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Original Article

Period and Repeatability of Blood Sampling in Reared Grasscutter (*Thryonomys Swinderianus* Temminck. 1827) in Benin

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Abstract

The present study aims to clarify the period and repeatability of blood sampling in subadult grasscutters to promote its health monitoring through blood tests. Eight subadult male grasscutters aged 6 of months from Benin were used. Through blood samples, haematological and biochemical parameters were determined. Lymphocytes were increased more in the morning compared in the evening. In contrast, glucose, total protein and triglycerides were lower in 6 hours compared to 18 hours. For repeatability, MCH and MCHC showed high values in 3 ml compared to 6 ml. Total protein, total cholesterol, transaminase (GPT) and phosphorus were lower for 6 ml compared to 3 ml that is contrary to glucose. The study showed that there are many changes in haematological and biochemical parameters according to the period and repeatability of blood sampling in reared subadult grasscutters. For this purpose, the period of blood sampling which is the most suitable is the morning. In addition, subadult grasscutters can be taken more than once for a blood volume of 3 ml which is not the case for blood volume 6ml.

Key words: Grasscutters, blood samples, period-repeatability, blood nutrients, benin-côte d'ivoire.

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Introduction

Animal health is a major preoccupation for farm operators and plans for monitoring public health programs. Several ways are being explored for monitoring animal welfare. Among these, include blood tests (Saun, 2000). Blood is a liquid tissue very important for the major functions of the body. Its main role is to lead nutrients to organs and remove waste from the organism. Knowledge of quantitative and qualitative components of the blood helps to control and monitor animal health (Bernard *et al*., 1996).

During recent decades, grasscutter (*Thryonomys swinderianus* Temminck 1827) breeding is booming in Africa, south of the Sahara (Mensah, 1983; Addo, 1998; Mensah and Ekue, 2003; Opara, 2010a). Like the conventional stockfarms the "aulacodiculteurs" (grasscutter breeders) endeavor to ensure better health for grasscutters. Works of Okeke *et al*. (2008), Opara (2010b) and Opara and Fagbemi (2010c) showed in grasscutters mainly infectious diseases. The control, monitoring and the fight against these diseases may be through the blood route. In blood, the changes of blood cells through the blood count is evaluated to establish a diagnosis in animals

In addition, blood allows distinguishing infectious microorganisms in animals. Moreover, the dynamics of blood concentrations of various nutrients associating at least one inflammatory protein is determined in order to assess the health status of animals (Opara, 2010b; Opara, 2010d). To do this, blood samples should be carried out at very different specific times and frequencies (repeatability). Period or time of blood sampling influences blood parameters for several reasons. Therefore, the choice of the blood sampling period must be based on the feeding attitude of animals (Low *et al*.. 1980). In general, blood samplings are done on fasting morning to avoid nycthemeral variations (Lee *et al*., 1978; Tornquist *et al*., 1999; Balkaya *et al*., 2001; Azar *et al*., 2008). If for conventional animal species, the period and frequency of blood sampling are clearly defined, in grasscutters, unconventional animal species, they are not yet established. Grasscutter is indeed a mammal which rests for 19 hours and active for five hours. In addition, the grasscutter has a nocturnal feeding attitude (Yewadan, 1992; Mensah, 1995; *Ekue and Mensah, 2003). Should we make his* blood sampling in the morning like other animals? This is in response to this concern. a study is carried out on blood samples from readed grasscutters through two blood samplings at different times of the day.

This is the morning to six hours and in the evening at 18 hours. According to actogram of grasscutters at 18 hours we are halfway between the day and night. Another parameter that has an impact on changes of blood parameters is the repeatability of blood samples.

We evaluate the repeatability across the blood sample volume in reared grasscutters, which should be done according to the total blood volume of animals (Bruckner-Kardos and Wostman, 1974; Evans, 1994). To our knowledge, the total blood volume of grasscutters has never been studied. As part of our investigation, we chose two blood volumes namely three ml and six ml samples in grasscutters. Furthermore from the literature synthesis, no investigation has ever been conducted over the period and repeatability of blood samples in reared grasscutters. This study aims to clarify the period and repeatability of blood samples in reared grasscutters through the evaluation of the blood cells and blood nutrients changes. In addition, the health of animals is expressed using the C-reactive protein. We would specifically through this study:

- Evaluate and characterize the eventual variations of hematological and biochemical parameters between six hours and 18 hours on the one hand and secondly between the three ml and six ml blood volumes.
- Determine the possible interaction of blood samples' period and repeatability on hematological and biochemical parameters.
- Estimate the health of reared grasscutters during experimentation through various correlations between C-reactive protein and haematological and biochemical parameters.

Materials and Methods

Animals and experimental design

Eight aulacodinets (grasscutter subadult males) from six months of age and means metabolic weight between 1.4 kg and 2.1 ± 0.4 ± 0.1 kg were used for the study. To obtain animals from the same environment and have undergone the same breeding practices grasscutters were collected with Mr Gilbert TANKPINOU a professional grasscutter rearer in Plateau Department to southeastern Benin. Grasscutters were divided into two groups of four each one. Each grasscutter was bred in an individual numbered rectangular parallelepiped form cage (0.7 m x 0.7 m x 0.4 m) which was performed of cement. The experiment was carried out in Non Conventional Animal Species Breeding Sub-Programm (S-PEEANC) of Zootechnical. Veterinary and Halieutic Research Laboratory (LRZVH) in Agricultural Research Centre of Agonkanmey (CRA-Agonkanmey) in the National Institute of Agricultural Research of Benin (INRAB). A feeder and waterer have daily been laid available to each animal to be fed and watered *adlibitum*.

Blood samples and determination of biological parameters

In each of eight grasscutters, a blood sample was taken by cardiac puncture without the vital prognosis of the animal is engaged. Different collected blood volumes were three ml and six ml. selected on the basis of blood sampling's principles and codes in animal laboratories (McGuill and Rowan, 1989; Diehl *et al*.. 2001). Grasscutter blood was collected on an empty stomach with Vénojet vacuum tubes with anticoagulant (Ethylene-Diamine-Tetra-acetic Acid / EDTA) and without coagulant (dry tubes) at six and 18 hours a day. Vénojet tubes with anticoagulant (Ethylene-Diamine-Tetra-acetic Acid or EDTA) were used to blood sample and determined immediately haematological parameters by automatic counter analyzer (Sysmex KX21N) at National Centre of University Hospital (CNHU/Cotonou, Benin) in the Laboratory of the Science and Conscience Research Group (GRSC).

Blood samples obtained through dry tubes were centrifuged at 3000 rpm for five minutes and the serum collected was used for the determination of glucose. lipid and protein profiles. They were determined by an automatic device (HITACHI 902) through reagents kits from the company Spinreact SA (Ctra.Santa Coloma. Spain) specific biochemical parameter by a colorimetric method and immunoturbidimetric immunoassay. The determination of these biochemical parameters was carried out at the Centre of University Hospital (CHU/Cocody. Abidjan/Côte d'Ivoire) in the Biochemical Laboratory, Department of Immuno-Hematology.

Statistical Analysis

Statistical treatments were performed in order to compare the different searched biological parameters between blood volumes and chosen periods for blood sampling. This was the Wilcoxon test for paired variables and MANN-WHITNEY test for unpaired data between two independent groups. In addition, KRUSKAL WALLIS test was used to compare the biological parameters between more than two independent groups. Relationship between grasscutters' C-reactive protein and Laboratory parameters was determined by estimating the coefficients of SPEARMAN. All these statistical analyses were performed with the computer program Statistica Statsoft Windows version 7.1 (Statsoft, 2005). The level of significance was set to a p value less than 0.05.

Results

The work presented here deals with the measurements of hematological and biochemical parameters of blood samples from eight subadult grasscutters. Some blood samples could not be performed due to the observed mortality in some grasscutters to 18 hours and some coagulation of blood samples. It has been able to explain the observed number of blood samples that varies at each selected group of our study.

Influence of the blood sampling period on blood nutrient

The description of the haematological parameters of all animals collected at 6 hours and 18 hours is shown in Table 2. The results in Table 2 showed no significant difference ($p > 0.05$) between grasscutters collected at 6 hours and those to 18

hours except of the lymphocytes rate. Of the study, it appears that the rate of lymphocytes was higher in the morning (72.1 ± 13.2 %) compared to evening (47.3 ± 15.1 %). In the same vein, other haematological parameters showed a decreasing trend in grasscutters to 18 hours although this decrease was not significant. In contrast, the rate of leukocytes and thrombocytes increased to 18 hours compared to 6 hours (Table 2).

Values describing the biochemical parameters were summarized in Table 3. The results of the study showed statistically significant differences between the grasscutters collected at 6 hours and those of 18 hours for glucose ($p = 0.04$), total protein ($p = 0.03$) and triglycerides ($p = 0.03$). Thus, total protein and triglycerides were significantly lower in grasscutters collected at 18 hours compared to six hours. In contrast, glucose was increased more for grasscutters collected in the morning compared to the evening (Table 3). No significant difference ($p>0.05$) was observed between these two groups of animals as regards other determined biochemical parameters.

Impact of Blood Samples Repeatability on Nutrient Blood

The description of the haematological parameters per blood collected volume from the eight grasscutters to observe the repeatability of blood sampling is shown in Table 4. The results of the present work showed no significant difference ($p > 0.05$) but only at the mean corpuscular hemoglobin/MCH ($p = 0.04$) and mean corpuscular hemoglobin/MCHC ($p = 0.02$) between the two groups of animals. Thus, grasscutters collected for a volume of 3 ml reported higher values of MCH (19.5 ± 0.7 pg) and MCHC (34.6 ± 1 mg/dl) compared with 6 ml (18.5 ± 0.7 pg and 33 ± 0.8 mg/dl respectively).

The mean values of biochemical parameters in blood volume collected from eight grasscutters to observe the blood sampling frequency are listed in Table 5. The comparison of these biochemical parameters between grasscutters collected for a blood volume of three ml and six ml, indicated significant differences in glucose ($p = 0.02$), total protein ($p = 0.04$), total cholesterol ($p = 0.04$), transaminase/GPT ($p = 0.03$) and phosphorus ($p = 0.03$). Indeed, the concentrations of total protein (101.4 ± 6 mg/dl against 78 ± 52.3 mg/dl), total cholesterol (120.9 ± 26.3 mg/dl against 87.3 ± 9.6 mg/dl), transaminase/GPT (71 ± 13.2 U/l against 43.3 ± 15 U/l) and phosphorus (89.1 ± 9 mmol/l against 78.2 ± 9 mmol/l) was lower in 6 ml compared to 3 ml. In contrast, glucose (213.1 ± 68.9 mg/dl ± 27.6 against 275.8 mg/dl) was higher in grasscutters collected for 6 ml of blood compared to those taken for 3 ml (Table 5).

Interaction of period and repeatability blood sampling on nutrient blood

Table 6 summarizes the comparisons which were conducted between haematological parameters grasscutters collected for volumes of 3 ml and 6 ml for periods of 6 hours and 18 hours. These comparisons performed intra-period and inter-period, presented threshold values of probability (p^3) generally higher than 0.05.

From the aroused various statistical tests, the results of studies have revealed that no significant interaction ($p > 0.05$) in the period and frequency of blood sampling was observed on haematological parameters (Table 6). The same comparisons for biochemical parameters are summarized in Table 7. In view of the threshold values of probability (p3) in the Table 7, only glucose and triglycerides indicated values below 0.05. Therefore, the results of the study reported that for all biochemical parameters, only glucose and triglycerides have been an interaction between the period and repeatability of grasscutters' blood sampling. For this purpose, the glucose concentration is markedly elevated to 6 hours for a blood sampling of 6 ml. In contrast, the concentration of glucose is significantly reduced for a second blood sampling to 18 ml of 6 hours (12 hours after the first sampling). For triglyceride, concentrations were also high to 6 hours for a blood sampling of 6 ml. However, concentrations of triglycerides are lowered to a second blood sampling from of three ml to 18 hours.

Correlation between C-reactive protein and haematobiochemical parameters

The results of work summarized in Table 8 showed the correlation coefficients between C-

reactive protein and hematological parameters. These SPEARMAN correlation coefficients showed no significant relationship ($p>0.05$) between C-reactive protein and all determined haematological parameters in the grasscutters collected for different periods and blood volumes.

Conversely, some determined SPEARMAN coefficients were significant between the biochemical parameters and C-reactive protein (Table 9). Indeed, creatinine (3 ml to 18 hours and 6 ml to 6 hours) and triglycerides (6 ml to 6 hours) showed probability thresholds ($p = 0.04$. $p = 0.04$. $p = 0$. 02 respectively) significant for their correlation coefficient of SPEARMAN (all corresponding to 0.09). Correlations were positive between creatinine, triglycerides and C-reactive protein. By implication, creatinine and C-reactive protein have reported an increased positive relation to 18 hours for 3 ml and to 6 hours for 6 ml of blood (Table 9). In the same vein, for triglycerides an increased relation was observed with C-reactive protein to six hours for 6 ml of blood. In addition, the results of the study also indicated in Table 9 that the observed significant positive correlations were high because of the coefficients which are very close to 1 (Table 9).

Discussion

This study shows in subadult grasscutters that haematological values are statistically similar at six and 18 hours of blood sampling. However, the rate of lymphocytes differs between the two groups of animals at different chosen periods of the investigation. This difference is explained by the chosen period to perform blood sampling. According to the work of some authors in rat (*Rattus norvegicus*), the favorable time of day to carry out the blood sampling is the morning to avoid diurnal variations of blood parameters. From the work of another researcher also in rats, the number of leucocytes increases in the light and decreases in the dark (Matsuzawa *et al,* 1993). The present findings are not in line with the results obtained by theses researchers. In addition, a transient decrease in lymphocyte counts was reported in rats (Ulich and Del-Castillo, 1991). This change is justified by the stress factors or anesthesia at the time of blood sampling. These factors could explain the observed lymphopenia at 18 hours in subadult grasscutters of our work. Moreover, on the whole all values of haematological parameters are normal compared to other works in Nigeria on grasscutters (Ogunsanmi et al., 2002; Opara et al., 2006; Byanet et al., 2008). However, the obtained red blood cells during this sudy is considerably below than those reported by Nigerian authors in young grasscutters and wild grasscutters (Ogunsanmi *et al.,* 2002; Opara *et al.*, 2006). In contrast, the number of lypmhocytes is highly increased in subadult grasscutters of this investigation (61.7 ± 18.5 %) as compared to those obtained with young grasscutters (38.8 % with 27 and 46 % as extreme values) (Byanet *et al.*, 2008).

The difference in age between the two groups of grasscutters justifies this discrepancy. For the repeatability characterized by the variation of volume blood samples, the results show that MCH and MCHC) are changed. These two hematological parameters are lower for 6 ml compared to 3 ml of blood. The MCH and MCHC are respectively defined as the contained amount of hemoglobin in a red blood cell and average concentration of hemoglobin in these cells. They are related to hematocrit and red blood cell count so the occupied volume by them in a specified volume of blood (Williams, 1983). This reflects their change when the volume of blood sample is increased. Other factors such as stress, explains the modification of both haematological parameters.

Grasscutters, according some studies, are very sensitive animals to the environment (Mensah, 1997). In addition, some authors have reported changes in haematological parameters in rats following repeatability and the collected amount of blood. In fact, the collection of quantity greater than or equal to 10 % (10, 20, 30 or 40 %) of the total blood volume, divided into ten taken samples on day leads a sharp drop in the number of RBCs.. in hematocrit and hemoglobin concentration. These changes are characteristic of acute blood loss which is always followed by the release of immature cells into the circulation of rats. Conversely, an increase in the number of leukocytes was observed. This transient leukocytosis is rather an impact of marginal leukocyte mobilization in response to

stress (Ulich and Del-Castillo, 1991). No significant change in the rate of thrombocytes in rats is revealed (Berger, 1986; Kurata *et al.*, 1997; Scipioni *et al.*, 1997). This finding is similar to that reported in this study subadult grasscutters. Moreover, the interaction of period and repeatability of blood samples on hematological parameters of subadult grasscutters is not significant. This result is contrary to those revealed in the rats (*Rattus norvegicus*) and the Sprague-Dawley rats (Rowan, 1989; McGuill and and Muharrem *et al.*, 2001; Descat, 2002).

Table 1: Weight characteristics of the study grasscutters

Animals	Grasscutters taken from three ml of blood	Grasscutters taken from six ml of blood
Initial body liveweight	1.6 ± 0.6	1.8 ± 0.1
Final body liveweight	1.9 ± 0.6	2.1 ± 0.1
Initial metabolic liveweight	1.4 ± 0.4	1.5 ± 0.08
Final metabolic liveweight	1.6 ± 0.4	1.7 ± 0.1
Weignt gain/Body liveweight	0.3 ± 0.1	0.4 ± 0.1
Weignt gain/metabolic liveweight	0.2 ± 0.1	0.2 ± 0.08

Table 2: Changes in haematological parameters according to blood sampling period

Haematological parameters	Total samples N = 12	Samples at 6 hours N = 7	Samples at 18 hours N = 5	p values
Blood red cell count				
Red cells blood (1012/l)	7.2 ± 1.5	7.4 ± 1.1	6.7 ± 1.9	0.4 (NS)
Hemoglobin (g/dl)	13.7 ± 2.8	14.4 ± 1.8	12.7 ± 3.8	0.3 (NS)
Hematocrit (%)	40.2 ± 8.2	42 ± 5.6	37.7 ± 11.2	0.4 (NS)
Erythrocyte indices				
MCV (fl)	56.2 ± 1.8	56.5 ± 2	55.8 ± 1.8	0.2 (NS)
MCH (pg)	19.1 ± 0.8	19.4 ± 0.8	18.7 ± 0.7	0.3 (NS)
MCHC (g/dl)	34 ± 1.2	34.4 ± 1.3	33.6 ± 0.9	0.3 (NS)
Leukocyte parameters				
Leucocytes (106/l)	8.2 ± 3.8	7.2 ± 4.2	9.5 ± 3.1	0.2 (NS)
Lymphocytes (%)	61.7 ± 18.5	72.1 ± 13.2	47.3 ± 15.1	0.01 (S)
Thrombocyte parameters				
Thrombocytes (106/l)	293.3 ± 162.3	245.8 ± 131	359.8 ± 193.1	0.2 (NS)

N: Total number of used blood samples, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration
S: Statistically significant for a $p < 0.05$, NS: Not statistically significant for a $p > 0.05$

For the biochemical parameters, the observed values are abnormal compared to works carried out in Nigeria (Ogunsanmi *et al.*, 2002; Opara *et al.*, 2006; Byanet *et al.*, 2008). According to these workers, values of glucose concentration were very low compared to obtained current values. The results indicate high concentrations of glucose at 18 hours compared to 6 hours for a blood sampling of 6 ml compared to 3 ml. Several factors are indicators of this increase in glucose of the grasscutters aged 6 months. This is the age of the grasscutters which is correlated with the concentrations of nutrients (Serrano *et al.*, 2008). This determinant is influenced by the nutritional timing and frequency of blood sampling. This result is similar to that obtained from blood samples of goats (Bas *et al.*, 1980). These authors showed that several biochemical parameters such as blood glucose, triglycerides, total lipids and urea decreased significantly between 7 and 16 hours. This observation is contrary to that which we obtained. However, similar results are shown for total protein, triglycerides, total cholesterol, transaminase (GPT) and phosphorus in subadult

grasscutters in 18 hours and blood samples from 6 ml. Indeed, the 18-hour period is halfway between day and night in grasscutters (Mensah and Ekue, 2003).

Table 3: Variation in biochemical parameters according to blood sampling period.

Biochemical parameters	Total samples N = 14	Samples at 6 hours N = 8	Samples at 18 hours N = 6	p values
Glucide				
Glucose (mg/dl)	240 ± 62.3	228.1 ± 50.6	255.8 ± 77.3	0.04 (S)
Proteins				
total protein (mg/dl)	91.4 ± 55.4	105.4 ± 64.7	72.7 ± 37.3	0.03 (S)
urea (mg/dl)	28.6 ± 10.6	27.4 ± 9.3	30.2 ± 12.9	0.9 (NS)
Creatinine (mg/dl)	13.8 ± 2.3	13.9 ± 2.6	13.7 ± 2	0.5 (NS)
Lipids				
Triglycerides (mg/dl)	56.4 ± 31.8	70.8 ± 36.2	37.3 ± 11.1	0.03 (S)
Total cholesterol (mg/dl)	106.5 ± 26.5	110.6 ± 29.8	101 ± 22.9	0.2 (NS)
HDL cholesterol (mg/dl)	35.5 ± 6.2	36.8 ± 5.3	33.8 ± 7.4	0.7 (NS)
Enzymes				
Transaminase GOT (U/l)	132.3 ± 47.9	122.5 ± 33	145.3 ± 64	0.6 (NS)
Transaminase GPT (U/l)	39.9 ± 12.7	36.5 ± 9.4	44.5 ± 15.9	0.4 (NS)
Inflammation				
C réactive Protein	4.4 ± 0.5	4.4 ± 0.5	4.3 ± 0.5	0.6 (NS)
Ions				
Sodium (mmol/l)	144.4 ± 3.6	144.6 ± 3.4	144 ± 4.1	0.7 (NS)
Potassium (mmol/l)	10.1 ± 0.9	9.9 ± 0.9	10.3 ± 1	0.4 (NS)
Calcium (mmol/l)	167.8 ± 14.8	170 ± 15.6	164.8 ± 14.5	0.2 (NS)
Chlorinee (mmol/l)	107 ± 2.3	107 ± 2.3	107 ± 2.5	0.8 (NS)
Magnesium (mmol/l)	27.9 ± 3.2	27.7 ± 3.6	28 ± 2.9	0.9 (NS)
Phosphorus (mmol/l)	84.4 ± 9.5	81.1 ± 11.2	88.8 ± 4.6	0.1 (NS)

N: Total number of used blood samples, HDL: High density lipoprotein. GOT: Glutamyl oxaloacetic transaminase or glutamate transaminase oxalacetat. GPT glutamyl pyruvic transaminase or glutamate transaminase Pyruvat. S: Statistically significant for a $p < 0.05$. NS: Not statistically significant for a $p > 0.05$

The time's passage of food through the digestive tract of the grasscutters measured with a method of marker (carmine red) is an average of 38.6 ± 5 hours (Lawani, 1989). For this, the variation of some observed blood nutrients during the study has its justification. Moreover, an interaction is found between the period and frequency of blood sampling during the study for the glucose and triglycerides. This interaction can be improved for a 3 ml blood volume at 18 hours (12 hours after the first). This result is contrary to that revealed in reared goats (Hem *et al.*, 1998; Balkaya *et al.*, 2001). As the haematological parameters, the factor that clarifies these observed changes in blood nutrients during this work is stress. Some authors showed in rats a variation of glucose and corticosterone after several blood samples at the jugular vein due to stress (Vachon and Moreau, 2001). The method of blood sampling also justifies the changes of haematological and biochemical parameters (Leforban and Vannier, 1989; Nau and Schunck, 1993). This factor also corroborates the different correlations between creatinine, Triglycerides, C-reactive protein and haematobiochemical parameters of grasscutters. Similar observations are indicated in some captive grasscutters and in the male african Grasscutters (Nyameasem and Karikari, 2009; Ajayi *et al.*; 2012).

Table 4: Evolution of haematological parameters based on collected volume of blood.

Haematological parameters	Total samples N = 12	Samples for 3 ml N = 8	Samples for 6 ml N = 4	p values
Blood red cell count				
Red cells blood (1012/l)	7.2 ± 1.5	6.9 ± 1.6	7.7 ± 1.1	0.4 (NS)
Hemoglobin (g/dl)	13.7 ± 2.8	13.4 ± 3.1	14.2 ± 2.4	0.7 (NS)
Hematocrit (%)	40.2 ± 8.2	38.9 ± 9	42.9 ± 6.9	0.5 (NS)
Erythrocyte indices				
MCV (fl)	56.2 ± 1.8	56.3 ± 2.2	55.9 ± 0.9	0.8 (NS)
MCH (pg)	19.1 ± 0.8	19.5 ± 0.7	18.5 ± 0.7	0.04 (S)
MCHC (g/dl)	34 ± 1.2	34.6 ± 1	33 ± 0.8	0.02 (S)
Leukocyte parameters				
Leucocytes (106/l)	8.2 ± 3.8	8.4 ± 4.7	7.7 ± 1.5	0.8 (NS)
Lymphocytes (%)	61.7 ± 18.5	60 ± 21.2	65.3 ± 13.5	0.7 (NS)
Thrombocyte parameters				
Thrombocytes (106/l)	293.3 ± 162.3	265.7 ± 192.6	348.5 ± 62.9	0.4 (NS)

N: Total number of used blood samples, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

S: Statistically significant for a $p < 0.05$. NS: Not statistically significant for a $p > 0.05$

Table 5: Changes in biochemical parameters depending on collected volume of blood

Biochemical parameters	Total samples N = 14	Samples for 3 ml N = 8	Samples for 6 ml N = 6	p values
Glucide				
Glucose (mg/dl)	240 ± 62.3	213.1 ± 68.9	275.8 ± 27.6	0.02 (S)
Proteins				
total protein (mg/dl)	91.4 ± 55.4	101.4 ± 6	78 ± 52.3	0.04 (S)
urea (mg/dl)	28.6 ± 10.6	31.1 ± 12.4	25.2 ± 7.3	0.2 (NS)
Creatinine (mg/dl)	13.8 ± 2.3	13.9 ± 1.7	13.7 ± 3.1	0.9 (NS)
Lipids				
Triglycerides (mg/dl)	56.4 ± 31.8	52.5 ± 32.7	61.7 ± 32.7	0.8 (NS)
Total cholesterol (mg/dl)	106.5 ± 26.5	120.9 ± 26.3	87.3 ± 9.6	0.04 (S)
HDL cholesterol (mg/dl)	35.5 ± 6.2	33.9 ± 7.3	37.7 ± 3.9	0.09 (NS)
Enzymes				
Transaminase GOT (U/l)	132.3 ± 47.9	136.6 ± 56.6	126.5 ± 37.7	0.9 (NS)
Transaminase GPT (U/l)	39.9 ± 12.7	71 ± 13.2	43.3 ± 15	0.03 (S)
Inflammation				
C réactive Protein	4.4 ± 0.5	4.3 ± 0.5	4.5 ± 0.5	0.7 (NS)
Ions				
Sodium (mmol/l)	144.4 ± 3.6	143.9 ± 3.2	145 ± 4.3	0.9 (NS)
Potassium (mmol/l)	10.1 ± 0.9	10.5 ± 0.7	9.4 ± 0.9	0.1 (NS)
Calcium (mmol/l)	167.8 ± 14.8	166.5 ± 17.5	169.5 ± 11.8	0.9 (NS)
Chlorinee (mmol/l)	107 ± 2.3	107.5 ± 2.3	106.3 ± 2.3	0.1 (NS)
Magnesium (mmol/l)	27.9 ± 3.2	28.7 ± 4.1	26.7 ± 1	0.3 (NS)
Phosphorus (mmol/l)	84.4 ± 9.5	89.1 ± 9	78.2 ± 9	0.03 (S)

N: Total number of used blood samples, HDL: High density lipoprotein, GOT: Glutamyl oxaloacetic transaminase or glutamate transaminase oxalacetat, GPT glutamyl pyruvic transaminase or glutamate transaminase Pyruvat, S: Statistically significant for a $p < 0.05$. NS: Not statistically significant for a $p > 0.05$

Table 6: Interaction of blood sampling's period and blood volumes on haematological parameters.

Parameters hématologiques	Period and repeatability of blood sampling						
	6 Hours		p1 values	18 Hours		p2 values	p3 values
	3 ml (N = 4)	6 ml (N = 3)		3 ml (N = 4)	6 ml (N = 1)		
Blood red cell count							
Red cells blood (1012/l)	7 ± 1	8 ± 1.1	> 0.05	6.8 ± 2.2	ND	ND	> 0.05, > 0.05
Hemoglobin (g/dl)	14 ± 1.7	14.9 ± 2.2	> 0.05	12.9 ± 4.4	ND	ND	> 0.05, > 0.05
Hematocrit (%)	39.8 ± 4.2	44.9 ± 6.7	> 0.05	38 ± 13	ND	ND	> 0.05, > 0.05
Erythrocyte indices							
MCV (fl)	56.7 ± 2.7	56.2 ± 0.9	> 0.05	55.9 ± 2	ND	ND	> 0.05, > 0.05
MCH (pg)	19.9 ± 0.5	18.7 ± 0.6	< 0.05	19 ± 0.5	ND	ND	> 0.05, > 0.05
MCHC (g/dl)	35.2 ± 0.9	33.3 ± 0.7	< 0.05	33.9 ± 0.5	ND	ND	> 0.05, > 0.05
Leukocyte parameters							
Leucocytes (106/l)	6.8 ± 5.8	7.8 ± 1.8	> 0.05	10.1 ± 10.1	ND	ND	> 0.05, > 0.05
Lymphocytes (%)	75.4 ± 12.4	67.6 ± 15.6	> 0.05	44.5 ± 15.9	ND	ND	< 0.05, > 0.05
Thrombocyte parameters							
Thrombocytes (106/l)	172.9 ± 118	343 ± 75.8	< 0.05	358.5 ± 222.9	ND	ND	< 0.05, > 0.05

N: Total number of used blood samples. MCV: Mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin concentration. ND: Not determined. 1: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml at 6 hours. 2: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml at 18 hours. 3: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml for different blood sampling periods of 6 hours and 18 hours

Table7: Interaction of blood sampling's period and blood volumes on biochemical parameters.

Biochemical parameters	Period and repeatability of blood sampling						
	6 Hours		p1 values	18 Hours		p2 values	p3 values
	3 ml (N = 4)	6 ml (N = 4)		3 ml (N = 4)	6 ml (N = 2)		
Glucose (mg/dl)	190.5 ± 36.	265.8 ± 29.3	< 0.05	235.8 ± 91.4	148.5 ± 205.8	< 0.05	< 0.05, < 0.05, < 0.05, < 0.05
total protein (mg/dl)	120.8 ± 72	90 ± 62.8	< 0.05	82 ± 44.1	54 ± 9.9	< 0.05	< 0.05, < 0.05, > 0.05, < 0.05
urea (mg/dl)	29.5 ± 11.6	25.3 ± 7.4	> 0.05	32.8 ± 14.8	25 ± 9.9	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Creatinine (mg/dl)	13.3 ± 1.7	14.5 ± 3.5	> 0.05	14.5 ± 1.7	12 ± 1.4	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Triglycerides (mg/dl)	69.3 ± 41.3	72.3 ± 34.3	> 0.05	35.8 ± 6	40.5 ± 21.9	> 0.05	< 0.05, < 0.05, < 0.05, < 0.05
Total cholesterol (mg/dl)	129.5 ± 32.8	91.8 ± 6.2	< 0.05	112.3 ± 18.2	78.5 ± 10.6	< 0.05	> 0.05, < 0.05, > 0.05, > 0.05
HDL cholesterol (mg/dl)	34 ± 5.7	39.5 ± 3.4	> 0.05	33.8 ± 9.5	34 ± 0	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Transaminase GOT (U/l)	140.8 ± 39.3	104.3 ± 9.9	< 0.05	132.5 ± 76	171 ± 29.7	< 0.05	> 0.05, < 0.05, < 0.05, < 0.05
Transaminase GPT (U/l)	36.5 ± 12.2	36.5 ± 7.5	> 0.05	38.3 ± 11.7	57 ± 19.8	> 0.05	> 0.05, < 0.05, > 0.05, < 0.05
C reactive Protein	4.3 ± 0.5	4.5 ± 0.6	> 0.05	4.3 ± 0.5	4.5 ± 0.7	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Sodium (mmol/l)	144.5 ± 4	144.8 ± 3.3	> 0.05	143.3 ± 2.5	145.5 ± 7.8	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Potassium (mmol/l)	10.4 ± 0.5	9.4 ± 1	> 0.05	10.6 ± 0.9	9.6 ± 1.1	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Calcium (mmol/l)	167.3 ± 19.8	172.8 ± 12.6	> 0.05	165.8 ± 17.8	163 ± 9.9	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Chlorinee (mmol/l)	108.5 ± 1.7	105.5 ± 1.9	> 0.05	106.5 ± 2.6	108 ± 2.8	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Magnesium (mmol/l)	28.7 ± 5.2	26.7 ± 1.1	> 0.05	28.8 ± 3.2	26.6 ± 1.1	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05
Phosphorus (mmol/l)	87.3 ± 10.1	75 ± 9.4	> 0.05	91 ± 2.9	84.5 ± 4.9	> 0.05	> 0.05, > 0.05, > 0.05, > 0.05

N: Total number of used blood samples. HDL: High density lipoprotein. GOT: Glutamyl oxaloacetic transaminase or glutamate transaminase oxalacetat. GPT glutamyl pyruvic transaminase or glutamate transaminase Pyruvat. 1: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml at 6 hours. 2: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml at 18 hours. 3: Threshold values of probability (p) for comparisons between grasscutters taken for 3 ml and 6 ml for different blood sampling periods of 6 hours and 18 hours.

Table 8: Correlation coefficients between C reactive protein and haematological parameters.

Haematological parameters	*Correlation coefficients* C reactive Protein			
	3 ml/6H	3 ml/18H	6 ml/6H	6 ml/18H
Blood red cell count				
Red cells blood (1012/l)	-0.5 (0.4)	0.3 (0.7)	0.9 (0.3)	ND
Hemoglobin (g/dl)	-0.6 (0.4)	0.3 (0.7)	0.9 (0.3)	ND
Hematocrit (%)	-0.7 (0.3)	0.8 (0.2)	0.9 (0.3)	ND
Erythrocyte indices				
MCV (fl)	-0.05 (0.9)	0.8 (0.2)	0.9 (0.3)	ND
MCH (pg)	0.02 (0.9)	0.8 (0.2)	0	ND
MCHC (g/dl)	0.1 (0.9)	-0.8 (0.2)	0	ND
Leukocyte parameters				
Leucocytes (106/l)	-0.6 (0.4)	-0.3 (0.7)	0	ND
Lymphocytes (%)	0.9 (0.1)	0.8 (0.2)	0.9 (0.3)	ND
Thrombocyte parameters				
Thrombocytes (106/l)	-0.6 (0.4)	0.8 (0.2)	0	ND

ND: Not determined; The p values are indicated in brackets, MCV: Mean corpuscular volume
MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

Table 9: Correlation coefficients between C reactive protein and biochemical parameters.

Biochemical parameters	Correlation coefficients C reactive Protein			
	3 ml/6H	3 ml/18H	6 ml/6H	6 ml/18H
Glucide				
Glucose (mg/dl)	0.1 (0.9)	0.5 (0.5)	0.3 (0.7)	ND
Proteins				
total protein (mg/dl)	0.7 (03)	0.5 (0.5)	-0.5 (0.5)	ND
urea (mg/dl)	-0.4 (0.6)	0.4 (0.6)	0.7 (0.3)	ND
Creatinine (mg/dl)	-0.1 (0.9)	0.9 (0.04)	0.9 (0.01)	ND
Lipids				
Triglycerides (mg/dl)	0.2 (0.8)	0.1 (0.8)	0.9 (0.02)	ND
Total cholesterol (mg/dl)	-0.3 (0.7)	-0.6 (0.4)	0.4 (0.6)	ND
HDL cholesterol (mg/dl)	-0.7 (0.3)	0.2 (0.8)	0.2 (0.8)	ND
Enzymes				
Transaminase GOT (U/l)	0.04 (0.9)	-0.03 (0.9)	-0.9 (0.1)	ND
Transaminase GPT (U/l)	0.2 (0.8)	0.1 (0.9)	0.4 (0.6)	ND
Ions				
Sodium (mmol/l)	0.1 (0.9)	0.7 (0.3)	-0.3 (0.7)	ND
Potassium (mmol/l)	-0.5 (0.5)	-0.4 (0.6)	-0.3 (0.8)	ND
Calcium (mmol/l)	0.4 (0.6)	0.8 (0.2)	0.9 (0.1)	ND
Chlorinee (mmol/l)	0.2 (0.8)	0.6 (0.4)	-0.3 (0.7)	ND
Magnesium (mmol/l)	-0.6 (0.4)	-0.3 (0.7)	0.7 (0.3)	ND
Phosphorus (mmol/l)	0.4 (0.6)	-0.9 (0.1)	-0.9 (0.1)	ND

HDL: High density lipoprotein, GOT: Glutamyl oxaloacetic transaminase ou glutamat oxalacetat transaminase, GPT: Glutamyl pyruvic transaminase ou glutamat pyruvat transaminase, ND: not determined. The p values are indicated in brackets

Conclusion

The results of this study showed that the measured haematological and biochemical parameters vary depending on the period and the number of blood sampling in subadult grasscutters. Thus, haematological parameters such as mean corpuscular concentration and mean corpuscular concentration hemoglobin (MCH and MCHC) and lymphocyte counts are degraded. Glucose, total protein, triglycerides, total cholesterol, transaminase (GOT) and phosphorus are also altered by period and repeatability of blood sampling in reared subadult grasscutters. The main reasons for these changes in blood parameters of reared subadult grasscutters are age, stress and blood sampling method. In view of our findings, we recommend performing blood sampling in grasscutters (subadult) in the morning at 6 o'clock. In addition, a blood sampling can be done twice to 3 ml volume of blood per sample in reared subadult grasscutters with an interval of 12 hours. Conversely, it is advisable to perform a single blood sample at the subadult grasscutters for a total blood volume of 6 ml. To explore the best period and repeatability of blood sampling in reared subadult grasscutters, it should consider all age physiological stages and method of sampling. Moreover, it will need to know the total blood volume in the reared grasscutters which is not yet the case.

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