

# Simultaneous detection of animal products from ruminant, poultry and pig in feed by LC-MS/MS

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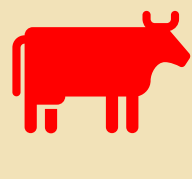
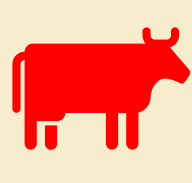

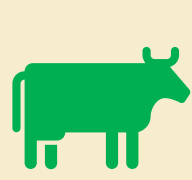

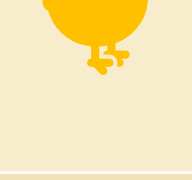
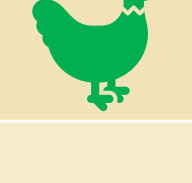
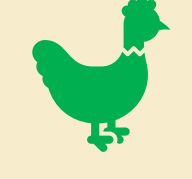

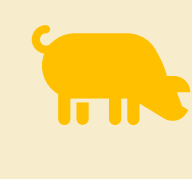
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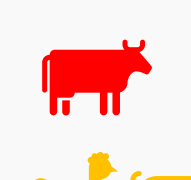


## Introduction

Caused by TSE as a health risk the European Parliament established a feed ban concerning the prohibition of mammal proteins in feedingstuff in 1994. This ban has been relaxed gradually since 2007 and processed animal proteins (PAPs) from non-ruminant origin, from insects and from pig and poultry are authorized now under certain considerations. Ruminant PAPs and blood products are still unauthorized but gelatine and dairy products are authorized in certain feedingstuffs. The official control method for the determination of animal proteins in feedingstuff (light microscopy and PCR) is no longer sufficient as it does not allow tissue-specific differentiation within the species. This analytical gap can be closed by LC-MS based methods.

## Results

Tab. 1: Overview tissue specific proteins with unique peptides of PRM method for ruminants, poultry and pig

Species	Tissue	Protein	UniProt ID	Peptide sequence	authorized / non-authorized
Ruminantia	Blood	Hemoglobin subunit beta	<a href="#">P04245</a>	AAVTFWVK EFTPVLQADFQK	
Bovinae			<a href="#">P02769</a>	LFTFHADICTLPDTEK DAFLGSFLYEYSR	
Cervidae/Caprinae		Albumin	<a href="#">P14639</a>	HLVDEPQNLIK DVFLGSFLYEYSR	
Ruminantia	Muscle/ Connective tissue	Prolargin	<a href="#">Q9GKN9</a>	ISSVPAISSR IEAIPSGYFK	
Ruminantia	Gelatine	Cathelicidin-4	<a href="#">P33046</a>	LLELDPPPKNEDLGR AVDQLNELSSEANLYR	
Ruminantia			<a href="#">P02465</a>	IGQPGAVGPAGIR GEPGAVGAVGAVGPR	
Ruminantia	Dairy products	Beta-lactoglobulin	<a href="#">P02754</a>	TPEVDDEALEK VYVEELKPTPEGDLEILLQK	
Cervidae/Caprinae			<a href="#">P67976</a>	TPEVDNEALEK VYVEELKPTPEGNLEILLQK	
Ruminantia		Alpha-S1-casein	<a href="#">P02662</a>	HQGLPQEVLENLLR YLGYLEQLLR	
Cervidae/Caprinae			<a href="#">P18626</a>	FVVAPFPEVFR	
Gallus gallus	Blood	Hemoglobin subunit alpha-D	<a href="#">P02001</a>	LIQQAWEK FLSAVSAVLAEK	
Gallus gallus			Albumin	<a href="#">P19121</a>	
Gallus gallus	Muscle/ Connective tissue	Creatine kinase M-type	<a href="#">P00565</a>	FSAEEFPDLSK LSVEALNSLEGEFK	
Gallus gallus			Myosin-11	<a href="#">P10587</a>	
Gallus gallus	Gelatine	Collagen alpha-2(I) chain	<a href="#">P02467</a>	VGPIGPAGNR GEIGPAGNVGPTGAPGPR	
Gallus gallus			Ovalbumin	<a href="#">P01012</a>	
Gallus gallus	Egg products	Apovitellenin-1	<a href="#">P02659</a>	AGQFLLDVSVQTTVVSGIR DWLVIPDAAAAYIEAVNK	
Sus Scrofa	Blood	Hemoglobin subunit Beta	<a href="#">P02067</a>	VLQSFSDGLK LLGNVIVVVLAR	
Sus Scrofa			Albumin	<a href="#">P08835</a>	
Sus Scrofa	Muscle/ Connective tissue	Carbonic anhydrase 3	<a href="#">Q551S4</a>	GGPLTAAYR HDPSSLPWATASYDPGSAK	
Sus Scrofa			Protein S100-A12	<a href="#">P80310</a>	

 non-authorized  
 authorized under certain considerations / for certain feedingstuffs  
 authorized

## References

European Commission. 94/381/EC: Commission Decision of 27 June 1994 concerning certain protection measures with regard to bovine spongiform encephalopathy and the feeding of mammalian derived protein. *Off. J. Eur. Communities* **1994**, L 172, 23–24

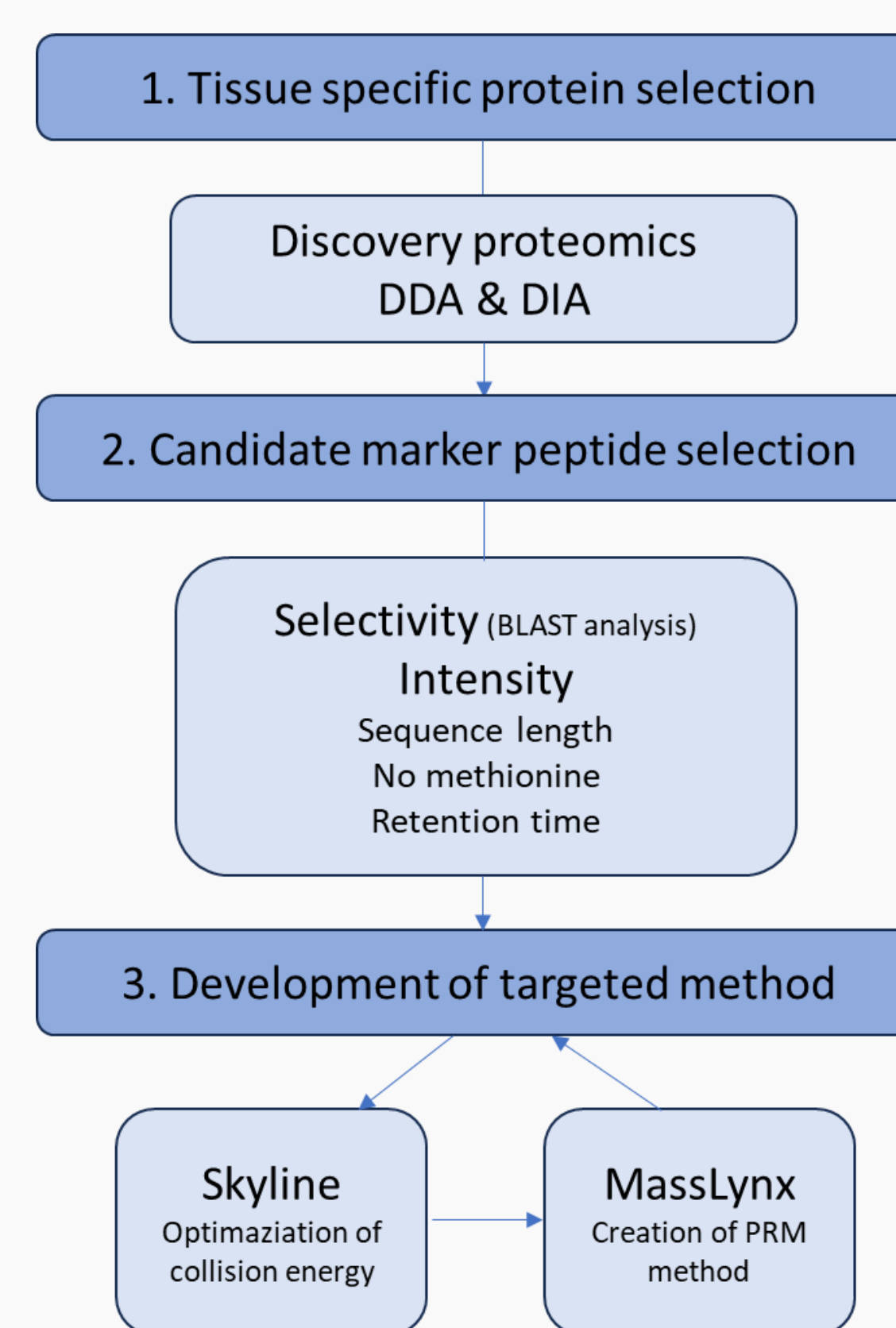
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## Conclusion

- Development of a **targeted PRM** (parallel reaction monitoring) method for animal products of ruminant (*Ruminantia*), poultry (*Gallus gallus*) and pig (*Sus scrofa*) in feed
- Identification of **tissue specific proteins** with **unique peptides** for ruminants, poultry and pig (tab.1) resulting in the clear identification of species
- **Distinction** between PAPs from **cattle (*Bovinae*)** and **goat or sheep (*Cervidae/Caprinae*)**
- Distinction between **authorized and non-authorized animal products** within species *Ruminantia*, *Gallus gallus* and *Sus Scrofa*
- BSA of *Ruminantia* not suitable to distinguish between blood and dairy products

## Material and Methods



### Sample preparation:

- Extraction by 2 M urea, 200 mM Tris-HCl (pH 9.2)
- Pre-digestion treatment (denaturation, reduction, alkylation)
- Digestion by Trypsin (47°C, 4 h)
- SPE purification

### LC-MS:

- Waters UPLC-System ACQUITY Bio H-Class equipped with a CSH C18 (1 mm x 150 mm, 1.7 μm) column
- Xevo G2 XS QTOF mass spectrometer from Waters

Fig. 1: Workflow PRM method development