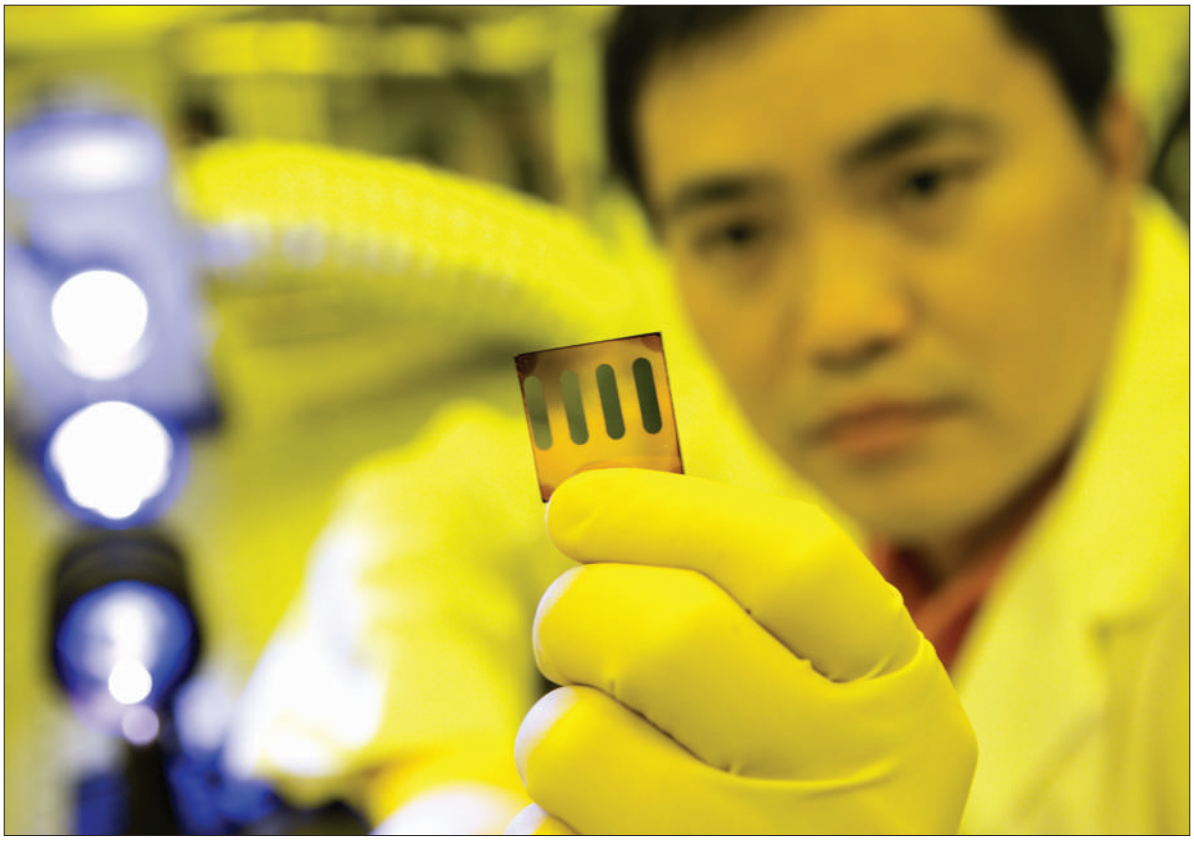

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Research Article
Effect of Duration and Storage Temperature on Haematological and Biochemical Parameters of Clinically Healthy Gudali Zebu in Ngaoundere, Cameroon

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Abstract

Background and Objective: Haematology and biochemical results are often influenced by the time between blood sampling and measurement, as well as storage conditions. The present study was carried out in Adamawa region of Cameroon to investigate the influence of various duration and storage temperature conditions on haematological and biochemical parameters of zebu bovine blood. **Materials and Methods:** Blood samples collected from 30 clinically healthy Gudali zebu were divided into 5 equal portions and stored at ambient (26-31°C), fridge (4±2°C) and coolers using ice (5-29°C) and gel packs (4-29°C) temperatures for determination of haematological and biochemical parameters after 0, 6, 12, 24, 48 and 72 h later. Parameters determined at 0 h were considered as reference or baseline values. **Results:** Red blood cell count increased significantly after 48 h for all storage temperatures except at 4°C and after 72 h for all storage temperature conditions. White blood cell count and blood glucose concentration decreased significantly after 24 h in blood stored at room temperature and after 48 h for all storage temperature conditions. The GGT activity increased while urea levels decreased significantly after 24 h in blood stored at room temperature, in ice and gel packs. **Conclusion:** The study showed that haematological and biochemical parameters of blood from clinically healthy Gudali zebu in Ngaoundere-Cameroon are not significantly affected if the sample is stored in the fridge, in ice packs and gel packs for up to 24 h and at room temperature up to 12 h.

Key words: Gudali zebu blood, haematology, blood biochemistry, storage temperature, duration of storage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Blood parameters such as haematological and biochemical values have been known to be good indices in assessing the physiological, nutritional and pathological status of animals[1]. The significance of determining these indices and changes of these parameters in domestic ruminants is well documented[2], with observed indices between breeds of goats[2-4]. Thus, it may be difficult to formulate a universal profile test for ruminants and particularly cattle. The need to establish appropriate physiological baseline values for various breeds of livestock in Cameroon, which could help in realistic evaluation of the management practice, nutrition and diagnosis of health condition cannot be overemphasized.

Though correct interpretation of haematological and biochemical analyses is crucial for precise diagnosis and management of disease problems in animal production systems, they are usually delayed during shipment of samples. The conditions of storage and transportation may not be appropriate particularly in poor developing countries. Also, considerable distances between farms and clinical laboratories, ill-equipped laboratories and transport duration exceeding 72 h particularly for shipment including weekends are common in Cameroon. Climatic conditions in Cameroon vary with agro-ecological zones and ambient temperature of21-38°C in the North and Far North[5] and 23-32°C in the Adamawa regions[6] have been recorded. Apart from these environmental constraints, electricity cuts, type of storage, delay in analysis of haematological and biochemical parameters of blood can occur and may lead to changes in blood parameters and erroneous laboratory results[7].

The nature and extent of the changes of haematological indices due to duration and temperature of storage have been widely reported in avian turkey, human and porcine blood[8-11]. Similarly, normal serum activity and isoenzyme patterns in animals differ greatly between species[11].

Adamawa region is the highest producer of cattle in Cameroon and Gudali zebu, reared traditionally at extensive levels, is the predominant breed. The region is relatively humid and favours high prevalence of diseases including ectoparasites (eg tse-tse fly, ticks) and vector borne blood diseases[12]. There is dearth of information on haematological and biochemical indices of indigenous animal species for different blood storage conditions in Cameroon. Reference parameters obtained for exotic breeds reared under different conditions are wrongly used for clinical laboratories interpretation in the country. The obtained data could be used for diagnosis of disease, for criteria of adaptability as well as to elucidate physiological mechanisms in the Gudali zebu. This study was therefore, carried out to determine baseline data and evaluate the effect of duration and temperature of storage on haematological and biochemical parameters of Gudali zebu in Ngaoundere, Cameroon.

MATERIALS AND METHODS

Study area: The study was carried out from January to June, 2017 in Ngaoundere (7°09'-7°70'N and 13°52'-13°70'E) in the Vina Division of the Adamawa Region of Cameroon (Fig. 1). The Adamawa region is located in the Savannah Guinean highland, in the mid to high altitude zones of Cameroon with average annual precipitations of 1200-1600 mm in the rainy season which runs from around mid-March to October. Mean temperature ranges from 23-26°C with a maximum[5,6] of up to 32°C. The region is the principal cattle production zone in the country and major beef cattle supplier to the southern zones[13].

Selection of animals and sample collection: Selection of individual cattle for the study was done in the Ngaoundere municipal abattoir using a described systematic random sampling technique reported by Ayoola *et al.*[14]. Clinically healthy adult (4-6 years old) Gudali zebu, both sexes with the current behavioural status (animal showed high alertness of its environment, high social activity with other animals, faecal texture is normal) were included in the study. Further inclusion criteria were based on clinical ante-mortem examination and included normal physiological parameters, state and alignment of hair coat (shinny/glossy and well aligned). Estimation of ages was done by dental inspection and examination of horn rings for animals without teeth (especially old/adult females) while the breed of the animals was obtained as previously described by Blench[15]. The body condition score was assessed as previously described by Natumanya *et al.*[16].

Apart from procedural restraining manipulations for safety purposes and jugular venipuncture for blood sampling ($\geq$15 mL) using sterile vacutainer, the animals were not subjected to suffering. Briefly, for each selected animal, blood samples were collected in 5 test tubes containing ethylene diamine tetra-acetic acid and transported with in 30 min in a closed, dark container to the Veterinary Research Laboratory of the Institute of Agricultural Research for Development (IRAD), Wakwa Regional Center, Ngaoundere, Cameroon. The 5 test tubes corresponded to the different study conditions including (1) Immediate analysis without storage and analysis after storage at the following temperatures, (2) Room temperature (29±3°C), (3) In refrigerator (4±2°C), (4) In a cooler flask containing ice packs (5±2°C) and (5) In a cooler flask containing gel packs (4±2°C) of equal masses (1.5 kg) kept in deep freezer (-20°C) for about 18 h. Blood samples in

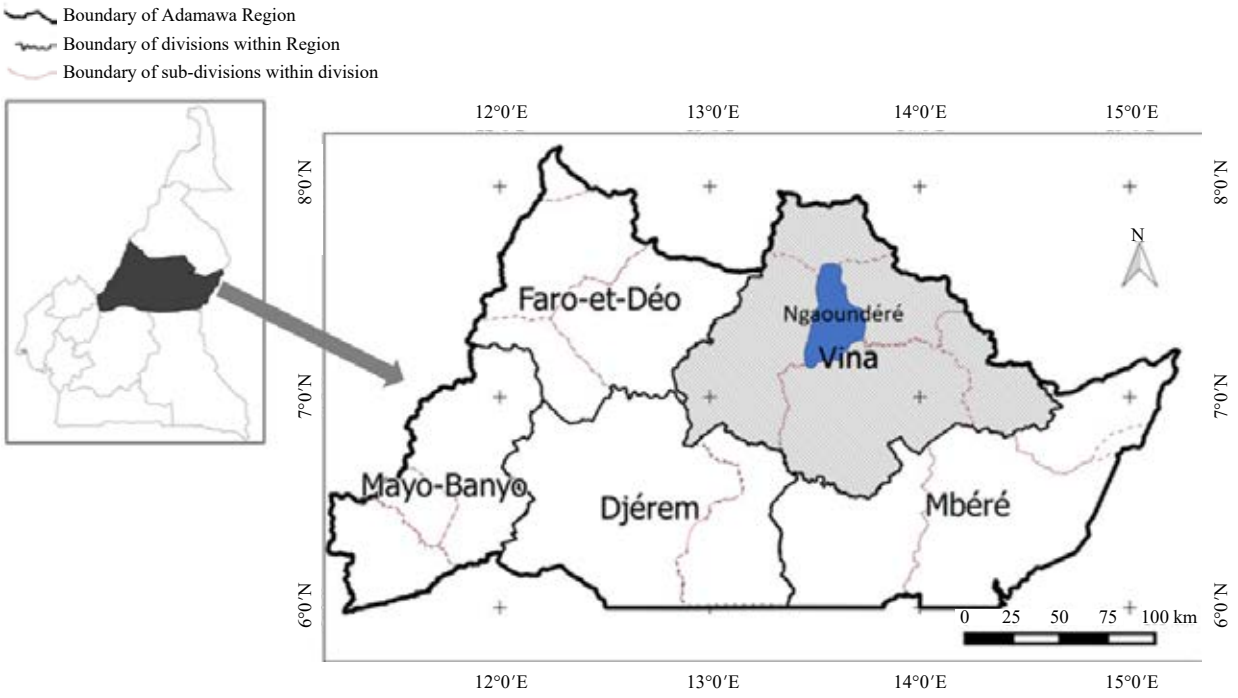

Fig. 1: Map showing study area (Ngaoundere) in Vina Division of Adamawa Region in Cameroon
Source: Awah-Ndukum *et al.*[5]

the test tubes for the 4 storage temperature conditions were mixed, by gently slanting and rotating the tubes and further dispensed into 5 tubes of 2-3 mL each corresponding to 5 analysis as after the following time durations of storage: 6, 12, 24, 48 and 72 h. However, the samples that did not require storage were immediately analyzed within an hour of sampling as described by De Baca *et al.*[17] to obtain a baseline value (BV) of each parameter. The temperatures in the different storage conditions were measured at the time of analysis with the aid of an analogue alcohol thermometer. Electricity cuts were not experienced and the refrigerator equipped with a functional thermostat worked without ceasing during the study period in the IRAD Veterinary Research Laboratory of Wakwa Ngaoundere.

Evaluation of haematological and biochemical parameters: In this study, whole blood samples were used to estimate haematological parameters such as red blood cells (RBC), white blood cells (WBC) count and packed cell volume (PCV) and biochemical parameters such as glucose, gamma-glutamyl transferase and urea following standard procedures. The RBC and WBC counts were determined using Malassez haemocytometer (Assistant, Germany) while PCV was determined using microhematocrit method[18] by means of a microhematocrit centrifuge and reader (Hawksley, England) and expressed as percent (%). Glucose was assessed using enzymatic-colorimetric method[19], Gamma-Glutamyl Transferase (GGT) was analyzed using Optimized Colorimetric Kinetic Colour test[20] and urea was assessed using Urea-Berthelot Enzymatic-Colorimetric test[21].

Statistical analysis: The data was registered in Microsoft excel 2016 (Microsoft Corporation, Redmond, WA, USA) and analyzed using Statgraphic centurion version 17.1. Student test was used to compare the means. Data were presented as means±standard deviation. Differences were considered statistically significant based on $p<0.05$.

RESULTS

Haematological parameters according to duration and temperature of storage: The results showed that the red blood cell count increased significantly ($p<0.05$) after 48 h for all storage temperatures except at 4°C and after 72 h for all storage temperature conditions compared to the baseline value (Table 1). White blood cell count and blood glucose concentration decreased significantly ($p<0.05$) after 24 h in blood stored at room temperature and after 48 h for all storage temperatures conditions compared to the baseline value (Table 2). The pack cell volume showed no significant ($p = 0.05$) change in values with respect to the duration and storage temperature conditions (Table 3).

Table 1: Changes in red blood cell of cattle blood samples at different storage temperatures and duration of storage

	Red blood cells ($\times 10^{12}$ L^{-1}) of cattle blood stored at different temperature (storage temperature°C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline value)	7.68±0.10 (29±3)	7.68±0.10 (4±2)	7.68±0.10 (5±2)	7.68±0.10 (4±2)
6	7.68±0.10 (31±4)	7.91±0.11 (4±2)	7.73±0.44 (5±3)	7.67±0.16 (4±2)
12	7.21±0.19 (31±4)	7.95±0.22 (4±2)	7.96±0.21 (6±2)	7.71±0.26 (5±3)
24	7.97±0.10 (31±2)	7.46±0.11 (4±2)	8.02±0.10 (9±2)	7.81±0.10 (6±4)
48	9.23±0.22 (31±2)*	8.01±0.28 (4±2)	9.95±0.15 (28±2)*	9.16±0.16 (27±4)*
72	9.63±0.23 (30±4)*	9.20±0.64 (4±2)*	10.17±0.58 (29±3)*	10.95±0.21 (28±4)*

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

Table 2: Changes in white blood cell of cattle blood samples at different temperatures and duration of storage

	White blood cells ($\times 10^{9}$ L^{-1}) of cattle blood stored at different temperature (storage temperature°C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline value)	10.46±0.29 (29±3)	10.46±0.29 (4±2)	10.46±0.29 (5±2)	10.4 6±0.29 (4±2)
6	8.31±0.48 (31±4)	8.60±0.52 (4±2)	10.58±0.26 (5±3)	10.37±0.62 (4±2)
12	7.81±0.35 (31±4)	9.94±0.41 (4±2)	9.79±0.37 (6±2)	10.37±0.40 (5±3)
24	6.86±4.77 (31±2)*	9.54±0.81 (4±2)	10.50±0.10 (9±2)	10.50±0.09 (6±4)
48	5.35±0.44 (31±2)*	5.02±0.32 (4±2)*	5.63±0.29 (28±2)*	5.93±0.21 (27±4)*
72	4.84±0.15 (30±4)*	4.80±0.18 (4±2)*	4.65±0.32 (29±3)*	4.82±0.21 (28±4)*

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

Table 3: Changes in packed cell volume of cattle blood samples at different temperatures and duration of storage

	Packed cell volume (%) of cattle blood stored at different temperature (storage temperature°C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline values)	32.40±0.56 (29±3)	32.40±0.56 (4±2)	32.40±0.56 (5±2)	32.40±0.56 (4±2)
6	33.37±1.37 (31±4)	33.30±1.42 (4±2)	33.60±1.50 (5±3)	33.87±1.74 (4±2)
12	32.40±0.56 (31±4)	32.40±0.56 (4±2)	32.40±0.56 (6±2)	32.40±0.56 (5±3)
24	32.40±0.56 (31±2)	32.40±0.56 (4±2)	32.40±0.56 (9±2)	32.40±0.56 (6±4)
48	34.17±1.69 (31±2)	33.34±1.31 (4±2)	32.87±1.60 (28±2)	33.30±1.38 (27±4)
72	33.57±1.33 (30±4)	33.57±1.33 (4±2)	32.87±1.60 (29±3)	33.30±1.38 (28±4)

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

Table 4: Changes in glucose concentration of cattle plasma at different temperatures and duration of storage

	Glucose level (mg L^{-1}) of cattle plasma stored at different temperature (storage temperature°C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline value)	128.99±7.69 (29±3)	128.99±7.69 (4±2)	128.99±7.69 (5±2)	128.99±7.69 (4±2)
6	120.86±3.90 (31±4)	122.41±35.03 (4±2)	128.09±19.34 (5±3)	127.32±12.59 (4±2)
12	119.69±10.90 (31±4)	121.25±4.76 (4±2)	127.61±5.82 (6±2)	123.03±12.05 (5±3)
24	86.01±7.69 (31±2)*	121.12±7.69 (4±2)	118.80±7.69 (9±2)	122.77±7.69 (6±4)
48	73.70±10.94 (31±2)*	98.38±20.20 (4±2)	71.14±11.45 (28±2)*	82.49±16.89 (27±4)*
72	59.94±10.3 (30±4)*	73.63±10.89 (4±2)	55.05±9.59 (29±3)*	62.24±11.42 (28±4)*

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

Biochemical parameters according to duration and temperature of storage: Blood glucose concentration decreased significantly (p<0.05) after 24 h in blood stored at room temperature and after 48 h for all storage temperature conditions compared to the baseline value (Table 4). The GGT activity increased while urea levels decreased significantly (p<0.05) after 24 h in blood stored at room temperature, in ice and gel packs compared to the baseline value (Table 5). Urea decreased significantly (p<0.05) at 24 h in the same condition (Table 6).

Table 5: Changes in gamma glutamyl transferase concentration of cattle plasma at different temperatures and duration of storage

	Gamma glutamyl transferase (U L^{-1}) of cattle plasma stored at different temperature (storage temperature °C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline value)	15.02±7.05 (29±3)	15.02±7.05 (4±2)	15.02±7.05 (5±2)	15.02±7.05 (4±2)
6	14.91±11.13 (31±4)	15.23±61.84 (4±2)	15.83±46.74 (5±3)	15.39±53.81 (4±2)
12	15.20±5.62 (31±4)	15.90±3.39 (4±2)	15.81±7.61 (6±2)	15.96±18.63 (5±3)
24	17.77±3.21 (31±2)	15.98±5.12 (4±2)	16.12±1.12 (9±2)	16.23±0.12 (6±4)
48	30.04±5.50* (31±2)	16.34±4.64 (4±2)	33.88±8.16* (28±2)	29.52±7.87* (27±4)
72	32.43±3.56* (30±4)	16.61±5.63 (4±2)	46.30±2.84* (29±3)	36.76±2.84* (28±4)

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

Table 6: Changes in blood urea concentration of cattle plasma at different temperatures and duration of storage

	Urea (mg L^{-1}) of cattle plasma stored at different temperature (storage temperature °C)			
Duration of storage (h)	Room temperature	Refrigerator	Ice packs**	Gel packs**
0 (baseline value)	21.49±5.62 (29±3)	21.49±5.62 (4±2)	21.49±5.62 (5±2)	21.49±5.62 (4±2)
6	19.81±3,76 (31±4)	20.2±3.43 (4±2)	19.57±5.25 (5±3)	19.65±3.36 (4±2)
12	18.88±4.12 (31±4)	19.60±3.58 (4±2)	19.01±5.54 (6±2)	19.65±3.17 (5±3)
24	18.98±1.96 (31±2)	18.84±1.96 (4±2)	18.25±1.96 (9±2)	19.34±1.96 (6±4)
48	17.20±3.35 (31±2)*	18.65±2.81 (4±2)	17.41±2.75 (28±2)*	17.50±2.04 (27±4)*
72	17.20±2.23 (30±4)*	18.46±2.69 (4±2)	17.17±2.46 (29±3)*	17.36±1.95 (28±4)*

*Significantly difference compared to baseline values at p<0.05, **Temperature in Ice packs (5-29°C) and gel packs (4-29°C) significantly increased with increase in duration of storage, data shown as Mean±SE

DISCUSSION

The present study provides substantial evidence that changes in blood parameters occur after storage of whole blood in different duration and temperature of storage conditions. The findings agree with Mahmoodi *et al.*[22], who reported significant increase in RBC count after 24 h of human blood and stored at room temperature (25-37°C). The increased RBC count was explained by loss of plasma volume due to the evaporation of liquid/water in the sample thus increasing the concentration of red blood cells. The ice packs and gel packs were still significantly cold after 24 h and the indices for samples stored in the conditions were comparable with the baseline values. However, from 48 h the RBC counts was significantly different from the baseline value and similar to samples stored at room temperature since the temperature of the ice and gel packs had increased to values similar to that of the room temperature. The RBC counts of refrigerated samples were significantly higher than the baseline value at 72 h of storage indicating cold drying of samples in the refrigerator. However, Hirase *et al.*[23] demonstrated human blood sample stability after 1 week of storage at 4°C in Iran.

The WBC counts in the study decreased when blood was stored at room temperature for 24 h. The study agrees with Wood *et al.*[24], who reported similar findings using human blood in Washington, USA whereas and is contrary to Obeidi *et al.*[25] and Kirmizigul *et al.*[26] in Iran and Turkey, respectively who observed no significant change in WBC count when stored at room temperature. The significant decrease in WBC was associated to cellular degeneration due to the duration of storage[27,28]. It is worth noting that Ihedioha and Ibeachu[29] and Ihedioha and Onwubuche[30] had reported in Nigeria that refrigeration of bovine blood during 24 h of storage decreased the white blood cells (WBCs) but had no change on red blood cells (RBCs).

There was no significant change in PCV values in the 4 temperature storage conditions suggesting that reliable PCV values maybe obtained up to 72 h in all the storage conditions. Mahmoodi *et al.*[22] also reported similar findings in human samples, contrary to Ihedioha *et al.*[31] and Furlanello *et al.*[32], who observed a significant increase in PCV values after 12 h of storage at room temperature (24°C) and refrigeration temperature (4°C) in canine blood samples using ADVIA 120 haematology analyzer. The low increases in PCV of all the blood samples in this study can be attributed to the fact that PCV is a combined measure of blood cell numbers and sizes, though the number of RBCs do not increase, the sizes do, as a result of degenerative swelling occasioned by progressive loss of ability to control fluid entry into the cell and consequent uncontrolled ingress of fluid in to the RBC, the cell membranes become weak as a result of hypoxia during keeping/storage of the blood[33]. Though WBC degenerated during storage, WBC particles still contribute to the bulk during centrifugation and packing of materials for determination of PCV in stored samples.

The significant decrease of glucose concentration during storage was related to sensitivity of glucose to higher temperatures[34]. In the present study, glucose was least stable at room temperature (<12 h) but was stable up to 24 h when stored in coolers (ice packs and gel packs) and fridge at 4±2°C. The general significant drop in glucose concentration in the 4 storage conditions was associated to *in vitro* glycolysis activities that continued within the blood cells causing the glucose concentration to fall. Marjani[34] observed that when blood samples were left at room temperature without being centrifuged, the concentration of glucose droppeds from 5-10% h^{-1}.

Gamma glutamyl transferase and Urea were found to be stable for a period of 24 h when stored at room temperature, in ice and gel packs and for up to 72 h at 4°C. The difference in the duration of sample stability of GGT when stored at room temperature and when using ice and gel packs were due to the increase in temperature within these storage conditions. Increase in temperature within the test tube and temperature in ice and gel packs increases the rate of collision between gamma glutamyl transferase (enzyme) and gamma glutamyl (substrate) thus increasing the rate of GGT activity. Though temperature increase in gel packs was slower than in cooler containing ice packs, temperatures in the 2 coolers were relatively stable for a period of 24 h and gradually increased to room temperature conditions by 48 h. The result of this study is contrary to Divya and Jayavardhanan[35], who reported that GGT activity remained stable for 2 days at room temperature. As recommendation, refrigeration at 4°C should be done if samples are to be analyzed within 48 h except white blood count and glucose concentration analysis that should be done in less than 24 h. In the absence of a refrigerator, sample storage in ice or gel packs should not exceed 24 h. Samples Stored at room temperature should be analyzed within 12 h except for PCV that can be done within 72 h.

CONCLUSION

Depending on the blood parameter to be estimated, refrigeration at 4°C is the choice storage condition for analysis of samples within 48 h except for white blood count and glucose concentration which should be estimated within 24 h. However, in the absence of a refrigerator, ice and gel packs maybe used to store samples for up to 24 h. Samples stored at room temperature should be analyzed within 12 h except for PCV that can be done within 72 h.

SIGNIFICANCE STATEMENT

This study discovers for how long and under what storage conditions values of blood parameters of samples can remain stable in Savannah Guinean highlands of Cameroon. These findings are beneficial to researchers or laboratories dealing with blood samples analyses. This study will help researchers uncover for how long and at what storage conditions they can rely on values obtained from blood sample analysis. Thus a new theory on duration and storage conditions of bovine blood samples, may be arrived at.

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