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Comparative Study of the Thyroid Gland of Juvenile Male Guinea Pigs and Grass-cutters: Hematological and Hormonal Profile

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ABSTRACT

This study presents a comparative examination of the hematological parameters, structure, and function of the thyroid gland between two rodent species: guinea pigs (*Cavia porcellus*) and grasscutters (*Thryonomys swinderianus*), which are taxonomically distinct. They are important for research and agriculture, yet little is known about their comparative thyroidal morphology, physiology, and hematological profiles. The study utilized six grasscutters and six guinea pigs with an average weight of 1800±530 g and 210±20 g, respectively. The thyroid gland of the guinea pigs is compact, while the grasscutters are bilobed without an isthmus joining the lobes. The average weight of the guinea pigs' and the grasscutters' thyroid gland weight was found to be 1.69±0.26 g and 2.81±0.37 g, respectively. The relationship between thyroid hormones (triiodothyronine [T3] and thyroxine [T4]) of the guinea pigs and grasscutters was found to be in line with that of most mammals, with the T4 concentration being significantly higher than the T3 concentration. The differences in the Red Blood cell properties of the guinea pigs and grasscutters were found to be significant at $P<0.05$, being significantly higher in grasscutters. The differences in the guinea pigs' and grasscutters' white blood cell indices were not statistically significant at $P<0.05$. This study contributes to a deeper understanding of the comparative endocrinology and hematology of guinea pigs and grasscutters, with implications for their use in physiological, toxicological, and pharmacological studies.

Keywords: grasscutters; guinea pigs; hematological parameters; thyroid gland

INTRODUCTION

Comparative anatomy explores the anatomical similarities as well as differences between various species, thereby understanding evolution and phylogeny across these species[1,2], as it indicates that various organisms share a common ancestor. Moreover, it assists scientists in classifying organisms based on similar characteristics of their anatomical structures. Comparative anatomy supports Darwin's theory of descent with modification, also known as evolution. Virchow's statement is as wise today as it was over a century ago. various animal species, even Homo sapiens, are similar, such that knowledge gained in one species

benefits all, leading to the concept of "One Medicine." Hence, knowledge of the comparative anatomy of different species can help develop certain therapies for humans[3,4]. The one medicine modality leverages various species similarities as few diseases affect exclusively one group of animals. Thus, knowledge of a particular disease in the lower vertebrates, such as guinea pigs, can help manage or treat some diseases in humans.

Blood is considered the fluid of life, growth, and health because it transports respiratory gases (oxygen and carbon dioxide), nutritive substances, hormones, and waste products, as well as protects the body against diseases [5]. The majority of these functions are carried out by the cells, which are the formed elements of the blood; for example, oxygen and carbon dioxide are transported by the hemoglobin found in the red cells, and the white blood cells protect the body against diseases [6]. The thyroid gland, however, is one of the major glands in the body in the sense that it produces the thyroid hormones, which regulate the overall metabolic rate of the body[7]. Adequate knowledge of hematology and thyroid functions in other mammals can help boost veterinary care and advance comparative biomedical research, which in turn can support the One Medicine approach emphasizing shared health principles across species. Moreover, hyperthyroidism has been reported to be common in guinea pigs[8].

This study explored the hematological parameters, thyroid structure, and function in male juvenile guinea pigs and grass cutters to deepen our knowledge of their physiology as well as provide reference for further studies that involve these species.

MATERIALS AND METHODS

Animals

Six male juvenile grasscutters with an average weight of 1800±530 g and six juvenile guinea pigs with an average weight of 210±20 g were obtained in Ganmo (Kwara state) and Araromi (Oyo state), respectively. The grasscutters and guinea pigs were euthanized by the respective administration of 20 mg/kg of ketamine intramuscularly and intraperitoneally. Animal handling was in line with guidelines recommended by the Animals Ethics Committee of the University of Ilorin.

Blood collection for hematological parameters, thyroid, and thyroid-stimulating hormonal assay

The euthanized animals were dissected to assess their hearts. Blood was collected from their right ventricle using a scalp vein needle connected to a 5ml syringe and immediately transferred into EDTA and heparinized bottles for hematological and hormonal analysis, respectively. Blood cells were counted manually using a Neubauer counting chamber. The blood collected into heparinized bottles was centrifuged immediately at 3000 rpm for 15 minutes. The plasma was collected and analyzed for thyroid (T3 and T4) and thyroid stimulating hormonal assay using a vast ELISA microwell kit with the code 8025-300 as described below. Furthermore, the animals were perfused with normal saline and 4% paraformaldehyde. The thyroid gland was removed and post-fixed in paraformaldehyde for gross study.

Analysis of the Hematological Parameters

Evaluation of the red blood cell count and its indices

The amount of red blood cells was determined using a Neubauer counting chamber. 20 µl of the blood collected into the EDTA bottle was diluted with an isotonic fluid (mixture of 100 ml of 1% formalin with 3 g sodium citrate) to prevent blood lysis during analysis. After the dilution, the diluted blood was then used to charge the counting chamber and left for 3–5 minutes for the RBC to settle. The cells were then counted under the light microscope in five groups of 16 small squares in the central ruled area of the chamber; the cells in the upper horizontal line and right vertical lines were also counted [9].

Some RBC absolute indices were further calculated to obtain more information from the RBC, as these indices are markers for RBC's functions; these indices are the mean cell volume (MCV); the average volume or size of individual red cells, mean cell hemoglobin concentration (MCHC); the quantity of hemoglobin in individual cells but in a

given volume of red cells, and mean cell hemoglobin (MCH); the average quantity of hemoglobin in an individual cell. They were calculated as shown below;

MCH(pg) = Hb in g per litre/ RBC per litre.

MCV (fl) = PCV in litres per liter/ RBC per litre.

MCHC (g/dl) = Hb in g per litre/ PCV litre per litre.

Determination of packed cell volume

The packed cell volume (PCV) measures the RBC's percentage, which is a strong indicator of blood function in respiration. Briefly, three-quarters of hematocrit tubes (with one closed end) were filled with anticoagulated blood and centrifuged for five minutes. The PCV was then read on a microhematocrit reader.

Estimation hemoglobin concentration

The hemoglobin estimation was done using Sahli's method. 20 cu. mm of anticoagulated blood was diluted with 10 cu. mm of prepared 0.1N HCl, after which the diluted blood was allowed to sit for 5 mins for the formation of acid hematin. The mixture was then further diluted with drops of distilled water, after which the color was compared with the standard against a white background. Two readings were taken; the first was taken when the diluted blood was slightly darker than the standard, while the second was taken when the diluted blood was lighter than the standard. The average of these two readings was taken as the hemoglobin concentration.

Total and differential white blood cell count

The white blood cells were also counted using the Neubauer counting chamber. 20 µl of blood in anticoagulant was diluted with 0.4 ml of diluting fluid (2% acetic acid with about 2 drops of Gentian violet stain) and allowed to sit for 2-3 minutes for adequate lysis of RBC by the acetic acid in the dilution fluid. All the white cells in the four corners of a 1mm x 1mm large square were counted, and the average was taken. The differential WBC count was done using a blood smear stained with a Leishman stain. The white cells were then counted under a high dry x40 objective lens until about 200 cells were counted, marking each as recognized in the appropriate column in a table from which the percentages of each type of cell present were then calculated.

Evaluation of thyroid stimulating hormone and thyroid hormones

Thyroid hormones and TSH were assayed using VAST ELISA kits, a product of Monobind Inc. Lake Forest, CA 92630 USA (8025-300). The procedure includes the arrangement of the microplate wells for the control, calibrators, and samples. 25 µl of calibrator, samples, and control were dispensed into T4 designated wells, while 50 µl were added to the T3 and TSH wells. 50 µl of T3 or T4 enzyme conjugate solution were then added to the appropriate wells, while 100 µl of TSH enzyme reagent was added to TSH wells. Furthermore, 50 µl of biotinylated T4 or T3 - antibody conjugates were added to the respective wells and allowed to incubate for 60 minutes at room temperature, after which the contents were aspirated and washed using wash buffer. 100 µl of working substrate solution was then added to each well and gently mixed for 15-20 seconds. Finally, absorbance was read at 450 nm using a microplate reader.

Statistical analysis

Charts were plotted as the mean ± standard error of the mean (SEM). Differences between the groups were considered significant if the p-value was less than 0.05, using an unpaired t-test. The statistical analysis was performed using GraphPad Prism version 7.0.

RESULTS

Physical observations

The grasscutters appeared stronger and bigger than the guinea pigs. The grasscutters have scanty furs on their ventral surface compared to their dorsal surface, while the guinea pigs have abundant furs on their' ventral and dorsal surfaces (Figure 1). The

guinea pigs are tailless rodents, while the grass cutters have short and fragile tails. The grass cutters have well-developed muscles when compared to the guinea pigs.

The guinea pigs' thyroid gland is a compact structure located in the anterior part of the neck. The grasscutters' thyroid gland lacks isthmus, each (right and left) occupying its compartment anterior to the trachea. The grasscutters' thyroid glands are located more superficially than guinea pigs, which lie deep in certain neck muscles.

Figure 1: Physical observation of the animals and thyroid gland; the upper left image depicts the abundant fur and the size of the guinea pigs while the upper right image shows the ventral surface of the grasscutter with scanty fur. The lower images (left) show the compact thyroid gland of a guinea pig while the (right) shows the bilobed grasscutter's thyroid gland.

Guinea pigs have a higher thyroid gland weight-body-weight ratio.

To explore the influence of the thyroid-body weight ratio on thyroid hormonal levels and TSH levels on juvenile guinea pigs and grass cutters, the body and thyroid weights were measured, and the thyroid gland weight-to-body weight ratio was assessed. It was observed that grass cutters had significantly higher total body weight and thyroid gland weight (Figure 2(a) and (b). however, juvenile guinea pigs' thyroid gland to body weight ratio was significantly higher (Figure 2c) than that of the grasscutters ($p<0.05$).

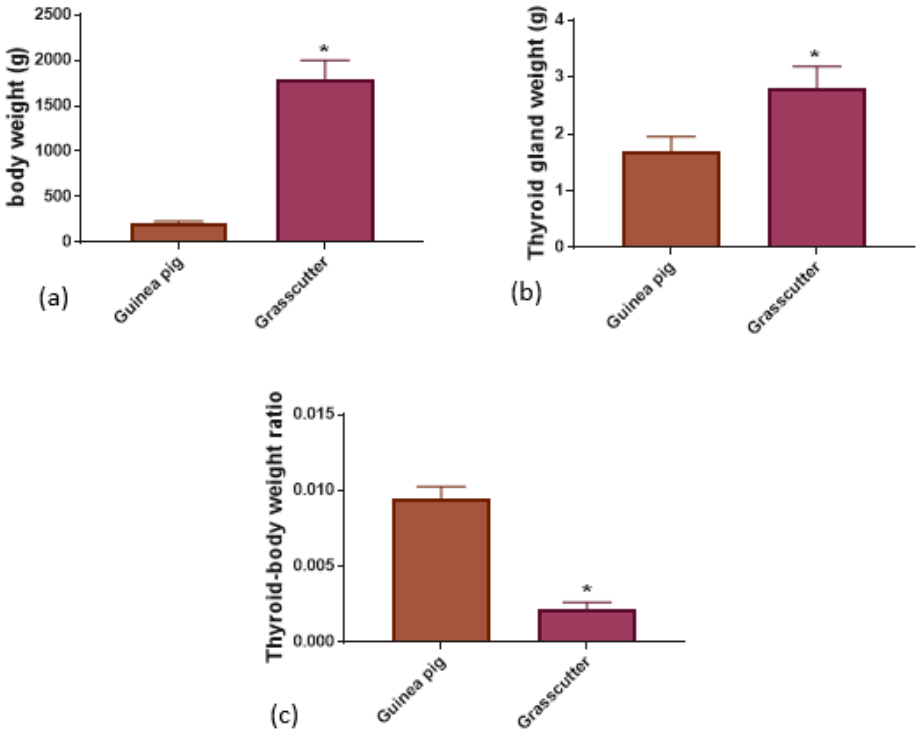

Figure 2: (a) body weight (b) thyroid gland weight (c) thyroid gland-body weight ratio. * indicates a significant difference from guinea pigs at $p<0.05$.

Grass cutters have higher red blood cell indices.

Blood was analyzed for the RBC indices of juvenile guinea pigs and grass cutters to compare the RBC indices. It was observed that grasscutters had significantly higher RBC indices compared to guinea pigs (Figure 3 (a) to (e) except for the mean cell hemoglobin concentration that was higher in guinea pigs (Figure 3 (f))

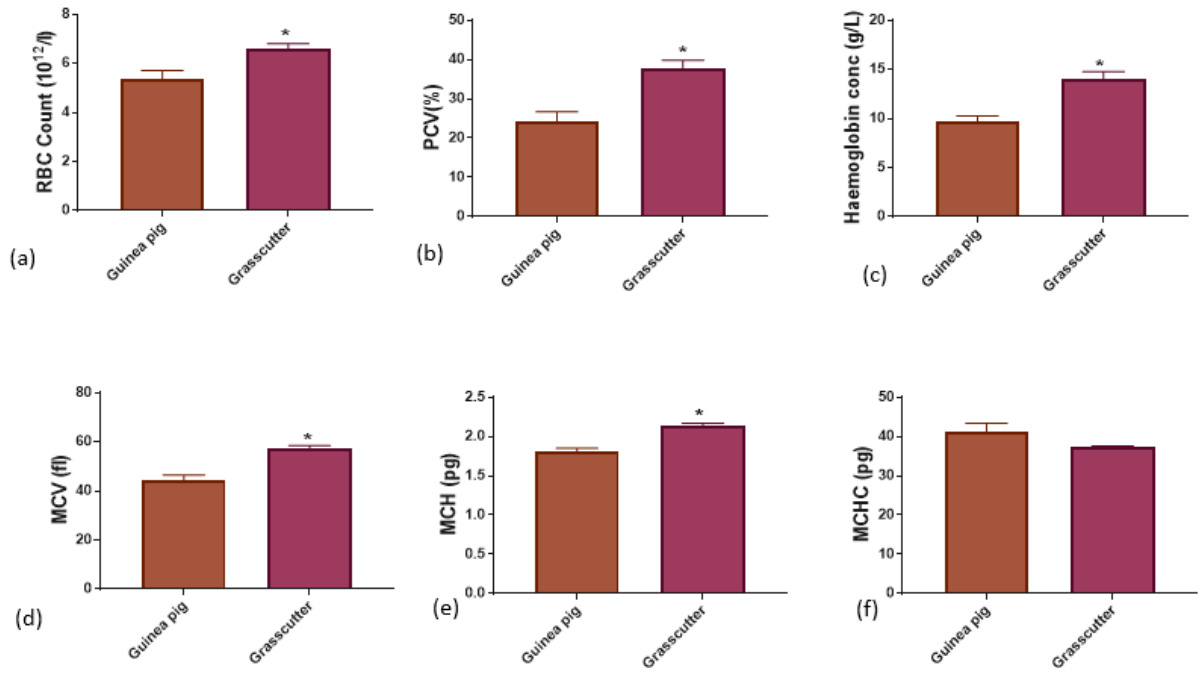

Figure 3: Red blood cell indices of guinea pigs and grasscutters. *indicates a significant difference from guinea pigs at $p<0.05$.

The total white blood cell counts and differential counts of guinea pigs and grass cutters

The WBC indices were not significantly different between the two species of animals (Figure 4 (a) to (d)), though most of them were slightly higher in guinea pigs than in grass cutters.

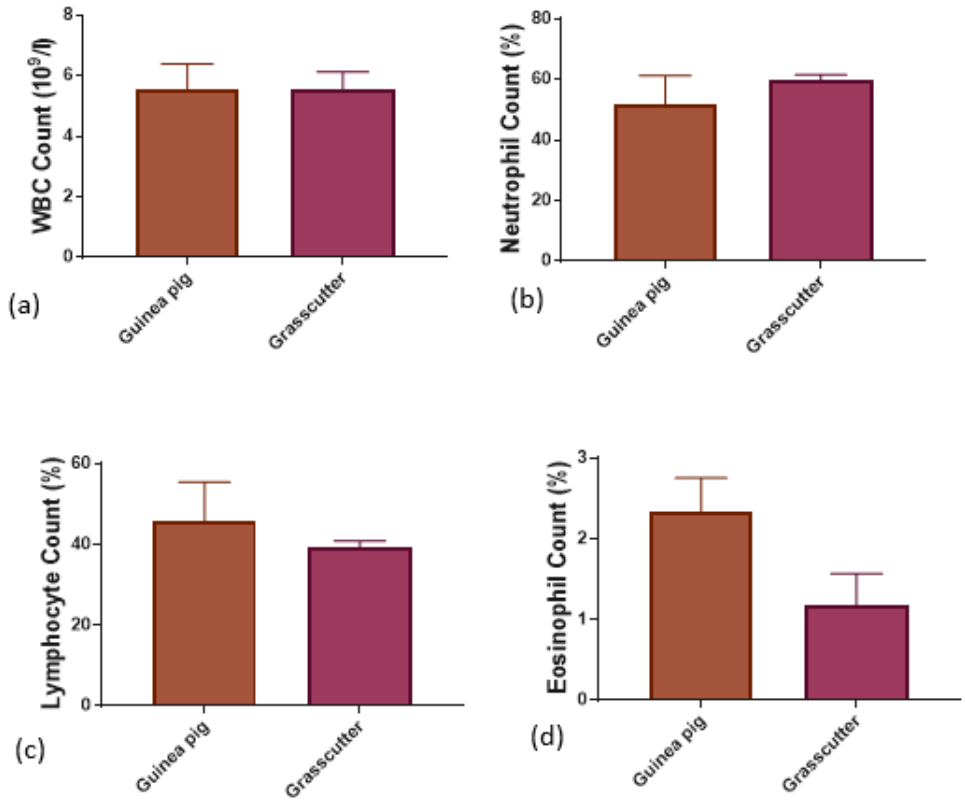

Figure 4: White blood cell indices of guinea pigs and grass cutters. There was no significant difference between the indices of the two species of animals.

The evaluation of thyroid stimulating hormone and thyroid hormones of grasscutters and guinea pigs

The guinea pigs' T3 and T4 concentrations, as well as thyroid-stimulating hormones, appeared to be higher. However, these differences were not statistically significant (Figure 5).

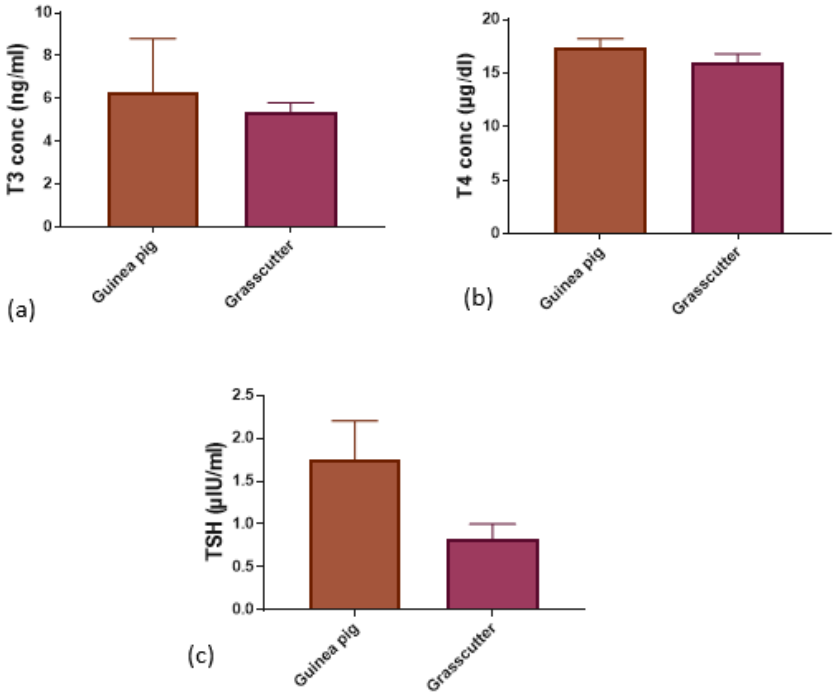

Figure 5: The levels of thyroid hormones and thyroid stimulating hormones.

Histological Observations

The representative photomicrographs of the blood smear of the animals. Their observation supported the findings from the blood cell count, as the blood smear of the guinea pigs had fewer red blood cells and abnormally shaped red blood cells (Figure 6) when compared to that of the grass cutters.

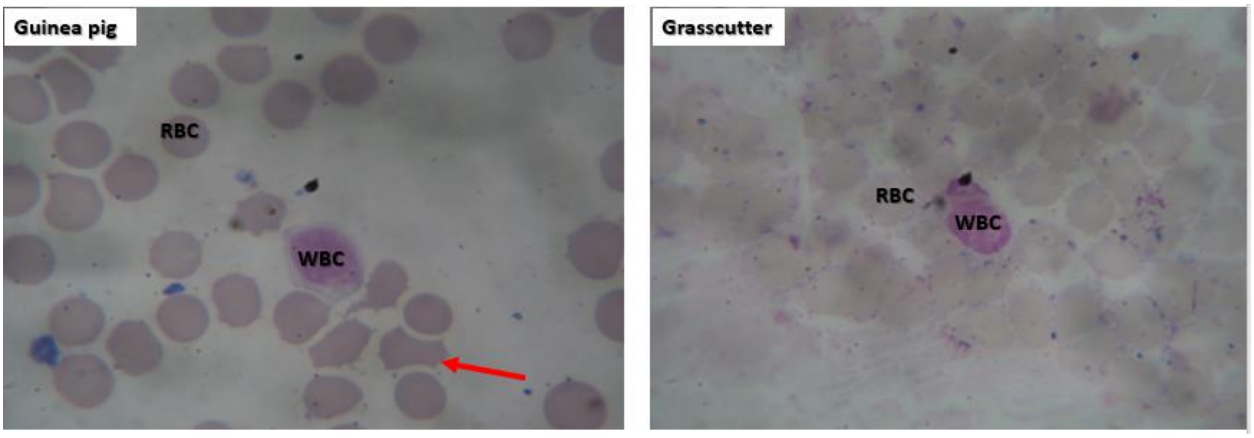

Figure 6: Representative photomicrographs of the blood smears of guinea pigs and grass cutters. ×400. Leishman stain. RBC: Red blood cells, WBC: White Blood Cells (WC), red arrow points to abnormally shaped RBC

DISCUSSION

The knowledge of the comparative anatomy of other mammals has deepened our understanding of evolution and improved the care and the use of these animals as pets, livestock, and human disease models. This study supports that grasscutters are mammals with well-built muscles as opposed to guinea pigs, which are tailless and, of course, have significantly lower body weight when compared to grasscutters. However, the ratio of thyroid weight to body weight was higher in guinea pigs compared to grass cutters.

The use of blood examination as a way of assessing the health status of an individual has been documented [10]. This is because it is vital for the physiological, nutritional, and pathological status of organisms [11]. They range from giving the level of the blood to detecting ailments or disorders through them. Hematological profiles, both in humans and in animal sciences, are an important index of the physiological state of the individual. The ability to interpret the state of blood profile in normal and diseased conditions is among its primary tasks. Many researchers have seen that there is a definite change in the profile of the blood cells throughout life [12]. Not only does the blood change with the advancement of age, but it also varies with certain conditions such as stress, bacterial infection, viral infection, and intoxication [13].

One of the important components of the blood is its formed element, i.e., the blood cells. The differences in the means of the RBC properties (PCV, Hemoglobin estimation, RBC count, and red cell indices) of guinea pigs and grasscutters were found to be statistically significant ($p<0.05$) excluding the MCHC. In summary, RBC properties are considerably higher in grasscutters compared to guinea pigs; this can be ascribed to the fact that grasscutters are more active; hence, their well-built muscles require an adequate supply of oxygen compared to guinea pigs, which are less active. This study supports the fact that PCV has lower RBC indices compared to other mammals [14] emphasizing their suitability for anemia research and adding to their suitability for scurvy research. The differences in the means of the WBC properties (total WBC count, neutrophils count, lymphocyte count, etc.) of guinea pigs and grasscutters demonstrate no statistical significance. However, the lymphocyte and eosinophil counts appeared to be slightly higher in guinea pigs. From this observation, it is revealed that guinea pigs have more capability to fight against parasites than grass cutters since eosinophils have a unique defense mechanism against parasites. As reported by Richardson, guinea pigs are often infected by parasites such as lice and mites, hence there is a need for adequate intrinsic defense against parasites which might account for the reason why there is a considered high eosinophilic count in guinea pigs compared to grasscutters[15].

In addition to the quantitative hematological parameters, the shape of the blood cells is also vital for their function, which can be deduced from blood smears. Evidence from the blood smears of the guinea pigs and grasscutters indicate that the guinea pigs have scanty RBCs when compared to that of grasscutters, supporting the RBC indices, particularly the PCV. This finding is in line with previous findings [16]. Additionally, certain irregularly shaped RBCs are seen in the guinea pigs' blood smear in contrast to those of the grasscutters which appeared uniform [16]. The higher lymphocyte count in guinea pigs can be linked to their mode of life in the sense that guinea pigs are timid, easily handled, and rarely bite and are therefore prone to both physical hazards and viral infections[17]. Hence, the high lymphocytic count serves as a defense mechanism against viral infection [14].

The thyroid glands of the two animals are located in the anterior part of the neck. That of the guinea pig has a compact structure, while the grass cutters lack isthmus, unlike most other mammals, such as humans, which are joined by an isthmus. Hence, each thyroid gland of a grasscutter (right and left) occupies its compartment lateral to the trachea. This is in line with the findings of Igbokwe [18]. The thyroid gland of the grasscutter is more superficial in contrast to that of the guinea pig, which is located deep in the anterior neck muscles.

The thyroid gland is the body's largest endocrine gland. It produces thyroid hormone, which controls

the rate of metabolism, and calcitonin, a hormone controlling calcium metabolism [17]. An optimal thyroid hormone level is, therefore, important for the overall health of an animal. The difference in means concentration of the thyroid hormones and thyroid stimulating hormone of guinea pigs and grasscutters are not statistically significant. However, these hormones were higher in the guinea pigs despite the difference in their mean thyroid gland weights and their total body weight. Hence, it can be deduced that more thyroid hormones are required and secreted by the thyroid gland of the guinea pigs. This might be linked to the fact that guinea pigs feed on their feces[17]. They produce special soft pellets from their feces called cecotropes, which recycle B vitamins, fiber, and bacteria required for proper digestion; in essence, more thyroid hormones are required to metabolize their feces for proper digestion. Additionally, the fact that guinea pigs are prone to hyperthyroidism[19] also supports our results as the thyroid function of less active guinea pigs is similar to that of active grass cutters. Lastly, from this study, the concentration of thyroxine was found to be more than that of tri-iodothyronine, which is also in line with that found in humans, where thyroxine forms about 90% of the total thyroid secretion[20].

This study has been able to demonstrate the relationship between certain hematological parameters, structure, and function of the thyroid glands of these two species. These two animals belong to a class of mammals and show certain features that are common to the mammalian class; this is evident in some of their features, such as the proportion of their thyroid hormones, where thyroxin concentration is considerably higher than that of tri-iodothyronine. This study also reveals the similarities between the hematological parameters of guinea pigs and grasscutters. Correction: "Hence, this study hopes to serve as a guide for further biomedical research studies involving guinea pigs and grasscutters."

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