Measurement of urinary zearalenone concentrations for monitoring natural feed contamination in cattle herds: On-farm trials


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ABSTRACT: The aims of the present study were to investigate the efficacy of measuring bovine urinary zearalenone (ZEN) concentrations by using a commercially available ELISA method in cattle kept under different feeding conditions to monitor the natural contamination of feeds at the farm level, and to investigate the effects of supplementation of a mycotoxin adsorbent (MA) product in the feed based on urinary ZEN concentration. First, Japanese Black cattle herds kept for breeding (4 herds) and fattening (4 herds) purposes were provided with similar feeding conditions. Then, urinary samples from 5 cows in each herd were collected and analyzed. Second, dairy cows from 1 herd fed with total mixed rations (TMR) were selected. After thorough mixing of the MA (40 g/d) with TMR, the supplemented TMR was fed according to the following schedule: with MA for 2 wk, without MA for 3 wk; then with MA for 2 wk and without MA for 6 wk. Urine samples were collected from cows (n = 6 to 7) and examined before and after each interval. Zearalenone concentrations were measured by the ELISA and liquid chromatography-tandem mass spectrometry methods. The concentration of ZEN and its metabolites was expressed after creatinine (Crea) correction [ZEN or metabolites (pg/mL)/Crea (mg/dL); pg/mg of Crea]. In the first experiment, the urinary concentrations of ZEN and its metabolites were variable in all herds, and significant differences were observed between herds. In 1 fattening herd, in particular, urinary ZEN concentrations were greater (P < 0.001) than in the other 3 herds. This might reflect significant natural ZEN contamination of the feed at the farm level. In Exp. 2, urinary ZEN concentrations displayed peculiar trends after supplementation with MA. After 2 wk of supplementation, a significant decrease of ZEN (P < 0.05) was observed. Zearalenone concentrations remained at a reduced amount during 3 wk without MA supplementation and 2 wk with MA supplementation. When MA was not added to the feed for the next 6 wk, the concentrations increased to the original quantity. These findings indicate the usefulness of measuring concentrations of urinary ZEN and its metabolites not only for monitoring the natural ZEN contamination of cattle feed at the farm level but also for in vivo evaluation of MA function after supplementing feeds with MA.

Key words: cattle, mycotoxin adsorbent, urine, zearalenone

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INTRODUCTION

Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin produced by Fusarium species in plants including pasture grasses. Zearalenone and its metabolites exhibit distinct estrogenic and anabolic properties that affect the reproductive system of several animal species (Kleinvoga et al., 2002; Fink-Gremmels and Malekinejad, 2007; Minervini and Dell’Aquila, 2008). Zearalenone can occur with other Fusarium toxins in animal feeds, and the amounts of these mycotoxins can be quite large (Avantaggiato et al., 2007; Binder et al., 2007). Animal feeding trials are commonly performed with 1 toxin under well-defined conditions (Binder et al., 2007; Zinedine et al., 2007). However, experimental conditions differ from farm conditions. A diagnosis of myco-
Toxicosis should be established not only based on feed sample analysis but also from parallel investigations of other animals having access to the same feed materials and from postmortem examinations (Binder et al., 2007). The concentrations of ZEN and its metabolites in urine samples of farm animals, including cattle, are considered to be potential biomarkers indicating ZEN exposure (Prelusky et al., 1989; Usleber et al., 1992; Kleinova et al., 2002). Analytical methods such as EIA, gas chromatography-mass spectrometry, liquid chromatography using UV fluorescence, or mass spectrometric detection are used for urine analysis (Seeling et al., 2005; Songsermsakul et al., 2006; Dusi et al., 2009; Kaklamanos et al., 2009).

This study aimed to examine the urinary concentrations of ZEN and its metabolites, collected from cattle under various feeding conditions at the farm level, using ELISA and liquid chromatography-tandem mass spectrometry with electrospray ionization (LC-ESI/MS/MS) assays. This study also aimed to monitor the urinary concentrations of ZEN and its metabolites in dairy cows fed with a diet supplemented with a mycotoxin adsorbent (MA) product.

**Materials and Methods**

Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Faculty of Agriculture, Kagoshima University).

**Chemicals and Solvents**

Zearalenone was purchased from MP Biomedicals (Heidelberg, Germany). The metabolites α-zearalenol (ZOL) and β-ZOL were purchased from Sigma (St. Louis, MO). Methanolic stock solutions with ZEN, α-ZOL, and β-ZOL concentrations of 1 µg/mL each were stored under light protection at 4°C. Ammonium acetate and HPLC-grade methanol were purchased from Wako Pure Chemical Co. Inc. (Tokyo, Japan), and Tris was purchased from Nakalai Tesque Inc. (Kyoto, Japan).

**Exp. 1**

**Herds of Japanese Black Cattle and Sample Collection.** Eight herds of Japanese Black cows kept for fattening (4 herds) or breeding (4 herds) in Kagoshima Prefecture, Japan, were included in this study. All cows were housed indoors, and forage and concentrates (top dressing method) were fed separately. Feeding and management systems were different in each herd. The contents of the feeds in each herd are detailed in Table 1. In each herd, 5 to 7 cows with the same age (fattening category) or similar BW (breeding category) were selected, and spontaneous urine samples were collected during natural urination after softly massaging the pudendum. The urine samples were immediately placed into a cooling box, protected from light, and transported to the laboratory. After centrifugation at 500 × g for 10 min at room temperature, the samples were frozen at −30°C until analysis of ZEN and creatinine (CREA) concentrations.

To confirm the effects of sampling time (before and after feeding) on the urinary ZEN concentration, additional samples were collected from cows in 2 fattening herds (F and H). Briefly, spontaneous urine samples were collected from 4 cows at 2 h before and after a morning feeding in herd F within the same day, and from 4 cows at 3 h before and after both morning and evening feeding in herd H within the same day, treated similarly as mentioned above. The samples were frozen at −30°C until analysis of ZEN and Crea concentrations.

**Table 1. Composition of feed provided to the herds kept for both breeding and fattening purposes (as-fed basis)**

<table>
<thead>
<tr>
<th>Purpose and herd</th>
<th>Forage feed, kg</th>
<th>Total, kg</th>
<th>Bran, %</th>
<th>Cereal, %</th>
<th>Oilseed meal, %</th>
<th>Other, %</th>
<th>TDN, %</th>
<th>CP, %</th>
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</thead>
<tbody>
<tr>
<td>Breeding</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Italian 1.7, straw 1.0, sorghum silage 1.0</td>
<td>2.1</td>
<td>26</td>
<td>37</td>
<td>9</td>
<td>28</td>
<td>&gt;69</td>
<td>&gt;14</td>
</tr>
<tr>
<td>B</td>
<td>Orchard grass 4.0</td>
<td>2.8</td>
<td>17</td>
<td>39</td>
<td>22</td>
<td>22</td>
<td>&gt;67</td>
<td>&gt;16</td>
</tr>
<tr>
<td>C</td>
<td>Fescue 6, native grass silage 5</td>
<td>2.3</td>
<td>18</td>
<td>26</td>
<td>4</td>
<td>52</td>
<td>&gt;61</td>
<td>&gt;14</td>
</tr>
<tr>
<td>D</td>
<td>Straw 2.0, Italian 1.0</td>
<td>2.2</td>
<td>57</td>
<td>20</td>
<td>4</td>
<td>18</td>
<td>&gt;64</td>
<td>&gt;14</td>
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<tr>
<td>Fattening</td>
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<td></td>
</tr>
<tr>
<td>E</td>
<td>Straw 2.0</td>
<td>8.0</td>
<td>26</td>
<td>70</td>
<td>4</td>
<td>0</td>
<td>75</td>
<td>12</td>
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<tr>
<td>F</td>
<td>Straw 2.0</td>
<td>9.0</td>
<td>17</td>
<td>77</td>
<td>5</td>
<td>1</td>
<td>&gt;75</td>
<td>14</td>
</tr>
<tr>
<td>G</td>
<td>Straw 1.5</td>
<td>8.0</td>
<td>19</td>
<td>77</td>
<td>1</td>
<td>3</td>
<td>&gt;74</td>
<td>11.5</td>
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<tr>
<td>H</td>
<td>Straw 1.0</td>
<td>8.0</td>
<td>15</td>
<td>80</td>
<td>3</td>
<td>2</td>
<td>&gt;76</td>
<td>&gt;14</td>
</tr>
</tbody>
</table>
ence of 8 µL of β-glucuronidase/arylsulfatase solution. Thereafter, the samples were loaded to a C18 solid phase extraction (SPE) column (Strata, Phenomenex, Torrance, CA), which had been preconditioned with 3 mL of methanol, followed by 2 mL of 20 mM Tris buffer (pH 8.5)/methanol (80:20). After washing the SPE column with 2 mL of 20 mM Tris buffer (pH 8.5)/methanol (80:20) and 3 mL of methanol (40%), the column was centrifuged for 10 min at 500 × g at room temperature to dry the column. Then, the analytes were eluted slowly (flow rate: 15 drops/min) with 1 mL of methanol (80%). The eluate was evaporated to dryness at 60°C by using a centrifugation evaporator. The dried residue was redissolved with 50 µL of methanol; then, 450 µL of sample dilution buffer was added, the solution was mixed thoroughly, and an aliquot of 50 µL was used for the ELISA assay. To determine the ZEN concentration in the urine sample, RIDA SOFT Win (R-Biopharm) was used for calculating the absorbance at 450 nm with a microplate spectrophotometer. The mean recovery rate of the ELISA assay based on the 3 trials was 84 ± 14%.

Urinary Crea concentrations were determined using a commercial kit (Sikarikit-S CRE, Kanto Chemical) according to the manufacturer’s instructions and measured with a clinical autoanalyzer (7700 Clinical Analyzer, Hitachi High-Tech, Tokyo, Japan). In the present study, urinary ZEN concentrations were expressed as their ratio to urinary Crea (urinary ZEN/urinary Crea [pg/mg of Crea]) as described previously (Miles et al., 1996; Padilla et al., 2007).

To confirm the concentrations of ZEN measured by ELISA, and to measure the ZEN metabolites in bovine urine, the urine samples measured by ELISA were reanalyzed by LC-ESI/MS/MS. The extraction method for the urine samples was similar to the above-described ELISA method, with some minor modifications. Briefly, 0.5 mL of a urine sample was mixed with 3.0 mL of 50 mM ammonium acetate buffer (pH 4.8) and 8 µL of glucuronidase/arylsulfatase solution, and incubated for 12 h at 37°C. After the incubation, 1.5 mL of distilled water was added into each sample solution. The solution (5.0 mL in total) was loaded onto a C18 SPE column, which was preconditioned with 3 mL of 100% MeOH and 2 mL of Tris buffer, followed by the addition of 2 mL Tris buffer and 3 mL of 40% MeOH. After washing the SPE column with approximately 1 mL of 80% MeOH, the volume of the eluted solution was adjusted to exactly 1 mL. Then, the 20-µL amount of the reconstituted solution was injected into the liquid chromatography-tandem mass spectrometry (LC/MS/MS) system.

Analyses with LC-ESI/MS/MS were performed on an API 2000 LC/MS/MS system (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface and an HPLC system (1200 Series, Agilent Technologies, Santa Clara, CA). Briefly, chromatographic separation was achieved on an Inertsil ODS-3 (4.6 i.d. × 150 mm, 5 µm; GL Sciences, Tokyo, Japan) at 40°C. The mobile phase consisted of methanol (A) and water (B), and the gradient elution was applied to separate the analyte as follows: solvent A was increased from 50 to 100% in 5 min along a linear gradient curve, and the isocratic elution was held for 10 min at a flow rate of 200 mL/min. Re-equilibration of the column was isocratically performed with 50% of solvent B for 7 min at a flow rate of 1,000 mL/min. The injected sample volume was 20 µL. For the LC/MS/MS analysis, a multiple reaction monitoring system was used for the transition of ZEN (m/z 317.0 to 130.5) and α/β-ZOL (m/z 319.0 to 129.9) in a negative mode. Instrumental parameters were optimized for each analyte by analysis of the corresponding standard solution (1.0 mg/L in methanol) at a flow rate of 10 mL/min, using a syringe pump integrated in the API-2000 mass spectrometer. The electrospray conditions for ZEN and α/β-ZOL were as follows: curtain gas, 20 psi; ion-spray voltage, −4.500 V; turbo temperature, 500°C; collision energy, −48.0 eV for ZEN and −44.0 eV for α/β-ZOL; declustering potential, −36.0 V for ZEN and −51.0 V for α/β-ZOL; focusing potential, −260 V for ZEN and −280 V for α/β-ZOL; entrance potential, −9.0 V for ZEN and −8.5 V for α/β-ZOL. Nitrogen was used as a nebulizer, curtain, and collision gas. The mean recovery rates for ZEN, α-ZOL, and β-ZOL were 116, 121, and 56%, respectively.

**Exp. 2**

**Application of the Urinary ZEN Monitoring System for Dairy Herd Fed With and Without MA Supplementation.** To validate the urinary ZEN monitoring system, an additional field trial was conducted in which MA was applied in 1 dairy herd. Briefly, a total of 7 mid-lactation multiparous Holstein cows from a private dairy farm (n = 20) in Yamaguchi Prefecture, Japan, were used in this trial. The average BW of experimental animals was 650 ± 7 kg, and the average daily milk production of the herd was approximately 40 ± 1 kg/cow. Diets were fed as total mixed rations (TMR; 12 kg/d) based on nutrient concentrations that meet nutritional requirements for lactating dairy cows according to the Japanese Feeding Standards for Dairy Cattle. Animals were randomly assigned to tie stalls with individual feeders and obtained feed and water ad libitum. The scheme of the MA supplementation and urinary sampling conducted in the present study is shown in Figure 1. Mycotoxin adsorbent (a commercially available product that contains a mixture of minerals and biological constituents, including enzymes, yeast cell wall, clay, and plant extracts; Marroquin-Cardona et al., 2009) was supplemented and thoroughly mixed as TMR (0.33%) with a feed mixer. The MA supplementation was given in intervals of 18 d on and 17 d off and then 19 d on and 38 d off. Urine samples were collected from individual cows at the end of each experimental period, approximately 3 h after morning feeding. The urine samples were immediately
placed in a cooling box, protected from light, and transported to the laboratory. After centrifugation at 500 × g for 10 min at room temperature, the samples were frozen at −30°C until analysis. Urinary ZEN concentration was measured by ELISA. For confirmation, concentrations of urinary ZEN, α-ZOL, and β-ZOL were measured by LC/MS/MS, and the ZEN/Crea ratio was finally determined as described above. Additionally, data on milk somatic cell counts (SCC) of each examined cow were retrieved from the farm records routinely collected at the middle of each month. The measurements were taken with an automatic milk component analyzer (Foss system, Foss Electric Inc., Hillerød, Denmark). In the present study, the mean SCC derived from examined cows were measured before, during, and after the treatment periods to evaluate the effect of MA on the SCC.

**Statistical Analysis**

Zearalenone concentrations and SCC were expressed as mean ± SEM. The concentrations of urinary ZEN and its metabolites in Exp. 1 and 2 (fattening, reproduction, and dairy cows), and SCC in Exp. 2, were analyzed by a 1-way ANOVA followed by a post hoc test, using the StatView program (Abac Concepts Inc., Berkeley, CA). Additionally, the urinary concentrations of ZEN and its metabolites before and after feeding of the 2 examined herds were analyzed by a paired t-test, using SPSS statistics software (IBM version 16.0, SPSS Inc., Chicago, IL). Probability values less than 0.05 were considered to indicate a statistically significant difference.

**RESULTS**

**Urinary ZEN Concentrations from Different Herds Measured by ELISA and LC/MS/MS**

Figure 2A shows the urinary ZEN concentrations of each herd in the fattening and breeding cattle groups as measured by ELISA. Figure 2B shows the urinary ZEN, α-ZOL, and β-ZOL concentrations of the same samples as measured by LC/MS/MS. Differences (P < 0.05) were observed in some herds from both groups when measured by ELISA and LC/MS/MS. The ELISA ZEN concentration of the herd with the greatest value (herd D: 249 pg/mg of Crea) was approximately 4 times greater than that of the herd with the least value (herd A: 63 pg/mg of Crea) in the breeding herd group. On the other hand, because the urinary ZEN concentrations of all samples from 1 herd (herd F) of the fattening cows measured by ELISA were beyond the standard maximum range values (4,050 pg of ZEN/mg) of the kit, the urinary ZEN concentrations of this herd was calculated as 4,050 pg/mg of Crea, without repeating ELISA measurements by using diluted samples. Even by excluding this value, the ZEN concentration of the herd with the greatest concentration (herd G: 145 pg/mg of Crea) was approximately 2 times greater than the value of the herd with the least concentration (herd E: 72 pg/mg of Crea), with a significant difference (P < 0.05) observed among the 3 other fattening herd groups. Results of LC/MS/MS analyses by which the concentrations of ZEN and its metabolites were measured corresponded to some extent with the results of the ELISA assay, although all measured ZEN concentrations were less than the values obtained with ELISA. In the breeding herds, significant differences among all concentrations of ZEN and its metabolites were observed between the 2 herds. Herds B and D showed greater (P < 0.05) concentrations than herds A and D. In the fattening category, 1 herd (herd F) showed greater concentrations of ZEN (642.5 pg/mg of Crea; approximately 18 to 250 times), α-Zol (236.0 pg/mg of Crea; approximately 16 to 150 times), and β-Zol (1,738.3 pg/mg of Crea; approximately 32 to 540 times) when compared with the other 3 herds (P < 0.05). This indicates increased absorption of ZEN from the intestines. However, no significant differences (P > 0.05) were observed among the other 3 herds (herds E, G, and H).

Figure 3 shows the results of cross-validation between ELISA and LC/MS/MS assays when the same urinary samples (n = 31) were measured. In general, a good correlation (r = 0.813) was observed between the ELISA and LC/MS/MS assays; however, absolute values differed considerably.

**Effects of Urinary Sampling Time to ZEN Concentrations Measured by ELISA and LC/MS/MS**

Table 2 shows the mean urinary ZEN concentrations of before and after feeding in each farm measured by ELISA and LC/MS/MS. Because the ZEN concentrations of urinary samples from herd F measured by ELISA were beyond the standard maximum range values of the kit, the urinary ZEN concentrations for evaluating the effects of feeding were measured using samples that were 100 times diluted with distilled water. Measurement with ELISA and LC/MS/MS assays in both herds showed no significant differences (P > 0.05) in the urinary concentrations of ZEN and its metabolites, α-ZOL and β-ZOL, collected from the samples between...
2 and 3 h before and after feeding within the day for urinary collection.

Changes of Urinary ZEN Concentrations During the Periods With or Without Supplementation of MA into the TMR

Figure 4A shows the urinary ZEN concentrations during the experimental periods with and without MA supplementation into the TMR as measured by ELISA. Before MA supplementation, the urinary ZEN concentration of the herd was 600 pg/mg of Crea. Zearalenone concentrations decreased \((P < 0.05)\) to 400 pg/mg of Crea after the first treatment period of 2 wk. No significant differences \((P > 0.05)\) were observed during the next 5 wk without (3 wk) and with (2 wk) MA supplementation when compared with the ZEN concentration of the first supplementation period. Finally, after 6 wk without MA supplementation, the urinary ZEN concentration of the examined dairy herd increased to the initial quantity (600 pg/mg of Crea; observed at the beginning of the experiment). Figure 4B shows the urinary ZEN, \(\alpha\)-ZOL, \(\beta\)-ZOL, and total ZEN (\(\Sigma\)ZEN; ZEN + \(\alpha\)-ZOL + \(\beta\)-ZOL) concentrations of the same urinary samples as measured by LC/MS/MS. A significant decrease in concentration \((P < 0.05)\) of urinary ZEN and its metabolites was observed after 2 wk of treatment. Similar results were observed with the ELISA assay. Additionally, the concentrations of urinary ZEN and its metabolites, especially \(\alpha\)-ZOL, significantly \((P < 0.05)\) increased to the original quantity of the herd after 6 wk without MA supplementation. During the whole experimental period, the \(\beta\)-ZOL concentrations were greater than the ZEN and \(\alpha\)-ZOL concentrations, and \(\alpha\)-ZOL was not detected at d 14, 38, and 92 of the experiment.
Changes of SCC During the Trial Involving a Dairy Herd

Figure 5 shows SCC changes of the herd during the experimental periods with and without MA supplementation, including changes of the concentrations of urinary ZEN and its metabolites as measured by LC/MS/MS. The concentrations of ZEN and its metabolites correspond with the concentrations depicted in Figure 4B. The SCC at the pre-experimental periods (d –38 and –8) were 14.1 ± 6.3 (×10⁴/mL) and 15.6 ± 8.0 (×10⁴/mL) somatic cells, respectively, and large variations were observed among individual cows in the herd. After the treatment periods, a decrease of SCC [5.4 ± 1.3 (×10⁴/mL)] was observed along with the reduced concentrations of urinary ZEN and its metabolites on d 50, although no significant difference (P > 0.05) was observed when these values were compared with the SCC values during the pre-experimental period. On d 112 (58 d after MA supplementation), the mean SCC of the herd increased (P < 0.05) to 19.4 ± 5.1 (×10⁴/mL) when compared with the SCC value measured at d 50.

DISCUSSION

Zearalenone and its metabolites can be detected in urinary samples from cattle with several methods such as ELISA, GC/MS, and LC/MS/MS; these methods are also suggested to be useful tools for residue control programs (Plasencia et al., 1990; Usleber et al., 1992; Dusi et al., 2009). It must be emphasized that this study was conducted as a field trial on a farm; thus, it does not objectively measure the carryover ZEN concentrations from contaminated feeds because the amount of ZEN in the feed was not known. Nevertheless, the results of
this study clearly indicate for the first time that exposure to ZEN via naturally contaminated feeds can be monitored at the farm level by measuring urinary ZEN concentrations. We observed significant differences in ZEN concentrations among the herds, which reflect different quantity of Zen intake in each herd. The urinary ZEN monitoring system may also be a useful tool for the objective evaluation of the effects of adsorbents or other mycotoxin mitigation agents. To the best of our knowledge, this is the first field study that detected significant differences in urinary ZEN concentrations in cattle, and confirmed the significant decrease in urinary ZEN concentration after supplementation of the feed with an MA product at the farm level.

In the present study, we evaluated urinary ZEN, α-ZOL, and β-ZOL concentrations normalized via their ratio with Crea. The urinary ZEN concentrations measured by ELISA tended to be greater than those measured by LC/MS/MS for the same samples. Similar to previous findings (Usleber et al., 1992), the anti-ZEN antiserum of the ELISA assay used in the present study recognized all other ZEN analogs at different amounts. Therefore, the urinary ZEN concentrations measured by ELISA represent not only ZEN but also its metabolites in the urinary samples. This partly explains the differences between the ELISA and LC/MS/MS results. Additionally, based on the results of the cross-validation between the ELISA and LC/MS/MS methods, an acceptable correlation was obtained between the 2 methods, which could further validate the effectiveness of the commercially available ELISA kit used in our study for monitoring ZEN concentrations. Our results also indicate that the urinary ZEN concentration adjusted by the Crea concentration might not be significantly affected by the feeding time, and that the urinary ZEN/Crea ratio may be stable within each cattle herd, which may reflect the natural contamination of ZEN. Moreover, our results showed no significant difference in the urinary concentrations of ZEN and its metabolites among the different sampling times, except for within at least 2 h before and after feeding, and among the different ZEN contamination levels of the herds examined. Therefore, our findings suggest that the ZEN monitoring system used in our study is a useful and practical method for monitoring naturally ZEN-contaminated feeds in on-farm conditions. Although the routine use of LC/MS/MS for this purpose does not seem to be economically viable, it may be useful for the final confirmation of the quantity of the intake of ZEN and its metabolites, even in on-farm conditions. Malekinjad et al. (2006) reported differences between species in the hepatic biotransformation of ZEN. Malekinjad et al. (2006) also demonstrated that β-ZOL is the dominant hepatic metabolite in cattle. In our study, the urinary concentration of β-ZOL was greater than those of both parent ZEN and α-ZOL in all cases. Moreover, the recovery rate of β-ZOL (56%) from bovine urinary samples was less than that of α-ZOL (121%) in our LC/MS/MS assay, thus supporting the findings of Malekinjad et al. (2006).

Several methods have been proposed for the mitigation of mycotoxin contamination of feeds during the preharvest, harvest, and postharvest periods (Kabak et al., 2006; Schatzmayr et al., 2006; Igawa et al., 2007; Zinedine et al., 2007; Boudra and Morgavi, 2008; Murata et al., 2008). Given the various limitations of these methods, it was suggested that the use of MA, MA-related mitigating agents, or both as feed additives is one of the most promising and widely used approaches to reduce the risk of mycotoxicoses in farm animals (Ramos et al., 1996; Huwig et al., 2001; Sabater-Vilar...
et al., 2007). Several in vitro and in vivo studies on the efficacy of MA have reported its beneficial effects (Avantaggiato et al., 2005, 2007; Korosteleva et al., 2007; Sabater-Vilar et al., 2007). The results of the present field study clearly confirm the significant reduction of urinary ZEN concentrations after a period of MA supplementation and the subsequent increase to the original concentration after cessation of MA supplementation. Although the number of examined herds was relatively small, our findings clearly indicate the efficacy of MA in preventing the absorption of ZEN from cattle intestines. In contrast, findings with MA supplementation revealed that it was not possible to adsorb ZEN completely from naturally contaminated feeds, even if supplied with a maximum dose of MA to the TMR, for more than 2 wk as recommended by the manufacturer. Additionally, although 1 type of MA was applied in our study, several types of MA, such as mineral clays, humic substances, and yeast cell wall, are currently commercially available (Avantaggiato et al., 2005; Sabater-Vilar et al., 2007). Therefore, further investigations may be required to determine the appropriate indications of MA based on biological samples from animals (e.g., urine).

Ruminants are known to be relatively less sensitive to ZEN exposure than are nonruminant animals because presystemic elimination of ZEN by their microbial rumen flora seems to reduce the internal dose of ZEN (Seeling et al., 2005; Fink-Gremmels and Malekinejad, 2007). In contrast, rumen acidosis occurs in beef and dairy cattle fed for greater production, resulting in rumen stasis and the destruction of a large percentage of the normal rumen microflora (Kersting and Thompson, 1999). Therefore, it is expected that the microflora will fail to completely eliminate ZEN in many high-production cattle herds. In the present field study, using the LC/MS/MS assay, we detected extremely high concentrations of urinary ZEN and its metabolites in 1 herd (herd F) compared with the other herds of the fattening category.

Moreover, various reports describe the occurrence of ZEN with other Fusarium toxins, such as deoxynivalenol (DON) or nivalenol, in animal feeds (Avantaggiato et al., 2007; Richard, 2007). Although the clinical history of the herd was not investigated in detail in our study, changes in the concentrations of urinary ZEN and its metabolites with and without MA supplementation were accompanied by changes in the milk concentration of ZEN and its metabolites in the urine.

Figure 5. Changes in somatic cell counts (SCC) of the dairy herd during the experimental periods with and without mycotoxin adsorbent (MA) supplementation, including the changes in concentrations of urinary zearalenone (ZEN) and its metabolites as measured by liquid chromatography-tandem mass spectrometry. The concentration data of ZEN and its metabolites correspond with the concentrations depicted in Figure 4B. Letters a and b in SCC were significantly different (P < 0.05). ZOL = zearalenol; ΣZEN = total ZEN (ZEN + α-ZOL + β-ZOL). Crea = creatinine.
SCC. Increased SCC are generally accepted to be the most significant indication of the onset of subclinical mastitis in dairy cows. The most important factor affecting SCC in an individual cow is the number of quarters infected with a major or minor pathogen (Radostits et al., 2000). Although ZEN is only known as a nonsteroidal estrogenic mycotoxin, Korosteleva et al. (2007, 2009) recently reported that feeds naturally contaminated with Fusarium mycotoxins could affect the metabolic variables and immunity in dairy cows, especially through the effect of DON contamination. One possible reason for the significant SCC changes after MA supplementation in our study is the amelioration of the effects of DON in these animals, which was not measured. Therefore, as previously suggested (Prelusky et al., 1989), routine monitoring of urinary ZEN concentrations in beef and dairy herds may be one of the important variables for monitoring not only ZEN but also the occurrence of other Fusarium mycotoxins in the same feed. Additionally, routine monitoring of urinary ZEN concentration may provide useful information and serve as a diagnostic tool to differentiate between mycotoxicosis-associated metabolic conditions and metabolic diseases resulting in rumen acidosis.

In conclusion, the results of our field trials indicate that monitoring of urinary ZEN concentrations with a small sample volume (0.5 mL) by using commercially available ZEN kits seems to be a useful tool for predicting exposure of animals to ZEN and other Fusarium toxins at the farm level. Additionally, it was confirmed that the urinary concentrations of ZEN and its metabolites were stable in each farm beyond 2 h after feeding, irrespective of the ZEN contamination levels of the herds examined. By applying the urinary ZEN monitoring system, we could detect mycotoxicosis in a cattle herd, which showed a critical level (based on the previously reported ZEN challenge study) of urinary ZEN concentration, for the first time in Japan. Moreover, the results of this study revealed that objective evaluation of MA supplementation in the feeds at the farm level enabled the estimation of the urinary ZEN concentrations of the herd. Thus, the use of bovine urine as a biological sample might be a more useful tool for evaluating the efficacy of MA than are in vitro MA evaluation methods. However, further studies on urinary ZEN concentrations that involve a greater number of cattle herds are required.

LITERATURE CITED


