GROSS AND HISTOLOGICAL STUDIES ON SOME LYMPHOID ORGANS OF THE GRASSCUTTER (*Thyronomysswinderianus)*

BY

Rachel Mngu-suur KORZERZER, DVM (UniMaid, 2009)
(MSc/VET-MED/14746/2010-2011)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTSFOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY ANATOMY

DEPARTMENT OF VETERINARY ANATOMY,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

JUNE, 2014

DECLARATION

I hereby declare that the work in this thesis, titled "GROSS AND HISTOLOGICAL STUDIES ON SOME LYMPHOID ORGANS OF THE GRASSCUTTER *(Thyronomysswinderianus)"* was carried out by me in the Department of Veterinary Anatomy under the supervision of Professors J. O. Hambolu, S. O. Salami and S. B. Oladele.

The information derived from various literatures has been duly acknowledged in the text and the list of references provided. No part of this thesis has previously been presented for another degree or diploma in any University.

....

Name of Student Signature Date

CERTIFICATION

This thesis, titled "GROSS AND HISTOLOGICAL STUDIES ON SOME LYMPHOID ORGANS OF THE GRASSCUTTER *(Thyronomysswinderianus)*" by Rachel Mngu-suur KORZERZER meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. J. O. Hambolu Chairman, Supervisory Committee	..... Signature	..... Date
Prof. S. O. Salami Member, Supervisory Committee,	..... Signature	..... Date
Prof. S. B. Oladele Member, Supervisory Committee,	..... Signature	..... Date
Prof. J. O. Hambolu The Head, Department of Veterinary Anatomy, Ahmadu Bello University, Zaria	..... Signature	..... Date
Prof. A. A. Joshua Dean, School of Postgraduate Studies, Ahmadu Bello University, Zaria	..... Signature	..... Date

DEDICATION

This work is dedicated to my lovely son, Emmanuel Ordedoo Aondokator Ukpo, and all those who wish to have an insight into the wonders of God's creation.

ACKNOWLEGEMENTS

I give all glory to the almighty God who has given me life, good health and the inspiration to do this work. The road seemed rough from the beginning,but by His grace I found succour. May His name alone be praised forever!

Worthy of mention are my supervisors, who were justa wonderful team to work with.

Prof. J. O. Hambolu,made time out of his tight schedules to ensure the success of this work.Thanks a million for the Amscope camera, the histology textbooks and other items you provided.Prof. S. O. Salami was always there to assist any time I called and Prof. S. B. Oladelewas diligent in supervising this work. Iam also thankful to my elders in the profession; Prof. D. V. Uzawho gave me every needededencouragement and support, Prof. P. A. Onyeyili, Former Dean, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Professors J. O. Ayo, I. U. Ate and C. Kudi who have encouraged me in diverse ways.

I would like to acknowledge Mr. D. I.Bogoro, Pastor Z. Jangber and all the administrative staff of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria for the constant assistance rendered to me.I would also like to thank all thehistology laboratory technologistsand all the staff of the Gross Anatomy Laboratory, Ahmadu Bello University, Zariafor the assistancethey gave me when I was doing the laboratory work.

I am thankful toDr. T. Dzenda for assisting me in the statistical analysis, Dr. O. Obadiahand Dr. U. E. Umanafor the time they spent to help me in preparing the photomicrographs.

All staff and postgraduate students of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and all staff of the Department of Veterinary Anatomy, Federal University of Agriculture, Makurdi are also acknowledged with thanks for their various contributions.

Thanks to my darling husband, Moses LubemUkpo (Esq) and our dear son,Emmanuel A. Ukpofor standing by me through all the challenging moments. Without your love, cooperation and understanding, this work would not see the limelight. I also acknowledge my wonderful parents, Hon. and Mrs. David Korzerzer Gborigo Achaku(JP). I appreciate you for laying the solid foundation. I appreciate my siblings, inlaws and other family members for their constant prayers and support. You folks have contributed in diverse ways to the success of this work. This work has assistance from the Tertiary Education Trust Fund (TETFUND). Thanks to the management of University of Agriculture, Makurdi.

ABSTRACT

The present study analyses the gross and histological features of some lymphoid organs in the grasscutter. Twelve, apparently, healthy grasscutters,between the ages of three and seven months were obtained from Benue State and used for this study. The animals were transported in cages to the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria where they were rested for 14 days before been sacrificed using chloroform inhalation anaesthesia. After dissection, the thymus, mesenteric lymph node and spleen were exposed, separated from surrounding tissue and photographed for gross assessment. Tissue samples were routinely prepared for histology using H & E as general histological stain and Rhodamine B-aniline methylene blue as special stain. Grossly, the thymus was bilateral on either side of the thoracic midline. Mean ± SEM values for thymic weights were 1.23 ± 0.048g, 0.53 ± 0.019g and 0.30 ± 0.042g at three, five and seven months of age, respectively. Histologically, the thymus showed loss of lymphoid cells as the grasscutters advanced in age.The spleen was dark red in colour, ovoid in shape and the size increased with age, having maximum value at seven months of age in both males and females. Mean ± SEM values for splenic weights were 0.67 ± 0.09g, 1.65 ± 0.35g and 2.31 ± 0.06g at three, five and seven months of age, respectively. The percentage ratio of splenic weight to body weight was 0.1%. The mesenteric lymph nodes were constantly bean shaped and appeared as opaque white masses which resembled fat, but were distinguished from fat by their consistent shape. The mesenteric lymph node weights were 0.056 ± 0.005g, 0.143 ± 0.034g and 0.1600 ± 0.023g at three, five and seven months of age, respectively.In conclusion, this study revealed that while the general morphologies of the thymus, spleen and mesenteric lymph nodes in the grasscutter are similar to that in other mammals, the relative amount of white to red pulp in the spleen showed some specie-specific characteristics, the thymus also showed a

constant decrease in weight and size with advancing age, and at seven months there was no Hassal's corpuscle and cortex-medulla distinction.

TABLE OF CONTENT

Title page.....i

Declaration.....ii

Certification.....iii

Dedication.....iv

Acknowledgements.....v

Abstract.....vii

Table of Contents.....viii

List of Figures.....xi

List of Tables.....xii

List of Plates.....xiii

List of Abbreviations.....xvi

CHAPTER 1 : INTRODUCTION

1.1 Background of the Study.....1

1.2 Statement of Research Problem.....3

1.3 Justification of the Research.....4

1.4 General Aim of the Study.....5

1.5 Specific Objectives of the Study.....5

1.6 Research Hypothesis.....5

CHAPTER2 : LITERATURE REVIEW

2.1 Background Knowledge of the Grasscutter.....6

2.1.1The grasscutter and its uses.....7

2.1.2 General Anatomy of the Lymphoid Organs.....8

2.1.3The Bone Marrow.....9

2.1.3.1 Gross Anatomy of the Bone Marrow.....9

2.1.3.2 Histology of the Bone Marrow.....9

2.1.4The Thymus.....10

2.1.4.1 Gross Anatomy of the Thymus.....11

2.1.4.2 Histology of the Thymus.....11

2.1.5The Spleen.....11

2.1.5.1 Gross Anatomy of the Spleen.....12

2.1.5.2 Histology of the Spleen.....12

2.1.6The Lymph Nodes.....14

2.1.6.1 Gross Anatomy of the Lymph Nodes of Mammals.....15

2.1.6.2 Histology of the Lymph Nodes of Mammals.....15

2.1.7The Mesenteric Lymph Node of Rodents and Immunity.....16

2.1.8Peyer's Patches of Mammals.....17

2.1.9 Mucosal-Associated Lymphoid Tissue.....18

2.1.9.1 Gut-Associated Lymphoid Tissue.....18

2.1.9.2 Bronchus-Associated Lymphoid Tissue.....18

2.1.9.3 Nasal cavity-Associated Lymphoid Tissue.....19

2.1.9.4 Conjuctival-Associated Lymphoid Tissue.....19

2.1.9.5 Vascular-Associated Lymphoid Tissue.....19

2.2Immunity in Mammals.....20

2.2.1Cells of the Immune Response in Mammals.....21

2.2.1.1 Natural Killer Cells.....21

2.2.1.2 Cytokines.....21

2.2.1.3 B Lymphocytes.....22

2.2.1.4 T Lymphocytes.....23

2.2.2 Interaction of Lymphocytes and Macrophages.....23

CHAPTER 3: MATERIALS AND METHODS

3.1 Animal Source.....25

3.2 Morphologic and Morphometric Studies.....26

3.3 Histological Studies.....26

3.4 Statistical Analysis.....27

CHAPTER 4: RESULTS

4.1 The Thymus.....28

4.1.1 Gross Observations of the Thymus.....28

4.1.2 Histological Observations of the Thymus.....28

4.2.1 Gross Observations of the Mesenteric Lymph Node.....44

4.2.2 Histological Observations of the Mesenteric Lymph Node.....44

4.3.1 Gross Observations of the Spleen of the Grasscutter.....52

4.3.2 Histological Observations of the Spleen of the Grasscutter.....52

CHAPTER5 : DISCUSSION.....66

CHAPTER 6 : CONCLUSION AND RECOMMENDATION.....72

6.1 Conclusion.....72

6.2 Recommendations.....73

REFERENCES.....74

APPENDICES.....83

LIST OF FIGURES

Figure **Page**

Figure 1: Relationship between body weight (g) and thymic weight (g).....64

Figure 2: Relationship between body weight (g) and splenic weight (g).....65

Figure 3: Relationship between body weight (g) and mesenteric lymph node weight (g).....66

LIST OF TABLES

Table **Page**

Table 1. Effect of age on body weight and weight and circumference of thethymus, spleen and mesenteric lymph node of the grasscutter (Mean ± SEM).....61

Table 2. Effect of sex on body weight and weight and circumference of the thymus, spleen and mesenteric lymph node of the grasscutter (Mean ± SEM).....62

Table 3. Correlation coefficients between age and some morphometric parameters of the grasscutter (n=12).....63.

LIST OF PLATES

Plate **Page**

Plate I.The grasscutter.....2

Plate IIGross presentation of thymus of a 3-months-old grasscutter.....31

Plate III Gross presentation of thymus of a 5-month-old grasscutter.....32

Plate IV Gross presentation of thymus of a 7-month-oldgrasscutter.....33

Plate V Photomicrograph of the thymus of a 3-month-old male grasscutter.....34

Plate VI Photomicrograph of the thymus of a 3-month-old female grasscutter.....35

PlateVII Photomicrograph of the thymic cortex of a 3-month-old grasscutter.....36

Plate VIIIPhotomicrograph of the thymus of a 3-month-oldmale grasscutter.....37

Plate IX Photomicrograph of the thymus of a 3-month-old male grasscutter.....38

Plate X Photomicrograph of the thymus of a 5-month-old female grasscutter.....39

Plate XI Photomicrograph of the thymic cortex of a 7-month-old femalegrasscutter.....40

Plate XII Photomicrograph of the thymus of a 7-month-old male grasscutter.....41

Plate XIII Photomicrograph of the thymus of a 7-month-old female grasscutter.....42

PlateXIVPhotomicrograph of thethymus of a 7-month-old male grasscutter.....43

Plate XV Photomicrograph of thethymus of a 7-month-old female grasscutter.....44

PlateXVI Photograph of the mesenteric lymph node of a grasscutter.....46

Plate XVII Photomicrograph of a 3-month-old male grasscutter.....47

Plate XVIII Photomicrograph of mesenteric lymph node of 3-month-old female grasscutter....48

Plate XIX Photomicrograph of mesenteric lymph node of a 5-month-old male grasscutter....49

Plate XX Photomicrograph ofmesenteric lymph node of a 7-month-old female grasscutter.....50

Plate XXI Photomicrograph ofmesenteric lymph node of a 7-month-old male grasscutter.....51

Plate XXII Photomicrographof mesenteric lymph node of a 7-month-old female grasscutter.....52

Plate XXIII Photograph of the spleen of grasscutter.....54

Plate XXIVPhotomicrograph of the spleen of a 3-month-old femalegrasscutter.....55

Plate XXVPhotomicrograph of the spleen of 3-month-old male grasscutter.....56

Plate XXVI Photomicrograph of the spleen of a 3-month-old female grasscutter.....57

Plate XXVIIIPhotomicrograph of the spleen of a 5-month-old female grasscutter.....58

Plate XXVIII Photomicrograph of the spleen of a 5-month-old malegrasscutter.....59

Plate XXIX Photomicrograph of the spleen of a 5-month-old female grasscutter.....60

LIST OF ABBREVIATIONS

Ag = Antigen

Ab= Antibody

Cm =Centimeter

µ= Micron

µm = Micrometer

□= Less than

≤ = Less than or equal to

IgG = Immunoglobulin G

IgM = Immunoglobulin M

IgA =Immunoglobulin A

PP = Peyer's Patches

H &E =Haematoxilin and Eosin

SEM = Standard Error of the Mean

g = gram

kg = kilogram

NRC= National Research Council

% = Per cent

^{0} = Degree

USA = United States of America

Fig.= Figure

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The grasscutter (*Thryonomys swinderianus)* is a wild herbivorous rodent, regarded by some people as a large version of the rat (Asibey and Addo, 2000). It is found mostly in West Africa (Ntiamoa-Badu, 1998), where it has been hunted aggressively for its meat (Yeboah and Adamu, 1995). The grasscutter meat has a high protein and mineral content with low fat content (Asibey, 1974), and is one of the most popular game reserves in West Africa, whose meat has contributed to the economy of most West African countries (NRC, 1991; Ntiamoa-Baidu, 1998).

Grass is known to provide the main habitat and food for the rat in Africa (Adu *et al*., 2000). In South Africa, it is known to be associated with cane fields and goes by the name 'cane rat' (NRC, 1991). The grasscutter has been documented as the second largest African rodent after the porcupine (Eben, 2004). It is well-adapted to small scale farming with lower economic risk and quick return. The rodent, *Thryonomys swinderianus* is therefore suitable for agricultural extension programme in rural areas that are well connected to urban areas (Jori and Cooper, 2001). The grasscutter skin is covered with spines instead of hairs, consequently, the veins are not superficially detectable and so not clinically relevant for blood aspiration or infusion therapy (Baptist and Mensah, 1986).

An important area of research that would contribute greatly to the success of grasscutter farming in Nigeria is the study of diseases and the health condition of these animals while in captivity.



Plate I. The grasscutter. Note the spines that cover the skin. Mag. X40.

Few reports actually exist concerning disease and health of grasscutters in captivity (Adu *et al*., 2000; Jori *et al*., 2001; Jori and Cooper, 2001). There is an evidence of the role of this rodent as a vector of diseases such as leptospirosis (Baylet and Van Riel, 1971), hepatic capillariasis (Malekani *et al*., 1994) and salmonellosis (Oboegbulem and Orowonko, 1990). Although it seems that these diseases do not pose a serious risk to the consumers of the meat of this rodent, they represent a potential risk to persons handling their carcasses (Jori and Cooper, 2001).

Generally, few reports exist on the anatomy of wild rodents undergoing domestication (Dijkstra *et al.,* 1990; Ibe *et al*., 2010). However, little or no information exist on the lymphoid organs of the grasscutter. With the current level of its domestication and captive rearing, coupled with the possible emergence of diseases in captivity, information on the lymphoid organs in the grasscutter has become imperative.

1.2 Statement of Research Problem

Domestication of the grasscutter is yet to be established perfectly (Addo, 1997). With the drive towards economic returns from grasscutter farming, there is need to intensify production practices. It is a well-known fact that associated with domestication of wild life species are the burden and stress that go to compromise their health status (Van Veen *et al*., 1974).

Stress has been reported to bring about changes in immune function, leading to a higher susceptibility to disease (Cowan and Curry, 2002). Among the health challenges to grasscutter farming are sudden deaths, worm infestation, haemoparasites and respiratory diseases. It has also been reported that grasscutter harbour a host of parasites, including *Trypanosoma, Babesia and Plasmodium* species in their blood without showing clinical signs (Opara and Fagbemi, 2010).

Information about the lymphoid system of the grasscutter is lacking, and this makes its exploitation difficult (Adamu *et al*., 1993; Yeboah and Adamu, 1995). Literature search on the lymphoid system of the grasscutter revealed no information. This study was undertaken to provide the base line information on both the gross anatomy and histology of the lymphoid organs such as the thymus, mesenteric lymph node and spleen of the grasscutter in order to understand and predict its dynamics in normal and disease situations.

1.3 Justification of the Research

The understanding of the anatomical structures of the lymphoid organs of the grasscutter will further give insights into functions of these organs and may also help in the diagnosis of diseases in the animal.

Grasscutter meat is a favourite one and accounts for the greater proportion of bush meat sold in most West African countries (NRC, 1991). The high dependence of consumers on this single specie provides a justification for the promotion of the grasscutter domestication programme. Domestication of this rodent can provide sound solutions to the needs of the people through income generation, bush meat production, and conservation of forests (Eben, 2004).

Grasscutter domestication can also help to combat poverty by creating more revenues for local people. It is clear that any success in combating poverty through such a concept would help discourage local people from becoming involved in poaching activities (Asibey and Addo, 2000).

1.4 General Aim of the Study

The aim of this study was to conduct gross and histological studies on the thymus, spleen and mesenteric lymph node of the grasscutter.

1.5 Specific Objectives of the Study

The objectives were to:

i. Evaluate the gross and histological architecture of the thymus, mesenteric lymph node and spleen of the grasscutters.

ii. Assess the lymphoid cells of these organs.

iii. Compare the findings above between the age groups and with related rodents.

1.6 Research Hypotheses

H_0: The gross anatomy and histology of thymus, spleen and mesenteric lymph node of the grasscutter is similar to other rodents

H_1: The gross anatomy and histology of thymus, spleen and mesenteric lymph node of grasscutter is not similar to other rodents

H_0: There are no age related change in the thymus, lymph node and spleen of the grasscutter.

H_2; There are age related changes in the thymus, lymph node and spleen of the grasscutter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Knowledge of the Grasscutter

The grasscutter has two species (*Thryonomys swinderianus* and *T. gregorianus*), which are both found only in Africa (Baptist and Mensah, 1986). The two species possess varied colours, which are thought to be influenced by ecological habitat and can be distinguished primarily by their size, *Thryonomys swinderianus* is larger in size than *Thryonomys gregorianus* (Baptist and Mensah, 1986), with an average body weight of 4.5 kg at 1 year old and 9.5 kg attainable subsequently, while the maximum weight attainable by *T. gregorianus* is 2.5 kg (Rosevear, 1969).

The grasscutter *(Thryonomys swinderianus)* is variously known as the marsh cane-rat, ground hog, and in francophone West Africa as the aulacode. In Nigeria, it is known as *Oya* among the Yorubas, *Mchi* among the Ibos and *Iviha* among the Tivs. It's origin has been traced down to the Savannah ecological zone (Pich and Peters, 1990). In West Africa where grass provides its main habitat and food, it is commonly known as the grasscutter or the cutting grass. In other parts of Africa particularly in Southern Africa, where it is closely associated with cane fields, it is called the 'cane rat'. The grasscutter is also found in grasslands and wooded Savannah throughout the humid and sub-humid areas, south of the Sahara (NRC, 1991), specifically from Senegal to parts of the Cape Province in South Africa (Rosevear, 1969). The cane rat can also be found anywhere there are dense reedy grasses growing in damp or wet places (Ajayi, 1971; Abioye *et al*., 2008).

They do not inhabit the rain forest, dry scrub or desert regions (NRC, 1991). Its distribution is determined principally by the availability of adequate or preferred grasses for food (NRC, 1991). *Thryonomys swinderianus* is a promising proposition for domestication, especially for the purpose of animal protein supply in Africa. In Nigeria, it has been noted that the depletion of wild game by unrestricted hunting is constituting a constraint to the sustainability of the wild source and so, its domestication and captive rearing should be encouraged (Abioye *et al*., 2008).

2.1.1 The grasscutter and its uses

The grasscutter is an important source of animal protein and is regarded as the most preferred bushmeat in West Africa (Ntiamoa-Baidu, 1998). Unlike some animals which may not be killed or touched because of traditional taboos and religious dictates, the grasscutter *(Thryonomys swinderianus)* transcends both traditional and religious prohibitions. For example, muslims who do not consume certain animals consume the grasscutter.

Grasscutter contributes to both the local and export earnings of Ghana (Asibey, 1974; Baptist and Mensah, 1986; NRC, 1991; Ntiamo-Baidu, 1998). At the local market level, for example, approximately 73 tons of grasscutter meat, representing more than 15,000 animals, can be sold in a year (NRC, 1991). A survey by Ntiamo-Baidu (1998) reported that the grasscutter dominates the bush meat trade. The rodent, therefore, has great potentials for alleviating poverty in countries where its farming is practised (Asibey and Addo, 2000).

2.1.2 General anatomy of the lymphoid organs

The lymphoid organs of mammals include: the thymus, spleen, lymph nodes (which could be best described as structured masses of lympho-reticular tissue, occurring at intervals along the lymphatic vessels), tonsils and appendix; the diffuse and organised masses of lymphocytes associated with mucosal surfaces, bone marrow; the diffuse and organised masses of lymphocytes associated with connective tissue (Banks, 1982), mucosa-associated lymphoid tissue.

Lymphoid tissues are those tissues whose major functions are associated with adaptive immune responses. They consist of dense accumulations of lymphocytes and are widely distributed in the body of an animal (Junqueira *et al*.,1998). The central lymphoid organs, also known as primary lymphoid organs which are responsible for synthesis and maturation of immunocompetent cells (lymphocytes). The peripheral organs also called secondary lymphoid organs, are the sites where these immunocompetent lymphocytes are exposed to antigen. It is the site where lymphocytes localise, recognise foreign antigen and mount response against it. These organs include the spleen, lymph nodes, tonsils and Peyer's patches (Gulmez and Alsan, 1999). At the histological level, lymphoid organs form delicate framework of reticular fibres, while the cytology reveals an easily recognisable nuclei and weakly eosinophilic cytoplasm (Junqueira *et al*., 1998).

The lymphoid system has been studied in many terrestrial species of animals. In ruminants, the system is fully developed at birth, while in rodents, full development of the lymphoid organs requires exposure to environmental antigens (Burkitt *et al*., 1993). Some lymphoid organs like

the spleen appear to be active throughout life, while others such as the thymus involute relatively early in life (Burkitt *et al*., 1993).

2.1.3 The bone marrow

The bone marrow is a primary lymphoid organ and is the site where all the cells of the immune system are produced through a process known as haematopoiesis. It is responsible for the production of lymphocytes, granulocytes, monocytes and immature thymocytes as well as red blood cells and platelets (Hoffman *et al*. , 2000).

2.1.3.1 Gross anatomy of the bone marrow

The bone marrow is found within the central cavities of axial and long bones (Picker and Siegelman, 1999) as a soft, gelatinous tissue that is either red or yellow in colour, depending on the preponderance of haematopoietic or fatty tissue, respectively. In adult rats, it accounts for approximately 3 % of the body weight (Schermer, 1967), approximately 2 % in adult dogs, (Jain, 1986), in adult humans about 5 % (Picker and Siegelman, 1999).

2.1.3.2 Histology of the bone marrow

The bone marrow consists of haematopoietic tissue islands and adipose tissue cells surrounded by vascular sinuses, interspersed within a meshwork of trabeculae bone (Wickramasinghe, 1992; Abboud and Lichtman, 2001).

Bone marrow innervations occur with myelinated and non-myelinated nerves that enter through the nutrient canals. Some innervations also occur through epipheseal and metaphyseal foramina. The haematopoietic tissue is composed of a variety of cell types including the blood cells and

their precursors, adventitia cells, adipocytes and macrophages (Wickramasinghe, 1992). The haematopoietic tissue cells are not randomly arranged but demonstrate a particular organisation within the tissue. Lymphopoiesis occurs within the bone marrow microenvironment of adult mammals. B-lineage cells derived from the bone marrow can be identified by sequential changes in cell size and expression of immunoglobulin chains (Abboud and Lichtman, 2001).

Large pre-B cells are early precursors and contain heavy chains within the cytoplasm. Morphologically, the rat or mouse bone marrow do not have, nor develop lymphoid cell aggregates or structures resembling follicles, even after immunization (Abboud and Lichtman, 2001). It is generally considered that, in the dog, normal bone marrow contains about 50 % fat and 50 % haematopoietic tissue, in rats and mice about 70-80 % of the bone marrow being haematopoietic tissue (Valli et al., 2002).

2.1.4 The thymus

The thymus is considered a primary lymphoid organ due to its role as a major site for the development of T cells, from where they are dispersed throughout the body to perform immune functions. While lymphocytes are produced through out life, the mammalian thymus usually undergoes atrophy and involutes at about the time of sexual maturity. At involution, connective and adipose tissue invades and replaces the thymic parenchyma resulting in reduction of its size (Dijkstra and Sminia, 1990). Dijkstra and Smnia (1990) has reported that captivity increases the severity of thymus involution in wild animals.

2.1.4.1 Gross anatomy of the thymus

In man, the thymus is located in the anterior mediatinum and is composed of two lobules joined along the thoracic midline by a connective tissue. It has various levels of extension of one or both lobes into the cervical region in rats (Haley, 2003), and in the guinea pig, it is found in the cervical region (Dijkstra and Sminia, 1990).

In the dog, the thymus is pale yellowish-gray in colour and situated between the second and sixth intercostal spaces in the pericardial mediastinum on the sternum (Yalcin and Tipirdamaz, 2002).

2.1.4.2 Histology of the thymus

The thymus is surrounded by a thin connective tissue capsule, which sends trabeculae into the thymic parenchyma forming incomplete lobules (Pearse, 2006). Each lobule consists of an outer cortex and an inner medulla. The cortex stains darker than the medulla and consists of densely-packed and matured lymphocytes. Lymphocytes of the superficial cortex are larger than those of the deeper cortex, and are fewer in the lighter staining medulla. In the centre of the medulla are eosinophilic structures known as the Hassall's corpuscles that represents degenerated epithelial cells. Several studies conducted on mice thymus have shown that it reacts to artificially-induced situations such as swim test or injections of stress hormones (Pearse, 2006).

2.1.5 The spleen

The spleen is a secondary lymphoid organ present in all vertebrates, and situated in the left upper quadrant of the abdominal cavity. Being the largest single lymphoid organ, it is the site where

aged platelets and erythrocytes are destroyed. This organ contains about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens (Kuper *et al.*, 2002; Nolte *et al.*, 2002; Balogh *et al.*, 2004). It filters blood and synthesizes antibodies which are released into the circulation. The spleen acts by capturing foreign antigens as blood passes through it. It is equally involved in phagocytosis of bacteria and the destruction of worn-out red blood cells. This is why splenectomised subjects are highly susceptible to infections with bacteria (Cesta, 2006).

2.1.5.1 Gross anatomy of the spleen

The macroscopic features and size of the spleen varies with species (Cesta, 2006). In dogs, for example, the spleen is dumb-bell shaped, while in mice and rats, it is more uniform along the longitudinal axis. The spleen in dog is able to expand to store large numbers of erythrocytes, but can also contract rapidly. Therefore, its gross appearance is quite variable, ranging from large and dark red to blue-black to smaller and lighter red (Losco, 1992). The spleen is supplied by the splenic artery, which pierces the capsule at hilum and divides into smaller branches that are surrounded by fibrous trabeculae (Cesta, 2006).

2.1.5.2 Histology of the spleen

The spleen is composed of a fibrous capsule with muscular trabeculae, extending inward to subdivide the spleen into lobules. It consists of two types of tissue, the red pulp and the white pulp (Balogh *et al*., 2004). The red pulp contains vascular sinusoids, large number of red blood cells (RBC), resident macrophages, dendritic cells, granulocytes, few plasma cells and lymphocytes. The white pulp contains the lymphoid tissue clustered around small arterioles and is known as a periarteriolar lymphoid sheath (PALS). From its inner surface, branching and

anastomosing connective tissue trabeculae project into the organ to form its skeleton. The red pulp forms the greater part of the spleen in most species of mammals, and consists of reticular cells with their fibres forming the splenic cords. Between the cords are sinusoids lined by endothelial cells. The capsule and trabeculae of the spleen of dogs contains more smooth muscle than that of mice and rats, so the spleens of rodents do not contract as rapidly and tend to vary less in their gross appearance (Valli *et al*., 2002).

The splenic artery also differs among species. In dogs, it branches into as many as 25 smaller branches prior to entry into the spleen (HoganEsch and Hahn, 2001), while in the rat, there are as many as eight branches (Satodate *et al*., 1986). Vascular arrangements are perhaps the greatest source of species variation in splenic architecture. The spleen has been classified into two groups, based on species variation in the structure and morphology of the venous sinuses; sinusal spleens and non-sinusal spleens (Schmidt *et al*., 1985). Sinusal spleens are found in rats and dogs and non-sinusal spleens are found in mice (Schmidt *et al*., 1985). The venous sinuses of sinusal spleens are larger, more abundant, have numerous anastomoses, and a characteristic wall structure, relative to the venous sinuses of non-sinusal spleens (Schmidt *et al*., 1985). The larger venous sinuses of the rat spleen are far more conspicuous than those of the mouse spleen. The spleen has also been classified by Banks (1982) into two basic types according to the arrangement of capsular and trabeculae smooth muscle fibres. Defensive type of spleen has few trabeculae and smooth muscle fibres, but abundant lymphatic tissue, as in man. As opposed to storage spleens in horse, dog and cat that have more trabeculae and smooth muscle fibres but less lymphatic tissue (Banks, 1982).

2.1.6 The lymph nodes

Lymph nodes are located along lymphatic vessels but clusters are strategically stationed in the neck, groin, axillae, and abdominal cavity as cervical, inguinal, axillary and mesenteric respectively, being the key lymph nodes. The lymphoid lobule is the basic anatomical and functional unit of the lymph node. The smallest lymph nodes may contain only a few lobules or even just one, while large lymph nodes may contain as many as possible.

Lobules were described in lymph nodes as early as 1975 (Kelly, 1975) although some authors have described them as physiological compartments (Belisle and Sainte-Marie, 1999) but were not described in detail and their relevance to understanding lymph node function and pathology not widely elucidated. Current standard guidelines for the examination of a lymph node calls for a detailed examination of the cortex, paracortex and medulla (Haley *et al*., 2005). These compartments contain specific lobular structures, so, changes in them reflect alterations in the lobules. The lymphoid lobule is as potentially useful and necessary in understanding lymph node function and pathology as the hepatic lobule is to the understanding of liver function and pathology. Ruminant lymph nodes are found to have a system of smooth walled channels in the periphery of the deep cortical units (Belisle and Sainte-Maire, 1981). Birds do not have lymph nodes, instead, there are accumulations of lymphoid tissue in their bone marrow. Studies have been done to investigate the gross and histological aspects of superficial lymph nodes in cattle, sheep, goat and pigmy hog (Haley *et al*., 2005) and there is information on the mesenteric lymph nodes of rat (Dijkstra *et al.,* 1990).

2.1.6.1 Gross anatomy of the lymph node

Lymph nodes of mammals are either oval, bean or kidney-shaped, depending on their location in the body and the species of mammal. They appear as white masses, which could sometimes resemble fat. Their sizes are variable ranging from a few millimetre, to more than 2 centimeters depending on the size of the animal (Haley *et al*., 2005).

2.1.6.2 Histology of the lymph node

The parenchyma of a lymph node is classified into two morphologically and functionally-distinct zones; the cortex and medulla. In the cortex, two sub zones can also be distinguished; the paracortex and the deep cortex. The lymph node medulla is in the form of dense cords and contains lymphocytes and plasma cells (Picker and Siegelman, 1999). Lymphocytes are the parenchymal cells of the lobules. They dominate the histological appearance of lymph nodes as they obscure the reticular meshwork. Lymphocytes recirculate continually, entering lymphoid lobules via specialized blood vessels, called high endothelial venules and exiting via specialized sinuses called paracortical sinuses. They leave the lymph node in the efferent lymph and are returned to the circulation via the thoracic duct to start the cycle again. Thus, quite unlike parenchymal cells in other organs, the lymphoid parenchymal cells move around inside the lymph nodes, relocate from one node to another and even move from one organ to another (Worbs *et al*., 2006). The visceral nodes contain variable amounts of smooth muscle, depending on their location. The mesenteric nodes are the most muscular of all the node groups (Worbs *et al*., 2006) and the muscular layer occurs as a component of the thick capsule, as well as part of

the trabeculae. In addition, fascicles of smooth muscle occur in a loosely interwoven or basket-weave pattern in the mesenteric nodes (Husby *et al*., 1994). The most distinctive feature of the mesenteric lymph nodes is the antler-like branching of the blunt collagenous trabeculae, a pattern which occurs within a short distance from the capsule. Both somatic and visceral node groups have the typical structure of sinuses and cords, and in responding to antigenic challenge will produce prominent follicles with germinal centres (Worbs *et al.*, 2006).

2.1.7 The mesenteric lymph nodes of rodents and immunity

The surface of the intestinal mucosa is constantly assaulted by food antigens and a vast number of commensal microbes and their products. According to Husby *et al* (1994), the mesenteric lymph nodes are the key sites for tolerance-induction to food proteins and a barrier to live commensal intestinal bacteria penetrating the immune system. It has been known for nearly a century that feeding rodents with a protein prevents subsequent responses to systemic challenge with the same protein (Wells, 1911). This phenomenon is termed oral tolerance and has been extensively studied using soluble proteins fed to rodents (Husby *et al*., 1994). New insights into the anatomy of intestinal tolerance was reported by Worbs *et al*., (2006) by showing that tolerance is induced exclusively in the mesenteric lymph nodes after migration of antigen-loaded dendritic cells from the intestinal mucosa. Oral tolerance can give insight into the crosstalk between mucosal surfaces and the systemic immune system, in particular may show how hypersensitivity to food components is normally avoided.

2.1.8 Peyer's patches; Gross anatomy of the small intestine

Peyer's patches (PP) are organised lymphoid nodules named after the 17th century Swiss anatomist Johann Conrad Peyer. They are aggregations of lymphoid tissue usually found in the sub-mucosa along the lowest portion of the small intestine, the ileum, where they can serve as land marks differentiating the ileum from the duodenum and jejunum. Peyer's patches are observable as elongated thickenings of the intestinal epithelium measuring a few centimetres in thickness. The duodenum can be identified by the Brunner's glands. The jejunum has neither Brunner's glands nor Peyer's patches (Jung *et al*., 2010).

The lumen of the gastrointestinal tract is exposed to the external environment, therefore much of it is populated with potentially-pathogenic microorganisms. Peyer's patches thus establish their importance in the immune surveillance of the intestinal lumen and in facilitating the generation of the immune response within the mucosa. The Peyer's patches have been intensely investigated in many species because of its importance (Jung *et al*., 2010).

2.1.9 Peyer's patches; Histology of the small intestine

Mature Peyer's patches consist of collections of large B-cell follicles and intervening T-cell areas (Debard *et al*., 1999). The lymphoid areas are separated from the intestinal lumen by a single layer of columnar epithelial cells, known as the follicle-associated epithelium (FAE) and a more diffuse area immediately below the epithelium known as sub-epithelial dome (SED). Peyer's patches are generally oval or rectangular in shape (Jump and Levine, 2002).

2.1.10 Mucosal associated lymphoid tissue

The Mucosal Associated Lymphoid Tissue (MALT) also known as Mucosal Associated Lymphatic Tissue are the diffused concentrations of lymphoid tissue found in various sites of the body, such as the gastrointestinal tract, eye and skin. Approximately, more than 50 % of lymphoid tissue in the body is found associated with the mucosal system (Croitoru and Bienenstock, 1994). As it is with most lymphoid tissues, MALT is characterised by the presence of lymphocytes such as the B and T cells, as well as plasma cells and macrophages, each of which is well situated to encounter antigens passing through the mucosal epithelium thereby regulating mucosal immunity. According to the anatomical and physiologic characteristics of the different mucosae, MALT can be classified into gut associated lymphoid tissue (GALT), bronchus associated lymphoid tissue (BALT), nasal cavity associated lymphoid tissue (NALT), conjuctival associated lymphoid tissue (CALT), larynx associated lymphoid tissue (LALT), trachea associated lymphoid tissue (TALT), lacrimal duct associated lymphoid tissue (LDALT) and vascular associated lymphoid tissue (VALT) (Casteleyn, 2010).

2.1.10.1 Gut Associated Lymphoid Tissue (GALT): This is an important part of the peripheral mucosal immune system, which provides localised immunity in the intestinal tract for coping with ingested materials that are potentially antigenic and pathogenic (Whitehead and Skinner, 1978). The GALT is the largest and best defined part of MALT (Calsen *et al*., 2002).

2.1.10.2 Bronchus Associated Lymphoid Tissue (BALT): This tissue has major functional and morphologic similarities to Peyer's patches found in the gut. Both contain a high percentage of cells bearing IgA surface immunoglobulin; both appear in (the apparent) absence of direct antigen stimulation; both possess a lymphoepithelium with selective

antigen sampling properties and both can repopulate the bronchial and gut lamina propria with IgA-containing cells. There is a great deal of species variability in BALT. For example, BALT is normally absent in dogs, cats and hamsters (Kiyono and Fukuyama, 2004).

2.1.10.3 Nasal cavity Associated Lymphoid Tissue (NALT): The NALT means the presence of organised lymphoid tissue in the nasal mucosa (Fujimura, 2000). In rodents, NALT has been shown to be an important inductive site for priming of immunocompetent cells to induce antigen-specific mucosal immune responses in the upper respiratory tract and other mucosal effector tissues (Shimoda *et al*., 2001). Additionally, potential inductive sites for mucosal B cells are associated with the naso and oro-pharynx such as the tonsils in humans. Rodents do not have tonsils but paired MALT structures are present on both sides of the nasopharyngeal duct dorsal to the cartilaginous soft plate (Kiyono and Fukuyama, 2004).

2.1.10.4 Conjuctiva Associated Lymphoid Tissue (CALT): The conjunctiva is provided with a system of specific immune response, in the form of Mucosa Associated lymphoid Tissue (MALT), which detects antigens and induces an immune response by the direct action of the lymphatic cells or the secretion of soluble antibodies (Liang *et al.,* 2012). The presence of Mucosa Associated Lymphoid Tissue in the conjuctiva is important, because, it maintains ocular surface homeostasis (Liang *et al.,* 2012).

2.1.10.5 Vascular Associated Lymphoid Tissue (VALT): Accumulations of immunocompetent and antigen presenting cells have been recognised in the arterial walls of mammals. Focal accumulations of mononuclear cells in the arterial walls of healthy humans at

predilection sites for artherosclerotic lesions have been described as Vascular Associated Lymphoid Tissue (VALT) (Bobryshev and Reginald, 2001).

Apart from the MALT sites, there are aggregations of lymphoid cells present in the lamina propria of many other tissues such as the genital tract (Brandtzaeg, 2010).

2.2 Immunity in Mammals

An important feature of the innate reaction is recruitment and activation of neutrophils at the site of infection to eradicate pathogens. During the very early stages of infection or tissue damage, there is release of cytokines from activated macrophages. Two of these, granulocyte and granulocyte macrophage colony stimulating factors, stimulate division of myeloid precursors in the bone marrow, releasing millions of cells into the circulation and causing a characteristic neutrophil leucocytosis (Muzio *et al*., 2000). Neutrophils, like most cells involved in immune responses, are not static within a particular compartment, but are mobile cells that travel round the body. They normally flow freely within the blood as the circulating pool, or roll along the vascular endothelium as the marginating pool. To locate a site of infection, neutrophils use a multistep process involving proinflammatory mediators, adhesion molecules, chemo attractants, and chemokines. Most leucocytes, including lymphocytes use this system of localisation. The recruited neutrophils phagocytose organsisms by making pseudopodia which form a membrane-bound vesicle (phagosome) around the particle. This fuses with neutrophil cytoplasmic granules to form the phagolysosome (Mackay, 2001).

2.2.1 Cells of the immune response in mammals

2.2.1.1 Natural killer cells

Natural killer cells have the morphology of lymphocytes but do not bear a specific antigen receptor. They bear immunoglobulin receptors and bind antibody coated targets leading to antibody-dependent cellular cytotoxicity. If on interaction with a cell, this receptor is not bound, the natural killer cell is programmed to lyse the target (Mackay, 2001). This is achieved by secretion of perforins onto the surface of the cell to which the natural killer cell has adhered. Perforins make holes in the cell membrane and granzymes are injected through the pores (Muzio *et al*., 2000). Natural killer cells do not attack invading microbes directly. They kill cells of the body that have been infected with viruses. They kill not by phagocytosis, but rather by creating a hole in the plasma membrane of the target cell. Proteins, called *perforins,* are released from the natural killer cells and insert into the membrane of the target cell, forming a pore. This pore allows water to rush into the target cell, which then swells and bursts (Muzio *et al*., 2000).

2.2.1.2 Cytokines

Cytokines are small molecular weight messengers secreted by a cell to alter the behaviour of itself or another cell. Cytokines send intracellular signals by binding to specific cell-surface receptors. Although most are soluble, some may be membrane-bound, making the differentiation between cytokine and receptor difficult (Mackay, 2001). Cytokines are produced by virtually all cells and have a wide variety of functions. The biological effect depends on the cytokine and the cell involved, but typically these molecules will affect cell activation, division, apoptosis, or movement. They act as autocrine, paracrine, or endocrine messengers. Cytokines produced by

leucocytes and having effects mainly on other white cells are termed interleukins. Cytokines that have chemoattractant activity are called chemokines (Muzio *et al*., 2000). Those that cause differentiation and proliferation of stem cells are called colony-stimulating factors. Those that interfere with viral replication are called interferons (Mackay, 2001).

2.2.1.3 B Lymphocytes

B cells produce antibody. This serves to neutralise toxins, prevents organisms adhering to mucosal surfaces, activates complement, opsonizes bacteria for phagocytosis, and sensitizes tumour and infected cells for antibody dependent cytotoxic attack by killer cells (Weiss, 2010). Thus antibody acts to enhance elements of the innate system. Although ultimately antibody is the secreted product of activated B cells with the functions listed, early in B-cell development it is a membrane bound molecule that acts as the B-cell receptor. In this role it internalises antigen and processes it to act as an antigen-presenting cell for T-cell responses (Lintermann *et al*., 2009). Different classes of antibody predominate at different compartments of the body (IgM being intravascular, IgG the main antibody of the blood and tissues, IgA in secretions). Mucosa associated lymphoid tissue consists of lymphoid tissue at several mucosal sites (bronchus, gut, urogenital tract etc). A response generated at one site will induce immune responses to the same antigen at other sites. This effect can be used therapeutically because vaccination at one mucosal site can potentially induce generalised mucosal immunity. For example, an oral vaccine could induce vaginal and rectal immunity (Mosmann *et al*., 2005).

2.2.1.4 T Lymphocytes

Mature T lymphocytes, known as naive T cells, circulate through blood and the lymphatic system, and reside in secondary lymphoid organs (Wing and Sakaguchi, 2010). Naive T cells are those that have not yet encountered foreign antigen and have not yet been activated. Antigenic peptides are presented to the naive T lymphocyte in secondary lymphoid organs by dendritic cells (Mosmann *et al*., 2005). Dendritic cells are the most efficient "professional" antigen presenting cells since they also provide stimulatory signals for effective T cell activation. Dendritic cells acquire antigen in non-lymphoid tissues throughout the body and migrate into secondary lymphoid organs guided by inflammatory stimuli and cytokines (Weiss, 2010). Activated T cells rapidly proliferate (clonal expansion), migrate through the tissues to the sites of antigen presence, and perform effector functions such as cell-mediated cytotoxicity and production of various cytokines (soluble mediators of the immune response) (Linterman *et al*., 2009). Cytotoxic CD8+ T cells are very effective in direct lysis of infected or malignant cells bearing the Ag, while CD4+ T helper cells produce cytokines that can be directly toxic to the target cells or can stimulate other T cell effector functions and B cell antibody production, as well as mobilize powerful inflammatory mechanisms (Matzinger, 2002).

2.2.2 Interaction of lymphocytes and macrophages

When microorganisms invade the body, they trigger a wide sequence of biological events (Askonas and Lehner, 1981). Here, the macrophage plays a key role. The functions of macrophage include in addition to its known microbial killing, antigen presentation to T cells, secretion of lymphoregulatory molecules (Unanue, 1978; Rosenthal, 1980) and the role as an accessory cell in polyclonal B cell stimulation. Close physical association between macrophages

and lymphocytes is required for the expression of a variety of the physiological processes of lymphoid cells.

Thus, the promotion of lymphocyte viability *in vitro* (Chen and Hirsch, 1972), the functional maturation and differentiation of immature thymocytes *in vitro* (Mosier and Pierce, 1972), and the enhancement of the bactericidal capacity of macrophages by antigenically stimulated lymphocytes (Simon and Sheagren, 1972) are all accomplished most efficiently when actual physical approximation develops between lymphocytes and macrophages. In addition, macrophage-lymphocyte cooperation is integrally involved in the development of specific immune phenomena, such as the *in vitro* induction of primary (Mosier, 1969) and secondary (Katz and Unanue, 1973) antibody responses, as well as the antigen-mediated *in vitro* initiation of immune lymphocyte proliferation (Waldron *et al*., 1973).

In these systems, macrophages are thought to be the cells which initially must interact with antigen for effective lymphocyte triggering. In view of the inhibition of *in vitro* immune responses noted when lymphocytes and macrophages are separated mechanically, and the demonstration of clustering of proliferating and/or antibody-producing lymphocytes about antigen-bearing macrophages (Hanifin and Cline, 1970), it is likely that the way macrophages present antigen to lymphocytes involves direct physical contact between these two cell types (Waldron *et al*., 1973).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Animal Source

Twelve grasscutters, comprising 6 males and 6 females, were purchased from local breeders in Benue State (Longitude 7^0 47□ and 10^0 0□ East and Latitude 6^0 25□ and 8^0 8□ North), Nigeria. The animals purchased were between three and seven months of age and transported in cages to the research laboratory of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria. The animals were acclimatised for 14 days and were given access to fresh elephant grass (*Pennisetum purpureum)* and water *ad libitum.* The grasscutters were then divided into three groups according to their ages, with four animals in each group. During this period, the animals were observed for signs of ill-health such as anorexia, diarrhoea, nasal and ocular discharges, skin lesions and emaciation, which were absent. Faecal and blood samples were collected in sample vials, stored in ice pack and then transported to Parasitology and Microbiology Laboratories for detection of haemo- and endo-parasites. Laboratory findings yielded no positive results in all the animals. Biosecurity measures such as wearing of protective clothings and disinfection of working equipment and surroundings were observed, bearing in mind the risk of zoonoses.

3.2 Morphologic and Morphometric Studies

The animals were euthanized using chloroform inhalation anaesthesia. Body weights post euthanasia of the animals were taken using Ohaus weighing balance (USA). Thereafter, the animals were placed on dorsal recumbency to expose the cervical- thoracic- abdominal regions. Then, a ventral mid-line incision was made, extending from the cervical to the pelvic region. The skin and superficial tissues were carefully reflected to locate and harvest the lymphoid organs designated. The thymus was located at the cranial mediastinum, just above the heart, close to the thoracic inlet, the spleen was located at the left hypochondral region, adjacent to the greater curvature of the stomach and the mesenteric lymph node was identified as a bean shaped structure within the folds of the mesentery. Photographs of these organs were taken in situ to show their anatomical position and relationship with other structures. Observations of size, shape and colour were made and recorded. Each organ was weighed prior to fixation following removal of adjacent fat and connective tissue using G & G® Electronic Scale (Model JJ1000 USA). Both lengths and widths of the glands were determined using Venier calipers, with the graduation of 1/10 cm with 1/100 cm Venier. All the glands were studied grossly with the aid of a hand lens and the anatomical features and observable differences at the different ages were recorded.

3.3 Histological Studies

The organs were labelled and fixed for 48 hours in Bouin's solution. Tissue specimen were taken from the lymphoid organs for routine histological preparation using Drury and Wallington (1980) method. Prior to fixing, each organ was examined critically and the excess fat and tissues

removed, the organs were thereafter dabbed before fixing. Dehydration was done through graded alcohol (70 %, 80 %, 90 %, 95 % and 100 %). They were then cleared in xylene and infiltrated with molten paraffin wax. Sections of 5 µ thick were cut using the Jung microtome (West Germany). The sections were then mounted on grease-free glass slides and stained with haematoxylin and eosin (H & E) stain for routine histological studies (Drury and Wallington, 1980). Rhodamine B-aniline-methylene blue for lymphoid tissues was used to study the architecture of reticular fibres in the tissues. The prepared sections were examined using a light microscope (Olympus, CHB Tokyo Japan). Variables were studied by using both low and high power objectives (between x 4 and x 40) and viewed with x 10 eyepiece. Photomicrographs of the tissues were taken using AmScope MT series Microscope Camera (USA), connected to a computer (Compaq Presario).

3.4 Statistical Analysis

GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, California, USA) was used. The data obtained were expressed as mean ± standard error of the mean (Mean ± SEM). Age differences in mean values were established by employing one way analysis of variance, followed by Tukey's multiple comparison *post-hoc* test; sex differences were established using two-tailed Student's *t* test. Pearson's correlation analysis was used to determine relationships between age, body weight and weight of the thymus, spleen and mesenteric lymph node of the grasscutters. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 The Thymus

4.1.1 Gross observations of thymus

The thymus of the grasscutters was found at the cervical region, located around the cranial mediastinum, just above the heart, close to the thoracic inlet. It was found as a bilateral milky coloured lump-like structure (Plates II and III). The size of the organ decreased consistently with age, and at seven months of age, it was much smaller (Plate IV). The mean weight of the thymus in these grasscutters were 1.23 ± 0.05 g, 0.53 ± 0.02 g and 0.30 ± 0.04 g for three, five and seven months, respectively (Table 1).

4.1.2 Histological observations of the thymus

The thymus of the grasscutters comprised two distinct zones; the outer cortex and an inner medulla. The cortex stained more intensely than the medulla which appeared much lighter (Plates V, VI, IX and X). Thymic corpuscles also known as Hassal's corpuscles were seen scattered over the medulla, and appeared as eosinophilic structures in the medulla. The corpuscles were observed to be more numerous and larger at three months of age (Plate V and VI), with a reduction in their size at five months (Plate X) was observed and no corpuscle by seven months (Plates XII and XIII). It was observed that the sharp delineation of the cortex from

medulla became less distinct with advance in age. At three months of age, there was a sharp contrast between the cortex and medulla (Plate V and VI), at five months, it became less distinct (Plate IX and X), and at seven months there was no observable cortex and medulla distinction (Plate XII and XIII). They contained more of epithelial structures and adipose tissue, with the capsule observed to be thin compared to those at three and five months of age. Rhodamine B aniline methylene blue stain revealed matrix fibres of the organ (Plate VIII, XV).

The reticular fibres appeared as the supporting framework of the thymic tissue. The staining technique revealed a fine pattern of arrangement of the reticular fibres, with the presence of collagen bundles at the periphery of the cells (Plate XV). At three months of age, the histology of the grasscutter thymus showed the presence of many small lymphocytes. The principal morphologic change observed in the thymus with advancing age was the depletion of cortical lymphoid cells or lymphocytes (Plate VII and XI). From this study, the histological findings on the thymus can be summarised as follows:

(a) Three-month-old animals: The thymi were active, lobulated and showed sharp demarcation of the cortex from the medulla. In the cortex, lymphocyte population was high while in the medulla, only few lymphocytes were scattered over the epithelial Hassal's corpuscles and epithelial cysts (Plates V and VI).

(b) Five-month-old animals: The thymi here were observed to be moderately involuted, with faint lobulation and reduced thickness of the cortex. The demarcation of the cortex from the medulla was also faint (Plates IX and X).

(c) Seven-month-old animals: In this group, there was a marked involution of the thymi, with no demarcation between the cortex and medulla. The lymphocyte numbers were drastically reduced and mostly replaced by adipocytes (Plates XII and XIII).

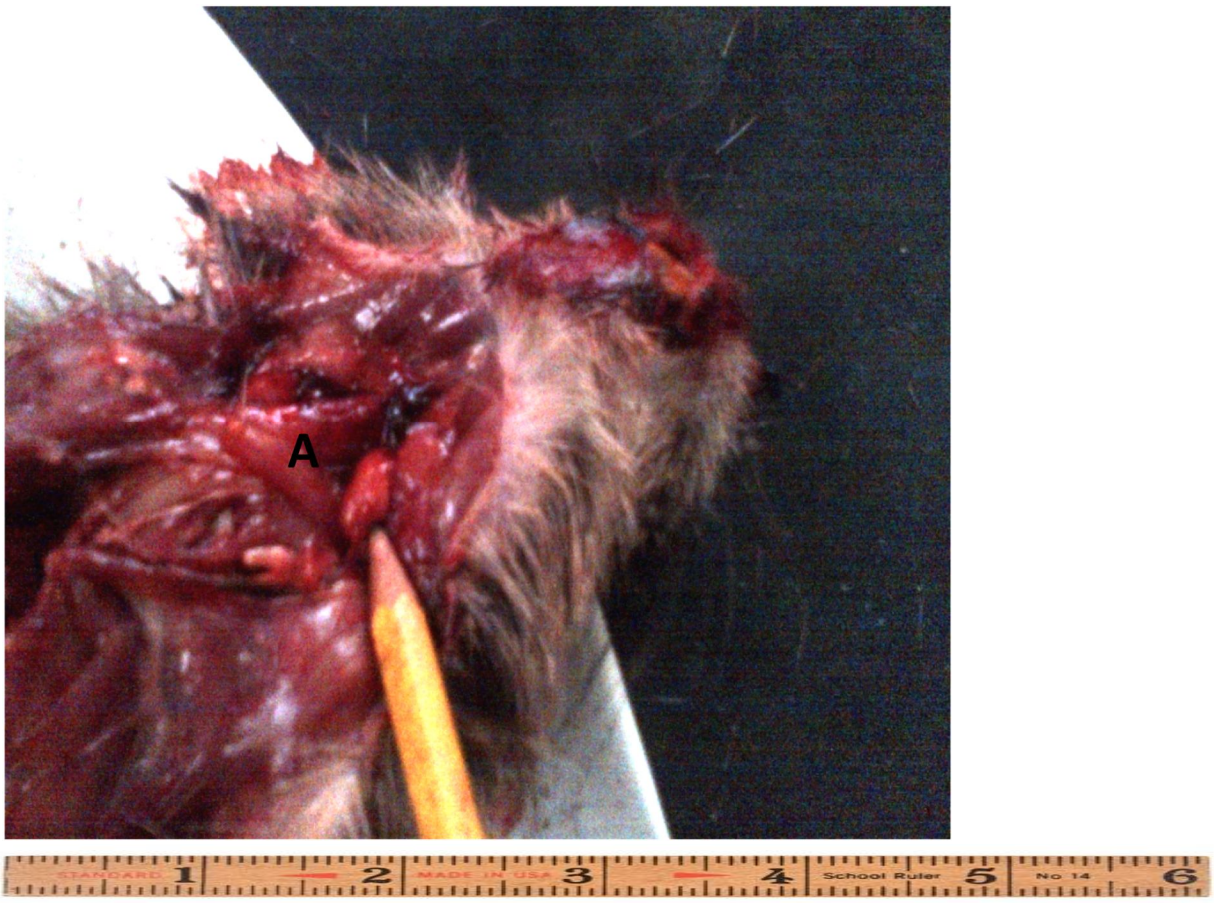


Plate II The thymus of a grasscutter at 3 months shown by pointer. The aorta is designated as **(A)** Mag. X 40.

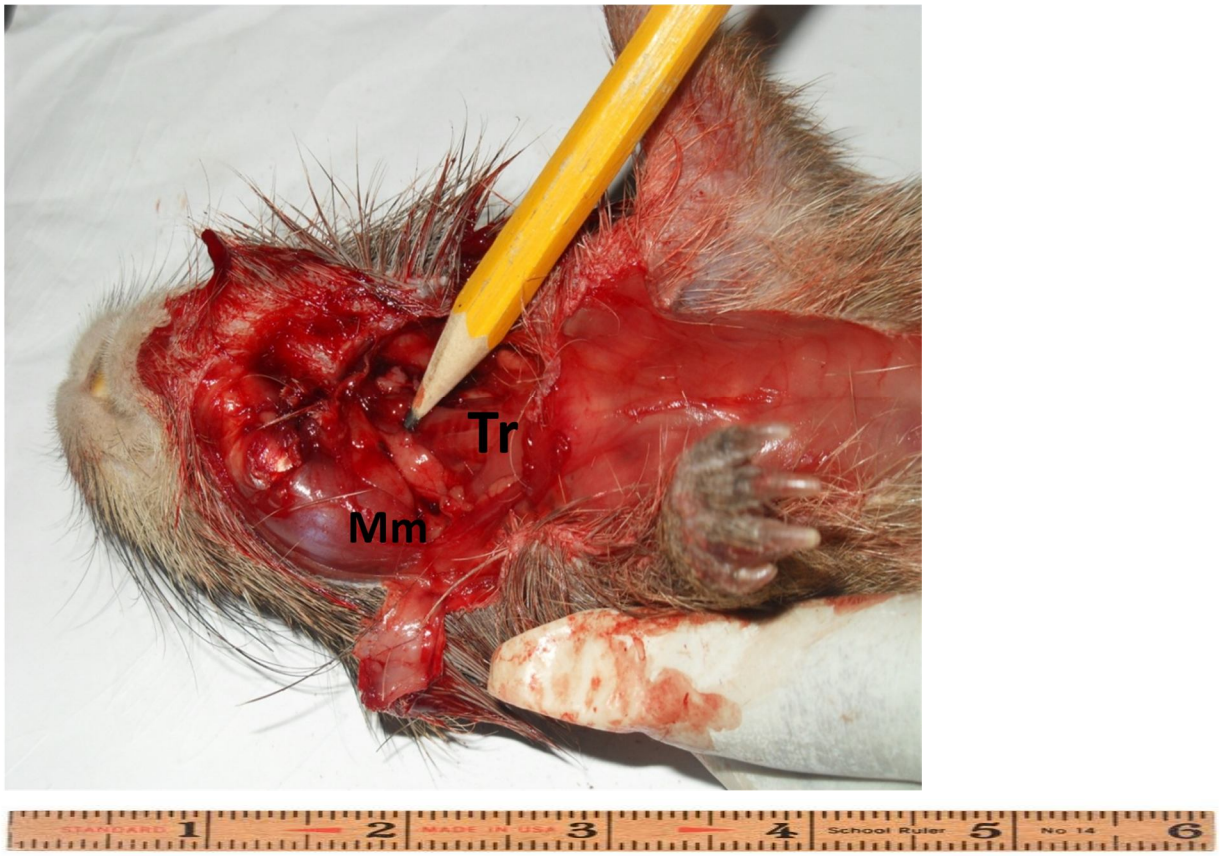


Plate III. The thymus of a grasscutter at five months shown by pointer. Note **(Mm)** Masseter muscle, and the **(Tr)** trachea. Mag. X40.



Plate IV. The thymus of a grasscutter at 7 months shown by arrow, and the Trachea **(Tr).** Mag.X 40

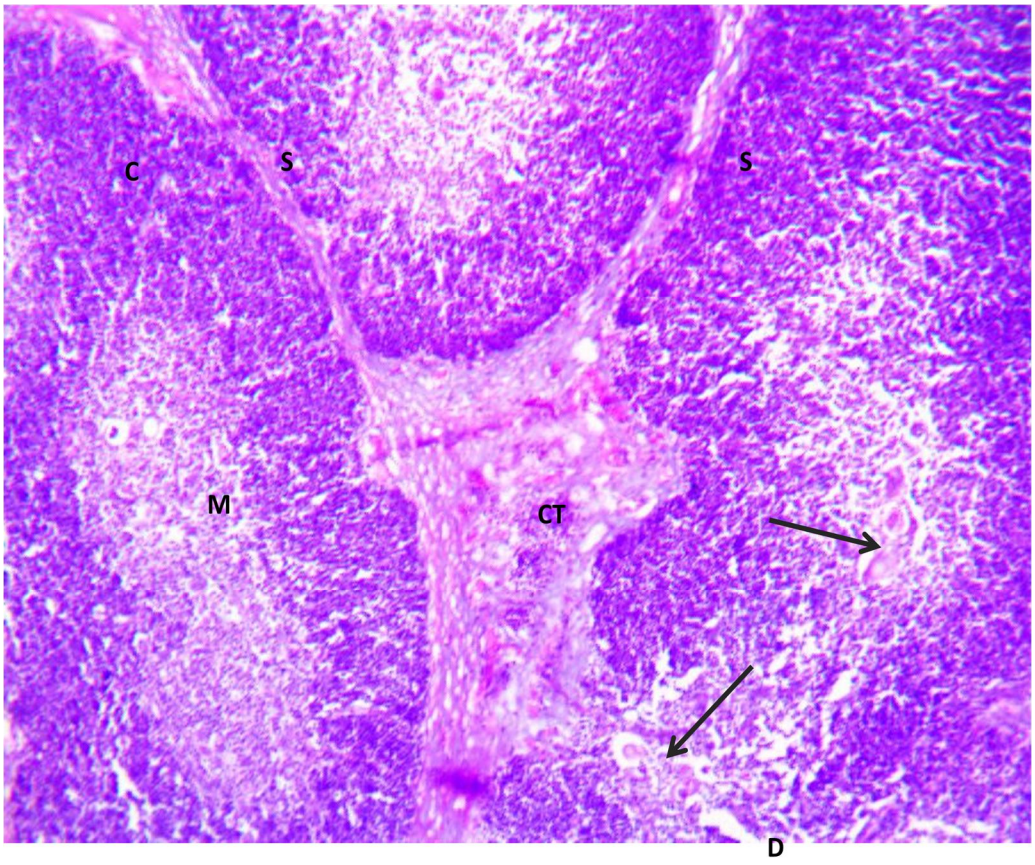


Plate V. Photomicrograph of the thymus of a 3 month old male grasscutter showing the distinct medulla (**M)**, and cortex (**C**), interlobular septa **(S)**, Hassal's corpuscles indicated by arrows. H & E. X100.

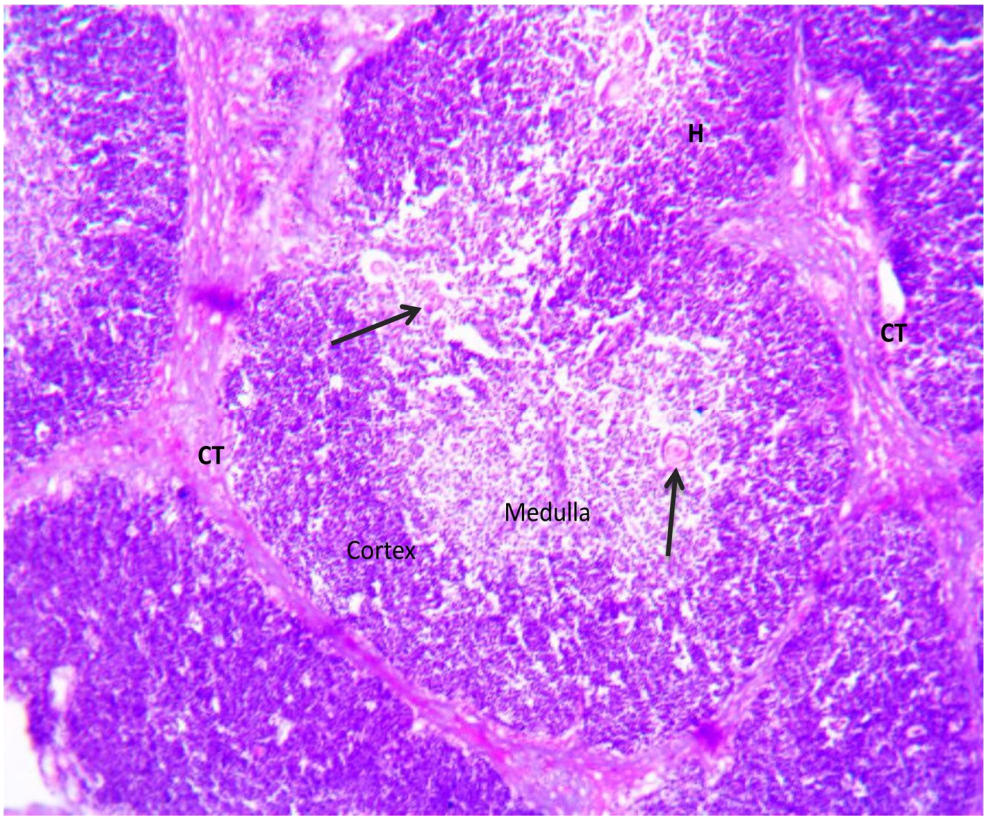


Plate VI. Photomicrograph of the thymus of a 3 month old female grasscutter showing connective tissue **(CT),** which divides the tissue into lobules, Hassal's corpuscles are shown by the arrows, medulla. H & E. X250.

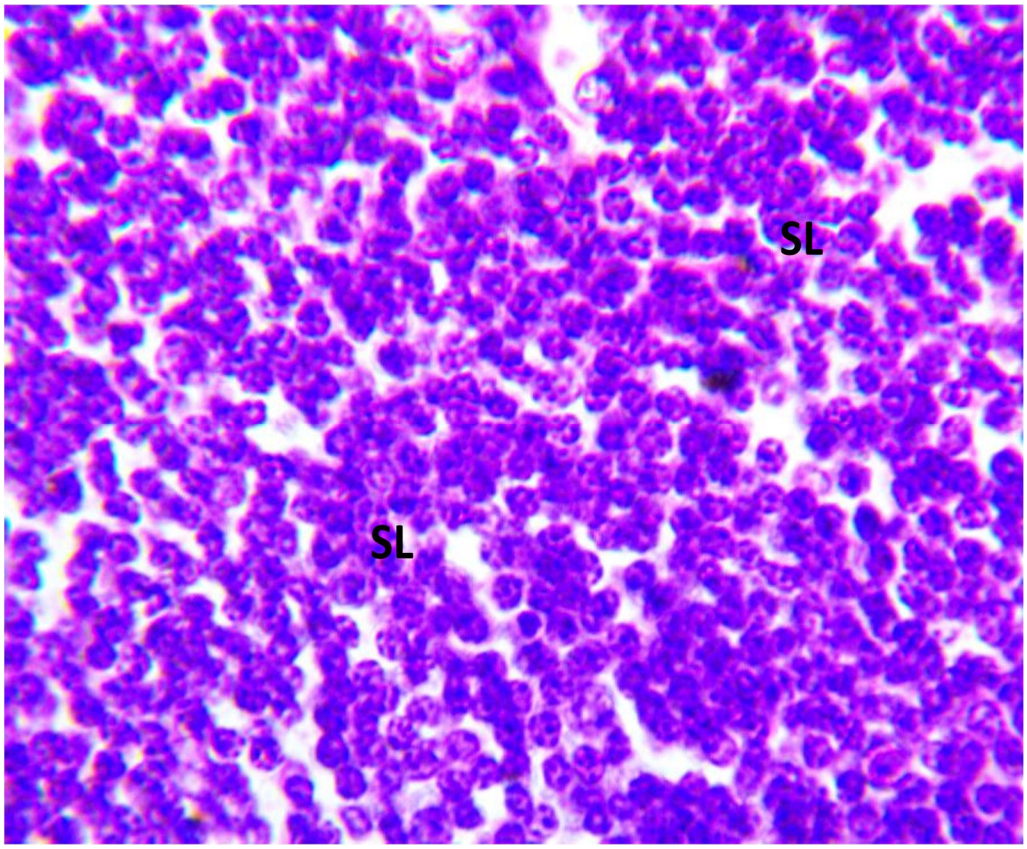


Plate VII. Photomicrograph of the thymic cortex of 3-month-old grasscutter, showing small lymphocytes **(SL)**. Note heavier population of lymphocytes at three months. Compare with **Plate XI**. H & E X250.

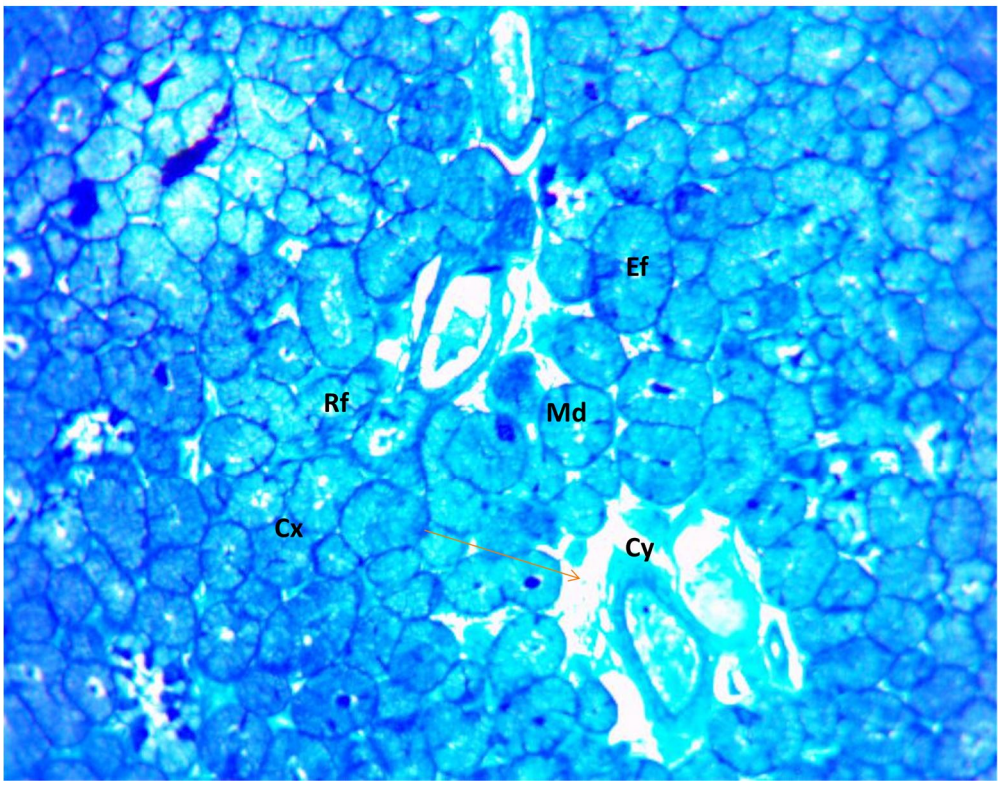


Plate VIII. Photomicrograph of the thymus of a 3-month-old grasscutter showing reticular fibres **(Rf)**, elastic fibres **(Ef)**, cortex **(Cx)**, medulla **(Md),** secretory vessel shown by arrow and the secretory substance **(Cy)**. Rhodamin B-aniline- methylene blue X 400.

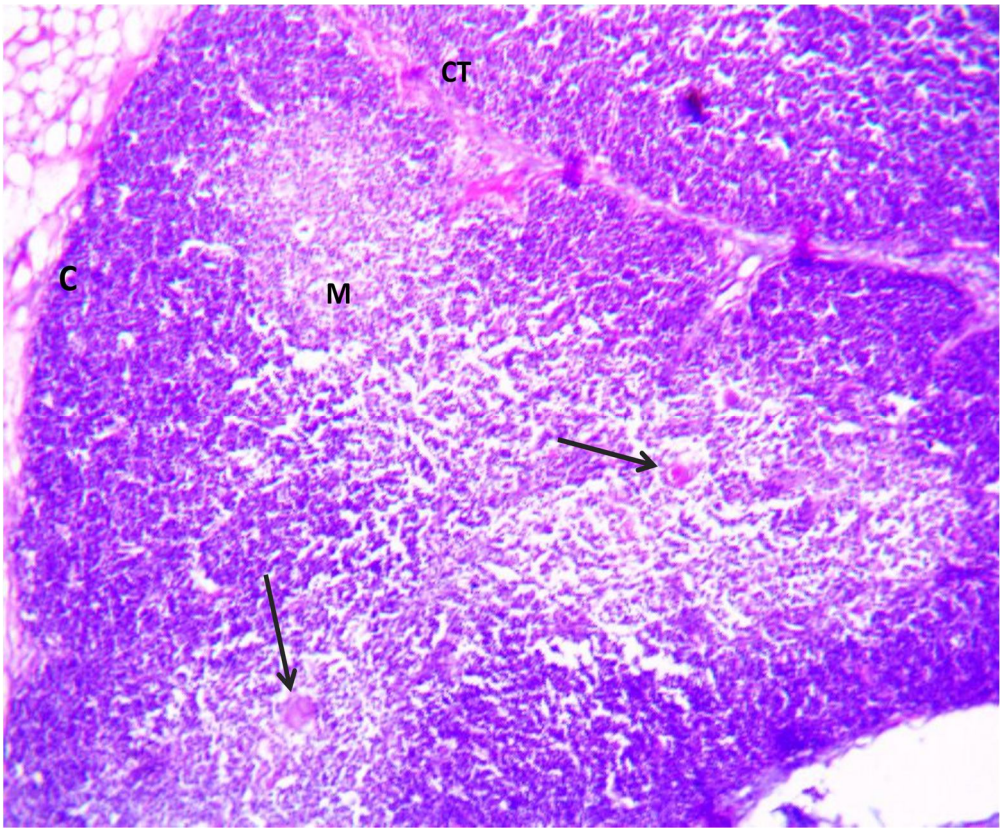


Plate IX. Photomicrograph of the thymus of a 5 month old male grasscutter showing the lighter staining medulla (**M)**, the darker staining cortex (**C)**, connective tissue septa **(CT)** which divides the thymus into lobules and Hassal's corpuscles shown by the arrows. Note reduction in size of thymic corpuscles as compared to that of younger grasscutter (see Plates V and VI) and reduced distinction between cortex and medulla. X100 H & E.

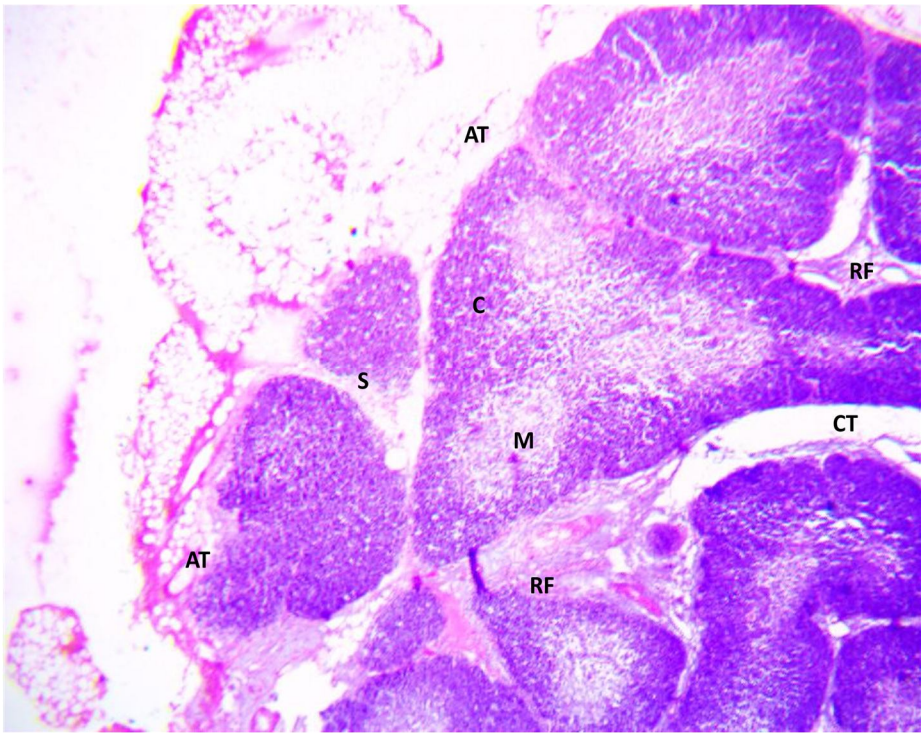


Plate X. Photomicrograph of the thymus of a 5-month-old female grasscutter showing adipose tissue (**AT)** infiltrations, cortex (**C**) medulla , (**M**), connective tissue (**CT**), reticular fibres (**RF**) and septa (**S**). H & E. X40.

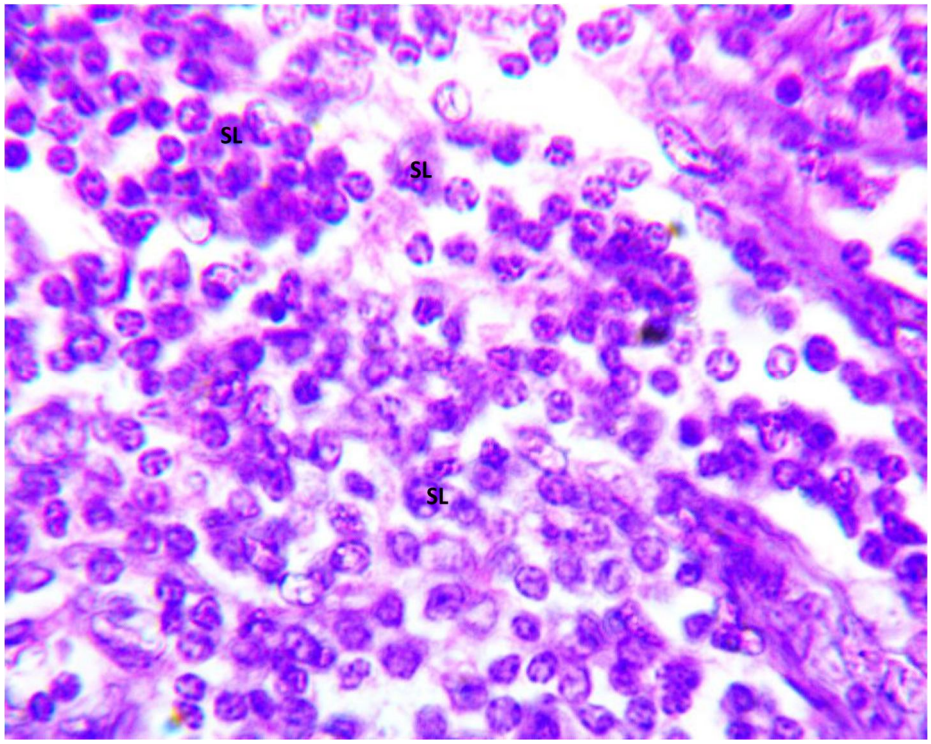


Plate XI. Photomicrograph of the thymic cortex of a 7-month-old grasscutter showing small lymphocytes **(SL).** H & E X 250. Note marked depopulation of lymphocytes here. Refer to **Plate VII**.

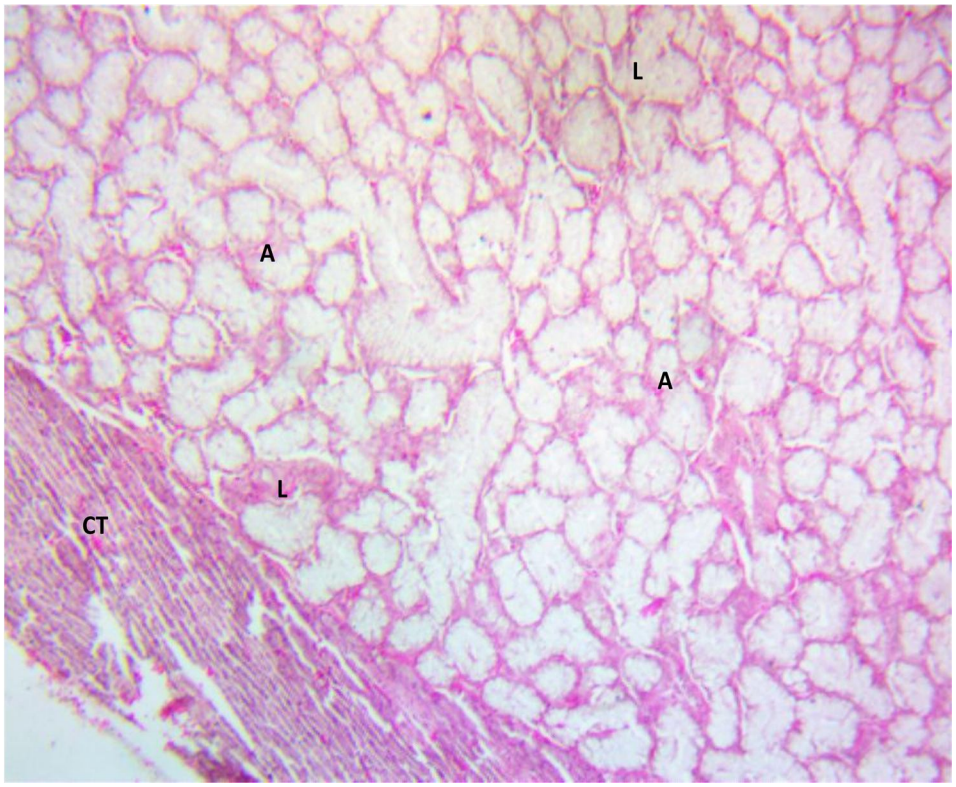


Plate XII. Photomicrograph of the thymus of a 7-month-old male grasscutter showing massive infiltration of the tissue by adipose tissue (**A**), connective tissue (**CT**) and remnants of lymphoid tissue (**L**), surrounded by much adipose tissue. Note total absence of Hassal's corpuscle and lack of distinction between cortex and medulla X100 H & E.

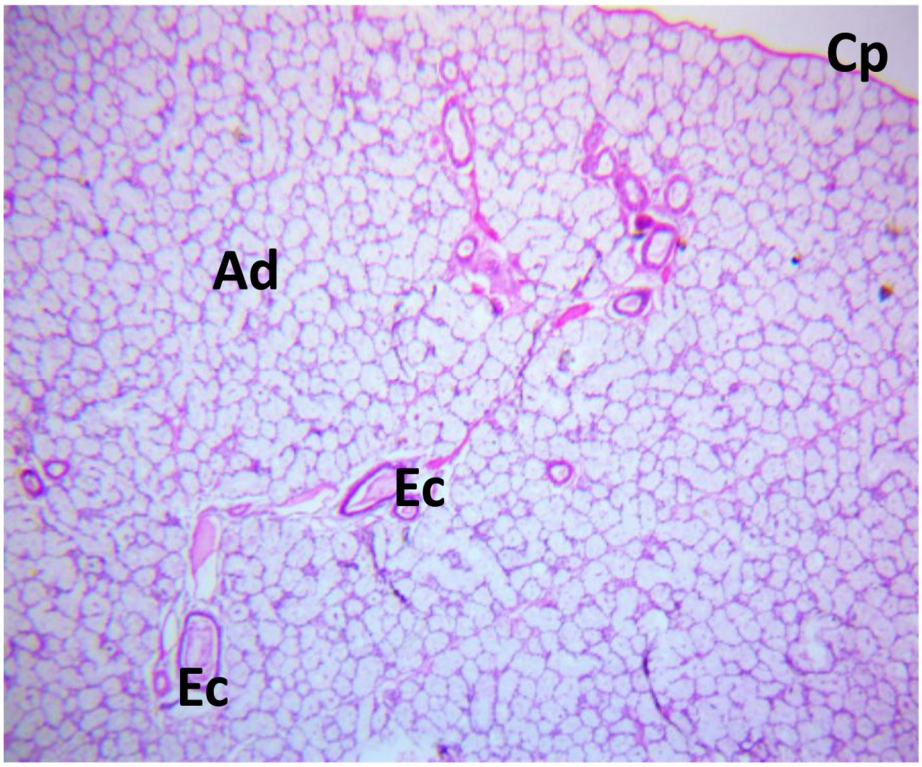


Plate XIII. Photomicrograph of the thymus of a 7-month-old female grasscutter showing the numerous epithelial cells towards the centre **(Ec)**, numerous adipose cells **(Ad)**, and the capsule **(Cp)**. H & E X40. Note total absence of Hassal's corpuscle and lack of distinction between cortex and medulla

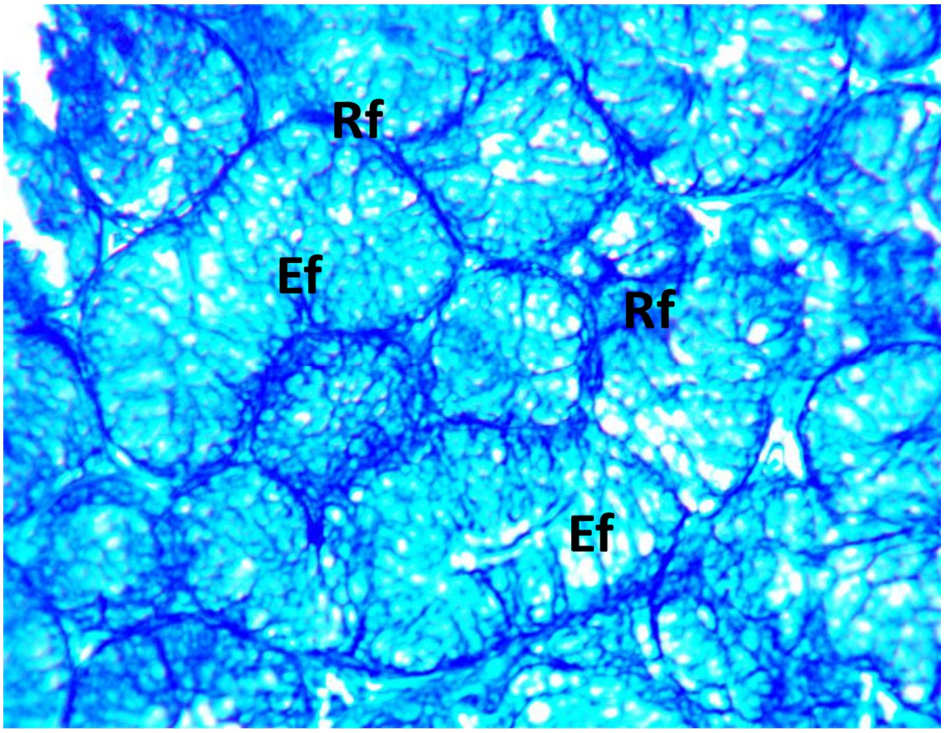


Plate XIV. Photomicrograph of the thymus of a 7-month-old male grasscutter. Note the reticular fibres **(Rf)** and elastic fibres **(Ef)**. Rhodamine- aniline-methylene X400.

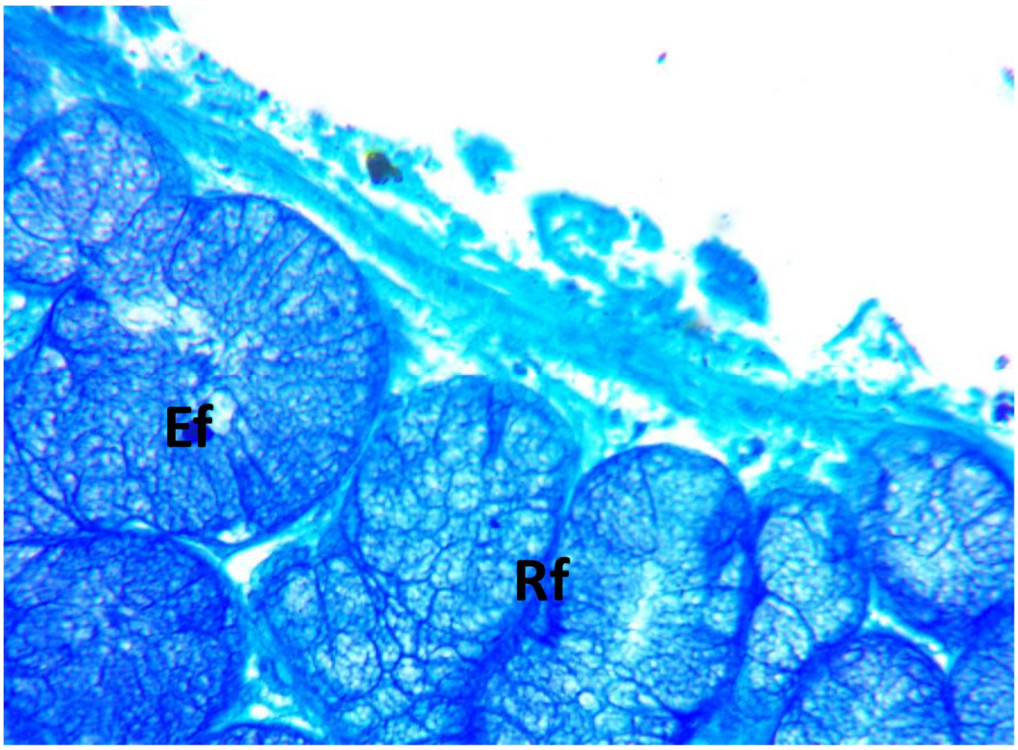


Plate XV. Photomicrograph of the thymus of a 7-month-old female grasscutter showing reticular fibres **(Rf)**, elastic fibres (**Ef)** Rhodamin B-aniline-methylene blue stain X400.

4.2 Mesenteric Lymph Nodes

4.2.1 Gross observations

The mesenteric lymph nodes were found within the folds of the peritoneum along the mesentery of the small intestine. They appeared as minute structures, which were distinguished from surrounding fat by their pearly gloss nature and consistent bean-shaped. (Plate XVI). The weight (Mean ± SEM) of the caudal mesenteric lymph nodes for these grasscutters were 0.05 ± 0.005 g, 0.14 ± 0.034 g and 0.16 ± 0.023 g at three, five and seven months, respectively (Table 1). The mean circumference was 0.36 ± 0.006 cm, 0.55 ± 0.08 cm and 0.64 ± 0.058 cm at three, five and seven months respectively (Table 1).

4.2.2 Histology

The mesenteric lymph nodes studied had a collagenous capsule. The connective tissue trabeculae from the capsule penetrate and incompletely divide the lymph node parenchyma into distinct lobules (Plate XVII). The cortex of the mesenteric lymph nodes were indistinguishable from medulla at all the ages (Plates XIX and XX). Bundles of collagen were observed in the tissue parenchyma at three months (Plate XVIII). Medullary cords were present (Plate XXII).

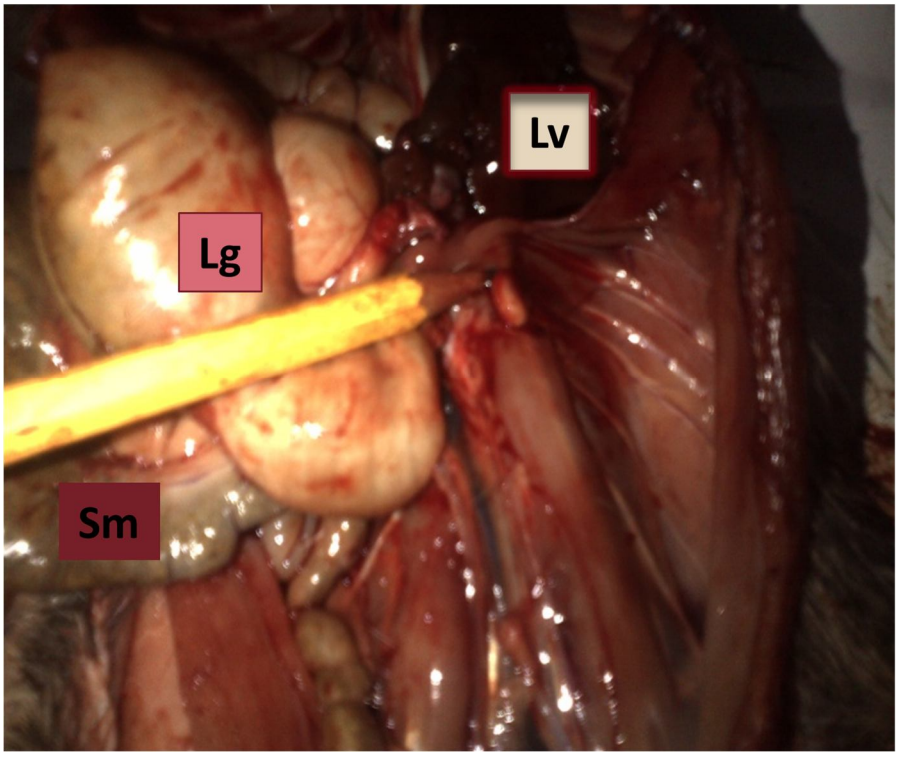


Plate XVI. Photograph of the mesenteric lymph node of a grasscutter being shown with the aid of a pencil. Also note, liver **(Lv)**, small intestine **(Sm)** and large intestine **(Lg)**.

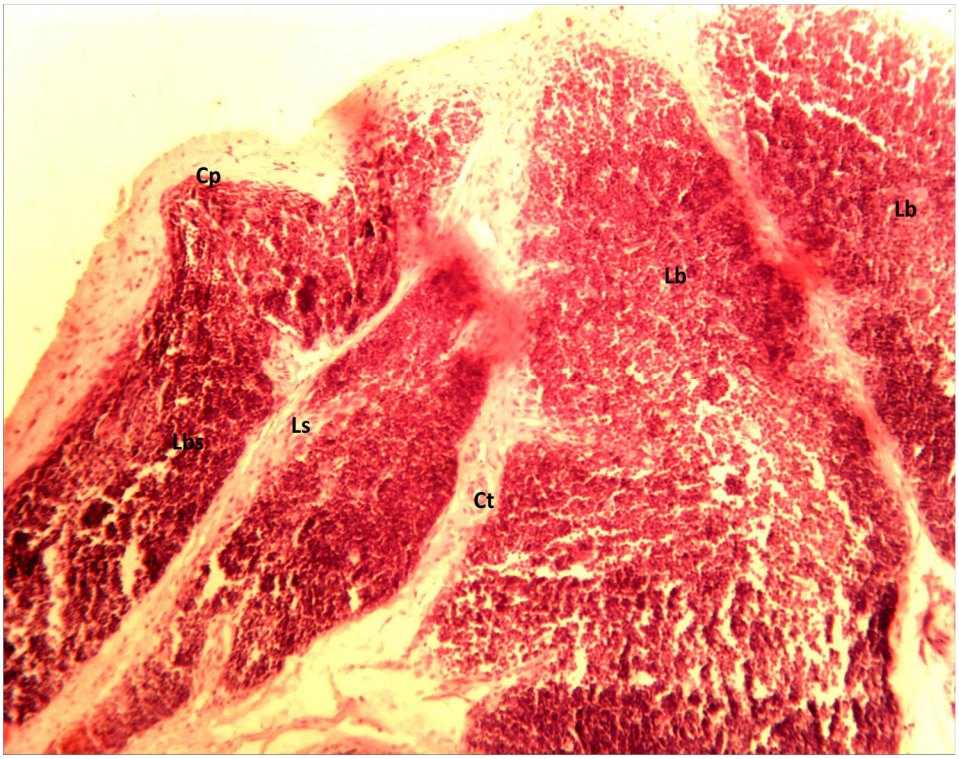


Plate XVII. Photomicrograph of a mesenteric lymph node of a three-month-old male grasscutter showing the capsule **(Cp)** the lobes **(Lb)**, separated by connective tissue septa **(Ct)** and lobules (**Ls**) which are subdivided by the lobular septa H & E X100.

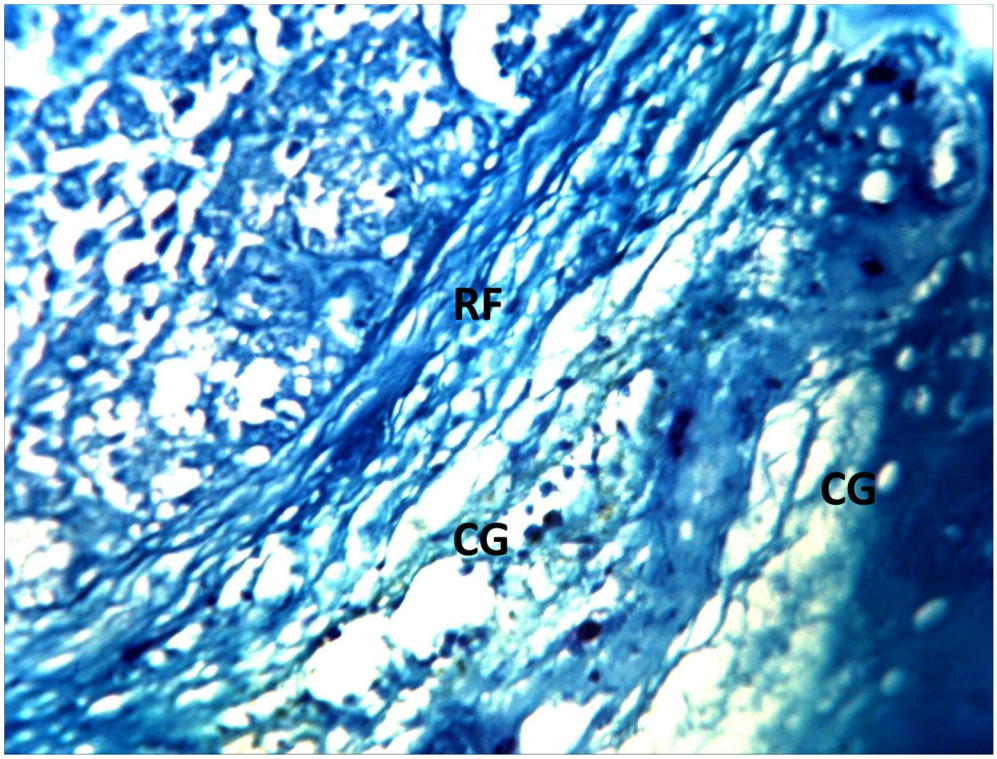


Plate XVIII. Photomicrograph of the mesenteric lymph node of a three-month-old female grasscutter showing collagen **(CG)** and reticular fibre **(RF)** framework of the lymphoid tissue. Rhodamin B-aniline-methylene blue X 250.

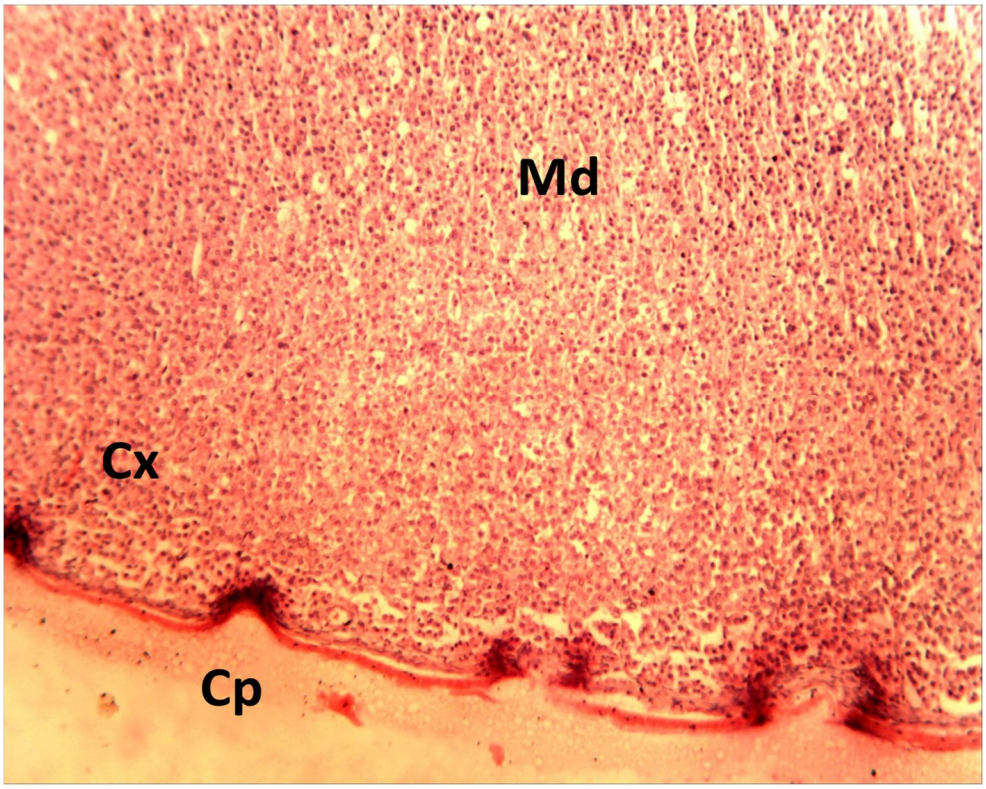


Plate XIX. Photomicrograph of the mesenteric lymph node of a five-month-old male grasscutter, showing the cortex **(Cx)**, medulla **(Md)**, the collagenous capsule **(Cp)**. Note absence of cortex-medulla distinction. H & E. X100.

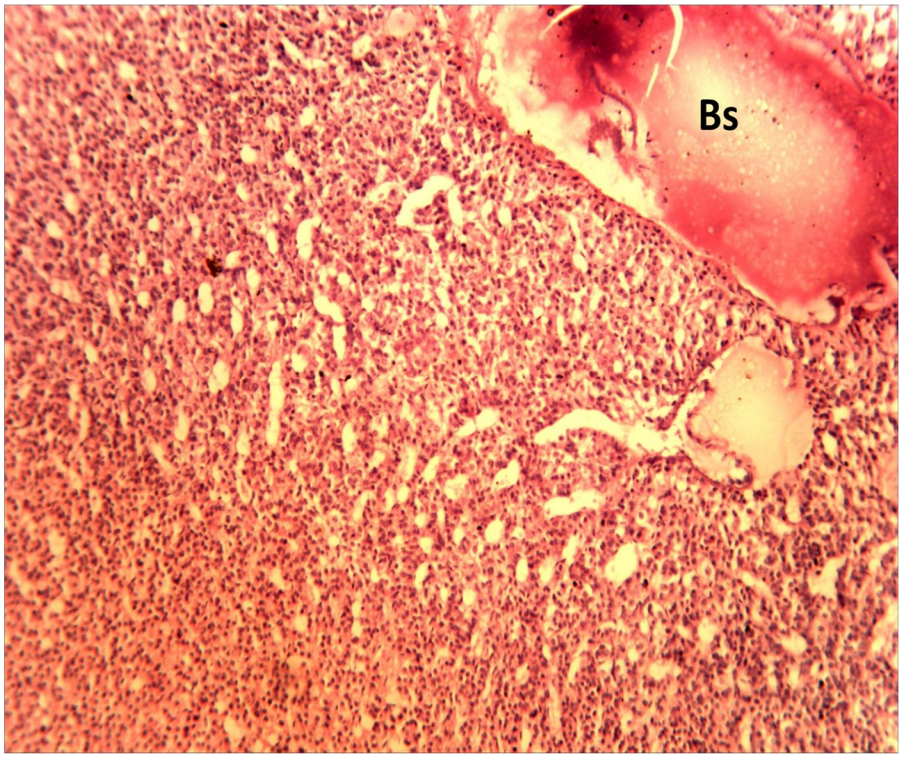


Plate XX. Photomicrograph of the mesenteric lymph node of a seven-month-old female grasscutter showing a blood space **(Bs)**. Note absence of cortex-medulla distinction. H & E. X100.

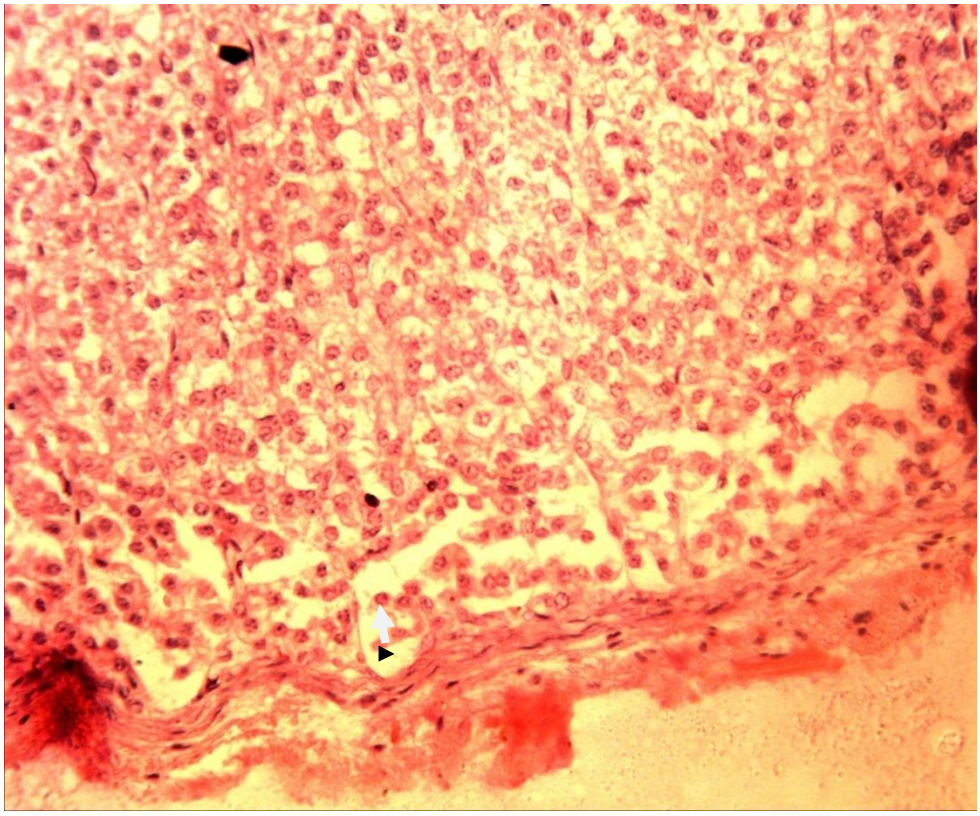


Plate XXI. Photomicrograph of the mesenteric lymph node in a seven-month-old male grasscutter. H & E X 250. Note, no cortex-medulla distinction here.

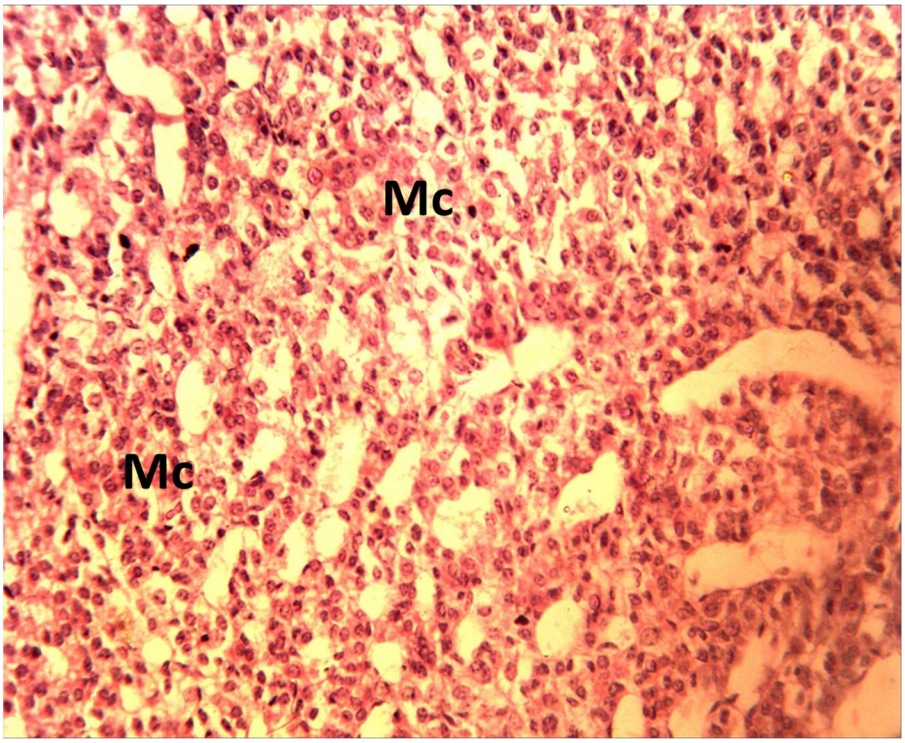


Plate XXII. Photomicrograph of the mesenteric lymph node of a seven-month-old female grasscutter showing the medullary cords **(Mc)**. Note absence of cortex-medulla distinction. H & E X100.

4.3 The Spleen

4.3.1 Gross observations

The spleen of the grasscutter was located at the left hypochondral region, adjacent to the greater curvature of the stomach within the omentum was ovoid in shape (Plate XXIII). The mean circumference was 4.20 ± 0.59 cm, 6.77 ± 0.30 cm, and 7.51 ± 0.15 cm at three, five and seven months respectively (Table 1). The spleen`was dark red in colour (Plate XXIII). The percentage ratio of splenic weight to body weight was 0.1% irrespective of their sexes or age. The only exception to this were the male grasscutters at five months of age which was 0.2% (Appendices 1,2,3).

4.3.2 Histology

The parenchyma of the spleen was observed to consist of two different tissues, the more abundant white pulp and the less abundant red pulp (Plates XXIV, XXVI and XXVIII). With rhodamine B-aniline methylene blue stain, elastic and reticular fibres were observed (Plate XXV). With H & E, the pulp, was observed to contain a few arteries found mostly at the germinal centres (Plate XXIV, XXVI and XXVIII). The germinal centre presented three layers; the germinal centre, followed by the cortical layer and then the marginal zone (Plate XXIV). The marginal zone of the spleen of grasscutter was large, not uniform in outline, but clearly demarcated from the Periarteriolar Lymphoid Sheath (Plate XXIV). Elastic and reticular fibres were observed within the tissue parenchyma (Plate XXV). Ellipsoids were scattered over the white pulp of the spleen (Plates XXIV). Haemosiderin deposits were found in the white pulp of some of the subjects (Plates XXVII, XXVIII and XXIX).

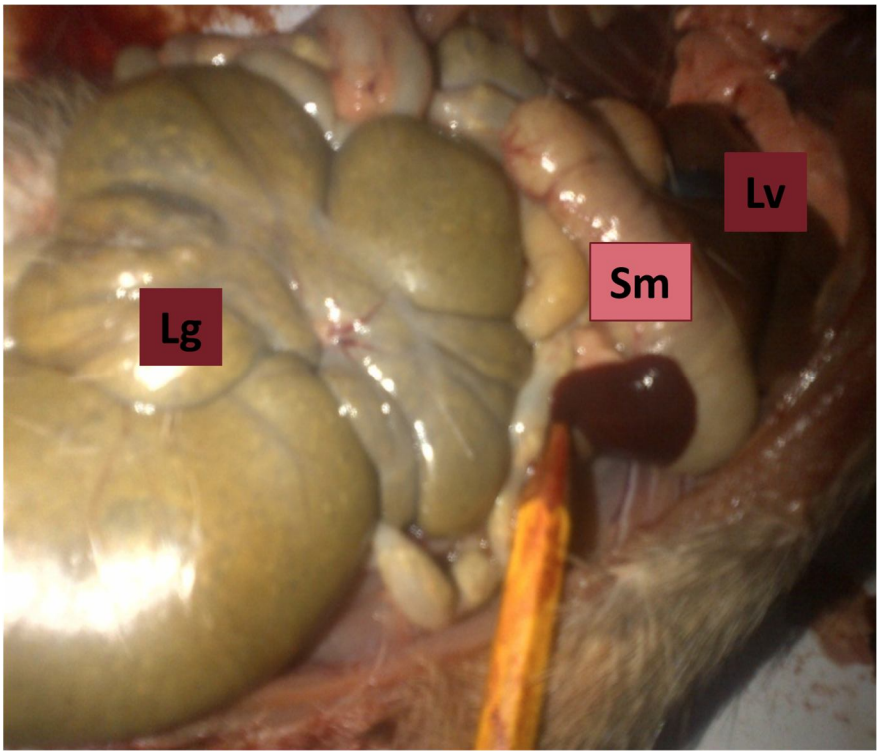


Plate XXIII. Photograph of the spleen of a grasscutter showing its location *in situ* by pointer. Also note, liver **(Lv)**, large intestine **(Lg)** and small intestine **(Sm).**

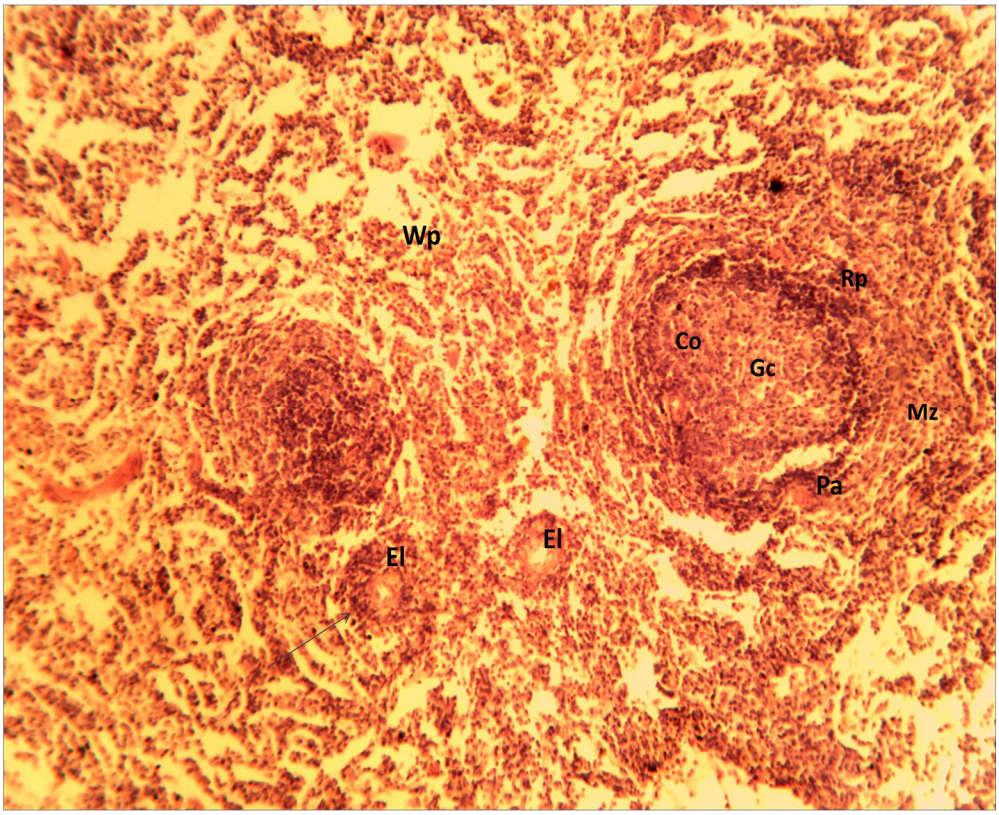


Plate XXIV. Photomicrograph of the spleen of a three-month-old female grasscutter showing the more abundant white pulp **(Wp)** and the less abundant red pulp **(Rp)**, ellipsoids (**El)**, germinal centre (**Gc**), coronary layer **(Co)**, marginal zone (**Mz**), pulp artery **(Pa)**. H & E X 250.

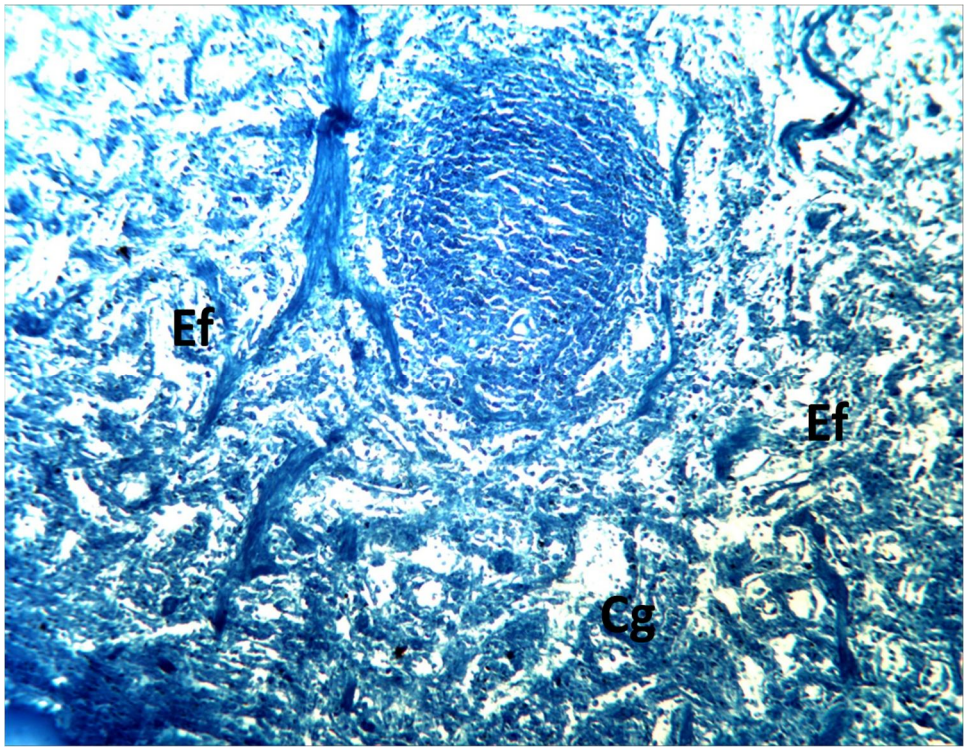


Plate XXV. Photomicrograph of the spleen of a three-month-old male grasscutter showing elastic fibres (**Ef)** and collagen (**Cg)**. RhodaminB-aniline- methylene blue X 250.

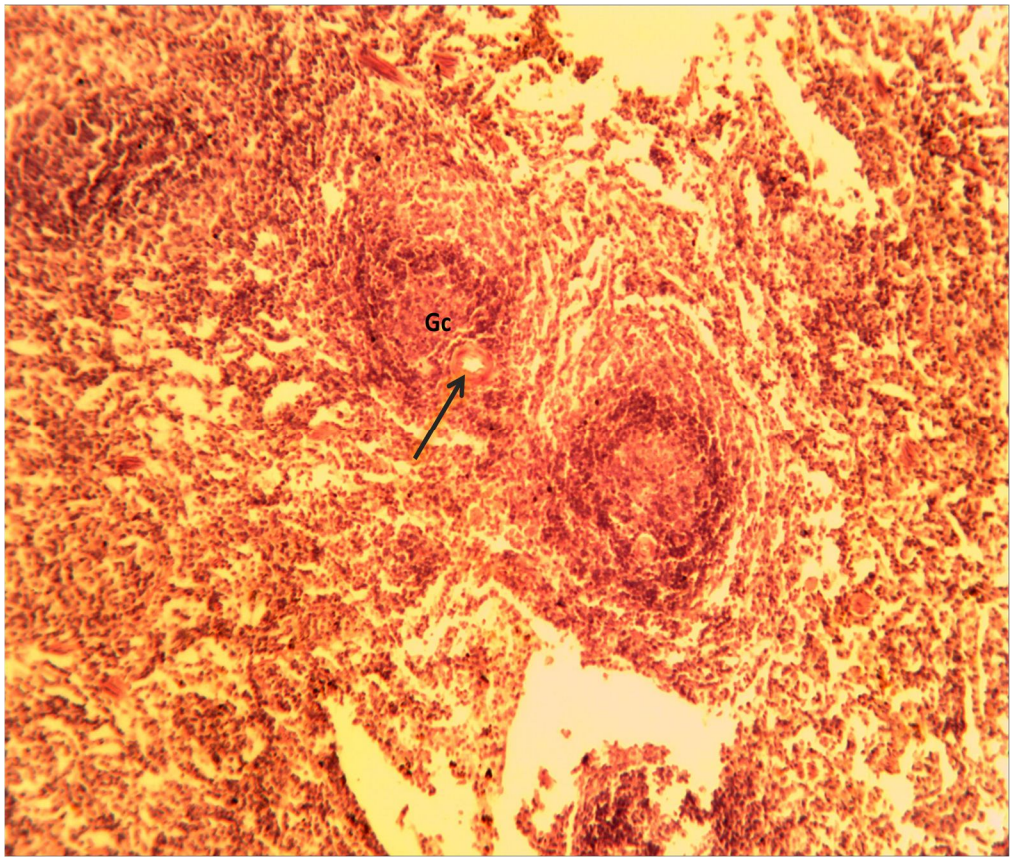


Plate XXVI. Photomicrograph of the spleen of a three-month-old female grasscutter. The arrow points to a pulp artery. Note Germinal centre (**GC)**. H & E X100.

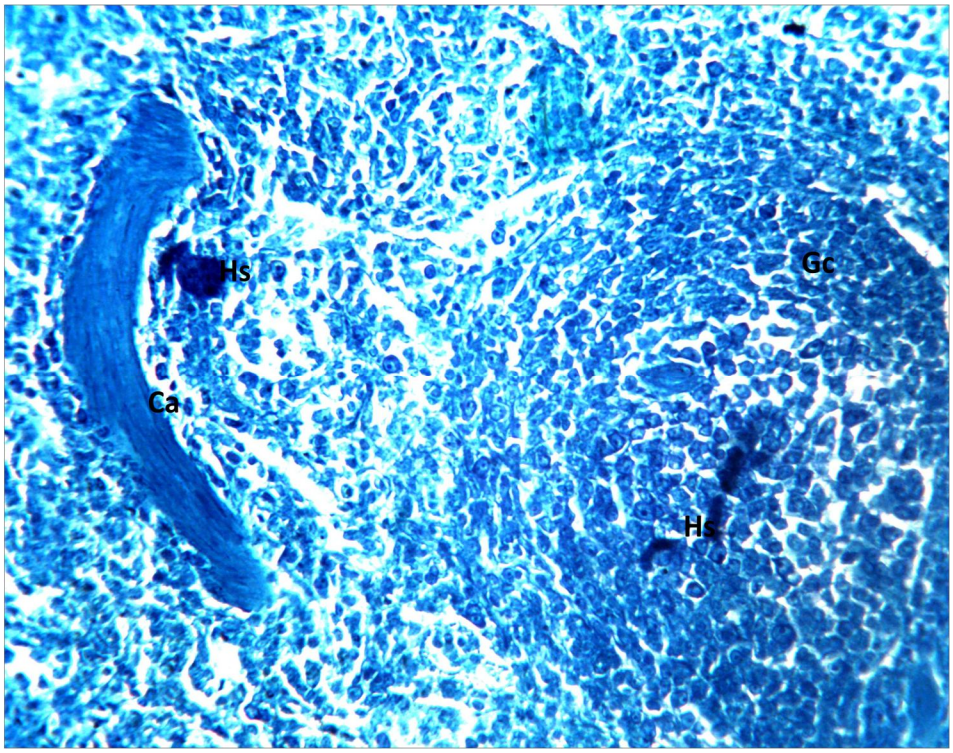


Plate XXVII. Photomicrograph of the spleen of a five-month-old female grasscutter showing germinal centre **(Gc)** and the pigment haemosiderin (**Hs)**. Rhodamine B-aniline-methylene blue X 250.

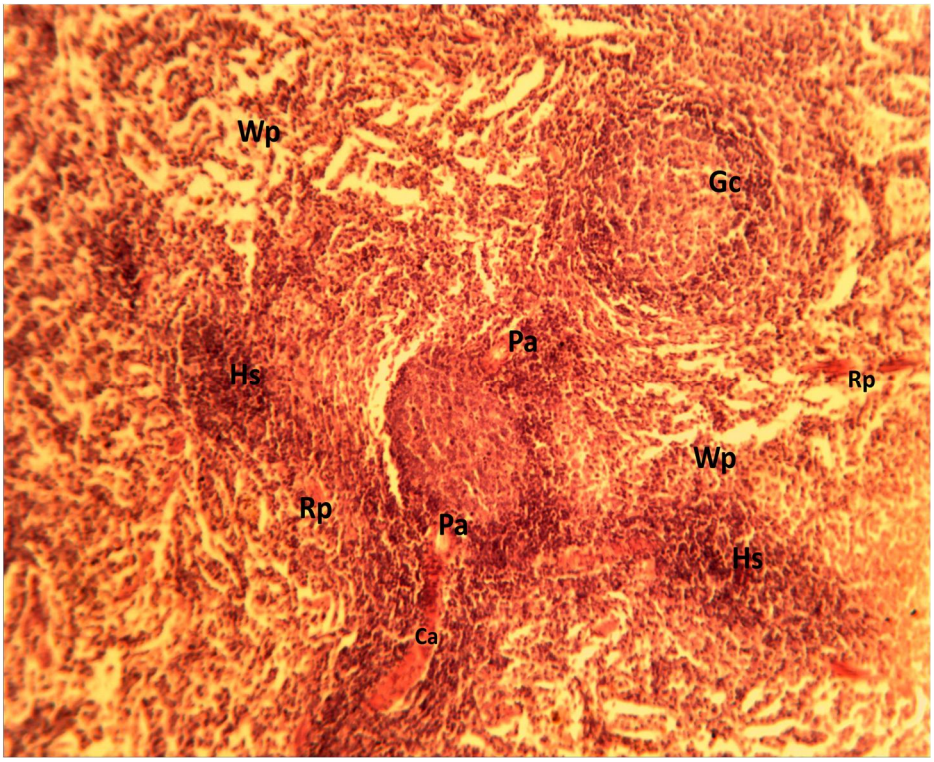


Plate XXVIII. Photomicrograph of the spleen of a five-month-old male grasscutter showing the pulp artery (**Pa)**, red pulp **(Rp)**, germinal centre **(Gc)**, haemosiderin (**Hs)**, central arteriole **(Ca)** and the white pulp **(Wp)**. H & E X 100.

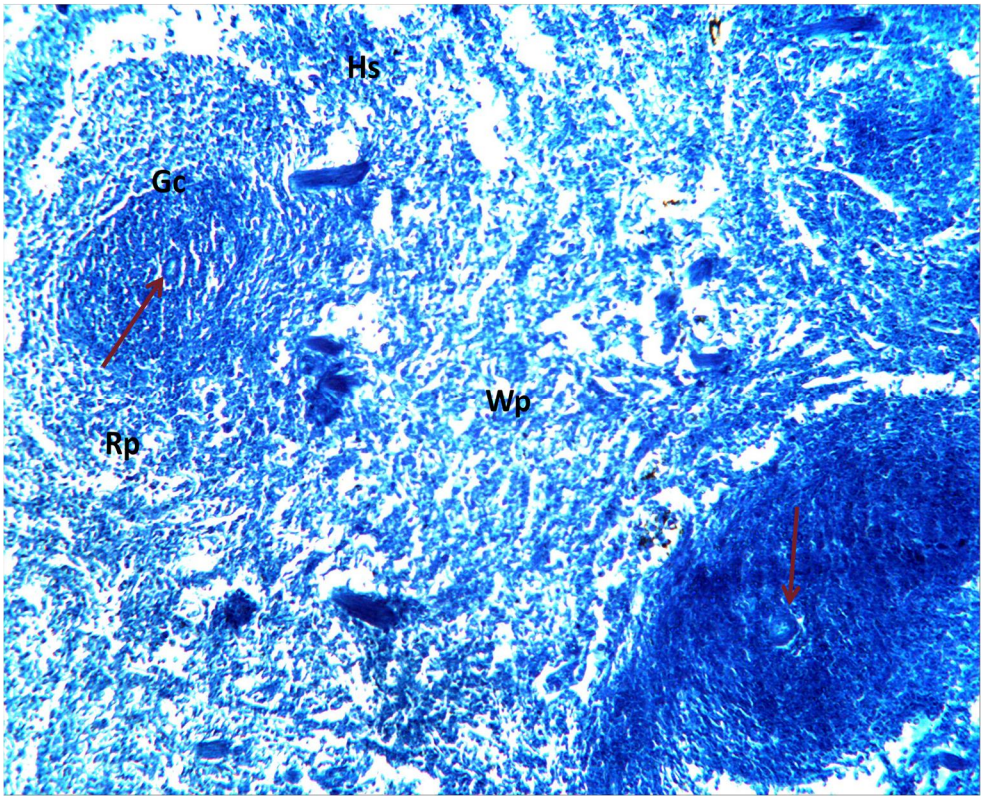


Plate XXIX. Photomicrograph of the spleen of a five-month-old female grasscutter showing the white pulp **(Wp)**, red pulp **(Rp),** germinal centre (**Gc)**, haemosiderin (**Hs)**, arrows point to pulp artery. Rhodamine B aniline methylene blue X100.

Table 1. Effect of age on body weight, weight and circumference of the thymus, spleen and the mesenteric lymph node of the grasscutter (mean ± SEM)

Parameter	Age of Grasscutter		
	3 months (n = 4)	5 months (n = 4)	7 months (n = 4)
Body weight (g)	415.0 ± 13.23[a]	940.0 ± 38.08[b]	1725 ± 85.39[c]
Thymic weight (g)	1.225 ± 0.048[a]	0.525 ± 0.019[b]	0.3025 ± 0.042[c]
Thymic circumference (cm)	5.330 ± 0.406[a]	2.218 ± 0.048[b]	0.4550 ± 0.017[c]
Mesenteric lymph node weight (g)	0.055 ± 0.005[a]	0.143 ± 0.034[b]	0.1600 ± 0.023[b]
Mesenteric lymph node circumference (cm)	0.363 ± 0.006[a]	0.552 ± 0.080[a,b]	0.6375 ± 0.058[b]
Splenic weight (g)	0.668 ± 0.094[a]	1.648 ± 0.358[b]	2.308 ± 0.059[c]
Splenic circumference (cm)	4.200 ± 0.584[a]	6.770 ± 0.305[b]	7.515 ± 0.152[c]

a, b, c = Mean values in the same row with different superscript letters are significantly different ($P < 0.05$)

Table 2. Effect of sex on body weight and weight and circumference of the thymus, spleen and the mesenteric lymph nodes of the grasscutter (mean ± SEM)

Parameter	Male (n = 6)	Female (n = 6)
Body weight (g)	1055 ± 243.2	998.3 ± 244.9
Thymic weight (g)	0.693 ± 0.200	0.675 ± 0.154
Thymic circumference (cm)	2.750 ± 0.931	2.585 ± 0.906
Splenic weight (g)	1.562 ± 0.344	1.520 ± 0.345
Splenic circumference (cm)	6.432 ± 0.646	5.892 ± 0.737
Mesenteric lymph node weight (g)	0.130 ± 0.031	0.1083 ± 0.023
Mesenteric lymph node circumference (cm)	0.550 ± 0.071	0.4850 ± 0.062

Mean values of the same parameter are not significantly different ($P < 0.05$) between the sexes.

Table 3. Correlation coefficients between age and some morphometric parameters of the grasscutter (n = 12).

Correlated Parameters	Pearson r
Age and body weight	0.9786****
Age and thymic weight	-0.9447****
Age and thymic circumference	-0.9677****
Age and splenic weight	0.8684***
Age and splenic circumference	0.8604***
Age and mesenteric lymph node weight	0.6902*
Age and mesenteric lymph node circumference	0.7402**

* = P < 0.05; ** = P < 0.01; *** = P < 0.001; **** = P < 0.0001

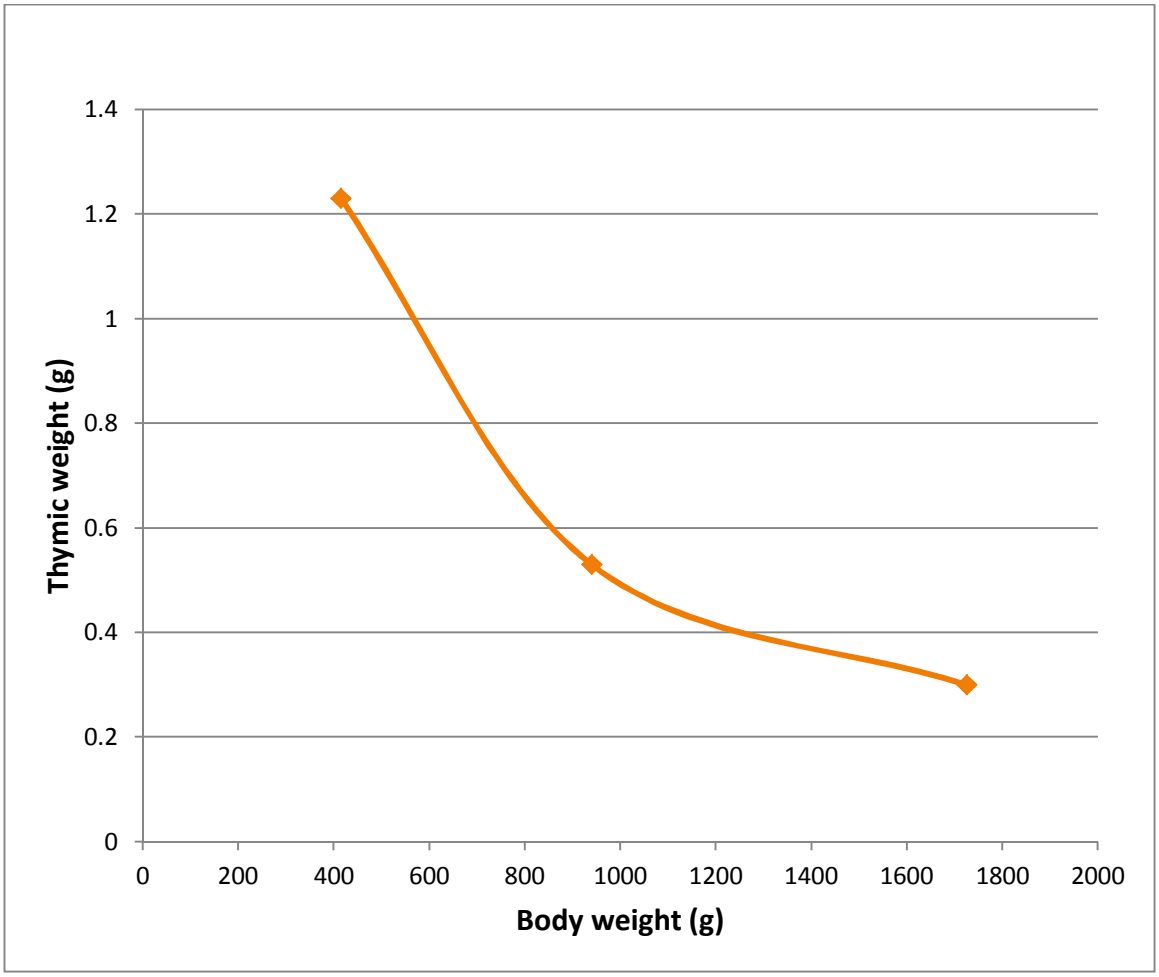


Fig. 1. Relationship between body weight (g) and thymic weight (g)

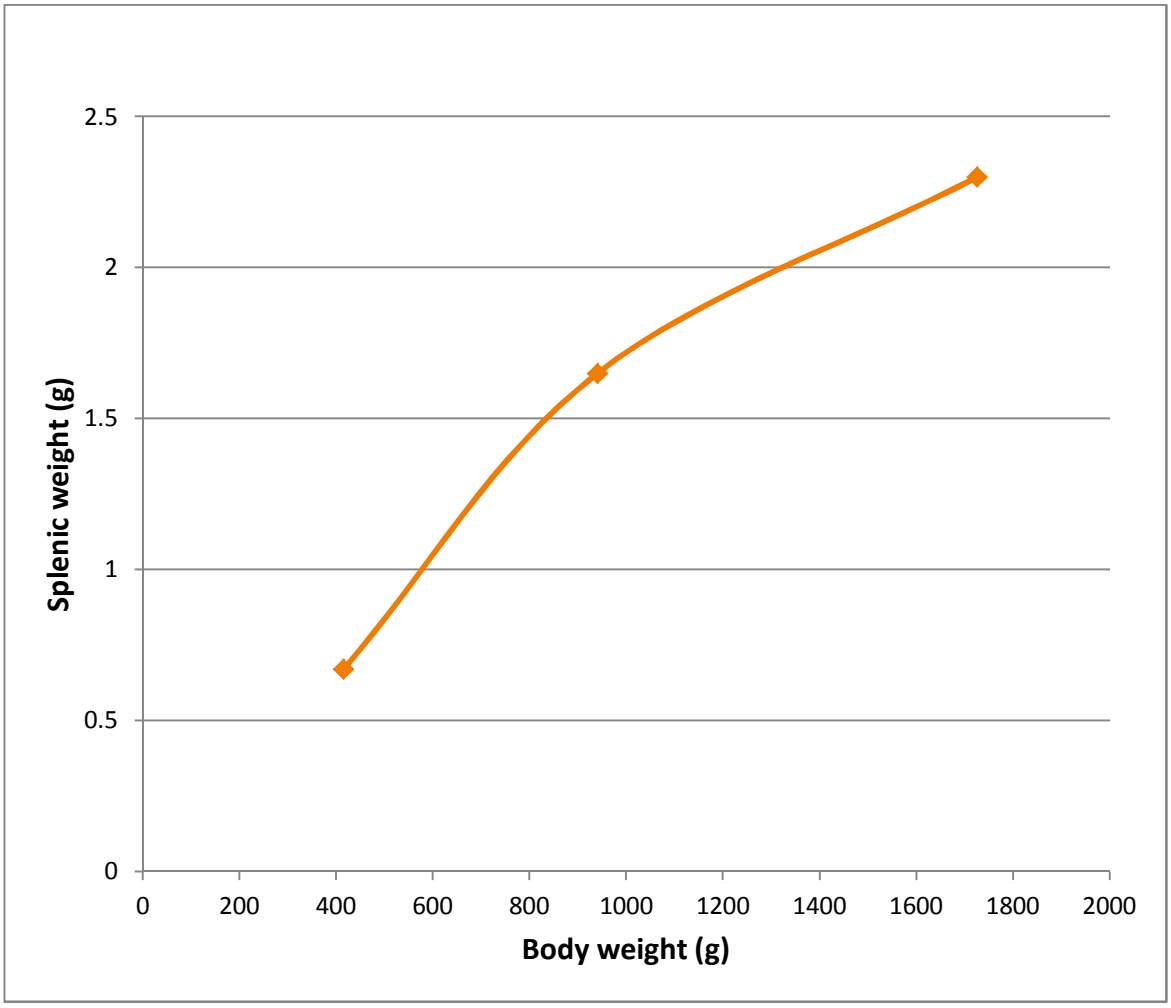


Fig. 2. Relationship between body weight(g) and splenic weight (g)

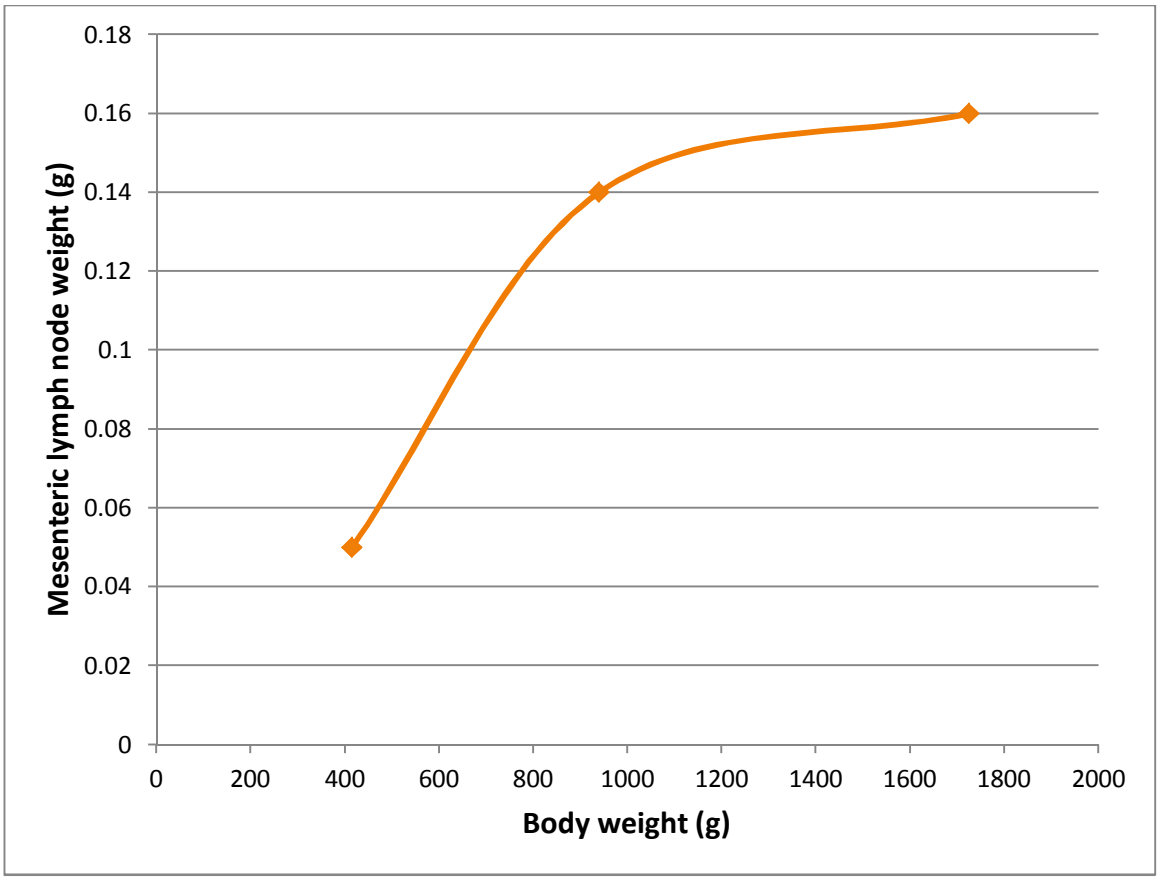


Figure 3. Relationship between body weight (g) and mesenteric lymph node weight (g).

CHAPTER FIVE

DISCUSSION

The decrease in size and weight of the thymus with increase in age observed in this study agreed with the findings of other researchers in other species of animals (Dougherty, 1952; Cowan and Smith, 1999; Gulmez and Alsan, 1999). This was attributed to the fact that lymphoid cells become depleted with age, reducing the volume and mass of the organ (Dasgupta *et al.*, 2013).

Previous studies in the bottlenose dolphin (*Tursiops truncatus)* by Cowan and Smith (1999) also showed a similar finding that the thymus loses lymphocytes progressively with increasing age and acquires a strong propensity to develop cysts from the epithelial cells which may replace it (Cowan and Smith, 1999). Dasgupta *et al*. (2013) also reported that immunological efficacy of the thymus can be directly attributed to the weight of the organ. It, therefore, means that as the weight of the thymus drops with advancing age, its immune competence also begins to decrease. It has been previously reported by Dasgupta *et al*. (2013) that the thymic and body weights in early life of mice had a correlation coefficient of 1. A histological investigation by Rankin (1954) on mice thymus during early maturity showed no correlation between body weight and weight of the thymus. These results have clearly shown that there are changes in the weight and function of the mammalian thymus as an animal advances in age.

The histologically darker cortex and lighter medulla of the grasscutter was similar to the finding in rats by Dijkstra and Sminia (1990) and Pearse (2006). In the ostrich, Song *et al*. (2012) reported that lobulations were not obvious, and the number of lobes per thymus were small,

whereas chicken thymus contained 14 lobes and duck thymus 10 lobes arranged like beads on a stringe (Song *et al*., 2012). With the aid of special staining (Rhodamine B aniline methylene blue stain), the elastic fibres were seen as twisted cords rich in collagen surrounded by the reticular fibres, without any barrier between the cortex and medulla. Goldstein and Whittigham (1966) found that the thymic medulla in guinea pig contained many Hassal's corpuscles which were occasionally cystic with even dispersion of small lymphocytes. Collagen plays a role in maintaining structural integrity and the elasticity of thymic tissues, and therefore, aids the secretory process (Rich and Whittaker, 2005).

At three months of age, the histology of the grasscutter thymus, showed the presence of many small lymphocytes, suggesting that it was very active. Small lymphocytes have been reported by Snell *et al.* (1961) to have a basic function of defending against foreign tissues. For those at five months, adipose tissue was gradually replacing the normal parenchyma, meaning that the process of involution had begun, and at seven months, the histological picture clearly showed marked replacement of the lymphoid tissue with adipose tissue. This observation agreed with what was reported by Dougherty (1952). The factors responsible for this involution among others include hormonal influences (Dougherty, 1952) where adrenocortical hormones were implicated as the most effective involution inducer. The stress of captivity can induce the release of these hormones in the captive-reared grasscutters resulting in "early" thymic involution. Frequent mating in the males have been reported to cause early involution of the thymus (Ernest *et al*., 1978). Adipose cells which usually surround the lobes of the thymus serve as energy source to the thymic lymphocytes and replace the thymic parenchyma in old age (Ernest *et al.*, 1978).

The mesenteric lymph nodes are located in the mesentery, appearing as minute off-white and bean-shaped structures. The nodes are distinguished from surrounding fat by their pearly gloss nature. This is similar to the finding of Dijkstra *et al.* (1990). The glossy nature of these nodes is the function of the rich supply of collagen fibres at the capsule and within the entire parenchyma as earlier stated in the result section.

The weight of the mesenteric lymph nodes did not show any specific proportion to body weight, but was observed to increase slightly with age. The size of the mesenteric lymph nodes of rodents vary depending on the specie and level of distention. A mesenteric lymph node faced with an antigenic challenge would be larger than that without a challenge. Histologically, the lymph nodes are composed of a capsule and parenchyma. The parenchyma tissue was not distinguishable into cortex and medulla. With Rhodamine B methylene blue stain, reticular meshwork of the mesenteric lymph nodes were observed to consist of thin bundles of collagen in the parenchyma and continued to the fibrous capsule. Ushiki *et al.* (1995) reported a similar finding in the mesenteric lymph nodes of rats, by using scanning electron microscope to study the reticular meshwork of the mesenteric lymph nodes of rats.

The reticular fibres were seen to be continous with the fibrillar sheaths of collagen as well as the capsule of the nodes. These fibres help and maintain the shape and intergrity of the mesenteric lymph nodes. The mesenteric lymph nodes of rodents produce tolerance to food antigens (Worbs *et al*., 2006) and are the first line of defense against pathogens that may be present in the type of food these rodents are fed with in captivity.

Grossly, the spleen of the grasscutter was observed to be similar in both appearance and location to what was described in rats and other rodent species (Valli *et al*., 2002; Cesta, 2006; Ibe *et al*.,

2010). In rats and mice the spleen has been reported to be ovoid in shape, dark-red in colour and located in the left cranial abdomen (Cesta, 2006). The spleen is bean-shaped in the alligator (Rooney *et al*., 2003) and guinea fowl (Onyeanusi, 2006). In dogs, the spleen is dark-red to blue-black in colour and dumb-bell shaped (Cesta, 2006). The spleen of dogs which ranges from large and dark-red to small and blue-black is due to its rapid contraction (Valli *et al*., 2002). In bottlenose dolphin, the spleen is greyish blue in colour and located in the left cranial abdomen (Cowan and Smith, 1995).

The percentage ratio of splenic weight to body weight was fairly constant at 0.1%, irrespective of the age. The exception to this were the male grasscutters at five months of age, in which the percentage ratio of splenic weight to body weight was 0.2%. In the rat, this ratio has been reported to be 0.2 % (Losco, 1992), and 0.3 % in the African giant pouched rat (Ibe *et al*., 2010). The varying proportion of the splenic weight to body weight among the rodents could be a compensatory mechanism for the spleen in relation to their habitat (Ibe *et al*., 2010). The lower splenic size in the grasscutter may be a compensation for its grassland habitat, where oxygen tension is very high and the spleen in grasscutter may conserve less blood than that of the rat and African giant pouched rat (Ibe *et al*., 2010).

Histologically, the splenic parenchyma is similar to what was reported in other rodents, being surrounded by a capsule, containing elastic fibres and some smooth muscle fibres. (Valli *et al*., 2002). The limited amount of smooth muscle fibres in rodents' splenic capsule makes rapid splenic contraction impossible. As a result, it appears to be dark red in colour consistently. Unlike the spleen of dogs that changes from dark red to blue black because of its rapid contraction(Valli *et al*., 2002). In the splenic parenchyma, the white pulp was observed to be more abundant than the red pulp. Based on this observation, the grasscutter spleen may be

classified as defensive rather than storage type. Those with abundant lymphatic tissue, few trabeculae and smooth muscle fibres are the defensive type, while those with less lymphatic tissue, abundant trabeculae and smooth muscle fibres are the storage ones (Banks, 1982). According to Cesta (2006), extra-medullary haematopoiesis is a common finding in rodent red pulp, whereas, the spleen in adult humans can only be reactivated to carry out haematopoiesis under severe haemolytic anaemia (Cormack, 1987). Most of the spleens studied had germinal centres, which stained faintly.

The germinal centres were large and had three distinct layers; the inner most which is the germinal centre proper, the middle coronary layer and the outer most marginal zone, and as the germinal centre continue to proliferate it pushed the pulp arteries off the side. The marginal zone of the spleen of grasscutters was large, not uniform in outline but clearly demarcated from the periarteriolar lymphoid sheath (PALS). Similar reports have been documented for the rat spleen, where the marginal zone is large, clearly demarcated from the PALS and somewhat uniform in outline (Cesta, 2006). The marginal zone is a unique region of the spleen and situated at the interface of the red pulp and follicles. Being located at a strategic point where it comes into contact with blood entering the spleen, it screens the systemic circulation for antigens and pathogens as the blood passes through it. The role of the marginal zone in antigen processing has also been documented (Kuper *et al.*, 2002; Mebius and Kraal, 2005). A pulp artery and vein were noticed consistently just by the side of each germinal centre. As reported earlier by Cesta (2006), the spleen is a highly vascular organ which suggests its role as a blood filter. Central arterioles were also present in some of the grasscutter spleens studied. Germinal centres represent a reactive state and are not always present depending on the state of an animal (Nakamine *et al*., 1992).

With rhodamine B –aniline-methylene blue stain, reticular fibres were prevalent at the capsule and sparsely in the pulp region of the spleen of the grasscutters. Collagen was clearly seen to be one of the major composition of these fibres. Saito *et al* (1998) studied the reticular fibres in spleen of mammals and has described the reticular cells of being composed of collaginous and elastic fibres, microfibrils, reticular cell basal laminae and unmyelinated adrenergic nerve fibres. The reticular cells are considered to play a role in splenic contraction (Saito *et al*., 1998).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From this study, it is conclusive that:

1. The histology of the thymus of the grasscutters reveal the Hassal's corpuscle at three and five months of age, which is a rare finding in other species of rodents.
2. The histology of the spleen of the grasscutter reveals the presence of germinal centres at at three, five and seven months of age
3. The percentage splenic to body weight ratio is 0.1 %
4. The histology of the mesenteric lymph nodes of the grasscutter lacks cortex-medulla distinction at three, five and seven months of age.

6.2 Recommendations

In order to build on this foundation of research, the following are the recommendations;

1. Hormonal assays for the evaluation of the effects of cortisol hormone on the lymphoid organs of grasscutters will have to be carried out.

2. Also, the use of anti-stress remedies such as the administration of vitamin C for a period of time to the grasscutters prior to commencement of research is also recommended. This will give more information about the effect of stress on these lymphoid organs.

REFERENCES

Abboud, C. N. and Lichtman, M. A. (*2001*). *Structure of the Marrow and the Haematopoietic Microenvironment, In: Williams' Haematology,* editions: Beutler E., Lichtman M. A., Coller, B. S., Kipps, T. J. *and* Seligsohn, U. *(*McGraw-Hill, New York*),* **6,** *Pp* 29–58.

Abioye, F. O., Uda, A. C., Opara, M. N., Aju, P. C. and Onyema, M. C. (2008). Adaptability of grasscutter *(Thryonomys swinderianus)*. *Livestock Resource,* 14 **:** 8-13.

Adamu, E. K., Alhassan, W. S. and Nelson, F. S. (1993). A survey on basic management practices in grasscutter (*Thryonomys swinderianus*) farming in Southern Ghana. *In*: *Annual Report of the Animal Research Institute, Ghana* Pp 220-234.

Addo, P. G. (1997). Detection of mating, pregnancy and imminent parturition in the grasscutter (*Thryonomys swinderianus*) *Livestock Resource,* 14: 8-13

Adu, E. K., Alhassan, W. S., Nelson, F. S. (2000) Smallholder farming of the greater cane rat, *Thryonomys swinderianus,* Temminck, in southern Ghana: a baseline survey of management practices. *Tropical Animal Health and Production,* 31(4) : 223-232.

Ajayi, S. S. (1971). Wild life as a source of protein in Nigeria; Some priorities for development. *Nigerian Field,* 36 **:** 115-127.

Asibey, E. O. A. (1974). The grasscutter, *Thryonomys swinderianus,* Temminck-1827, in Ghana. *Symposium of the Zoological Society of London,* 34: 161-170.

Asibey, E. O. A. and Addo, P. G. (2000). Grasscutter, promising animal for meat production. *In*: Turnham, D. (Ed.). *African Perspectives, Practices and Supporting Development. Scandinavian Seminar Policies College, Denmark, in Association with Weaver Press, Harare, Zimbabwe,* pp 120-128.

Askonas, B. A. and Lehner, T. (1981). Immune function in infectious diseases. *Journal of Medicine,* 74 pp 382-399.

Balogh, P., Horvath, G., and Szakal, A. K. (2004). Immunoarchitecture of distinct reticular fibroblastic domains in the white pulp of mouse spleen. *Journal of Histochemistry and Cytochemistry,* 52 : 1287–1298.

Banks, K. L. (1982). Host defense in the newborn animal. *Journal of the American Veterinary Medical Association,* 181: 1053-1056.

Baptist, R. and Mensah, G. A. (1986). Benin and West Africa: The cane rat farm, animal of the future. *World Animal Revolution,* 60**:** 2-6.

Baylet, R. and Van Riel, S. (1971). *Cricetomys gambianus*, nouvel hôte de conservation de leptospires. *Médécine d'Afrique Noire,* 18 (11): 803-805.

Belisle, C. and Sainte-Marie, G. (1981). Topography of the deep cortex of the lymph nodes of various mammalian species. *Anatomy Record,* 201: 553-561.

Bobryshev, Y. V. and Reginald, L. S. A. (2001). S-100 Positive cells in human arterial intima and in atherosclerotic lesions. *Cardiovascular Resource,* 29: 689–696.

Brandtzaeg, P. (2010). Function of Mucosa Associated Lymphoid Tissue in antibody formation. *Immunological Investigations,* 39(4-5):303–355.

Burkitt, H. G., Young, B. and Heath, J. W. (1993). Anatomy of the lymphoid organs: *Wheater's Functional Histology*, 3rd edition, New York: Churchill Livingstone. pp. 211-213.

Casteleyn, C. (2010). Morphological characteristics of the ovine tonsils—a study of their volume and surface area, lymphoid tissue organization, lining epithelia and vascular architecture Ph.D. thesis, Faculty of Veterinary Medicine, Ghent University. Pp 6.

Carlsen, H. S., Baekkevold, E. S., Johansen, F. E., Haraldsen, G. and Brandtzaeg, P. (2002). B cell attracting chemokine 1 (CXCL13) and its receptor CXCR5 are expressed in normal and aberrant Gut Associated Lymphoid Tissue. *Gut,* 51: 364-371.

Cesta, M. F. (2006). Normal structure, function and histology of the spleen. *Toxicology and Pathology*, 34: 455- 465.

Chen, C. and Hirsch, J. G. (1972). The effects of mercaptoethanol and of peritoneal macrophages on the antibody-forming capacity of non adherent mouse spleen cells *in Vitro*. *Journal of Experimental Medicine*, 136 : 602-623.

Cormack, D. H. (1987). Histology of the lymphoid organs. *Ham's Histology,* 9th edition., J. B. Lippincott, Philadelphia, Pp 255-261.

Cowan. D. F. and B. E. Curry. (2002). Histopathological assessment of dolphins necropsied onboard vessels in the eastern tropical Pacific tuna fishery. Administrative Report 02-24C, NMFS, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037. Pp 455-468.

Cowan, D. F. and Smith T. L. (1995). Morphology of complex lymphoepithelial organs of the anal canal (`anal tonsil ') in the bottlenose dolphin, *Tursiops truncatus*. *Journal of Morphology* 223 : 263-268.

Cowan, D. F. and Smith, T. L. (1999). Morphology of lymphoid organs of the bottlenose dolphin (*Tursiops truncates). Journal of Anatomy,* 194 **:** 505-519.

Croitoru, K. and Bienenstock, J. *(*1994*).* Characteristics and Functions of Mucosa-associated Lymphoid Tissue*, In: Handbook of Mucosal Immunology, editions* Ogra, P. L.*,*

Mestecky, J., Lamm, M. E., Strober, W., McGhee, J. R. *and* Bienenstock, J. Academic Press, San Diego, *pp* 141–151.

Dasgupta, H., Bhattacharya, K., Majumda, S., Gupta, P. N. and Bhattacharya, S. (2013). A study on morphological and histological relations between thymus gland and body hair. *Indian Journal of Applied and Medical Research,* 6 (2) : 483-500.

Debard, N., Sierro, F. and Kraehenbuhl, J. P. (1999). Development of Peyer's patches, follicle-associated epithelium and M cell; lessons from immunodefecient and knockout mice, *Journal of Immunology* 11 : 183-191

Dijkstra, C. and Sminia, T. *(*1990*).* Normal anatomy, histology, immunohistology, ultrastructure of rat, *In:* Haematopoietic System. *Monographs on pathology of laboratory animals,* Springer-Verlag, Berlin, *pp* 249–256.

Dijkstra, C., Kamperdijk, E. W. A. and Veerman, A. J. P. (1990). Normal anatomy, histolog, immunohistology and ultrastructure, lymph node of rat. *Monographs on Pathology of Laboratory Animals,* Pp 129-136.

Drury, R. A. and Wallington, E. A. (1980). Carleton's Histology Technique, 4th Edition, Oxford University Press, Pp 45-51.

Dougherty, T. F. (1952). Histological changes in the mouse thymus following administration with corticosteroid hormone. *Physiological Review*, 32 : 379-388.

Eben, A. B. (2004). Grasscutter: Importance, habitat, characteristics, feed and feeding, breeding and diseases. *Centre for Biodiversity Utilization and Development (CBUD,)* Kumasi Ghana. Pp.1-4

Ernest, E. L., Nancy C. G., Fresia G., Lawrence A. M. and Robert G. (1978). Energy metabolism in thymic lymphocytes in normal and leukaemic AKR mice. *Cancer Research,* 38: 1113-1119.

Fujimura, Y. (2000). Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. *Virchows Archive,* 436:560–566.

Gretz, J. E., Anderson, A. O. and Shaw, S. *(*1997*).* Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunology Review, 156:11–24.*

Gulmez, N. and Alsan, S. (1999). Histological and histometrical investigations on Bursa of Fabricius and Thymus of Native Geese. *Tropical Journal of Veterinary and Animal Sciences,* 23 **:** 163-171.

Haley, P. J. (2003). Species differences in the structure and function of the Immune System. *Toxicology,* 188:49–71.

Hanifin, J. M ., and Cline, M. J. (1970). Human monocytes and macrophages : interactions with antigen and lymphocytes . *Journal of Cell Biology*, 46:97-111.

Hoffman, R., Benz, E. J., Shattil, S. J., Furie B, Cohen HJ, Silberstein, L. E. and McGlave, P. *(*2000*)*. Basic Principles and Practice. *Hematology,* 3*:*66-72.

HoganEsch, H. and Hahn, F. F. (2001). Anatomy, Development, and Age-related Changesof the thymus. In: *Pathobiology of the Aging Dog* (Mohr, U., Carlton, W. W., Dungworth, D. L., Benjamin, S. A. and Capen, C. C. editors.), Iowa State University Press, Ames. *The Lymphoid Organs,* 1:127–135.

Husby, S., Mestecky, J., Moldoveanu, Z., Holland, S. and Elson, C.O. (1994). Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *Journal of Immunology,* 152: 4663–4670.

Ibe, C. S., Onyeanusi, B. I., Salami, S. O., Ajayi, I. E. and Nzalak, J. O. (2010). On the structure of the spleen in the african giant pouched rat (Cricetomys gambianus, water house 1840) *Veterinary Research,* 3 : (4) :70-74.

Jain, N. J. *(*1986*)*. The hematopoietic system In: *Schalm's Veterinary Haematology*, *(Lea and Febiger, Philadelphia, PA),* 4*: pp* 350–387.

Jori, F., Cooper, J. E. and Casal, J. (2001). A survey of pathology on captive cane rats (*Thryonomys swinderianus)*. *The Veterinary Record*, 148 : 624-628.

Jori, F**.** and Cooper, J. E. (2001). Spontaneous neoplasms in captive cane rats *(Thryonomys swinderianus*). *Veterinary Pathology*, 38 (5) : 102-104.

Jump, R. and Levine, A. D. (2002). Murine Peyer's patches favour development of an IL-10 secreting region of T cell population, *Journal of Immunology* 168 : 6113-6119

Jung, A. G., Labarrera, C., Jansen, A. M., Qvortrup, K., Wild, K., Kjaeruff, O. (2010). The submergence tolerance regulator Sub 1A mediates stress responsive expression of AP2/ ERF transcription factors. *Plant Physiology* 152:3 pp 1674-1692.

Junqueira, L. C., Carneiro, J. and Kelley, R. O. (1998). Hematopoiesis. In: (Junqueira, L. C., J. Carneiro, R. O. Kelley, Eds.) *Basic Histology* 9th ed. Appleton and Lange, USA. Pp. 234-247.

Katz, D. H., and Unanue, E. R. (1973). Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. *Journal of Experimental Medicine,* 137:967-975 .

Kelly, R. H. (*1975*). Functional anatomy of lymph nodes I. The paracortical cords. *International Archive of Allergy and Applied Immunology,* 48*:*836*–*849.

Kiyono, H. and Fukuyama, S. (2004). Nasopharynx Associated Lymphoid Tissue versus Peyer's Patch Mediated Mucosal Immunity. *National Revolutionary Immunology,* 4: 699-710.

Kuper, D. F., de Heer, E., Van Loveren, H., and Vos, J. G. (2002). Immune System. *In*: Handbook of Toxicologic Pathology (Haschek, W. M., Rousseaux, C. G. and Wallig, M. A. editors), Academic Press, San Diego, 2 : 585–646.

Liang, H., Baudouin, C., Labbe, A., Riancho, L. and Brignole-Baudouin, F. (2012). *Conjunctiva-Associated Lymphoid Tissue (CALT) Reactions to Antiglaucoma Prostaglandins with or without BAK-Preservative in Rabbit. Acute Toxicity Study.* Plus One, **7**(3) : 455-512.

Linterman, M. A., Rigby, R. J., Wong, R. K., Yu D, Brink, R., Cannons, J. L. (2009). Follicular helper T cells are required for systemic autoimmunity. *Journal of Experimental Medicine* 206: 561–576.

Losco, P. (1992). Normal Development, Growth, and Aging of the Spleen. *In*: Pathobiology of the Aging Rat. Mohr, U., Dungworth, D. L. and Capen, C. C. (Editions), ILSI Press, Washington, D.C. 1 Pp 75–94.

Mackay, C. R.(2001) Chemokines: Immunology's high impact factors. *National Journal of Immunol*ogy, 2**:** 95–101.

Mackay, I. and Rosen, F., (2000). Innate immunity. *New England Journal of Medicine*, 343:338–344.

Magnusson, G. and Majeed, S. (1978). Histochemichal study of mesenteric lymph nodes in old rats. *Laboratory Animals,* 12 : 99-102.

Malekani, M., Kumar, V., Pandey, V. S., (1994). Hepatic capillariasis in edible Cricetomys spp. (Rodentia : Cricetidae) in Zaïre and its possible public health implications. *Annals of Tropical Medicine and Parasitology*, 88 (5) : 569-572.

Matzinger, P. (2002). The danger model: a renewed sense of self. *Science,* 296: 301–305.

Mebius, R. E., and Kraal, G. (2005). Structure and function of the spleen. *National Review Immunology,* 5: 606–616.

Mosier, D. E. (1969). Cell interactions in the primary immune response in vitro : a requirement for specific cell clusters. *Journal of Experimental Medicine*, 129 :351-366 .

Mosier, D. E., and Pierce C. W. (1972) . Functional maturation of thymic lymphocyte populations in vitro . *Journal of Experimental Medicine*, 136:1484-1510.

Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. and Coffman, R. L. (2005). Two types of murine helper T cell clone I. Definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology,* 175: 5–14.

Muzio, N., Polentarutti, N., Bosisio, D., Prahlada, M. K. P. and Mantovani, A. (2000). Toll-like receptors: A growing family of immune receptors that are differentially expressed and regulated by different leukocytes. *Journal of Leukocyte Biology* , 67: 450–56.

Nakamine, H., Nagata, S., Yonezawa, M. and Tanaka, Y. (1992) The whale (Odontoceti) spleen: a type of primitive mammalian spleen. *Acta Anatomica Nippon,* 67 : 69-81.

Nolte, M. A., Hamann, A., Kraal, G., and Mebius, R. E. (2002). The strict regulation of lymphocyte migration to splenic white pulp does not involve common homing receptors. *Immunology,* 106: 299–307.

National Research Council (NCR), (USA) (1991). *Microlivestock.* Luthe-Kwnown small animals (grasscutter) with promising economic future. National Academy Press, Washington, D.C. Pp. 449-453.

Ntikamoa-Baidu, Y. (1998). Sustainable use of Bush meat Wild life Development plan:(1998 – 2003). *Wildlife Department, Accra*, Vol. 6, pp. 78.

Oboegbulem, S. I. and Orowonko, I. (1990). Salmonellae in the African great cane rat *(Thryonomys swinderianus)*. *Journal of Wildlife Diseases,* 26: 119-121.

Onyeanusi, B. I. (2006). The guinea fowl spleen at post hatching and embryonic periods. *Anatomia, Histologia, Embryologia,* 35: 3 pp 140-143.

Opara, M. N. and Fagbemi, B.O. (2010). Patho-physiological effects of experimental *Trypanosoma congolense* and *Trypanosoma vivax* infections in the grasscutter (*Thryonomys swinderianus*, Temminck). Science, 8: 88-101.

Palmer, M. T. and Weaver, C. T. (2010). Autoimmunity: increasing suspects in the CD4+ T cell line up. *National Immunology,* 11: 36–40.

Pearse, G. (2006). Normal structure, function and histology of the thymus. *Toxicologic pathology,* 34: 504-514.

Pich, S. and Peters, K. J. (1990). *Possibilities for using grasscutter (Thryonomys swinderianus) for meat production in Africa. Institute of Animal Husbandry and Genetics.* University of Totingen. Pp 46.

Picker, L. J. and Siegelman, M. H. *(*1999). Lymphoid tissues and organs*, In: Fundamental Immunology, (Lippincott-Raven, Philadelphia, PA),* 4 *:* 479–531.

Rankin, J. J. (1954). Lack of correlation between thymus and body weights during adolescence and early maturity. *Journal of physiology,* 125 : 316-325.

Rich, L. and Whittaker, P. (2005). Collagen and picrosirius red staining: a polarized light assessment of fibrillar hue and spatial distribution. *Regular Paper,* 24 : 320-328.

Rooney, A. A., Bermudez, D. S. and Guillettee, L. J. (2003). Altered histology of the thymus and spleen in contaminant exposed juvenile American alligators. *Journal of Morphology,* 256: 349-359.

Rosevear, D. R. (1969). The rodents of West Africa. Trustees of the British Museum (National 1-list) N0 677, London). Pp 50-61.

Rosenthal, A. S. (1980). Regulation of the immune response; role of the macrophage. *National England Journal of Medicine,* 303**:**1153-1178.

Saito, H., Yokoi, Y., Watanabe, S., Tajima, J., Kuroda, H., Namihisa, T. *(*1998*)*. Reticular meshwork of the spleen in rats studied by electron microscopy. *American Journal of Anatomy,* 181*:* 235*–*252.

Satodate, R., Tanaka, H., Sasou, S., Sakuma, T., and Kaizuka, H. (1986). Scanning electron microscopical studies of the arterial terminals in the red pulp of the rat spleen. *Anatomy Record,* 215: 214–216.

Schermer, S. *(1967). The Blood Morphology of Laboratory Animals (Davis, Philadelphia, PA),* ***3****, pp 43–49.*

Schmidt, E. E., MacDonald, I. C., and Groom, A. C. (1985). Microcirculation in mouse spleen (nonsinusal) studied by means of corrosion casts. *Journal of Morphology,* 186: 17–29.

Shimoda, M., Nakamura, T. and Takahashi Y. (2001). Isotype-specific selection of high affinity memory B cells in nasal cavity associated lymphoid tissue. *Journal of Experimental Medicine,* 194:1597–1607.

Simon, H. B. and Sheagren, J. N. (1972). Migration inhibitory factor and macrophage bactericidal function. *Journal of immunology,* 6:101-118.

Snell, G.D., Winn, H.J., Stimpfling, J.H. and Parker. S.J. (1961). Depression of antibody of the immune response to homografts and its role in immunological enhancement. *Journal of Experimental Medicine* 112: 2 pp 293-314.

Song, H., Peng, K., Li, S., Wang, Y., Wei, L., Tang, L. (2012). Morphological characterisation of the immune organs in ostrich chicks. *Turkey Journal of Veterinary and Animal Science,* 36 (2) : 89-100.

Unanue, E. R. (1978). The regulation of lymphocyte functions by the macrophage. *Immunologic Review,* 40 : 227-229.

Ushiki, T., Ohtani, O. and Abe, K. (1995). Scanning electron microscopic studies of reticular framework in the rat mesenteric lymph node. *Anatomy Record,* 241 (1) : 113-122.

Valli, V. E., McGrath, J. P., and Chu, I. (2002). Hematopoietic System. *In*: Handbook of Toxicologic Pathology (W. M. Haschek, C. G. Rousseaux and M. A. Wallig, editions.), Academic Press, San Diego, 2 : 647–679.

Van Veen, S., Shonekan, R. A. O. and Fabiyi, J. P. (1974). A host-parasite checklist of helminth parasites of domestic animals in Northern Nigeria. *Bulletin for Animal Health and Production African Journal*, 23 : 269-288.

Waldron, J. A., Horn, R. G. and Rosenthal, A. S. (1973). Antigen-induced proliferation of guinea pig lymphocytes *in vitro*. Obligatory role of macrophages in the recognition of antigen by immune T-lymphocytes . *Journal of Immunology,* 111:58-65.

Weiss, A. (2010). The right team at the right time to go for a home run: tyrosine kinase activation by the TCR. *National Immunology,* 11: 101–104.

Wells, H. G. (1911). Studies on the chemistry of anaphylaxis III. Experiments with isolated proteins. *Journal of Infectious Diseases,* 8:147–171.

Whitehead, R. and Skinner, J. M. (1978). Morphology of the Gut Associated Lymphoid System in health and disease: A review. *Journal of Pathology,* 10: 3-16.

Wickramasinghe, S. N. *(*1992*). The* Bone marrow*, In: Histology for Pathologists, Sternberg, S. S. Editions, Raven Press, New York, pp 1–31.*

Wing, K. and Sakaguchi, S. (2010). Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *National Immunology,* 11: 7–13.

Worbs, T., Boke, U., Yan, S., Hoff mann, M. W., Hintzen, G., Bernhardt, G., Förster, R. and Pabst, O. (2006). Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *Journal of Experimental Medicine,* 203: 519–527.

Yalcin, H. And Tipirdamaz, S. (2002). A macroanatomic investigation on the arterial vessels of the canine thymus. *Revue Méd. Vet* 153:3 173-175

Yeboah, S. and Adamu, E. K. (1995). The cane rat. *Biologist,* 42: 86-87.

APPENDICES

Appendix 1. Morphometric indices of lymphoid organs of three months old grasscutters

Structure	Animal number			
	Female		Male	
	1	2	3	4
Body Weight (g)	390	400	420	450
Thymic Weight (g)	1.1	1.3	1.2	1.3
Splenic Weight (g)	0.48	0.55	0.75	0.89
LN Weight (g)	0.05	0.05	0.07	0.05
Thymic Cmf (cm)	4.05	4.60	4.19	4.60
Splenic Cmf (cm)	3.24	3.50	4.22	5.84
LN Cmf (cm)	0.35	0.36	0.38	0.36

Splenic to body weight ratio = 0.48/390 by 100%

= 0.1%

Appendix 1 above showed that the body weight varied between the animals even at the same age. The males had a higher body weight than the females. There was no significant variation in the thymic, splenic and lymph node weights between the sexes.

Appendix 2. Morphometric indices of lymphoid organs of five months old grasscutters

Structure	Animal number			
	Female		Male	
	1	2	3	4
Body Weight (g)	830	1000	950	980
Thymic Weight (g)	0.54	0.57	0.48	0.51
Splenic Weight(g)	1.10	0.98	2.09	2.42
LN Weight(g)	0.08	0.09	0.18	0.22
Thymic Cmf (cm)	2.20	2.24	2.10	2.33
Splenic Cmf (cm)	5.86	7.00	7.10	7.12
LN Cmf (cm)	0.38	0.48	0.60	0.75

Appendix 2 showed that the body weight varied between the animals even at the same age. There was no significant variation in the thymic, splenic and lymph node weights between the sexes.

Appendix 3. Morphometric indices of lymphoid organs of seven months old grasscutters

Structure	Animal number			
	Female		Male	
	1	2	3	4
Body Weight (g)	1800	1500	1700	1900
Thymic Weight (g)	0.28	0.33	0.20	0.40
Splenic Weight (g)	2.35	2.45	2.18	2.25
LN Weight (g)	0.18	0.09	0.19	0.18
Thymic Cmf (cm)	0.48	0.43	0.50	0.46
Splenic Cmf (cm)	7.25	7.28	7.88	7.65
LN Cmf (cm)	0.70	0.47	0.65	0.73

Appendix 3 showed that the body weight varied between the animals even at the same age. There was no significant variation in the thymic, splenic and lymph node weights between the sexes.