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Comparison of biuret and refractometery method for serum total protein measurements in cattle and goat

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Abstract

Objectives The biuret method is frequently used to determine serum total protein. On the other hand refractometer, a quicker and less expensive option, is available to determine serum total protein. However, there is no study conducted in Ethiopia to compare serum total protein measurement in veterinary settings. Therefore, this study was conducted to compare the serum total protein concentration measurement in cattle and goats obtained by the biuret method and refractometer.

Results Serum samples from 60 cattle and 60 goats were assayed by both methods and data were analyzed with a paired t-test, Pearson's correlation, and Bland-Altman plots. There was a strong positive correlation between the total protein values determined with the refractometer and the biuret method in cattle ($r = 0.93$) and goats ($r = 0.97$). There were no significant differences ($p > 0.05$) in the protein values measured with the refractometer and those evaluated with the biuret method in both species. Bland-Altman plots showed that biases indicating the analytic and user error were 8.33% in both species which is below the acceptable total error (< 10%). Thus, refractometer can be used in place of biuret method since it is valid enough to measure serum total protein in cattle and goats.

Keywords Biuret, Cattle, Goat, Refractometer, Total protein

Introduction

Proteins are the most abundant constituents of the blood having important physiological functions [1]. Proteins are important for biological functions; some of them support connective tissues structurally, while others are crucial in biochemical reactions. Moreover, proteins serve as buffers, helping in maintaining of colloid osmotic structure and the acid-base balance. Some of them also play a role in the control of cellular activity and the immune system, as well as serving as carriers of lipids, vitamins, hormones and minerals in the circulatory system [2]. The measurement of serum proteins is an essential diagnostic tool for the detection, diagnosis and monitoring of various disease and pathological processes. These includes renal diseases, liver cirrhosis, nephrotic syndrome, chronic malnutrition, gastrointestinal diseases, protein-losing enteropathy, internal parasitism, acute inflammation (acute phase response) and immune-mediated disorders [3].

Quantifying the quantity of total serum protein is the initial step in the investigation of protein patterns. Numerous techniques have been created for determining them, each of which is based on a distinct analytical technique [4]. The measurement of serum total protein

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is frequently performed in reference and in-clinic laboratories using the biuret method [5]. The biuret method depends on the formation of copper chelates via protein peptides linkages that have been enolized at an alkaline pH [6]. This technique is mostly used and is frequently cited as the reference method for total protein quantification in research [7]. Refractometery is another method for measuring total protein that is often used in veterinary laboratories. Refractometery offers a quick and economical assessment in a range of fluids by measuring the angle of refraction between air and aqueous solution [8].

Different studies have demonstrated that refractometery determines serum protein comparable to biuret method. However, refractometery needs to be reevaluated for determining serum total protein, as evidenced by studies that produced results that were both higher [9, 10] and lower than those obtained with the biuret method [11, 12]. Therefore, the objective of this study was to compare the serum total protein concentration obtained by refractometery and the biuret method in cattle and goats to determine whether the refractometery is accurate enough to assess serum total protein.

Methods

Study design and animals

The study was developed using guidelines for evaluation of clinical chemistry methods [13] and National Committee for Clinical Laboratory Standards (NCCLS) Approved guideline for method comparison and bias estimation [14]. The American Society of Veterinary Clinical Pathology (ASVCP) guidelines: allowable total error guidelines for biochemistry were utilized for bias estimation [15]. Sample collection and animal use was approved by the institutional research ethics review committee of college of veterinary medicine and agriculture (reference no. VM/ERC/09/01/12/2020).

Study animals and area

The cattle and goats owned by farmers used for conducting the study were males aged greater than one year. They were selected while visiting veterinary clinics of Arba Minch, a city located 500 km south of Addis Ababa, Ethiopia. The animals were selected using a simple random sampling method. The animals used in the study were released after blood collection.

Sample size determination

The sample size was determined based on the concepts and practices in the evaluation of clinical chemistry methods [13] and National Committee for Clinical Laboratory Standards (NCCLS) Approved guideline for method comparison and bias estimation using patient samples. It could be evaluated utilizing 40 to 100 samples analyzed using both methods under investigation (two field methods), or using one tested method and a reference method, or using both instruments on the same day over a length of 8 to 20 days (preferably within 4 h) [14]. Accordingly, the current study utilized 120 serum samples, 60 from each animal species.

Blood Collection and Processing

Blood samples (5 ml) were aseptically collected from jugular vein of cattle and goats by sterile 20- gauge needle using blood collection vacutainer. After clotting, the serum was separated by low-speed centrifugation at 2500 revolution per minute for 10 min. The serum samples that don't have any obvious abnormalities (clots, hemolysis) were transferred into sterile cryogenic vials that bear the species name and number.

Laboratory analysis

Instrumental setup

Spectroscopic analysis was conducted using the instrument EMP-168 semi-automated chemistry analyzer (EMP-168 Chengdu Empsun Medical Technology Co., Ltd., China). The instrument was calibrated using calibrator and quality control samples for normal (N) and pathological (P) were run for validation before running samples for tests. While for refractometery, portable refractometer with triple scale that give specific gravity, total serum protein and refractive index was used. The refractometer had a scale interval of 0.2 g/dl and a measuring range of 0 to 12 g/dl. Prior to measuring serum protein, distilled water was used to verify the refractometer's calibration. The interval between the serum protein determinations with the refractometer and the biuret method for each sample was less than two hours and each sample was analyzed twice with each method. All measurements were taken at room temperature.

Refractometery

Refractometery is based on measurement of refractive index produced by a serum sample due to the combined concentration of all its solute. A drop (10 µl) of serum is used for this measurement, and the angle corresponds to the border line between the dark and the light area, which is measured by the image detector. The measured angle is converted to total serum protein concentration in grams per deciliter (g/dl) [8].

Biuret method

This method is based on colorimetric principle, in which the copper ions from the biuret reagent react with the amide groups from the proteins at strong alkaline pH, creating a violet color. The total protein concentration in the sample was calculated using formula: optical density of sample divided by optical density of standard and

the result was multiplied by standard concentration. The standard concentration used was 6 g/dl [16, 17].

Data management and analysis

The data generated from laboratory investigation was recorded in a Microsoft Excel spreadsheet and analyzed using SPSS version 20 (IBM Corp.). Serum protein concentration was expressed as mean ± standard deviation (g/dl). 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. The normality of the data was tested with the Shapiro–Wilk normality test. For both species, scatterplots and Bland-Altman plots were generated. A t-test for paired samples was run to evaluate the significance of differences between total protein values determined by the biuret method and the refractometer for both cattle and goats.

Results

The present study evaluates refractometery as an alternative to biuret method for measurement of total protein in cattle and goat serum. The data obtained from biuret and refractometery method for cattle ranged from 2.6 to 8.7 g/dl and 3.1 to 8.3 g/dl respectively. While for goats data from biuret and refractometery methods ranged from 2.5 g/dl to 7.8 g/dl and 2.8 g/dl to 7.80 g/dl respectively. The mean ± SD concentrations of total serum protein by the biuret method in cattle and goat were 5.49 ± 1.69 g/dl and 5.86 ± 1.39 g/dl respectively, while by using refractometery 5.82 ± 1.29 g/dl and 5.82 ± 1.29 g/dl respectively. There was non-significant ($p>0.05$) difference between total serum protein determined from Biuret and refractometery method from paired t test $p=0.413$ for cattle and $p=0.281$ for goat (Table 1).

Bland-Altman plots demonstrated that the mean ± SD bias for total protein concentrations in the cattle was −0.068 ± 0.64 g/dl (95% CI of the difference, −0.234 to 097 g/dl) and in goat was 0.045 ± 0.32 g/dl (95% CI for difference, −0.037 to 0.127 g/dl). Such biases are considered to be clinically and analytically insignificant as they are less than 10% in both animal species (5/60), 8.33%.

Pearson correlation coefficient (r), determines the direction and intensity of a linear relationship between two variables. The biuret reaction and the refractometery technique proved to be highly correlated; $r=0.93$ for cattle and $r=0.97$ for goat (Fig. 1).

Discussion

The current research was conducted to compare serum total protein concentrations in cattle and goats measured by refractometer and biuret method. The mean serum protein determined with the refractometer were not significantly differed with those measured with the biuret method in both cattle ($p=0.413$) and goats ($p=0.281$), supporting the results obtained in a study by sheep serum [12], horse serum [18] and serum from *Podocnemis expansa* (Podocnemididae) and *Phrynops geoffroanus* (Chelidae) [19]. Refractometery has previously produced total protein values that were both higher and lower than those obtained using the biuret method in a variety of animal species [8]. In contradiction to our findings, refractometery produced much greater total protein concentrations than the biuret technique in different investigations [9, 10]. This is due to the contribution of total solids to the refractive index.

According to Cohen's standards $r=0.10$, $r=0.30$, and $r=0.50$ were recommended to be small, medium, and large in magnitude, respectively, for the purposes of interpreting the magnitude of a correlation as well as estimating power [20]. The precision, which is represented by the Pearson correlation coefficient, was discovered through the investigation of concordance correlation coefficients, was $r=0.93$for cattle and 0.97 for goats, suggesting that biuret method has strong and positive correlation with refractometer, which means with the increase protein concentration in biuret method also result in increase in protein concentration with refractometers in both species. The existing information supports the speculation that the two methods are highly correlated in cattle [21, 22], in sheep [12, 23] and goats [12] serum samples; however, the correlation coefficients from the current study were larger than those from prior studies. Given that the refractometer's design is constant the variable correlations appear to be due to changes in biuret results [8]. This deviation could be attributed to changes in aspects of the biuret method, such as the reagents' composition, the standards employed, or the reaction circumstances [24].

According to the analysis of Bland-Altman plots (Fig. 2), the bias suggesting analytical and user error was 8.33% in both species. Since this bias was less than the maximum total allowable error (TE_a) of 10% for determining total protein, it is regarded as clinically and analytically insignificant [14].

Table 1 Mean total serum protein concentration ± SD (g/dl) measured by the biuret method and by refractometery in cattle and goats using the paired t-test

Method	*N*	Cattle (g/dl)					Goat (g/dl)				
		Mean ± SD	SE	CV	Range	*p*-value	Mean ± SD	SE	CV	Range	*p*-value
Biuret	60	5.49 ± 1.69	0.21	2.88	6.1	0.413	5.86 ± 1.39	0.18	1.94	5.3	0.281
Refractometery	60	5.82 ± 1.29	0.17	1.91	5.2		5.82 ± 1.29	0.16	1.67	5.0	

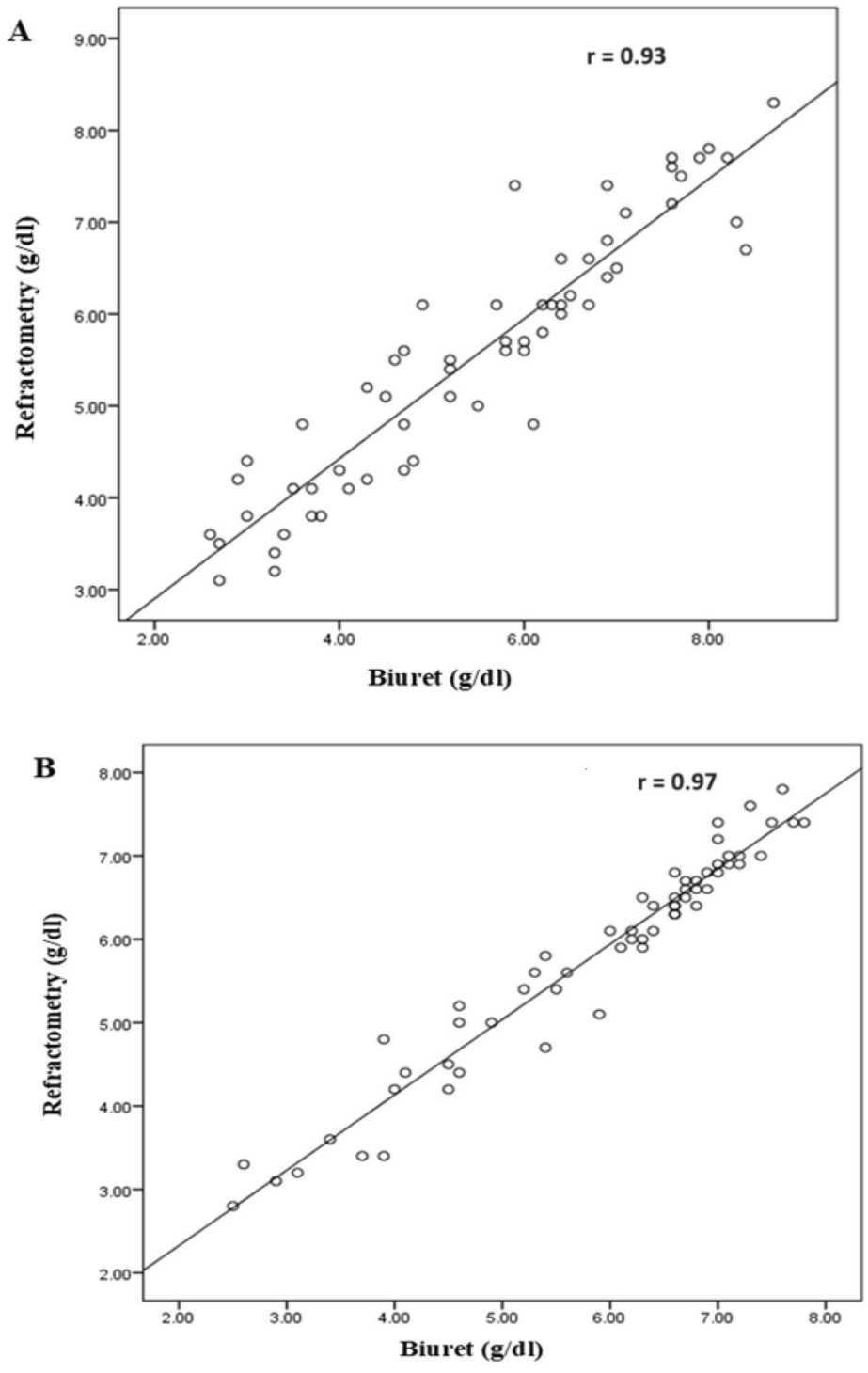

Fig. 1 Scatterplots demonstrating the correlation between biuret method and refractometery in cattle and goats (A = Cattle and B = Goat)

Conclusion and recommendation

It can be said that as there was no statistically significant difference between the serum protein readings from the refractometer and the biuret method, so the performance of refractometers under the study was sufficiently accurate for the determination of serum total protein concentrations in cattle and goats. Therefore, in a clinical context, total serum protein can be quantified rapidly, easily, and affordably with refractometers compared with

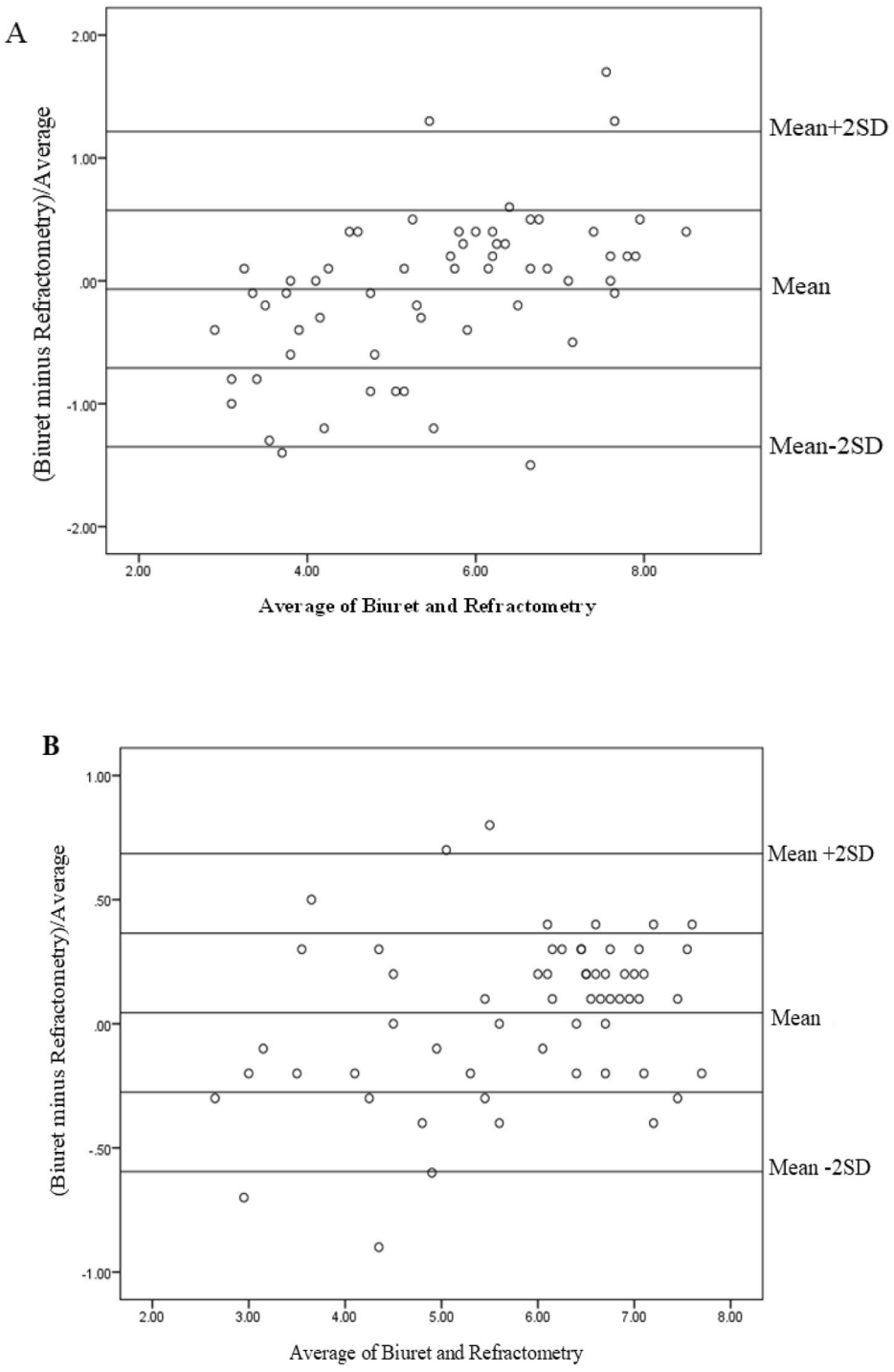

Fig. 2 The Bland-Altman plots showing the difference between total protein concentrations obtained with biuret method and refractometery plotted against the mean of the two methods. (**A**) cattle ($n=60$), (**B**) Goat ($n=60$)

a biuret reaction so that it leads to a faster diagnosis for the patient and facilitate field analysis.

It should be used in caution in samples exposed to different pre-analytical conditions including storage, which might change the accuracy of results.

Limitations

The study has some limitations. One is the study unable to conduct comparison studies on a variety of pre-analytical conditions due to logistic constraints. Breed and disease state were also not taken into consideration during sampling. Finally the study was limited to only male

goats and cattle. Therefore, considering the limitations for future research is recommended.

Abbreviations

AAU	Addis Ababa University
ASVCP	American Society of Veterinary Clinical Pathology
CV	Coefficient of Variation
SD	Standard deviation
SE	Standard Error
TE_a	Total allowable error
g/dl	Grams per deciliter

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Author contributions
SM and YC conceived and designed the study, responsible for data integrity, analysis, and interpretation. SM and YC drafted and revised the manuscript. All the authors read and approved the final manuscript.

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Data availability
The data used to support this study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
The study obtained research ethical clearance approved by the institutional animal research ethics committee of College of Veterinary Medicine and Agriculture, Addis Ababa University, (Reference No VM/ERC/09/01/12/2020). All methods in the study were performed in accordance with the institutional animal research ethics committee of College of veterinary medicine and Agriculture, Addis Ababa University.

Consent to publish
Not Applicable.

Competing interests
The authors declare no competing interests.

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