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Research Article

GROSS AND HISTO-MORPHOLOGICAL STUDIES ON THE LARGE INTESTINE OF RABBIT

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ABSTRACT

The present study was undertaken to explore the gross and histo-morphological details of the large intestine of rabbit. The study was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 kg. The tissue samples were processed, sectioned and subjected to specific stains to reveal histological details. General architecture was similar to other mammals with few peculiarities: Caecum was highly developed sacculated structure with numerous spiral leaf-like foldings for microbial fermentation. Grossly, the colon was divided into a sacculated and smooth part on the basis of external features. The vermiform appendix was well developed with numerous lymphatic nodules within the tunica submucosa. It constituted a part of the organ of immune response in this species of animal. In the colon, the goblet cell count was highest which could be correlated with caecotrophy nature of this species. The mean thickness of tunica mucosa was significantly more in the colon because of the presence of relatively long crypts.

Keywords: Gross, Histo-morphology, Large intestine, Rabbit

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The morphology and gross disposition of the digestive tract has been extensively studied and reported in various species of mammals like sheep, cattle, pigs, horses, dogs (Sisson and Grossman, 1953), man (Harold, 1992) and birds (Devyn *et al.*, 2000). Regarding laboratory animals, detailed information is still lacking. As a classical experimental animal model, rabbits are extensively used in the laboratories for various experiments (McNitt *et al.*, 2011). Rabbit meat is high in protein and low in fat. Sodium and caloric content of the meat is also low (McNitt *et al.*, 2011). Hence it is a good source of protein for children, sick, aged and health-conscious people. It has the capacity to produce the above commodities on feeds not fit for human consumption as well as not relished by other species of livestock that signifies the importance of its gastrointestinal tract (Handbook of Animal Husbandry, 2008).

Interestingly, similar to horses, rabbits are typical hindgut fermenters, monogastric with a well developed caecum. The specific gross anatomy of the gastrointestinal tract (i.e. the size of the large intestine) reflects its functional importance. Moreover, rabbits are caecotrophic in nature, i.e. they directly feed on their own soft faeces during night time. These caecotrophes are a good source of proteins, vitamins and minerals (Halls, 2008). The distinctiveness in the functioning of the large intestine of rabbits in microbial fermentation of food particles and formation of caecotrophes defines its anatomical capabilities. Hence, the present work was focused on gross and histomorphological details of the large intestine of rabbits.

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MATERIALS AND METHODS

The present study was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 kg after approval of the Institutional Animal Ethics Committee (IAEC). After the topographic study, gross parameters for length and diameters were recorded for different components of the large intestine. Tissue specimens from every 10 cm interval were taken from different segments of the large intestine i.e. caecum, colon and rectum and fixed in 10% Neutral buffered formalin (10% NBF) and Bouin's fixatives. The samples were processed by acetone-benzene schedule and these 5-6 μm thick paraffin sections were stained with haematoxylin and eosin (H & E) for routine morphology, Masson's trichrome method for collagen fibers, Gridley's method for reticular fibers and Weigert's method for elastic fibers (Luna, 1968). Micrometry was done by using Leica Qwin Image Analyzer software in Leica DM 2000 microscope. The data collected were analyzed statistically (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Gross Anatomy

The large intestine comprised of three segments: caecum, colon and rectum. The caecum had a blind terminal end; the vermiform appendix (VA). The colon had sacculated and smooth parts.

Caecum: The caecum was a tubular structure about 49.75 ± 2.9 cm in length and 29.773 ± 0.5 mm to 19.842 ± 1.28 mm in diameter from cranial to caudal direction. It was located on the abdominal cavity floor and was always found coiled into a loop of one and a half turns in a counterclockwise direction as viewed from the ventral surface. The sacculated part of the colon was seen between the caecal coils. The caecum occupied almost the entire abdominal floor (Fig. 1). It was related ventrally to the abdominal wall, dorsally to the part of the sacculated colon, duodenum, jejunum, ileum and a part of the smooth colon. Caudally, it was related to the urinary bladder and cranially to the stomach and liver. The spiral grooves were apparent on the external surface, which corresponded to the spiral fold within the lumen of the caecum from the base to the appendix (Fig. 1). Perez *et al.* (2007) also reported similar observations in rabbit.

The complicated structure of the caecum was readily investigated only after removal from its *in situ* position and detachment of the connecting mesentery. Macroscopically, the caecum was roughly divided into three major portions: the bulbous base (basis ceci), the body (corpus ceci) and the terminal portion, vermiform appendix (Fig. 2 & 3). The basis ceci gave passage to the sacculated part of the colonby forming a ceco-colic fold (Fig. 2). The corpus ceci provided attachment to the ileum via sacculus rotundus (SR). The SR opened dorsally on the second gyrus of the caecum and was guarded by ileocecal valve (Fig. 2). Approximately, the caecum had 27 gyri. The blind terminal end of the caecum was a well developed structure, the vermiform appendix (Fig. 3). These findings were in accordance with the observations of Perez *et al.* (2007) and Saleh (2012).

Vermiform Appendix: The vermiform appendix was well developed and about 8.333±0.40 cm in length and 9.199 ± 0.30 mm in diameter. Its colour was lighter than the caecum because the ingesta were not visible through the highly lymphoid wall (Fig. 3). It began just ventral and caudal to the right kidney and extended caudo-medially to the level of 6^{th} lumbar vertebrae. It then turned cranio-laterally to the left of the median plane.

Malla (2003) also reported that morphologically distinct vermiform appendix was found in the rabbit only, whereas no other laboratory animals showed any definite vermiform processes. O'Malley (2008) stated that the rabbit had the largest appendix within the entire animal kingdom. The vermiform appendix was provided with numerous lymphoid follicles. Saleh (2012) also reported that the appendix was large and formed a terminal blind end of the rabbit caecum. It was bright in color and characterized by a thick wall and numerous lymphoid follicles. All these observations correlated with the present findings.

Colon: The colon of rabbit had sacculated and smooth portions (Fig. 1). The sacculated colon started from the caecum and formed the proximal part of colon.

Sacculated Colon: The sacculated colon was a highly corrugated structure about 27.833 ± 1.05 cm in length and 10.937 ± 0.15 mm in diameter. It originated from the base of the caecum at the ceco-colic junction and passed between the coils of the caecum (Fig. 1 & 2). In the right flank region, it turned dorso-caudally then caudo-medially. It was closely attached to the root of the caecal mesentery. Rows of sacculations were present on the external surface of the colon all along its course (Fig. 1). The present observations were in accordance with the reports of Huffman (1958).

Smooth Colon: The smooth colon appeared as a tubular structure about 73.0 ± 4.03 cm in length and 4.522 ± 0.42 mm in diameter. This part of the colon was observed ventral to the fourth lumbar vertebra. The smooth colon passed caudally for a short distance and then passed between the descending duodenum and the body of the caecum (Fig. 1). It then passed transversely caudal to the stomach. The transverse segment was closely attached to the dorsal abdominal wall. It then progressed near the median plane to the pelvic cavity, where it continued as rectum. Perez *et al.* (2007) in rabbit reported similar observations which were in accordance with our present findings.

Rectum: The rectum was a tubular structure approximately 5.333 ± 0.17 cm in length and 5.398 ± 0.38 mm in diameter. It progressed caudally in the dorsal part of the pelvic cavity to the anus. The caudal few centimeters were retroperitoneal. Longitudinal folds of the mucous membrane were present. Just cranial to the anus, there were two or three circular folds in the mucous membrane. The present observations were in accordance with the reports of Agarwal *et al.* (2002).

Histo-morphology

Microscopically, this segment of intestine was characterized by the presence of mucosal folds instead of prominent villi, a characteristic feature of the small intestine.

Caecum: The histo-architecture of basis ceci and corpus ceci was same, while the vermiform appendix varied in its microscopic structure than the other two portions.

In the basis and corpus ceci, the tunica mucosa had numerous folds lined by simple columnar epithelium with striated free borders (Fig. 4). The mucosal folding was different at the point of constriction, which appeared as tall spiral leaf-like folding of different sizes (Fig. 5), whereas in the sacculus part it continued as spiral fold. These foldings contained all the lamina of tunica mucosa *viz.* lamina epithelialis, lamina propria and lamina muscularis

Table 1

Mean ± S.E. of micrometrical observations of different regions of Large intestine (µm)

Region	Tunica mucosa	Tunica submucosa	Tunica muscularis	Tunica serosa
Caecum	247.35 ± 17.54^{a}	33.01 ± 2.65^{ab}	129.09 ± 14.13^{ab}	33.91 ± 3.47^{ab}
Sacculated colon	600.84 ± 16.07^{d}	45.92 ± 7.38^{b}	86.98 ± 9.77^{a}	40.89 ± 3.40^{b}
Smooth colon	375.94 ± 18.83^{b}	22.30 ± 1.91^{a}	164.27 ± 22.32^{b}	26.34 ± 3.48^{a}
Rectum	481.83 ± 30.94^{c}	77.15 ± 9.69^{c}	505.30 ± 24.11^{c}	57.76 ± 5.92^{c}

N.B. – Mean Values bearing different superscripts in a column differ significantly, where P<0.05

(Fig. 4). Huffman (1958) also reported similar observations. In the present study, the mean thickness of tunica mucosa of caecum was significantly less than that of the other segments of large intestine that might be due to the presence of relatively short and straight cryptal glands within the lamina propria (Fig. 4, Table 1). Goblet cells were found interspersed with the columnar absorptive cells (Fig. 4) but the goblet cell concentration was more in the depressed area between the mucosal foldings. Intra-epithelial lymphocytic infiltration was also observed (Fig. 4, Inset). The present findings were in accordance with the reports of Grant and Specian (2001) in rabbit.

Microscopic observations revealed that simple columnar epithelium without free borders and basally located elongated nucleus lined the crypts. Goblet cells were also present within the cryptal epithelium; however, the goblet cell count was significantly lower in caecum than the other segments of large intestine (Table 2). Apart from the mucosal glands, the lamina propria also contained collagen fibers, fibrocytes, numerous lymphocytes, plasma cells and blood capillaries. The lamina muscularis was thin and separated the lamina propria from the underlying tunica submucosa. The tunica submucosa was a connective tissue layer with abundant collagen fibers showing a wavy appearance (Fig. 4). The average thickness of the tunica submucosa of caecum varied non-significantly with that of the sacculated and smooth colon (Table 1). The report of Huffman (1958) was in accordance with the present findings.

The tunica muscularis had an inner circular and outer longitudinal smooth muscle fiber layer, the thickness of which varied non-significantly (Fig. 4, Table 1). The tunica serosa was similar in structure to that of the other segments of the intestine.

VermiformAppendix: Histologically, the vermiform appendix appeared to be a lymphoid organ and constituted a part of the gut-associated lymphoid tissue (GALT) in rabbit as had been reported by Saleh (2012).

The tunica mucosa formed an umbrella-shaped tree structure over the lymphatic nodules present within the submucosa. The stem of the tree structures were lined by simple columnar epithelium with few goblet cells. The lamina propria was similar in structure to the other segments of intestine. Crypts with numerous goblet cells were abundant in the upper part of the structure (Fig. 6). Huffman (1958) also reported similar observations in the rabbit vermiform appendix.

Table 2

Goblet cell count/10,000 µm² area in different segments of intestine

Structure	Caecum	Colon	Rectum
Cryptal epithelium	9.6 ± 1.24^{a}	21.4 ± 0.92^{c}	12.9 ± 0.82^{b}
Mucosal epithelium	6.2 ± 0.48^{a}	7.6 ± 0.65^{a}	6.4 ± 0.37^{a}

N.B. – Mean Values bearing different superscripts in a row differ significantly, where P<0.05

The lymphatic nodules were encapsulated by connective tissue fibers. The inter-follicular space was absent. Within the nodule, the centrally positioned germinal center was well differentiated from the peripheral darker zone (corona). The corona of the superficial lymph node was further covered by the apical part of the lymph nodule (dome). The dome was lined by follicle associated epithelium that was cuboidal to low columnar with numerous infiltrated lymphocytes but lacked the goblet cells (Fig. 6). The entire nodule was filled with lymphocytes and some plasma cells. Saleh (2012) also reported similar observations regarding the architectural arrangement of lymph nodules, however, in contrast to the present observation; he reported a wide interfollicular area between neighboring follicles.

The huge lymphocytic aggregation within the nodules implied its role of local immune response, especially in case of the rabbit. In this regard, the experiment conducted by Meuwissen *et al.* (1969) substantiates our observations which reported that the higher number of cells involved in DNA synthesis was present in rabbit appendix.

The tunica muscularis and serosal layer were similar in structure to other segments of intestine. The connective

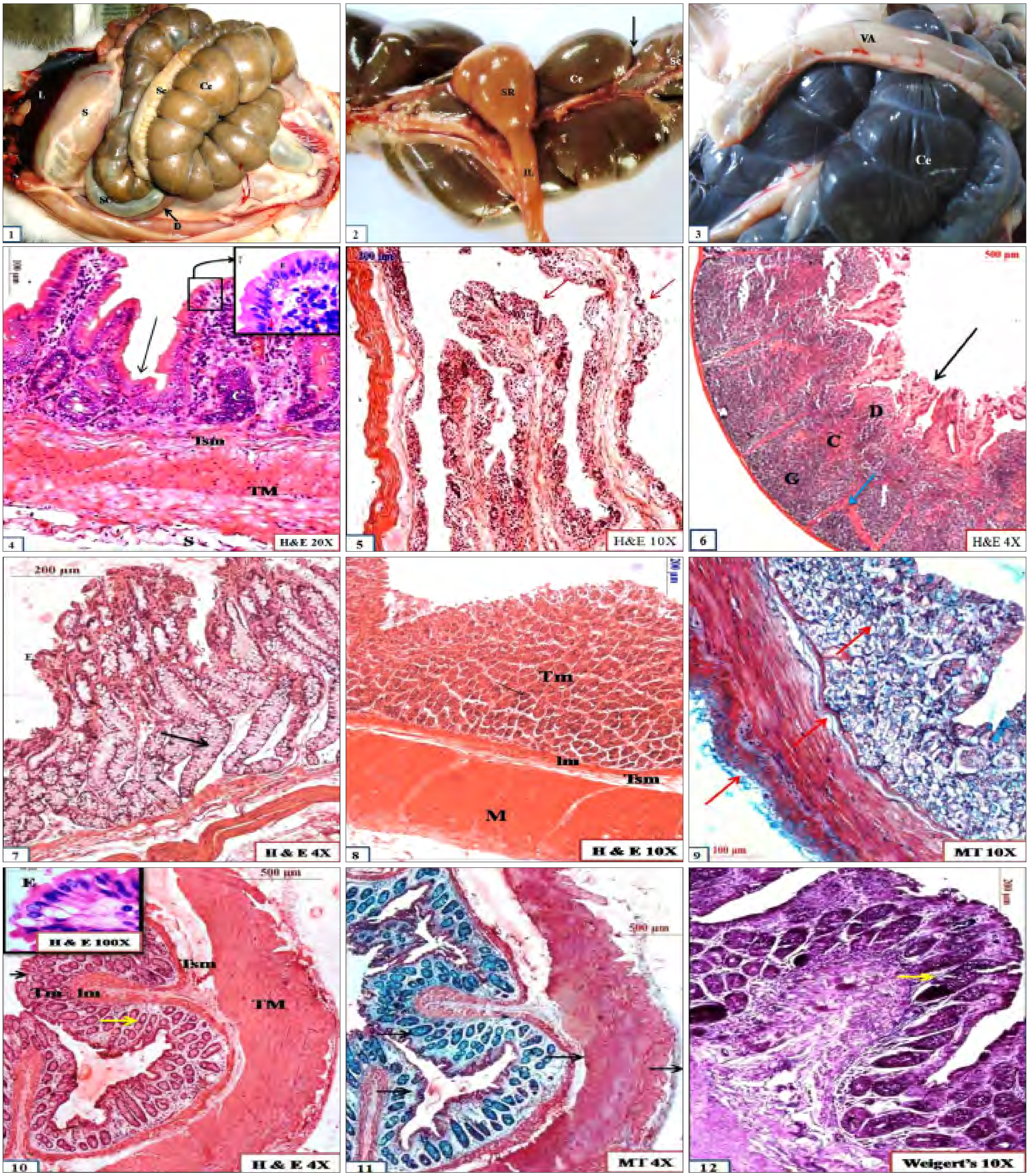

Fig. 1-12. (1) Photograph showing caecum (Ce) into a loop of one and a half turns in a counter clock wise direction Sacculated colon (Sc) lied between the caecal coils, smooth colon (Sc) passing caudally for a short distance between the descending duodenum (D) and the body of the caecum and abdominal wall laterally. Other structures are cranially placed stomach (S) liver (L); **(2)** Photograph showing the ileum (IL) terminating into the Sacculus rotundus (Sr), opening dorsally on the second gyrus of the body (Corpus ceci) of Caecum (Ce) and initiation of Sacculated Colon (Sc) from the bulbous base (basis ceci), arrow indicating caeco-colic junction. **(3)** Photograph showing the well developed blind end of caecum (Ce) i.e. vermiform appendix (V.A) after exposing it from the viscera. Colour is lighter than the caecum because the ingesta i not visible through the highly lymphoid wall. **(4)** Photomicrograph of caecum showing mucosal folds (arrow) lined by simple columnar epithelium (E; [inset] and numerous lymphocytes (red arrow) in the lamina propria. H&E 100 X), crypts (C), submucosa (Tsm), Tunica muscularis (TM) and serosa (S). H&E 20 X; **(5)** Photomicrograph showing leaf shaped spiral foldings (red arrow) of tunica mucosa corresponding to the sacculations on the external wall of caecum present grossly. H&E 10X; **(6)** Sections of vermiform appendix showing umbrella shaped tunica mucosa (black arrow) at the top and lymph nodules in submucosa having germinal centre (G), Corona (C) and Dome (D) surrounded by collagen fibers (blue arrow). H&E 4X; **(7)** Section of sacculated colon showing simple columnar epithelium (E) and long cryptal glands (arrow). The tunica submucosa had a fine wavy network of collagen fibers. The tunica muscularis had an inner circular and an outer longitudinal smooth muscle fiber. H&E 4X; **(8)** Section of smooth colon showing tunica mucosa (Tm) with short cryptal glands (arrow), lamina muscularis (lm), wavy tunica submucosa (Tsm) and thick tunica muscularis (M). H&E 10X; **(9)** Section of smooth colon showing collagen fibers present in serosa, submucosa and lamina propria (red arrow). Masson's Trichrome 10 X; **(10)** Section of rectum showing longitudinal folds of tunica mucosa (Tm), simple columnar epithelium (E; inset), short cryptal glands (yellow arrow), lamina a propria (lm) forming the core of the mucosal fold, wavy tunica submucosa (Tsm), thick tunica muscularis (Tm) and thin serosa. H&E 4X; **(11)** Section of rectum showing the presence of collagen fibers in serosa, submucosa and lamina propria (black arrow). Masson's Trichrome 4X; **(12)** Section of rectum showing longitudinal folds of tunica mucosa with few elastic fibers (yellow arrow) around the short cryptal glands. Weigert's 10X.

tissue fibers from the submucosa often penetrated the muscularis layer.

Sacculated Colon: The tunica mucosa was folded and lined by simple columnar epithelium with striated free borders. Numerous goblet cells were interspersed between the lining columnar cells (Fig. 7). The mean thickness of this tunic was significantly higher than the tunica mucosa of other segments of the large intestine that might be due to the presence of very long and straight crypts of Lieberkûhn present within the lamina propria (Table 1). Eurell and Frappier (2006) had also reported similar observations.

The mucosal glands extended deep into the lamina propria upto the level of lamina muscularis. These glands had numerous goblet cells,which were significantly high than other segments of the large intestine (Table 2). The lamina muscularis consisted of smooth muscle fibers that separated the lamina propria from that of the underlying tunica submucosa. Grant and Specian (2001) had also reported similar observations. Eurell and Frappier (2006) also reported the increased number of goblet cells in the colon of mammals compared to the small intestine.

The tunica submucosa had a fine wavy network of collagen fibers and fibrocytes, the mean thickness of which varied non-significantly with caecum but was significantly higher than that of the smooth colon (Table 1). Numerous large blood vessels were observed having prominent elastic fibers in their walls, especially in the inner elastic lamina. No lymphocytic aggregation was noticed throughout its length. The thickness of tunica muscularis, with an inner circular and an outer longitudinal smooth muscle layer, varied non-significantly with the caecum but was significantly less than the smooth colon and rectum (Table 1). The outer longitudinal muscle layer formed thick bands in some areas, whereas it remained as a thin layer covering the inner circular layer. The tunica serosa was similar in structure to that of the other segments of the intestine. Huffman (1958) also reported similar observations.

Smooth Colon: In the present study, it was noticed that the tunica mucosa was characterized by numerous foldings. The surface epithelium was thick and invaginated upto the lamina muscularis in many areas, forming extremely folded epithelium. The cellular makeup of surface epithelium consisted of absorptive columnar epithelial cells with striated free borders and numerous goblet cells (Fig. 8). The mean thickness of this tunic was significantly less than that of the sacculated colon that might be due to the presence of relatively short crypts of Lieberkûhn within the lamina propria (Table 1). These glands contained numerous goblet cells secreting acidic mucins. Grant and Specian (2001) also reported the presence of extremely folded epithelium with numerous goblet cells and short crypts. Oliver *et al.* (2011) reported similar observations in the colon of the African giant rat.

The lamina muscularis was found in between lamina propria and submucosa, which was composed of smooth muscle fibers. The mean thickness of the underlying tunica submucosa was significantly less than that of the sacculated colon and consisted of a loose network of collagen fibers with few large blood vessels having elastic fibers in its laminae (Fig. 9, Table 1). The present finding was in accordance with the reports of Huffman (1958).

The tunica muscularis with an inner circular and outer longitudinal smooth muscle layers was significantly thicker than that of the sacculated colon (Table 1). Oliver *et al.* (2011) also reported the same in African giant rat. The thickening of the muscularis was correlated with the temporary storage and expulsion of fecal materials from this area (Fig. 8). The tunica serosa consisting of collagenous connective tissue was similar in structure to that of the other segments of the intestine (Fig. 9, Table 1).

Rectum: The tunica mucosa had numerous longitudinal folds with surface epithelium comprising of simple columnar cells with striated borders and numerous mucous containing goblet cells (Fig. 10). The core of the longitudinal folds was occupied by thick lamina muscularis that appeared multilayered and tunica submucosa comprising of densely packed collagen and elastic fibers. The lamina propria had numerous short crypts, just like that of the smooth colon, consisting of goblet cells (Fig, 10 & 11). The mean thickness of tunica mucosa was significantly higher than that of the caecum and smooth colon but not with that of the sacculated colon that might be due to the presence of relatively short crypts as compared to that of the sacculated colon (Fig. 10, Table 1). The tunica submucosa was also relatively thicker than that the caecum and colon (Table 1) that might be due to more compact arrangement of connective tissue fibers. The present finding was in accordance with the reports of Agarwal *et al.* (2002). Apart from this, the lamina propria had an abundance of collagen and elastic fibers, lymphocytes and few smooth muscle cells and fibers (Fig. 11 & 12). Eurell and Frappier (2006) had also reported abundant elastic fibers in the rectum of horse and cattle.

The mean thickness of tunica muscularis was significantly higher than other segments of the large intestine (Fig. 10, Table 1). Similar observations were reported by Eurell and Frappier (2006) in horses and cattle where the rectal wall was thicker than that of the colon wall.

The tunica serosa was significantly thicker and similar in structure to that of the other segments of intestine containing densely arranged collagen fibers (Fig. 11) which was later replaced by tunica adventitia in the retroperitoneal part of the rectum. Similar observations had been reported by Agarwal *et al.* (2002) in rabbit and Eurell and Frappier (2006) in other mammals.

CONCLUSION

The present study revealed significant gross and histological differences between the three segments of the large intestine of rabbit, which signifies its functional aspects. Moreover, the histological architecture of vermiform appendix marked its significant role as a primaryorgan for immune response forming a part of the gut-associated lymphatic tissue (GALT) in rabbits.

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