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Histomorphology of Thyroid Gland and Thyroid Hormonal Changes in *Pati* ducks (*Anas platyrhynchos domesticus*) of Assam with Age

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Abstract

The study was conducted on 36 number of Assam *Pati* ducks divided into 6 groups to study the histomorphological characteristics of thyroid gland and Thyroid hormones from 2 weeks to 40 weeks of age. The thyroid glands were collected from 6 birds in each group. After recording the gross parameter values the material was fixed and processed for histological studies. Blood samples of about 2-3 ml were collected from the wing veins of each bird before sacrifice of each age group and immediately transferred into blood collection tubes without adding any anticoagulant for biochemical study. The paired thyroid glands in the *Pati* ducks were located on either side of the trachea close to the vascular angle formed by the subclavian artery and common carotid artery. The microscopic structure of thyroid glands of *Pati* ducks revealed primarily the capsule enclosing the follicles that contained colloid in the H & E sections. The interfollicular area contained the parafollicular cells. A homogenous translucent colloidal mass filled the thyroid follicles in all age groups. C cells were present in the interfollicular zone of the thyroid gland. The average serum triiodothyronine level in 2 weeks old ducks was 2.706 ± 0.049 nmol/L. The mean serum level of thyroxine in 2 weeks old ducklings was 53.981 ± 0.590 nmol/L and in 40 weeks ducks was 71.198 ± 2.337 nmol/L.

Keywords: Pati ducks, thyroid, histomorphology, gross, thyroid hormone, age

The thyroid gland is a unique endocrine gland which plays an important role in carbohydrate, protein, lipo-regulatory mechanisms etc. relevant to growth. It is a critical organ for maintaining general metabolic rate and controlling pre

and post natal growth and differentiation of many organ systems. Thyroid hormones in birds regulate body weight, plumage growth, fertility, secondary sex characteristics and lipid metabolism, (Lucy *et al.* 2009). Literature on the histomorphology of thyroid gland and thyroid hormone level in ducks is scanty and therefore, this work was undertaken to form the basis for correlating the possible functions of thyroid gland in relation to the growth and production of layer ducks, deficiency syndrome and detection in any abnormality of the thyroid gland.

Materials and Methods

In this study, a total of 36 *Pati* ducks were utilized. The ducks were randomly divided into seven (6) groups consisting of six (6) birds in each group. The randomly selected 36 birds were divided into 6 groups and reared under open range system. The six groups of birds were divided into 6 different age viz. day old, 2 weeks, 4weeks, 8weeks, 20weeks, 30weeks and 40 weeks. The experimental birds were sacrificed according to the method of Gracy (1986). Blood samples of about 2-3 ml were collected from the wing veins of each bird of each age group and immediately transferred into blood collection tubes without adding any anticoagulant before the scarifice. After the serum was separated, it was kept in plastic vials and stored in deep freeze at -20^{o}C for estimation of blood thyroid hormone levels.

Table 1: Micrometric parameters of follicular diameter at different age groups in *Pati* ducks

Age groups	Diameter of follicles (μm)
2 week	94.155 ± 0.150^{f}
4 weeks	96.296 ± 0.114^{e}
8 weeks	98.921 ± 0.249^{d}
20 weeks	125.390 ± 0.149^{c}
30 weeks	142.535 ± 0.100^{b}
40 weeks	153.886 ± 0.162^{a}

Means with different superscripts are significantly different from each other ($P<0.0001$).

After slaughter, the birds were placed on a clean dissecting table, mid-ventral incision was put; clavicle along with breast muscles were cut and were reflected carefully without disturbing the organs of the region. The thoracic cavity of each bird was exposed by making a ventro-median incision and then the thoracic muscular layers and air sac of the clavicle were reflected.

The location and relative topographic in-situ position of the thyroid gland was recorded. For histological and micrometrical study thyroid gland samples were collected from different age group of *Pati* ducks. The tissue samples were fixed in 10% neutral buffered formalin and standard procedures were adopted for histomorphological studies. Different micrometrical parameters were recorded on Hematoxylin and eosin stained sections by means of standard method of micrometry using Nikon E 200 camera mounted microscope and Image Pro Express Ver-2.0 Software.

Table 2: Morphometry of the thyroid glands in Assam *Pati* ducks at different age groups

Experimental groups	Age		Organ Weight (g)	Length (cm)	Breadth (cm)	Thickness (cm)
I	2 Weeks	Left	0.053 ± 0.004^{f}	0.420±0.002^{f}	0.260 ± 0.002^{f}	0.213 ± 0.002^{f}
		Right	0.056 ± 0.004^{f}	0.426±0.003^{f}	0.265 ± 0.003^{f}	0.216 ± 0.002^{f}
II	4 Weeks	Left	0.120 ± 0.003^{e}	0.476±0.014^{e}	0.388 ± 0.005^{e}	0.258 ± 0.003^{e}
		Right	0.120 ± 0.003^{e}	0.473±0.004^{e}	0.380 ± 0.005^{e}	0.258 ± 0.003^{e}
III	8 Weeks	Left	0.338 ± 0.004^{d}	0.568±0.003^{d}	0.438 ± 0.004^{d}	0.316 ± 0.003^{d}
		Right	0.331 ± 0.004^{d}	0.566±0.003^{d}	0.438 ± 0.004^{d}	0.310 ± 0.002^{d}
IV	20 Weeks	Left	0.738 ± 0.003^{c}	0.653±0.003^{c}	0.576 ± 0.004^{c}	0.558 ± 0.003^{c}
		Right	0.733 ± 0.002^{c}	0.651±0.003^{c}	0.576 ± 0.004^{c}	0.550 ± 0.003^{c}
V	30 Weeks	Left	0.853 ± 0.003^{b}	0.776±0.003^{b}	0.636 ± 0.004^{b}	0.700 ± 0.005^{b}
		Right	0.853 ± 0.003^{b}	0.776±0.003^{b}	0.636 ± 0.004^{b}	0.700 ± 0.005^{b}
VI	40 Weeks	Left	0.981 ± 0.004^{a}	0.983±0.002^{a}	0.778 ± 0.004^{a}	0.820 ± 0.004^{a}
		Right	0.981 ± 0.004^{a}	0.986±0.002^{a}	0.778 ± 0.004^{a}	0.821 ± 0.003^{a}

Means with different superscripts are significantly different from each other (P<0.0001).

Serum hormonal parameters like T_3 and T_4 shall be estimated with the standard procedure of Radio Immuno Assay (RIA), using RIA kits supplied by Immunotech, France. The tracer I-125 was used in the estimation technique which involved competition between free and isotope tagged hormones for binding to the limited antibody sites and subsequently quantification was made through calibration curve. The estimation was done in 6 well Automatic gamma counter, Startec, West Germany. The intra and inter assay co-efficient of variation were 6.3% and 7.7 % for Triiodothyronine and 6.2 % and 8.6 % for thyroxine.

The data were analysed using the Statistical Analyses System version 9.3 (SAS 2012) for Microsoft Windows.

Results

The paired thyroid glands in the *Pati* ducks were located on either side of the trachea close to the vascular angle formed by the subclavian artery and common carotid artery. The thyroid glands were round, oval or elliptical and were reddish brown in colour.

Table 3: Showing serum Triiodothyronine (T_3) and Thyroxine (T_4) level in Assam *Pati* ducks at different age groups

Age groups	Triiodothyronine (T_3) (nmol/L)	Thyroxine (T_4) (nmol/L)
2 weeks	2.706 ± 0.049^{a}	53.981 ± 0.590^{a}
4 weeks	2.653 ± 0.055^{a}	49.040 ± 0.454^{b}
8 weeks	2.593 ± 0.006^{b}	46.708 ± 0.401^{b}
20 weeks	2.353 ± 0.011^{c}	56.281 ± 0.996^{a}
30 weeks	1.610 ± 0.029^{d}	82.071 ± 1.284^{c}
40 weeks	1.311 ± 0.032^{e}	71.198 ± 2.337^{d}

Means with different superscripts differ significantly.

The microscopic structure of thyroid glands of *Pati* ducks revealed primarily the capsule enclosing the follicles that contained colloid in the H & E sections. The interfollicular area contained the parafollicular cells. Thy thyroid glands were invested by a thin capsule that enclosed numerous thyroid follicles which were well differentiated in the day-old ducklings itself. The capsule was made up of collagen and reticular fibers with scanty elastic fibers. Collagen fibers showed an increasing trend with age. The reticular fibers showed an increasing trend with age. The interfollicular space was relatively devoid of elastic fibers except the last two groups comprising of scanty elastic fibers. The nerves fibers were present in all age groups.

Parenchyma of thyroid gland was composed of follicles which were separated from each other by interfollicular connective tissue made up of collagen and reticular fibers.

The follicles were closely packed together and their shape varied from oval to polyhedral in all the age groups. As the age advanced, the size of the follicles increased with a mean diameter of 94.155 ± 0.150 μm in 2 weeks old to 153.886 ± 0.162 μm in 40 weeks old birds. Micrometrical values of follicular

diameter for all the age groups are presented in Table 2. The trabeculae extending from capsule to interior carried numerous blood vessels. Follicles were lined by single layer of epithelial cells and type of epithelium depends upon their functional status. Therefore, based on the type of epithelium and nature of colloid, the follicles were categorized as active follicles and inactive follicles. The active follicles increased upto 8 weeks of age and then a gradual decrease was seen until the end of the study. The inactive follicles increased with the advancement of age. This might be related with the activity of the thyroid gland with advancement of age. The active follicles were lined by simple cuboidal epithelium, while the inactive follicles were lined by simple squamous epithelium.

A homogenous translucent colloidal mass filled the thyroid follicles in all age groups. The quantity of colloid varied according to the activity of the thyroid gland. In the inactive follicles, it was more and homogenous due to accumulation of large amount of colloid, whereas in active follicles it was lesser and non-homogenous. This might be due to regular production and consumption of the colloid. The solid form of the colloid observed in the older age groups might probably be due to prolonged stasis of colloid in the follicle.

In addition to the connective tissue fibers, nerves fibers and capillaries, the parafollicular or interfollicular or C cells were present in the interfollicular zone of the thyroid gland. These cells were present from day old ducklings to 40 weeks old birds.

The serum triiodothyronine hormone level showed a decreasing level from 2 weeks old birds up to the adult birds. The average serum triiodothyronine level in 2 weeks old ducks was 2.706 ± 0.049 nmol/L and 40 weeks old adult ducks was 1.311 ± 0.032 nmol/L. The serum thyroxine level showed a decreasing trend up to 8 weeks, while the level again increased on 30 weeks and again decreased on adult birds. The mean serum level of thyroxine in 2 weeks old ducklings was 53.981 ± 0.590 nmol/L and in 40 weeks ducks was 71.198 ± 2.337 nmol/L. . The serum triiodothyronine (T_3) and thyroxine (T_4) level in Assam *Pati* ducks from 2 weeks old ducklings to 40 weeks old are shown in table 3. There was highly significant difference ($P<0.0001$) between the various age groups in serum triiodothyronine (T_3) and thyroxine (T_4) level.

Discussion

The paired thyroid glands in the *Pati* ducks were located on either side of the trachea close to the vascular angle formed by the subclavian artery and common carotid artery. Similar observations were made by Balasundaram

(2000) in domestic fowl. The thyroid glands were round, oval or elliptical and were reddish brown in colour. Similar findings were observed by Hodges (1974) in poultry. The capsule was made up of collagen and reticular fibers with scanty elastic fibers. Similar finding was reported by Hodges (1974) and Balasundaram and Mookkappan (2004) in domestic fowl and Sathyamoorthy and Vijayaragavan (1997) in Japanese quail. The interfollicular space was relatively devoid of elastic fibers except the last two groups comprising of scanty elastic fibers. The nerves fibers were present in all age groups. Similar findings were observed by Lucy *et al*. (2009) in Kuttanad ducks. As the age advanced, the size of the follicles increased with a mean diameter of 90.715 ± 0.003 µm in day old to 153.886 ± 0.162 µm in 40 weeks old birds which was similar to the findings observed by Lucy *et al*. (2009) in Kuttanad ducks. The active follicles were lined by simple cuboidal epithelium, while the inactive follicles were lined by simple squamous epithelium which was similar to the findings of Hodges (1974) in fowl, Enura *et al*. (1977), Wight and Shannon (1985) in quail. C cells were present from day old ducklings to 40 weeks old birds which was similar to the findings were reported by Lucy *et al*. (2009) in Kuttanad ducks.

The serum triiodothyronine hormone level showed a decreasing level from 2 weeks old ducks till attainment of adult age. This might be due to the physiological decline of thyroid gland activity with advancement of age. The average serum triiodothyronine level in day old ducks was found to be 2.706 ± 0.049 nmol/L and in 40 weeks old adults ducks was 1.311 ± 0.032 nmol/L. Stojevic (2000) also reported that the concentration of T_3 in 28 day old chicken varied between 1.2nmol/l and 2.0 nmol/l, averaging 1.58±0.07 nmol/l, at the age of 35 days plasma concentration of T_3 varied between 1.3 nmol/l and 2.3nmol/l with a mean of 1.85±0.11nmol/l and at the age of 42 days, T_3 concentration ranged between 1.8nmol/l and 3.9nmol/l, with a mean of 2.48±0.17nmol/l.

The serum thyroxine level showed a decreasing trend up to 8 weeks, while the level again increased up to 30 weeks of age and thereafter found decreased level at the age of 40 weeks. The decreased trend might be due to decrease in activity of thyroid gland, while the increased level in 30 weeks old ducks might be related with egg laying stage in which the requirement of thyroid hormones might be higher for higher metabolic demand. The mean serum level of thyroxine in day old ducklings was 53.981 ± 0.590 nmol/L and in 40 weeks old ducks was 71.198 ± 2.337 nmol/L. In chicken, Stojevic (2000) recorded that the concentration of plasma T_4 in 28 day old ranged between 16.00 and 38.00

nmol/l with a mean of 27.8 ± 2.21 nmol/l while at 35 days of age, values varied between 18.00 and 42.00 nmol/l, with mean value 30.11±2.88nmol/l and in 42 day old chicken concentration of plasma T_4 ranged between 24.00 and 38.00 nmol/l, amounting to a mean of 31.00 ± 1.55nmol/l.

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