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Study of basic haematological parameters: indicators of the general state and immune competence in the male grasscutter (*Thryonomys swinderianus*, Temminck, 1827) bred in captivity in Côte d'Ivoire

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1 SUMMARY

The study of basic haematological parameters as indicators of the general state and immune competence in the male grasscutter (*Thryonomys swinderianus*, Temminck, 1827) bred in captivity was conducted. The study included 720 male grasscutter divided into three age groups (young, young adults and adults grasscutter) of 240 animals each. A sample of 5 ml of blood per animal fasting is collected in a tube containing an anticoagulant. The sample is analyzed using an automated haematology analyzer.

The explored parameters are: Hematocrit (HCT), hemoglobin (HB), red blood cell count (RBC), Red cell Distribution Width (RDW), Mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), the number of white blood cells (WBC), the percentage of neutrophils (Neu), eosinophils (Eos), basophils (Bas), lymphocytes (Lym), monocytes (Mon) and the platelets count (PLT). With the exception of the index distribution of red blood cells, mean corpuscular hemoglobin content, the number of white blood cells, the percentage of lymphocytes and monocytes, all other parameters have shown a strong individual variability. Significant correlations were present between RBC, HB, HCT, MCV, MCHC, PLT, Neu, Eos, Bas and the age of the grasscutter. Blood of the grasscutter was characterized by a significantly high platelet count (P< 0.05) very high (876±52 106/L) in adults. However, there is a very small amount of red blood cells (5.3 ± 0.6 1012/µL) in the young grasscutters. The rate of lymphocytes was very high in grasscutters of all ages (80.6 ± 3.6% in younger and 84.1 ± 7.5 in adults).

These results show that there is a physiological anaemia (erythrocytopenia) marked in young grasscutter. The excessive production of platelets and lymphocytes respectively characterized physiological thrombocytosis and leukocyte reversed in grasscutter.

2 INTRODUCTION

Blood is a fluid tissue that circulates in the veins and arteries and is with lymph and interstitial fluids, the internal environment in which others live anatomical elements. It is composed of three major types of cells: red blood cells or erythrocytes, White blood cells or leukocytes and Platelets or thrombocytes. The red blood cells or erythrocytes are involved in respiratory gas exchange. White blood cells or leukocytes ensure the defence of the body against pathogens. Platelets or thrombocytes are involved in the process of

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haemostasis helping maintain blood vessels inside of stopping bleeding. Thus, the exploration of haematological parameters in animals is rich in experimental and practical interests. Some haematological parameters can be suitably used to determine the physiological level in the animal of a harmful event, as in the case of a stress, pollution or a disease state (Adams *et al.* 1993). However, outside of experimental research, exploration of haematological parameters in animals remains an uncommon practice. One possible reason for this neglect is the difficulty of obtaining reliable reference standard values; especially if we consider that the individual biological variability of haematological parameters can be significant and require a considerable sampling effort (Michel *et al.* 1984). Studies dealing with the infestation of animals by various pathogens, management and exploitation of animal resources are clearly needed to have reliable hematologic standards (Obi and Oduye, 1985, Merlin, 1986; Ahmed *et al.* 1988; Aba-AduluBCa and Joshua, 1990). Both blood those gastrointestinal parasites, nutritional deficiencies conditioning livestock productivity can be validly assessed by blood parameters (Zouh Bi *et al.*, 2013 Soro *et al.*, 2013). Since then, nearly three decades, research on grasscutters has increased considerably, so that from the state of hunting in 1983, the grasscutter has become a domestic animal (Mensah *et al.*, 2011). Its intensive breeding in close captivity is a source of protein and additional income for grasscutter breeders. In grasscutter rearing, only one male is used for several females, hence the importance of having male in very good health for reproduction. This work aims to establish the values of haematological baseline in males reared grasscutter in captivity.

3 MATERIAL AND METHODS

3.1 Animals: The grasscutters were from a modern farm in the rural area in Côte d'Ivoire. They were housed in good conditions for breeding with good ventilation and in natural lighting. The temperature and humidity of the building respectively of 26-28 ° C and 50-60% (figure 1). The study ran from October 2012 to September 2013.

Figure 1: House of grasscutters breeding

The grasscutters are selected from a month, just after weaning. This selection reflects the good health status of the grasscutter (normal temperature 38 ° C, shiny hair, normal droppings.).The grasscutter are divided into three classes: the young grasscutters (figure 2) aged 1 to 3 months with a maximum weight of 1.5 kg. Young adults (figure 3) ages 4 to 7 months (>1, 5 and 3 kg) and adults (figure 4) between the ages of 8 months and

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more (>3 kg). This study involved two hundred and forty grasscutters aged 1 to 3 months, two hundred and forty grasscutter from 4 to 7 months of age and two hundred and forty other 8 months of age and older. They are all male, housed in pens staged under conditions of intensive livestock (figure 1). The youngest are grouped between 4 and 10 animals by compartmentalized paddock with an area of 2 m^2 (figure 2). From 4 months of age, they are installed in individual enclosures of 0.8 m^2 of surface. They are all fed with a diet consisting of 70% of green fodder and 30% concentrate food and dewormed every month (Mensah and ebony, 2003; Fantodji and Soro, 2004, Soro, 2007).


Figure 2: Young Grasscutter


Figure 3: Young adult Grasscutter


Figure 4: adult Grasscutter

3.2 Blood samples: In order to facilitate the collection of blood, a general anaesthetic was required. The animals were given an injection of the anaesthetic mixture intramuscularly at the base of the tail. Anaesthesia was composed of hydrochloride xylazine (ROMPUN®) and ketamine (KETAVET®) hydrochloride. The two products are used in equal volume in the same syringe at a dose of 0.1 ml/kg of body weight. A few minutes (5-10 min) after, the collection was done by cardiac puncture with Venoject needles and tubes containing Ethylene Diamine Tetra - acetic (EDTA) vacutainer. A quantity of blood of at least 3 to 5 ml was taken.

3.3 Haematology Analyzer : The sample is analyzed at the Laboratory of Animal Biology and Cytology (LBCA) Unit of Training and Research (UFR) Nangui Abroagoua University (UNA) (Côte d'Ivoire) with an automaton haematology MINDRAY 2800 BC.

3.4 Studied parameters: The PLC of Haematology contains within itself an erythrogramme, a leucogramme and a thrombogramme to know the (NFS) blood count and platelet count.

3.4.1 Red line parameters measured: The parameters measured by the erythrogramme are hematocrit (HCT), hemoglobin (HB), red blood cell count (RBC) and Red cell

Distribution Width (RDW). Mean corpuscular volume (MCV), the particle concentration mean hemoglobin (MCHC) and globular content mean hemoglobin (significant).

3.4.2 White line parameters measured : The leucogramme counts and gives us the formula of total leukocytes. His study is useful in the diagnosis of inflammation, viral or bacterial infection during or parasitic infestation. It corresponds to the total leukocyte count (WBC), the leukocyte count (percentage of neutrophils (Neu), eosinophils (Eos), basophils (Bas), lymphocytes (Lym) and monocytes (Mon).

3.4.3 Parameters measured platelet lineage: The Thrombogram gives us platelet count (PLT) is the number of platelets per unit of blood volume.

3.5 Statistical Analysis : Different statistical tests were used with the R software to check for any variations between the values of the same age group and between age groups. This is the coefficient of variation (CV) that can analyze the distribution of values in each age group. An analysis of variance (ANOVA) with a factor (age) and Bartlett's test were used to characterize changes in blood parameters grasscutters between different age groups of the threshold $P < 0.05$.

4. RESULTS

Table 1 shows the variability of the values of haematological parameters around the mean in the different age groups and between ages. Thus, the majority (78.6%) of the values of haematological parameters of young grasscutter (1-3 months) are less dispersed and more homogeneous (CV < 15%). Only three of the fourteen parameters: the number of platelets, neutrophils and lymphocytes have very heterogeneous values (CV > 15%). At the level of young adults, saying the same thing is observed in addition to a great dispersion of the values of the rate of lymphocytes and monocytes present (CV>15%). The opposite phenomenon is observed at the level of adults with 71.4% of haematological parameters ranging from heterogeneously (CV>15%). Except, the values of parameters such as mean corpuscular volume, index of distribution of red blood cells, the rate of polynuclear eosinophils and basophils have a low dispersion (CV<15%). Furthermore, it was noted that a marked influence of age on a number of haematological parameters. More than half (64.3%) values of haematological parameters (RBC, HB, HCT, MCV, MCHC, PLT, Neu, Eos, Bas) increase with age grasscutter. In the normal state, in children aged 1 to 3 months grasscutter young, the amount of red blood cells (GR) varies from 4.8 to 6.5 x 1012 per microliter of blood with an average of 5.3 ± 0 6 x 1012 / microl. The analysis reveals a significant mean difference between the amount of erythrocytes (RBC) in young grasscutter and the young adult and adult grasscutter (9.2 ± 1.6 x 1012 / microl). No significant difference is observed at the level of the amounts of the RBC in young adults and adults. The average value of haemoglobin (9, 7±0, 3%) among the young grasscutter is significantly different (P< 0.05) from those of young adults (15, 4±1, 7%) and adults (18, 6±0, 7%). However, no significant different (P >0.05) is found between the grasscutter young adult and adult. Share of the volume occupied by red blood cells in the blood (HCT) this a significant difference (P < 0.05) depending on the age of the grasscutter. The normal physiological state in the grasscutter, it is low among the young is averaging 35, 3±1, 5%, and high in young adults (42, 2±1, 5%) and adults (46, 4±1, 8%). The average size of erythrocytes by the mean corpuscular volume (MCV) is 66, 6±0, 6 fentolitres (fl) at the young grasscutters with values extreme very few scattered (62.5 to 68.7 fl) and significantly different among young adults (54, 1±2, 6 fl) and adults (50, 4±0, 7 fl). The average size of erythrocytes by the mean corpuscular volume (MCV) is 66, 6±0, 6 fentolitres (fl) at the young grasscutters with values extreme very few scattered (62.5 to 68.7 fl) and significantly

different among young adults (54, 1±2, 6 fl) and adults (50, 4±0, 7 fl). Concentration Mean Corpuscular Hemoglobin in or MCHC which represents the amount of hemoglobin in 100 ml of erythrocytes varies with age of the grasscutter. It is significantly ($P < 0.05$) higher in adults (40 ± 2.5) and young adult (36.5 ± 1.7) and low in young (27.5 ± 4.6). The number of platelets or platelets involved in blood coagulation varies greatly with age of grasscutter. It is significantly ($P > 0.05$) lower in young grasscutter 620 ± 46 x 10^6 per litre and higher in young adults and adults, respectively 780 ± 78 x 10^6 / L and 876 ± 52 x 10^6 / L. In the grasscutter, neutrophils are the most numerous of granulocytes. The rate of polymorphonuclear neutrophils, involved in immune reactions presents a significant difference ($P< 0.05$) with a high rate (67.4 ± 15.9%) among adults. Eosinophils and basophils involved in immune responses change with the age of the grasscutter. Their number is significantly lower ($P< 0.05$) among the young grasscutter from young adults and adults. It is 0 to 1% for eosinophils and 0 to 0.5% for basophils youth and 0 to 5%, and 0 to 1% eosinophils and basophils in young adults as in adults respectively. No significant difference ($P>0, 5$) is observed at the level of the MCH, the RDW, the number of WBC, the percentages of lymphocytes and monocytes depending on the age of the grasscutter. The usual values in the male grasscutter in good health range from 17.8 to 22 pg for MCH, 12 to 16.1% for RDW, from 0.7 to 8.6 10^6/L WBC, from 7 to 93.8% for lymphocytes and from 2 to 8% monocytes. It is observed in grasscutter a preponderance of the lymphocyte population compared with other cell types of the white line. Lymphocytes are the most leukocytes (80.6 ± 3.6%) among young people.

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Table 1: Description the haematological parameters of grasscutters depending on the age

	young (1 - 3 months)			young adults **(4 - 7 months)**			**Adults (≥ 8 months)**		
haematological parameters	Mean ± standard deviation	Limits	Coefficient of variation (%)	Mean ± standard deviation	Limits	Coefficient of variation (%)	Mean ± standard deviation	Limits	Coefficient of variation (%)
RBC (10^{12}/µL)	5,3 ± 0,6 **a**	4,8–6,5	7,9	7,8±0,6 **b**	5,5-9	6,4	9,2±1,4 **b**	5,2-10	26
BH (g/dL)	9,7±0,3 **a**	9,5-12,1	6,4	15,4±1,7 **b**	10-18,2	13,2	18,6±0,7 **b**	9,2-22	17,2
HCT (%)	35,3±1,5 **a**	33-40,6	7,3	42,2±1,5 **b**	37,1-46,7	11	46,4±1,8 **b**	36,7-49,4	16,5
MCV (fl)	66,6±0,6 **a**	62,5-68,7	9,4	54,1±2,6 **b**	51,8-67,4	14,7	50,4±0,7 **b**	49,4-70,6	34,6
MCH (pg)	18,3±1 **a**	18,7-19,8	1,4	19,7±0,2 **a**	18,2- 20,2	2,8	20,4±1,9 **a**	17,8-22	4,5
MCHC (g/dl)	27,5±4,6 **a**	23,4-36,7	14,7	36,5±1,7 **b**	37,1-39 b	3,6	40±2,5 **b**	25,1-44,5	27
RDW (%)	15±1 **a**	13,6-16,1	3,9	14,9±0,5 **a**	12-15,5	4	15,6±0,1 **a**	14,2-15,9	2,7
Plaq (10^6/L)	620± 46 **a**	165-670	45,6	780±78 **b**	280-810	44,3	876±52 **c**	170-940	47,5
WBC (10^6/L)	5,3±0,3 **a**	4-6	2,4	6,6±0,2 **a**	1,5-7	27,4	4,1±0,5 **a**	0,7-8,6	17,8
Neu (%)	44±27 **a**	7-78	67	39,6±26,8 **a**	5-84	47,3	67,4±15,9 **b**	10-96	66,2
Eos (%)	0,7±0,2 **a**	0-1	6,7	2,1±1,7 **b**	0-5	9,2	3,3±1,2 **b**	0-5	8,9
Bas (%)	0,3±0,1 **a**	0-0,5	2,3	0,7±0,2 **b**	0-1	5,1	0,6±0,1 **b**	0-1	4,7
Lym (%)	80,6±3,6 **a**	12-87,9	56,1	78,4±8,1 **a**	17-91,6	67,5	84,1±7,5 **a**	7-93,8	62,5
Mon (%)	2,3±0,2 **a**	2-5	7,5	2,8±0,4 **a**	2-7	31,2	3,2±1 **a**	2-8	33,1

Mean values with different letters in the same row are significantly different ($p < 0.05$).
RBC: Red Blood Cells; HB: hemoglobin; HCT: hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red cell Distribution Width; PLT: Platelet count; WBC: White Blood Cell; Neu: Neutrophiles; Eos: Eosinophiles; Bas: Basophiles: Lym: Lymphocytes; Mon: Monocytes.

5. DISCUSSION

In this study, we examined the cytological composition of the blood of male grasscutter belonging to different age groups (from 1 to more than 8 months of age) to establish reference values that could form the basis of comparison between a normal physiological changes and pathological phenomenon. This work is carried out according to a well-established protocol where the experimental conditions were rigorously respected and reproduced. The samples are fairly representative and determination of different parameters is done immediately after sampling using a rigorous and always reproducible method. This set of conditions can be concluded that the results of this study are normal and reproducible. This study results have been compared with earlier work on animals other than the grasscutter. Indeed, the literature on the subject is inadequate or even absent. The results of this study revealed that some parameters vary widely from one individual to another in the same age and of an age to the other class. This internal change in the same age group could be explained by the nature of the grasscutter, because according Mensah (1997), are distinguished among the grasscutter born in captivity two animals (grasscutter docile or stubborn) that agree well in captivity. Grasscutters being domesticated wildlife (Mensah and Ekué, 2003) still in them the instinct to want to escape the man saw. Teixeira (2007) indicates that in nature, animals tend to avoid human contact. In captivity, docility or at least the loss of avoidance behaviour is a constraint to which the animals are forced to adapt. These constraints result a chronic stress. The consequences of this resistance to stress can have according to Boes (2010) an effect on the results of the hemogram. As to the variation of the haematological constants depending on the age of the grasscutter, it is consistent to that obtained by Bose (2003a), Stein and Walshaw (1996) the rabbit; Huerkamp (1996) in the Guinea pig; Strake (1996) in the chinchilla; Kishida (2002) and Vennam (2002) in the octodon; Lipman and Floltz (1996) in the hamster and Fallon (1996) in the domestic rat. The hemogram of grasscutter is characterized by a physiological thrombocytosis often with very high platelet counts ($78.10^6 \pm 780$/L) and oscillates between 280 and 810.10^6/L. The average number of blood platelet is very high compared to the results obtained by Bléyéré (2013) with young adult grasscutter fed a pelleted feed (640 ± 80.10^6 / L). However, extreme values are substantially similar to those of the same author (184 to 884 10^6 / L). This parameter reflects the specificities of a grasscutter blood which coagulates very quickly after collection. Another element of hemogram of the grasscutter is the presence of a physiological macrocytosis observed in the young grasscutter from grasscutter young adult and adult. Mean corpuscular volume is high (66, 6±0, 6 fl) level young people then gradually decreases with age to reach 54, 1±2, 6 fl in young adults and 50, 4±0, 7 fl in adults. These variations of mean corpuscular volume are similar to those obtained by Ogunsanmi *et al.* (2002) among the young grasscutters reared in captivity (66.8±10.47 fl) and 71.90±4.20 fl Opara *et al.*, 2006 and Opara, 2010b. Mean corpuscular volume is a particularly useful indicator in the diagnosis of anaemia. The number of red blood cells and low hemoglobin levels in young grasscutter is comparable to results obtained in most of the authors who have studied haematology rodents (Kaspareit *et al*, 1988; Akingbemi and Aire, 1994. - Boussarie, 1996,1997 and 1999, Al-Eissal, 2011). Furthermore, there is an anaemia (erythrocytopenia) marked at physiological grasscutters youth. Regarding the leukocyte count, eosinophils and basophils are low and sometimes zero. This rate is in no way disturbing, and it causes absolutely no condition or complication. These low rates mean that the blood concentration of these white blood cells often multiply in the presence of parasites and various allergens to neutralize. An increase in these rates is often caused by an allergic reaction or a parasitic infection. An inversion of the Leukocyte formula is observed in the grasscutter to all ages with lymphocytes almost twice more likely than neutrophils. These observations

are consistent with those observed in other rodents and rabbits (Kaspareit *et al.*, 1988; JONGH, 1993; Akingbemi and area, 1994; - Bose, 1999; Al - Eissal, 2011).

6. CONCLUSION

The grasscutter has specific haematological features quite interesting. Furthermore lymphocytosis common to other rodents physiological blood, it is characterized by anaemia (erythrocytopenia) Physiological marked in young grasscutter. It also presents a leukocyte reversed. In qualitative terms, this has enabled us to establish reference values for blood counts of grasscutter bred in captivity.

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