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Histomorphometry of Gastrointestinal Tract Mucosae of Sonali and Indigenous Chickens in Bangladesh

Nure Jannat Monisha[1], Shakhawat Hossain Sojol[1], Akash Saha John[1], Rafiqul Islam[1], Ziaul Haque[1], Abu Hadi Noor Ali Khan[2] and Mohammad Rafiqul Islam[1*]

[1]*Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
[2]*Department of Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

Abstract

The study was conducted to provide a histomorphmetric comparison between the mucosa of the gastrointestinal tract (GUT) and the distribution pattern of gut-associated lymphoid tissue (GALT) in two types of chickens in Bangladesh: Sonali and Indigenous chickens. The study included 20 male Sonali and indigenous chickens aged 10 to 11 weeks, divided evenly into two groups of 10 chickens each. Following humane slaughter, various segments of the GUT were collected for histomorphometric analysis with hematoxylin and eosin staining. The histological investigation revealed that the epithelial lining and mucosal layer of the esophagus were thicker in the Sonali than in the Indigenous chickens. Nonetheless, the Indigenous chickens had a bigger number of lymphocytes. Indigenous chickens had thicker lining epithelium in the proventriculus, whereas Sonali chickens had thicker mucosa. Indigenous chickens showed greater lymphocyte populations than Sonali. Indigenous chickens exhibited taller duodenal and jejunal villi, but Sonali chickens had taller ileal villi. The Sonali possessed wider duodenal and jejunal villi, while Indigenous chickens had wider ileal villi. Because of their scavenging activity, Indigenous people have greater lymphocyte populations in the small intestine. The Sonali possessed the most lymphatic nodules in Meckel's diverticulum, as well as a higher crypt depth and lymphocyte density. Indigenous chickens had thicker mucosa and Lieberkühn crypts in the caecum, whereas Sonali had a greater lymphocyte count. Indigenous chickens showed larger lymphatic nodules and crypts in the cecal tonsil, whereas Sonali had thicker mucosa, deeper crypts, and a higher lymphocyte population in the colon. Lymphocytes were found scattered throughout the tissues of Sonali and Indigenous chickens. The cells were primarily detected within the lamina propria. Further research with other types of chickens could be undertaken to measure lymphocytes, intraepithelial lymphocytes, and lymphatic nodules.

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Introduction

Livestock farming, especially poultry farming, holds significant promise for poverty reduction in Bangladesh. The livestock sector generated 1.85% of the total GDP of Bangladesh in 2022–2023 where the poultry sector was one of the major contributors (DLS, 2023). Bangladesh's poultry industry is quickly increasing, offering both a source of protein and employment. Interestingly, indigenous chickens still dominate the poultry sector in Bangladesh with an estimated population ranging from 168 to 224 million birds, as reported in a study in 2021

*Corresponding author e-mail: rafiqul.islam@bau.edu.bd

(Hennessey *et al.*, 2021). The Indigenous types, *e.g.* Hilly, Naked Neck, Aseel, Yeasine, Native Dwarf, Frizzled Plumage, and Common Native chickens of non-distinctive typical types are prevalent (Das *et al.*, 2008). Indigenous chickens are commonly known as dual-purpose birds due to their capacity to provide both meat and eggs for consumption (Barua *et al.*, 2019). Because they require less feed and exhibit greater resilience to diseases and heat stress, the nondescript Indigenous chicken is preferred by rural communities as a primary source of both meat and eggs (Barua *et al.*, 2019).

The Sonali chicken was produced in 1986 as a crossbreed between a Fayoumi and Rhode Island Red. The Sonali crossbred chicken is considered as a promising source of economic growth while also providing several job opportunities. Sonali chickens are well adapted to the country's environmental conditions, requiring less care and attention than other types of chicken. The Sonali crossbred rearing is easier than the broiler due to the country's favorable environment (Saleque and Saha, 2013). The Sonali is accounted for over 30% of the country's total broiler and layer production (Huque *et al.*, 2011).

The gut mucosa is extremely convoluted and adapted to allow for maximum absorption of food components. The epithelium is folded into villi, and epithelial cells have a brush border formed by a thick matting of microvilli. This raises the absorption surface area of the small intestine by around 600 times, resulting in a better nutritional absorption capacity. Changes in absorption capacity in chickens could be due to differences in absorptive epithelial development (Verdal *et al.*, 2010).

The chicken digestive tract is the body's primary source of antigenic challenge, as it is constantly exposed to foreign bodies and microbes (Mowat and Viney, 1997). The mucosa is occupied by a considerable fraction of the immune system's overall cellular population to deal with these problems. In these lymphoid tissues, known as Mucosa Associated Lymphoid Tissue (MALT), various cell types are present, including T lymphocytes, B lymphocytes, plasma cells, macrophages, dendritic cells, and non-professional antigen-presenting cells (APCs). Non-professional APCs refer to cells such as epithelial cells, endothelial cells, and fibroblasts, which, despite not being specialized for antigen presentation, are capable of presenting antigens to T cells (MacDonald and Spencer 1994). The MALT is well developed in most birds (Matsumoto and Hashimoto, 1999). The avian MALT consists mainly of lymphoid cells located in the lamina propria and submucosa layers of the intestinal and respiratory tracts (Casteleyn *et al.*, 2010). Therefore, it acts as the body's initial defense against potentially harmful antigens introduced through ingestion and respiration (Casteleyn *et al.*, 2007). The current study is important for understanding the histomorphometric differences in the gastrointestinal tract (GUT) and distribution of GALT between Sonali and indigenous chickens, shedding light on potential variations in gut immunity and digestibility between these breeds, with implications for better production practices.

The GALT that are found in the intestinal tracts of birds, is a kind of MALT (Casteleyn *et al.*, 2010). The avian digestive system includes pharyngeal tonsil, dispersed lymphoid tissue in the esophagus, an esophageal tonsil, scattered proventricular lymphoid tissues, Meckel's diverticulum, Peyer's patches, caecal tonsils, dispersed rectal lymphoid tissue, bursa of Fabricius, etc. All these lymphoid tissues are categorized as secondary lymphoid organs, except for the bursa of Fabricius (considered a primary lymphoid organ) (Pabst, 2007).

However, to our knowledge, no research has been carried out comparing the distribution pattern of GALT of Sonali and indigenous chicken in Bangladesh. Therefore, this investigation was undertaken to study the histological architecture of the GUT with special emphasis on the distribution pattern of GALT in Sonali and Indigenous chicken. This study will offer significant insights into the abundance and dispersion of lymphocytes in the digestive system of Sonali and Indigenous chickens in Bangladesh.

Materials and Methods

The research was undertaken at the Department of Anatomy and Histology, Bangladesh Agricultural University (BAU), located in Mymensingh and was carried out during the period from July 2022 to December 2022. A number of 20 Indigenous and Sonali chickens, 10 from each type, were used for the experiment. These chickens (aged between 10 to 11 weeks) were obtained from the BAU poultry farm. All chickens had good health without any types of deformity. The chickens were euthanized via cervical subluxation, following a two-hour period of fasting without access to food or water prior to sacrifice. The esophagus, the upper part of proventriculus, duodenum, jejunum, Meckel's diverticulum, ileum, cecum, cecal tonsil, and colo-rectum were collected and fixed in EMA (Ethanol-Methanol-Acetic acid). EMA fixed tissue samples were processed routinely. Hematoxylin and Eosin (H&E) stain was applied to these tissue samples for histomorphometric study. Epithelium height, mucosal thickness, villus height, villus width, depth of crypt of Lieberkühn, length and breadth of lymphatic nodules were measured by coulometer for morphometric analysis.

Statistical Analyses

The data collected in this study underwent comparison using the student t-test via SPSS software (IBM SPSS Statistics 22), with results presented as mean±standard deviation (mean±SD). Significance was determined at $P<0.05$ for all analyses.

Results and Discussion

Esophagus

Histomorphometric study was performed on a calibrated stage micrometer. The study revealed that the average height and mucosal thickness of the epithelium were higher in Sonali compared to indigenous chicken (Table 1). Clusters of lymphoid cells were observed within the mucosal layer, positioned closely adjacent to the mucous glands which was comparatively higher in number in Indigenous compared to Sonali chicken (Figure 1A, B). This observation was similar with Rahman *et al.* (2003) who reported non-keratinized stratified squamous epithelium as the lining epithelium of esophagus. The highest height and thickness of mucosa of the epithelium was seen in Sonali chicken. On the other hand, highest lymphocyte population was observed in Indigenous chicken. The present finding of lymphocyte population was consistent with Islam *et al.* (2008) who reported a significantly higher presence of lymphocytes both in the epithelial and lamina propria of different segments of GUT in native chickens, potentially attributed to their scavenging behavior. Present finding of the presence of lymphocytes in the GUT of Indigenous chicken is partially similar with Rahman *et al.* (2003) who observed isolatory lymphocytes as well as both isolatory and aggregated lymphatic nodules in the lamina propria around the esophageal glands at day 30.

Table 1. Histomorphometrical comparison of different segments of GUT in Sonali and Indigenous chickens

Segments of GUT	Parameters (µm)	Sonali	Indigenous
Esophagus	Epithelial height	485.75±35.34	435.00±37.94
	Mucosal thickness	1082.32±104.13	990.11±55.39
Proventriculus	Epithelial height	352.14±74.93	455.71±59.12
	Mucosal thickness	1354.84±172.89	1064.84±59.70
Duodenum	Villi height	1939.38±126.88	1504.38±126.88
	Villi width	191.67±37.16	174.00±42.33
	Depth of crypts of Lieberkühn	252.30±25.78	269.70±31.97

Segments of GUT	Parameters (µm)	Sonali	Indigenous
Jejunum	Villi height	942.50±55.37	930.42±224.17
	Villi width	96.67±12.79	87.57±31.59
	Depth of crypts of Lieberkühn	232.30±35.03	184.88±19.96
Meckel's diverticulum	Length of lymphatic nodules	108.75±50.75	87.00±0.00
	Breadth of lymphatic nodules	58.00±29.00	43.50±0.00
	Depth of crypts of Lieberkühn	145.00±8.37	130.50±8.37
Ileum	Villi height	652.50±33.09	634.38±23.40
	Villi width	58.00±4.83	145.00±41.86
	Depth of crypts of Lieberkühn	147.90±14.47	153.70±18.37
Cecum	Mucosal thickness	416.88±22.44	445.88±31.07
	Depth of crypts of Lieberkühn	186.08±12.65	256.17±22.04
Cecal tonsil	Length of lymphatic nodules	164.33±29.40	299.67±50.46
	Breadth of lymphatic nodules	116.00±22.15	198.17±19.33
	Depth of crypts of Lieberkühn	157.43±15.32	240.29±32.09
Colo-rectum	Mucosal thickness	385.57±30.24	326.25±34.56
	Depth of crypts of Lieberkühn	140.17±25.58	113.58±8.71

Data are presented as Mean±SD.

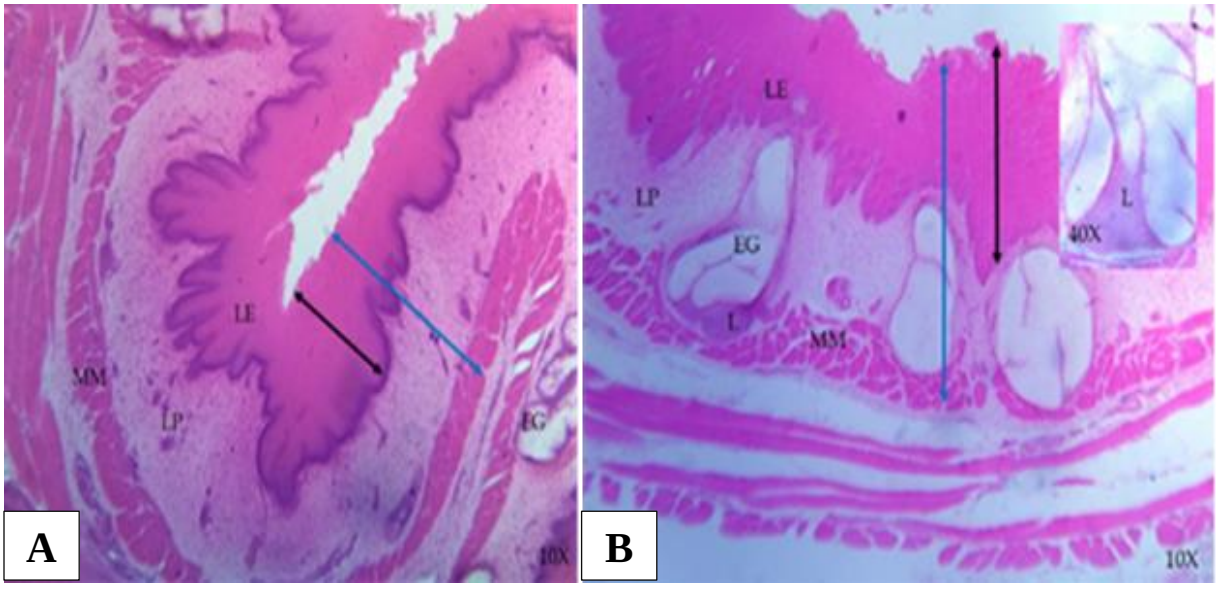

Figure 1 (A–B). Histomorphological observation of esophagus of Sonali (A) and Indigenous (B) chickens. Here, lamina epithelia (LE), lamina propria (LP), esophageal gland (EG), muscularis mucosae (MM), small aggregations of lymphoid cells in close apposition to the mucous glands (L), epithelial height (black line), and mucosal thickness (blue line) are shown. Stained with H & E. 10x magnification (inset histomicrograph is magnified at 10x).

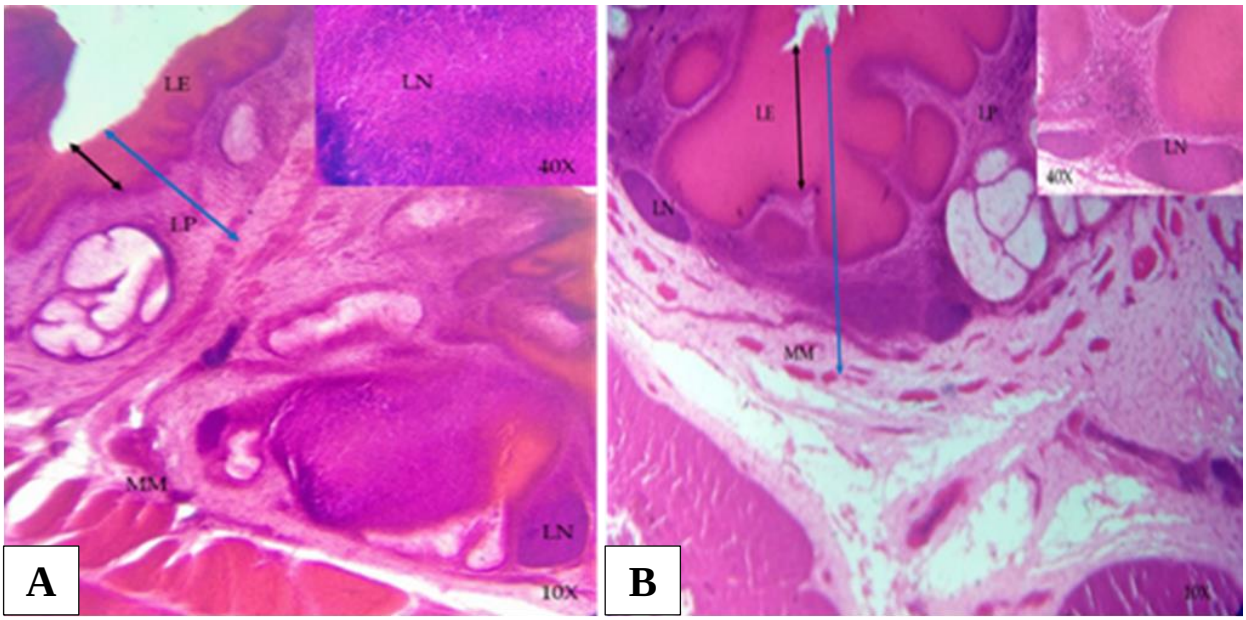

Figure 2 (A–B). Histomorphological observation of proventriculus of Sonali (A) and Indigenous (B) chickens. Here, lamina epithelia (LE), lamina propria (LP), lymphatic nodules (LN), muscularis mucosae (MM), epithelial height (black line), and mucosal thickness (blue line) are shown. Stained with H & E. 10x magnification (inset histomicrographs are magnified at 40x).

Proventriculus

The average height of the epithelium of upper part of proventriculus is higher in Indigenous chicken and the average mucosal thickness is less in Indigenous compared to Sonali chicken (Table 1). A small number of isolated and clustered lymphocytes were dispersed throughout the lamina propria which were comparatively less in Sonali compared to Indigenous chicken (Figure 2A, B). There was substantial difference in mucosal thickness of the upper part of the proventriculus between Sonali and Indigenous chicken (Table 1) although they were not statistically significant (p=0.005). The highest height of the epithelium and lymphocyte population was observed in indigenous chicken. The highest thickness of mucosa was found in Sonali. The present finding of the presence of lymphocytes of indigenous chicken was partially similar to Rahman *et al.* (2003), who reported diffuse and clustered lymphocytes, as well as nodular lymphocyte formations in the lamina propria on day 30.

Duodenum

The average height and width of the villi of duodenum is higher in Sonali than in the Indigenous chicken (Table 1). The average depth of crypts of Lieberkühn is higher in Indigenous compared to Sonali chicken (Table 1). Few isolated lymphocytes were observed in the epithelial layer and lamina propria which were comparatively less in number in Sonali than in Indigenous chicken (Figure 3A, B). There was a substantial difference in villi height of duodenum between Sonali and Indigenous chicken (Table 1) although they were not statically significant (*P*=0.047). This finding aligns with Aitken's (1958) observation that the surface epithelium in the small intestine was of a simple columnar type. Sonali chicken exhibited the tallest and widest villi, as well as the deepest crypts of Lieberkühn. However, Indigenous chicken had the highest lymphocyte count. According to Islam *et al.* (2008), lymphocytes were predominantly found in the villi core and lamina propria of the duodenum in both chicken types, consistent with Aitken's (1958) report that Brunner’s glands and Paneth cells were absent in the duodenum.

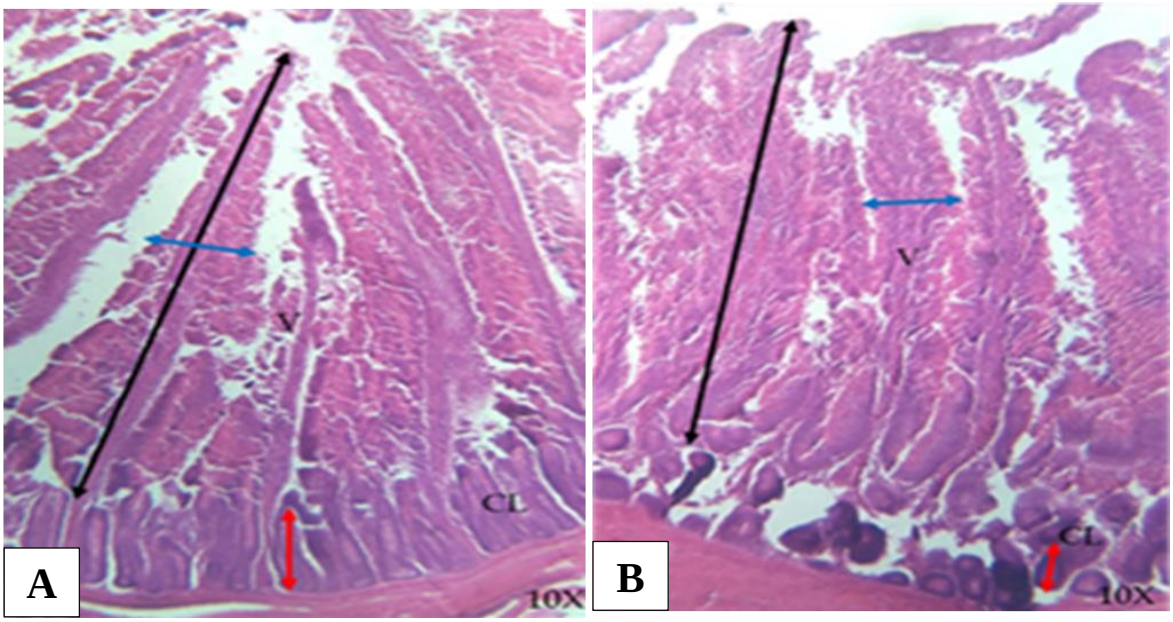

Figure 3 (A–B). Histomorphological observation of duodenum of Sonali (A) and Indigenous (B) chickens. Here, villi (V), crypts of Lieberkühn (CL), villi height (black line), villi width (blue line), depth of crypts of Lieberkühn (red line) are shown. Stained with H & E. 10x magnification.

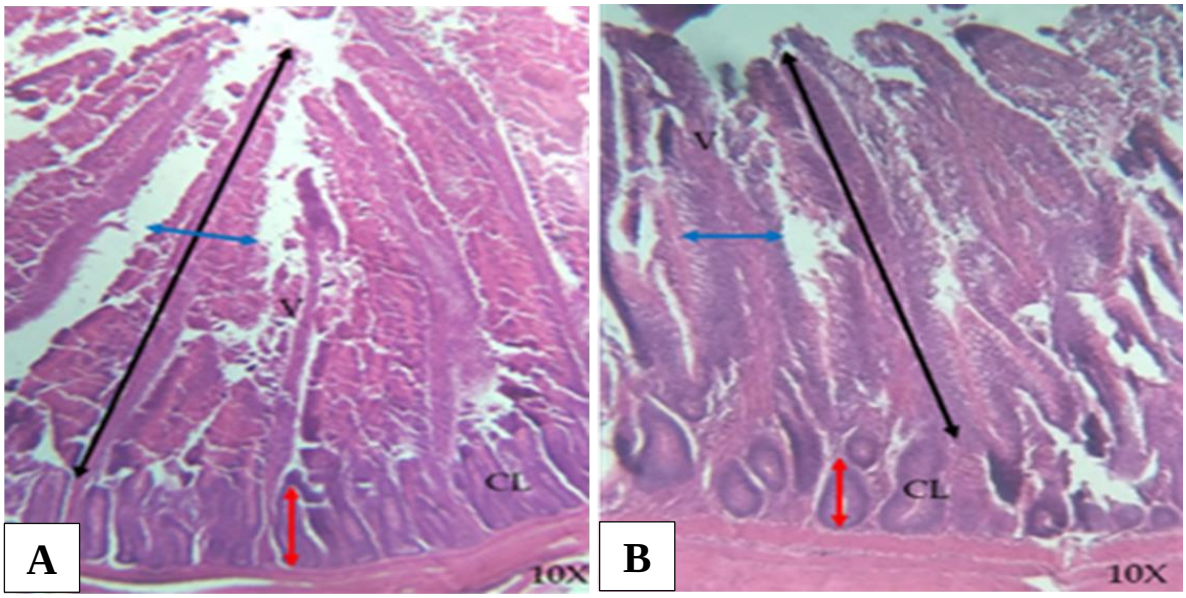

Figure 4 (A–B). Histomorphological observation of jejunum of Sonali (A) and Indigenous (B) chickens. Here, villi (V), crypts of Lieberkühn (CL), villi height (black line), villi width (blue line), depth of crypts of Lieberkühn (red line) are shown. Stained with H & E. 10x magnification.

Jejunum

The measurement by calibrated stage micrometer revealed that the average height, width, and crypt depth in the jejunum was higher in Sonali compared to Indigenous chicken (Table 1). A few isolated and scattered lymphocytes were observed both in the epithelium and the lamina propria, with lower

quantity in Sonali compared to Indigenous chicken (Figure 4A, B). There was non-significant (*P*=0.0001) difference in the villi height of jejunum between Sonali and Indigenous chicken (Table 1). According to Nasrin *et al.* (2012), the villi in the jejunum of the studied chickens were covered by a simple columnar epithelium, which was shorter and wider than that in the duodenum, a finding consistent with the current study. Sonali chickens exhibited the tallest villi and deepest crypts of Lieberkühn, while the indigenous chickens had the highest lymphocyte count. Islam *et al.* (2008) reported that lymphocytes were commonly found in the villi core and lamina propria of the jejunum in both chicken types, which corresponds with our findings. Rahman *et al.* (2003) observed isolated and scattered lymphocytes in deshi chickens, a result that aligns with our study.

Meckel's Diverticulum

The measurement by calibrated stage micrometer revealed that the average diameter of lymphatic nodules and crypt depth was higher in Sonali than in Indigenous chicken (Table 1). The lamina propria contained a high number of scattered lymphocytes and a small number of lymphatic nodules, which were relatively fewer in Indigenous compared to Sonali chicken (Figure 5A, B). The epithelial lining of the Meckel's diverticulum was composed of a simple columnar epithelium, as observed in a study by Rahman *et al.* (2003). Despite histological similarities between the diverticulum wall and the small intestine, distinct features were noted in Meckel's diverticulum. This corresponds with findings by Gofur (2020), who highlighted differences in its morphological structure, including a small number of crypts in the lumen. Gofur (2020) also noted the absence of villi in Meckel's diverticulum compared to the jejunum or ileum, along with a higher concentration of aggregated lymphoid follicles in its mucosal layer. Sonali chickens exhibited the greatest dimensions in lymphatic nodules, crypts of Lieberkühn, and lymphocyte population. In Indigenous chicken, the presence of lymphoid cells was consistent with observations by Islam *et al.* (2008), who reported higher frequencies of isolated, scattered, and clustered lymphoid cells and lymphoid nodules in the epithelium and lamina propria, particularly at day 90.

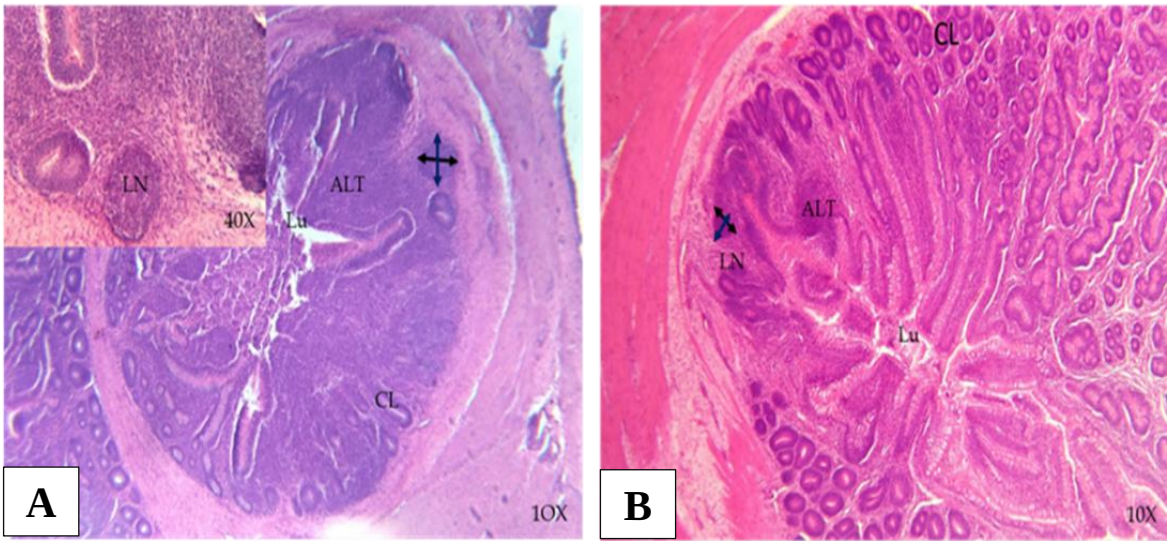

Figure 5 (A–B). Histomorphological observation of Meckel's diverticulum of Sonali (A) and Indigenous (B) chickens. Here, crypts of Lieberkühn (CL), lumen of Meckel's diverticulum (Lu), aggregated lymphoid tissue (ALT), lymphatic nodules (LN), mucosal fold (MF) are shown. Stained with H & E. 10x magnification (inset histomicrograph is magnified at 40x).

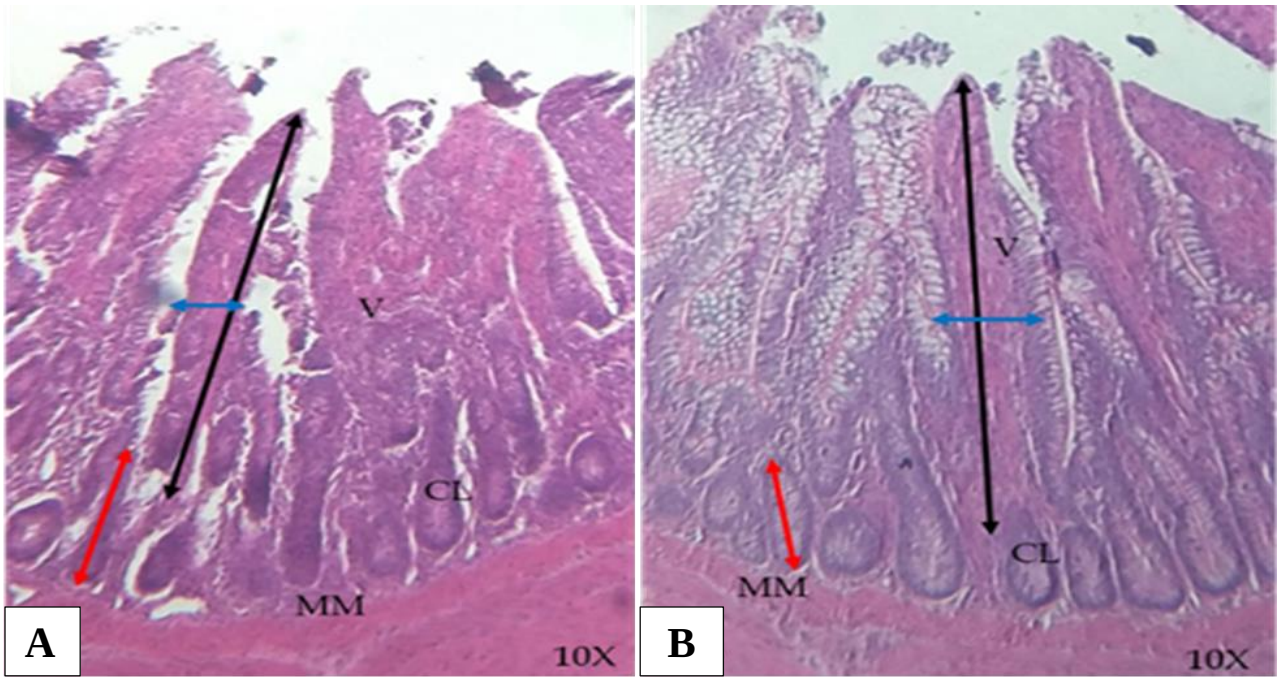

Figure 6 (A–B). Histomorphological observation of ileum of Sonali (A) and Indigenous (B) chickens. Here, muscularis mucosae (MM), villi (V), crypts of Lieberkühn (CL), epithelial and villi height (black line), mucosal thickness and villi width (blue line), depth of crypts of Lieberkühn (red line) are shown. Stained with H & E. 10x magnification.

Ileum

The measurement by calibrated stage micrometer revealed that the average height of the villi of ileum and crypt depth were higher in Sonali compared to Indigenous Chicken but the average villi width was higher in indigenous chicken (Table 1). In the lamina propria, there were isolated, scattered, and clustered lymphocytes, which were relatively more abundant in Indigenous compared to Sonali Chicken (Figure 6A, B). There substantial (P=0.043) difference in villi height and substantial (P=0.021) difference in villi width of ileum between Indigenous and Sonali chicken (Table 1). This finding corresponds to the observation of Rahman *et al.* (2003) in Indigenous chicken ileum, where the surface epithelium was noted as simple columnar. Sonali exhibited the tallest villi, while Indigenous chicken had the widest. Indigenous chicken also had the deepest crypts and the highest lymphocyte population.

Caecum

The mean thickness of the mucosal layer and the crypt depth in the caecum were greater in Indigenous compared to Sonali chicken (Table 1). The lamina propria contained numerous clustered lymphocytes and medium-sized lymphatic nodules, which were relatively more abundant in Indigenous compared to Sonali chicken (Figure 7A, B). There was substantial (P=0.004) difference in crypt depth of caecum between indigenous and Sonali chicken (Table 1). The current observation aligns with findings of Nasrin *et al.* (2012), where plicae were described as well-developed folds of the mucous membrane and muscularis mucosae extending along the inner surface of the caeca's distal two-thirds. Indigenous chicken exhibited the greatest thickness of mucosa and depth of Lieberkühn's crypts, while Sonali chicken had the highest lymphocyte count.

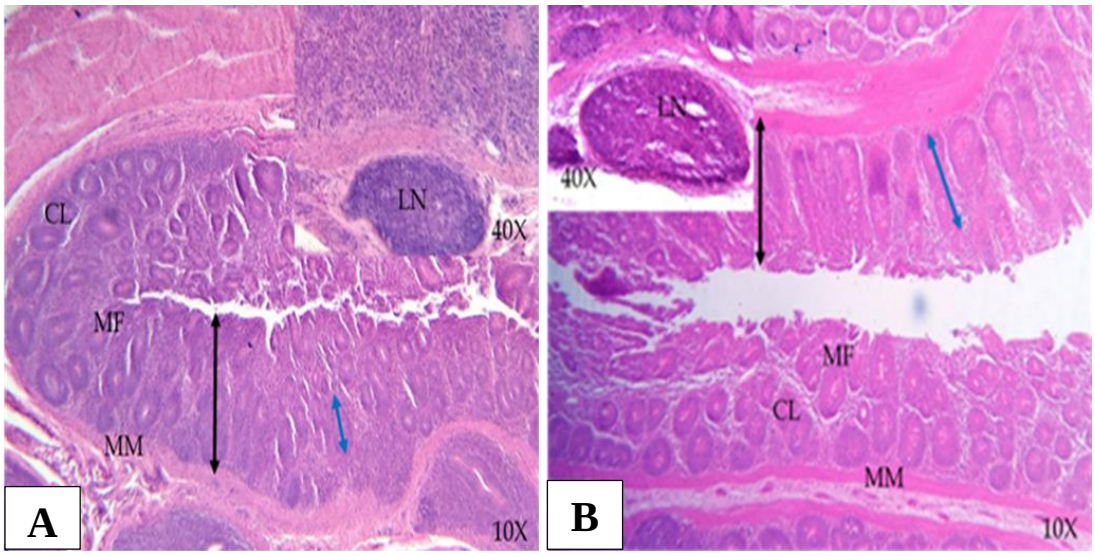

Figure 7 (A–B). Histomorphological observation of cecum of Sonali (A) and Indigenous (B) chickens. Here, muscularis mucosae (MM), crypts of Lieberkühn (CL), lymphatic nodules (LN), mucosal fold (MF), epithelial height (black line), mucosal thickness and villi width (blue line) are shown. Stained with H & E. 10x magnification (inset histomicrographs are magnified at 40x).

Cecal Tonsil

The measurement by calibrated stage micrometer revealed that the average length and breadth of lymphatic nodules and crypt depth of cecal tonsil were higher in indigenous compared to Sonali chicken (Table 1). Abundant scattered lymphocytes and numerous medium to large-sized encapsulated lymphatic nodules were found in the lamina propria, both within the fold and at its base, showing relatively higher numbers in Indigenous compared to Sonali chicken (Figure 8A, B).

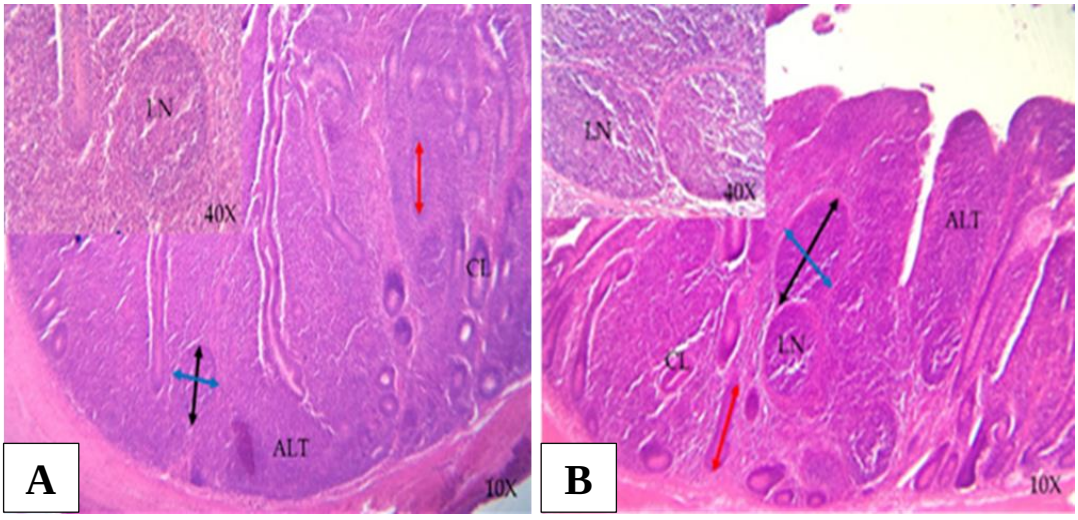

Figure 8 (A–B): Histomorphological observation of cecal tonsil of Sonali (A) and Indigenous (B) chickens. Here, crypts of Lieberkühn (CL), aggregated lymphoid tissue (ALT), lymphatic nodules (LN), epithelial height (black line), mucosal thickness (blue line), depth of crypts of Lieberkühn (red line) are shown. Stained with H & E. 10x magnification (inset histomicrographs are magnified at 40x).

There was a substantial (*P*=0.031) difference in the breadth of lymphatic nodules and depth of crypts of Lieberkühn of cecal tonsil between Sonali and Indigenous chickens (Table 1). The greatest dimensions in terms of length, breadth of lymphatic nodules, depth of Lieberkühn's crypts, and lymphocyte count were observed in indigenous chicken. Rahman *et al.* (2003) noted that isolated, scattered, and clustered lymphocytes, as well as lymphatic nodules in the cecal tonsil of Indigenous chicken, were predominantly distributed in the lamina propria, a finding consistent with our study. Ayman *et al.* (2021) reported that on day 42, the lengths and breadths of lymphatic nodules in the cecal tonsil of Sonali chicken were measured at 331.40±28.36 µm and 242.44±34.84 µm, respectively.

Colo-rectum

The examination using a calibrated stage micrometer showed that the average thickness of the mucosal layer and the average crypt depth in the colo-rectum of Sonali chickens were greater than in Indigenous chickens (Table 1). Indigenous chickens exhibited a lower number of isolated and scattered lymphocytes, as well as large-sized lymphatic nodules in the lamina propria, in comparison to Sonali chicken (Figure 9A, B). Sonali chicken exhibited the greatest mucosal thickness, depth of Lieberkühn's crypts, and lymphocyte count. According to Rahman *et al.* (2003), isolated and scattered lymphocytes, as well as isolated and clustered lymphatic nodules in the colo-rectum of Indigenous chicken, were predominantly distributed in the epithelial lining, lamina propria, and submucosa, a finding consistent with our study. Hodges (1974) and Schummer (1973) noted that the lamina propria mucosae of the rectum were extensively infiltrated by lymphoid cells, often forming small lymphoid follicles.

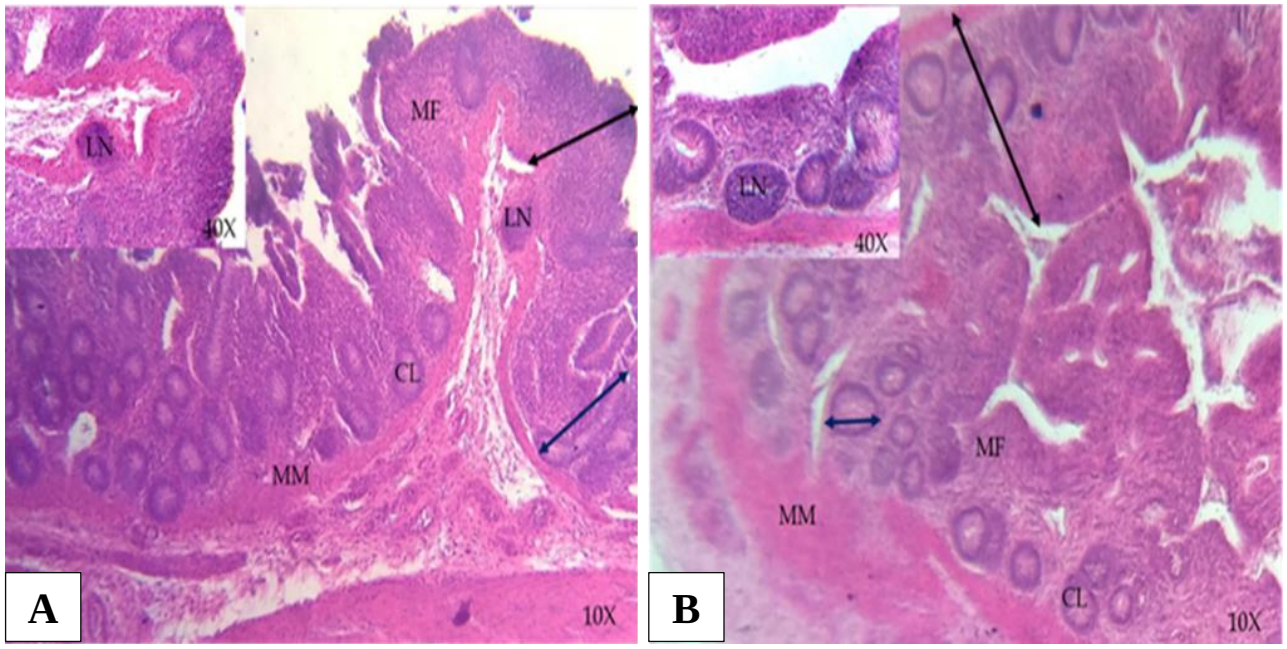

Figure 9 (A– B). Histomorphological observation of colo-rectums of Sonali (A) and Indigenous (B) chickens. Here, muscularis mucosae (MM), crypts of Lieberkühn (CL), lymphatic nodules (LN), mucosal fold (MF), epithelial height (black line) are shown. Stained with H&E. 10x magnification (inset histomicrographs are magnified at 40x).

Conclusions

The current study found that the heights of epithelial mucosal thickness in the esophagus and upper section of the proventriculus differed between Sonali and Indigenous chickens. Indigenous chickens had thicker mucosal layers and deeper crypts in the caecum. Furthermore, the lymphocyte population in the upper gastrointestinal tract and small intestine was higher in Indigenous chickens. Indigenous chickens exhibited longer and larger lymphatic nodules in their cecal tonsils, as well as deeper crypts than Sonali chickens.

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Declaration

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

Author's Contributions

MRI and AHNAK designed research; NJM, SHS, ASJ performed research works and wrote the manuscript; and RI and ZH critically reviewed the manuscript. The completed manuscript has been read and approved by all authors.

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