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A preliminary study on devising a hematological formula for estimation of hemoglobin from packed cell volume in beetal goats

[*Um estudo preliminar sobre a elaboração de uma fórmula hematológica para a estimativa da hemoglobina do volume de células embaladas em cabritos beetal*]

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ABSTRACT

The study was devised with the aim to evaluate the relationship between hemoglobin (Hb) concentration and Packed Cell Volume (PCV) in beetal goats being reared under pastoralism. It also aims to devise a hematological formula for estimation of Hb from PCV. Female (n=59) and male goats (n=41) were bled for PCV determination through microhematocrit method, and Hb estimation through Sahli's hemoglobinometer (HbD) as well as through calculation being 1/3rd of PCV (HbC). The HbD and HbC were statistically non-significant (P≥0.05) for male and female beetal goats. Overall, significantly (P≤0.01) positive correlation coefficient was noticed between HbD and PCV, and between HbD and HbC (r=0.75; adjusted r-square=0.57). As the overall model predicted that 57% variability in HbD could be deduced from PCV, hence, in order to enhance the prediction probability, the regression equation *i.e*. Hb concentration= 0.24(PCV) +1.5 was utilized to deduce corrected hemoglobin (CHb). The comparison of this CHb with HbD gave a non-significant (P≤0.05) difference. Similarly, linear regression of CHb with PCV gave a 99% prediction. We accordingly recommend a simplified pen-side hematological formula for deducing Hb concentration from PCV *viz*. Hb concentration= 0.24(PCV) +1.5 for beetal goats instead of its calculation as one-third of PCV.

Keywords: Cholistan desert; hematology; packed cell volume; hemoglobin; pastoralism

RESUMO

O estudo foi elaborado com o objetivo de avaliar a relação entre a concentração de hemoglobina (Hb) e o volume de células embaladas (PCV) em caprinos beetal em pastoreio. O objetivo também é elaborar uma fórmula hematológica para a estimativa da hemoglobina de PCV. Cabras fêmeas (n=59) e machos (n=41) foram sangrados para determinação da PCV através do método do microhematócrito, e a estimativa de Hb através do hemoglobinômetro Sahli (HbD), bem como através do cálculo sendo 1/3 do PCV (HbC). O HbD e HbC foram estatisticamente não significativos (P≥0.05) para caprinos machos e fêmeas beetal. De modo geral, foi observado um coeficiente de correlação positiva (P≤0.01) entre HbD e PCV, e entre HbD e HbC (r=0,75; r-quadrado ajustado=0,57). Como o modelo geral previa que 57% de variabilidade em HbD poderia ser deduzida do PCV, portanto, para aumentar a probabilidade de predição, a equação de regressão i.e. concentração de Hb= 0,24(PCV) +1,5 foi utilizada para deduzir a hemoglobina corrigida (CHb). A comparação desta CHb com HbD deu uma diferença não significativa (P≤0.05). Da mesma forma, a regressão linear de CHb com PCV deu uma previsão de 99%. Assim, recomendamos uma fórmula hematológica simplificada do lado da caneta para deduzir a concentração de Hb do PCV, ou seja, concentração de Hb= 0,24(PCV) +1,5 para cabras beetal em vez de seu cálculo como um terço do PCV.

Palavras-chave: Deserto de Cholistan; hematologia; volume de células embaladas; hemoglobina; pastoreio

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INTRODUCTION

Small ruminants constitute an essential economic and ecological segment in agriculture and small farming systems (Devendra, 2001). The goat (*Capra hircus*) is amongst the first species of small ruminants to be domesticated for meat and milk (Dubeuf and Boyazoglu, 2009; Galal, 2005). Although goats are distributed over all types of ecological zones worldwide, they are more concentrated in the tropical desert areas. About 97.3% of the global goat's population is found in the developing countries including Asia (65.9%), Africa (27.4%), Europe (3.5%) and Americas (3.0%) (Faostat, 2012).

The global goat population has gradually been increasing (~1045.916 million heads in 2018), resulting in about 200% rise during the last six decades (Faostat, 2020; Hegde, 2020). This significant growth in goat population in the arid and semi-arid regions is ascribed to their specific biological features such as small size, low metabolic necessities, feeding behavior and efficient reproductive potentials (Silanikove, 2000).

There are about 570 established breeds of goats worldwide (~25.6% from Asia) which can be categorized into four groups, *i.e*. milk, meat, dual-purpose and fiber (Hegde, 2020). Pakistan is blessed with 34 well-known indigenous breeds (~80.30 million heads) of goats (Pakistan Economic Survey, 2020-2021). Beetal is an elite dual-purpose breed native to Pakistan which is being reared in different parts of the country including pastures of Cholistan desert (Khan and Ashfaq, 2010).

Goats are usually reared by small-holder farmers and pastoralists in arid and semi-arid regions, where their contribution is highly valued in terms of feeding the population and poverty alleviation in under-privileged parts of the world (Boyazoglu *et al.* 2005). They are by nature browsers and the seasonal variations in the quantity and quality of food in arid and semi-arid zones may result into low productive and reproductive performance and increased vulnerability to the infections and parasitic infestations. The onset of parasitic infestations, which are generally high in tropical areas, causes anemia and results into massive financial losses.

The Hb and PCV can be used together or separately to track and judge the prevalence of anemia in goats. The thumb-rule of calculating Hb as a third of PCV (Hb=PCV/3) has widely been used in human clinical medicine to ascertain the prevalence of anemia (Quintó *et al*., 2006; Carneiro *et al.*, 2007; Rodríguez-Morales *et al*., 2007). Similarly, this convention (Hb = PCV/3) has also been validated in various avian orders (Velguth *et al.*, 2010) and indigenous cattle breeds (Turkson and Ganyo, 2015). However, in case of goats, no such study has yet been conducted to evaluate the validity of three-fold conversion between Hb and PCV. The present preliminary study is therefore, aimed to evaluate the relationship between PCV and Hb in beetal goats being reared in Cholistan desert under pastoralism. The ultimate outcome will be a pen-side hematological formula for quick assessment of Hb concentration from PCV under field conditions.

METHODS AND MATERIALS

The study was conducted in the Cholistan desert of Bahawalpur (27º42´& 29º45´N latitude and 69º52´& 75º24´E longitudes) which is located in the Southeast of Punjab province, Pakistan. The Cholistan desert is positioned at an altitude of 112m higher than the sea level and its climate is arid, hot sub-tropical and monsoonal with 180 mm, average annual rainfall and 28.33°C, mean annual temperature. The common flora of Cholistan desert comprises of the grasses (annuals and perennials) together with the sedge, herbs, shrubs, and trees (Farooq *et al.*, 2010). Research study was approved by the animal ethical committee of the Faculty of Sciences, the Islamia University of Bahawalpur. During the study work, all endeavors were made certain to curtail the animal distress. All the goat herders who were engaged for this study were well-informed and on-paper consent was also acquired from them.

Beetal goats (n=100) being reared in Cholistan desert under similar management and feeding conditions of pastoralism, identified based on their phenotypic characteristics, were randomly selected from nomadic herds for blood sampling irrespective of their age and sex. The overall health status of study animals was made certain via clinical symptoms and detailed anamnesis. The study animals which were lethargic, depressed, off feed, and isolated from the herd

were excluded from this study. Blood samples (~3 mL) were collected aseptically from the jugular vein of each animal into K_3-EDTA vacutainer tube during September to December 2020. To reduce the stress in study animals and to minimize the diurnal variations in blood samples together with normalization of the blood collection procedure, the same restraining technique with same personnel and time was practiced. Without delay, blood samples were transported in ice box to the Physiology Laboratory, Faculty of Veterinary & Animal Sciences, the Islamia University of Bahawalpur for hematological analysis.

The blood samples were analyzed for manual estimation of PCV and Hb concentration. The PCV was determined by using a heparinized capillary tube and centrifuging the blood in a microhematocrit centrifuge (Sigma Aldrich, Model 5254, Germany) for 5 min at 2,500 rpm (WHO, 2000). The hematocrit reading was taken through a hematocrit card-reader. For each blood sample, two hematocrit measurements were completed and mean of these two measurements was taken as PCV. The Hb concentration was determined through acid hematin (Sahli's hemoglobinometer) method and was termed as Hemoglobin Determined (HbD). For each blood sample, HbD was measured in triplicate and mean value was used for further analyses. The PCV reading was used for calculating Hb as its third (Hb=1/3rd of PCV) and was termed as Hemoglobin Calculated (HbC).

Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA) was used for data analysis. For analysis, all study animals were grouped as female (n= 59) stock and male (n= 41). By using the prescribed formulae, the means (±SE) and 95% CI for the blood parameters were calculated. The independent-sample t-test was used for deducing difference between hemoglobin calculated (HbC) as a third of PCV and hemoglobin determined (HbD), and between HbD and corrected Hb (CHb) for overall and group-wise data. Linear regression analyses were carried out and scatterplots were drawn for HbD versus PCV and HbD versus HbC for overall and group-wise data as prescribed earlier (Bland and Altman, 1999; Olayemi *et al.*, 2006). Trendline analysis was used to generate linear equations. Regression equation for overall results was used further for deducing CHb.

RESULTS

Mean (±SE) values and 95% CI for hematological attributes (HbD, HbC and PCV) in beetal goats (n=100) is presented in Table 1. The HbD and HbC were statistically non-significant (P≥0.05) for male and female beetal goats. The results for linear regression of all study groups are presented in Table 2.

Table 1.Mean (±SE) values and confidence intervals for hematological attributes in beetal goats (n=100)

Groups	Hemoglobin Determined (g/dL)		Hemoglobin Calculated (g/dL)		Sig.	Packed Cell Volume (%)	
	x±SE	CI	x±SE	CI		x±SE	CI
Female Stock (n=59)	6.8±0.1	6.5-7.1	7.1±0.1	6.7-7.4	0.276	21.4±0.5	20.3-22.5
Male Stock (n=41)	7.1±0.1	6.8-7.3	7.2±0.1	7.0-7.5	0.380	21.9±0.3	21.1-22.7
Overall	6.9±0.1	6.7-7.1	7.1±0.1	6.9-7.4	0.169	21.6±0.3	20.9-22.3

*Significant at P≤0.05 within rows for each group between hemoglobin determined and calculated.

Table 2.Linear regression between various hematological attributes for beetal goats (n=100)

Groups	Hemoglobin Determined versus Packed Cell Volume	Hemoglobin Determined versus Calculated	R	Adjusted r-Square
Female Stock (n=59)	y= 0.25; x+1.4	y= 0.76; x+1.4	0.80**	0.63
Male Stock (n=41)	y= 0.22; x+2.2	y= 0.66; x+2.2	0.61**	0.35
Overall	y= 0.24; x+1.5	y= 0.74; x+1.6	0.75**	0.57

**Significant correlation at P≤0.01.

Scatterplots for HbD versus PCV and CHb versus PCV are given in Fig. 1 and 2, respectively. Overall, significantly (P≤0.01) positive correlation coefficient was noticed between HbD and PCV, and between HbD and HbC (r=0.75; adjusted r-square=0.57). Female beetal goats showed highest positive correlation (r=0.80; adjusted r-square=0.63; P≤0.01). As the overall model predicted that 57% variability in HbD could be deduced from PCV, hence, in order to enhance the prediction probability, the regression equation *i.e*. Hb concentration= 0.24(PCV) +1.5 was utilized to deduce CHb. The comparison of this CHb with HbD gave a non-significant difference (P≤0.05). Similarly, linear regression of CHb with PCV gave a 99% prediction.

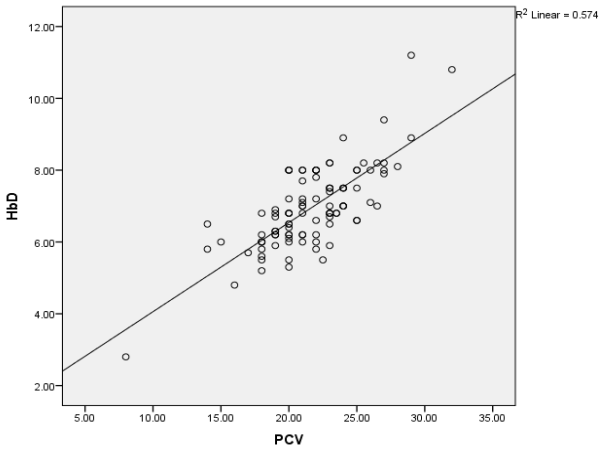

Figure 1.Scatterplot for hemoglobin determined (HbD) versus packed cell volume (PCV)

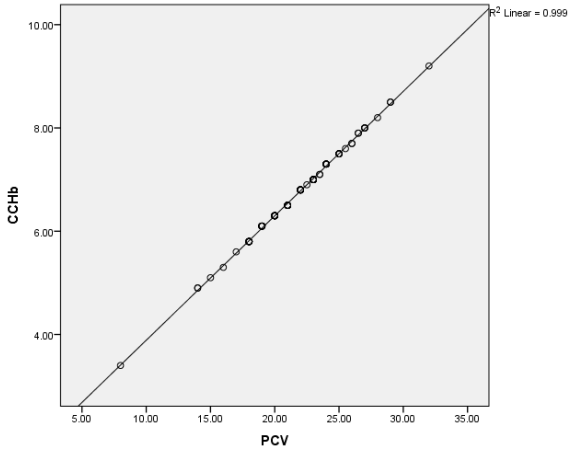

Figure 2.Scatterplot for corrected hemoglobin (CHb) versus packed cell volume (PCV)

DISCUSSION

Goat production adds a significant share to the livelihoods of low and medium-income growers in tropical belts of the developing countries, where production systems have changed to survive with the terrifying constraints imposed by the harsh natural and economic situations by adapting the incorporated crop/livestock production policies. As compared to other ruminants, the goat breeds perform better in their local harsh ecological settings. The large population of goats in the hot and dry ecosystems indicates most probably an improved adjustment of caprine species to such settings (Silanikove, 2000). In Asia, the goat production depends primarily upon grazing on communal/pastoral lands that barely offer the least nutrient supplies due to degradation and over-stocking (Escareño *et al*., 2012).

In terms of health issues, anemia due to poor nutrient quality and parasitic infestation is the major cut in livestock production performance

(Echavarría-Chairez *et al*., 2010).Various hematological parameters *i.e.* PCV, Hb, absolute RBC count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) are normally used to assess the health, nutritional and physiological status of ruminants (Gupta *et al*., 2007). It has long been established and validated in human clinical medicine that Hb concentration has a relation of being one-third of PCV and *vice versa* (Bland and Altman, 1999; Fairbanks and Tefferi, 2000; O'Connor *et al.*, 1994). Thus, this convention is extensively being used for anemia detection in humans. The present study is the first of its kind which reports an inter-relationship between PCV and Hb concentration in beetal goats and presents a pen-side hematological formula for estimating of Hb from PCV in these study animals.

In this study, hemoglobin determined (HbD) through the acid hematin method and hemoglobin calculated (HbC) as a third of PCV were statistically non-significant ($P \geq 0.05$) for both male and female beetal goats. The results are indicative of the fact that this validated convention in practice for human clinical medicine can be implied for caprine blood as well. A similar study on avian blood reported a strong linear relationship between Hb and PCV for birds in general, with good conformity of this relationship amongst 9 avian orders (Velguth *et al*., 2010). On the contrary, a study on blood of different indigenous African cattle breeds has annulled the validity of this convention (Turkson and Ganyo, 2015). In a malaria-endemic setting, this convention was also found invalid in children, and it was concluded that age, gender, season of sampling and physiological status of humans affects relationship between Hb concentration and PCV (Carneiro *et al*., 2007). It was hence dubbed impossible to deduce a validated mathematical formula for their relationship (Quintó *et al*., 2006).

In the present study, significant ($P \leq 0.01$) positive correlation was noticed between HbD and PCV and between HbD and HbC for male and female stock of beetal goats. Furthermore, the adjusted r-square was highest for female stock ($r=0.80$; adjusted r-square=0.63; $P \leq 0.01$). This endorses that the convention of Hb=PCV/3 may be implied for the blood samples of male and female beetal goats. Comparing this with a study conducted on African indigenous cattle breeds, it is noticed that no such relation was deduced for any of those breeds (Turkson and Ganyo, 2015). The linear regression equation *i.e*. Hb concentration (g/dL) = 0.24(PCV) +1.5 accomplished in the present study for the male and female beetal goats was utilized to deduce CHb which was statistically non-significant from HbD. We thus, recommend two pen-side hematological conversions *viz.* a) Hb Concentration (g/dL) = PCV/3 and b) Hb Concentration (g/dL) = 0.24(PCV) +1.5 for estimation of Hb concentration from PCV in male and female beetal goats, with higher predictability for the later.

CONCLUSION

The results of the present study conclude that the already validated 1/3rd relationship between Hb and PCV being used in human clinical medicine for the estimation of Hb from PCV is less valid in beetal goats. However, the mathematical formula *i.e.* Hb Concentration (g/dL)= 0.24(PCV) +1.5 reported in the present study is found to be more predictable and reliable for deducing Hb in beetal goats. It is expected that results of this pioneering study may perhaps assist to give information that can be applicable for the fieldwork along with clinical identification of anemia in beetal goats in specific and all other goat breeds in general. As it is a preliminary study, we recommend future research endeavors with larger populations towards the estimation of similar mathematical formulae and inter-relationships of various hematological attributes in beetal goats, as well as other livestock species and breeds of Pakistan.

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