crops and grain-based food along with modified forms which contribute to overall mycoestrogen exposure. As no data exist on the occurrence of ZEN in food commodities consumed in Bangladesh, we have analyzed ZEN and its main metabolites α-and β-zearalenol (α-ZEL, β-ZEL) by targeted LC-MS/MS method as biomarkers of exposure in urines (n = 62) from rural and urban residents in Rajshahi district collected in two seasons and from a pregnant women cohort (n = 20) in Dhaka district. Average levels of α-ZEL, the far more potent estrogenic metabolite, were clearly higher than those of ZEN and β-ZEL. Biomarker levels in urban and rural residents showed some seasonal fluctuation: In winter urines, ZEN mean level was 0.040 ± 0.037, α-ZEL 0.182 ± 0.047 and β-ZEL 0.018 ± 0.016 ng/mL; in summer urines, ZEN mean was 0.028 ± 0.015, α-ZEL 0.198 ± 0.025 and β-ZEL 0.013 ± 0.005 ng/mL. In pregnant women, mean levels were: ZEN 0.057 ± 0.041, α-ZEL 0.151 ± 0.026 and β-ZEL 0.055 ± 0.057 ng/mL, thus similar to levels found in the Rajshahi cohort in winter season. Estimates of probable dietary mycoestrogen intake in the Bangladeshi adults reveal an exposure below the tolerable daily intake of 0.25 µg/kg b.w. set by EFSA.

1. Introduction

The mycotoxin zearalenone (ZEN) is produced by several *Fusarium* species which infect primarily crops in the field, e.g. corn (maize), wheat, barley, and other cereals, including rice (Maragos, 2010; Zinedine et al., 2007). Contamination of cereal-based food and feed is of concern due to the estrogenic activity of ZEN and its major metabolites α- and β-zearalenol (α- and β-ZEL), and undesirable effects on the endocrine system of humans and animals (reviewed in (EFSA, 2011; Metzler et al., 2010). Based on a no-observed-effect level (NOEL) for estrogenic effects in female piglets and an uncertainty factor of 40 (to account for inter- and intra-species variability), the EFSA Panel on Contaminants in the Food Chain derived for humans a tolerable daily intake (TDI) for ZEN of 0.25 µg/kg b.w/day (EFSA, 2011). In 2011, EFSA conducted also a detailed assessment of chronic dietary exposure, based on ZEN occurrence in major food categories and on food consumption data for different consumer groups. But, there were major uncertainties in this assessment, due to important data gaps for ZEN occurrence data (mostly left censored analytical data) and little consideration of special consumer groups and of biotransformation (Lorenz

et al., 2019; Mally et al., 2016). Biotransformation is important as estrogenic potency is known to differ considerably *in vitro* (e.g. Frizzell et al., 2011) and *in vivo* (Everett et al., 1987) with α-ZEL being 60 times more potent than ZEN, whilst β-ZEL is 5 times less potent than ZEN.

Then in 2014, in light of some new data on the presence of modified forms of ZEN (i.e. glucose and sulfate conjugates of ZEN, and glucosides of its reduced forms α- and β-ZEL in contaminated food commodities), EFSA doubled its previous intake estimates for ZEN alone to account for such additional sources of mycoestrogen exposure (EFSA, 2014). This was based on findings that modified forms (mainly ZEN-14-Sulfate, ZEN-14-Glucoside, ZEN-16-Glucoside, α-ZEL-14-Glucoside, β-ZEL-14-Glucoside) are hydrolyzed during digestion to the estrogenic parent compound and its metabolites α- and β-ZEL (Dall'Erta et al., 2013; Gareis et al., 1990), now corroborated by more recent *in vivo* and *in vitro* studies (Binder et al., 2017; Yang et al., 2018). In light of the complex exposure scenario, EFSA reassessed in 2016 the TDI for ZEN and defined it now as "group TDI" for ZEN and its modified forms (EFSA, 2016). The current health-based guidance value was set by considering relative estrogenic potency factors for ZEN and its phase I metabolites and assuming that conjugated fungal and plant metabolites are cleaved

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Table 1
Demographic characteristics of the urine donors.

Characteristics	Adult cohort (Rajshahi)			Pregnant women cohort (Dhaka)		
	All	Rural	Urban	All	Rural	Suburban
Participant(n)	62	30	32	20	11	9
Male	31	15	16	–	–	–
Female	31	15	16	20	11	9
Age (years)	39 ± 11 (60)	41 ± 12 (60)	36 ± 9 (57)	26 ± 5 (36)	27 ± 6 (36)	24 ± 4 (30)
BMI (kg/m^2)	22.6 ± 3.2 (32.9)	21.4 ± 2.6 (27.3)	23.8 ± 3.2[a] (32.9)	20.6 ± 2.7 (25.5)	21.6 ± 1.9[c] (25.5)	19.3 ± 3.1 (24.4)
Creatinine (g/L)[*]						
Summer	0.40 ± 0.44	0.40 ± 0.41	0.41 ± 0.47	–	–	–
Winter	0.80 ± 0.55[b]	0.72 ± 0.41	0.87 ± 0.66	0.53 ± 0.34	0.51 ± 0.23	0.55 ± 0.46

[*]Value presented as Mean ± SD.

In Rajshahi district. [c]$p < 0.01$ when compared to suburban cohort in Dhaka district. P-value obtained from independent sample *t*-test.

Values in parentheses indicate the maximum of the respective parameter.

[a] $p < 0.001$ when compared to rural cohort.

[b] $p < 0.05$ when compared to summer season.

in the gastrointestinal tract to the unconjugated forms (EFSA, 2016). This pragmatic approach is described in more detail in a recent review along with further research needs (Table 1 in (Lorenz et al., 2019). In short, comprehensive estimates of external exposure to ZEN and its modified forms are presently hampered by insufficient data on their occurrence in food commodities and a lack of commercially available analytical standards for quantifying the levels of all modified forms.

Human biomonitoring (HBM), *i.e.* analysis of ZEN and its main metabolites in body fluids, is considered an attractive approach to assess overall internal exposure and obtain also more insight into the biotransformation of modified forms (Lorenz et al., 2019). HBM requires suitable methods for detecting biomarkers of exposure (parent compound and relevant metabolites) in human sample material (urine or blood) and also some knowledge on the toxicokinetics of the mycotoxin under investigation (Ali and Degen, 2018). ZEN itself is rapidly absorbed after oral intake and metabolized by α-and 3β-hydroxysteroid dehydrogenases to the stereoisomers α-ZEL and β-ZEL, the major phase I metabolites (Mally et al., 2016; Metzler et al., 2010; Zinedine et al., 2007). ZEN, α-ZEL and β-ZEL are converted by uridine diphosphate-glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes in liver and intestinal cells to polar conjugates (phase II metabolites), the predominantly excreted forms in urine. Other known metabolites, resulting in either from CYP-mediated aliphatic or aromatic hydroxylation of ZEN or from a reduction of the olefinic double bond in ZEN, α-ZEL and β-ZEL to the fully reduced forms (ZAN, α-ZAL, β-ZAL) are quantitative of minor importance (EFSA, 2017; EFSA, 2011).

Thus, analysis of ZEN, α-ZEL and β-ZEL (aglycones plus conjugates) in urine provides a good and comprehensive approach to monitor human (and animal) exposure to mycoestrogens. Methods applied for biomarker analysis in human urine (reviewed in Mally et al., 2016) can be grouped in two main categories, according to sample preparation prior to LC-MS/MS analysis in: a) direct 'dilute-and shoot' approaches without prior enzymatic hydrolysis of conjugates which minimize sample preparation, but require (commercially not available) standards for the glucuronide/sulfate conjugates, and b) indirect approaches which determine the total aglycones after enzymatic deconjugation with β-glucuronidase/sulphatase and sample clean-up with enrichment of analytes by immunoaffinity and/or solid phase extraction. Comparative analysis of human urines from a South African and a Nigerian cohort (by both approaches) found far higher percentages of positive detects for the indirect method due to higher sensitivity (Šarkanj et al., 2018; Shephard et al., 2013). Thus, in our recent study, a targeted LC-MS/MS-based indirect method has been used to determine ZEN and its metabolites α-ZEL and β-ZEL ('total ZEN') in urines from German adults (Ali and Degen, 2018).

Up to now, no data exist on the occurrence of ZEN and its modified forms in food commodities consumed in Bangladesh. Thus, the present study conducted biomonitoring by a targeted method to assess exposure to mycoestrogens in the Bangladeshi population. Samples collected earlier for biomarker-based studies of other mycotoxins (Ali et al., 2015b, 2015a; 2016a) were used now for ZEN biomarker analysis, *i.e.* urines from rural and urban residents in Rajshahi district collected in two seasons and from a pregnant women cohort in Dhaka district. Our new results will be discussed in the context of biomarker data from other countries and with regard to provisional dietary intake estimates for ZEN and its modified forms in relation to the current 'group TDI' set by EFSA.

2. Materials and methods

2.1. Chemicals and materials

Certified standard solutions of ZEN and U-[$^{13}C_{18}$] ZEN were purchased from Romer Labs (Tulln, Austria); α-ZEL and β-ZEL were obtained from Sigma–Aldrich (Taufkirchen, Germany). All standard solutions were stored at −80 °C. The β-glucuronidase/arylsulfatase (β-Gluc/ArylS) enzyme from *Helix pomatia* (with specific activity 5.5 U/mL β-Gluc, 2.6 U/mL ArylS at 37 °C) was from Roche Diagnostics (Mannheim, Germany) and used with 10-fold hydrolysis buffer (13.6 g sodium acetate hydrate, 1.0 g ascorbic acid, 0.1 g EDTA in 100 mL deionised water, adjusted to pH 5.0 with acetic acid 98%) for treatment of urine samples. The working standard solutions were prepared weekly as dilutions in methanol/water (1:1, v/v) in a range from 0.01 to 5 ng/mL. ZearalaTest™ (VICAM) columns were purchased from Ruttmann (Hamburg, Germany). Acetonitrile, methanol and acetic acid were of LC/MS grade (Merck, Darmstadt, Germany).

2.2. Sampling areas and study populations

Urine samples were collected in two seasons (May 2013, and February 2014) from adult volunteers who are either residents of a rural area (Mongol Para, Puthia) or an urban area (Rajshahi University region) in the Rajshahi district of Bangladesh. Overall, 62 volunteers provided urines in both sampling rounds to account for possible seasonal fluctuations in exposure (see Ali et al., 2016b, 2016a). Furthermore, urine samples from pregnant women of a rural and a suburban area of Savar region in Dhaka district were collected in winter (February–March 2014) for mycotoxin analysis (see Ali et al., 2016c, 2015a). Samples of the volunteers in Rajshahi district who enrolled in both sampling periods and the urine aliquots of pregnant women (n = 20) residents in Dhaka district were analyzed for ZEN biomarkers. All participants had been informed about the study and written consent was obtained prior to inclusion in the study. Individual's body weight and height were measured by trained personnel and recorded in a short

questionnaire form along with other anthropometric information of the participants such as age, gender, occupation and food habits. First-morning urine samples collected into non-sterile disposable containers were stored at $-20\,°C$ at Rajshahi University and then sent on dry ice to IfADo, Dortmund and stored there at $-20\,°C$ for subsequent biomarker analysis. The study was approved by the Institute of Biological Sciences of Rajshahi University, Rajshahi-6205, Bangladesh and by the Institutional Internal Review Board of IfADo, the Leibniz Research Centre for Working Environment and Human Factors, Germany.

2.3. Creatinine analysis

Creatinine concentration in urine was determined to correct for differences in urine dilution between individual spot urines by a modified Jaffe method (Blaszkewicz and Liesenhoff-Henze, 2002). Creatinine analysis was carried out in a 96 well plate reader with an automated chemistry analyzer (TecanGenios®, Salzburg, Austria). The mean levels of urinary creatinine are given in Table 1; levels of most individuals were in the range of values (0.3–3.0 g/L) considered acceptable by WHO for biomonitoring (WHO, 1996). The ZEN biomarker concentrations in urines are also expressed as ng/mg creatinine to facilitate a comparison with values reported in other studies.

2.4. Sample preparation

All steps involving enzymatic hydrolysis of urines, immunoaffinity column (IAC) extraction for sample clean-up and enrichment of analytes (aglycones) were performed in 2016 as described before (Ali and Degen, 2018). In brief, to cleave conjugates, samples were hydrolyzed by adding 250 μL of hydrolysis buffer (pH 5.0) and 40 μL of β-Gluc/ArylS to 5 mL urine aliquots with internal isotope labeled internal standard (U-[$^{13}C_{18}$] ZEN at 0.5 ng/mL) and incubation at 37 °C overnight before IAC extraction. After rinsing the column with 1 mL of water, the entire hydrolyzed urine sample was loaded on a ZearalaTest™ column at a flow rate of 1 drop/sec. The column was washed with 5 mL distilled water, then ZEN, α-ZEL and β-ZEL were eluted (flow rate 1 drop/sec) using 2 mL of methanol. The elute was evaporated to dryness under a stream of nitrogen at 45 °C; the residue was dissolved in 500 μL water/methanol (30:70 v/v), vortexed and filtered through a 0.45 μm pore size Teflon syringe filter prior to LC-MS/MS analysis. The analyte concentration factor was 10 compared to urine.

2.5. Biomarker analysis

Urinary ZEN and its metabolites α-ZEL and β-ZEL were determined in 2016 by an in-house validated method used also for analysis of German urines (Ali and Degen, 2018). Briefly, the instrumental analysis was performed with a Q-Trap 5500 (ABSciex) equipped with a Turbo V™ Ion Spray Source (ESI) and the HPLC system UFLC XR Prominence (Shimadzu). Chromatographic separation was carried out with 10 μL injection volume at 30 °C on a Nucleosil® 100-5 C18 HD 125 × 3 mm column under isocratic conditions with 30% water and 70% methanol as mobile phase at a flow rate of 0.3 mL/min. ESI–MS/MS was executed by multiple reaction monitoring (MRM) in negative ion mode, with the following settings: source temperature 500 °C, curtain gas 30 psi, gas1 50 psi, gas2 50 psi, and ion spray voltage at -4500 V. The specific transitions of precursor ion and product ion were as follows: $317.1 \rightarrow 174.9$ m/z (quantifier) and $317.1 \rightarrow 130.9$ (qualifier) for ZEN. The optimized collision energy (CE) was 38 and 42 eV, respectively. For both α-ZEL and β-ZEL, the transitions of precursor and product ions were $319.1 \rightarrow 275.1$ (qualifier) and $319.1 \rightarrow 160.0$ m/z (quantifier) with an optimized CE of 28 and 38 eV. For internal standard ($^{13}C_{18}$ZEN), the transitions of precursor and product ions were $335.1 \rightarrow 185$ (quantifier) and $335.1 \rightarrow 139.6$ (qualifier) m/z with an optimized CE of 20 and 26 eV in that order. The retention times for ZEN and its metabolites α-ZEL and β-ZEL were 6.2, 5.8 and 4.5 min, respectively. Data analysis

was done with Analyst software 1.6.1 (ABSciex). Calibration with pure standards in mobile phase showed linearity in the range of 0.1–20 ng/mL for all analytes, and analysis in spiked blank urines yielded limits of detection (LOD) of 0.01 ng/mL and limits of quantification (LOQ) of 0.025 ng/mL urine for ZEN, α-ZEL and β-ZEL (Ali and Degen, 2018). Biomarker quantification in sample extracts was based on IS (U-[$^{13}C_{18}$-ZEN]), to account for a possible loss of analytes during sample preparation and correcting for matrix effects.

2.6. Statistical analyses

Statistical analysis was performed using IBM SPSS, version 23. The data are presented as means ± standard deviation or medians and interquartile ranges. Urines containing the analyte ≥ LOD were used for calculation of mean and median values. Correlation between biomarker levels with age and body mass index (BMI) was assessed by Pearson's correlation coefficient test (two-tailed). Gender group comparison was made by independent sample *t*-test. The box plot represents the central data of distribution where upper and lower limits of the box indicate 25th and 75th percentiles (first quartile or Q1 and third quartile or Q3, respectively) and the median value is presented as a line inside box. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of the urine donors

The baseline characteristics of the study participants are given in Table 1. The number of male and female urine donors was equal in rural and urban residents in Rajshahi district. Their mean age was 39 ± 11 years with no significant differences between gender or region. Mean BMI of the urban (23.8 ± 3.2 kg/m²) was higher than that of the rural (21.4 ± 2.6 kg/m²) cohort (*p* < 0.001). Urinary creatinine levels were not significantly different between rural and urban residents, whilst a significant (*p* < 0.05) difference was found between summer (0.40 ± 0.44 g/L) and winter (0.80 ± 0.55 g/L) urines, related to more fluid intake in the hot season. The mean age of pregnant women in Dhaka district was 26 ± 5 years (range 19–36 years) with no significant difference between rural and suburban residents. Their mean BMI was 20.6 ± 2.7 kg/m², with women in the rural subgroup having clearly higher BMI (21.6 ± 1.9 kg/m²) than those in the suburban (19.3 ± 3.1 kg/m²) group (*p* < 0.01). The mean urinary creatinine level of this cohort was 0.53 ± 0.34 with no significant difference between subgroups.

3.2. Urinary levels of ZEN and its metabolites by cohort and season

Our biomarker analysis documents widespread dietary exposure of Bangladeshi adults to ZEN and its plant/fungal modified forms. The urinary levels of ZEN, α-ZEL and β-ZEL of all individuals in the rural and urban Rajshahi cohort in two sampling periods and those of the pregnant women cohort from Dhaka are depicted in Fig. 1. Plotting data for individual urine donors illustrates best biomarker variability within a given cohort along with possible influences of region and season on analyte levels and composition. Overall, it is readily apparent that levels of α-ZEL far exceed those of ZEN whilst β-ZEL is only a minor metabolite in Bangladeshi adults, albeit more present in pregnant women urines. Moreover, stacking graphs summing up all analytes ('total ZEN') help to identify individuals with a higher than average exposure to ZEN and its metabolites. For instance, the majority of urines collected in summer season from rural and urban participants contained no more than 0.25 ng/mL 'total ZEN'; in winter season more urines were found to contain higher analyte levels, in particular, those of the rural cohort. Also several of the pregnant women urines (8 of 20) contained 'total ZEN' levels above 0.25 ng/mL. The individual biomarker data (Fig. 1) and the box-plot for 'total ZEN' in the Bangladeshi
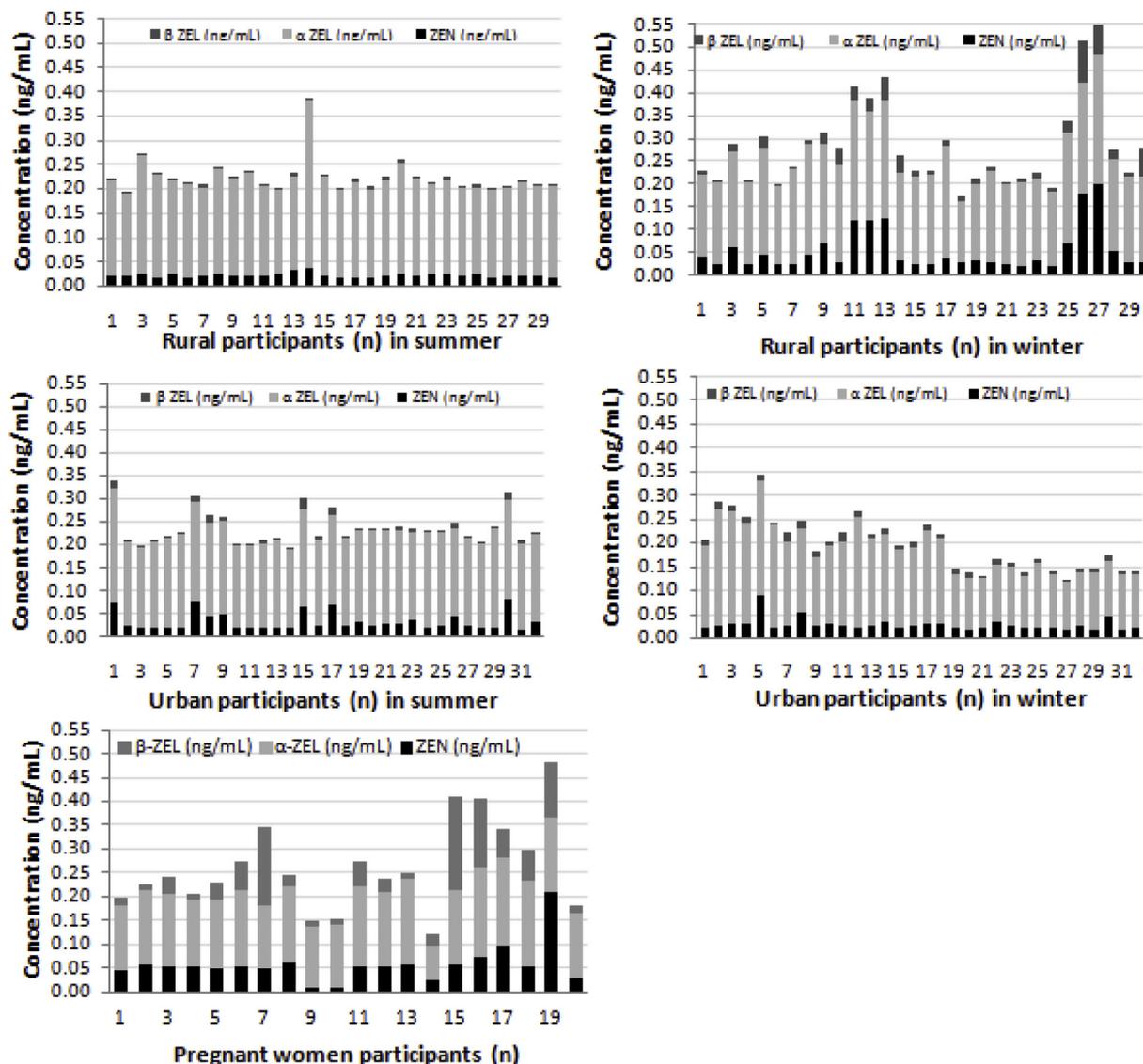
Fig. 1. Urinary exposure biomarkers ZEN, α-ZEL and β-ZEL and their sum in ng/mL. Only positives samples included in the graph. In rural participants 1–15 are males and 16–30 are females; in urban participants 1–16 are males and 16–32 are females. In pregnant women participants 1–11 are rural and 12–20 are suburban participants.

cohorts (Fig. 2) suggest region of residence and season of sampling as variables which can affect urine analyte levels, with some statistically significant differences between the median values (ng/mL). On the other hand, when adjusting 'total ZEN' levels for urinary creatinine content, differences are no longer statistically significant.

The analytical data for ZEN and its metabolites α-ZEL and β-ZEL are summarized in Table 2 for the Rajshahi cohorts, grouped by region, gender and season. Biomarker levels are given in ng/mL and ng/mg creatinine, along with descriptive statistics. Biomarker values for male and female urine donors were not significantly different. Yet, some differences, marked by superscripts, were observed for other sub-categories. For example, in summer, mean levels for ZEN and β-ZEL in the urban cohort were apparently higher than in the rural cohort, yet the difference was not significant when comparing median levels (ng/mL) or crea-adjusted (ng/mg) values. Mean levels in winter urines of the rural and urban subgroup showed also some differences, yet not significant for median and crea-adjusted values. Also, seasonal differences in analyte levels were comparatively small. As summer urines were on average more dilute than winter urines (Table 1) this may explain apparent seasonal differences in the crea-adjusted biomarker values (Table 2).

What is consistently found in all samples of the Rajshahi cohorts (for both regions and seasons) are the high urine concentrations of metabolite α-ZEL compared to parent mycotoxin, present at up to 7.1-fold higher levels in summer and 4.6-fold higher levels in winter than ZEN. This underlines the importance of α-ZEL as a biomarker of mycoestrogen exposure, despite some individual variability in the ratio of ZEN to α-ZEL and β-ZEL in our cohorts (Fig. 1). Yet, the analyte composition was quite similar in male and (non-pregnant) female urine donors. Furthermore, there was no significant correlation of urinary biomarkers with age and BMI of the study participants from Rajshahi district.

3.3. Urinary levels of ZEN and its metabolites in pregnant women cohort

Whilst the analyte composition in either male or (non-pregnant) female urine donors in Rajshahi district is rather similar, the pattern of ZEN biomarkers in pregnant women (of Dhaka district) differs apparently (Fig. 1): Although α-ZEL and ZEN are also main analytes, β-ZEL is present in each urine sample, and often found at clearly higher concentrations than in the other cohorts. On the other hand, the levels of 'total ZEN' biomarkers excreted by pregnant women resemble those of the other cohorts (Fig. 2), indicative of an overall similar dietary
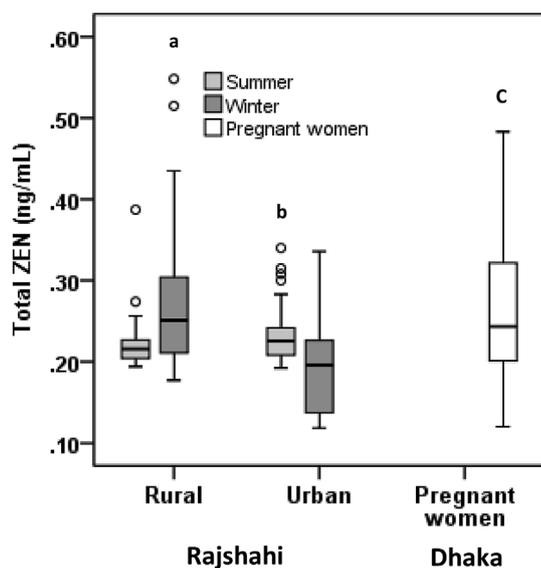
Fig. 2. Urinary biomarker levels of total ZEN (ZEN+ α-ZEL +β-ZEL) in samples from rural and urban cohorts in Rajshahi district and the pregnant women cohort in Dhaka district.
[a] $p < 0.01$ when compared to summer in rural cohort and also compared to winter in urban cohort.
[b] $p < 0.01$ when compared to winter in urban cohort.
[c] $p < 0.01$ when compared to winter in urban cohort.
p-value obtained from independent sample t-test. Only positive samples considered in the graph.

mycoestrogen exposure. Table 3 summarizes results for ZEN, α-ZEL and β-ZEL in the pregnant women cohort: Biomarker levels for the subgroups are given in ng/mL and ng/mg creatinine. Although levels of all three analytes tend to be slightly higher in residents of the suburban than those of the rural area, the difference is not significant.

4. Discussion

Biomonitoring is an effective approach to investigate human mycotoxin exposure by various routes and from all dietary sources (Degen, 2011; Marin et al., 2013; Vidal et al., 2018). It is particularly useful for developing countries such as Bangladesh where food contaminant data is rather scarce (Bhuiyan et al., 2013; Dawlatana et al., 2002; Roy et al., 2013). Biomonitoring studies on aflatoxins, citrinin, deoxynivalenol, and on ochratoxin A provide now data on exposure to these mycotoxins in the Bangladeshi population, also in relation to cohorts of other countries, e.g. Germany (Ali et al., 2018, 2016d; 2016a, 2016b, 2016c, 2016a; 2015a, 2015b; 2014; Gerding et al., 2015).

The new data document widespread exposure to ZEN and its modified forms and shed also some light on the variability among Bangladeshi adults. Urines from individuals in Rajshahi district collected in winter and summer, showed some variation in analyte levels between seasons (Fig. 1), more pronounced in rural than urban residents. On the other hand, no significant gender-related differences in biomarker levels were found between male and female urine donors in Rajshahi district (Table 2). Notably, in their urines, α-ZEL levels far exceed those of ZEN whereas β-ZEL is only a minor metabolite, albeit more present in urines of the pregnant women cohort. The levels of 'total ZEN' biomarkers excreted by pregnant women resemble those of the other cohorts (Fig. 2), indicative of an overall comparable dietary exposure to mycoestrogens. When we assume similar food habits and thus similar external exposure to contaminants for pregnant women and the other cohort, the observed 'shift' in urine analyte pattern (with an increase in β-ZEL) deserves a comment: ZEN is a substrate of α-and β-hydroxysteroid dehydrogenases (Malekinejad et al., 2006), enzymes

of the aldo-keto reductase superfamily which metabolize various endogenous steroid hormones (Penning and Drury, 2007; Thomas et al., 2004). Since endogenous steroid hormone levels change in pregnancy compared to non-pregnant females, this ('hormonal status') may affect the conversion of the (alternative) substrate ZEN. Yet, also a change of food habits in pregnancy and/or the food contaminant pattern might have influenced the observed analyte composition.

ZEN biomarker analysis in human samples reflects both, the intake of ZEN and its modified forms (fungal/plant metabolites) with food commodities, but also ZEN conversion to α-ZEL and β-ZEL in the organism (Ali and Degen, 2018). In other words: The analyte pattern detected in a particular matrix (here: urine) is the net result of the composition of the external exposure (the 'pattern' of ZEN forms in food) and internal metabolism in a given individual or species (Lorenz et al., 2019). Thus, the pattern observed in Bangladeshi (or German) urines, with α-ZEL as the predominant analyte, could be due mainly to ZEN metabolism in the human subjects and/or result from α-ZEL forms present in food (Ali and Degen, 2018). Either way, regarding overall exposure, it is indicated to consider not ZEN alone, but the sum of urine biomarkers determined ('total ZEN'). Summing up ZEN, α- and β-ZEL in individual urine samples can also identify persons with a higher than average mycoestrogen exposure (Fig. 1; also Fig. 2 in Ali and Degen, 2018). Yet, the analyte pattern is also important, in light of pronounced differences in estrogenic potency between ZEN and its reduced metabolites, with α-ZEL being 60 times more potent in vivo than ZEN, whilst β-ZEL is 5 times less potent than ZEN (Everett et al., 1987; EFSA, 2016).

Biomonitoring data, compiled earlier, document a wide range of ZEN biomarker levels and variable patterns in cohorts from different countries (Ali and Degen, 2018; Mally et al., 2016). An overview of this data, updated by present study and more recent studies (Li et al., 2018; Mitropoulou et al., 2018), is given in the Supplementary Material (Supplementary Table 1). The highest exposure to mycoestrogens was found in Nigeria, with ZEN as the major analyte in urine (Šarkanj et al., 2018), and in a cohort of female farmers in Transkei with ZEN, α-ZEL and β-ZEL (Shephard et al., 2013). The biomarker concentrations found in European cohorts from Germany (Ali and Degen, 2018), Italy (Solfrizzo et al., 2014), and Sweden (Mitropoulou et al., 2018; Wallin et al., 2015), are clearly lower than those in the African cohorts. The analyte patterns vary, yet α-ZEL levels often exceed those of ZEN and β-ZEL, similar to our Bangladeshi cohorts who present overall lower biomarker levels than German adults. A study in Henan province, China, reported the occurrence of ZEN in 71.1% of urines (Li et al., 2018); the reported mean value of 0.24 ng/mL and the range (between 0.02 and 3.7 ng/mL) were higher than those found in Bangladeshi adults.

The observed ranges in urine biomarker levels for various countries indicate high or low mycoestrogen exposures, likely a result of differences in ZEN contamination of major crops and differences in food habits of the populations studied: For instance, in African countries, maize is a staple food, and known to be frequently contaminated by Fusarium toxins. Lower levels of ZEN biomarkers found in European countries are in accord with exposure estimates based on ZEN occurrence and food consumption data (EFSA, 2011). The new finding that total ZEN exposure is apparently lower in Bangladesh than in Europe is in line with the following: ZEN is mainly a contaminant of wheat, maize, barley and derived products (EFSA, 2011), commodities which are consumed far more frequently in Europe than in Bangladesh, where rice is the main staple food and wheat or maize intake is still low (Ali et al., 2017, 2016a; FAOSTAT, 2013). ZEN has been found in South Korean rice samples which contain only small amounts of deoxynivalenol (DON), but higher levels of nivalenol (Lee et al., 2011; Ok et al., 2014). Thus, rice could be a source of ZEN and co-occurring Fusarium toxins in Bangladesh.

DON biomarker levels in Bangladeshi cohorts are far lower than in German adults, indicative of low dietary intake with rice or other crops in Bangladesh, and well below the TDI set for this Fusarium toxin (Ali

Table 2
Occurrence and urinary levels of ZEN and its metabolites in rural and urban adult cohorts in Rajshahi district.

Cohorts	Gender	N	Summer				Winter			
			Positive n (%)	Mean ± SD (ng/mL)	Median (range) (ng/mL)	Mean ± SD (ng/mg crea)	Positive n (%)	Mean ± SD (ng/mL)	Median (range) (ng/mL)	Mean ± SD (ng/mg crea)
Rural										
ZEN	Male	15	15 (100)	0.023 ± 0.005	0.020 (0.018–0.036)	0.082 ± 0.051	15 (100)	0.054 ± 0.037	0.040 (0.023–0.123)	0.094 ± 0.095
	Female	15	15 (100)	0.021 ± 0.003	0.020 (0.018–0.026)	0.108 ± 0.067	15 (100)	0.054 ± 0.057	0.031 (0.019–0.201)	0.086 ± 0.058
	All	30	30 (100)	0.022 ± 0.004	0.020 (0.018–0.036)	0.095 ± 0.060	30 (100)	0.054 ± 0.047[e]	0.032 (0.019–0.201)	0.090 ± 0.077
α-ZEL	Male	15	15 (100)	0.208 ± 0.042	0.196 (0.171–0.346)	0.755 ± 0.461	15 (100)	0.214 ± 0.031	0.211 (0.170–0.267)	0.377 ± 0.252
	Female	15	15 (100)	0.190 ± 0.014	0.185 (0.178–0.231)	1.040 ± 0.700	15 (100)	0.200 ± 0.039	0.191 (0.133–0.286)	0.434 ± 0.327
	All	30	30 (100)	0.199 ± 0.032	0.193 (0.171–0.346)	0.895 ± 0.599	30 (100)	0.207 ± 0.035[f]	0.197 (0.133–0.286)	0.406 ± 0.288
β- ZEL	Male	15	1 (6.7)	0.010	0.010 (nd–0.010)	0.013	12 (80)	0.023 ± 0.013	0.022 (nd–0.048)	0.034 ± 0.025
	Female	15	nd	nd	nd	nd	14 (93)	0.026 ± 0.026	0.013 (nd–0.090)	0.043 ± 0.036
	All	30	1 (3.3)	0.010	0.010 (nd–0.010)	0.013	26 (87)	0.024 ± 0.021[g]	0.017 (nd–0.090)	0.039 ± 0.031
Urban										
ZEN	Male	16	16 (100)	0.033 ± 0.021	0.021 (0.018–0.078)	0.112 ± 0.068	16 (100)	0.031 ± 0.018	0.024 (0.021–0.090)	0.044 ± 0.024
	Female	16	16 (100)	0.033 ± 0.018	0.027 (0.017–0.084)	0.153 ± 0.093	16 (100)	0.023 ± 0.007	0.020 (0.015–0.043)	0.037 ± 0.016
	All	32	32 (100)	0.033 ± 0.020[a]	0.024 (0.017–0.084)	0.132 ± 0.083	32 (100)	0.027 ± 0.014	0.023 (0.015–0.090)	0.041 ± 0.020
α-ZEL	Male	16	16 (100)	0.194 ± 0.020	0.189 (0.170–0.250)	0.895 ± 0.695	16 (100)	0.193 ± 0.032	0.182 (0.146–0.245)	0.336 ± 0.246
	Female	16	16 (100)	0.198 ± 0.09	0.198 (0.184–0.215)	1.041 ± 0.688	16 (100)	0.124 ± 0.027	0.113 (0.100–0.198)	0.214 ± 0.113
	All	32	32 (100)	0.196 ± 0.016[b]	0.194 (0.170–0.250)	0.968 ± 0.684	32 (100)	0.159 ± 0.046	0.164 (0.100–0.245)	0.275 ± 0.198
β- ZEL	Male	16	5 (31)	0.015 ± 0.004	0.016 (nd–0.022)	0.019 ± 0.017	15 (94)	0.013 ± 0.004	0.010 (nd–0.021)	0.022 ± 0.017
	Female	16	5 (31)	0.012 ± 0.006	0.010 (nd–0.019)	0.043 ± 0.038	14 (87)	0.011 ± 0.001	0.010 (nd–0.013)	0.018 ± 0.010
	All	32	10 (31)	0.013 ± 0.005[c]	0.010 (nd–0.022)	0.031 ± 0.030	29 (91)	0.012 ± 0.003	0.010 (nd–0.021)	0.020 ± 0.014
Both cohorts										
ZEN	All	62	62 (100)	0.028 ± 0.015	0.021 (0.017–0.084)	0.114 ± 0.075	62 (100)	0.040 ± 0.037[h]	0.027 (0.015–0.201)	0.064 ± 0.060
α-ZEL	All	62	62 (100)	0.198 ± 0.025[d]	0.194 (0.170–0.346)	0.993 ± 0.640	62 (100)	0.182 ± 0.047	0.183 (0.100–0.286)	0.338 ± 0.252
β-ZEL	All	62	11 (18)	0.013 ± 0.005	0.013 (nd–0.022)	0.023 ± 0.029	55 (89)	0.018 ± 0.016[i]	0.011 (nd–0.090)	0.029 ± 0.025

nd: Samples below the detection limit or not detected.
Positive sample refer to urines containing the analyte ≥ limit of detection (LOD: 0.01 ng/mL).Only positive samples were used for calculation of mean and median values.

[a] $p < 0.01$, [c] $p < 0.05$ when compared to respective biomarker level of rural cohort in summer season. [e] $p < 0.01$, [f] $p < 0.001$, [g] $p < 0.01$ when compared to respective biomarker level of urban cohort in winter season.
[b] $p < 0.001$, [d] $p < 0.05$ [e] $p < 0.05$ [f] $p < 0.001$, [h] $p < 0.05$, [i] $p < 0.001$ when compared to respective biomarker level between summer and winter cohort, p-value obtained from independent sample t-test.

Table 3

Occurrence and urinary levels of ZEN and its metabolites in pregnant women in Dhaka district.

Cohorts	N	Positive n (%)	Mean ± SD ng/mL	Median (range) ng/mL	Mean ± SD ng/mg crea
Rural					
ZEN	11	11 (100)	0.044 ± 0.018	0.051 (0.009–0.061)	0.123 ± 0.098
α-ZEL	11	11 (100)	0.147 ± 0.014	0.143 (0.129–0.170)	0.385 ± 0.263
β-ZEL	11	11 (100)	0.040 ± 0.045	0.023 (0.010–0.166)	0.103 ± 0.117
Suburban					
ZEN	9	9 (100)	0.072 ± 0.056	0.056 (0.024–0.209)	0.262 ± 0.242
α-ZEL	9	9 (100)	0.157 ± 0.036	0.158 (0.073–0.188)	0.677 ± 0.646
β-ZEL	9	9 (100)	0.074 ± 0.066	0.064 (0.012–0.198)	0.204 ± 0.165
Both subgroups					
ZEN	20	20 (100)	0.057 ± 0.041	0.052 (0.009–0.209)	0.185 ± 0.187
α-ZEL	20	20 (100)	0.151 ± 0.026	0.156 (0.073–0.188)	0.516 ± 0.484
β-ZEL	20	20 (100)	0.055 ± 0.057	0.032 (0.010–0.198)	0.148 ± 0.146

Only positive samples (analyte ≥ LOD 0.01 ng/mL) were considered during calculation of mean and median values.

et al., 2016b). As a high fraction of ingested DON is excreted in urine (about 70% per day), conversion of biomarker levels to provisional daily intake gives reasonably accurate estimates. For ZEN and its modified forms, such calculations involve far more uncertainty, since present knowledge on the toxicokinetics of ZEN and its derivatives in humans is very limited (Ali and Degen, 2018; Lorenz et al., 2019). One male volunteer who ingested 100 mg ZEN has excreted ZEN, and its reduced metabolites α-ZEL and β-ZEL, mainly as glucuronides (Mirocha et al., 1981). Based on this urine analyte data, it was estimated that about 10–20% are excreted within a day (Metzler et al., 2010). Another study measured ZEN excretion in a male volunteer exposed to a daily dose of 10 μg ZEN (about 2/3 of the TDI of 0.25 μg/kg b.w.) in a naturally contaminated diet (Warth et al., 2013). During the exposure phase (4 days), ZEN levels in urine ranged between 0.30 and 0.59 ng/mL (mean 0.39 ng/mL); this corresponded to an excretion rate of 7.0–13.2% (mean 9.4%), yet without α-ZEL and β-ZEL. These studies which inform on a) ZEN urine levels found during exposure below the current TDI value and b) urine excretion rates of 10–20% for the sum of ZEN, α-ZEL and β-ZEL, helped to assess mycoestrogen intake of German adults: The mean level for 'total ZEN' in the German cohort of 0.32 ng/mL corresponds to an exposure below the 'group TDI' of 0.25 μg/kg b.w. set by EFSA; yet an individual with a 'total ZEN' urine level of up to 1.6 ng/mL had a higher intake (details in Ali and Degen, 2018). As mean ZEN biomarker levels in the present study, measured in two seasons and in different regions (Tables 2 and 3), as well as 'total ZEN' concentrations in all individuals (Fig. 1 max. 0.55 ng/mL) are lower than those in German adults, the exposure of Bangladeshi adults to ZEN and its modified forms is apparently lower than the present group TDI set by EFSA. Yet, one has to keep in mind that these results are a 'snapshot' of two seasons a few years ago, and the situation might be different nowadays due to the mutability of mycotoxin levels in food. Annual variability of food contamination, as well as dietary habits, should be considered when comparing various biomarker data sets on human exposure to mycotoxins.

Moreover, exposure to contaminants can vary between age groups, and has been found to be higher in young consumers (infants, toddlers, children) as compared to adults, which is explained by a higher food intake per kg body weight in younger age groups (EFSA, 2011; Mally et al., 2016; Marin et al., 2013). A new biomonitoring study from Sweden reported more frequent detection of ZEN, α-ZEL and β-ZEL in urines of children (age 11–12 years) and at higher mean concentrations than in urines from adults (Mitropoulou et al., 2018). It is of interest to conduct also biomonitoring in young (er) children of other countries, including Bangladesh, to assess mycoestrogen exposure in vulnerable groups of the population.

Conflicts of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.036.

References

Ali, N., Blaszkewicz, M., Al Nahid, A., Rahman, M., Degen, G.H., 2015a. Deoxynivalenol exposure assessment for pregnant women in Bangladesh. Toxins 7, 3845–3857. https://doi.org/10.3390/toxins7103845.

Ali, N., Blaszkewicz, M., Alim, A., Hossain, K., Degen, G.H., 2016a. Urinary biomarkers of ochratoxin A and citrinin exposure in two Bangladeshi cohorts: follow-up study on regional and seasonal influences. Arch. Toxicol. 90, 2683–2697. https://doi.org/10.1007/s00204-015-1654-y.

Ali, N., Blaszkewicz, M., Degen, G.H., 2016b. Assessment of deoxynivalenol exposure among Bangladeshi and German adults by a biomarker-based approach. Toxicol. Lett. 258, 20–28. https://doi.org/10.1016/j.toxlet.2016.06.006.

Ali, N., Blaszkewicz, M., Hossain, K., Degen, G.H., 2017. Determination of aflatoxin M1 in urine samples indicates frequent dietary exposure to aflatoxin B1 in the Bangladeshi population. Int. J. Hyg Environ. Health 220, 271–281. https://doi.org/10.1016/j.ijheh.2016.11.002.

Ali, N., Blaszkewicz, M., Manirujjaman, M., Degen, G.H., 2016c. Biomonitoring of concurrent exposure to ochratoxin A and citrinin in pregnant women in Bangladesh. Mycotoxin Res. 32, 163–172. https://doi.org/10.1007/s12550-016-0251-0.

Ali, N., Blaszkewicz, M., Manirujjaman, M., Perveen, R., Al Nahid, A., Mahmood, S., Rahman, M., Hossain, K., Degen, G.H., 2014. Biomonitoring of ochratoxin A in blood plasma and exposure assessment of adult students in Bangladesh. Mol. Nutr. Food Res. 58, 2219–2225. https://doi.org/10.1002/mnfr.201400403.

Ali, N., Blaszkewicz, M., Mohanto, N.C., Rahman, M., Alim, A., Hossain, K., Degen, G.H., 2015b. First results on citrinin biomarkers in urines from rural and urban cohorts in Bangladesh. Mycotoxin Res. 31, 9–16. https://doi.org/10.1007/s12550-014-0217-z.

Ali, N., Degen, G.H., 2018. Urinary biomarkers of exposure to the mycoestrogen zearalenone and its modified forms in German adults. Arch. Toxicol. 92, 2691–2700. https://doi.org/10.1007/s00204-018-2261-5.

Ali, N., Hossain, K., Blaszkewicz, M., Rahman, M., Mohanto, N.C., Alim, A., Degen, G.H., 2016d. Occurrence of aflatoxin M1 in urines from rural and urban adult cohorts in Bangladesh. Arch. Toxicol. 90, 1749–1755. https://doi.org/10.1007/s00204-015-1601-y.

Ali, N., Hossain, K., Degen, G.H., 2018. Blood plasma biomarkers of citrinin and ochratoxin A exposure in young adults in Bangladesh. Mycotoxin Res. 34, 59–67. https://doi.org/10.1007/s12550-017-0299-5.

Bhuiyan, M.N.H., Hassan, M.T., Begum, M., Ahan, M., Rahim, M., 2013. Occurrence and seasonal trends of aflatoxin in rice, maize and wheat in Bangladesh. Int. J. Sustain. Agril. 9, 8–14.

Binder, S., Schwartz-Zimmermann, H., Varga, E., Bichl, G., Michlmayr, H., Adam, G.,

Berthiller, F., 2017. Metabolism of zearalenone and its major modified forms in pigs. Toxins 9, 56.

Blaszkewicz, M., Liesenhoff-Henze, K., 2002. Creatinine in Urine [Biomonitoring Methods]. MAK-Collect. Occup. Health Saf. Annu. Thresholds Classif. Workplace 169–184. Wiley VCH, Weinheim, Germany. https://doi.org/10.1002/3527600418.bi6027urie0012.

Dall'Erta, A., Cirlini, M., Dall'Asta, M., Del Rio, D., Galaverna, G., Dall'Asta, C., 2013. Masked mycotoxins are efficiently hydrolyzed by human colonic microbiota releasing their aglycones. Chem. Res. Toxicol. 26, 305–312.

Dawlatana, M., Coker, R.D., Nagler, M.J., Wild, C.P., Hassan, M.S., Blunden, G., 2002. The occurrence of mycotoxins in key commodities in Bangladesh: surveillance results from 1993 to 1995. J. Nat. Toxins 11, 379–386.

Degen, G., 2011. Tools for investigating workplace-related risks from mycotoxin exposure. World Mycotoxin J. 4, 315–327.

EFSA, 2017. Risks for animal health related to the presence of zearalenone and its modified forms in feed. EFSA J. 15, e04851. https://doi.org/10.2903/j.efsa.2017.4851. 123 pp.

EFSA, 2016. Appropriateness to set a group health-based guidance value for zearalenone and its modified forms. EFSA J. 14, e04425. https://doi.org/10.2903/j.efsa.2016.4425. 46 pp.

EFSA, 2014. Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. EFSA J. 12, 3916. https://doi.org/10.2903/j.efsa.2014.3916. 60 pp.

EFSA, 2011. Scientific Opinion on the risks for public health related to the presence of zearalenone in food. EFSA J. 9, 2197. https://doi.org/10.2903/j.efsa.2011.2197. 124 pp.

Everett, D.J., Perry, C.J., Scott, K.A., Martin, B.W., Terry, M.K., 1987. Estrogenic potencies of resorcyclic acid lactones and 17ß-estradiol in female rats. J. Toxicol. Environ. Health 20, 435–443.

FAOSTAT, 2013. Food and Agriculture Organization Statistics. Food Consumption Data for Bangladesh. http://www.fao.org/faostat/en/#data/CC, Accessed date: 21 January 2019.

Frizzell, C., Ndossi, D., Verhaegen, S., Elliott, C.T., Eriksen, G.S., Sorlie, M., Ropstadt, E., Conolly, L., 2011. Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis. Toxicol. Lett. 206, 210–217.

Gareis, M., Bauer, J., Thiem, J., Plank, G., Grabley, S., Gedek, B., 1990. Cleavage of zearalenone-glycoside, a "masked" mycotoxin, during digestion in swine. J. Vet. Med. Ser. B 37, 236–240.

Gerding, J., Ali, N., Schwartzbord, J., Cramer, B., Brown, D.L., Degen, G.H., Humpf, H.-U., 2015. A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. Mycotoxin Res. 31, 127–136. https://doi.org/10.1007/s12550-015-0223-9.

Lee, T., Lee, Soo-Hyung, Lee, Seung-Ho, Shin, J.Y., Yun, J.-C., Lee, Y.-W., Ryu, J.-G., 2011. Occurrence of Fusarium mycotoxins in rice and its milling by-products in Korea. J. Food Prot. 74, 1169–1174.

Li, C., Deng, C., Zhou, S., Zhao, Y., Wang, D., Wang, X., Gong, Y.Y., Wu, Y., 2018. High-throughput and sensitive determination of urinary zearalenone and metabolites by UPLC-MS/MS and its application to a human exposure study. Anal. Bioanal. Chem. 410, 5301–5312.

Lorenz, N., Dänicke, S., Edler, L., Gottschalk, C., Lassek, E., Marko, D., Rychlik, M., Mally, A., 2019. A critical evaluation of health risk assessment of modified mycotoxins with a special focus on zearalenone. Mycotoxin Res. 35, 27–46.

Malekinejad, H., Maas-Bakker, R., Fink-Gremmels, J., 2006. Species differences in the hepatic biotransformation of zearalenone. Vet. J. 172, 96–102.

Mally, A., Solfrizzo, M., Degen, G.H., 2016. Biomonitoring of the mycotoxin Zearalenone: current state-of-the art and application to human exposure assessment. Arch. Toxicol. 90, 1281–1292.

Maragos, C.M., 2010. Zearalenone occurrence and human exposure. World Mycotoxin J. 3, 369–383.

Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V., 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem. Toxicol. 60, 218–237.

Metzler, M., Pfeiffer, E., Hildebrand, A., 2010. Zearalenone and its metabolites as endocrine disrupting chemicals. World Mycotoxin J. 3, 385–401.

Mirocha, C.J., Pathre, S.V., Robison, T.S., 1981. Comparative metabolism of zearalenone and transmission into bovine milk. Food Cosmet. Toxicol. 19, 25–30.

Mitropoulou, A., Gambacorta, L., Lemming, E.W., Solfrizzo, M., Olsen, M., 2018. Extended evaluation of urinary multi-biomarker analyses of mycotoxins in Swedish adults and children. World Mycotoxin J. 11, 647–659.

Ok, H.E., Kim, D.M., Kim, D., Chung, S.H., Chung, M.-S., Park, K.H., Chun, H.S., 2014. Mycobiota and natural occurrence of aflatoxin, deoxynivalenol, nivalenol and zearalenon in rice freshly harvested in South Korea. Food Control 37, 284–291.

Penning, T.M., Drury, J.E., 2007. Human aldo–keto reductases: function, gene regulation, and single nucleotide polymorphisms. Arch. Biochem. Biophys. 464, 241–250.

Roy, M., Harris, J., Afreen, S., Deak, E., Gade, L., Balajee, S.A., Park, B., Chiller, T., Luby, S., 2013. Aflatoxin contamination in food commodities in Bangladesh. Food Addit. Contam. B 6, 17–23.

Šarkanj, B., Ezekiel, C.N., Turner, P.C., Abia, W.A., Rychlik, M., Krska, R., Sulyok, M., Warth, B., 2018. Ultra-sensitive, stable isotope assisted quantification of multiple urinary mycotoxin exposure biomarkers. Anal. Chim. Acta 1019, 84–92.

Shephard, G.S., Burger, H.-M., Gambacorta, L., Gong, Y.Y., Krska, R., Rheeder, J.P., Solfrizzo, M., Srey, C., Sulyok, M., Visconti, A., 2013. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food Chem. Toxicol. 62, 217–225.

Solfrizzo, M., Gambacorta, L., Visconti, A., 2014. Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. Toxins 6, 523–538.

Thomas, J.L., Duax, W.L., Addlagatta, A., Kacsoh, B., Brandt, S.E., Norris, W.B., 2004. Structure/function aspects of human 3β-hydroxysteroid dehydrogenase. Mol. Cell. Endocrinol. 215, 73–82.

Vidal, A., Mengelers, M., Yang, S., De Saeger, S., De Boevre, M., 2018. Mycotoxin biomarkers of exposure: a comprehensive review. Compr. Rev. Food Sci. Food Saf. 17, 1127–1155.

Wallin, S., Gambacorta, L., Kotova, N., Lemming, E.W., Nälsén, C., Solfrizzo, M., Olsen, M., 2015. Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis. Food Chem. Toxicol. 83, 133–139.

Warth, B., Sulyok, M., Berthiller, F., Schuhmacher, R., Krska, R., 2013. New insights into the human metabolism of the Fusarium mycotoxins deoxynivalenol and zearalenone. Toxicol. Lett. 220, 88–94.

WHO, 1996. Biological Monitoring of Chemical Exposure in the Workplace: Guidelines. World Health Organization, Geneva.

Yang, S., Zhang, H., Zhang, J., Li, Yanshen, Jin, Y., Zhang, S., De Saeger, S., Li, Yi, Zhou, J., Sun, F., 2018. Deglucosylation of zearalenone-14-glucoside in animals and human liver leads to underestimation of exposure to zearalenone in humans. Arch. Toxicol. 92, 2779–2791.

Zinedine, A., Soriano, J.M., Molto, J.C., Manes, J., 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem. Toxicol. 45, 1–18.