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ORIGINAL ARTICLE

Histomorphometry of the gastrointestinal tract of the broiler and cock chicken in Bangladesh

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Abstract

Background: The digestive system of birds is crucial for providing their bodies with the nutrition they require for upkeep, development, and reproduction. The study was conducted to investigate the histological variation of the mucosa of the digestive tract and the distribution pattern of gut-associated lymphoid tissue (GALT) in broiler and cock.

Methods: A total of 20 (6-7 weeks old) broiler and cock were divided into two equal groups (n=10). After cervical subluxation, different segments of gastrointestinal tract were collected and stained with Haematoxylin and Eosin stain.

Results: The histomorphological study revealed that the proventriculus lamina epithelium was higher in broiler, whereas the esophageal lamina epithelium and tunica mucosa were thicker in cock. The cock had the highest level of proventriculus's mucosal thickness, villi height and width of jejunum and ileum and small intestine crypt depth. The lymphocyte population in the upper part of the proventriculus and Meckel's diverticulum were highest in the broiler. The highest length, breadth of lymphatic nodules, and crypt depth of Meckel's diverticulum were found in cock. The mucosal thickness and depth of the broiler's caecum and cecal tonsil crypts were the highest. The lymphocyte population of the cecal tonsil and thickness of tunica mucosa, and depth of the crypts of Lieberkuhn's of colo-rectum were highest in broiler than cock. The lamina propria, lamina epithelium, core of the esophageal villi, the upper part of proventriculus, from duodenum to ileum, Meckel's diverticulum, caecum, cecal tonsil and colo-rectum of the broiler and cock all had high lymphocyte population frequencies.

Conclusion: The study's results indicate significant histological variations in the gastrointestinal tracts and GALT between broiler and cock, potentially impacting their general well-being and productivity.

Keywords: Broiler, Cock, Histology, Lymphocyte population, Crypts of Lieberkuhn's

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Introduction

In Bangladesh, livestock, particularly poultry, is a viable source of poverty relief. The poultry industry contributes approximately 1% of the country's GDP (DLS, 2023). Bangladesh's poultry sector is rapidly expanding, providing both a source of nourishment and employment. The commercial chicken industry is based on the genetic development of high-yielding breeds designed for egg and meat production (Athrey et al., 2018).

Male layer chickens are considered as cock. They are known as meat-productive poultry breeds. They also raised commercially for meat production. Bangladesh's Department of Livestock Service (DLS) imported Arbor Acres' broiler parent stock in the late 1980s (Hassan et al., 2016). Any chicken reared and raised primarily for meat production is called a broiler. Cornish crosses and rocks, for example, are modern commercial broilers purposely selected and bred for large-scale, efficient meat production (Fanatico et al., 2005). They are known for their rapid growth rates, high feed conversion ratios, and low activity levels. Modern commercial broilers are developed to achieve a slaughter weight of approximately 2 kg in 5 to 7 weeks (Joshua et al., 2022). Intestinal development significantly impacts the growth rate of broilers selected for rapid growth (Zavarize et al., 2012).

The avian digestive tract is essential for converting ingested food into the nutrients their bodies need for maintenance, growth, and reproduction (Salem et al., 2023). The glandular stomach (proventriculus), gizzard (ventriculus), and intestine make up the chicken's gastrointestinal tract (small and large). Food is mixed with these digestive liquids in the glandular stomach, lined by glands that secrete pepsin, hydrochloric acid, and mucus (Rodrigues I et al., 2018). The gizzard is the second stomach chamber, where forceful contractions crush food. Feed ingredients are digested as simple molecules in the small intestine, especially free short peptides, amino acids, free fatty acids, and monosaccharides (Svihus et al., 2011). The gut mucosa is highly convoluted and adapted to maximize food components' absorption. The epithelium is folded into villi, and epithelial cells have a brush border formed by a thick matting of microvilli (Schneeberger et al., 2018). This raises the absorption surface area of the small intestine by around 600 times, resulting in a better nutritional absorption capacity (Alshamy et al., 2018). Changes in absorption capacity in birds could be due to differences in absorptive epithelial development (De Verdal et al., 2010). The thickness of a chicken's mucosa, villus height, villus surface area, and other factors influence its growth and development (Ravindran & Abdollahi, 2021).

The chicken digestive tract is the body's primary source of antigenic challenge, as it is constantly exposed to antigens and commensal microbes (Mowat & Viney, 1997). The mucosa is occupied by a considerable fraction of the immune system's overall cellular population to deal with these problems. T lymphocytes, B lymphocytes, plasma cells, macrophages, dendritic cells, and non-professional antigen-presenting cells (APCs) are all found in these lymphoid tissues, which are commonly referred to as Mucosa Associated Lymphoid Tissue (MALT) (MacDonald and Spencer, 1994). Most birds' MALT is well-developed (Matsumoto and Hashimoto, 2000). This avian MALT comprises lymphoid cells mostly found in the intestinal and respiratory tracts' lamina propria mucosae and tela submucosa (Casteleyn et al., 2010). As a result, it serves as a first line of defense against potentially hazardous antigens that enter the body by eating and breathing (Casteleyn et al., 2007). Depending on the region, it can be found as aggregations of lymphoid cells or structured in lymphoid follicles. The so-called GALT in the avian intestinal tract is a kind of MALT (Casteleyn et al., 2010). The avian intestinal tract has a pharyngeal tonsil, diffuse lymphoid tissue, and lymphoid follicles in the cervical and thoracic parts of the esophagus, an oesophageal tonsil, diffuse lymphoid tissue in the proventriculus, a pyloric tonsil, Peyer's patches, Meckel's diverticulum, two caecal tonsils, diffuse lymphoid tissue in the rectum, the bursa of Fabricius and diffuse lymphoid tissue in the wall of the proctodeum. All of these lymphoid tissues are secondary lymphoid organs except the bursa of Fabricius, which is a primary lymphoid organ (Pabst, 2007).

However, no research has been carried out regarding the distribution pattern of GALT in cock and broiler in Bangladesh. Hence, this study was undertaken to understand the histological architecture of the gastrointestinal tract with particular emphasis on the distribution pattern of GALT in cock and broiler. This work will provide valuable information regarding the population and distribution of lymphocytes in the digestive tract of cock and broiler in Bangladesh.

Materials and methods

Ethics Statement

The current study was solely conducted following the institutional guidelines for using the animal model for research purposes. The experimental procedure was authorized by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University (BAU), Bangladesh. The authorization number is - AWEEC/BAU/2024(29).

Animal Model

The study took place in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, from September 2020 to December 2021. This comparative study included a total of 20 cock and broiler breeds. These chicken varieties were acquired from the Nourish Poultry & Hatchery Ltd. at Bhaluka in Mymenshing. The chickens were all between 6 and 7 weeks old. All the birds appeared to be in good health and free of deformities. Ten male chickens from each breed were used in this investigation.

Sampling and Measurements

They were sacrificed by cervical subluxation following adequate anesthetic. Food and water were withheld two hours before the sacrifice. The esophagus, upper part of the proventriculus, duodenum, jejunum, Meckel's diverticulum, ileum, cecum, cecal tonsil, and colorectum were collected and fixed in EMA (Ethanol-Methanol-Acetic acid). EMA-fixed tissue samples were processed following the standard protocol (Rahman et al., 2022). The tissue sections were stained with Hematoxylin and Eosin (H&E) for histological evaluation (Sultana and Islam, 2022). The epithelium height, mucosal thickness, villus height, villus width, depth of Lieberkühn's crypt, and length and breadth of lymphatic nodules are measured in micrometer (µm) and eye estimation of lymphatic tissues were gathered for histomorphological and morphometric analysis. One-way analysis of variance (ANOVA) with posthoc Duncan’s multiple range test was done to compare all the data obtained in this study by using Statistical Package for the Social Sciences (IBM SPSS; version 22.0, IBM Corp., Armonk, NY, USA) software.

Results

Esophagus

Based on the findings of the study, the average height and mucosal thickness of the epithelium in cock was higher than in the broiler, as determined by a calibrated stage micrometer measurement. Little clusters of lymphoid cells, which were less common in broilers than in cocks, were discovered in the tunica mucosa near the mucous glands (Figure1). The cock and broiler chickens' esophageal epithelial heights differed significantly ($p=0.022$) (Table 1).

Table 1: Histomorphometric comparison of the various organs of Broiler and Cock

Organ	Parameters (µm)	Broiler	Cock
Esophagus	Epithelial height	396.33±38.01	512.33±26.68
	Mucosal thickness	947.68±43.80	1090.09±47.68
Upper part of proventriculus	Epithelial height	269.29±43.94	253.75±34.48
	Mucosal thickness	611.72±26.03	725±62.79
Duodenum	Villi height	1593.87±67.28	1975.63±54.38
	Villi width	159.27±54.57	197.53±79.18
	Depth of crypts of Lieberkühn	217.5±28.27	324.8±44.36
Jejunum	Villi height	939.75±21.73	1534.58±52.67
	Villi width	119.18±40.54	91.83±26.91
	Depth of crypts of Lieberkuhns	170.38±12.38	239.25±42.89
Meckel's diverticulum	Length of lymphatic nodules	195.75±7.25	224.75±21.75
	Breadth of lymphatic nodules	108.75±7.25	159.5±0
	Depth of crypts of Lieberkuhns	130.5±22.15	178.83±26.91
Ileum	Villi height	775±25.63	788.44±70.00
	Villi width	141.33±19.33	67.67±4.83
	Depth of crypts of Lieberkuhns	108.75±13.01	155.15±15.89
Cecum	Mucosal thickness	264.63±10.88	242.88±18.76
	Depth of crypts of Lieberkuhns	152.25±11.07	120.83±12.79
Cecal tonsil	Length of lymphatic nodules	291.30±27.59	265.83±49.05
	Breadth of lymphatic nodules	193.56±18.57	251.33±25.58
	Depth of crypts of Lieberkuhns	300.36±37.93	300.36±26.04
Colo-rectum	Mucosal thickness	672.72±44.63	181.25±15.46
	Depth of crypts of Lieberkuhns	215.08±35.40	103.92±6.92

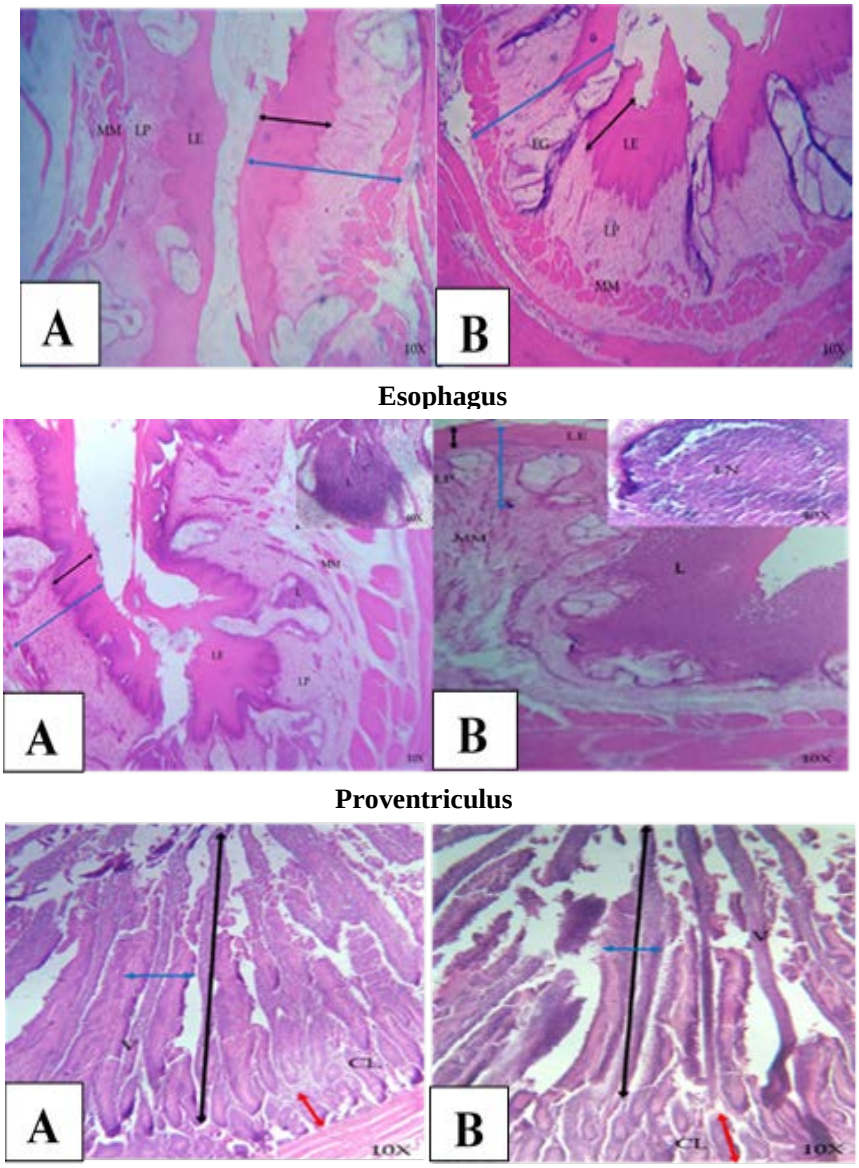

Figure 1: Histomorphological observation of the Esophagus, Proventriculus and Duodenum of Broiler (A) and Cock (B). Here, Lamina epithelia (LE), Lamina propria (LP), Esophageal gland (EG), Muscularis mucosae (MM), Small aggregations of lymphoid cells in close apposition to the mucous glands (L), Lymphatic nodules in lamina propria (LN), Villi (V), Crypts of Lieberkühn (CL), Epithelium height (Black line), Mucosal thickness (Blue line) for Esophagus and Proventriculus and Villi height (Black line), Villi width (Blue line), Depth of crypts of Lieberkuhns (Red line) for Duodenum are shown. H & E stain (10X and 40X).

Proventriculus

When comparing broiler to cock, the average proventriculus had a larger average height (Table 1). In the lamina propria, there were fewer isolatory and aggregated lymphocytes, which were more prevalent in broilers than cock (Fig. 1).

Duodenum

A calibrated stage micrometer demonstrated that the thickness of the tunica mucosa was lower, and the epithelium of the upper section of the average height of the villi, width of the villi, and depth of the crypts of Lieberkuhns were higher in cock than in the broiler. There was no significant difference in villi breadth between breeds. The depth of crypts of Lieberkuhns differed significantly (p=0.037) between cock and broiler (Table 1).

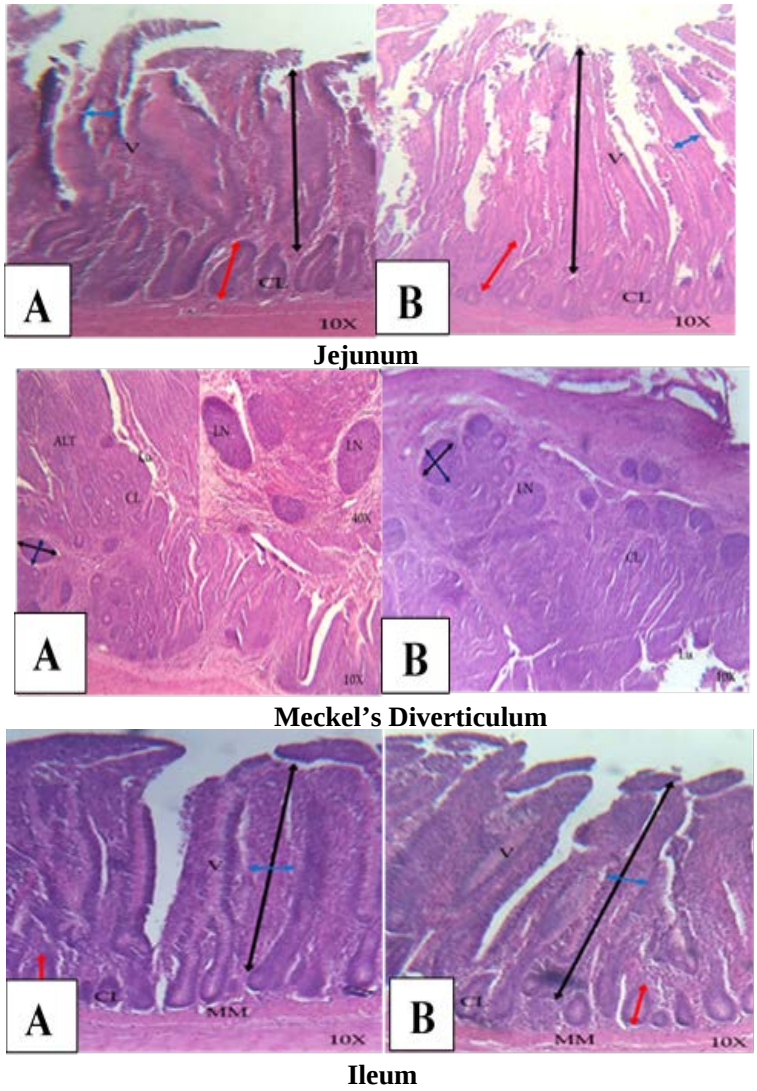

Figure 2: Histomorphological observation of Jejunum, Meckel's Diverticulum and Ileum of Broiler (A) and Cock (B). Here, Villi (V), Crypts of Lieberkuhns (CL), Lumen of Meckel's diverticulum (Lu), Aggregated lymphoid tissue (ALT), Lymphatic nodules (LN), Muscularis mucosae (MM), Villi height (Black line), Villi width (Blue line), Depth of crypts of Lieberkühn (Red line) for Jejunum and Ileum and Length of lymphatic nodule (Black line) and Breadth of lymphatic nodule (Blue line) for Meckel's Diverticulum are shown. H & E stain (10X and 40X).

Jejunum

The average height of the villi and depth of the Lieberkuhns crypts were larger in cock than in the broiler, according to the calibrated stage micrometer measurements. However, the average width of the villi broiler was more than that of the cock (Table 1). There was a significant (p=0.0001) difference in villi height between cock and broiler breeds but not in villi width (Table 1). The depth of Lieberkuhns crypts did not differ significantly between breeds (Table 1).

Meckel's diverticulum

The average length, breadth, and depth of Lieberkuhns crypts of Meckel's diverticulum were greater in cock than in broiler. The lamina propria contained abundant diffuse lymphocytes as well as a considerable number of tiny and medium-sized lymphatic nodules, which were more numerous in broiler than in cock (Fig. 2). The depth of Lieberkuhns crypts did not differ significantly

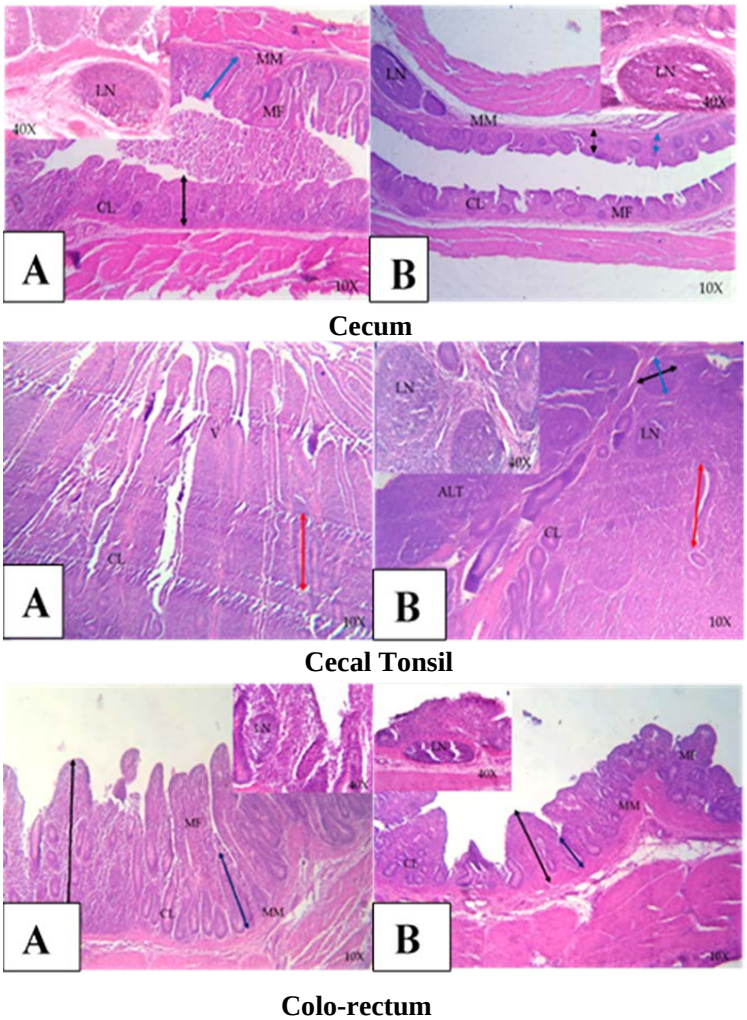

Figure 3: Histomorphological observation of the Cecum, Cecal Tonsil and Colo-rectum of Broiler (A) and Cock (B). Here, Mucosal fold (MF), Crypts of Lieberkuhns (CL), Muscularis mucosae (MM), Lymphatic nodules (LN), Villi (V), Aggregated lymphoid tissue (ALT), Thickness of mucosa (Black line) and Depth of crypts of Lieberkühn (Blue line) for Cecum and Colo-rectum and Length of lymphatic nodule (Black line) and Breadth of lymphatic nodule (Blue line), Depth of crypts of Lieberkuhns (Red line) for Cecal Tonsil are shown. H & E stain (10X and 40X).

Ileum

The average height of the villi and the depth of the Lieberkühn ileum crypts were found to be higher in cock, according to the measurement made with a calibrated stage micrometer. However, the average width of villi was greater in broiler than cock (Table 1). The depth of the Lieberkuhns crypts and the villi of the broiler and cock differed significantly (p=0.042 and p=0.047, respectively) (Fig. 2).

Caecum

When comparing the broiler to cock, the average thickness of the tunica mucosa and depth of the Lieberkühn crypts were larger in the broiler (Table 1). The Lieberkuhns of the caecum's crypts did not significantly change in depth between cock and broiler (Fig. 3).

Cecal tonsil

Broiler tonsils had longer lymphatic nodules on average than those in the cock's tonsils (Table 1). Conversely, Table 1 shows that the average breadth of lymphatic nodules was greater in cock compared to broiler. Between the breeds, the average depth of Lieberkuhns crypts was nearly equal. Fig. 2 shows that broiler chickens had less diffuse lymphocytes than cocks, although they still had lymphatic nodules in the lamina propria. The length of the lymphatic nodules in the cecal tonsils did not significantly differ between the broiler and cock chicken (Fig. 3).

Colo-rectum

The average thickness of tunica mucosa and depth of crypts of Lieberkuhns were higher in the broiler compared to that in cock (Table 1). Few numbers of large sized lymphatic nodules in the lamina propria were found in case of broiler compared to cock chicken (Fig. 3). Large number of medium-sized lymphatic nodules were found in the lamina propria which were comparatively higher in number in cock compared to broiler (Figure 3). There was substantial (p=0.0001) difference in mucosal thickness and was also substantial (p=0.002) difference in depth of crypts of Lieberkühn between cock and broiler (Figure 3).

Discussion

The findings showed that the esophageal surface epithelium was nonkeratinized stratified squamous epithelium. The maximum epithelial height and mucosal thickness were seen in the cock, but the highest lymphocyte population was recorded in the broiler. The highest epithelial height and lymphocyte population was recorded in broilers, followed by cock. Few isolatory and aggregated lymphocytes in the lamina propria of both broiler and cock (Islam *et al.,* 2006). The current lymphocyte population observation was consistent with that. However, these were more noticeable in the broiler. The maximum height and width of the villi were recorded in cock. Duodenal villi were thin and longer in broilers than in cocks (Islam *et al.,* 2006). The current study yielded similar results. The average length and width of villi in the duodenum were 870.75 ± 2.287 µm and 123.25 ± 0.629 µm at D28, which is similar to the current findings in broiler villi (Nasrin *et al.,* 2012). Lymphocytes were more commonly seen in both chicken strains' villi core and duodenal lamina propria (Islam *et al.,* 2008). This observation was similar to the finding that the duodenum lacked Paneth cells and Brunner's glands (Aitken, 1958). The villi of the chicken's jejunum were bordered by simple columnar epithelium that was shorter and broader than those of the duodenum (Nasrin *et al.,* 2012). The current study yielded similar results. The average length and width of villi in the jejunum were 652.625 ± 1.700 µm and 108.187 ± 51.096 µm, respectively, at D28 (Nasrin *et al.,* 2012). The current findings in height and width of broiler villi are partially similar. Cock has the deepest Lieberkühn crypts, followed by broiler. According to Lymphocytes were most commonly found in the core of the villi and the lamina propria of the jejunum in both strains of chickens (Islam *et al.,* 2008). The surface epithelium of Meckel's diverticulum was a simple columnar epithelium. Though the wall of the diverticulum was constructed similarly to the small intestinal wall, Meckel's diverticulum had several different features. This observation was similar to that of Gofur (2020), who pointed out that Meckel's diverticulum differs from the digestive system, especially in morphologic structure, due to its moderate number of crypts in the lumen. The tunica mucosae of Meckel's diverticulum had a larger proportion of aggregated lymphoid follicles and fewer villi than the ileum or jejunum (Gofur, 2020). Meckel's diverticulum had lymphoid follicles with lymphocyte profiles, which were more common in broilers (Gofur, 2020). The results of the current investigation were comparable. The ileum's surface epithelium was simple columnar, and the ileum villi of broilers were longer and thinner than those of native chickens (Saleh *et al.,* 2022). This observation aligned with those findings. At D28, the average

ileum villi length and width were 1161 ± 4.203 µm and 137.9375 ± 0.819 µm, respectively (Nasrin *et al.,* 2012). The width and height of the broiler villi were found to be somewhat comparable. In both chicken strains, lymphocytes were more frequently discovered in the ileum's lamina propria and the center of the villi (Islam *et al.,* 2008). Plicae are well-developed folds of mucous membrane and muscularis mucosae that run along the inner surface of the distal two-thirds of the caeca (Nasrin *et al.,* 2012). The recent broiler discovery is compatible with their description. The mucosal folds, or villi, had simple columnar epithelium as the surface epithelium, thick bases, and rounded or pointed apexes (Akter *et al.,* 2006). The current study of broiler is similar with it. Disorganized lymphatic nodules and more extensive lymphoid tissue were found in the mucosa and submucosa of broilers, and the lymphatic nodules in the broiler's cecal tonsils measured 255.20 ± 20.46 µm and 186.08 ± 24.90 µm at 28 days old (Akter *et al.,* 2006). The current study yielded somewhat similar results. The mucosal folds appeared as numerous long, flat, leaf-shaped structures that filled a large proportion of the lumen (Nasrin *et al.,* 2012). A similar result was found in the present research. The lamina propria mucosae of the rectum was strongly infiltrated by lymphoid cells that are often organized into small lymphoid follicles (Hodges, 1974, Nickel R *et al.,* 1973).

Conclusions

This study showed variations in the esophageal epithelium and the height of proventriculus among the broiler & cock chicken. The esophageal mucosal thickness and epithelium height were highest in cock. Broiler chickens have the maximum mucosal thickness and depth in the Lieberkühn crypts of the colo-rectum. The crypts of Lieberkuhns of the duodenum, jejunum, and ileum had the highest villi height and depth in the cock. The lymphatic nodules' length, width, and depth within Meckel's diverticulum's Lieberkuhn’s crypts were all highest in the cock. Broiler chickens have the largest lymphocyte populations in their cecal tonsil and Meckel's diverticulum.

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Conflict of Interest

The authors have declared no conflicts of interest.

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