Poster 19

AUTOMATION OF A METHOD TO DETERMINE ACID SOLUBLE GLYCOPROTEIN IN PORCINE SERUM AFTER ACID PRECIPITATION

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Introduction

After perchloric acid precipitation, the acid soluble glycoprotein (ASG) fraction left in supernatant can be easily measured using Coomassie brilliant blue (Nagahata et al 1989) or bicinchoninic acid (Eckersall et al., 1996). In most species, the acute phase protein alpha-1 acid glycoprotein (AGP) is the predominant protein in ASG, so determination of ASG can reflect the acute phase status of pigs (Lampreave et al 1994, Eckersall et al 1996). The purpose of this work was to validate an automated protocol for the measurement of ASG after acid precipitation in porcine serum and prove its usefulness to detect acute phase response in pigs.

Material and Methods

All analyses were performed as follows: samples were precipitated using perchloric acid. After centrifugation, total protein concentration in supernatant was determined using bicinchoninic acid in Cobas Mira Plus analyzer. Calibration was performed using a secondary pooled porcine serum standard with a concentration of ASG of 19.15 g/L, diluted to 9.58, 4.79, 2.39, 1.2, 0.6 and 0.0 g/L. These concentrations were established using human ASG (Sigma Chemical) as primary standard. For analytical validation of the method, 3 porcine serum samples with high ASG value, and 3 with low ASG concentration were used. Within and between run coefficients of variation were determined by repeated measurements performed the same day and in different days, respectively. Accuracy was determined by linearity under dilution method, diluting samples at 50, 25 and 12.5%. Limit of detection was determined by repeated analysis of sample diluent. To assess clinical validation, 10 porcine samples from healthy animals and 12 from animals with different diseases were measured using the same protocol described above. A non parametrical statistic analysis was performed using SPSS for Windows.

Results

Within and between run coefficients of variation were lower than 5% and 10%, respectively. Linearity under dilution showed a coefficient of linear regression higher of 0.99 in all the samples tested. Limit of detection estimated for the test was 0.23 g/L. Values for healthy samples ranged between 2.71 and 5.32 g/L, with median of 3.57 g/L; for pathologic samples values ranged between 4.46 and 11.45 g/L, with median of 8.35 g/L. Statistical differences were seen between healthy and pathologic samples ($p<0.001$).

Conclusion

The automated method described can be used for an accurate, cheap, rapid and easy quantification of ASG in porcine serum samples, and it could contribute to a wider use of ASG as acute phase protein in porcine medicine.

References.

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