COMPARISON OF ALBUMIN MEASUREMENTS BY ELECTROPHORESIS AND THE BROMOCRESOL GREEN METHOD IN CHICKEN PLASMA
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Introduction

Avian species are renowned for their ability to conceal clinical evidence of disease until the pathological process is advanced. As in mammalian species, total protein (TP) measurement forms part of any routine biochemistry profile, often evaluated in conjunction with other biochemical and haematological parameters to assess the health status of the bird. However, significant variation in protein fractions may occur in the absence of alterations in TP, creating a need for a reliable method of evaluating the individual components.

In mammalian species the bromocresol green (BCG) colorimetric method is considered the most accurate and commonly used clinical laboratory procedure for albumin evaluation (Duncan & others 1994), but it is of questionable reliability in birds. This method involves the binding of albumin to a dye in an acid medium which results in a colour change proportional to the concentration of albumin in the sample.

Protein electrophoresis (PE) is generally considered the 'Gold Standard' for evaluation of all protein fractions, including albumin, in avian species. PE is based on the principal that proteins can be separated by charge and mass by migration through a support medium when an electric field is applied. This enables the various protein fractions to be differentiated and quantified. Therefore, assuming that PE provides repeatable results, accuracy of BCG albumin measurements will be reflected by how closely the measurements compare to this Gold Standard.

In healthy animals, albumin is the largest PE peak and can constitute 35-65% of TP in psittacines (Cray 2003). On PE, albumin is usually a single spike in mammals although in some avian species, another spike corresponding to pre-albumin is visualised prior to that of albumin (Figure 1). The only known function of pre-albumin at present is the transport of thyroid hormones (Kaneko 1997). In recent years there has been an increase in the number of publications describing the use of PE in certain avian species. However, data on the comparison of the different albumin determination methods remains sparse and largely anecdotal. Recently, limitations in the interpretation of electrophoresis have become evident. Hence determination of the worth and reliability of PE is important to justify use of this more expensive method for albumin determination.

The aim of the study was to determine whether PE and the BCG method agree for avian plasma albumin calculation. Albumin in paired plasma samples was measured by the BCG dye-binding method, using an ILab 600 Clinical Chemistry System at CSD Diagnostic Laboratories, and calculated from PE at IDEXX. A between laboratory comparison of plasma TP measurement, was also made utilizing the biuret reagent.

Figure 1. Electrophoresis Trace Illustrating the Different Protein Fractions
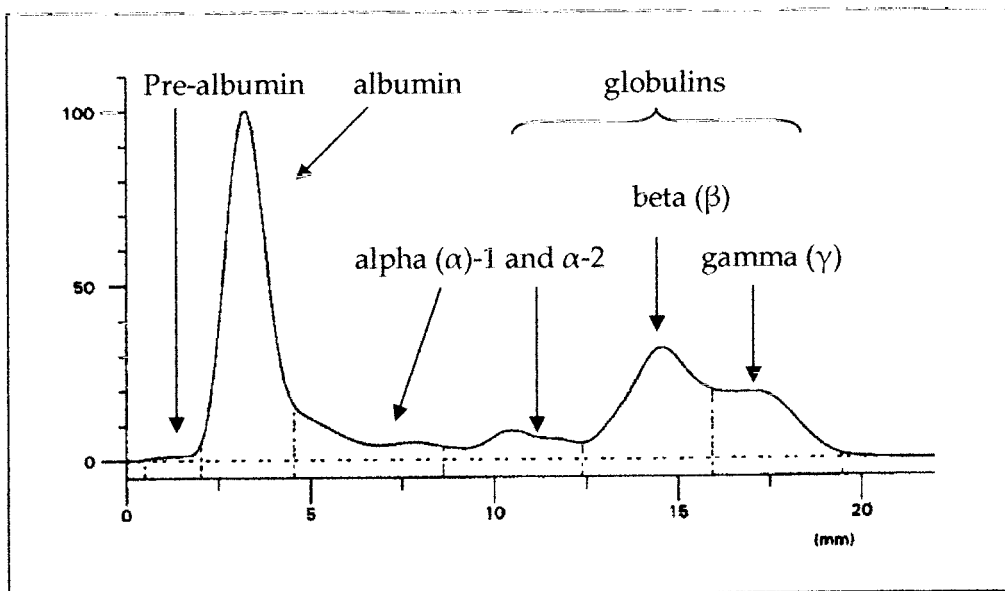
Method and Materials

Blood from 40 chickens was collected into 1ml lithium heparin tubes immediately post stunning and neck-cutting at a poultry processing plant. All samples were centrifuged within two hours and 0.1ml plasma was transferred to cups in the ILab 600 Clinical Chemistry System for albumin and TP measurement. The remainder of each sample was pipetted into numbered plain tubes and sent to IDEXX for PE and TP evaluation.

Albumin measurements obtained by the BCG method were first compared to only the albumin fraction from PE and then, by combining the pre-albumin and albumin fractions, to total albumin concentrations. Statistical tests were used to determine whether a significant difference existed between albumin measurements made by the two methods. Data found by the Shapiro-Wilk test to be Normal (Gaussian) in distribution was analysed using a paired t-test, whereas the non-parametric Wilcoxon Signed Ranks test was used to evaluate data that did not follow the Normal distribution.

Agreement was assessed by calculating the difference between albumin concentrations obtained by PE and BCG for each sample and plotting this against the average albumin value for the two methods. The mean albumin difference (meanDiff) was calculated to determine how well the methods agreed on average. To establish how well the paired results compared for each individual sample, the meanDiff + 2 standard deviation of the difference (SDdiff) were used to calculate the 'Limits of Agreement'.

Results

All samples were included in the study since there was no evidence of haemolysis or coagulation. Paired plasma albumin concentrations were not identical for any of the 40 samples. The population was not Normal in distribution so Limits of Agreement could not be calculated. However, the


Figure 2. Comparison of Plasma Albumin Measurements by BCG and Electrophoresis
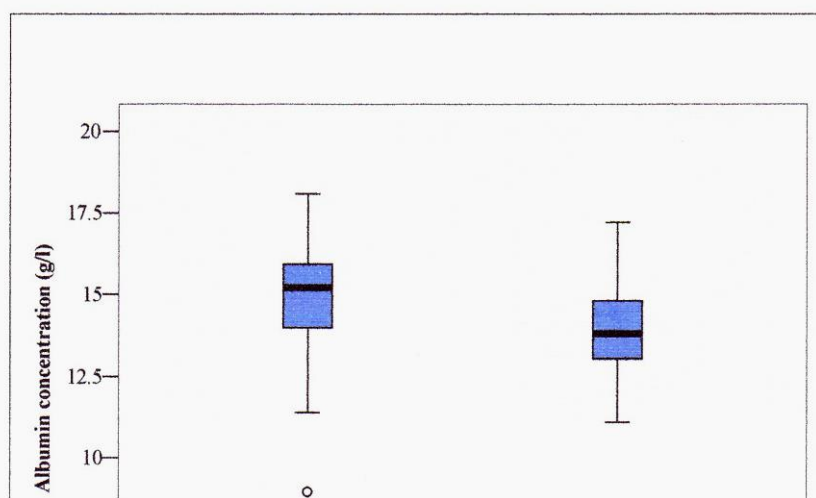
One sample was highlighted as an outlier on a box-plot was created to illustrate the distribution of albumin values for each method (Figure 2). Possible causes for the outlier were subsequently investigated. When the electrophoretogram traces obtained for each sample were carefully studied, it became obvious that large protein fraction alterations existed in the 'suspect' sample (Figure 3).

Additionally, the TP value obtained for this sample was significantly greater. The sample was excluded on the basis that it did not appear to belong to the 'healthy' chicken population.

Figure 3. **Electrophoretogram of the 'Suspect' Sample**
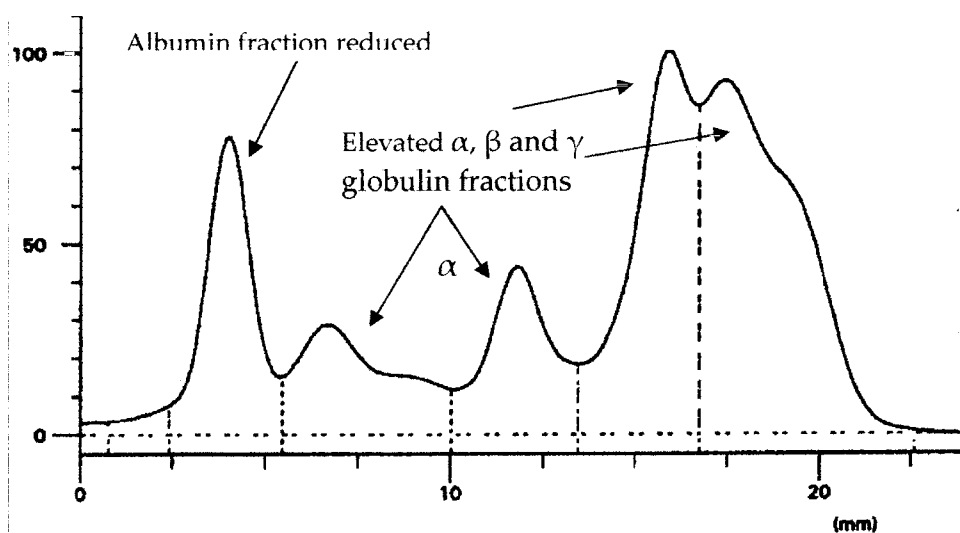
Following removal of the outlier, the data was re-analysed and was found to be Normal in distribution. Mean plasma albumin concentration was 13.98 g/1 for the BCG method (minimum 11.10 g/1) and 14.85 g/1 (minimum 11.39 g/1) as determined by PE. The mean albumin difference was found to be significant (P = 0.000) at the 95% confidence level, indicating that there is a statistical difference in plasma albumin measurements made by the two methods. Limits of Agreement were -0.81 to 2.53 g/1 (Figure 4) with a confidence interval of approximately ± 0.5 g/1, suggesting a difference of up to 3.0 g/1 in albumin measurements between methods.

The pre-albumin fraction was found to be negligible on the PE traces, ranging from 0.17 to 4.44% of 1'1' (Figure 1). However, PE total albumin values also demonstrated a statistical difference to BCG albumin concentrations irrespective of outlier inclusion. The Limits of Agreement (-0.34 to 3.0 gil ± 0.5 g/1) were equally wide to those of the albumin fraction only but repositioned in the positive direction. The limits suggest that values from PE may be up to 3.5 g/1 greater than for BCG.

Figure 4. Agreement for PE and BCG Albumin Measurements with the Extreme Value Removed.
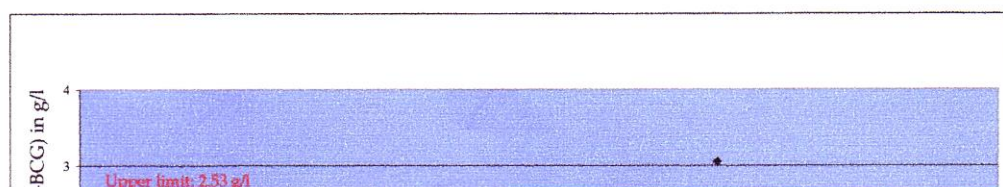
Plasma TP varied from 26.9 - 59.8 g/1 for the ILab 600 Clinical chemistry System and 25.7 to 59.2 g/1 for IDEXX. The difference of the means was not statistically different (P = 0.447). Limits of Agreement indicated that plasma '1'1' values are expected to differ by less than 2 g(1 between laboratories.

Discussion

The mean plasma albumin concentration determined by BCG was lower than those obtained by electrophoresis, which is similar to results from other studies (Lumeij & others 1990; Spano & others 1988). However, the results from the other studies are confusing since they are not consistent. It is difficult to draw meaningful conclusions when comparing the findings of those studies with my own since serum rather than plasma was utilised and the sample sizes were considerably smaller. Electrophoresis was originally designed to analyse serum (Cray 2000) and consequently a large proportion of the literature evaluating proteins by electrophoresis relates to serum utilisation. Although it has been suggested that serum and plasma are equally suitable (Werner & others 1999), ideally plasma should be used as it contains fibrinogen and other coagulation proteins. Plasma was used in this study to make it as relevant to the clinical situation as possible since plasma is the preferred sample type in avian medicine.

The difference between the means was unaltered by either outlier removal or inclusion of the pre-albumin spike, which may support the possibility of a true difference in albumin measurements between methods. It was considered likely that a difference in albumin concentration of up to 3.0 g/l, as indicated by the 'Limits of Agreement', could have implications for the appreciation of health status. In a bird with a mean albumin concentration of approximately 14 g/l, 3.0 g/1 represents a substantial potential inaccuracy between methods.

Concurrent assessment of other clinical data, such as physical examination findings, remains important when any test is performed since a significant test result does not necessarily infer clinical relevance. Using a sample size of 40 provides a 90% probability that 90% of the population lie within the upper and lower limits of the observed values (Walton 2001). Analysis of a greater number of samples may have minimised any influence from biological variation. It could be argued, however, that the detection of a significant difference despite a relatively small sample size aids in reinforcing the possibility of a true difference. The poor agreement between methods indicates that they should not be used interchangeably for albumin measurement in birds. Therefore PE rather than BCG is recommended for albumin determination.

The between laboratory TP measurements can be considered to show excellent agreement on average as the mean difference is small (Altman 1991). The noted variation in TP measurements is unlikely to be clinical important since it constitutes a minimal proportion of the TP value.

Future studies could assess whether the observed lack of method agreement can be applied to other avian species, particularly those with substantial pre-albumin fractions. A concurrent assessment of reliability could be performed to aid in confirming the reliability of electrophoresis in avian species since it has recently been questioned (Rosenthal & others 2005).

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