ACUTE PHASE VARIABLES TO ASSESS HEALTH IN VARIOUS SPECIES

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Introduction

During tissue damage, infection and inflammation, pro-inflammatory cytokines are liberated. This first reaction of the body to immunological stress is the innate, non-specific, response preceding specific immune reactions. Within a few hours after infection the pattern of protein synthesis by the liver is drastically altered (1). Some proteins show an increase in concentration, the positive reactants (such as C-reactive protein (CRP)), whereas others decrease, the negative ones (such as albumin, retinol binding protein (RBP) and alpha-a1 lipoprotein). Individual variability in reactivity occurs and depending on energy balance, the negative blood variables may be more indicative of a changed metabolism than the positives. The positive APPs are regarded as having general functions in opsonisation and trapping of micro-organisms and their products, in activating complement, in binding cellular remnants like nuclear fractions, in neutralising enzymes, scavenging free haemoglobin and radicals, and in modulating the host's immune response. Measurement of positive acute phase variables in combination with negative ones and calculation of an index results in a rather sensitive method to analyse the nutritional and inflammatory state of an individual.

The index has been used as prognostic inflammatory and nutritional index (PINI) for human patients (2,3) and as acute phase index (API) for cattle (4). When well chosen it becomes a *nutritional and acute phase indicator* (NAPI). Such index enhances sensitivity and specificity in comparison to single APPs remarkably detect non-healthy subjects in populations of normal animals, as was shown for cattle and finishing pigs at slaughter (4-6) and was favoured by findings in experimental pigs with *Streptococcus suis* infection (5).

Results from measurements in cattle and pig will be shown as model for other species including man. Furthermore preliminary results from milk analyses are presented. Milk is described to contain a specific isotype of SAA, measurable in mastitis cows at an earlier moment compared to the elevation of SAA found in blood (7). Isoelectric focus (IEF) was used for a time curve of samples from cows intra-mammarily infected with *E. coli* 0:157 (30 cfu in one quarter) for detecting SAA isoforms in blood and milk. With these findings an easy and reliable to determine, early determent of mastitis might be found.

Materials and Methods

The index was calculated as a combination of positive and negative reacting proteins. The format used is stated below:

$$(N)API = \frac{\text{value of a rapid positive APP} \times \text{value of a slow positive APP}}{\text{value of a rapid negative APP} \times \text{value of a slow negative APP}}$$

The data used were obtained from previous performed well-described experiments (4-6). In those studies several blood values were determined. These values are calculated here.

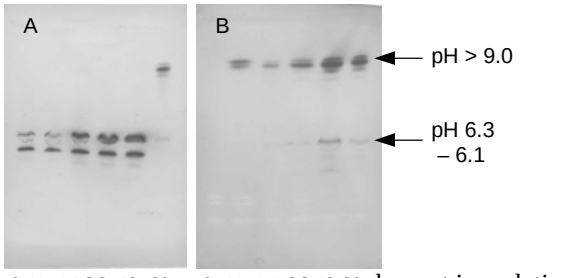
Isoelectric focus (IEF) and Western blot (WB) techniques were performed routinely. For detecting SAA isoforms on the WB after IEF, the biotinylated anti human antibody from a commercial kit (TP 802, Tridelta, Ireland) in 1:100 dilution was used, followed by Streptavidin-Horse Radish Peroxidase, 1:4000. (DAKO, Denmark), and visualization by incubating for 10 to 15 minutes with a TBS solution containing 0.5 mg/ml 3,3'-diaminobenzidin (Sigma-Aldrich, USA) and 0.02% of 30% hydrogen peroxide (Merck, Germany).

Results

When compared 21 healthy control cows with 233 clinical patients the API of the normal cows revealed a value of 0.001 whereas the non-healthy group had a API value of 2.9. The API calculated for these cows contained haptoglobin and SAA as positive reacting proteins and albumin and alpha-2-macroglobulin as negative ones.

In the case of pigs experimentally infected with Streptococcus suis the measurements before time point of infections were used as control. The API used contained CRP and Haptoglobin as positive reacting proteins and Albumin and Vitamin A (reflecting RBP) as negative one. The API value at the time points before infecting was 0.005, whereas this value increased up to 9.1 at day 3 post-infection.

Figure 1. Western blot of IEF of time series of plasma (A) and milk (B) samples after inoculation with E.coli 0:157 (30 cfu in one quarter). The 60 hours sample from the milk was run on both gels to allow comparison between them.



Discussion

The findings presented indicate the calculated index to be more powerful in discriminating between normal and diseased animals.

Furthermore the results indicate milk SAA to be a marker for infectious mammary disorders. The milk isoform has a different pI, compared to the plasma isoform and it was measurable at an earlier time point compared to the blood plasma SAA.

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