Detection of active compounds and their stability in feeds

Lessons learnt from SMEthane

Hamid Boudra[1], Ignacio Martin Garcia[2]
[1]INRA (UMR 1213 Herbivores, 63122 Saint-Genès-Champanelle, France)
[2]Instituto de Nutrición Animal, Estación Experimental del Zaidín (CSIC)

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Ideal additive:

1- Definable = active molecules can be analysed

2- Stable under practical conditions of use

1- Analytical method:

☞Why?

- **To quantify** concentration in commercial *products* and *feeds*

- **To trace** them in *animal products and in the environnment*

1- Analytical methods:

- Developed methods for **12** natural or synthetic additives provided by **5** industrial partners
- Additives were analysed either by LC or GC-MS/MS
- LC-MS/MS was developed with objective to analyse all the additives **simultaneously**.

1- LC-MS/MS method:

For simultaneous detection:

1- Extraction

➔ achieved with **aqueous acetonitrile**

2- MS Detection

➔ **Ionization** (positive or negative)

➔ **Acquisition (**MRM, SIR, ..)

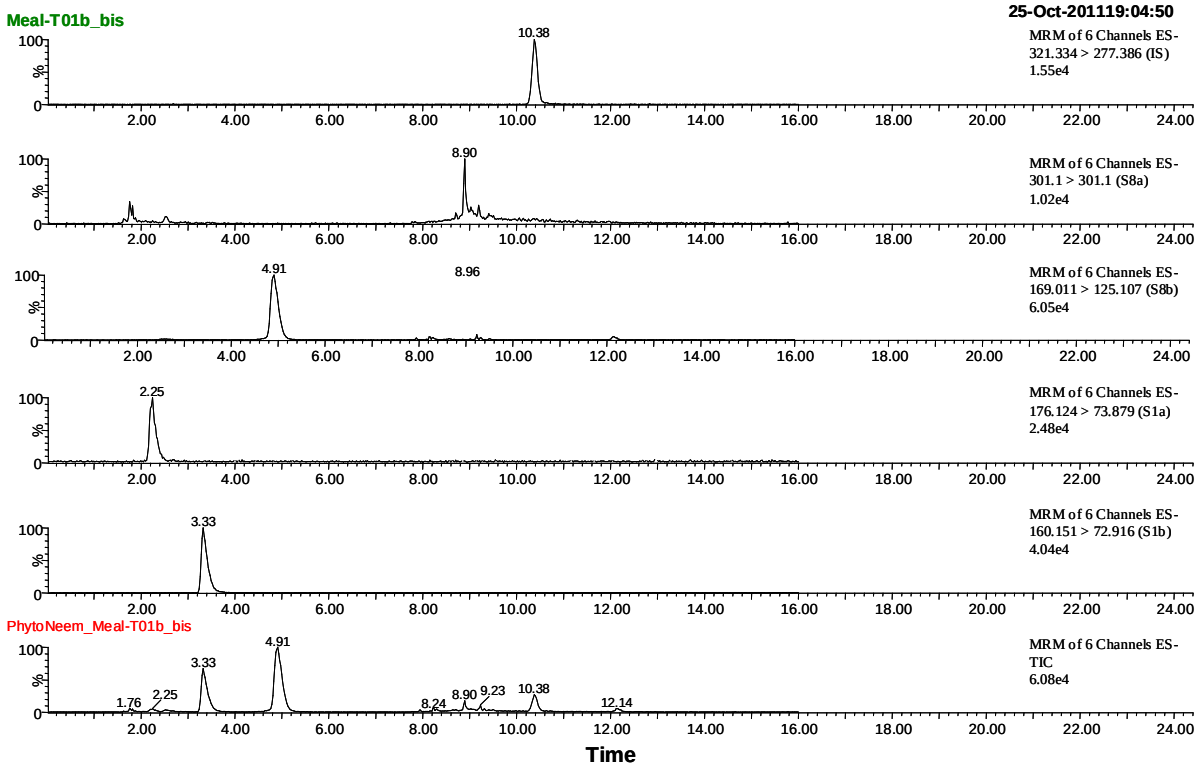
	Extraction	**Detection**	
Compounds		ESI mode	MRM
S1a	ACN-DW	Negative	
S1b	ACN-DW	Negative	
S8	ACN-DW	Negative	
S11	ACN-DW	Positive	
S12	ACN-DW	Positive	

Conclusion:

Analysis was done in 2 different methods

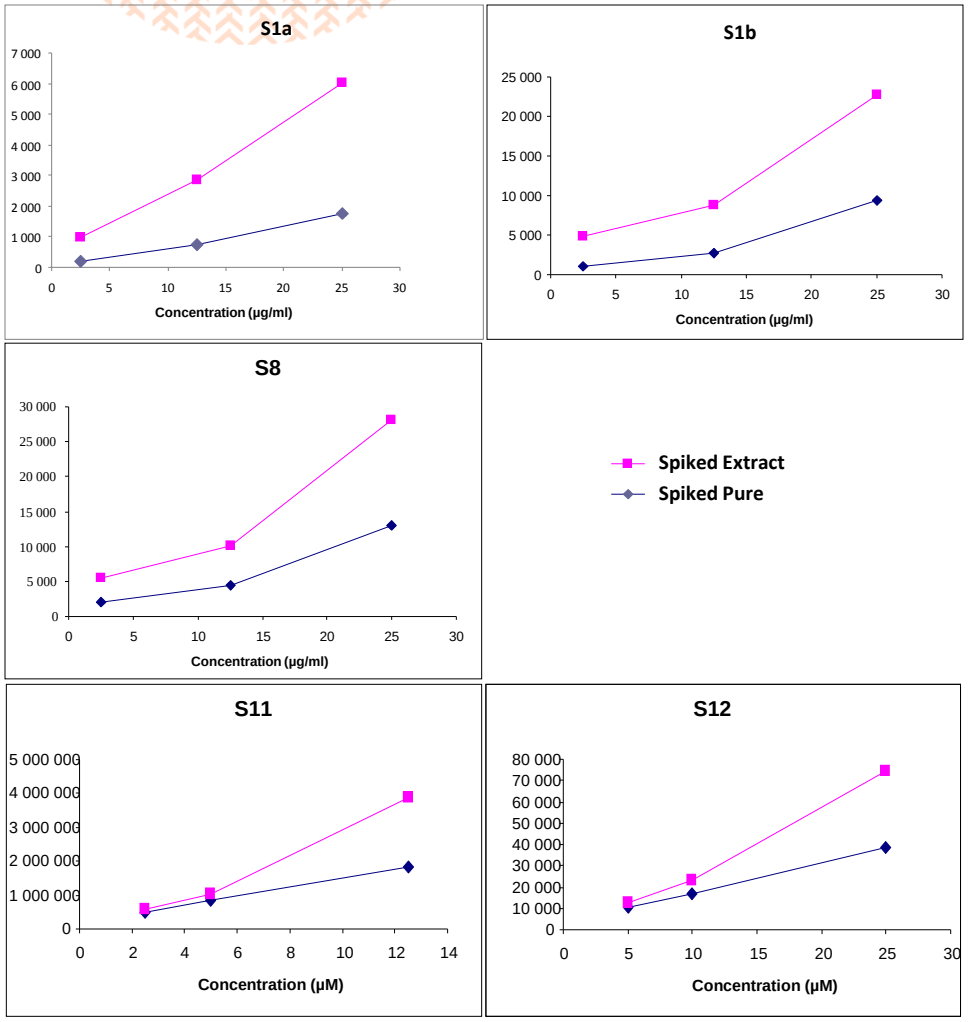
1- LC-MS/MS method: How?

Validation: linearity, specificity and variability



1- LC-MS/MS method: How?

Matrix effect



➔ **Matrix-matched calibration should be used**

2- Stability:

Why we should test the stability?

- To know the effect of storage conditions
  - humidity
  - temperature
  - length of storage

- To make recommendations on
  - packaging
  - Processing
  - best before use date

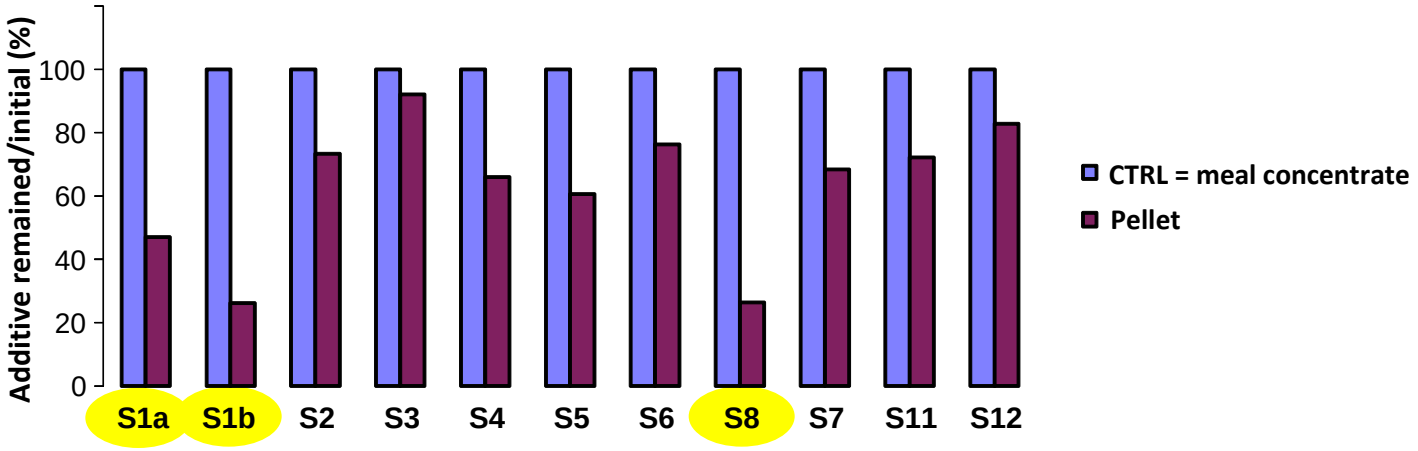
2- Stability:

☞ Preparation of spiked meal and pellet

- Done by Spain team: **same substrate** and **preparation**
- **Homogeneity** tested by analysing 5 replicates
- Feeds were aliquoted in several plastic bottles.
  - One set analysed immediately (**control**)
  - The rest stored at different temperatures: **+4** (as reference), **15 and 30 °C**.
  - Analysed at **0**, **1 and 2 months**

2- Stability: Results

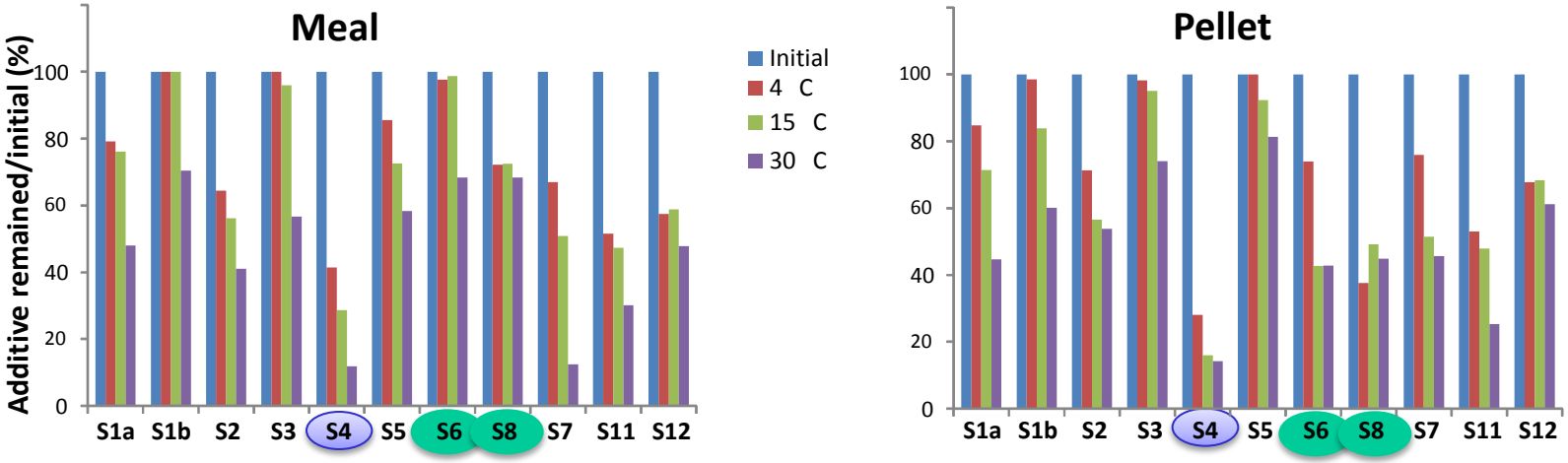
☛ Effect of pelleting process



- **Loss of all additives**
- **Marked loss for 3 additives ➔ 53 up to 74%**

2- Stability

☛ Effect of 1 month storage and temperature



- No differences between **meal** and **pellet,** except S6 and S8*
  - ➔ **Fungal growth**
- Loss increase with **temperature**
- S4 was unstable

Conclusion: What we learned from this project?

☞From analytical method

- **Multi detection is possible** depending on chemical structure
- **Matrix enhanced the response in LC-MS method**
- **Sample homogeneity** can be disturbed during storage

Conclusion: What we learned from the stability study?

- Stability highly variable: nature of additive and temperature of storage
  - Not recommended to store in tropical and subtropical areas or during the hot season in temperate areas
- **Pelleting process** affected negatively stability
- **Residual humidity** in feeds can promote mold development, especially at high temperature.
- The absence of fungal growth in **pellet-S1a & b, S11 and 12** could be explained by antimicrobial and antifungal activities of additives (*Yabuki et al. 2010)*