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Comparative anatomical, histological and morphometric study of the thyroid gland in Egyptian Mulard duck (*Cairina moschata* × *Anas platyrhynchos*) and Egyptian turkeys (*Meleagris gallopavo*)

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ABSTRACT

The thyroid gland of the Mulard duck and turkey is a bilateral endocrine organ that is located in the thorax. The current study aimed to describe the topographical, anatomical and histological picture of the thyroid gland in ducks and turkeys. Eeighteen healthy adult males of ducks and Egyptian turkeys were selected. Gross morphology and histological analysis of thyroid tissue was performed. Grossly, thyroid glands appeared dark red oval bodies near the carotid artery. Histologically, glands contained thousands of follicles within a collagenous capsule. Follicle shapes ranged from spherical to elliptical. In ducks, follicular epithelium was squamous to cuboidal. In turkeys, epithelium was cuboidal to columnar based on activity. Colloid stained strongly positive in duck follicles but moderately to weakly in turkeys on PAS reaction. Connective tissue and follicular lining showed weak PAS positivity in both species. This study characterized thyroid gland location, gross anatomy and microscopic features in ducks and turkeys. Histological analysis revealed inter-species similarities and differences at the tissue and cellular level.

Introduction

Birds are of very great economic importance for humans. Some of them represent a source of meat, egg and feather production. The endocrine system in birds plays an important role in controlling growth and differentiation of many organ systems. Thyroid gland is one of the most important glands of the endocrine system due to secretion of triiodothyronine (T3) and thyroxine (T4). The function of thyroid hormones in birds similar to the function of thyroid hormones in mammals; regulate body weight, body temperature, plumage growth, fertility, sex characteristics and lipid metabolism (Danforth and Burger, 1984; Wentworth and Ringer, 1986; Whittow, 2000). Hyperplasia in the thyroid gland can form in many avian species due to the foods containing iatrogenic agents as well as to goiter, septicemic diseases, and toxication. As a result of mechanical pressure on the adjacent organs, vomiting, weight loss, respiratory distress, convulsions; even sudden death may be observed (McLelland, 1990; Butcher and Beck, 1993). The thyroid, parathyroid and ultimobranchial endocrine glands are situated in a descending manner closely applied to common carotid artery, supplied with arteries arising as one trunk from it (Kameda, 2002). There is individual intra-species variation in the positions of the parathyroid glands relative to the thyroid gland among gallus (Abdel-Magied and King, 1978). The thyroid gland in birds is located at vascular angle formed by subclavian and common carotid arteries inside the thoracic inlet or slightly cranially to it, as two separate glands at the right and left. They are placed on the surfaces of the common carotid artery and jugular vein, usually rostrally to the syrinx and laterally to the esophagus and trachea (Nike *et al*., 1977; Epple, 1993;Orosz, 1997). The weight of thyroid gland increases with age and its size changes in different seasons and temperatures. Low temperatures increase the secretion of thyroid hormone (Dar *et al*., 2012). The current study threw insights on the normal topographical, anatomical, and histological picture of thyroid gland in Mulard ducks and turkeys.

Materials and methods

Sample collection

The present work was performed on 22 adult males of healthy Egyptian moulard duck (or "Mulard") and Egyptian local turkey (*Meleagris gallopavo*). Mulards are one of the most popular duck types. These ducks are hybrids between male of Muscovy ducks (*Cairina moschata*) and female of White Pekins (*Anas platyrhynchos*). All specimens were obtained from Assiut governorate, Egypt. The average body weight of the ducks and turkeys were about 3000 - 4000 g respectively. All of birds were slaughtered by using Halal method of slaughtering.

Ethical approval

The Ethics committee of Assiut University and Veterinary authority, Egypt has approved this study.

Gross examination

Four birds from each species were used for gross anatomical studies. The thoraco-abdominal cavity of each bird was exposed by making a ventro-median incision. The shape, color, location and relative topographic in-situ position of thyroid glands were recorded.

Histological examination

Ten birds (five birds from each species) were used for the light microscopical analysis. Thyroid glands were dissected carefully using a scalpel immediately after incision and they were fixed in Bouin's solution. The fixed specimens were dehydrated in ascending grades of ethanol, cleared in methyl benzoate, embedded in paraffin wax. Finally, paraffin blocks

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of the processed samples were prepared. Thin sections (5-6 µm thick) were cut, dried in an electrical incubator and stained with Harris's hematoxylin and eosin (H&E) for detection of general structure of the gland, Periodic Acid-Schiff (PAS) for detection of natural mucopolysaccharides, Crossmon's trichrome for detection of collagenous fibers (Bancroft and Gamble, 2002).

Semi-thin and transmission electron microscopy

Two birds of each species were used for semi-thin and transmission electron microscopy. Small specimens from thyroid gland of the two bird species were preserved in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.3 for 4 hours at 4ºC. They were washed in the same buffer used and then post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in ethanol and embedded in Araldite-Epon mixture. Semi-thin sections (1µm in thickness) were cut and stained with Toluidine blue and examined under light microscope (Spurr, 1969).

Morphometric and statistical analysis

Morphometric analysis was applied on Hematoxylin and Eosin-stained sections of thyroids of six birds (three birds from each species). Five paraffin sections were randomly selected from thyroid of each bird. In each section, four microscopic fields were randomly selected, observed photomicrography and analyzed using Image J. software. The morphometrical measurements included: Thickness of thyroid capsule, Diameter of thyroid follicles and Height of follicular epithelium.

All the data were expressed as mean ± SD, and statistically analyzed using One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. These data were expressed by Graph Pad Prism (version 6.05; International Scientific Community). Differences were significant at $P<0.05$ and highly significant at $P<0.01$.

Results

Gross morphology and topographic location

The duck and turkey have a pair of thyroid glands situated on both sides of the trachea, closely related to common carotid artery, and cranial to the junction of the subclavian and common carotid arteries (Figs. 1, 2). The thyroid glands of duck located caudally to the thoracic inlet near the syrinx (Fig. 1 A), while in turkey, they are located within the thoracic inlet rostrally to the syrinx (Fig. 2). The right thyroid gland closely approaches to esophagus in ducks and turkeys (Figs. 1, 2). The left thyroid gland of turkeys surrounded by anterior and posterior parathyroid glands from the cranial, caudal and dorsal aspects (Fig. 2). They are oval-shaped glands, red to dark red in color with a bright appearance. The bilateral thyroids of both species were symmetrically located in the thorax (Figs. 1, 2).

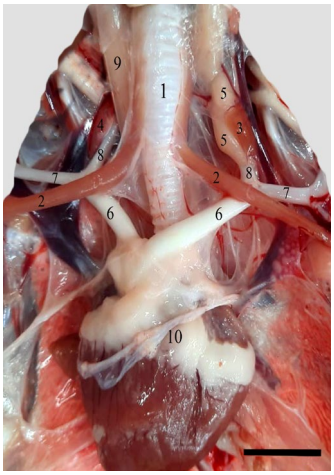

Fig. 2. In situ ventral view of the thyroid and parathyroid glands in male turkey showing:1. Trachea; 2. Sternotrachealis muscles; 3. Left thyroid gland; 4. Right thyroid gland; 5. Parathyroid glands; 6. Innominate artery; 7. Subclavian artery; 8. Common carotid artery; 9. Esophagus; 10. Heart.

Histological, morphometric, and statistical findings

Thyroid glands of mallard ducks and turkeys were composed of thousands of glandular follicles as structural unit surrounded by a thin connective tissue capsule that composed mainly of collagen fibers. In both species, the large sized follicles commonly are towards the center of the gland and small sized follicles are near to the connective tissue capsule with some exceptions. Each follicle is surrounded by a basement membrane (Figs. 3 and 4). In ducks, the follicular wall is seen from simple squamous to simple cuboidal epithelium (Figs. 3A and 5A). The large active follicle wall has a simple squamous epithelium, whereas the small low active simple wall has a cuboidal epithelium (Fig. 3B). In turkeys, the follicular wall is seen from simple cuboidal to columnar epithelium depending on the degree of the activity of the follicles (Figs. 4A and 5B). The average height of the lining epithelium of thyroid follicles in turkeys was 156±11 µm whereas in ducks the height of follicular epithelium was 146±17 µm (Table 1). Follicles of turkey's thyroid characterized by numerous vacuoles of various sizes were seen at the periphery of follicles (Fig. 4B). The connective tissue capsule, inter-follicular areas and follicular lining showed a weakly PAS positive reaction in ducks and a moderately PAS positive reaction in turkeys (Fig. 6). The height of the lining epithelium of thyroid follicles in turkeys is significantly higher than the lining epithelium of thyroid follicles in ducks (Fig. 8). The colloid substance inside the follicle is seen as a uniform homogeneous substance. In ducks, the colloid in active follicles were predominantly eosinophilic while the colloid in low active follicles were basophilic in H & E-stained sections (Fig. 3A). It also showed a strongly PAS positive reaction (Fig. 6A). In turkey's thyroid, the colloid was less in amount with vacuolation in the periphery of follicles (Fig. 4B). It was showed a moderate to a weak PAS positive reaction (Fig. 6 B). Under the capsule of the thyroid glands of both species, there are many blood vessels (Figs. 3 & 4A).

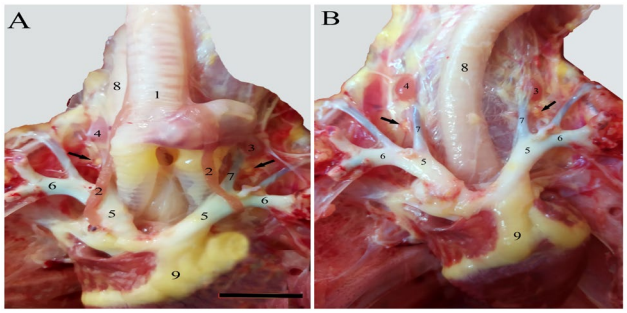

Fig.1. In situ ventral views of the thyroid glands in male Mulard duck; (A) in presence the syrin and (B) after removal the syrinx showing: 1. Syrinx; 2. Sternotrachealis muscles; 3. Left thyroid gland; 4. Right thyroid gland; 5. Innominate artery; 6. Subclavian artery; 7. Common carotid artery; 8. Esophagus; 9. Heart; Ultimobranchial glands (black arrowheads).

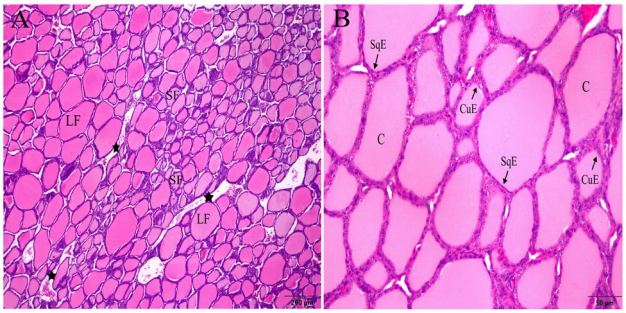

Fig. 3. Paraffin sections stained with H&E in thyroid gland of male mallard duck showing: A: Large follicle (LF), Small follicle (SF), blood vessels in thyroid parenchyma (black stars). B: Cuboidal Follicular Epithelium (CuE), Squamous Follicular Epithelium (SqE) and Colloid (C).

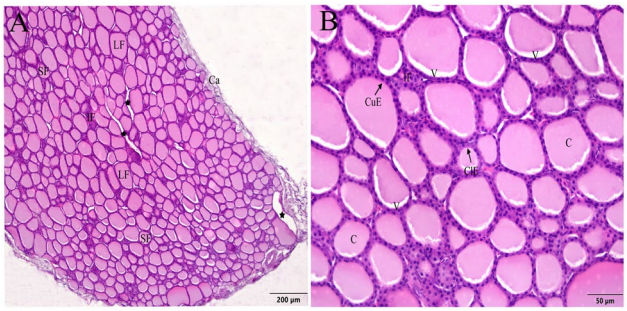

Fig. 4. Paraffin sections stained with H&E in thyroid gland of male turkey showing: A: Large follicle (LF), Small follicle (SF), Inter-follicular cells (IF), connective tissue Capsule (Ca), blood vessels in thyroid parenchyma (black stars). B: Cuboidal Follicular Epithelium (CuE), Columnar Follicular Epithelium (ClE), Inter-follicular cells (IF), Vacuoles (V) and Colloid (C).

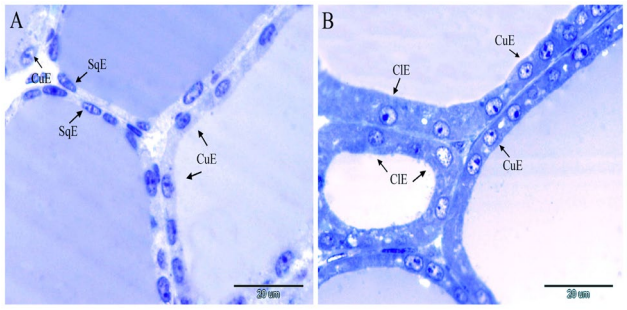

Fig. 5. Paraffin sections stained with PAS stain in thyroid gland of male mallard duck (A) and male turkey (B) showing: The colloid (C) exhibits a strong PAS raction in thyroid follicles of duck, while a moderate to a weak PAS positive reaction in thyroid follicles of turkey. The connective tissue capsule (Ca), inter-follicular areas (IF) and follicular lining (FL) showed weakly PAS positive reaction in both species.

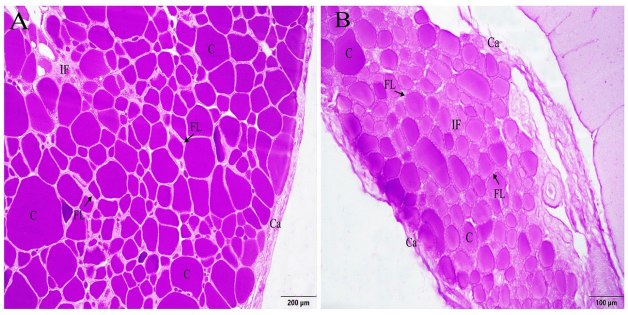

Fig. 6. Paraffin sections stained with Crossmon's Trichrome stain in thyroid gland of male Mulard duck (A) and male turkey (B) showing: Thyroid follicles surrounds by connective tissue capsule (Ca) that composed mainly of collagen fiberous (black arrowheads).

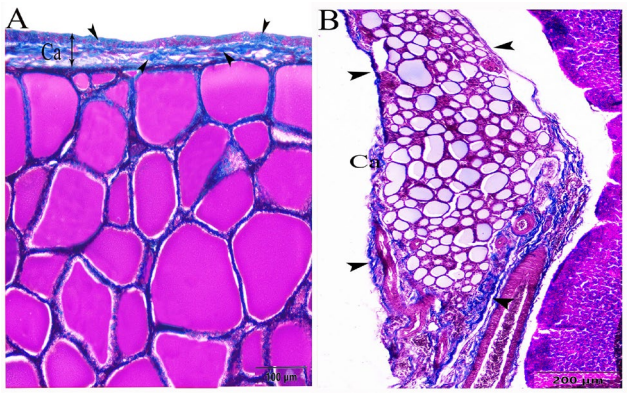

Fig. 7. Semithin sections stained with toluidine blue in thyroid gland of male mallard duck (A) and male turkey (B) showing: A: The lining epithelium of thyroid follicles in ducks varies from simple squamous epithelium (SqE) to simple cuboidal epithelium (CuE). B: The lining epithelium of thyroid follicles in turkeys varies from simple cuboidal epithelium (CuE) to simple columnar epithelium (ClE).

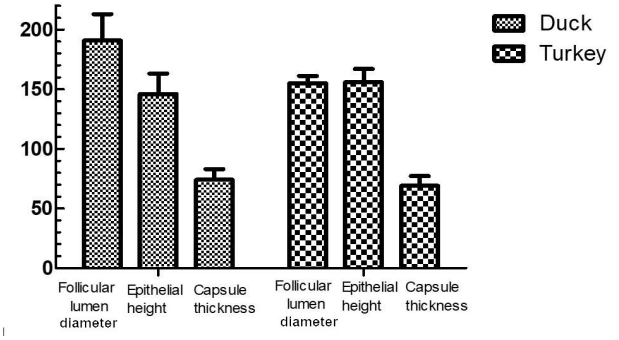

Fig. 8. Histomorphometry graph showing; The diameter of follicular lumen, the height of the lining epithelium of thyroid follicles as well as thickness of thyroid capsule in ducks and turkeys.

The thickness of thyroid capsule of ducks is significantly higher than the thickness of thyroid capsule in turkeys (Fig. 8). The average thickness of the thyroid capsule of ducks was 74.0±9.0 µm while in turkeys the thickness was 69.0±8.0 µm (Table 1). The connective tissue capsule in ducks consisting of two layers, upper dense and lower loose connective tissue layers (Fig. 7A), whereas, it's a delicate capsule in turkeys (Fig. 7B). The follicles in thyroid glands of ducks and turkeys are found in various shapes and sizes, often spherical to elliptical with very little interstitial tissue between them (Figs. 3 and 4). The diameter of follicular lumens in ducks is significantly higher than the diameter of follicular lumens in turkeys (Fig. 8). The average diameter of follicular lumens in ducks was 191.0±22.0 µm but in turkeys the diameter was 155.0±6.0 µm (Table 1).

Discussion

The thyroids are paired organs in ducks and turkeys in the current investigation as described earlier by McLelland (1990) in birds, Firdous *et al.* (2012) in Kuttanad ducks, Sinha *et al.* (2016) in Pati ducks, Ali and Mirhish (2015) in Iraqi turkey, Paul *et al.* (2011) in chicken and Onuk and Kabak (2012) in long-legged buzzard.

The topographical findings of the thyroids of ducks and Egyptian turkeys in this study were supported by Yonkova *et al.* (2023) who stated that both thyroids located cranially from this vascular angle formed by subclavian and common carotid arteries in mallard ducks, North Caucasian turkeys and broiler chickens and by Radek and Piasecki (2007) in Accipitres and Falcones. Dissimilar from the observation of Vishen *et al.* (2019) and Paul *et al.* (2011) in Chabro chicken and indigenous chicken respectively, which recorded that the thyroids located just caudal to the junction of subclavian and common carotid arteries.

The anatomical results in this investigation showed that, the pairs of thyroid glands are oval dark red in color, symmetrically located within the thorax in both species. These results are similar to those of guinea fowls (Moghanlo and Mohammadpour, 2019) and Iraqi turkeys (Hanaa and Shakir, 2015). Dissimilar observation was recorded by Radek and Piasecki (2007) in Accipitres and Falcones and Yonkova *et al.* (2023) in broiler chicken and North Caucasian turkey which stated the left thyroid gland is placed more cranially than the right one as well as by Teresa and Tomasz (2004) in budgerigar which reported that right thyroid gland is placed more cranially than the left one.

In the present study, The thyroid gland of ducks and turkeys have a thin connective tissue capsule which completely surrounds the gland that composed mainly of collagen fibers, which was in agreement with the previous studies of Parchami and Dehkordi (2012) and Hanaa and Shakir (2015) on ducks and turkeys. Our investigation showed that the thyroid capsule of ducks consisted of two layers, dissimilar from observation of Paul *et al.* (2011) in indigenous chickens which recorded that the thyroid capsule consisted of three layers, an outer, middle and an inner layer. The thyroid follicles are found in various shapes and sizes. Commonly, large sized follicles are towards the center of the gland and small sized follicles

Table 1. Histometric measurements on diameter of follicular lumen, height of the lining epithelium of thyroid follicles and thickness of thyroid capsule. Values represented as (means±SD).

Birds	Diameter of follicular lumen (µm)	Height of the lining epithelium of thyroid follicles (µm)	Thickness of thyroid capsule (µm)
Duck	191.0±22.0	146.0±17.0	74.0±9.0
Turkey	155.0±6.0	156.0±11.0	69.0±8.0

are near to the connective tissue capsule with some exceptions. These findings are compatible with those in quail (Suryawanshi *et al*., 2009), in indigenous chicken (Paul *et al*., 2011) and in white leghorn chicken (Singh and Bharadwaj, 1982), whereas antagonistic from the results of (Sekuli *et al*., 2007; Šoši -Jurjevi *et al*., 2006) in rats and pigs who recorded that the larger thyroid follicles are mostly located at the periphery of the thyroid lobes. Dehkordi and Parchami (2011) reported that in dogs, unlike rats and pigs, no statistically significant differences between the peripheral and central thyroid regions.

The shape of the follicular cells, together with the size and shape of the follicles, depends upon the activity of the thyroid gland (Hodges, 1974). Results obtained from the present investigation showed that the large active follicle wall has a simple squamous epithelium, whereas the small low active simple wall has a cuboidal epithelium in mallard duck. This come in agreement with previous studies on indigenous chicken (Paul *et al*., 2011), white pekin duck (Arneja *et al*., 1986) and white crowned sparrow (Oakeson and Lilley, 1960). On the other hand, this study revealed that the follicular wall in turkey is seen from simple cuboidal to columnar epithelium, which was in agreement with Hanaa and Shakir (2015). According to Yonkova *et al*. (2023), there is a specific microstructural in thyroid follicles of North Caucasian turkey, numerous vacuoles of various sizes were seen at the periphery of follicles, which was in agreement with our results in Egyptian turkey. Yonkova *et al*. (2023) reported that presence of one or more thymus penetrations in thyroid parenchyma of broiler chickens and North Caucasian turkeys, while these thymic penetrations are absent in the thyroid parenchyma of mallard ducks. This finding similar to our results on mallard ducks, whereas dissimilar from our results that revealed absence of thymus penetrations in turkeys.

The current study supported the previous finding recorded by Paul *et al*. (2011) in indigenous chicken which revealed that the colloid in active follicles are predominantly eosinophilic, while the colloid in low active follicles are basophilic with H & E stain. The colloid in present work showed a strong PAS positive reaction in ducks, similar observation was also stated by Prasad *et al*. (1999) and Sinha *et al*. (2016) as well as Yonkova *et al*. (2023) in previous studies on ducks and by Balasundaram (2005) in domestic fowls. Our results support the findings stated by Yonkova *et al*. (2023) which revealed that thyroid colloid reacted positively with PAS stain in North Caucasian turkey.

Conclusion

The anatomical study on the thyroid gland of ducks and turkeys, showed that the thyroids symmetrical located in the thorax on both sides of trachea, just cranial to the junction of the subclavian and common carotid arteries. Histomorphometrically, there are differences between two species in diameter of follicular lumens, height of the lining epithelium of thyroid follicles and thickness of thyroid capsule.

Conflict of interest

The authors declare no conflict of interest.

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