A novel sandwich ELISA for the measurement of insulin in bovine serum and plasma

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CONCLUSION
This insulin ELISA provides high performance, precision and reliability as well as high in vivo relevance.

This method enables the performance of additional mechanistic investigations, which may contribute to a more complete understanding of the development of insulin resistance in the cow.

INTRODUCTION
It is well known that ruminants show typical insulin resistance compared to monogastric animals. During lactation, glucose is effectively re-directed to the mammary glands as a result of an increase in insulin resistance in the peripheral tissues. Furthermore, the level of insulin secretion also differs depending on the physiological states of dairy cattle.

The mechanisms underlying the development of insulin resistance are currently unclear and further investigations are warranted.

METHODS

Monoclonal antibodies directed against different antigenic determinants on the bovine insulin molecule were evaluated and chosen by care. Optimal reaction condition was established where the samples and calibrators were allowed to react simultaneously with the capture antibody and the HRP-conjugated detection antibody. A highly purified bovine insulin preparation was used as calibrator material. A representative set of samples from apparently healthy cows (n=33) was analyzed for the purpose of determining the appropriate measuring range.

RESULTS

Hook dose effect
Studies on hook dose effect showed that samples with a concentration of up to 1000 µg/L can be measured without giving falsely low results.

Calibration
Mercodia Bovine Insulin ELISA was calibrated against an in-house reference preparation of native bovine insulin.

Sensitivity
The sample volume in the Bovine assay is 25 µL and the calibrator concentration ranges from 0.05 µg/L to 3.0 µg/L.

Samples from healthy cows showed that more than 95% of insulin measurements fell between the insulin concentrations of calibrator 2 and calibrator 4, which confirms that the assay is sensitive and has an accurate dynamic range.

Detection limit
The detection limit was determined to 0.025 µg/L according to the methodology described in ISO 11843-Part 4.

Specificity
Recovery
Recovery upon addition was 86-107% (mean 96%). Recovery upon dilution was 91-131% (mean 107%).

Cross reactivity
No crossreactivity with proinsulin or C-peptide.

Clinical validation

Swedish study
Healthy cows in the middle of lactation (n=62) showed an insulin range of 0.24-4.5 µg/L.

Danish study
Healthy cows before and after feeding (n=40) showed an insulin concentration of 0.46 µg/L (0.07-0.99 µg/L) before feeding and 0.70 µg/L (0.14-2.27 µg/L) after feeding.

US study
Heat stressed cows (n=12) showed an insulin range of 0.23-3.45 µg/L where the thermal neutral basal levels were 0.81 µg/L.

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