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# Histomorphological, histochemical and micrometrical studies on the proventriculus of Haringhata black chicken

**Rupam Malakar, Arun Kumar Mandal and Nirmal Kumar Tudu**

**Abstract**
The investigation was carried out on 30 Haringhata Black breed of chickens divided into five group viz. day old, 15 days, 30 days, 45 days and 60 days old with six birds in each age group. The present research about the histomorphological, histochemical and micrometrical study of the proventriculus of the Haringhata Back chickens revealed that the proventriculus was made up of four distinct histological layers. The tunica mucosa presented various longitudinal mucosal folds of varying height lined by tall columnar epithelium. The proventricular glands in the submucosa varied in shape from oval or round to elongated. A framework of collagen, reticular and elastic fibres supported the glands. Serosa was the outermost layer composed of connective tissue, blood vessels and mesothelium. The thickness of the different layers was most in 60 days old birds whereas it was least in 0 day old birds. The epithelial cell and nucleus showed less variation in height, width and diameter with the progression of age.

**Keywords:** Histomorphological, histochemical, micrometrical, proventriculus, serosa, mesothelium

## 1. Introduction
The Haringhata Black (HB) chicken is a native breed of West Bengal state of India. It has originated from the Haringhata and Chakdah blocks of Nadia district and nearby areas like Amdanga and Bongaon blocks in North 24 Pargana district of West Bengal. It is a medium sized chicken with compact body and is reared for both meat and eggs. They have black feathers with red coloured single comb, ear lobe and wattle. These birds are genetically superior to most other chickens and have a very good resistance to diseases. They can be reared in the backyard poultry farming system with minimum investment and also in commercial farms in the deep litter system. The proventriculus is referred as the true stomach of birds because of the presence of the glands and digestion primarily begins here. The present study was undertaken to understand the histological organisation as well as histochemical composition of the said organ in the Haringhata Black chicks of various age.

## 2. Materials and Methods
For observing the structural organization of the proventriculus of Haringhata Black chickens, a total of thirty birds were used and they were procured from State Poultry Farm-1, Mohanpur, Nadia, West Bengal. The research was duly approved by the Institutional Ethical Committee. The flock was maintained in the same farm and divided into 5 groups, viz. day old, 15 days, 30days, 45 days and 60 days of age respectively. On reaching the respective age, six birds were selected randomly and sacrificed by cervical dislocation after anaesthetising them with chloroform vapour. The proventriculus was then dissected out after removing the feathers manually and opening the body cavity of the birds along the mid-ventral border. Tissue samples from the proximal, middle and distal part of the proventriculus were collected and fixed in 10% neutral buffered formalin immediately for 48 hours. Tissue samples were processed for paraffin embedding (Luna, 1968) [1]. 5µ thick tissue sections were obtained with the aid of Leica DM rotary microtome and taken on clean grease-free glass slides.

### 2.1 Following stains were performed
a. Haematoxylin and Eosin (Luna, 1968) [1] for general histology and histometry.
b. PAS-AB (Luna, 1968) [1] for mucopolysaccharides (mucins).
c. Masson's Trichome (Luna, 1968) [1] for connective tissue elements (collagen fibres).

The slides were then visualised under light microscope (Leica DM 2000) fitted with camera and microphotography was done. For each parameter 10 observations were taken and the average of these micrometrical observations was presented in tabulated form after statistical analysis.

## 3. Results and Discussion

In the present study, the proventriculus of 0, 15, 30, 45 and 60 days old Haringhata Black birds were composed of four tunics or layers: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inside to out. The tunica mucosa again consisted of the lamina epithelialis, lamina propria and lamina muscularis. The thickness of the tunica mucosa of proventriculus in 0, 15, 30, 45 and 60 days old birds was 284±22.318 µm, 358±18.322 µm, 438.5±20.012 µm, 523±321 µm and 648±46.155 µm respectively (Table No. 1). The average thickness of tunica mucosa of proventriculus in day old, 7 days, 28 days and 112 days old Kadaknath birds was 323±24.722 µm, 354±18.390 µm, 677.5±20.052 µm, and 712±46.13507 µm respectively as reported by Das *et al.,* (2017) [2]. The mucosal lining of the proventriculus presented numerous longitudinal folds of variable height. These folds were intervened by depressions which were called as sulci (Fig.1). The same findings were documented by Abumandour (2013) [3] in falcon, Deka *et al.*, (2017) [4] in pati duck and Parisa *et al.,* (2019) [5] in pheasants. The average height of the mucosal folds of the proventriculus in 0, 15, 30, 45 and 60 days old birds was 247.3±8.234 µm, 302.1±10.754 µm, 352±13.221 µm, 402.4±612 µm and 464±11.152 µm respectively (Table No 2). Rocha and De Lima (1998) [6] stated that mucosal folds in proventriculus of burrowing owl were lined by the simple columnar epithelium which is in accordance with our findings. The height of the epithelium was more towards the tip of the folds and less towards the base of the folds. Similar finding was reported in Kadaknath birds by Das *et al.,* (2017) [2]. The average height of the epithelial cell of the proventricular mucosa in 0, 15, 30, 45 and 60 days old birds was 7.7±0.321 µm, 8.3±0.453 µm, 8.9±0.312 µm, 9.2±0.222 µm and 9.6±0.112 µm respectively (Table No 2). The average breadth of the epithelial cell of the proventricular mucosa in 0, 15, 30, 45 and 60 days old birds was 4.22±0.315 µm, 4.96±0.212 µm, 5.33±0.412 µm, 5.81±0.334 µm and 5.9±0.124 µm respectively (Table No 2). The average nucleus diameter of the proventricular epithelium in 0, 15, 30, 45 and 60 days old birds was 3.02±0.215 µm, 3.82±0.716 µm, 4.21±0.373 µm, 4.52±0.534 µm and 4.87±0.256 µm respectively (Table No 2). The epithelium of the mucosa showed strong PAS positive reaction indicating the presence of mucins (Fig.3). Das *et al.,* (2017) [7] and Al-Saffar *et al.,* (2016) [8] also reported the same in Kadaknath fowls and domestic pigeon respectively. In the mucosal layer, multiple numbers of scattered papillae were seen around which the mucosal folds were arranged in a concentric ring manner (Fig.2).The excretory ducts of the proventricular glands passed through the centre of papillae.

The lamina propria invaded the central portion of the mucosal folds and was composed of connective tissue fibres. Large lymphoid foci were observed in the lamina propria (Fig.2). Rahman *et al.,* (2003) [9] observed the same in Bangladeshi deshi chicken. Collagen fibres were seen predominantly in between the proventricular glands and in the lamina propria (Fig.4). Simple tubular glands were found in the lamina propria layer. These glands were more pronounced and separated by smooth muscle fibres of muscularis mucosa.

The muscularis mucosa was made up of smooth muscle fibres and was visible between the lamina propria and submucosa and was also scattered among the proventricular glands.

The tunica submucosa was made up of thin layer of connective tissue containing large number of proventricular glands (Fig.5). The same findings were observed before in guinea fowl by Selvan *et al.,* (2008) [10], in duck by Hassan and Moussa (2012) [11] and in chickens by Nasrin *et al.,* (2012) [12]. The average thickness of the tunica submucosa of proventriculus in 0, 15, 30, 45 and 60 days old birds was 1610±23.356 µm, 1728±51.323 µm, 1860±28.071 µm, 2271±45.283 µm and 2620±44.162 µm respectively (Table No. 1). Each proventricular gland was almost oval to round to elongated in shape and presented numerous lobules which were round, oval, hexagonal or polygonal in shape. The glands and lobules varied in size and diameter. The lobules were separated by thin perilobular connected tissue and few smooth muscle fibres. The average diameter of the proventricular glands in 0, 15, 30, 45 and 60 days old birds was 660±10.283 µm, 721±35.036 µm, 960±153.592 µm, 1250±647.345 µm and 1520±221.341 µm respectively (Table No. 2). With the advancement of age the size of the proventricular glands increased may be due to the fusion of two or more glands together. The proventricular glands were lined by low columnar to cuboidal epithelial cells. Each lobule was composed of numerous secretory alveoli or tubules opening together into a wide central cavity from which a wide duct originated. Duct from several lobules joined together to form a short main duct which was connected to the apex of the raised mucosal papillae and opened into the lumen of the proventriculus (Fig.5).Das *et al.,* (2017) [2] reported that the tubular alveoli of the proventricular glands in Kadaknath fowl were lined by oxyntico-peptic cells which agreed with our findings (Fig.6). These cells are responsible for the production of HCL. The ducts were lined by the simple columnar epithelium. The submucosal connective tissue layer consisted of a narrow band of fibrous connective tissue composed of collagen, reticular and few elastic fibers. The submucosa reacted very weakly to the PAS-AB stain (Fig.3).

The tunica muscularis of the proventricular wall consisted of an inner circular and an outer longitudinal muscle layers. Loose connective tissue and blood vessels and nerves were observed in between the muscle layers. The average thickness of the inner circular muscle layer of the proventriculus in 0, 15, 30, 45 and 60 days old birds was 36.2±4.554 µm, 48.2±6.932 µm, 72.4±5.615 µm, 90.2±9.331 µm and 112.1±8.945 µm respectively and the average thickness of the outer longitudinal muscle layer in 0, 15, 30, 45 and 60 days old birds was 76.2±4.318 µm, 120.15±10.222 µm, 138.6±3.116 µm, 162.15±2.222 µm and 184.7±12.448 µm respectively (Table No 1).

The tunica serosa was composed of thin layer of loose connective tissue composed of collagen, reticular and few elastic fibers. The tunica serosa layer was covered by mesothelium as well as blood vessels. The average thickness of the tunica serosa of proventriculus in 0, 15, 30, 45 and 60 days old birds was 12.35±2.162 µm, 24.51±2.961 µm, 32.25±3.639 µm, 45.32±4.131 µm and 62.05±4.832 µm respectively (Table No 1).

**Table 1:** Average Thickness (Mean±SE) of the Various Layers of Proventriculus (µm)

| Layers of proventriculus | | Age Group of birds | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 15 days | 30 days | 45days | 60 days |
| Tunica Mucosa | | 284±22.318 | 358±18.322 | 438.5±20.012 | 523±321 | 648±46.155 |
| Tunica submucosa | | 1610±23.356 | 1728±51.323 | 1860±28.071 | 2271±45.283 | 2620±44.162 |
| Tuinica muscularis | Inner circular | 36.2±4.554 | 48.2±6.932 | 72.4±5.615 | 90.2±9.331 | 112.1±8.945 |
| | Outer longitudinal | 76.2±4.318 | 120.15±10.222 | 138.6±3.116 | 162.15±2.222 | 184.7±12.448 |
| Tunica serosa | | 12.35±2.162 | 24.51±2.961 | 32.25±3.639 | 45.32±4.131 | 62.05±4.832 |

**Table 2:** Mean±SE of the diameter of proventricular glands, height of mucosal folds, mucosal epithelial cell height, breadth and nucleus diameter (µm)

| Parameters | | Age groups of birds | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 15 days | 30 days | 45 days | 60 days |
| Height of mucosal folds | | 247.3±8.234 | 302.1±10.754 | 352±13.221 | 402.4±612 | 464±11.152 |
| Diameter of proventricular glands | | 660±10.283 | 721±35.036 | 960±153.592 | 1250±647.345 | 1520±221.341 |
| Mucosal epithelium | Height | 7.7±0.321 | 8.3±0.453 | 8.9±0.312 | 9.2±0.222 | 9.6±0.112 |
| | Breadth | 4.22±0.315 | 4.96±0.212 | 5.33±0.412 | 5.81±0.334 | 5.9±0.124 |
| Nucleus diameter | | 3.02±0.215 | 3.82±0.716 | 4.21±0.373 | 4.52±0.534 | 4.87±0.256 |

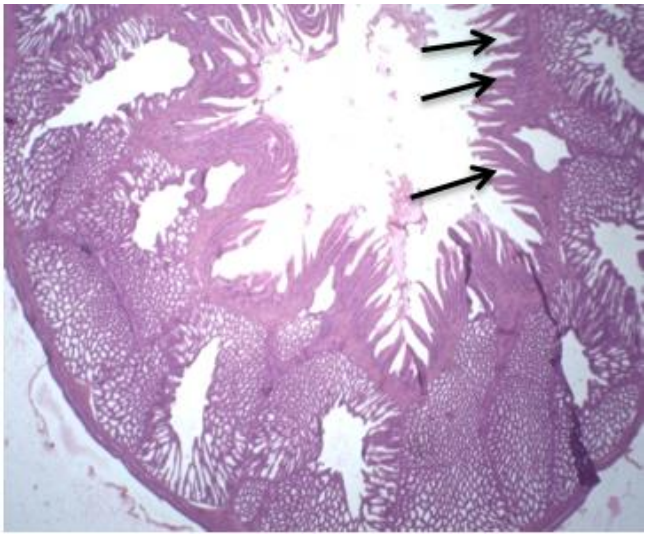

**Fig 1:** Photomicrograph of proventriculus of day old Haringhata Black chick showing Sulci (arrow) between the mucosal folds. H&E X4

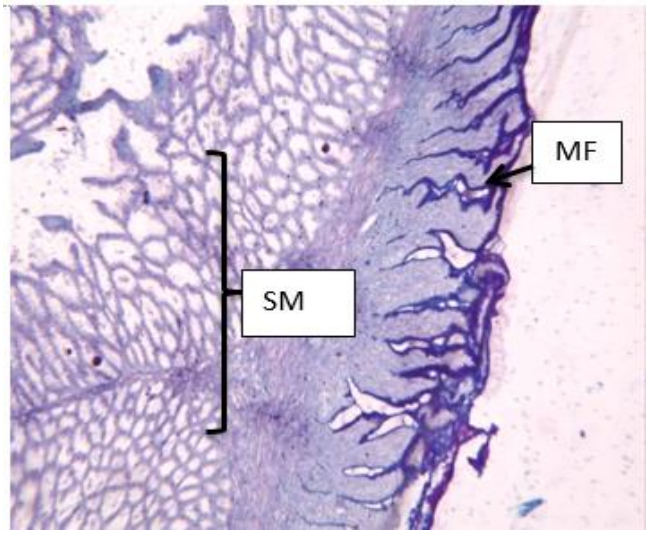


**Fig 3:** Photomicrograph of proventriculus of 30 days old Haringhata black chick showing PAS positive stained epithelium of mucosal folds (MF) and weakly stained sumucosa (SM). PAS-AB X10

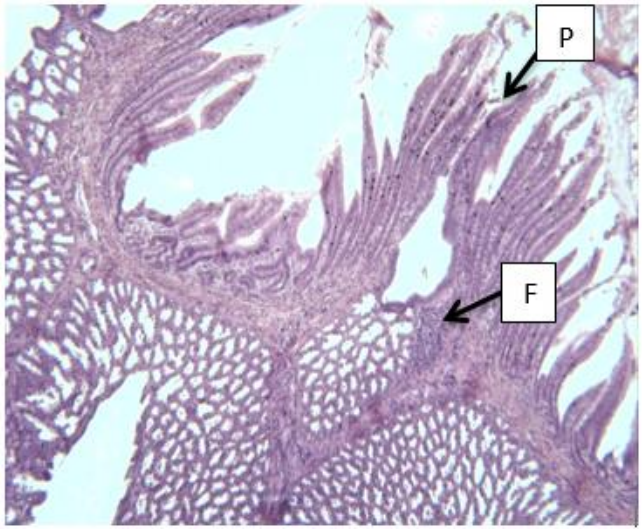


**Fig 2:** Photomicrograph of proventriculus of day old Haringhata Black chick showing lymphoid foci (F) in the lamina propria andthe mucosal folds arranged in concentric manner around the papillae (P). H&E X10

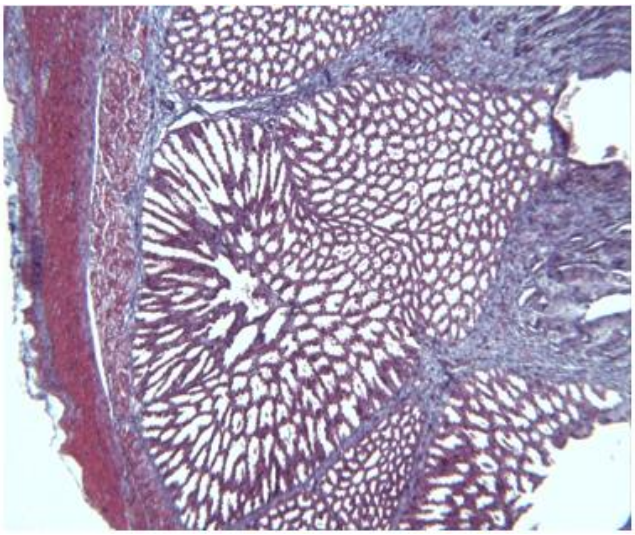

**Fig 4:** Photomicrograph of proventriculus of 0 days old Haringhata black chick showing collgen fibres (blue colour) in the lamina propria and between the submucosal glands. Masson's Trichome X10

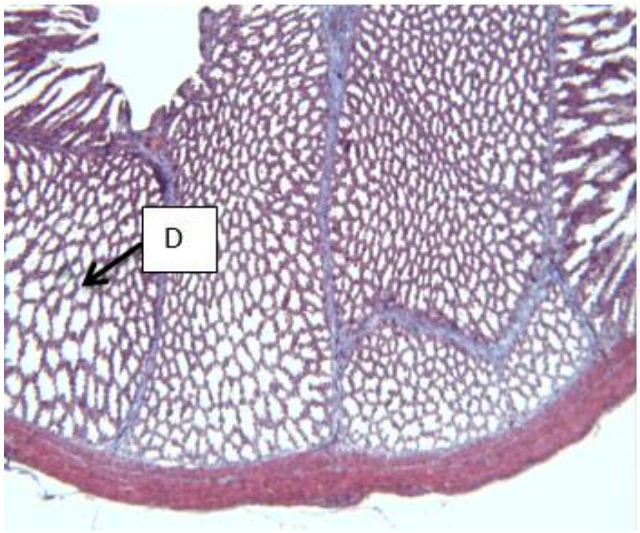


**Fig 5:** Photomicrograph of proventriculus of 0 days old Haringhata black chick showing submucosal glands with lobules and ducts (D) of the lobules.PAS-AB X10

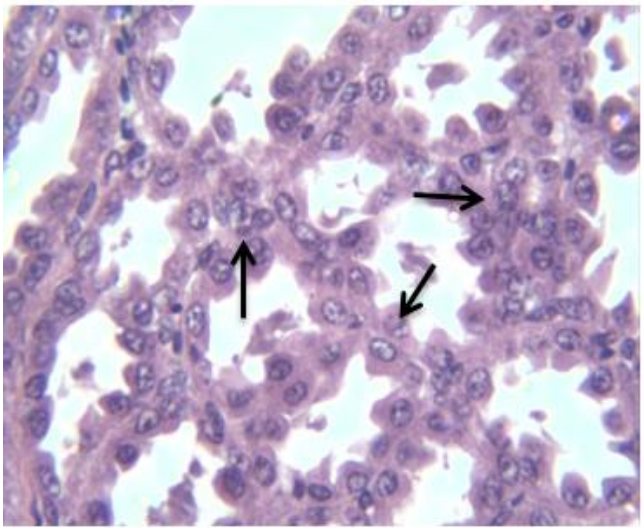

**Fig 6:** Photomicrograph of proventriculus of 15 days old Haringhata black chick showing oxyntico-peptic cells (arrows) in the alveoli of the lobules of proventricular glands. H&E X100

## 4. Conclusion

The present investigation revealed that the proventriculus of the Haringhata Black chickens was made up of four definite histological layers. The mucosa presented numerous folds which were lined by tall columnar epithelium. The lamina propria consisted of several tubular glands and the muscularis mucosa was composed of smooth muscle fibres and was evident between the mucosa and submucosa. Large lymphoid foci were observed in the propria. The submucosa was the thickest layer due to the presence of round, oval or elongated proventricular glands with their duct system. The tunica muscularis layer was composed of an inner circular and an outer longitudinal sub-layer. The serosa made up of mesothelial cells was the outermost layer. The present study will be helpful in better understanding of the proventriculus of this breed of chicken and also to compare with other breeds of chicken which have already been studied.

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## 6. References

1. Luna LG. Manual of Histologic Staining Method of the Armed Forces Institute of Pathology. 3rd edn., McGraw Hill Book Company, New York; c1968. p. 36-37, 168-169, 185-186, 199-200.
2. Das S, Dhote BS, Singh GK, Sinha S. Histomorphological and micrometrical studies on the proventriculus of Kadaknath fowl. Journal of Entomology and Zoology Studies. 2017;5(3):1560-1564.
3. Abumandour MM. Morphological studies of the stomach of falcon. Scientific Journal of Veterinary Advances. 2013;2(3):30-40.
4. Deka A, Sarma K, Rajkhowa J, Sarma M, Ahmed J. Macro and Micro anatomical studies on Oesophagus, Proventriculus and Gizzard of Pati Duck (*Anas platyrhynchos domesticus*) of Assam International Journal of Chemical Studies. 2017;5(2):443-445.
5. Parisa B, Khojaste B, Mahdi S. Morpho-histology of the alimentary canal of pheasant (*Phasianus colchicus*). Online Journal of Veterinary Research. 2019;3(6):615-627.
6. Rocha DOS, De Lima MAI. Histological aspects of the stomach of burrowing owl (*Speotyto cunicularia*, Molina, 1782). Rev. Chil. Anat. 1998;16(2):191-197.
7. Das S, Dhote BS, Singh GK, Sinha S. Histochemical studies of Proventriculus and Gizzard of Kadaknath Fowl. The Pharma Innovation Journal. 2017;6(5):25-28.
8. Al-Saffar FJ, Al-Sawamy ERM. Histomorphological and histochemical study of stomach of domestic pigeon (*Columba livia domestica*). The Iraqi Journal of Veterinary Medicine. 2016;40(1):89-96.
9. Rahman ML, Islam MR, Masuduzzaman M, Khan MZI. Lymphoid tissues in the digestive tract of deshi chicken (*Gallus domesticus*) in Bangladesh. Pak. J Biol. Sci. 2003;(6):1145-1150.
10. Selvan PS, Ushakumary S, Ramesh G. Studies on the histochemistry of the proventriculus and gizzard of post-hatch Guinea fowl (*Numida meleagris*). International Journal of Poultry Science. 2008;7(11):1112-1116.
11. Hassan SA, Moussa EA. Gross and microscopic studies on the stomach of domestic duck (Anas platyrhynchos) and domestic pigeon (*Columba livia domestica*). Journal of Veterinary. Anatomy. 2012;(5):105-127.
12. Nasrin M, Siddiqi MNH, Masum MA, Wares MA. Gross and histological studies of digestive tract of broilers during postnatal growth and development. Journal of Bangladesh Agriculture University. 2012;10(1):69-77.