Poster 11

DEVELOPMENT OF A RAPID BIOSENSOR METHOD FOR DETERMINATION OF HAPTOGLOBIN IN BOVINE MILK

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Introduction

The somatic cell count (SCC) in bulk tank milk is widely used in milk quality programmes, serving as one of the parameters for quality payment of the raw milk. The dairy cooperatives often give farmers premium quality payment to encourage a low SCC although there is no clear scientific data defining the level of SCC in bulk tank milk with additional benefits in terms of milk quality. Recent research on alternative markers for inflammatory processes in the lactating cow includes the acute phase proteins haptoglobin (Hp) and serum amyloid A (SAA). So far these proteins have been studied from the diagnostic perspective, with little attension regarding their role in relation to milk composition and technological properties. The aim of the present study was to develop a rapid biosensor method to determine Hp in milk and to compare the results of the assay with results obtained by a commercially available immunoassay.

Materials and Methods

An affinity sensor assay based on the interaction between Hp and haemoglobin was developed using surface plasmon resonance (SPR) biosensor technology (Biacore AB, Uppsala, Sweden). The resulting assay was used to analyse Hp in composite milk samples from cows without any clinical signs of mastitis (n=43) collected at the dairy research herd at Jälla, Uppsala. In addition, quarter milk samples from cows with clinical mastitis (n=23) were obtained from the National Veterinary Institute, Uppsala. All milk samples were also analysed with a commercial ELISA for determination of Hp in milk (Tridelta Development, Ltd., Co. Kildare, Ireland) for comparison with the biosensor assay. According to the manufacturer, the limit of detection of the ELISA was 0.3 µg/ml.

Results

The detection limit (LOD) of the developed biosensor assay was determined to 1 µg/ml. With-in and between-day variations (CV) determined at 4 µg/ml were 3.6 % and 5.9 %, respectively. In the analysis of Hp in composite milk samples from cows without clinical signs of mastitis, 36 of the 43 samples did not contain detectable levels of Hp. The ELISA detected Hp in the remaining 7 samples (1-12 µg/ml) whereas the developed biosensor assay only detected Hp in the 3 samples with highest concentrations. In the analysis of quarter milk samples from cows with mastitis, 19 of the 23 samples had Hp concentrations below 50 µg/ml (1-48 µg/ml) and 4 samples contained higher Hp levels (51-143 µg/ml). For these samples the results obtained by the biosensor assay agreed satisfactory with the results in the ELISA.

Conclusions

Detection of sub-clinical cases of mastitis in the herd is of great importance, not only to the producer but also to the dairy industry. Milk from cows with sub-clinical mastitis has altered composition and processing properties, which will affect the quality of dairy products. New indicators of inflammation are needed to allow early identification of infected quarters. The overall aim of our work is to study if the acute phase proteins can be used as indicators not only for udder health but also for raw milk quality. For such studies we need rapid and simple methods. To our knowledge this study is the first application of an optical biosensor for determination of acute phase proteins e.g. Hp in milk. The main advantage of the biosensor in comparison with ELISA is the automated and rapid detection; each analysis requiring approximately 8 minutes. The results of this study show that the developed assay has potential to be useful in screening of Hp in milk, however, further optimization is required to achieve a lower detection limit.