Poster 20

MEASURING THE NEGATIVE ACUTE PHASE REACTANT TRANSTHYRETIN IN PORCINE SERUM

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Introduction

Transthyretin (TTR) is a thyroxine binding protein found in blood which forms a complex with retinol binding protein for the transport of vitamin A. TTR is a negative acute phase reactant in humans and the concentration of TTR in serum falls due to decreased synthesis in inflammation, infection or trauma. Serum levels of TTR are measured as a health status indicator using commercially available assays. We have developed an assay for TTR in pig serum the method and use of which is described below.

Materials and Methods

Microtiterplates (96 well, Corning Costar, Cambridge UK) were coated with pig serum samples or purified human prealbumin (TTR) (Sigma-Aldrich, Poole, UK) diluted in 50 mM NaHCO3 (pH9.6), 100 µl/well for 20 hours at 4°C. The samples were then decanted and unbound sites blocked by adding 250µl of 5% (w/v) non fat dried milk in assay buffer, (0.12 M NaCl, 0.02 M Na2HPO4, 0.1% (v/v) Tween 20, pH 7.4) at room temperature for 30 minutes. The plates were washed with assay buffer and then 100 µl of sheep anti-human TTR (ICN Biomedicals, Basingstoke UK) diluted 1/1000 in assay buffer was added to each well and the plates incubated for 1 hour at 37°C. The wells were then decanted and washed three times in assay buffer. Then 100µl of HRP conjugated anti-sheep IgG (Sigma, Poole, UK) 1:2000 in assay buffer was added to each well and incubated for 30 minutes at 37°C. After washing three times with assay buffer the wells were filled with 100 µl freshly prepared TMB substrate solution (KPL, Guildford, UK) the reaction was stopped after 30 min by the addition of 50µl of 1 M HCl and then the absorbance was read at 450nm. The TTR concentration in the serum samples after a 1:400 dilution was compared to a standard curve of human TTR over a range of 0.03-2.00 µg/ml. This assay was used to determine TTR concentrations in porcine serum samples from individual pigs in order to give an idea of the range of concentrations of TTR present in porcine serum.

Results

The lower detection limit for porcine serum TTR was determined as 0.031 µg/ml. Detection limits were determined by measuring 15 replicates of saline and replicates of the lowest concentration standard and calculating the mean concentration of saline +2 SD of the lowest concentration using human TTR as standard. The interassay precision determined by calculation of coefficient of variance (CV) of 8.4 % at 129.4 µg/ml and 13.8% at 83.6µg/ml was obtained by measuring the same two porcine serum samples in 18 separate assays. The intraassay CV was ascertained to be 2.2 % being the mean of the CVs of 28 duplicate samples in the same assay at a concentration range of 117 – 292 µg/ml.

Serum samples taken from individual pigs (n=272) were assayed for TTR and values for the individual pig serum samples measured ranged from 32 to 690 µg /ml with a median of 129.5 µg /ml.

Conclusions

We have developed a means to measure TTR in pig serum. Measuring the levels TTR in serum in addition to other acute phase proteins to monitor pig health status may provide extra information particularly as its may be a useful monitor of growth related phenomena as it is in man.

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