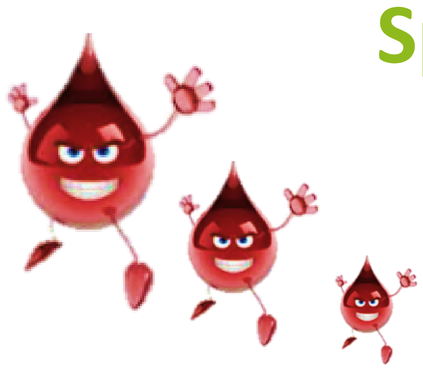

Specific detection of blood derived products in animal feed using UPLC-MS/MS

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Introduction

Context: Blood derived products are valuable animal products used in feed for their nutritional value and their positive effects on growth and health. Nevertheless, since the **BSE crisis**, the use of **animal by-products** is strictly regulated. The identification of the species of origin and the protein type is therefore crucial to ensure **feed safety.**

The objective was to set-up a **routine mass spectrometry method** [1] for the **specific** detection of blood meal and hemoglobin powder**.** This method has to reach the limit of detection (LOD) of 0.1 % w/w imposed by the European Commission (EC) for the detection of **processed animal proteins**.

Materials & study design

1. LOD evaluation

- ✓ Feed matrices: Pig feed and aquafeed
- ✓ Adulterant: mixed blood meal (80 % ruminant, 20 % porcine blood)

Adulteration levels : 0.05 % to 1 %

2. Prospective study on five commercial aquafeeds (AQF)

- ✓ Aquafeeds containing porcine hemoglobin powder. Their analysis using official PCR method gave:
  - ✓ positive result for ruminant DNA for AQF#1
  - ✓ negative results for ruminant DNA for the other AQF (AQF#2, AQF#3, AQF#4 and AQF#5)
- ✓ By official methods (PCR and light microscopy), it is impossible to determine whether ruminant DNA originates from authorised or non-authorised by-products.

Method

Biomarkers:

- ✓ 8 ruminant + 1 porcine hemoglobin peptides identified by high resolution MS [2]
- ✓ 3 – 4 transitions were selected for each biomarker

Sample preparation protocol:

- ✓ Extraction: TCA/aceton precipitation, Dige labelling buffer (DLA) extraction
- ✓ Purification: 2-D Clean-Up Kit (GE Healthcare, USA)
- ✓ Digestion: trypsin

UHPLC-MS/MS:

- ✓ Waters Acquity system (C18 BEH130 Column; 2.1 x 150 mm)
- ✓ Waters Xevo TQS triple quadrupole

Results

1. LOD evaluation

- LODs were evaluated for two biomarkers (one ruminant + one porcine) on two feed matrices adulterated with a mixed ruminant/porcine blood meal.
- Signal-to-noise ratio (S/N) was calculated for the two most intense multiple reaction monitoring (MRM) transition of each biomarker.

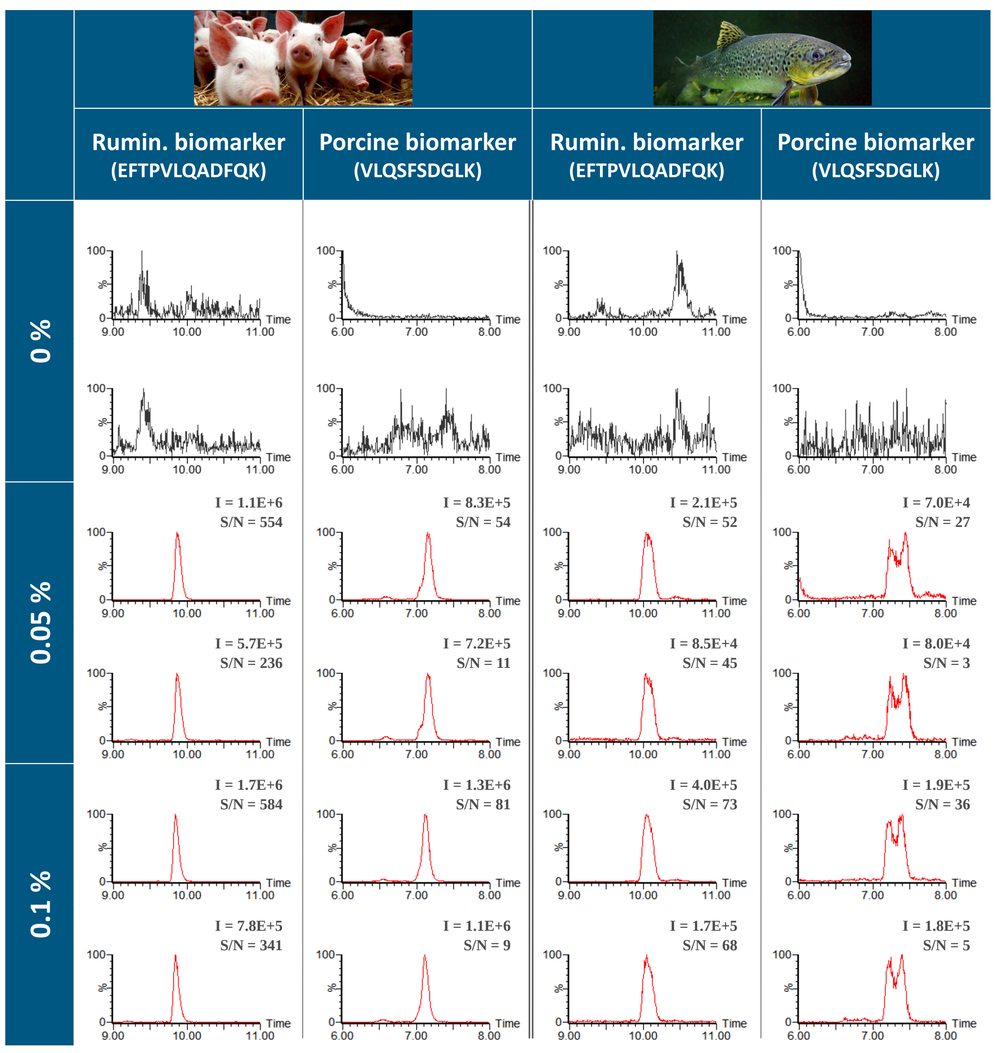

Figure 1: Chromatograms of the 2 higher MRM transitions of ruminant biomarker EFTPVLQADFQK (711.9 > 1041.6 & 711.9 > 736.4) and porcine biomarker VLQSFSDGLK (547.3 > 753.4 & 547.3 > 881.4) in pig or aqua- feed.

- **The method is highly specific: no signal was obtained in blank feeds.**
- **LODs were defined as the minimum concentration giving a S/N ratio > 3 for the most intense MRM transition.**
- **The method is sensitive: 0.05 % (w/w) of a mixed ruminant/porcine blood meal was detected. This level corresponds to ~ 0.04 % ruminant blood meal and ~ 0.01 % porcine blood meal.**
- **S/N varied depending of the feed. Matrix effect can affect the LOD.**

2. Prospective study on five commercial aquafeeds

- Five aquafeeds containing porcine hemoglobin powder as feed material were analysed to assess the potential of the method in explaining the origin of the PCR positive result for ruminant DNA for AQF#1.
- The same MRM transitions as for point 1. were used.

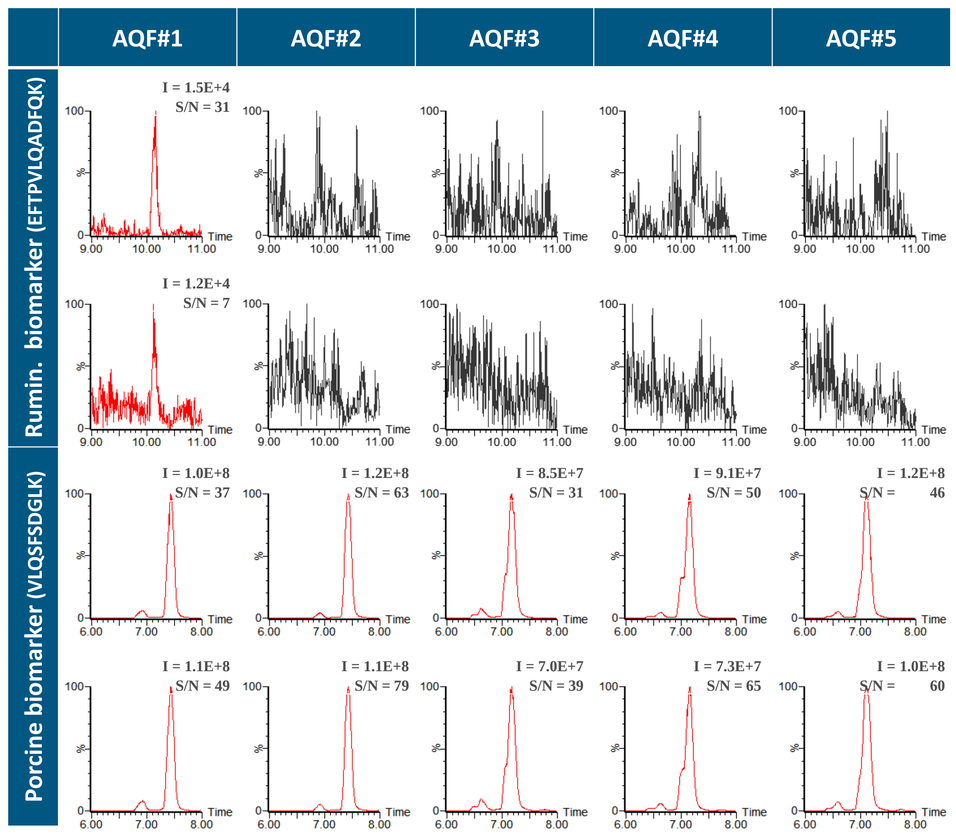

Figure 2: Chromatograms of the 2 higher MRM transitions of ruminant biomarker EFTPVLQADFQK (711.9 > 1041.6 & 711.9 > 736.4) and porcine biomarker VLQSFSDGLK (547.3 > 753.4 & 547.3 > 881.4) in 5 aquafeeds.

- **Porcine blood derived products are allowed to be used in aquafeed.**
- **By using UHPLC/MS-MS, blood derived products were easily detected in feeds at the concentration level usually used in feed for fish (~ 10 % w/w). This shows the applicability of the method on real commercial samples.**

- **Ruminant blood derived products are not allowed to be used in animal feed.**
- **Ruminant blood was detected in AQF#1 while it was absent in the other AQFs.**
- **UHPLC/MS-MS could be an efficient method to confirm positive results obtained by PCR, indicating in addition the type of by-product used and therefore the presence or not of non-authorised animal by-products.**

Conclusion and perspectives

In first estimation, the method would be able to detect ruminant and porcine blood at levels of **0.05 % w/w which is below the 0.1 % LOD required by the EC** for animal proteins detection methods. The combination of these biomarkers could give an accurate answer about the feed material used and the species origin of the proteins in support to the official methods.

References

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