HISTOMORPHOMETRIC STUDY OF THYROID GLAND IN TIMORENSIS DEER

By

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement of the course DVT 55204 – Research Project.

This study aims to explore the gross morphology and the histomorphometric characteristics of the thyroid gland of Timorensis deer in Malaysia and assess their adaptability towards environment with high temperaturePhysiologically, the thyroid activity is highly influenced by the temperature which could be assess through histomorphometric study of thyroid follicles. Six (6) samples from Kelantan, Terengganu and Perak were collected from deer farms that performed slaughtering for meat purposes. The samples collected were brought to the laboratory for histology for gross morphology measurement and sample preparation. The histology slides were prepared and stained by using Hematoxylin and Eosin. From the histology slide, morphometric studies were conducted to observe and measure the micro features of the thyroid follicle's distribution, shape and diameter. The study revealed that the size of thyroid follicles in deer is 2.5 ± 0.46 cm in length and 2 ± 0.14 in width while the size of follicles recorded as 65.49 ± 8.79 (small), 168.53 ± 13.78 (medium) and 317.42 ± 16.58 (large). The size of the follicle in deer is larger compared to other ruminants reflecting higher activity and adaptability in deer. With the understanding and findings from this study, it provides strong evidence on the ability of the deer to withstand the high local temperature of Malaysia making it the next choice for ruminant agriculture of choice among the farmers in Malaysia, due to its high market value.

ABSTRAK

Abstrak daripada kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai keperluan sebahagian daripada kursus DVT 55204 – Projek Penyelidikan.

Kajian ini bertujuan mengkaji morfologi kasar dan ciri histomorfometrik kelenjar tiroid rusa Timorensis di Malaysia dan kebolehtahanan mereka terhadap suhu tinggi. Aktiviti kelenjar tiroid sangat dipengaruhi oleh suhu di mana ia dapat diperhatikan melalui kajian histomorfometrik tiroid folikel. Enam (6) sampel dari Kelantan, Terengganu dan Perak telah diambil dari ladang rusa yang melakukan penyembelihan untuk tujuan daging. Sampel yang dikumpul dibawa ke makmal untuk penyediaan sampel histologi dan ukuran morfologi kasar. Slaid histologi yang telah siap di stain menggunakan Hematoxylin dan Eosin. Kajian morfometrik telah dijalankan untuk memperhatikan taburan ciri mikro, bentuk dan diameter folikel tiroid. Kajian menunjukkan bahawa saiz folikel tiroid pada rusa adalah 2.5 ± 0.46 cm panjang dan 2 ± 0.14 lebar manakala saiz folikel direkodkan sebagai 65.49 ± 8.79 (kecil), 168.53 ± 13.78 (sederhana ± 1.78) dan 31.). Saiz folikel dalam rusa adalah lebih besar berbanding ruminan lain yang menggambarkan aktiviti yang lebih tinggi dan kebolehsuaian dalam rusa. Dengan pemahaman dan penemuan daripada kajian ini, ia memberikan bukti kukuh tentang keupayaan rusa untuk menahan suhu tempatan yang tinggi di Malaysia menjadikannya pilihan seterusnya untuk pertanian ruminan pilihan dalam kalangan penternak di Malaysia, kerana nilai pasarannya yang tinggi.

CERTIFICATION

This is to certify that we have read this research paper entitled **'Histomorphometric Study of Thyroid Gland in Timorensis Deer'** by **Nur Insyirah binti Muhammad**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course DVT 55204 – Research Project.

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My family

Deer farm, Besut, Terengganu

Deer farm, Tanah Merah, Kelantan

Deer farm, Machang, Kelantan

Deer farm, Sungai Siput, Perak..

Staff of Teaching Farm and lab assistants of FPV UMK

DVM 5 Class of 2020/2025

Thank You

DEDICATIONS

This thesis is dedicated to my beloved parents Muhammad bin Abdullah and Rahimah binti Shafiee, whose unwavering love, support, and encouragement have been my greatest source of strength.

To my mentors and friends, thank you for your guidance and inspiration throughout this journey.

First and foremost, all praises be to Allah, The Almighty, who deserves the utmost gratitude and favour for endowing me with the fortitude necessary to conclude this thesis successfully. Having been granted the fortitude to conclude this final-year project successfully. I would like to express my deepest gratitude to my beloved parents, Muhammad bin Abdullah and Rahimah binti Shafiee, for their continuous love and support and to my siblings who cheer up this life.

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LIST OF ABBREVIATIONS AND SYMBOLS

H&E	Hematoxylin and Eosin
ID	Identification
NBF	Neutered Buffered Formalin
TFCs	Thyroid Follicular Cells
SD	Standard Deviation
T	Trachea
C	Cricoid cartilage
L	Left lobe of thyroid
R	Right lobe of thyroid
T3	Triiodothyronine
T4	Thyroxine
ºC	Degree Celsius
µm	Micrometer
Cm	Centimeter
Kg	Kilogram
±	Plus Minus
×	Times
%	Percent

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

This study is conducted to assess the histomorphometric features of the deer's thyroid gland towards understanding how they contribute in adaptation to heat from global warming due to climate change. Therefore, this study is significant as it provides anatomical and histological insight and details of the thyroid gland towards its functional secretion of hormones that aid in resilience and adaptation to extreme environmental heat stress due to climate change thus encouraging farmers, especially in the country with high temperature to invest in deer farming determining suitability of imported breeds which are adaptable to and highly productive under tropical climate such as Malaysia.

In Malaysia, there are 3 common breeds of deer where Sambar deer (*Rusa unicolor*) is categorized as a large breed in Malaysia while Timorensis deer (*Rusa Timorensis*) is considered more negligible. On the other hand, axis deer (*Axis axis*) is a breed that can be easily recognized due to its distinctive white spots scattered throughout the brown coat. This breed originated from the Indian subcontinent.

Previous studies recognize the anatomical and histological structure of thyroid gland in other species such as Surti goats (Menaka et al., 2023), Jaffarabadi buffalo (Sharma et al., ,2020), albino rat (Eman & Amira, 2019) and white - tailed deer (Hoffman & Robinson, 1966). However, there is still a lack of study and limited knowledge regarding the histomorphometric studies of the thyroid glands in deer, which play vital roles in the ability to adapt to the high temperature. Deer were reported to show morphological variations in their structural location and relation with the trachea, thus also having heterogeneity of the follicle location and diameters (Maltseva et al., 2021).

1.1 RESEARCH PROBLEM STATEMENT

Alteration in the climate due to industrialization and urbanization leads to changes in weather and temperature, representing the significant effect of climate change, which directly impacts the livestock farming industries. High ambient temperatures eventually reduce the productivity of livestock. Identifying animals with silent resilience adaptive structures to temperature changes and withstand high temperatures is significant in ensuring the continuous advantages of the economic value in the farming industry.

Generally, small ruminants concede better adaptation ability towards the adverse effects of climate change than large ruminants, enabling them to maintain reproductive performance and production yield (Danmaigoro et al., 2014). A recent study shows deer can withstand both low and high temperatures. However, the ability is correlated with hormonal and physical features in small ruminants (). This is influenced by the morphological features against the high temperature (Danmaigoro et al., 2024). The adaptation ability collectively enables deer to adapt to various temperatures and thrive in diverse environments.

Previous studies show an essential correlation between the morphometrics of the thyroid gland and the ability of the deer to adjust and survive during the high temperature of summer and lower temperature during the winter (Hoffman & Robinson, 1966). Hence, further study on histomorphometric indices is important to maximize the application of knowledge for the sustainability of livestock production. Since there is a paucity of data on the histomorphometric features of the deer thyroid gland that will provide clues to the hormonal secretive potential of the gland in the deer toward silence and adaptation process to harsh environmental conditions.

1.2 Research Questions

1. What is the histomorphology of the thyroid gland of the deer?
2. What are the histometric indices of the thyroid gland follicle in the deer?
3. Is there any variation in the histometric indices of the thyroid gland of adult deer.

1.3 Research Hypothesis

The histomorphology and histomorphometric induce of the thyroid gland in Timorensis deer exhibit distinct structural adaptations that are reflective of their unique environmental, and physiological requirements.

1.4 Research Objectives

1. To evaluate the histomorphology of the thyroid gland of adult deer,
2. To determine the baseline value of histometric indices of the thyroid gland of adult deer includes the distribution and diameter size of the thyroid follicles
3. To analyze the variation in the histometric indices of the thyroid gland of adult deer.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Anatomical Structure of Thyroid Gland

The thyroid gland is embryologically developed as a protrusion or outgrowth from the oral cavity, which subsequently merges at the fifth pharyngeal pouch (Samuelson, 2007). Specifically, it is in the ventrolateral neck region (Dyce et al., 2010), bilaterally alongside the most cranial part of the trachea and larynx (Horst et al., 2014). Thyroid glands are present as bi-lobed in most species except in a pig, where a lobed thyroid united ventrally (Swindle et al., 2012). The right and left lobes are connected by an isthmus, which provides structural support and cohesion within the neck (Dyce et al., 2010). However, isthmus may be absent in some species of animals (Joshi & Mathur, 2015).

Some studies show an array of species with thyroid glands that vary in size, shape, and anatomical positioning in relation to the trachea and esophagus (Meyer & Peterson, 2017). Generally, both lobes extend approximately between the 1st and the 6th tracheal rings (Joshi & Mathur, 2015). However, Menaka et al. (2023) reported variation in the location of the thyroid gland between the 2nd to 7th tracheal ring in prenatal, depending on the fetal age. This thyroid forms a strong attachment to the trachea via a group of laterally positioned connective tissue known as Berry's ligament (Allen & Fingeret, 2023)

The thyroid gland possesses fine granular and exhibits a brick-red coloration in mature animals (Dyce et al., 2010). On the other hand, the colour of the thyroid could be dark reddish and brownish in goats (Joshi & Mathur, 2015). In small ruminants, the shape of thyroid glands is described as a spindle to cylindrical-shaped lobes without isthmus (Horst et al., 2014). In ruminants such as goats, the thyroid gland resembles inverted pyramids positioned laterally below the cricoid cartilage, where the right lobe is positioned more caudally (Joshi & Mathur, 2015).

The thyroid gland is irregularly surrounded by a capsule consisting of collagen and a few elastic connective tissues, extending into the thyroid parenchyma and encasing the follicles. The fibrous architecture is denser in older animals (Nandeshwar et al.,2024). Harvesting the thyroid gland is particularly challenging due to its anatomical position and location, as it is located beneath three layers of muscles: the sternothyroideus, sternomandibularis, and sternomastoideus muscles (Mahmud et al., 2021). After the slaughtering procedure, there is also a possibility for glands to be displaced and become asymmetrically located.

2.2 Physiological Function of Thyroid Gland

The mammal thyroid glands are critical endocrine organs regulating numerous physiological processes. The gland synthesizes and secrete thyroxine (T4) and triiodothyronine (T3). These hormones play crucial roles in regulating animal metabolism, growth, development, and energy balance (Peterson & Drucker, 2015). T4 serves as the precursor hormone, while T3 is the biologically active form of the hormone that exerts most of the physiological effects. These hormones are produced and stored by the thyroid follicular cells in the colloids (Eurell et al., 2006).

Across the mammalian species, thyroid glands play vital roles in regulating the metabolism and body temperature (Peterson & Drucker, 2015). The seasonal and temperature changes show insignificant effects on external parameters such as the topographic position, shape, and colour (Sharma et al., 2021). On the other hand, the consistency of the thyroid during the summer and rainy seasons was found to be increased compared to the winter (Sharma et al., 2021).

2.3 Histology and Distribution of the Colloid Follicles

Thyroid glands are covered by dense irregular connective tissue layers where the thin septa divide the parenchyma into smaller lobules (Mahmood, 2023). The thickness of the capsule varies across the species; *Bubalus bubalis* buffalo consists of a thyroid gland with an inner layer consisting of loose collagen fibers, adipose tissue, and elastic fibers, while the outer layer is made of collagen bundles, elastic fibers and few muscle cells (Mahmood, 2023). According to a study in goats by (Nandeshwar et al., 2024), the thickness of the capsule was reported to have a significant increase with the age of the animals. Thyroid follicles can be described as a hollow structural unit of the thyroid gland surrounded with numerous thyroid follicular cells (TFCs). These irregular-sized - follicles are filled with mucilaginous substances known as colloids (Mahmood, 2023). In most glands, thyroid follicles may be observed with a high variety of shapes, sizes and distribution (Mahmood, 2023). In a normal animal, the thyroid follicles appear as round homogenous lumen surrounded by cuboidal follicular cells (Liu, 1984).

Morphological differences between species also exist in the heterogeneity of the location of follicles with different diameters to each other. In the dapple deer, large-diameter follicles are located in the center of the organ, and smaller-diameter follicles are located both in the center and periphery (Maltseva, 2021). The colloid volume within the thyroid follicles is highly dependent on the current physiological status, and high volume may be observed during inactive duration. In the case of iodine deficiency, no colloid was observed (Khan & Farhana, 2022).

2.4 Thyroid gland and Age variation

This study targeted sampling adult deer with fully developed thyroid glands within the age range of 1 -2 years. Thus, the gland's activity gradually decreases as more stromal elements develop, with an increase in follicle size and colloid storage capacity; however, this decreases the resorption process (Ovcharenko et al., 2016). Besides, the C-cell development is observed in deer that have attained the age of 6 months and above (Ovcharenko et al., 2016). Hence, it is very important to sample the animals within the specific age at which the thyroid gland is fully developed with uniform distribution of the follicles. Additionally, animals over 10 years old have bigger variations in either follicles or epithelium size (Pantic and Stosic, 1996). In addition, there is a significant relationship between the thyroid gland weight and the total body weight of the animals (Maltseva et al., 2021).

2.5 Influence of Environmental Temperature Towards Colloids Distribution

A study shows that the colloid of the thyroid gland reaches the maximum distribution at the mid or late winter in Maryland, United States. This was observed together with the minimum height of the thyroid cells (Hoffman et al., 1966). In addition, the average weight of the thyroid gland of deer during the winter season was recorded as 5.7g, 5.8g during spring and >8g during the summer (Pantic and Stosic, 1996). The thyroid gland shot during winter has less weight than the one shot during the summer.

CHAPTER 3

3.0 METHODOLOGY

3.1 Ethical Consideration

This research methodology does not require animal ethics consideration as the sample collection was performed on the fresh dead carcass immediately after the slaughtering process by the farmers. The slaughtering procedure was solely performed for meat harvesting purposes. No sample was collected from the live animals, and no live animals were sacrificed solely for sample collection.

3.2 Sample Collection and Preparation

A total of 6 thyroid samples of Timorensis deer were obtained from 3 different locations in Malaysia, including Kelantan (4), Terengganu (1), and Perak (1)after slaughtering procedure . The thyroid glands were harvested together with the cranial portion of the trachea, including the cricoid and thyroid cartilage up to 5th cartilage rings of the trachea, using a sharp surgical blade size 20 and scalpel holder size 4. Samples were secured in sample bags labeled with ID, collection date, sex, and weight of the animals. After that, samples were immediately stored within the polystyrene box packed with ice to maintain the samples below 4°C to prevent autolysis of the organs. On the other hand, samples collected from a distant area from the laboratory will be preserved in the freezer beforehand. Samples are then preserved in a polystyrene box packed with ice for transportation.

3.3 Thyroid Identification and Harvesting

Upon arrival at the anatomy laboratory, the tissue samples collected were presented on the table. The anatomical location of the thyroid was identified craniolaterally on the first fifth trachea where the underlying muscles were slowly removed to preserve the glands. The thyroid glands were carefully removed from the trachea by dissecting the ligament attachment using a scalpel blade and forceps. The covering fats layer was removed as much as possible to improve measurement accuracy. Then, a gross examination of the length, shape, and colour of the thyroid gland was done on the fresh samples while frozen samples were thawed.

3.4 Histology Processing

3.4.1 Tissue Fixation

After gross examinations, the thyroid gland was fixed in 10% neutral buffered formalin (NBF) for 48 hours in a tight, leak-proof container. The ratio volume of the formalin to specimen used is 10:1. Following the correct ratio and duration is essential in providing sufficient tissue fixation to retain the morphology composition of the tissue and preserve the colloidal material in accordance with the method of Gunasegaran, (2014). The container was properly labeled with ID, sex and date of fixation.

3.4.2 Specimen Transfer to Cassettes

The specimen ID and date of fixation were appropriately labeled on each cassette. After 48 hours, the samples were removed from the formalin solution and the fixation quality was observed. The properly fixed thyroid was sectioned into a smaller size with 0.3 cm of thickness by using a sharp scalpel blade and inserted into the cassette. The labeled cassettes containing samples were immersed in the new solution of 10% formalin.

3.4.3 Tissue Processing

Fixed samples were transferred to the histology laboratory for further tissue processing. This sample underwent three vital procedures: dehydration, clearing, and embedding machine by using automated tissue processing machine LEICA TP1020 Automatic Benchtop Tissue Processor, Semi-EnclosedWater and formalin content were removed gradually through ascending tissue immersion in different alcohol concentrations starting from 50%, 70%, 90%, and absolute alcohol for 30 - 60 minutes in each concentration (Gunasegaran, 2014). Due to the insoluble characteristics of paraffin wax, it is essential to remove the water content to enable paraffin wax penetration. A clearing process was performed to remove the alcohol. The commonly used organic solvent is xylene (Marinho & Hanscheid, 2023). Toluene can also be used as the paraffin solvent, where tissue will be treated for 2-3 hours, allowing the agent to penetrate (Gunasegaran, 2014). Then, the tissues were embedded in the paraffin wax by using embedding machine of LEICA HistoCore

Arcadia Embedding Center and allowed to solidify on the cold plate of LEICA HistoCore Arcadia C - Cold Plate prior to sectioning.

3.4.4 Sectioning

The solidify blocks were sectioned using a rotary microtome, producing thin tissue slices of approximately 5–7 µm-thick. The tissue ribbons were carefully collected, placed into a warm water bath, and left to float on the surface. The tissue ribbons were then scooped onto a clean slide by submerging it into the water. The microslides will be carefully labeled and left for drying upright at 37 ºC to melt the extra paraffin wax while maintaining the integrity of the tissue segment.

3.4.5 Staining

The microslide with thin tissue sections were stained using the combination of haematoxylin and eosin (H&E) to enhance contrast and evaluate the sample's cellular features. An automated staining machine of LEICA ST5010 Autostainer XL was used. A coverslip will be affixed to the slide following tissue staining using optical grade adhesive. Dried slides were sent to the R - NOMIC laboratory to be scanned by using Pannoramic midi II from 3DHISTEC Ltd and converted into soft copies. The scanned slides were observed by using Case Viewer software.

3.5 Data Collection and Interpretation

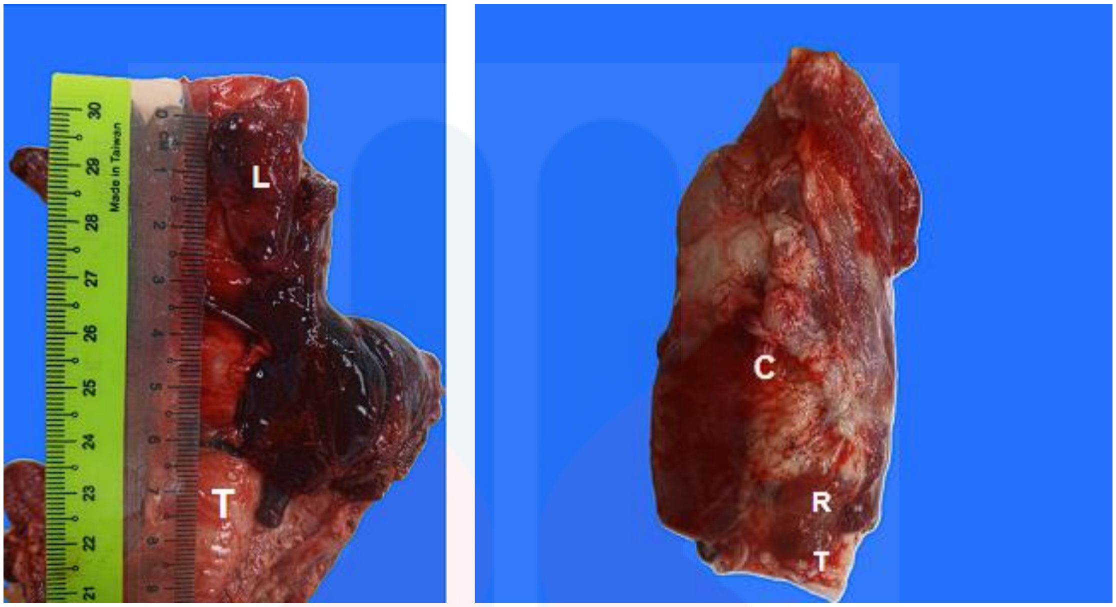
All the data required for the gross study observation were collected before tissue fixation. These numerical and descriptive data were tabulated in Microsoft excel according to the ID of the animals. IBM SPSS software was used to confirm the data analysis. All the numerical variables of the morphometric and histometric evaluation were recorded as mean, and standard deviation information will be recorded in the provided table to smoothen the process. Further data observation and collection were done on the prepared histology slide. Pictures of individual thyroid glands were scanned by using Pannoramic midi II from 3DHISTEC Ltd. The 3DHISTEC application was used for observation, recording and analyzing of the picture from he slides as the data will be subjected to descriptive analysis. The histomorphometric parameters such as the distribution of thyroid follicles, layers of the follicular cells and the homogeneous colloid (Eman & Amira Z, 2015).

CHAPTER 4

4.0 RESULTS

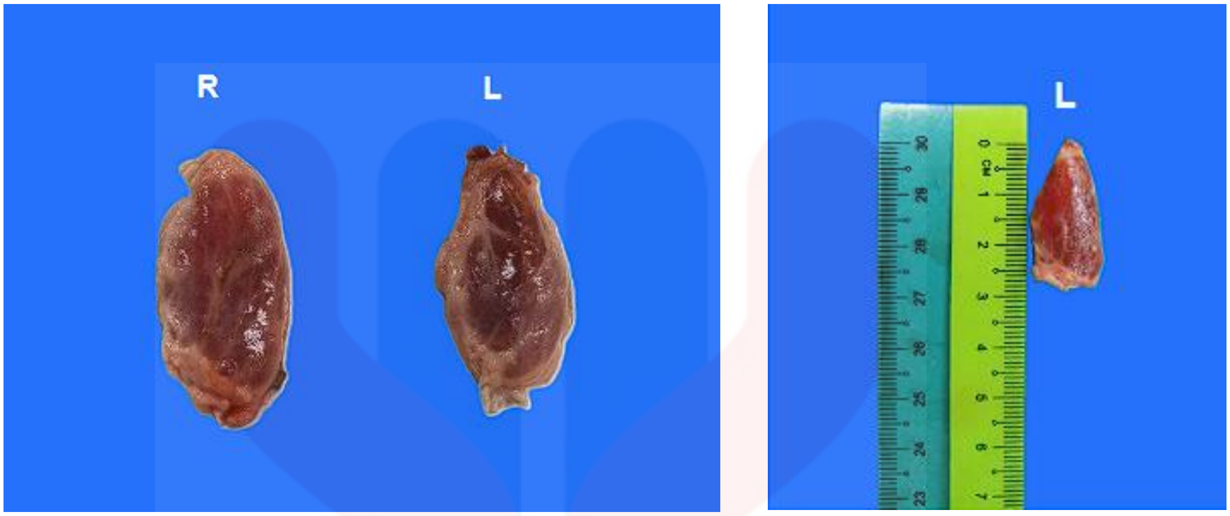
4.1 GROSS MORPHOLOGY OBSERVATION

A total of six samples were collected from 6 deer aged between 1 to 3 years old. The thyroid gland of deer can be observed lying under the layers of sternothyroideus, sternomandibularis, and sternomastoideus muscles. This thyroid is firmly attached to the cranial ring of the trachea below the cricoid cartilage. Despite the deep location underneath the muscles, thyroid can be differentiated from the surrounding muscles based on its exclusive characteristics. The thyroid gland can be clearly recognized as an almond shape gland with rounded caudal end covered by a firm extension layer of connective tissue provide attachment to the tracheal ring. Upon removal of the connective tissue, the colour was observed to be brownish to red.



a) b)

Figure 4.1 Photograph of topographic position of thyroid lobes a) - Left lobes (L) attaching to the cranial part of the trachea, b) - Right thyroid lobes (R) situated at the ventrolateral aspect of trachea caudal to the cricoid cartilage (C), T - Trachea.



a) b)

Fig 4.2 Photograph of dissected thyroid gland from TD01 a) - Almond shaped right lobe (R) and left lobe (L), b) - The measured length is 2.70 cm.

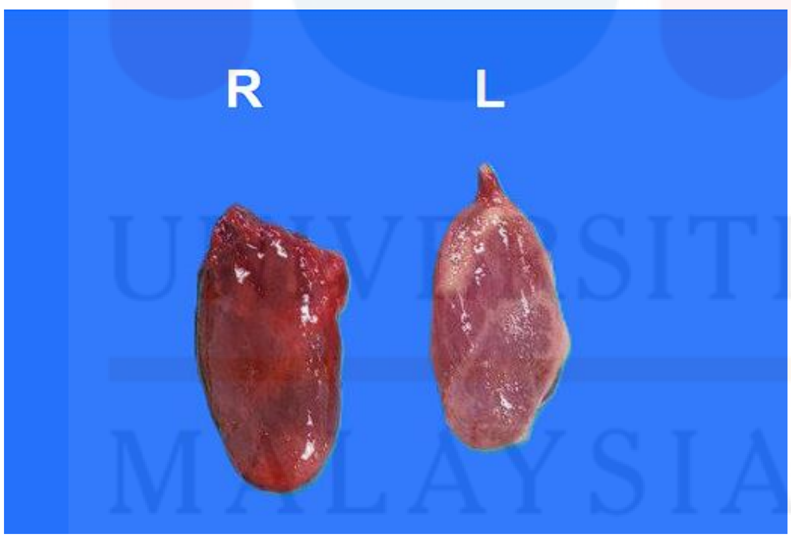


Figure 4.3 Photograph of dissected thyroid gland from TD03 - Shows bright red almond shaped right lobe (R) and slightly pale left lobe (L)

4.2 MORPHOMETRIC INDICES OF THE THYROID GLAND

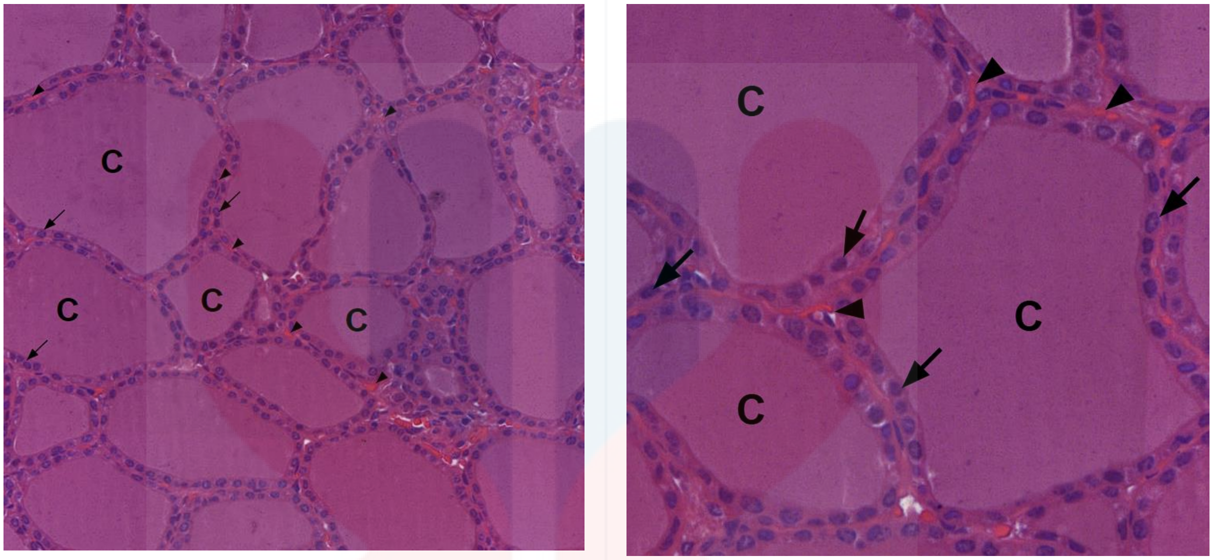
The average of the deer is 60.33 ± 8.66 kg, mean total length of the thyroid gland was 2.5 ± 0.46cm and average thyroid width was 2 ± 0.14 cm as shown in table 4.1 below.

Table 4.1 Morphometric Indices of Thyroid Gland

Parameter	**Weight of deer (kg)**	**Thyroid Length (cm)**	**Thyroid Width (cm)**
Adult	60.33 ± 8.66	2.5 ± 0.46	2 ± 0.14

4.3 HISTOLOGY OF THE THYROID GLAND OF THE DEER

The thyroid follicles can be observed to have irregular shape and sizes. These follicles are lined with a single layer of cuboidal follicular cells primarily exhibiting spherical nuclei.The Parafollicular cells were observed between thyroid follicles. The follicles were surrounded by blood capillaries Moderate amounts of collagen can be seen between follicles with thyroid follicles filled with homogenous acidophilic colloidas shown on Figure 4.3 below.



a)	b)

Figure 4.3 Photomicrograph of the thyroid gland shows follicular cells (arrow) lined the thyroid follicles. Parafollicular cells (arrowhead) observed between the thyroid follicles and colloids (C)filling the thyroid follicles a) - H&E × 20, b) – H&E × 40

4.4 HISTOMORPHOMETRIC EVALUATION

The mean value of the small diameter follicle is 65.49 ± 8.79 μm. It indicates that most of the small follicle diameters are close to the average value with only slight variation in diameter. The mean value of the moderate diameter of the follicle is 168.53 ± 13.78. The mean value of the large diameter on the other hand is 317.42 ± 16.58. This is an indication shown that most of the moderate and large follicle diameter are close to the average value with high variation in diameter as shown in table 4.2 below.

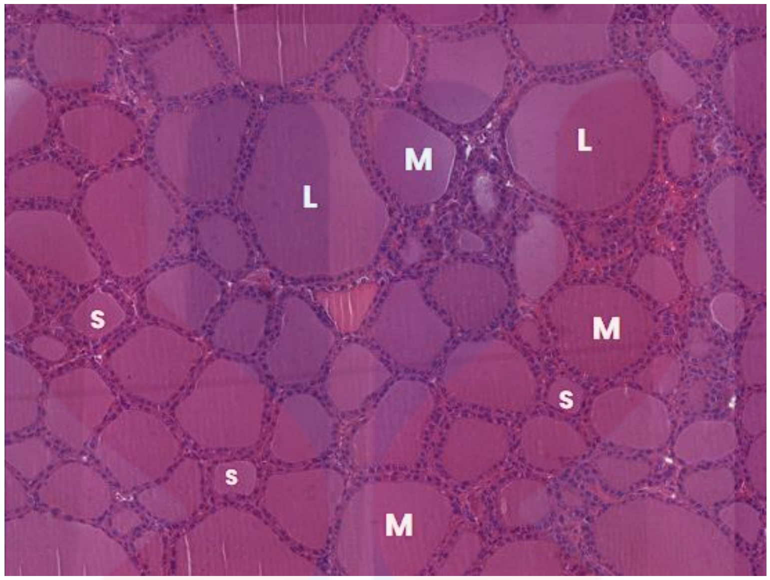


Figure 4.4 Photomicrograph of the thyroid gland with multiple size of the thyroid follicles large (L), moderate (M) and small (S) H&E × 20

Table 4.2: Mean follicular diameter of the thyroid gland of the deer (Mean ± SD)

Follicular diameter of thyroid gland	**Small (µm)**	**Moderate (µm)**	**Large (µm)**
	60.48 ± 10.5279	157.52 ± 13.0176	283.5 ± 6.3

CHAPTER 5

5.0 DISCUSSION

Identifying the thyroid glands from their anatomical location is challenging as this gland is firmly attached to the trachea, underlying a few layers of muscles. Anatomical knowledge regarding the exact location, consistency, and shape of the thyroid gland will be very useful in ensuring the correct sampling of the thyroid. However, during the slaughtering procedure, there is a possibility of the thyroid gland being cut as the process of extending the neck region will also cause movement of the thyroid gland from the original location. Upon palpation, thyroid glands share the same firm consistency as the surrounding muscles. However, thyroid gland was observed with absence of origin and insertion which are prominent in muscle.

The colour of the thyroid gland from some deer may be observed to be dark or bright red which is different compared to the other thyroid gland collected. This is caused by blood pooling on one side of the organ during the transportation before arrival at the laboratory which leads to slight variations in colour. The natural colour can be observed from the fresh sample that was immediately brought to the laboratory as soon as the samples were collected.

Histological slide preparation is a very important procedure determining proper histological tissue and staining. The histological staining procedure benefits the acidic and essential nature of the dye, enabling cellular observations of the tissue. In this study, H&E stain was used. Hematoxylin stains the acidic components such as the cell's nucleus, into blue or black, while eosin stains the basic components in the cytoplasm and colloid in pink. (Gunasegaran, 2014). Masson's Trichrome stain would also be used to demonstrate the collagen fibers of the thyroid gland (Eman & Amira, 2019). This special stain integrates the combination of three dyes: Weigert's iron hematoxylin, Biebrich scarlet and aniline blue enable the researcher to distinguish the nuclei, muscle fibers and erythrocytes, and collagen fibers, respectively (Van De Vlekkert et al., 2020).

Grossly, the thyroid gland of deer was observed individually on the right and left side of the dorsolateral part of cartilage with absence of isthmus. In comparison to the thyroid gland of goats, some of thyroid glands were attaching the right and left lobes. The size of the gland in goats that aged above 18 months was recorded as 2.15 ± 0.16 cm length and 1.39 ± 0.13 cm width (Nandeshwar et al.,2024). In contrast, the length of thyroid gland of deer that aged above 12 months is larger, recorded as 2.5 ± 0.46 cm length and 2 ± 0.14 cm width. Histologically, the average size of the follicles in goat was recorded as 90.46 ± 4.90 while the size of follicles in deer recorded as 65.49 ± 8.79 (small), 168.53 ± 13.78 (medium) and 317.42 ± 16.58 (large). Based on the result obtained from this study, the size of the thyroid gland and the thyroid follicle in deer is larger in comparison to the goat.

The baseline data collected from the study shows that the size of the thyroid gland of Timorensis deer show slight variation of size that could be affected by other factors such as the age and temperature of specific regions (Hoffman & Robinson, 1996) that varies according to sampling time that range from July to October. However, despite the slight distinction in the thyroid follicular sizes, it is considered to be larger compared to the other small ruminants indicate higher hormonal and metabolic activity which allow them to thrive in the region with high environmental temperature.

From the histological evaluation, all the thyroid samples showed complex deviations of sizes and shapes individually. This study's size distribution of thyroid follicles may be characterized into 2 patterns. In most thyroid glands, the small, moderate, and large follicles are well distributed from the central to the periphery region of the gland. In another way, the deer show larger diameter follicles distributed at the center, where the follicles are smaller towards the periphery region. These findings are supported by Maltseva et al., (2020) where large-diameter follicles are located in the center of the organ, and smaller-diameter follicles are located both in the center and on the periphery (Maltseva, 2021).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

This study provided baseline data and information on the size of thyroid gland and thyroid follicle, specifically in deer. In future, this project may be improved by testing a larger sample size being specific according to the breed of the deer to provide more accurate measures of the histomorphology and histomorphometric of the thyroid gland of Timorensis deer in Malaysia. The data collected from the study shows that despite the variation in the thyroid follicular sizes, the deer population in Malaysia, can adapt and withstand our local temperature. This insight provides a meaningful reason for the deer farming industry to be explored by ruminant farmers in Malaysia.

This study was performed under few limitations and challenges involving small sample size due to limited numbers and network with the deer farmers community. This study provided clearer picture on the distribution of small - scale deer farms in Kelantan and Terengganu. This network build at the end of this study would be beneficial to enhance the number of samples for the next study in future. As a result, precision and reliability of the data can be improved in providing results that represent the population of the specific area. Additionally, the deer may also be classified based on age group as it plays significant roles in determining the activity of the thyroid gland. Hence, the new study will emphasize and provide wide range knowledge on the size and the distribution of the thyroid follicles in correlation with age. This information will provide significant results and application in deer farming industry which then drive enhancement in deer farming industry in Malaysia.

APPENDIX

Figure A (1): The deer appear healthy, with well-groomed coats and alert postures.

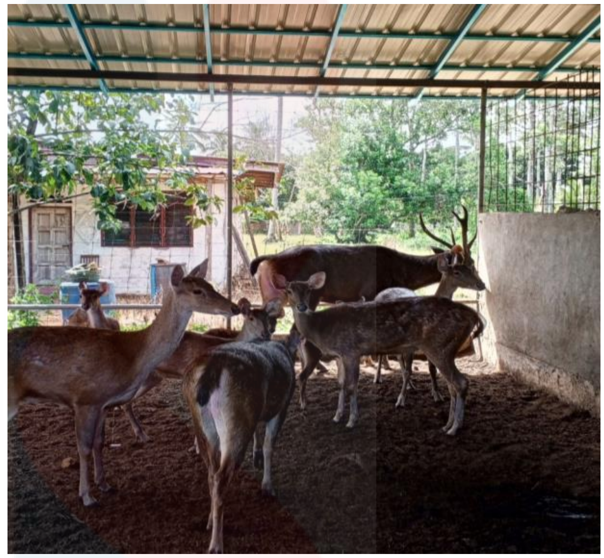


Figure A (2): Deer farm located at Besut, Terengganu

Figure A (3): Handling and restraint before slaughtering.

Figure A (4): Slaughtering process

Figure A (5): Fresh venison processed after the animal was slaughtered

Figure A (6): Sample preparation

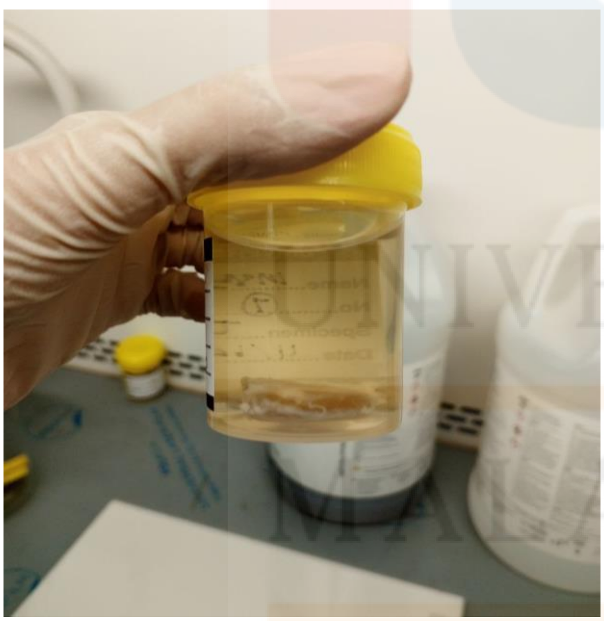


Figure A (7): The sample was preserved in a 10% formalin solution

Figure A (8): The sectioned thyroid placed in the cassette for histology processing.

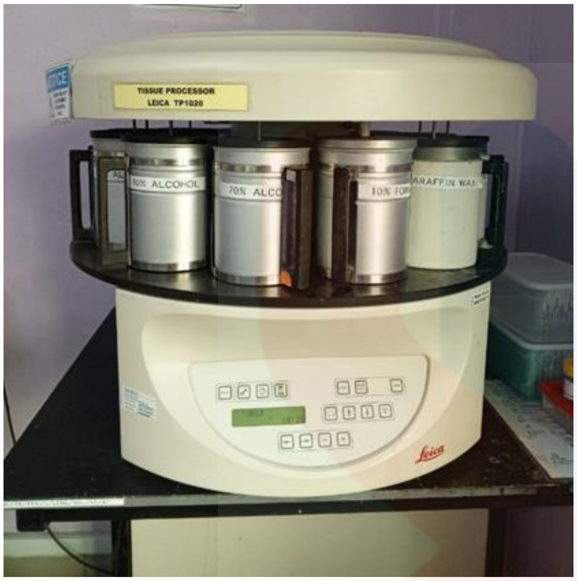


Figure A (9): Tissue processing machine LEICA TP1020 Automatic Benchtop Tissue Processor, Semi-Enclosed

Figure A(10): Microtome HistoCore BIOCUT - Manual Rotary Microtome

Figure A(11): Embedding machine of HistoCore Arcadia Embedding Center and cold plate of HistoCore Arcadia C - Cold Plate

Figure A(12): Staining machine of Leica ST5010 Autostainer XL

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