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Determination of Chicken and Turkey Plasma and Serum Protein Concentrations by Refractometry and the Biuret Method

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SUMMARY. Plasma and serum protein concentrations were determined in chickens and turkeys by refractometry (with human and veterinary refractometers) and by the biuret method. Chicken and turkey serum protein values were significantly lower than respective plasma protein values according to both methods. Refractometer readings for both plasma and serum correlated closely with the results of the biuret test ($r^2 = 0.72$ to 0.97). These findings indicate that plasma and serum protein values may be determined accurately in chickens and turkeys with a hand-held refractometer.

RESUMEN. Determinación de las concentraciones de proteínas séricas y plásmicas de pollos y pavos mediante la refractometría y el método de biuret.

Se determinaron las concentraciones de proteínas séricas y plásmicas en pollos y pavos mediante la refractometría (usando refractómetros para uso humano y en Medicina Veterinaria) y por el método de biuret. En ambos métodos, los valores de las proteínas séricas de pollos y pavos fueron significativamente menores que los respectivos valores de las proteínas plásmicas. Las lecturas en el refractómetro para las proteínas plásmicas y séricas correlacionaron estrechamente con los resultados de la prueba de biuret ($r^2 = 0.72$ a 0.97). Estos resultados indican que los valores de las proteínas séricas y plásmicas pueden determinarse con precisión en pollos y pavos usando un refractómetro manual.

Hand-held, temperature-compensated refractometers have been used for decades to determine plasma and serum protein values in humans and various domesticated animals (4,8,10). These instruments have provided reproducible values that correspond closely to those obtained with the biuret test, a chemistry method for standard determination of protein values (4,8,10). Refractometers are especially useful in a clinical setting, where plasma protein may be conveniently determined from the plasma contained in a centrifuged microhematocrit capillary tube. Despite the continued use of refractometers to determine plasma protein values in various species of birds (1), a recent study has suggested that refractometers are inaccurate for determi-

nation of serum and plasma protein concentrations in pigeons (5); however, refractometry produced values comparable to those of the biuret test in ducks (3). The purpose of this study was to evaluate the accuracy of human and veterinary models of hand-held, temperature-compensated refractometers in determining plasma and serum protein concentrations in chickens and turkeys as compared with the standard biuret test. We also wanted to determine whether significant differences exist between serum and plasma protein values in chickens and in turkeys. Finally, we wished to determine whether the human and veterinary model refractometers gave comparable results.

MATERIALS AND METHODS

Supported in part by a grant from the Poultry Disease Research Center, University of Georgia Veterinary Medical Experiment Station, using funds provided by the Georgia General Assembly by the request of the Georgia Poultry Federation.

Birds. Eleven young adult broiler breeder chickens and 12 young adult Nicholson white turkeys were used. All birds were fasted for 12 hours before blood samples were taken.

Table 1. Linear regression^A comparison of human and veterinary refractometers and the biuret method for determining protein in poultry serum and plasma.

Group	Sample	Refractometer type	ANOVA for linear regression <i>F</i> ratio	Correlation coefficient (<i>r</i>)	Coefficient of determination (<i>r</i> ²)
Chicken	serum	veterinary	193.9 ^B	0.98	0.96
		human	181.6 ^B	0.98	0.95
	plasma	veterinary	289.3 ^C	0.98	0.97
		human	265.8 ^C	0.98	0.97
Turkey	serum	veterinary	24.4 ^D	0.87	0.75
		human	27.1 ^D	0.88	0.77
	plasma	veterinary	25.5 ^E	0.85	0.72
		human	40.6 ^E	0.90	0.80

^AAnalysis of variance for linear regression.

^BRegression lines not significantly different (RLNSD). Slopes: $F = 0.04$, $df = 1/18$, not significant. Elevations: $F = 0.06$, $df = 1/19$, not significant.

^CRLNSD. Slopes: $F = 0.04$, $df = 1/18$, not significant. Elevations: $F = 0.07$, $df = 1/19$, not significant.

^DRLNSD. Slopes: $F = 0.05$, $df = 1/16$, not significant. Elevations: $F = 0.006$, $df = 1/17$, not significant.

^ERLNSD. Slopes: $F = 0.03$, $df = 1/20$, not significant. Elevations: $F = 0.14$, $df = 1/21$, not significant.

Venipuncture and sample handling. A 5-ml sample of blood was obtained from the wing vein and divided equally in two. One-half of the specimen was placed in a tube with ethylenediaminetetraacetic acid (disodium EDTA, 1.5 mg/ml blood). The remaining blood was placed in a glass clot tube. After the anticoagulated sample was centrifuged at $400 \times g$ for 10 minutes, the plasma layer was removed and placed in another test tube. The clot tubes were processed when coagulation appeared complete. After the clot was rimmed, the tubes were centrifuged at $2000 \times g$ for 10 minutes, and the serum was separated from the clot and placed in a second test tube. In some turkey samples, inadequate clot retraction was observed. The use of 2 NIH (National Institutes of Health) units of thrombin promoted complete clot retraction in these samples. Following clotting, these specimens were centrifuged once more and the serum was removed. Protein determinations were then performed.

Protein determinations. Plasma and serum protein concentrations were determined by the biuret method and by refractometry. The biuret test was performed at 25 °C; absorbance at 540 nm was determined using a computer-directed spectrophotometer (Stasar III and System 101, Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Human protein standards (American Dade, Aguada, Puerto Rico) were used for protein determinations. Quality control was maintained by assay of normal and abnormal control samples during each batch run (Gilford QCS Normal and Abnormal Control Sera, Ciba Corning Diagnostics Corp., Irvine, Calif.). Two hand-held, temperature-compensated refractometers were used. These instruments were both human- and veterinary-marketed instruments (Models 10400 and 10436; Reichert Scientific Instruments, Di-

vision of Warner-Lambert Technologies, Inc., P.O. Box 123, Buffalo, N.Y.). Instrument calibration was checked with distilled water (specific gravity 1.000) and 5% NaCl solution (specific gravity 1.022) before and after use.

Biostatistics. The data were tested for significance by an analysis of variance for linear regression. Coefficients of correlation (*r*) and determination (*r*²) were calculated. The slopes and elevations of regression lines were also compared using an analysis of variance.

RESULTS

For chickens and turkeys, the serum and plasma protein values obtained using the veterinary and human refractometers were regressed against the protein values derived from the biuret test (Table 1). The human- and veterinary-marketed refractometer values were compared, and no significant differences in performance were found (Table 1).

The slopes and elevations of chicken and turkey serum and plasma values obtained from the veterinary refractometer readings were compared using an analysis of variance. The slopes of the regression lines were not significantly different for chicken plasma and serum (slopes: $F = 0.57$, $df = 1/18$; elevations: $F = 14.51$, $df = 1/19$) or turkey plasma and serum (slopes: $F = 0.45$, $df = 1/18$; elevations: $F = 13.15$, $df = 1/19$). For chickens and turkeys, the regression lines for serum and plasma values were significantly dif-

ferent ($P < 0.005$) owing to differences in the y -intercept (Figs. 1, 2).

The chicken serum and plasma coefficients of correlation were highly positive values. The coefficients of determination indicated that between 95% and 97% of the variation in the refractometer values could be explained by differences in the biuret values (Table 1). For the turkey serum and plasma, the coefficients of correlation were moderately high. The r^2 values indicated that between 72% and 80% of the variation in the refractometric values could be explained by differences in the biuret values.

DISCUSSION

The biuret reaction has proven to be a reliable method of protein determination, and this methodology is commonly incorporated into biochemical profiles for mammalian and avian species (2,9). The biuret method is based upon measurement of the colored product that results when protein reacts with Cu^{2+} in an alkaline solution (2). Sample turbidity due to lipemia is the most significant source of error in biuret protein readings. Fasted birds were selected to eliminate this variable.

Refractometric values of serum and plasma proteins have been clinically reliable and are often used instead of chemistry methods such as the biuret reaction. In many species, plasma protein values are conveniently determined by refractometry using the plasma remaining in a microhematocrit capillary tube after centrifugation and determination of the packed cell volume. Accurate determination of refractometric protein values requires a temperature-compensated instrument that will yield correct readings over an ambient temperature range from 15.6 to 37.8 C with a protein value variation of only 0.05 g/dl (7). Uncompensated instruments will perform satisfactorily only over a narrow temperature range from 18.3 to 21.1 C, and the protein values may vary 0.50 g/dl. Generally, sample temperature has little or no effect on the protein reading, because the sample volume is so small that it

Figs. 1, 2. Regression lines of serum and plasma. 1) Chicken. Regression lines obtained by predicted points for chicken serum: $y = 0.2866 + 0.9597x$; chicken

plasma: $y = 0.8047 + 0.8946x$. The regression lines are significantly different ($P < 0.005$) at the y -intercept, therefore, the serum and plasma values are not comparable. 2) Turkey. Regression lines obtained by predicted points for turkey serum: $y = 0.7622 + 0.7964x$; turkey plasma: $y = 1.517 + 0.6520x$. The regression lines are significantly different ($P < 0.005$) at the y -intercept, therefore, the serum and plasma values are not comparable.

quickly equilibrates to the temperature of the instrument (7).

Therefore, determination of plasma and serum protein values with a temperature-compensated refractometer is based upon the assumption that any change in the reading is the result of a change in protein concentration. The presence of excessive nonprotein solutes such as glucose, cholesterol, triglycerides, sodium (chloride), or urea may lead to erroneous results (2,6). In birds, the presence of high glucose values has been suggested as the cause of inaccurate refractometer protein readings (5), but increasing the serum glucose concentration markedly (from 64 to 649 mg/dl) changes the refractometric protein value only slightly (from 8.0 to 8.4 mg/dl) (6). In mammals, excess urea may cause erroneous refractometric protein readings (6); however, uric acid and not urea is the by-product of the ammonia cycle in birds. Serum sodium values in chickens and turkeys are similar to those of mammals and would not be expected to have a significant effect on protein concentrations as determined by refractometry (6).

No significant differences in protein concentrations were found when the human- and veterinary-marketed refractometer were compared. Therefore, the veterinary refractometer would be preferred because it is less expensive.

This study demonstrates that chicken and turkey refractometric plasma and serum protein values are accurate when compared with values obtained by the biuret reaction. If the nonprotein solute values in birds were similar to the values in mammals, one would expect refractometric values to be comparable to chemistry protein determinations. However, the accuracy of refractometric protein values should be determined for each species, and an effort should be made to limit variables such as lipemia that would cause inaccurate results.

Difficulties in obtaining adequate clot formation and retraction in turkey whole blood samples also have been encountered by other investigators (B. G. Harmon, D.V.M., Ph.D., University of Georgia, 1988, personal communication). To obtain serum, some whole-blood turkey samples required the addition of thrombin for adequate clot formation. The cause of this phenomenon is unknown.

The regression lines for both chicken and turkey serum were significantly lower than the regression lines for plasma; therefore, plasma and serum values cannot be considered comparable in each species. When evaluating methods for protein determination, comparing serum to plasma values may result in erroneous conclusions (5). A difference in protein values is expected, since plasma includes fibrinogen and other clotting factor proteins that are absent in serum.

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