

# OCCURRENCE OF PRINCIPAL ERGOT ALKALOIDS IN SWINE FEEDING

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## Introduction

Ergot alkaloids (EAs) are secondary metabolites produced by fungi in the genus *Claviceps* that contaminate a large variety of cereals. The ingestion of contaminated cereals might cause adverse health effects in humans and animals, as pigs, cattle, sheep, and poultry. Focusing on pigs, these effects comprise poor performance, loss of appetite, agalactia, impact on reproduction, neonatal mortality, impaired liver function, and gangrenous, among others. To avoid that, European Union (EU) has established a maximum content of 1000 mg/kg of rye ergot sclerotia (*Claviceps purpurea*) in feed materials and compound feed containing unground cereals. However, the absence of sclerotia does not exclude the presence of EAs. Although EAs as such are still not regulated, feed industry recommends practical limits for EAs in pig feeds (from 200 to 500 µg/kg for lower and higher limit, respectively). This work describes an analytical method based on QuEChERS and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of the main EAs in 228 feed samples.

## Analytical method

### SAMPLES

Compound feed (226 samples):

71 for fattening pigs

42 for sows

111 for piglets

2 for gilts

(flour:183; pellets: 43)

Maize (grain, 2 samples)



### ANALYTES

Ergometrine (Em)

Ergosine (Es)

Ergotamine (Et)

Ergocornine (Eco)

Ergokryptine (Ekr)

Ergocristine (Ecr)

Ergometrinine (Emn)

Ergosinine (Esn)

Ergotaminine (Etn)

Ergocorninine (Econ)

Ergokryptinine (Ekcn)

Ergocristinine (Ecrn)

### SAMPLE TREATMENT

QuEChERS

- Sample: 1 g

- Extraction: 10 mL of acetonitrile : ammonium carbonate (85:15, v/v)

- Vortex (3 min) and centrifugation (9000 rpm, 5 min at 4°C)

- Clean-up: 5 mL of extract + 150 mg Z-Sep+ sorbent

- Vortex (3 min) and centrifugation (9000 rpm, 5 min at 4°C)

- Two mL of the upper layer was evaporated

- The residue was dissolved with 1 mL of methanol : water (50:50, v/v)

### DETERMINATION

LC-MS/MS

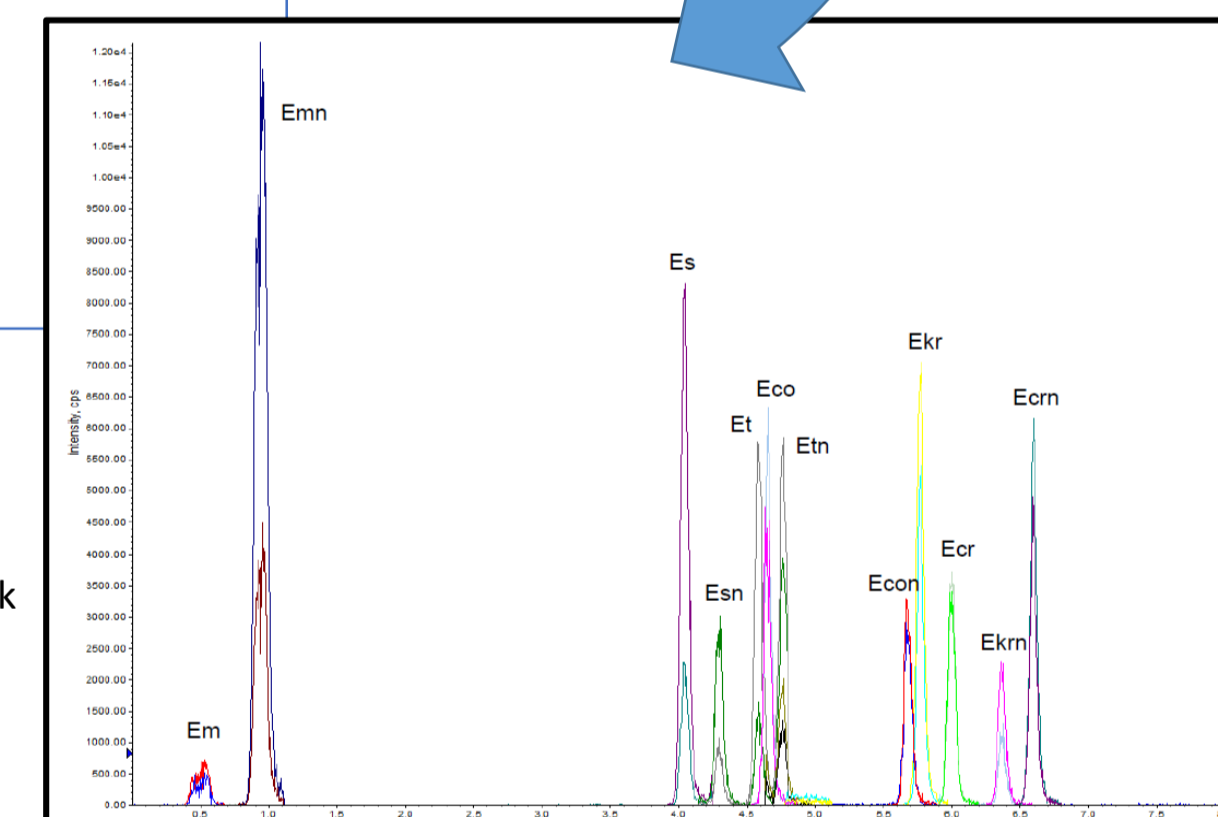
- C18 Zorbax Eclipse Plus RRHD column (50 x 2.1 mm, 1.8 µm)

- Mobile phase: A (water with 0.3% formic acid, v/v), and B (MeOH with 0.3% formic acid, v/v), gradient elution

- Multiple reaction monitoring (MRM) conditions

- Positive electrospray ionization (ESI+)

Chromatogram of a blank feed sample spiked with EAs at 100 µg/kg



## Results

### Performance parameters of the method

- ✓ Linearity:  $r^2 > 0.99$
- ✓ Limits of detection (LD): 0.6-5.2 µg/kg
- ✓ Limits of quantification (LQ): 2.1-22 µg/kg
- ✓ Precision < 13% (RSD)
- ✓ Recovery: 70-90%
- ✓ Matrix effect < 25%

### Analysis of feed samples

	Summary of EA occurrence in positive samples							
	Em	Emn	Et	Es	Esn	Ecr	Eco	Total EAs
Incidence <sup>a</sup>	18 (62%)	5 (17%)	8 (28%)	9 (31%)	11 (38%)	5 (17%)	1 (3%)	
Mean concentration <sup>b</sup> (µg/kg)	59.1	<LQ	9.5	9.8	10.7	12.2	<LQ	44.1
%RSD <sup>b</sup>	76.6	--	40.1	3.8	32.2	15.6	--	103.6
n <sup>b</sup>	14	--	8	2	11	5	--	25

<sup>a</sup> Considering samples with concentrations >LD; <sup>b</sup> Considering samples with concentrations >LQ

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## Conclusions

- ✓ EAs were detected in 29 samples (12.7%)
- ✓ Twenty-five samples (11%) showed concentrations >LQ
- ✓ Only 7 of 12 target EAs were detected
- ✓ The 65.5% of positive samples (19 from 29) revealed the co-occurrence of several EAs (up to 5 EAs).
- ✓ Possible synergic effect should be taken into account
- ✓ Total EA concentrations were between 5.9-158.7 µg/kg, lower than the recommended limits, indicating low risk due to the presence of EAs in pig feeds
- ✓ However, some samples showed concentrations higher than 100 µg/kg, which, according to some studies, could have negative effects on long term consumption