Development of an UPLC-MS/MS multi-method for the quantitative analysis of important mycotoxins in rumen fluid

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INTRODUCTION AND AIMS

In Belgium, the most important mycotoxins found in maize silage are deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), mycophenolic acid (MPA), roquefortine C (ROQ-C), citrinin (CIT) and enniatin B (ENN B)1,2,3. The detoxifying capacity of ruminal microbiota for the previously mentioned mycotoxins, especially in combinations, is not well known. To answer this question, a method to determine multiple mycotoxins in rumen fluid is needed. Therefore, a sensitive and specific analytical method for the quantitative determination of the mycotoxins DON, NIV, ZEN, MPA, ROQ-C, CIT and ENN B as well as their metabolites deepoxy-deoxynivalenol (DOM-1), α-zearalenol (α-ZOL), β -zearalenol (β-ZOL), zearalanone (ZAN), α-zearanol (α-ZAL) and β-zearanol (β-ZAL) in rumen fluid using UPLC-MS/MS was developed and validated.

MATERIALS AND METHODS

Sample extraction:

- Rumen fluid (cattle/sheep)
- 1) sample for extraction of CIT
- 2) sample for extraction of other mycotoxins

Chromatography:
- HPLC instrument: Acquity UHPLC system (Waters, Zellik, Belgium)
- UPLC-column: HSS T3 column (2.1 x 50 mm, d.p.: 1.8 μm) in combination with a VanGuard pre-column (2.1 x 5 mm, d.p.: 1.8 μm) both from Waters (Zellik, Belgium)
- Aqueous and organic mobile phases with gradient elution program

Validated according to European guidelines4,5

RESULTS

FIGURE 1: LC-MS/MS chromatograms of spiked rumen fluid (10 ng/mL)
- CIT (ESI -)
- NIV, DON, DOM-1, ENN B, ROQ-C & MPA (ESI +)
- ZEN and metabolites (ESI +)

Table 1: Evaluation of validation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All mycotoxins, except ROQ-C</th>
<th>ROQ-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Accuracy &amp; precision</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>0.05–0.6 ng/mL</td>
<td>0.1 ng/mL</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>10–280 pg/mL</td>
<td>4 pg/mL</td>
</tr>
<tr>
<td>Specificity &amp; selectivity</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Carry-over</td>
<td>✓</td>
<td>✗</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSIONS

The LC-MS/MS parameters resulted in good chromatographic properties (Figure 1). Furthermore, the method is validated according to European guidelines (Table 1). ROQ-C shows carry-over, but this can be countered by injecting the samples from low to high concentrations of ROQ-C. This analytical method can be used to determine the fate of multiple mycotoxins in rumen fluid on a specific and sensitive way. In vitro studies will be performed to determine the detoxifying capacity of ruminal microbiota when incubated with the mycotoxins DON, NIV, ZEN, MPA, ROQ-C, CIT and/or ENN B in different rumen conditions.

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REFERENCES