Bovine Blood Biomarkers as a Way of Processed Animal Proteins Detection in Feedingstuffs

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Introduction

Context: The prohibition of use of animal **by-products in feedingstuffs** depends on 2 factors : their nature (defined by the **tissue/cell type** and the **species of origin**) and **on their destination** (pets, fur animals or other farmed animals). Currently, the detection of unauthorised feed material is based on light microscopy and Polymerase Chain Reaction (PCR) methods. However, some cases require additional methodologies to identify the **processed animal proteins** present. For instance, in feed for farmed animals other than fur animals, **milk powder** is an authorised feed material while **bovine blood products** are prohibited. In this case, specific identification is not possible microscopically and both materials provide a similar DNA signature, making them analytically indistinguishable.
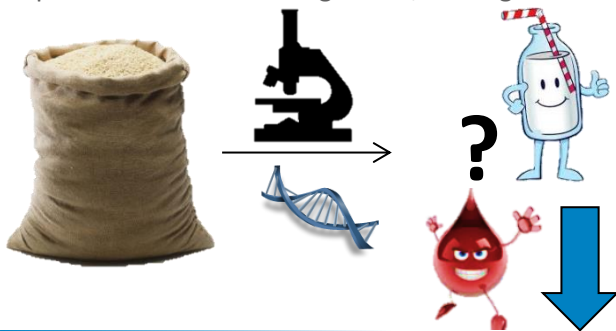
Objective: The nature of the feed material might be distinguished based on their protein content. **Proteomics** are therefore particularly well-suited for this purpose as it allows the identification of species and tissue-specific proteins.

The aim of this study was the identification and the selection of **specific peptide biomarkers** using **tandem mass spectrometry** for the detection of **bovine blood proteins** in animal feed.

Materials

Ingredients:
18 (bovine or porcine) blood products
11 (bovine, porcine or poultry) blood meals
3 milk products
2 fish meals

Adulterated feeds:
Horse feed + 1 % or 10 % (m/m) bovine blood products or blood meal

ESI-MS/MS

Method

Sample preparation:
TCA/acetone extraction
Purification (2D Clean-Up Kit, GE Healthcare)
In-solution trypsin digestion

Analysis:
Nano-UPLC**,** Electrospray ionisation (ESI), Q-TOF
Database search and protein identification
Dominant proteins selection
Specific peptide selection

Results

Four proteins of interest were highlighted as possible providers of specific peptide sequences:

Alpha-2-macroglobulin,   **Serotransferrin**,
Apolipoprotein A-1,   **Haemoglobin** (α and β chains).

From these proteins, **16 peptide sequences** of 9-28 amino acids in length were identified as potential bovine blood biomarkers in feed. Nine of them could be used for the detection of plasma powder and seven of them for haemoglobin powder or blood meal. The evaluation of these peptides by a search against NCBInr database revealed that some of them could also be used to detect blood of other ruminants such as sheep or goats.

Discussion

Preliminary results are promising. Efforts are now focused on the improvement of the protocol in order to increase the sensitivity of the method regarding the selected proteins.

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