

Refractometry as an alternative to the biuret method for measuring total serum proteins in *Podocnemis expansa* (Podocnemididae) and *Phrynosops geoffroanus* (Chelidae)

Lourdes Marina Bezerra PESSOA¹, Maíra Gonçalves da Mota LIMA¹, Filipe Tavares CARNEIRO¹, Nathalia Salgado ZANANI¹, Marcela Corrêa SCALON¹, Thamiris Figueiredo SILVA¹, Mariana Accioly LIMA¹, Maia Araújo ABRAHIM¹, Giane Regina PALUDO¹

¹ University of Brasília, Faculty of Agronomy and Veterinary Medicine, Federal District, Brazil.

* Corresponding author: lmarinabpesoa@gmail.com

ABSTRACT

Total serum protein is a significant indicator of health condition in animals. The aim of this study was to analyze the precision of the portable refractometer in determining the concentration of total serum proteins in *Podocnemis expansa* and *Phrynosops geoffroanus*. A total of 26 animals were used. The blood samples were collected from the supraoccipital sinus and stored in tubes without anticoagulant. Total serum protein was determined using both the biuret reaction and refractometry. The total serum protein mean concentration (g dL⁻¹) with biuret method and refractometry for *P. expansa* were 3.16 and 3.2; and for *P. geoffroanus* were 3.56 and 2.72, respectively. These results indicate that total serum protein values can be determined with precision in *P. expansa* and *P. geoffroanus* using a portable refractometer.

KEYWORDS: biuret method, refractometry, *Podocnemis expansa*, *Phrynosops geoffroanus*.

Refratometria como alternativa ao método do biureto para mensuração de proteínas séricas totais em *Podocnemis expansa* e *Phrynosops geoffroanus* (Podocnemididae, Chelidae)

RESUMO

A proteína sérica total é um indicador significativo do estado de saúde em animais. O objetivo desse estudo foi analisar a precisão do refratômetro portátil para determinar a concentração de proteínas séricas totais em *Podocnemis expansa* e *Phrynosops geoffroanus*. Foram utilizados um total de 26 animais. As amostras de sangue foram coletadas por punção do seio supraoccipital e armazenadas em tubos sem anticoagulante. A concentração de proteína sérica total foi determinada utilizando tanto a reação de biureto como um refratômetro portátil. A média da proteína sérica total (g dL⁻¹) pela reação de biureto e pela refratometria para *P. expansa* foram de 3,16 e 3,2; e para *P. geoffroanus* foram de 3,56 e 2,72, respectivamente. Estes resultados indicam que os valores de proteínas séricas totais podem ser determinados com precisão em *P. expansa* e *P. geoffroanus* usando o refratômetro portátil.

PALAVRAS-CHAVE: método do biureto, refratometria, *Podocnemis expansa*, *Phrynosops geoffroanus*.

The concentration of total proteins is used as a general indicator of clinical health, stress, and well-being of terrestrial and aquatic organisms (Riche 2007). It is also an important resource for a veterinarian who needs an urgent result to aid in the diagnosis of some diseases. The measurement of serum protein concentration is a frequently performed routine analysis for investigating electrolyte imbalances, inflammatory or infectious diseases, colostrum ingestion, tumors, besides other health conditions (Kaneko 1997), and is also an important supplemental component in the diagnosis of gastrointestinal, hepatic, and renal diseases (Kaneko *et al.* 2008). The relation between individual fractions of proteins reflects the functional, metabolic, and health status of animals. A number of methods are available for the determination of total serum proteins, but the most used are the colorimetric (biuret reaction) and the refractometric ones.

Colorimetric procedures are usually the preferred choice; however, they are more expensive, time-consuming, and cannot be easily performed in the field (Riche 2007). Due to the ease, speed of procedure, and small amount of material required, refractometry has been widely used to determine the total protein concentration in bovines, canines, equines, felines, fish, wild mammals, and birds (De Smet 1978; Alexander and Ingram 1980; George 2001).

The aim of this study was to analyze the precision of the portable refractometer in measuring the concentration of total serum proteins in *Podocnemis expansa* (Podocnemididae) and *Phrynops geoffroanus* (Chelidae) when compared with the biuret reaction.

The blood samples were collected from the supraoccipital sinus of 26 animals, 11 Amazon turtles (*Podocnemis expansa*) and 15 Geoffroy's side-necked turtles (*Phrynops geoffroanus*), of different ages, in tubes without anticoagulant. These specimens belong to the Zoological Brasília Foundation. The analyses were performed in the Clinical Pathology Laboratory of the College of Veterinary Medicine of the University of Brasília. The serum was obtained after centrifugation of blood samples at $600 \times g$ for 5 minutes. Total serum protein concentration was determined by the colorimetric method (biuret reaction) and by refractometry. Colorimetric measurements were performed in a semi-automatic biochemical analyzer (BIOPLUS 2000[®]) using a specific biochemical kit (Labtest[®], São Paulo, Brazil), following the manufacturer's recommendations. Next, the total serum protein concentration was measured by a portable refractometer (JSCP-Uridens[®], São Paulo, Brazil). For this measurement, a drop (10 μ l) of serum was placed in the portable refractometer and the total serum protein concentration expressed as grams per deciliter (g dL⁻¹) was read. Calibration of the refractometer was checked with distilled water before each series of measurement. All readings were made at room temperature (approximately 20°C). Serum

protein concentration was expressed as mean \pm standard deviation (g dL⁻¹). The statistical differences between both methods were analyzed using a paired t-test and the correlation was determined by Pearson's correlation test. A $P < 0.05$ was considered significant. All analyses were done using the software GraphPad InStat (version 3.00, GraphPad InStat Software Inc. 2000).

The experimental methods used in this project were evaluated and approved by the Ethics Committee for the Use of Animals, University of Brazil (CEUA-UNB; UnBDOC N.154161/2013) and the capture and handling of animals was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through the Biodiversity Authorization and Information System (SISBIO; N. 38444-4).

The mean concentration values for total serum protein concentration (\pm SD) for *P. expansa* and *P. geoffroanus* were 3.16 ± 1.20 and 3.56 ± 1.41 g dL⁻¹ respectively, with the biuret method, and 3.2 ± 1.45 and 2.72 ± 1.41 g dL⁻¹ respectively, by using refractometry (Table 1). No statistical differences were observed between the two methods in both the species with a paired t-test ($t = 0.06397$, $P = 0.9496$ for *P. expansa* and $t = 1.625$, $P = 0.1153$ for *P. geoffroanus*). A strong correlation between the biuret reaction and the refractometry method was observed ($r = 0.9717$ for *P. expansa*, and $r = 0.7248$ for *P. geoffroanus*).

Table 1. Mean total serum protein concentrations (\pm SD) (g dL⁻¹) measured by the biuret method and by refractometry in *Podocnemis expansa* and *Phrynops geoffroanus* using the paired t-test.

Method	<i>Podocnemis expansa</i>	<i>Phrynops geoffroanus</i>
	Mean concentration (\pm SD) (g dL ⁻¹)	Mean Concentration (\pm SD) (g dL ⁻¹)
Biuret	3.16 \pm 1.20	3.56 \pm 1.41
Refractometry	3.2 \pm 1.45	2.72 \pm 1.41
P	0.9496	0.1153

Portable refractometers are especially useful in a clinical setting, where total serum protein can be measured quickly, with simplicity, and at less cost compared with a biuret reaction. It may also lead to a faster diagnosis for the patient and facilitate field analysis. In the present study, refractometer readings of serum proteins correlated closely with the results of the biuret test and showed no statistical differences, supporting the results obtained in a study by Schmidt *et al.* (2011) in animals of the same order. This finding indicates that the total serum protein values in *P. expansa* and *P. geoffroanus* may be determined with precision using a portable refractometer, which can aid the field clinician in establishing diagnosis, as well as with *in situ* studies using these species.

Although there is some controversy in the literature about serum protein determinations in reptilian blood, refractometry may be used to estimate protein concentrations in *P. expansa* and *P. geoffroanus*. It is possible to reach the conclusion that the refractometric method for determination of plasma protein concentration in these species is reliable.

ACKNOWLEDGMENTS

The authors wish to thank MEC (Ministério da Educação) and Capes (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for their support with regard to scholarship funding.

REFERENCES

- Alexander, J.B.; Ingram, G.A. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. *Journal of Fish Biology*, 16:115–122.
- De Smet, W.H.O. 1978. The total protein content in the blood serum of vertebrates. *Acta Zoológica Pathologica Antverpiensia*, 70:35–56.
- George, J.W. 2001. The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: an historical and technical review. *Veterinary Clinical Pathology*, 30:201–210.
- Kaneko, J.J. 1997. Serum proteins and the dysproteinemias. In: Kaneko, J.J.; Harvey, J.W.; Bruss, M.L. (Ed.). *Clinical biochemistry of domestic animals*. San Diego: Academic Press, p.117–138.
- Kaneko, J.J.; Harvey, J.W.; Bruss, M.L. 2008. *Clinical biochemistry of domestic animals*. 6ed. Burlington: Academic Press, 932 p.
- Riche, M. 2007. Analysis of refractometry for determining total plasma protein in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) at various salinities. *Aquaculture*, 264:279–284.
- Schmidt, S.E.M.; Lange, R.R.; Paulillo, A.C. 2011. Determination of snake-necked turtle *Hydromedusa tectifera* (Cope, 1870) (Testudines: Chelidae) plasma protein concentrations by refractometry and the biuret method. *Comparative Clinical Pathology*, 20:487–489.

Received: 12/05/2016

Accepted: 25/07/2016

