DESIGN OF SRM BASED MASS SPECTROMETRY METHODS FOR QUANTITATIVE ANALYSES OF PORCINE ACUTE PHASE PROTEINS

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Introduction

Biomarker discovery:

- It is mandatory to validate candidate markers in a large amount of samples.
- Commercial kits are not always available for validation in farm animal species.
- SRM may be used as an alternative to commercial kits.

Acute phase proteins (APPs):

- Are widely recognized markers for inflammation, infection and welfare.
- In pigs the most important APPs are haptoglobin (Hp), C-reactive protein (CRP), the inter-α-inhibitor-heavy chain 4 (ITIH4), serum amyloid A (SAA) and apolipoprotein A-I (Apo A-I), but also fetuin A, albumin and transferrin.

Objectives

- The set up of SRM based mass spectrometry methods for the quantification of four porcine APPs: Hp, ITIH4, Apo A-I and fetuin A.
- The measurement of these four APPs in serum samples from sows subjected to moderate stress.
- The comparison of the APPs levels obtained using commercial kits and SRM.

Methods

- The protein sequences of the APPs were obtained from UniProt and pasted into the Skyline software (MacCoss Lab Software).
- Peptide and transition parameters were applied for filtering.
- Serum proteins were denatured, reduced, alkylated and digested with trypsin. Peptides were micropurified using C18 Stage Tips.
- 1.5 µg peptide of each sample was run in triplicate on an AB SCIEX QTRAP® 5500 System.

Results and discussion

1) Set up of SRM based mass spectrometry methods

- *Hp, ITIH4 and Apo A-I.* Four proteotypic peptides with intense transition signals were selected.
- *Fetuin A.* Only a single peptide with high transition signal was available.

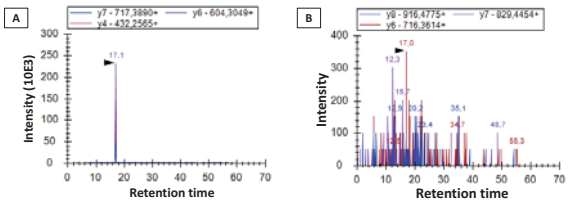

Figure 1. Transitions chromatogram after MS analysis. A) Good proteotypic peptide (intense transitions); B) Bad proteotypic peptide (non-intense transitions)

2) Quantification of porcine APPs in a welfare experiment

Porcine serum samples were run in triplicate. Ratios between post-stress and control days were calculated:

- *Positive APPs:*
  - Hp slightly increased after exposure to stress.
  - ITIH4 decreased after the challenge, but there was a large variability between animals.

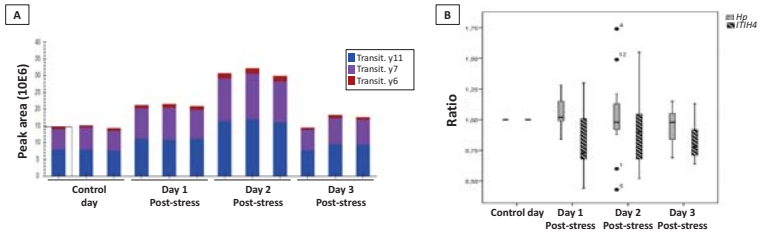

Figure 2. Analysis of the positive APPs in porcine serum samples. A) Peak areas of the three transitions of one of the targeted Hp's peptides (DIAPTLR) along the experiment. B) Box diagram of the positive APPs change ratios after exposure to stress.

Hp, but not ITIH4, behaved as expected during an acute phase response.

- *Negative APPs:*
  - Apo A-I and fetuin A were decreased after exposure to stress.

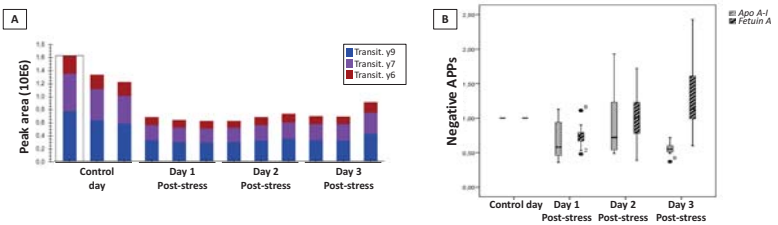

Figure 3. Analysis of the negative APPs in porcine serum samples. A) Peak areas of the three transitions of one of the targeted Apo A-I's peptides (VSILAAIDEASK) along the experiment. B) Box diagram of the negative APPs change ratios after exposure to stress.

The negative APPs behaved as expected during an acute phase response.

3) Comparison of the results obtained with commercial kits and SRM

Results from both techniques were compared:

Protein	Quantitative methods	R	*P*
Hp	Colorimetric assay vs SRM	0,397	**0,022**
ITIH4	ELISA vs SRM	0,847	**< 0,001**
Apo A-I	ELISA vs SRM	0,448	**0,028**
Fetuin A	ELISA 1 vs SRM	0,040	0,841
	ELISA 2 vs SRM	-0,269	0,137
	ELISA 1 vs ELISA 2*	0,130	0,432

Table 1. Pearson's correlations between techniques.
* Two different porcine fetuin A commercial kits didn't show the same results.

- The correlation of the APPs levels between both methods was significant when measuring Hp, ITIH4 and Apo A-I.
- ITIH4 showed the best correlation between techniques.

Conclusions

We conclude that:

- Commercial kits and SRM measures of porcine haptoglobin, ITIH4 and apoliproprotein A-I correlated well.
- SRM is an important alternative to commercial kits for the quantification of porcine proteins.