



# Long term detectability of recombinant bovine somatotropin in serum and milk

Nathalie GE Smits<sup>1</sup>, Marco H Blokland<sup>1</sup>, Bijol Bakhai<sup>1</sup>, Saskia S Sterk<sup>1</sup>, Merel A Nessen<sup>1</sup>, Leen A van Ginkel<sup>1</sup> & Michel WF Nielen<sup>1,2</sup>

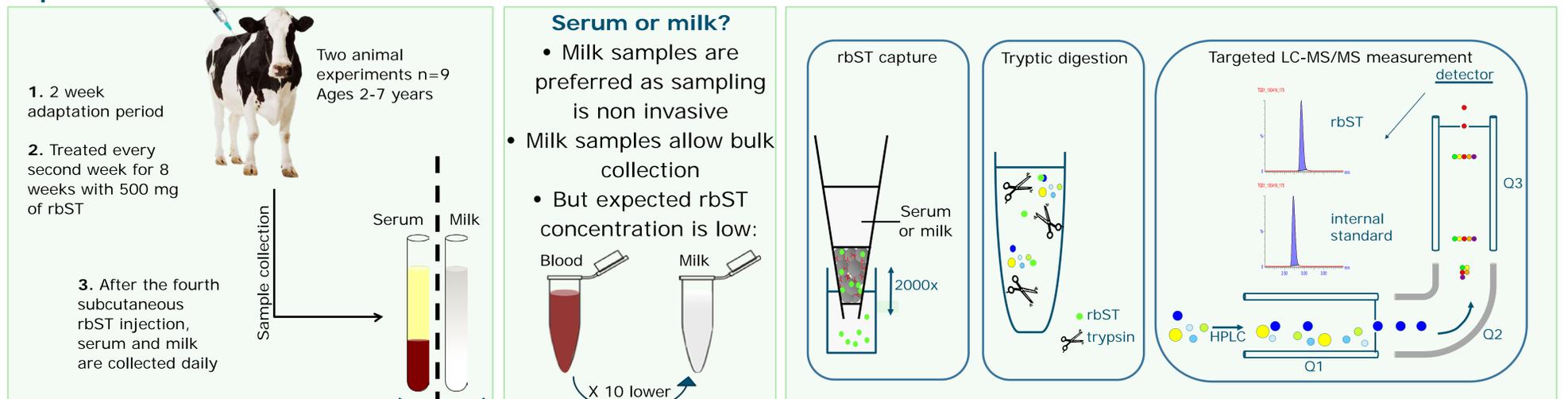
## Background

Recombinant bovine somatotropin (rbST) enhances milk production with approximately 25 %. Detection of rbST in blood and milk during the period of subsequent administrations is valuable for pharmacokinetic studies but also for control purposes as its use is forbidden in the European Union.

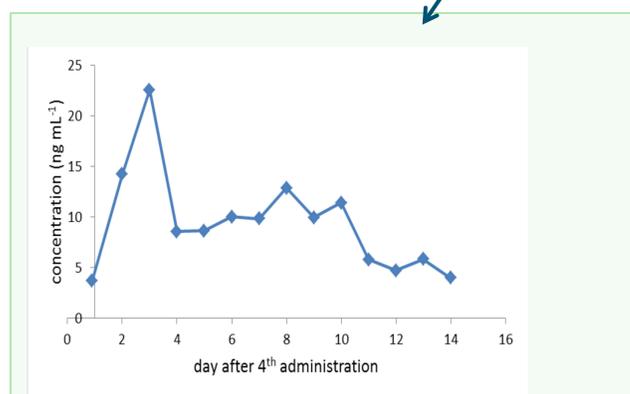
## Conclusions

- In the total period between subsequent administrations rbST is detectable in serum.
- Serum highest rbST concentration is found on the third day after rbST administration.
- rbST is also detectable in incurred milk samples, enabling pinpointing rbST abuse via milk samples.

## Experimental



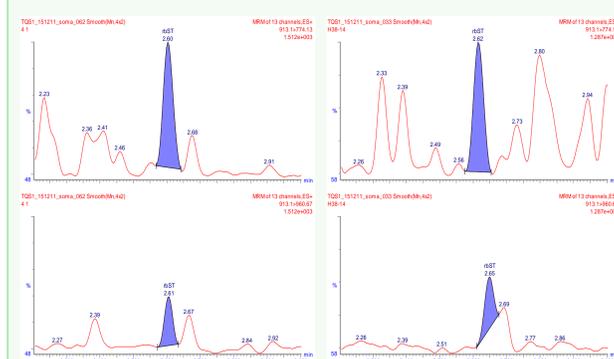
## Results



**Figure 1.** Daily average (n=9) serum rbST concentrations after rbST administration

- Serum rbST concentrations show high inter-individual differences. With extremes of 92 ng mL<sup>-1</sup> and 2 ng mL<sup>-1</sup> in the samples taken on the third day after administration.
- In the individual cows, a daily variation in serum rbST concentrations is seen between subsequent rbST administrations.
- General trend for all cows: the serum rbST concentration reduces from the fourth day onward after rbST administration (Fig. 1)
- **Future outlook:**
  - Extend data collection for a pharmacokinetic study

- Milk samples spiked with rbST showed a high intra-assay variation.
- Nevertheless, for the first time rbST is detected in incurred milk samples (Fig. 2).

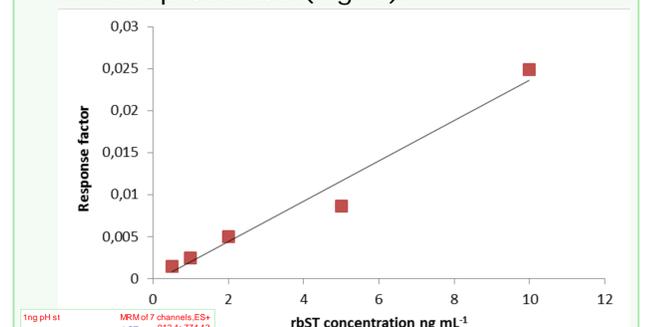


**Figure 2.** Chromatograms of two different transitions,  $m/z$  913> $m/z$  774 and  $m/z$  913> $m/z$  960 specific for the N-terminal peptide of rbST of a 1 ng mL<sup>-1</sup> rbST spiked milk sample (left) and an incurred milk sample of a cow, taken on day 4 after rbST administration (right).

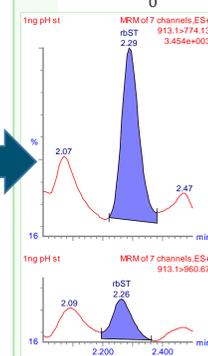
- **Future outlook:**
  - **Method robustness and sensitivity for rbST detection in milk should be improved**
  - Extend data collection for a pharmacokinetic study
  - Investigate applicability for bulk milk samples

## Preliminary Results

- Twofold sensitivity increment by reducing the internal diameter of the LC-column from 2.1 mm to 1 mm.
- Calibration curve shows R<sup>2</sup> of 0.97, with rbST determination at the lowest calibration point of 0.5 ng mL<sup>-1</sup> (Fig. 3).
- Chromatograms of calibration point 1 ng mL<sup>-1</sup> are presented (Fig. 4).



**Figure 3.** Constructing a linear calibration curve of the response factor (peak area ratio of rbST fragment and internal standard) with respectively 0.5, 1, 2, 5 and 10 ng mL<sup>-1</sup> of rbST spiked milk samples



**Figure 4.** Chromatograms of two different transitions,  $m/z$  913> $m/z$  774 and  $m/z$  913> $m/z$  960 specific for the N-terminal peptide of rbST of a 1 ng mL<sup>-1</sup> rbST spiked milk sample.



## Acknowledgements

This project was financially supported by the European Commission and the Dutch Ministry of Economic Affairs