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Determination of Ring-Necked Pheasant (*Phasianus colchicus*) Serum Protein Concentrations by Refractometry and the Biuret Method

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Abstract: The purpose of this study was to evaluate the accuracy of hand-held refractometer in determining serum protein concentrations in ring-necked pheasants (*Phasianus colchicus*) as compared with the standard biuret method. The results indicated that serum protein values may be accurately determined in ring-necked pheasants with a hand-held refractometer.

Key words: Refractometry, biuret method, serum protein, ring-necked pheasants

Introduction

Some studies have reported that the results of protein estimation by refractometry for domestic mammals and birds correlate well with those obtained by the biuret method, although others have reported both high and lower refractometric results compared with biuret results (George, 2001). According to George (2001) the most marked inconsistencies between refractometry and biuret results have been found for avian samples. According to Andreasen *et al.* (1989) and Lumeij (1997) 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.

Materials and Methods

A total number of 96 adult ring-necked pheasants were used. All birds were fasted for eight hours before blood samples were taken. Blood samples were collected from the ulnar superficial vein and placed in glass tubes without anticoagulant. The blood was immediately centrifuged following collection and the serum was promptly removed after centrifugation. Only clear serum samples were used, aiming to avoid lipemia or hemolysis. Serum total protein concentrations were determined by the biuret method and with a hand-held Goldberg-type refractometer and the instrument calibration was checked with distilled water. The biuret

test was performed at 25°C, 525 nm absorbance with an automated serum chemistry analyzer*. Values (g/dL) are expressed as mean (\pm standard deviation).

Statistics: The normality of data was verified by Bartlett's test, values were transformed into $\log(x+1)$ and analyzed by Student t-test, Spearman's correlation test and Pearson linear regression using BioEstat 5.0 (Ayres *et al.*, 2005). Statistical significance values were set at 0.05.

Results and Discussion

In biuret method, serum protein concentrations were 5.2479 (± 1.0848), while in hand-held refractometer were 5.5291 (± 1.4575), with no significant statistical differences observed at t-test**. Strong correlations ($r_s = 0.8061$, $p < 0.0001$; $r^2 = 0.8036$, $p < 0.0001$, $y = 1.6241 + 0.6042x$) also were observed. According to Andreasen *et al.* (1989), plasma and serum protein values may be determined accurately in chicken and turkeys with a hand held refractometer. However, Lumeij and Buijine (1985) suggested that refractometers are inaccurate for determination of serum and plasma protein concentrations in pigeons, probably because this species has high concentrations of blood glucose, although the cause of consistently greater values obtained by the refractometric method is uncertain. Thus, according to George (2001), because of known

interspecies variation, refractometer use should not be extended to samples from exotic species without correlation to standard protein methods. In this study, the refractometer readings for serum protein concentrations in ring-necked pheasants correlated closely ($r_s = 0.8161$) with the results of the biuret. Refractometers are especially useful in clinical routine and for experimental purposes, in which serum or plasma protein concentrations may be determined, because no chemical reagents are necessary and the results are rapidly obtained. These advantages may be useful for an application in massive avian production, improving the management of clinical abnormalities observed in the stock.

Conclusions: Refractometer readings for serum proteins are closely correlated with the results of the biuret test. These findings indicate that serum protein values may be accurately determined in ring-necked pheasants with a hand-held refractometer.

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