Biomarker discovery in milk for detection of recombinant bovine somatotropin abuse

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Background

Recombinant bovine somatotropin can be used to increase milk production in dairy cows, but the administration of this hormone is illegal in the EU (but approved in the US). Serum protein biomarker-based detection of rbST has been proven to be advantageous, because biomarkers remain altered for a longer period of time than the hormone itself can be detected. So far, only targeted biomarker discovery approaches were used in serum. To identify rbST-dependent protein biomarkers in milk, untargeted proteomics are used.

Experimental setup

- Milk samples from rbST-treated and untreated animals
- Depleting milk samples from major abundant proteins
- Performing two-dimensional differential gel electrophoreses
- Imaging of gels and identification of significantly up- or down-regulated protein spots
- Spot picking and in-gel digestion
- LC-MS/MS identifications of proteins (candidate biomarkers) from selected spots.

Animal study

- 8 rbST-treated and 2 untreated animals
- Treatment according to manufacturer's suggestions (Figure 1)

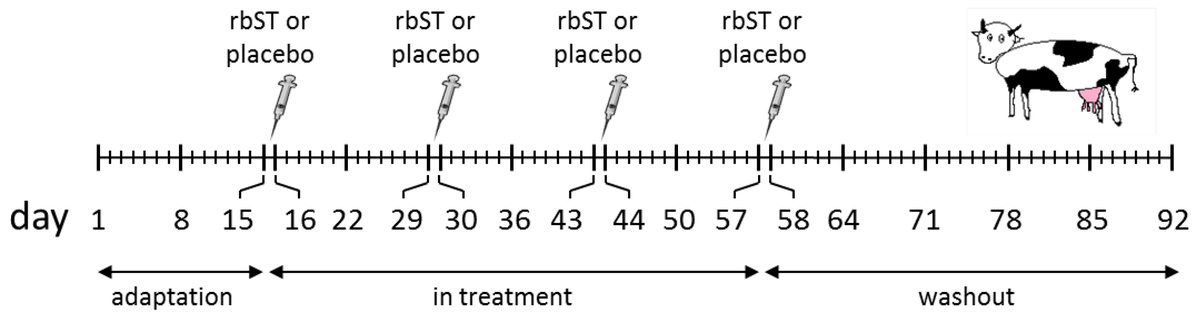

Figure 1. RbST treatment and sampling schedule.

Milk composition

- Milk consists of highly abundant proteins (caseins, β-lactoglobulin, α-lactalbumin, serum albumin) and low abundant whey proteins (Figure 2)
- Candidate biomarkers are expected in the low abundant fraction
- Therefore depletion of the major abundant proteins is necessary

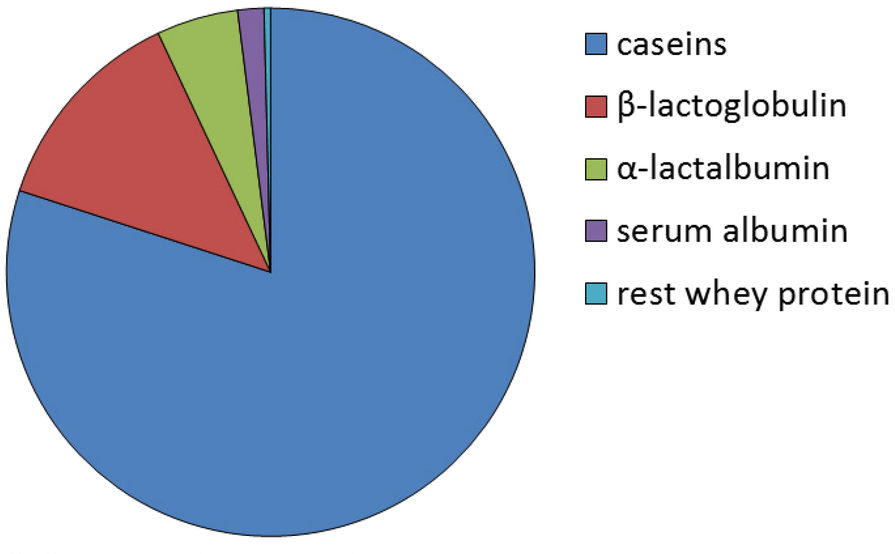

Figure 2. Cow milk protein composition

Strategy for depletion of major abundant proteins

Caseins

- Molecular weight ranging from 11 – 26 kDa
- Caseins are mainly insoluble
- They can therefore be depleted by ultracentrifugation (Figure 3.A)

β-lactoglobulin

- Molecular weight: 18.3 kDa
- β-lactoglobulin forms dimers and oligomers at pH 4.6
- β-lactoglobulin monomers were coupled to Sepharose beads and beads were incubated with the casein-depleted milk sample at pH 4.6 (Figure 3.B); then beads with oligomers were removed

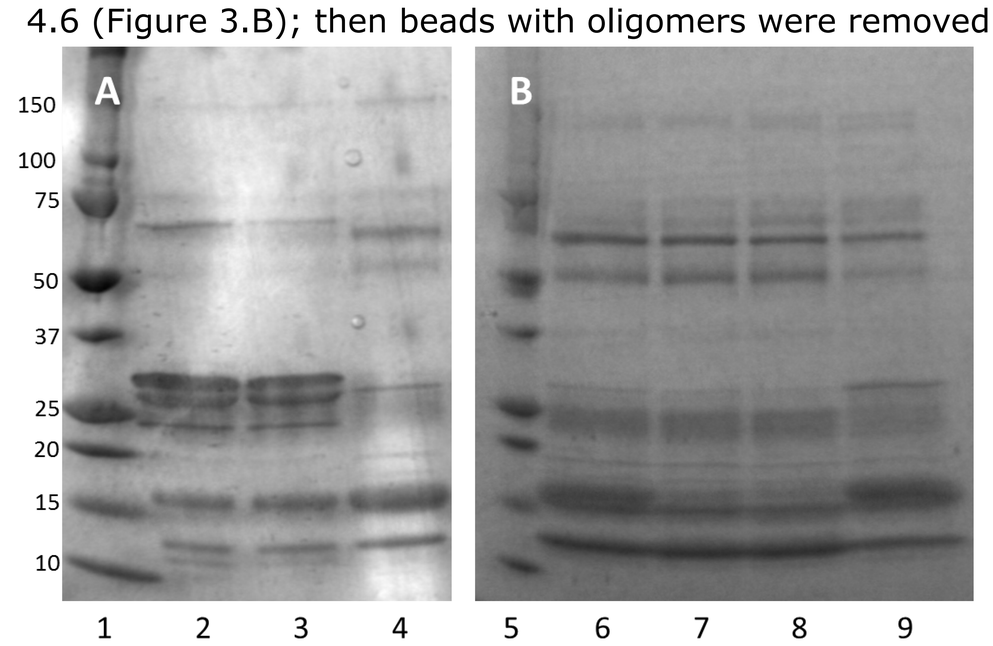

Figure 3. SDS-PAGE to control depletion efficiencies for casein (A) and β-lactoglobulin (B). 20 µg total protein were applied to each slot. Lane 1 and 5 – marker, lane 2 – defatted milk, lane 3 - 50,000 g ultracentrifugation for 30 min , lane 4 - 100,000 g ultracentrifugation for 60 min, lane 6 – sample to β-lactoglobulin Sepharose beads ratio 1:2, lane 7 - sample to β-lactoglobulin Sepharose beads ratio 1:4, lane 8 - sample to β-lactoglobulin Sepharose beads ratio 1:6, lane 9 – no β-lactoglobulin depletion.

Two-dimensional gel electrophoresis

- Depletion efficiency was tested on a two-dimensional gel (Figure 4)

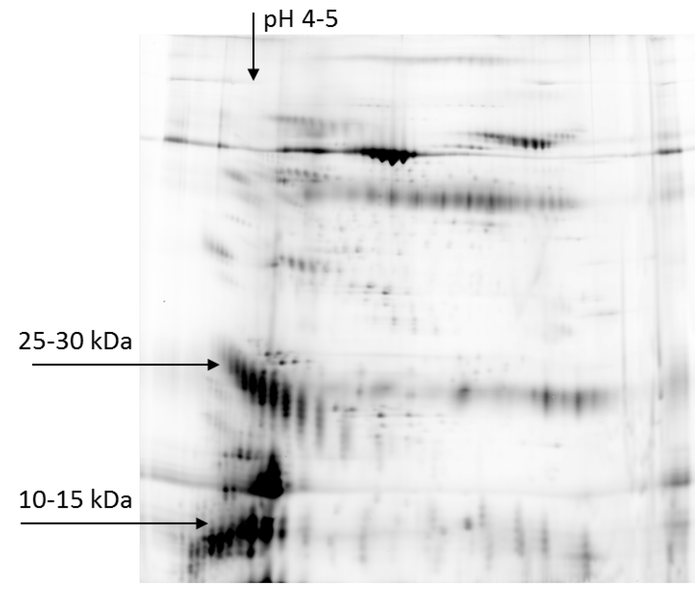

Figure 4. Two-dimensional gel electrophoresis separating based on charge and molecular weight.

Conclusions

Further depletion is necessary to avoid big spots, because they make quantification and sample to sample comparisons difficult.

Acknowledgements

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