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Research Article

HISTO-ARCHITECTURE OF THE SMALL INTESTINE OF GAROLE SHEEP

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ABSTRACT: Ruminants like sheep are prone to infections through ingestion of contaminated feed and water since they are reared on a free-ranging system. The gut immune system plays a major role in fighting the pathogens that gain entry through the oral route. The present study was conducted to explore the histological organization of the small intestine of the adult Garole sheep as it is the major site of absorption in the intestine. The intestine samples were collected from 10 healthy sheep. Tissues collected from the duodenum, jejunum, and ileum were fixed in a 10% neutral buffered formalin solution and processed for histological studies by following standard protocols. The small intestine revealed four distinct layers namely tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa inside out. The tunica mucosa consisted of lamina epithelialis, lamina propria with intestinal glands, and lamina muscularis. The tunica mucosa presented numerous villi of different shapes and heights lined by columnar epithelial cells with Goblet's cells, lymphocytes, Paneth's cells, etc. interspersed among them. Brunner's glands were present in the initial portion of the duodenum and Peyer's patches were observed in the middle ileum.

Key words: Tunica mucosa, Tunica submucosa, Tunica muscularis, Tunica serosa, Peyer's patches.

INTRODUCTION

Domestic sheep (*Ovis aries*) are quadrupedal ruminants and are members of the order Artiodactyla, the even-toed ungulates. Sheep is one of the oldest species to be domesticated for agricultural purposes across the globe. India ranks 3rd in sheep population after China and Australia (Sastry and Thomas 2015). Sheep are mostly reared for wool and meat and are occasionally for pelt, as dairy animals, or as model organisms for science.

Garole sheep are distributed over the Sunderban area of West Bengal, India, a salty region, and this region is believed to be their breeding tract. The breed is comparatively small in size and is very much known for its prolificacy and adaptation in the marshy land of Sunderban. Garole is a popular breed of sheep because of its capacity of giving birth twice a year and its resistance to diseases. Garole produces rough wool, good-quality skin, and mutton with a low-fat percentage.

Nutrition plays an important role in successful animal husbandry. Animals need the proper nutrition for growth, maintenance and to provide energy for work and vital functions. Supply of balanced food and active absorption of different nutrients through the gut is very important in this regard (Sawhney *et al.* 2022). Maintenance is the nutrition required for an animal to maintain its body weight. Energy is the ability of the body to perform physiological functions. For maintaining body temperature, producing milk, reproducing, and developing proper bone structures handsome nutrition is necessary. When proper nutrition is not provided animals can develop health-related problems resulting in treatment costs or even fatality.

Unlike human beings, animals consume large amounts of raw and contaminated feed materials and are often prone to food-borne infection. The intestine of herbivorous animals harbors a complex, dynamic, and fermentative microbial ecosystem that has several major functions (Chharang and Choudhary 2022). In the gut, over 1,000 unique microbial species have already been identified, making the gastrointestinal tract the most heavily colonized organ (Sekirov *et al.* 2010). The main function of the small intestine is to absorb the ingested materials by breaking them down through different enzymatic actions. The small intestine also has a defensive function against the harmful pathogens that enter the body along with feed materials apart from digestion and absorption.

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Keeping in view, the immense role of the small intestine and due to the paucity of relative literature, this study was undertaken for a better understanding of the micro-morphological environment of the small intestine of Garole sheep.

MATERIALS AND METHODS

The intestine of ten healthy sheep of the Garole breed was collected from the Haringhata meat slaughter unit, Govt. of West Bengal, Haringhata, Nadia. The research was conducted in the Department of Veterinary Anatomy of West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal with the permission of the institutional ethical committee (Ref. No. IAEC/49/V). The samples were collected immediately after slaughter and taken into the ice box after proper cleaning. Tissues collected from the duodenum, jejunum, and ileum were preserved in 10% neutral buffered formalin (NBF) for histological studies (Luna 1968). Preserved tissues were later dehydrated by processing through graded alcohol, cleared in xylene, and embedded in paraffin. Tissue sections of 5 mm thickness were procured by a manually operated rotary microtone machine. The sections were then stained by following staining methods for demonstration of the histological organization:

A) Mayer's Hematoxylin and Eosin (H&E) staining method for general histological observations (Luna 1968).

B) Masson's Trichome (MT) staining method for visualization of collagen fibers (Luna 1968).

C) PAS-Alcian Blue (PAS-AB) staining method for demonstration of mucopolysaccharides (Luna 1968).

D) Weigert'sResorcinFuschin (Weigert's) staining method for demonstration of elastic fiber (Sheehan and Hrapchak 1973).

Micrometry

Leica Qwin Images Analyser software in Leica DM 2000 Microscope, bearing serial number 290806-022008 manufactured in the year 2013, was used for taking micrometrical data and desired photographs from the stained sections.

RESULTS AND DISCUSSION

The small intestine of sheep was subdivided into duodenum, jejunum, and ileum which shared a similar basic histological organization although numerous modifications were identified among these regions. The histological orientation of the small intestine revealed four distinct layers which were tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa from inside out.

Duodenum

The duodenum was studied based on three anatomical portions. The initial portion extended from the pyloric end to the cranial flexure of the duodenum, the descending duodenum, and the ascending duodenum. The tunica mucosa consisted of lamina epithelialis, lamina propria with intestinal glands, and lamina muscularis. The lamina epithelialis of all the segments of the duodenum presented numerous villi lined by columnar epithelial cells with lymphocytes and PAS-AB positive goblet cells interspersed among them. The villi were long, and slender with wide tips in the initial and ascending duodenum while the tips were rounded in the descending duodenum. Mitotic figures were observed in some of the epithelial cells (Fig. 1). The average height of the villi was comparatively more in the initial part (Table 1) as this results in an increased surface area for absorption of the nutrients from the feed materials digested in the stomach which later passes to the duodenum for absorption. Lamina propria was composed of loose irregular connective tissues with a network of collagen, and elastic and reticular fibers. Lymphocytes, macrophages, plasma cells, and smooth muscle cells were distributed within the networks. The simple tubular intestinal glands (Crypts of Lieberkuhn) with bases arranged in different layers were packed in the propria (Fig. 2). Ducts of the glands open at the base of the villi. Glands were lined by columnar epithelial cells, goblet cells, Argentaffin cells, and Paneth's cells (Fig.1). The crypts showed a strong PAS-AB positive reaction (Fig. 5). The lamina muscularis was composed of a single layer of smooth muscle cells and connective tissue fibers. The submucosa was composed of dense connective tissue fibers, mainly the collagen fibers, blood vessels, lymphatics, and nerve plexuses (Fig. 6). The wall of the blood vessels was mostly made up of elastic fibers (Fig. 3). Brunner's glands were present in the submucosa of initial duodenum and absent in the other portions of the duodenum except in one area of the descending duodenum where the intestinal gland pushed into the submucosal area which might be considered as Brunner's gland (Fig. 4). The glands were confined only to the proximal or initial part of the duodenum in our subject of study as the glands secrete alkaline substances which contains mucins and that serve as a protective barrier to the intestinal epithelium because the feed materials that are digested by the stomach acids faces the duodenum first and then to the other parts of the intestine. Banks (1983) stated that Brunner's gland in small ruminants was confined to the initial or middle portion of the duodenum which is under our present finding. However, Stinson and Calhoun (1993) in gaddi goats, Ergun *et al.* (2010) in angora rabbit, Al-

Table 1. Histometricalfindings of different parts of the intestine of indigenous Garole sheep (N=10).

Parameters/ Parts of intestine	Height of villi (µm)	Cryptal depth (µm)	Thickness of inner circular layer (µm)	Thickness of outer longitudinal layer (µm)
Initial duodenum	365.1 ± 8.88	198.93 ± 10.18	143.2 ± 5.49	43.33 ± 1.17
Descending duodenum	354.13 ± 10.69	176.5 ± 8.33	228.52 ± 11.45	80 ± 0.94
Ascending duodenum	414.3 ± 6.05	205.87 ± 5.77	258.43 ± 13.32	85.33 ± 2.42
Initial jejunum	312.6 ± 2.31	193.47 ± 3.74	188.87 ± 2.98	124.73 ± 3.02
Middle jejunum	338.73 ± 8.65	238.43 ± 5.84	155.93 ± 6.75	105 ± 4.86
Terminal jejunum	316.33 ± 6.42	214.17 ± 7.52	234.2 ± 6.60	132.37 ± 2.80
Initial ileum	264.4 ± 2.57	162.53 ± 3.66	247.7 ± 5.26	83.5 ± 1.97
Middle ileum	240.06 ± 2.38	134.8 ± 3.08	250.33 ± 2.22	96.43 ± 0.97
Terminal ileum	251.46 ± 2.24	146.03 ± 3.53	256.17 ± 2.75	108.93 ± 4.65

kennany *et al.* (2012) in mice, Kumar *et al.* (2013) in sheep, Nzalak *et al.* (2015) in rats and Nag and Prasad (2016) in guinea pig reported presence of Brunner's gland in the submucosa of respective species. Solitary lymph nodules were absent in this layer. The nerve plexuses were scattered throughout this layer.

The tunica muscularis was composed of smooth muscle cells divided into two sub-layers; the inner sublayer was oriented circularly and the outer layer was arranged longitudinally. In this layer the Myenteric nerve plexus was observed between the two sub-layers (Fig. 6). Besides these, blood vessels, lymphatics, and connective tissue fibers were also found in between the muscle bundles. The thickness of the inner circular layer was more than the outer longitudinal layer (Table 1).

The tunica serosa, the outermost layer was made up of loose connective tissue fibers, blood vessels, and lymph vessels.

Jejunum

The basic histological arrangement of the jejunum was similar to that of the duodenum with certain modifications. Grossly, the total length of the jejunum was divided into three equal segments: initial jejunum, middle jejunum, and terminal jejunum for histological studies. The shape of the villi was variable but the height was almost similar in all the segments (Table 1). A similar observation was reported by Kumar *et al.* (2014) in sheep. Peyer's patches were absent throughout the length of the jejunum but Raju *et al.* (2012) documented the presence of Peyer's patch in the submucosa of the jejunum of sheep. Hasanzadeh and Monazzah (2011) and Kapoor and Singh (2015) also documented the presence of jejuna Peyer's patch in the submucosa throughout the whole length of the jejunum of river buffalo. The average cryptal depth was maximum in the middle portion of the jejunum and minimum at the initial part of the jejunum (Table 1). PAS-

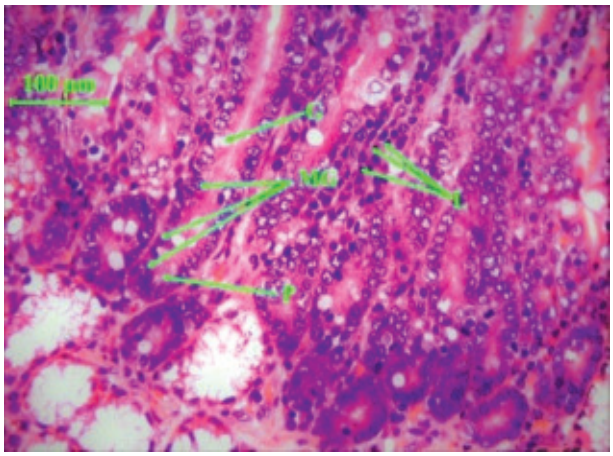

Fig. 1. Photomicrograph showing crypts of initial duodenum, goblet cell (G), intra-epithelial lymphocytes (L), mitotic figures in nuclei (MG) and Paneth's cell (P). H&E X20.

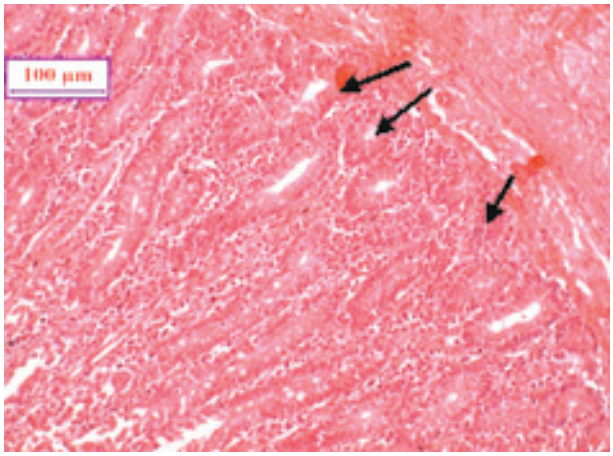

Fig. 2. Photomicrograph of duodenum showing intestinal glands (arrows) in the submucosa. H&E 20X.

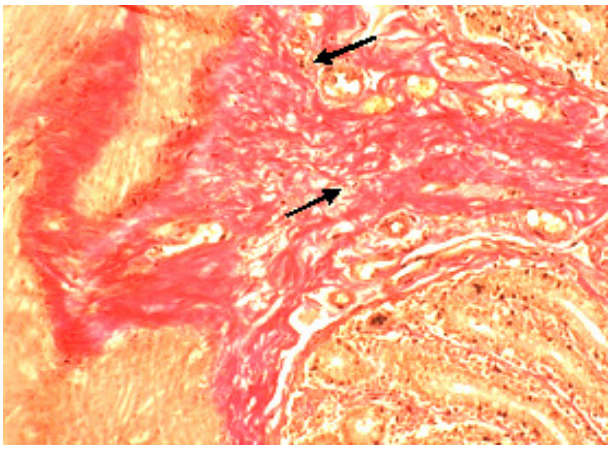

Fig. 3. Photomicrograph showing elastic fibres (arrows) in the walls of blood vessels of descending duodenum. Weigert's X20.]

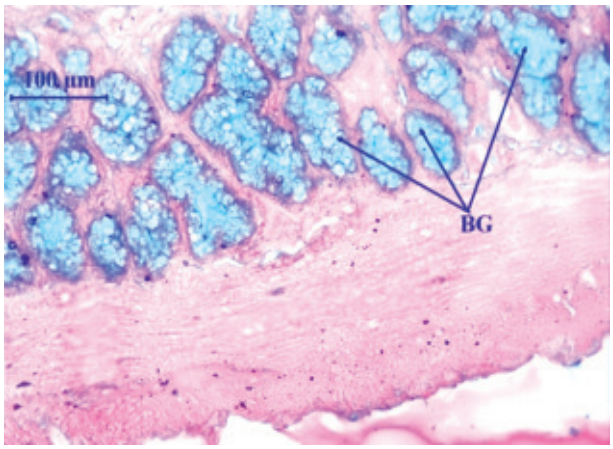

Fig. 4. Photomicrograph showing Brunner's glands (BG) in the submucosa of initial part of duodenum. PAS-AB X20.

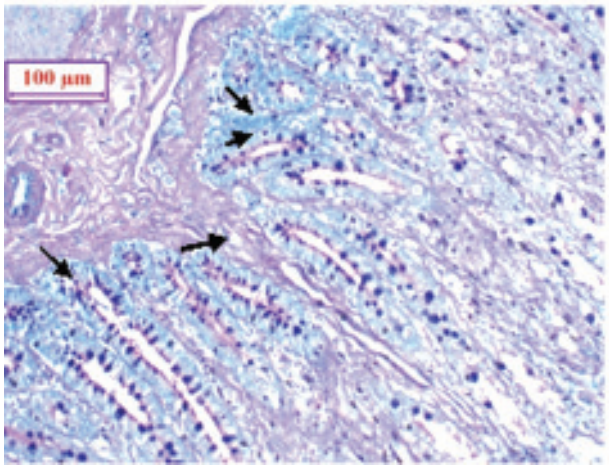

Fig. 5. Photomicrograph showing alcinophilic cells (arrows) in the glands of descending duodenum. PAS-AB X20.

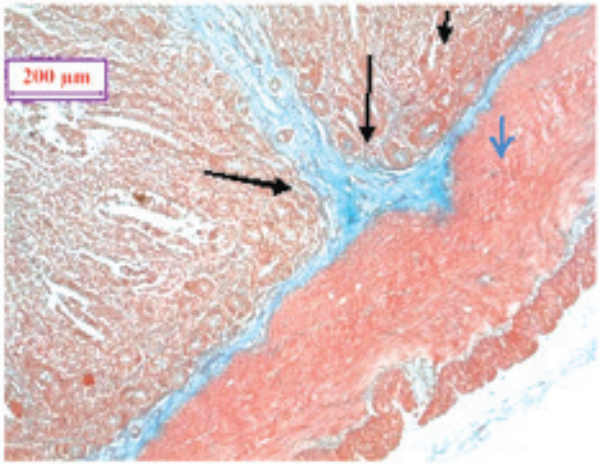

Fig. 6. Photomicrograph showing collagen fibres (black arrows) in thesubmucosa and Myenteric plexus (blue arrow)between two sub-layers of tunica muscularisof descending duodenum. MT X4.

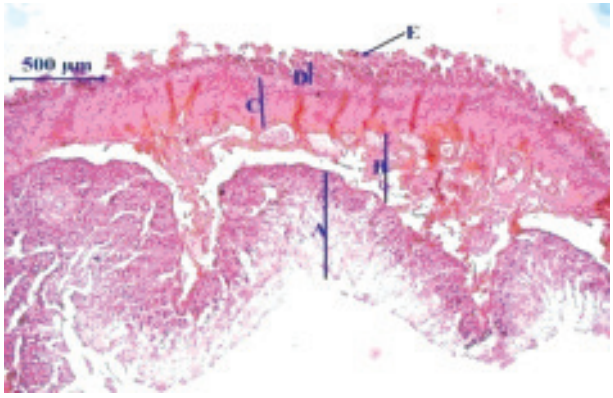

Fig. 7. Photomicrograph showing initial ileum, tunica mucosa (A), tunica submucosa (B), inner circular layer (C) and outer longitudinal layer (D) of tunica muscularis and tunica serosa (E) . H&E X4.

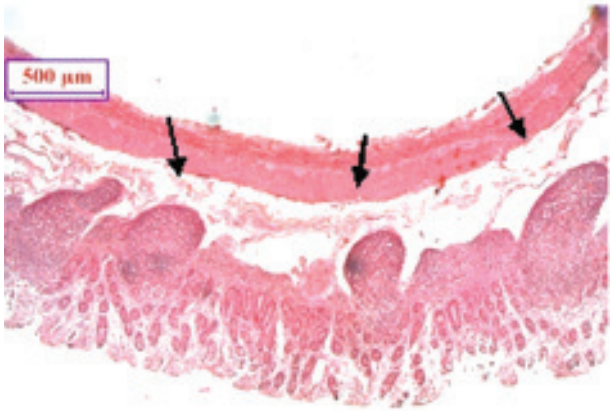

Fig. 8. Photomicrograph showing middle ileum, Peyer's patch (arrows) in the submucosa and invaginating into the submucosa. H&E X10.

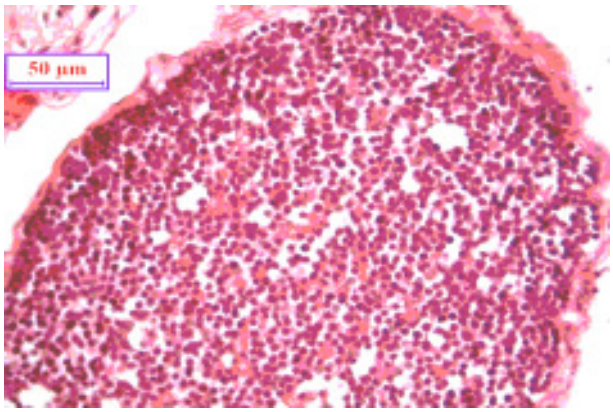

Fig. 9. Photomirograph of ileal Peyer's patch showing middle light zone encapsulated by a dark zone. H&EX40.

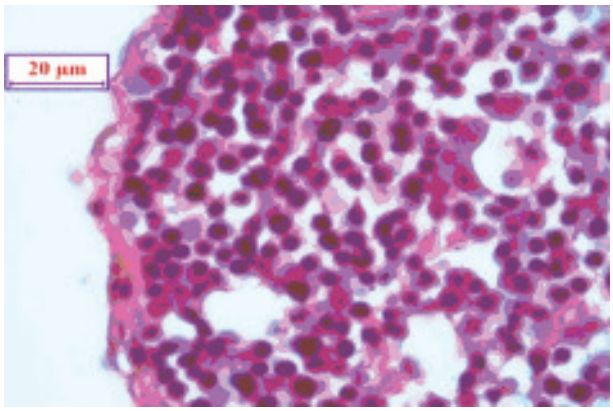

Fig. 10. Photomicrograph showing lymphocytes within the ileal Peyer's patch. H&E X100.

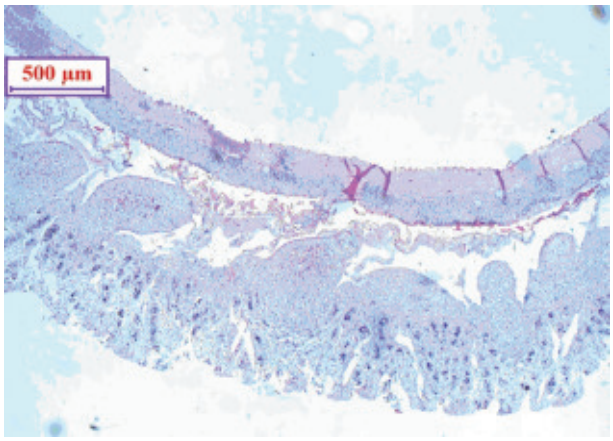

Fig. 11. Photomicrograph showing middle ileum, alcinophic cells (dark blue dots) in the mucosal glands. PAS-AB X4.

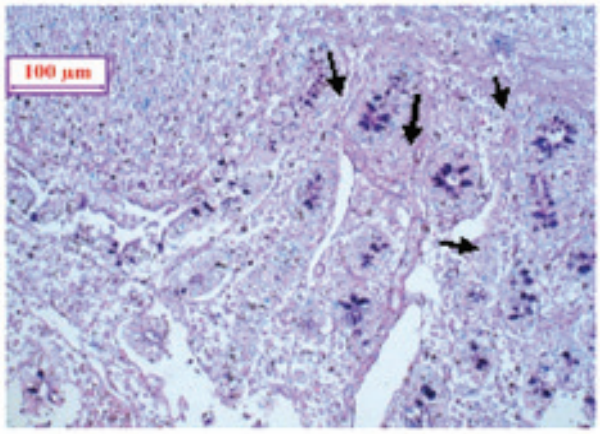

Fig. 12. Photomicrograph showing middle ileum, alcinophic cells (arrows) in the mucosal glands. PAS-AB X10.

AB reaction in the intestinal glands and goblet cells was stronger than in the duodenum. Hamza and Al-Mansor (2017) found moderate PAS-AB reaction in the crypts while in the goblet cells of crypts in the jejunum in indigenous gazelle the PAS-AB reaction was strong.

Ileum

Like jejunum, the total length of the ileum was equally divided into three regions of equal length for histological study. Like the other segments of the small intestine the ileal wall was also made up of four distinct histological layers (Fig.7). The villi of the ileum were long, slender, and with blind ends, and at the terminal part the villi were wide. The height of the villi and the crypt depth were more in the initial part of the ileum than in the other two parts (Table 1). The crypts of Lieberkuhn showed strong PAS-AB positive reactions due to the presence of mucopolysaccharides (Fig.11 and Fig. 12). Thesubmucosa of middle ileum, at about 9 cm onward from the ileo-jejunal junction first revealed the presence of pleomorphic Peyer's patch (Fig. 8). Kumar *et al.* (2015), Ranjan *et al.* (2016) and Andleeb *et al.* (2016) recorded that Peyer's patches were present in the ileum of adult sheep, rabbit, and gaddi goats respectively. Gahlot and Kumar (2018) documented the presence of ileal Peyer's patches in goats and they found that the Peyer's patches consisted of follicles of various shapes and sizes which was in agreement with our present finding. The patches had a well-differentiated middle light zone capped by a dark zone and late beneath the dome there was an elevated region overlying lymphatic nodules beyond the lamina muscularis and protruded into the propria (Fig. 9). The dome was lined by nodule-associated epithelium with numerous inter-epithelial lymphocytes of varying sizes with some plasma cells within it (Fig. 10). The nodules were separated by a wide inter-nodular area containing lymphocytes and blood capillaries. The nodules were encapsulated by connective tissue fibers, mainly collagen fibers.The tunica muscularis and tunica serosa was similar in orientation to that of duodenum and jejunum.

CONCLUSION

In the present study, we observed that the small intestine was made up of four distinct histological layers. However, different histomorphometric and histological orientations were revealed in different segments. The villi were absent in the large intestine. The goblet cell population within the enterocytes and crypts of Lieberkuhn gradually increased from pyloric to the aboral end. The Brunner's glands were present only in the submucosa of the initial portion of the duodenum. The Peyer's patches were identified in the submucosa of the middle and terminal portion of the ileum with their base embedded into the lamina propria. The Peyer's patches were irregular in shape and size. A large number of lymphocytes was observed within the epithelium, in the lamina propria, and the Peyer's patches.

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