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The Histochemical Study of the Thyroid Gland of Chabro Chicken Reared in Summer and Winter Seasons

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ABSTRACT

Background: Chabro is a strain of poultry birds especially designed for backyard farming and is more adoptive to climatic variations in the tropics. The thyroid gland plays an important role in controlling basal metabolic rate. Histochemical changes of the gland in association with seasonal changes have not been studied so far in Chabro chicken. The present study describes the season related variations in the histochemistry of thyroid gland.

Methods: The histochemical study was conducted on thyroid gland of eight to ten weeks old 24 apparently healthy Chabro chickens procured from Poultry Farm, DUVASU, Mathura. The birds were divided into two groups consisting of 12 chickens in each group reared in summer and winter seasons.

Result: The capsule showed mild reaction for PAS, protein and glycogen. Follicular cells showed intense reaction for bound lipids, protein and alkaline phosphatase. The colloid in summer season showed strong reaction for PAS at periphery and very strong reaction at center while in winter colloid showed strong PAS positive activity. The Chabro chicken thyroid was functionally more active in winter as compared to summer season because of increased diameter of all follicles and concentration of thyroid hormones level in winter season.

Key words: Chabro chicken, Histochemistry, Season, Thyroid gland.

INTRODUCTION

Thyroid is a bilobed structure situated on either side of trachea. It is the only gland in the body of both mammals and birds that stores hormone in its inactive form. The substance of the gland consists of roughly spherical thyroid follicles. The thyroid gland is a unique endocrine gland which plays an important role in controlling pre and post natal growth and differentiation of many organ systems. Chabro chicken was developed by Central Poultry Development Organization (CPDO) by crossing Barred Plymouth Rock with Red Cornish especially for the farmers of our country owing to more adoptive to climatic fluctuations and acclimatizes to the entire climatic zone. The study was conducted on thyroid gland because it plays an important role in basal metabolic rate, reproduction and development and growth of muscles and bones. On perusal of literature it is found that the histochemical studies in Chabro with special reference to season is very scanty. Hence, the present study is designed to record the seasonal behavior in thyroid gland.

MATERIALS AND METHODS

The present study was conducted in the year 2018 on thyroid gland of eight to ten weeks old 24 apparently healthy Chabro chickens procured from Poultry Farm, DUVASU, Mathura. The birds were divided into two groups consist of 12 chickens in each group (six male and six female) reared in summer and winter seasons. For histochemical observations, small pieces of the thyroid gland were collected and fixed in 10% neutral buffered formalin. Fixed tissues were processed by routine paraffin embedding technique (Luna, 1968) and 6µ paraffin sections were procured. For studying lipid and enzymes cryostat (15µ) sections were utilized. For histochemical and histoenzymic studies following staining procedures were carried out :

1.) Periodic Acid Schiff's (PAS) (Luna, 1968): For study of polysaccharides.
2.) Best's carmine method (Luna, 1968): For study of glycogen.
3.) Muller's Colloidal (hydrous) ferric oxide method (Luna, 1968): For study of Acid mucopolysaccharides.
4.) Acetone Sudan black method (Pearse, 1975): For study of bound lipids.
5.) The Calcium Cobalt method (Pearse, 1975): For study of Alkaline phosphatase.

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6.) Modified lead nitrate method (after Takeuchi and Tanoue) (Pearse, 1975): For study of Acid phosphatase.
7.) Feulgen's reaction (Singh and Sulochna, 1996): For study of DNA.

RESULTS AND DISCUSSION

In the present study the thyroid gland of Chabro chicken was composed of stroma and parenchyma. The stroma of the thyroid gland was composed of connective tissue supported the follicles and Parafollicular cells present in the parenchyma. In present study the thyroid gland was enclosed by a thin capsule composed of two layers, the outer and inner layer.

The capsule showed mild reaction and the wall of blood vessels exhibited strong reaction for presence of Polysaccharides (PAS) (Table 1). The nuclei of red blood cells showed intense Feulgen's reaction for presence of DNA (Photograph 8). Singh (1967) in chicken observed that the innermost capsula fibrosa exhibited positive reaction for PAS. Prasad *et al*. (1999) in domestic ducks, Balasundaram (2005) in domestic fowl, Firdous and Lucy (2015) in Kuttanad ducks and Sinha *et al.* (2016) in Pati ducks observed mild to intense positive reaction for PAS in the capsule. Sinha *et al.* (2016) in Pati ducks observed weak glycogen activity in capsule. Balasundaram (2005) in domestic fowl and Firdous and Lucy (2015) in Kuttanad ducks noticed accumulation of lipids in the capsule. These authors also mentioned the increased accumulation of lipids in the middle layer of the capsule with age. The mild alkaline phosphatase activity in the capsula fibrosa was observed by Singh (1967) in chicken.

The interfollicular connective tissue showed mild, moderate and negative reaction for PAS, glycogen and AMPS, respectively. The interfollicular space was moderately reactive for lipids (Table 1). The interfollicular area of thyroid gland showed weak PAS positive reaction in domestic ducks, domestic fowl and Pati ducks (Prasad *et al*., 1999; Balasundaram, 2005 and Sinha *et al*., 2016, respectively). Sinha *et al*. (2016) in Pati ducks observed weak glycogen activity in interfollicular space. Balasundaram (2005) in domestic fowl and Firdous and Lucy (2015) in Kuttanad ducks observed the accumulation of lipids in the interfollicular area of the thyroid gland.

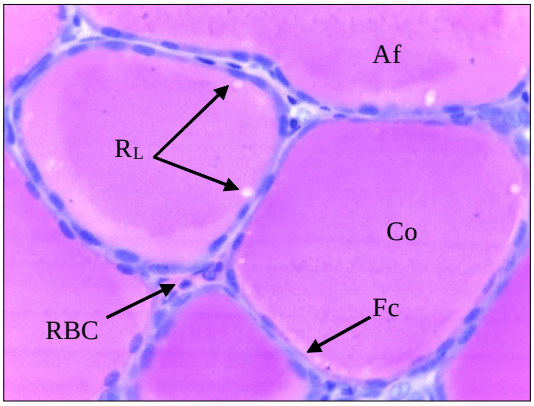
The follicular epithelium exhibited negative to mild reaction for acid mucopolysachharides, mild for PAS, alkaline phosphatase enzyme and moderate for acid phosphatase enzyme and bound lipids (Photograph 4, 1 and 2, 6, 7 and 5). The nuclei of the follicular cells exhibited intense positive Feulgen's reaction for DNA (Table 1) (Photograph 8). Prasad *et al.* (1999) in domestic ducks, Firdous and Lucy (2015) in Kuttanad ducks noticed intense and Sinha *et al*. (2016) in Pati ducks observed moderate PAS reaction in follicular cells of thyroid gland. Firdous and Lucy (2015) in Kuttanad ducks detected the glycogen granules in the follicular epithelial cells. Sinha *et al*. (2016) in Pati ducks observed moderate glycogen activity in follicular lining epithelium. Singh (1967) in chicken noticed a mild alkaline phosphatase activity at the apical border of the follicular cells. Gulati and Nangia (1974) in chicken, Prasad *et al.* (1999) in domestic ducks and Balasundaram (2005) in domestic fowl reported the presence of alkaline phosphatase activity in the follicular epithelium. The follicular epithelium in Kuttanad ducks had maximum activity of the alkaline phosphate at six weeks of age and thereafter the activity became moderate in the older age groups (Firdous and Lucy, 2015). Sinha *et al.* (2016) in follicular epithelium of Pati ducks found strong reactivity for alkaline phosphatase from day old to 20 weeks age. Singh (1967) noticed mild Feulgen's reaction in chromatin network of follicular cells in chicken.

The colloid was glycoprotein in nature when stained with PAS for polysaccharide because of its content of thyroglobulin, an iodinated glycoprotein (Banks, 1993 in domestic animals and Beyzai and Adibmoradi, 2010 in ostrich). The colloid of summer season showed strong reaction at periphery and very strong reaction at center for PAS while in winter colloid had strong PAS positive activity (Table 1) (Photograph 1 and 2). Follicular colloid exhibited PAS positive reaction in animals (Dellmann and Brown, 1987 and Banks, 1993). Singh (1967) in chicken and Firdous and Lucy (2015) in Kuttanad ducks recorded strong positive reaction for PAS in colloid of thyroid gland. Sinha *et al.* (2016) in Pati ducks observed strong glycogen activity in colloids. The colloid gave mild reaction for presence of glycogen (Photograph 3). Moderately positive alkaline phosphatase enzyme was present in colloidal content (Table 1)

Table 1: Various histochemical reactions of thyroid gland of 8 - 10 weeks old chabro chickens reared in summer and winter seasons.

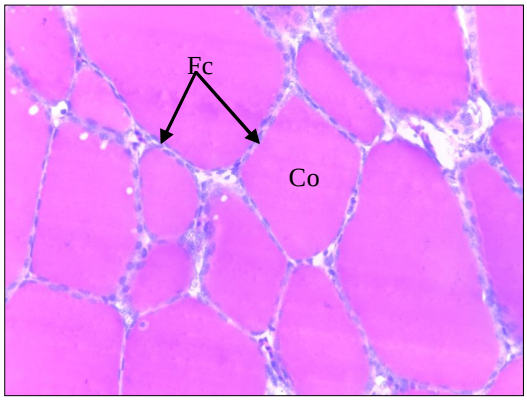
Characters	Cellular reactions				Nuclear reaction		
	PAS	AMPS	Glycogen	Lipid	Alkaline phosphatase	Acid phosphatase	Feulgen's reaction
Capsule	+	-	+	+ to ++	-	-	-
Epithelial cell	+* to ++#	- to +	++	++# to +++*	+++	+*	+++
Colloid	+++ to ++++		+	-	+ to ++	-	-
Interfollicular connective tissue	+	-	++	++	-	-	-
C-cells	+	-	+	-	-	-	+
Blood vessels	+++	++	++				+++
RBC	+++	-	++	-	-	-	+++

Note: - (+) mild, (++) moderate, (+++) strong, (++++) very strong, (-) negative, *(cell membrane), #(cell cytoplasm).

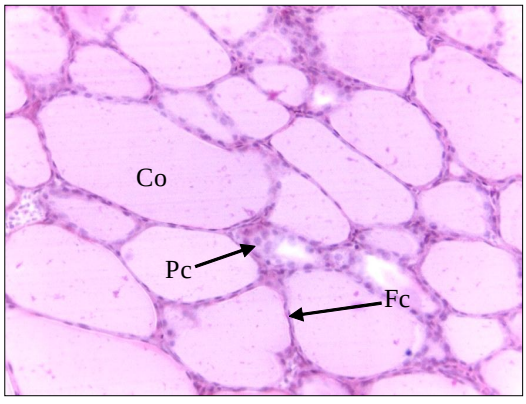


Photograph 1: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing the strong polysaccharide reaction for PAS in active follicle (A_f), strong reaction at periphery and very strong reaction at center in colloid (Co), mild to moderate reaction in follicular cells (Fc) and resorption lacunae (R_L) and strong reaction in red blood cell (RBC).

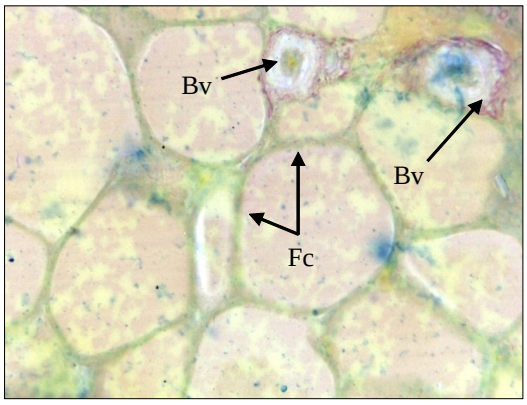
Periodic Acid Schiff's stain X1000



Photograph 2: Photomicrograph of the thyroid gland of Chabro chicken reared in winter season showing the mild to moderate polysaccharide reaction for PAS in follicular cells (Fc) and strong reaction in colloid (Co). **Periodic Acid Schiff's stain X400**

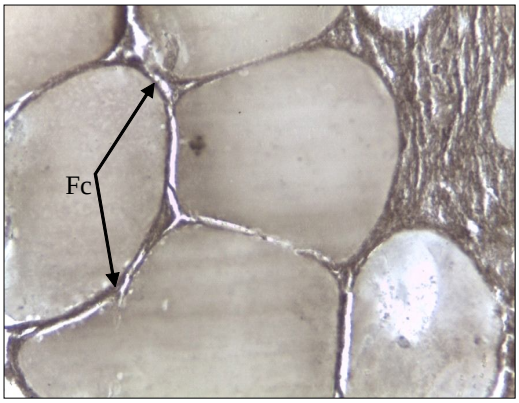


Photograph 3: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing the moderate reaction for glycogen in follicular cells (Fc), mild reaction in colloid (Co) and Parafollicular cells (Pc). **Best's carmine method X400**



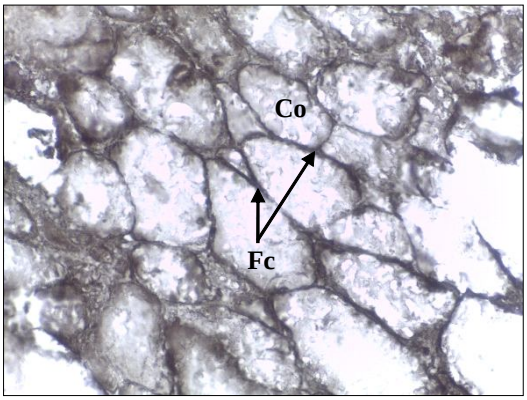
Photograph 4: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing the negative to mild reaction for Acid mucopolysaccharides in follicular cells (Fc) and moderate reaction in blood vessels (Bv) present in interfollicular space.

Muller's colloidal (hydrous) ferric oxide stain X400

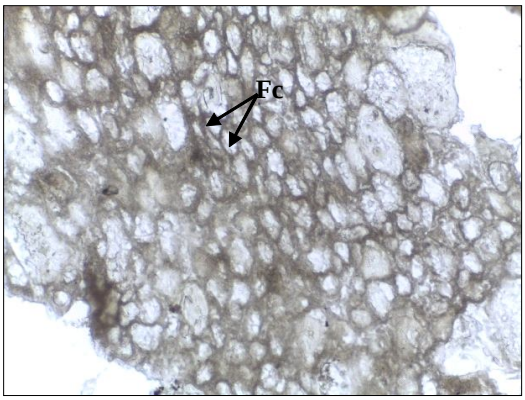


Photograph 5: Photomicrograph of the thyroid gland of Chabro chicken reared in winter season showing the moderate to strong reaction for bound lipid in follicular cells (Fc).

Acetone Sudan Black stain X400

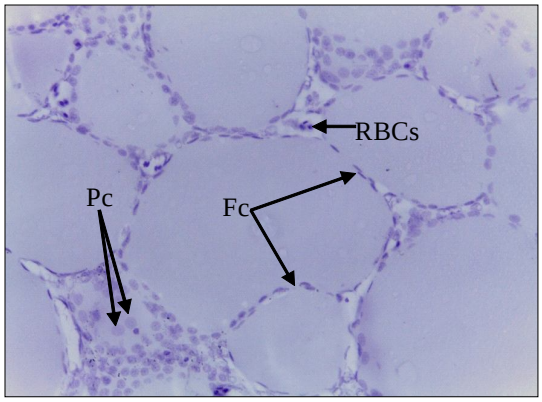


Photograph 6: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing intense reaction for Alkaline phosphatase in follicular cells (Fc) and mild to moderate reaction in colloid (Co). **Gomori's stain X200**



Photograph 7: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing the moderate reaction for Acid phosphatase in follicular cells (Fc).

Gomori's stain X200



Photograph 8: Photomicrograph of the thyroid gland of Chabro chicken reared in summer season showing the intense Feulgen's reaction in nuclei of follicular cells (Fc) and red blood cells (RBCs) and mild reaction in Parafollicular cells (Pc).

Feulgen's Reaction X400

(Photograph 6). Singh (1967) observed alkaline phosphatase activity at the periphery of colloid. Singh (1967) observed absence of acid phosphatase reaction in the colloid in chicken. Prasad *et al.* (1999) in domestic ducks; Balasundaram (2005) in domestic fowl; Firdous and Lucy (2015) in Kuttanad ducks and Sinha *et al.* (2016) in Pati ducks noticed strong acid phosphatase reaction towards the colloidal margin of the lining cells of the active thyroid follicle.

The nuclear chromatin of Parafollicular cells gave mild reaction with Feulgen stain for DNA (Table 1) (Photograph 8). The parafollicular cells of thyroid gland in Pati ducks (Sinha *et al.*, 2016) reported mild reaction for PAS. Ismail (1987) in Fayomi laying and non-laying hens reported positive reaction for PAS in parafollicular cells. Singh (1967) noticed mild Feulgen's reaction in chromatin network of parafollicular cells in chicken.

CONCLUSION

In the present study the follicular cells showed intense reaction for bound lipids, protein, alkaline phosphatase while moderate reaction was noted for PAS, glycogen and acid phosphatase. The nuclei of follicular cells exhibited intense positive Feulgen's reaction. The colloid in summer season showed strong reaction for PAS at periphery and very strong reaction at center while in winter colloid showed strong PAS positive activity. The colloid showed mild reaction for glycogen and moderate reaction for protein and alkaline phosphatase. Colloid cells of Langendorff were also encountered in few of the follicles within colloid. The colloid cells of Langendorff showed mild reaction for PAS. The nuclear chromatin gave mild reaction with Feulgen stain for DNA and the cytoplasm had mild PAS and glycogen reaction.

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