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BEHAVIOR OF MYCOTOXINS IN MAIZE SILAGE IN *IN VITRO* RUMEN SIMULATIONS

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INTRODUCTION

Mycotoxin contaminated feed is increasingly being associated with subclinical health problems in highly productive dairy cows, reflected by vague and non-specific symptoms and suboptimal milk production. 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). The detoxifying capacity of ruminal microbiota for the previously mentioned mycotoxins, as well as the binding capacity of those mycotoxins to silage is not well known. To answer these questions, *in vitro* bovine and ovine rumen incubations with maize silage, spiked with single mycotoxins, and possible degradation of these mycotoxins after incubation was studied. Furthermore, inactive (autoclaved) rumen fluid and UHPLC water was used as inoculum to determine the binding capacity to maize silage. In addition, the impact of mycotoxins on rumen fermentation was studied, and was monitored by volatile fatty acids and CH_4 production. To determine the mycotoxin concentrations in the samples, 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-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) in rumen fluid using UPLC-MS/MS was developed and validated.

MATERIALS AND METHODS

Spiked substrate:

- 50 mg maize silage
- spiked in triplicate with single mycotoxins at following concentrations:

CIT	DON	NIV	ENN B	MPA	ROQ-C	ZEN
0.5 µg/g	12 µg/g	12 µg/g	1 µg/g	6 µg/g	2 µg/g	3 µg/g

***In vitro* inoculum:**

- Fresh rumen fluid/buffer mixture (FRF):
  - mixture from 3 lactating cows
  - or mixture from 3 hamels
  - or from 4 individual cows (3x lactating, 1x non-lactating)

  → added immediately to spiked substrate

  → incubation for 1.5h at 39°C in shaking incubator
- Autoclaved rumen fluid (ARF) } added to substrate
- UHPLC water (H_2O) } 24h after spiking

Spiked matrix-matched control samples (n=3x3):
FRF: without incubation; ARF: 0h; H_2O: 0h, without substrate

Analysis of samples for mycotoxins:

- Sample preparation: salting-out liquid-liquid extraction (SALLE)[1]
- Quantitative analysis (UHPLC-MS/MS[1]) of CIT, DON, DOM-1, NIV, ENN B, MPA, ROQ-C, ZEN, α-ZOL, β-ZOL, ZAN, α-ZAL, β-ZAL

[1]Debevere et al. (2017). 39th Mycotoxin Workshop, Abstracts, p103

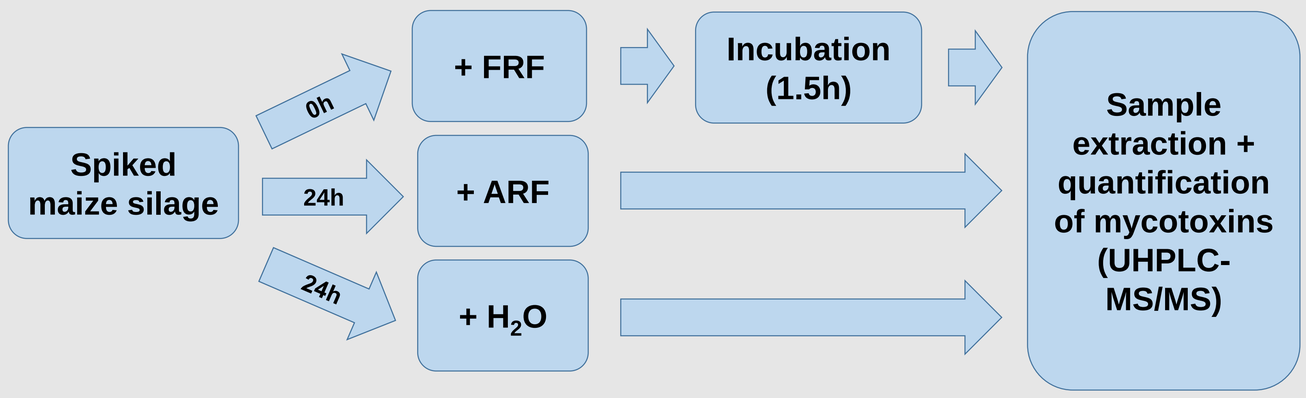

Figure 1. Setup of the *in vitro* rumen incubation study to determine the effect of rumen microbiota, inactive rumen fluid and substrate on recovery of mycotoxins.

RESULTS AND DISCUSSION

Table 1. Reduction of mycotoxins, expressed as % compared to control samples, when fresh rumen fluid (FRF), autoclaved rumen fluid (ARF) or UHPLC water was added to spiked maize silage according to the design in Fig. 1.

Disappearance compared to control (%)	FRF	ARF	H_2O
CIT	96±4	100±0	100±0
ROQ-C	57±13	76±9	54±5
DON	$24±9^{A}$	$10±1^{B}$	$4±3^{B}$
MPA	$21±13^{B}$	$42±10^{A}$	$52±3^{A}$
ENN B	$29±7^{A}$	$37±9^{A}$	$10±3^{B}$
ZEN	$25±15^{B}$	$46±10^{A,B}$	$51±4^{A}$
NIV	3±5	5±5	0±0

Values are presented as mean ± SD. Values with a different superscript are significantly different (P<0.05). No metabolites were detected in the samples (range LOQ mycotoxins: 0.05- 1.56 ng/mL; NIV: 36 ng/mL)

Volatile fatty acids (VFA) and CH_4 productions were normal during the *in vitro* incubations with FRF → good microbial activity, although the pH range of FRF and ARF was divergent: pH 5.36-6.55.

High **similiarities** when FRF, ARF or H_2O is used:

- **CIT:** (almost) complete disappearance (90-100%)
- **ROQ-C:** extended disappearance (>50%)
- **MPA, ENN B, ZEN**: moderate disappearance (10-50%)
- **NIV**: (almost) no disappearance (0-10%)

Some **differences**:

- **DON:** higher disappearance when FRF is used, which may be caused by rumen microbiota.
- **MPA, ZEN**: higher disappearance when (ARF or) H_2O is used, which may be due to longer exposure/binding time to substrate (24h)
- **ENN B**: higher disappearance when FRF or ARF is used, which may be caused by extra binding to particles in rumen fluid.

CONCLUSIONS

Degradation of DON by the microbiota is assumed, however, no DOM-1 was detected. In contrast, NIV showed no degradation. There was no effect seen on the rumen fermentation parameters tested. Binding to the maize silage substrate of the mycotoxins CIT (complete), ROQ-C (high), MPA, ENN B and ZEN (moderate) is concluded as disappearance of those mycotoxins in spiked substrate also occurs when no active rumen fluid is added. Future studies to determine the stability of this binding in the gastrointestinal tract of ruminants are necessary, as bound mycotoxins could be released post-ruminally (e.g. pH effects) and exert their toxic effects.

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