Full Length Article

HISTOLOGY AND HISTOCHEMISTRY OF THE GALL BLADDER IN SIRUVIDAI CHICKEN

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ABSTRACT

The present investigation aims to study the histological and histochemical details of the gall bladder in four different age groups viz.2,6,12 and 18 week-old Siruvidai chicken, an indigenous poultry ecotype of Tamil Nadu. Six healthy birds from each group were used. Gall bladder tissues were collected, fixed in various fixatives, processed by paraffin embedding, sectioned at 5–6 µm and subjected to histological and histochemicalstaining techniques. The gall bladder had three tunics. The innermost tunica mucosa was folded, forming short villous-like projections. It was lined by simple columnar epithelium which was PAS-positive and also showed alcianophilic reaction. The middle tunica muscularis consisted of circularly arranged smooth muscle fibres, while the outermost tunic, serosa in the free surface and adventitia in the attached surface of the gall bladder. Significant difference was observed in the mean thickness of all the three tunics and epithelial cell height across all the four age groups under study.

Keywords: Gall bladder, histology, histochemistry, Siruvidai chicken

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INTRODUCTION

Siruvidai chicken, an indigenous ecotype of Tamil Nadu, is prevalent in Ariyalur district of Tamil Nadu (Richard *et al.*, 2023). Although they are slow growers, Siruvidai chicken are increasingly preferred by poultry farmers due to their distinctive traits, including hardiness, adaptability to tropical climates, striking plumage that aids in predator evasion and strong mothering instincts (Vasanthi *et al*., 2022).

The gall bladder is a small sac lying on the visceral surface of the right lobe of liver in Siruvidai chicken (Hodges, 1974). It is one of the accessory digestive organsthat plays an important role in the storage of

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the bilesecreted by the liver (Kent,1987) found in chicken, duck and geese (Hamodi *et al*., 2013). However, it is absent in pigeon,parrots and ostriches (Hamodi *et a*l., 2013).

The purpose of the present study was to investigate the microscopic structureof gall bladder in various age groups of Siruvidai chicken and to determine thevariation in these features compared to other bird species.

MATERIALS AND METHODS

The present study was conductedin *Siruvidai* chicken of four different age groups- two week, six week, twelve week and eighteen week-old as per the approval by the Institutional Animal Ethics Committee, Faculty of Basic Sciences, Chennai, Ref. No - 20/SA/IAEC/2024 at the Department of Veterinary Anatomy, Madras Veterinary College. For histological studies, six apparently healthy *Siruvidai* chickenoffour different age groups were procured from Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai. Birds were sacrificed and the gall bladder was collected. Tissue samples of the gall bladder were fixed in 10 percent Neutral buffered formalin and Bouin's fluid for various histological and histochemical investigations. Fixed tissues were dehydrated through a graded series of alcohols, cleared in xylene and embedded in paraffin wax. Paraffin-embedded tissues were sectioned at 5-6 µm thickness and stained by standard Haematoxylin and Eosin (H&E) method (Bancroft and Stevens, 1996), Masson's trichrome method for collagen and muscle fibres (Luna, 1968) and Verhoeff method for elastic fibres (Bancroft and Stevens, 1996) for the histological study and Periodic acid Schiff (PAS) for neutral mucopolysaccharides (Singh and Sulochana,1996), Alcian blue for acidic mucopolysaccharides (Luna,1968), Combined Alcian blue-PAS (AB-PAS) technique for acidic and neutral mucins (Singh and Sulochana,1996) and Mercury- Bromophenol method for proteins (Pearse,1960).

RESULTS AND DISCUSSION

Light microscopic examination revealed that the wall of the gall bladder of Siruvidai chicken of all the four age groupsconsisted of three tunics. The inner most was tunica mucosa in which lamina propria was fused with underlying submucosa, the middle tunica muscularis and the outermost tunica serosa/adventitia (Fig.1) as mentioned by Hodges (1974) in domestic fowl. The surface epithelium was lined by simple tall columnar cells with microvilli giving a brush border appearance on its luminal surface (Fig.2), which is consistent with the findings of Al-Shaheen and Hussein (2019) in white eared bulbul. The nucleus was spherical to ovoid located at the base of the vacuolated cytoplasm. However, in some areas, the epithelium appeared pseudostratified columnar in type as mentioned by Abdulqader *et al*. (2023) in broiler fowl whereas, Mobini (2014) reported that it varied from simple columnar to stratified columnarin common quail.

In the present study, goblet cells were not found in the mucosal epitheliumin all age groups which is in accordance with Mobini and Poursafar (2019) in graylag goose and Abdulqader *et al*. (2023) in broiler fowl. However, Mobini (2014) in common quail, Abdulqader *et al.* (2023) in mallard duck and Aboghanima *et al*. (2025) in white Pekin duck observed goblet cells among the epithelial cells of the gall bladder mucous tunic. The mucosa was thrown into villous-like folds that varied in shape and size.Invariably, primary folds gave secondary folds (Fig.3) as reported by Al-Bakri and Al-Hamawandy (2020) in local adult chicken whereas, isometric folds were observed in chukar partridge (Mobini, 2012) and in common quail (Mobini, 2014).

In the current study, the average height of the epithelium in the gall bladder of two week-old Siruvidai chicken was 9.29± 0.31µm, which gradually increased to about 28.72±1.96µm in eighteen weeks of age. Significant differences ($p<0.01$) were observed in the epithelial height of the gall bladder mucosa among the four various age groups studied (Table1).

The epithelial cells in the gall bladder of Siruvidai chicken showed mild positive reaction to PAS with intense reactivity in the microvilli at the apical border, indicating the presence of neutral mucopolysaccharides (Fig.4) as observed by Sivagnanam and Geetha Ramesh (2008) in guinea fowl. Similar to the observations of Mobini (2012) in chukar partridge, the epithelial cells in the mucosa of gall bladder exhibited positive reaction to alcian blue, indicating the presence of acidic glycosaminoglycan complexes (Fig.5). In the combined alcian blue and PAS technique, the gall bladder mucosal epitheliumin Siruvidai chicken reacted positive for alcian blue and were Schiff negative indicating the predominance of acid mucins than the neutral mucopolysaccharides. The mucus secretion of the epithelium attributes to the water absorbing function of the gall bladder for concentration of stored bile (Hayward,1968).

Each villus had a connective tissue core of lamina propria and smooth muscle fibresrunning into it from the deeper layer of muscular tunic. Similar to the observations of Mobini (2012) in chukar partridge, Mobini (2014) in common quail and Mobini and Poursafar (2019) in graylag goose and Abdulqader et al. (2023) in mallard duck, there were invaginations or crypts formed by the mucosal epithelium,whichinvaded the lamina propria giving a tubular gland like structure (Fig.7). Lamina muscularis mucosae was absent in all the age groups of the gall bladder as mentioned by Dellmann and Eurell (1998) in mammals and Mobini (2012) in chukar partridge.

The lamina propriasubmucosa was made up of loose connective tissue fibres which consisted of nodular infiltration of lymphocytes, fibroblast cells and blood vessels (Fig.3) in agreement with the observations mentioned by Dellmann and Eurell (1998) in mammals. The average thickness of the tunica mucosa was 76.82 ± 4.91 µm in two week-old, 94.78 ± 2.02µm in six week-old, 121.81 ± 6.17µm in twelve

week-old and 168.38 ± 10.51µm in eighteen week-old Siruvidai chicken, which proved that the thickness of this layer varied significantly among the four different age groups studied ($p< 0.01$)(Table.1).

The tunica muscularis of the gall bladder in Siruvidai chicken comprised of smooth muscle fibres arranged only in circular fashion. Few strands of smooth muscle fibres from this tunic entered into the base of villous projections (Fig.7) as observed by Hodges (1974) in domestic fowl. However, this is in contrast to the findings of Calhoun (1954) who stated that this tunic had smooth muscle fibres arranged in anouter circular and inner, definite longitudinal layer. In the present study, between the muscle fibres of tunica muscularis, few connective tissue fibres were intermingled with predominantly collagen fibres and a few elastic fibres.Outer to this tunic, a thick connective tissue layer mostly of collagen fibres formed perimuscular connective tissuewhich corroborates with the observations made by Maya *et al*. (2011) in Kuttanad ducks.

The average thickness of the muscular tunic significantly increased ($p < 0.01$) with age, which was about 17.79± 1.20µm in two week, 37.31± 2.91µm in six week, 44.23± 1.01µm in twelve week and 48.40± 1.02µm in eighteen week-old Siruvidai chicken(Table.1). The muscular tunic showed mild positive reaction to mercury bromophenol blue staining indicating the presence of proteins.

The outermost tunica on the free surface of the gall bladder was lined by mesothelial cells forming the serosa, in which loose connective tissue was coated externally with simple squamous cells, whereas on the attached surface, an adventitia was present and mesothelium was absent. The loose connective tissue on the external surface contained blood vessels and nerves. These findings are in agreement with Hodges (1974) in fowl. The mean thickness of this tunic differed significantly at ($p < 0.01$) with increasing age, which was 5.02± 0.34µm in two week, 6.72± 0.30µm in six week, 8.44± 0.49µm in twelve week and 12.53± 0.39µm in eighteen week-old Siruvidai chicken (Table.1).

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Table 1. Micrometric values in µm (Mean ± SE) showing the thickness of all tunics and the epithelial height in the gall bladder of different age groups of Siruvidai chicken

Age group	Thickness (µm)			Height of epithelium (µm)
	T. Mucosa	T. Musclaris	T. Serosa / T. Adventita	
2 weeks	76.81 ± 4.90[a]	17.79± 1.20[a]	5.02± 0.34[a]	9.29± 0.31[a]
6 weeks	94.78 ± 2.01[a]	37.31± 2.91[b]	6.71± 0.30[b]	13.90± 0.98[b]
12 weeks	121.81 ± 6.17[b]	44.23± 1.01[c]	8.44± 0.49[c]	17.73 ± 1.54[b]
18 weeks	168.38 ± 10.50[c]	48.40± 1.02[c]	12.53± 0.39[d]	28.72± 1.96[c]
F value	35.95	61.35	68.42	37.81
p value	.000	.000	.000	.000

p> 0.05 - not significant; p < 0.05 – significant at 5 % level; p < 0.01 – significant at 1% level
Means with similar superscripts in columns do not differ significantly

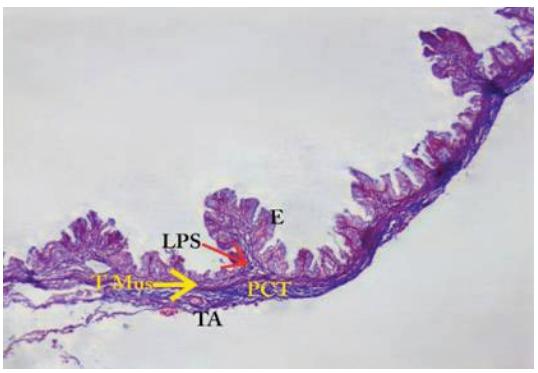

Fig.1. Photomicrograph of the cross section of the gall bladder in eighteen week-old Siruvidai chicken showing the three tunics. E- Epithelium LPS- Lamina Propria Submucosa TA-Tunica AdventitiaTMus – Tunica Muscularis PCT – Perimuscular Connective Tissue Masson's Trichrome x 40

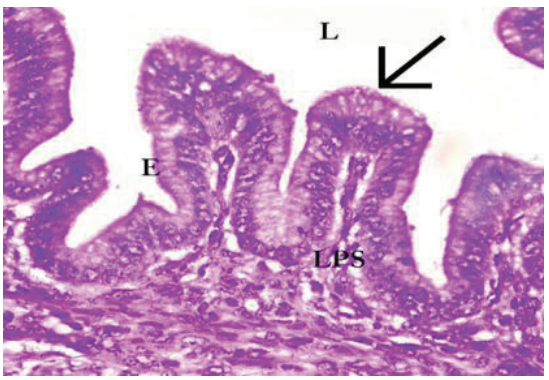

Fig.2. Photomicrograph of the cross section of the gall bladder in two week - old Siruvidai chicken showing simple columnar epithelium with brush border appearance (arrow). E- Epithelium LPS- Lamina Propria Submucosa L- Lumen H&E x 400

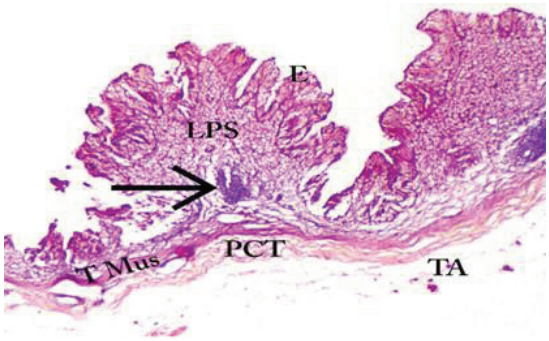

Fig.3. Photomicrograph of the cross section of the gall bladder in twelve week - old Siruvidai chicken showing mucosal folds with lymphatic nodule (arrow) in the lamina propria. E- Epithelium LPS- Lamina Propria Submucosa TA-Tunica Adventitia TMus – Tunica Muscularis PCT – Perimuscular Connective Tissue H&E x 100

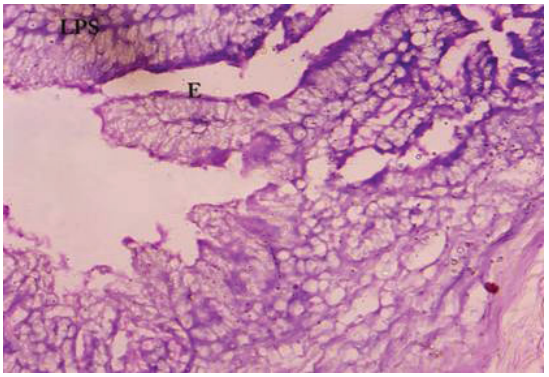

Fig.4. Photomicrograph of the cross section of the gall bladder in six week-old Siruvidai chicken showing the presence of neutral mucins in apical border of surface epithelial cells. E- Epithelium LPS- Lamina Propria Submucosa PAS x 400

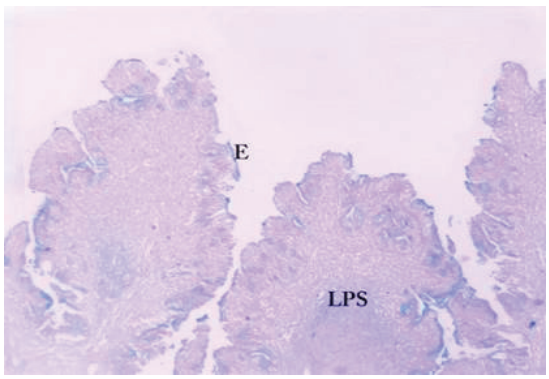

Fig.5. Photomicrograph of the cross section of the gall bladder in twelve week - old Siruvidai chicken showing acidic mucins in the apical part of the mucosal epithelium. E- Epithelium LPS- Lamina Propria Submucosa Alcian blue x 100

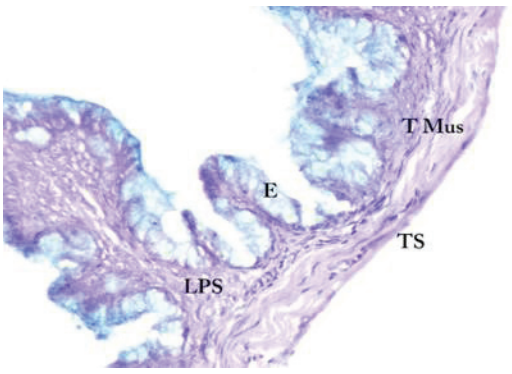

Fig.6. Photomicrograph of the cross section of the gall bladder in eighteen week - old Siruvidai chicken showing alcinophilic reaction in the mucosal epithelium. E- Epithelium LPS- Lamina Propria Submucosa T Mus – Tunica Muscularis TS – Tunica Serosa Combined alcian blue and PAS x 400

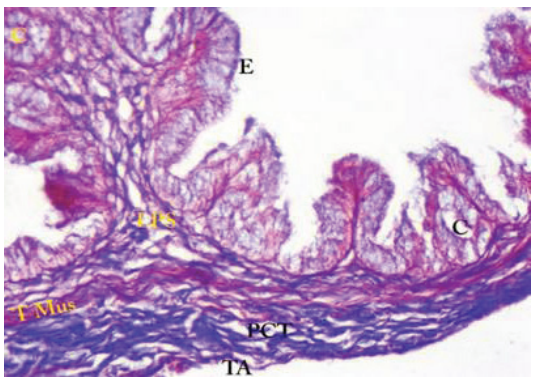

Fig.7 Photomicrograph of the cross section of the gall bladder in eighteen week-old Siruvidai chicken showing the mucosal invaginations that formed the crypts. E- Epithelium LPS- Lamina Propria Submucosa TA-Tunica Adventitia TMus – Tunica Muscularis PCT – Perimuscular Connective Tissue C- Crypts Masson's Trichrome x 400 Combined alcian blue and PAS x 400

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