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Histomorphological and Histochemical Characterization of Jejunum in Domestic Pig (*Sus Scrofa Domesticus*)

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ABSTRACT

The present research work was conducted on six healthy adult pigs of local mixed breed of either sex to study the microscopic anatomy of jejunum. The pointed tapering to blunt round ends of villi present in the tunica mucosa of jejunum, were lined by simple columnar epithelium with goblet cells. Lamina propria was comprised of irregular connective tissue, fine blood capillaries and intestinal glands. The tubulo-alveolar intestinal glands were lined by simple cuboidal to columnar epithelium. The acini presented strong PAS positive pattern for mucopolysaccharides. The lymphoid aggregates were also observed in the lamina propria. The descending population of enterochromaffin cells was observed as progressed from cranial to caudal part of the jejunum. Lamina muscularis mucosa was moderately thick and was interrupted at places due to invasion of lymphatic nodules into it. The thickness of submucosa increased in size towards the caudal part of the jejunum with isolate patches of lymphatic nodules. Thickness of inner circular and outer longitudinal layer of tunica muscularis was consistent throughout the jejunum. Tunica serosa lined by loose connective tissue covered with mesothelium.

Keywords: Histology, Histochemistry, Jejunum, Pig

INTRODUCTION

Among the various livestock species, piggery is the most potential source for meat production. Swine production in India has remained somewhat unexploited despite the country having an established pig population. In pigs, the jejunum is the workhorse section of the small intestine ensuring vital for digesting and absorbing the majority of nutrients, adapting to digestive challenges, and maintaining gut health and immunity. The jejunum accounts for nearly 80% of the pig's small intestine and is equipped with villi that greatly expand surface area, enabling efficient nutrient absorption. The lymphoid aggregates along with mucus layer of the jejunum forms a critical barrier to prevent pathogen invasion, and contributes to intestinal immune. A histological and histochemical study has been conducted on sheep (Kumar *et al*. 2013), camels (Korkmaz & Kum. 2016) and goat (Kumar, 2017). Keeping in view the importance of the intestine, the present study was done to describe the detailed microscopic anatomy of jejunum of pig.

MATERIALS AND METHODS

The tissue from the cranial, middle and caudal parts of the jejunum were collected from the six young pigs (8-10 months of age) immediately after their sacrifice from local slaughter house, fixed in 10% neutral buffered formalin and processed for routine paraffin technique. The paraffin sections of 5-6 µ were cut and stained by routine Harris haematoxylin and eosin stain (Luna, 1968), collagen fibres (Crossman, 1937), Gomori's method for reticular fibres and Weigert's method for elastic fibres. For Histochemical demonstration, McManus' method for glycogen (PAS), PAS-Alcian blue method for mucosubstances, Alcian blue for mucosubstances (pH 2.5) (Luna, 1968), Mayer's mucicarmine for mucin and Fontana method for enterochromaffin cells.

RESULTS AND DISCUSSION

Histo-architecture of the jejunum was comprised of tunica mucosa, tunica submucosa, tunica muscularis (Fig. 1) and tunica serosa which was in consistent with studies in pigs (Urmila *et al*., 2019), sheep (Kumar *et al*., 2013), goat (Kumar, 2017) and cattle, sheep and goat (Kapadnis and Thakur, 2018). The villi of tunica mucosa appeared more slender with pointed tapering ends along with blunt to round end. Gradually these became tall, slender and finger like as progressing further towards caudal part of jejunum where they attained maximum height (Figs.

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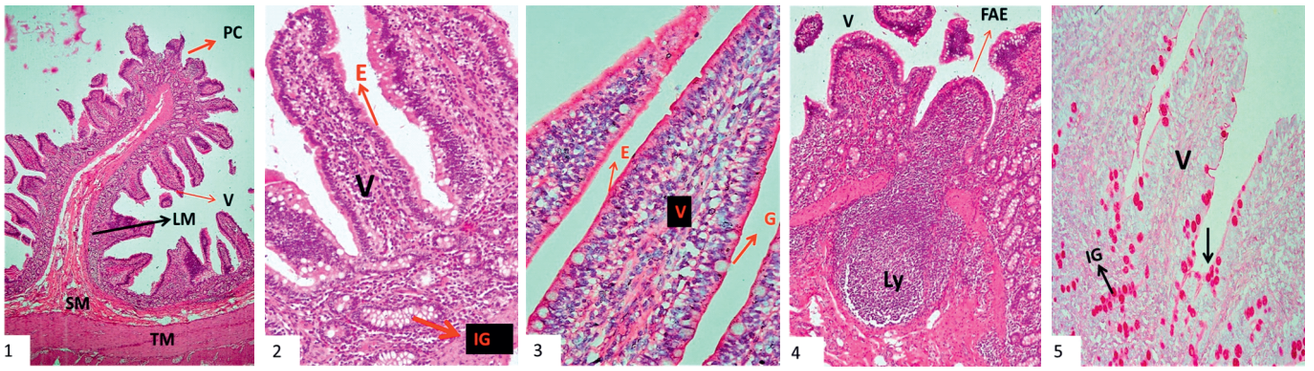

Fig. 1: Photomicrograph showing plica ciruclaris (PC), villi (V), goblet cells, Lamina muscularis mucosae (LM), tunica submucosa (SM) and tunica muscularis (TM) in cranial jejunum. H. & E. x 40; **Fig. 2:** Photomicrograph showing simple columnar epithelium (E) in villi (V) and intestinal glands (IG) of mid jejunum. H. & E. x 200; **Fig. 3:** Photomicrograph showing simple columnar epithelium (E) with goblet cells (↑) in villi (V) of caudal jejunum. H. & E. x 400; **Fig. 4:** Photomicrograph showing villi (V), Follicular associated epithelium (FAE) and underlying lymphoid follicle (Ly) in caudal jejunum. H. & E. x 100; **Fig. 5:** Photomicrograph showing distribution of glycogen in goblet cell (↑) in villi (V) and intestinal glands (IG) in cranial jejunum. McManus' PAS x 200.

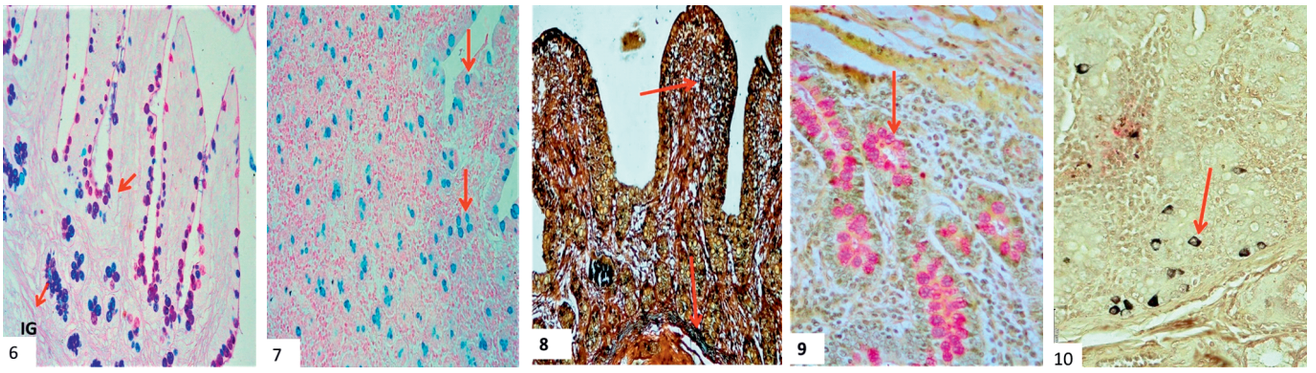

Fig. 6: Photomicrograph showing acidic mucopolysaccharides in goblet cell (↑) in villi and intestinal glands (IG) of mid jejunum. PAS-AB x 100; **Fig. 7:** Photomicrograph showing positive activity (Blue colour) for Alcian blue in goblet cells (↑) in villi and intestinal glands (↑) of crypts of cranial jejunum. Alcian blue x200; **Fig. 8:** Photomicrograph showing distribution of reticular fibres (↑) in the core of villi of caudal jejunum. Gomori's method x 200; **Fig. 9:** Photomicrograph showing mucin in goblet cell (↑) in crypts of caudal jejunum. Mayer's mucicarmine x 400; **Fig. 10:** Photomicrograph showing distribution enterochromaffin cells (Black colour) (↑) in crypts of Lieberkuhn of cranial jejunum. Fontana method x 200.

1-3). The present study was in accordance with the findings of Talukdar (1999), in pig, piglets (Rajkhowa and Baishya, 2013), sheep (Kumar *et al*., 2013), goat (Kumar, 2017) Gaddi goat (Andleeb *et al*., 2009), Red Sokoto goat (Bello and Danmaigoro, 2019), buffalo (Hasanzadeh and Monazzah, 2011). In buffalo villi were leaf shaped or tongue shaped but some villi were pointed at their tips (Rani, 1991). The villi were lined by simple columnar epithelium with goblet cells (Figs. 2-4). The number of goblet cells increased towards the caudal part of the jejunum concentrated more towards the base of the villi as reported in sheep (Kumar et al., 2013) and goat (Kumar, 2017). The columnar cells had elongated, oval and round basophilic nuclei present towards the base. The cytoplasm of the columnar cells was finely granular and strongly eosinophillic and accentuated towards the apical portion of the cells as reported in sheep (Kumar et al., 2013), Gaddi goat (Andleeb et al., 2009) and buffalo (Rani, 1991). The cytoplasm of goblet cells was lightly eosinophilic along with round to oval shaped basophilic nuclei pushed towards the base (Figs. 2-4). In between the simple columnar epithelium, the epithelium was modified and it drastically reduced in size and it was having varied type of epithelium which varied from simple cuboidal to low columnar epithelium. This patch was infiltrated with lymphoid cells and it was mainly associated with underlying lymphoid tissue of Peyer's Patches and this layer was called to be follicular associated epithelium (FAE) (Fig. 4) in the caudal part, as reported in sheep (Kumar *et al*., 2013), Gaddi goat (Andleeb et al., 2009) and buffalo (Rani, 1991). The columnar cells of the villi showed weak reaction for PAS, PAS-AB and Alcian blue which were similar to observations in dromedary camel (Korkmaz and Kum, 2016). However, Andleeb *et al*. (2009) reported that the

luminal border of columnar epithelium of jejunum of Gaddi goat showed weak to moderate reaction with PAS. The villi and the basement membrane of the epithelium showed moderate to weak reaction with Alcian blue stain at pH 2.5 throughout the intestine in Gaddi goat (Andleeb *et al*., 2009). Weak PAS and Alcian blue reactions by the columnar absorptive cells has been reported in goat (Kumar, 2017) sheep (Kumar *et al*., 2013) and cattle (Ohwada and Suzuki, 1992).The cytoplasm of goblet cells was lightly eosinophilic along with round to oval shaped nuclei as reported in sheep (Kumar *et al*., 2013). The goblet cells showed a strong PAS positive reaction for glycogen (Fig. 5) and also showed presence of acidic and neutral mucopolysaccharides with PAS-AB (Fig. 6) as reported in sheep (Kumar *et al*., 2013), Goat (Kumar, 2017), Gaddi goat (Andleeb *et al*., 2009) and dromedary camel (Korkmaz and Kum, 2016). The goblet cells showed strong Alcianophillic reaction indicating the presence of weakly acidic sulphated muscosubstances (Fig. 7). The performic acid-Alcian blue method showed the presence of more than 4% cysteine in goblet cells.

Lamina propria was having irregular connective tissue (Fig. 1) comprised of connective tissue cells (fibroblast, lymphocytes) and fibres (collagen, reticular) (Fig. 8) extending into core of villi along with few elastic fibres and fine blood capillaries. The lymphoid aggregates were observed, and they were scattered between the crypts of Lieberkuhn and villi (Fig. 4) as observed in sheep (Kumar *et al*., 2013) and goat (Kumar, 2017). In contrast, large number of lymphocytes was observed in lamina propria of buffalo (Rani, 1991). The intestinal glands were tubulo-alveolar; which were lined by simple cuboidal to columnar epithelium and having goblet cells (Fig. 2) which was similar to the observations in pig (Talukdar, 1999). In buffalo, glands were made up of undifferentiated columnar cells along with very few to nil goblet cells (Rani, 1991). The connective tissue cells also surrounded the glandular acini. The acini presented strong PAS positive pattern for glycogen especially in the goblet cells (Fig. 5). However, the PAS-AB reaction in superficially placed intestinal glands was showing more concentration of acidic mucopolysaccharides and concentration of neutral polysaccharides increased in basal part of the intestinal glands (Fig. 6) whereas in sheep they showed predominance of acidic mucopolysachharides (Kumar *et al*, 2013) and similar observations were found in dromedary camel (Korkmaz and Kum, 2016). Mayer's mucicarmine staining showed the presence of mucin in goblet cells (Fig. 9). The performic acid-Alcian blue method showed the presence of more than 4% cysteine in goblet cells. The number of enterochromaffin cells was decreased as progressed from cranial to caudal part of the jejunum and they were observed in the basal part of crypts and were having argyrophillic granules in the cytoplasm (Fig. 10), which was in agreement with to the earlier findings in buffalo (Rani, 1991). Alcainophilic activity was strong indicating the presence of weakly acidic sulphated mucosubstances (Fig. 7) as reported in sheep (Kumar *et al*., 2013), goat (Kumar, 2017), Gaddi goat (Andleeb *et al*., 2009) and dromedary camel (Korkmaz and Kum, 2016).

Lamina muscularis mucosa was moderately thick and regular and consisted of smooth muscle fibres (Fig. 1). This layer consisted of numerous collagen fibres running parallel to the smooth muscle fibres along with few reticular fibres which were similar to observations reported in pig (Talukadar, 1999), sheep (Kumar *et al*., 2013), goat (Kumar., 2017), buffalo (Rani, 1991) and other domestic animals (Stinson and Calhoun, 1993).

The submucosa was formed by loose irregular connective tissue having connective tissue cells, large blood capillaries (Fig. 1) and lymphatic's along with elastic, collagen and reticular fibers (Fig. 8). The submucosa increased in thickness towards the caudal part of the jejunum as it was occupied by lymphatic nodules of various shapes and sizes (Fig. 4) as observed in pigs (Singh et al., 2021), sheep (Kumar *et al*., 2013), river buffalo (Hasanzadeh and Monazzah, 2011) and Red skoto goat (Bello and Danmaigoro, 2019). Their shape varied from pear shape, elliptical, oval, round, some were even rectangular, hexagonal. Their size varied from small, medium to large as observed in sheep (Kumar et al., 2013), river buffalo (Hasanzadeh and Monazzah, 2011) and calves (Po Po *et al*., 2005). Some lymphoid follicles had lightly stained germinal center and darkly stained peripheral zone called corona and follicles were separated by interfollicular regions. The germinal centre contained densely packed lymphocytes, lymphoblasts, plasma cells, macrophages ad folliculo-dendritic cells which were supported by reticular fibres. The corona contained compactly arranged lymphocytes and deeply stained as compare to germinal centre as reported in goat (Gautam *et. al*., 2013) & buffalo calves (Kapoor and

Singh, 2015). These Peyer's patches had follicular area, interfollicular space and follicle associated epithelium (FAE). Similar observations were reported in calves (Po Po *et al*., 2005) and buffalo calves (Kapoor and Singh, 2015). The FAE was present between normal absorptive epithelial cells and devoid of goblet cells as reported in pigs (Urmila *et al*., 2019), sheep (Raju *et al*., 2012), buffalo calves (Kapoor and Singh, 2015), Caspian pony (Asadi *et al*., 2008), camel (Zidan and Pabst, 2008) and equines where goblet cells in FAE also reported (Lowden and Heath, 1995). The scattered bundles of collagen were also observed in parenchyma of Peyer's patches. The nodules were encircled by connective tissue capsule consisted of reticular, collagen and elastic fibres as reported in goat (Gautam *et al*., 2013) and buffalo calves (Kapoor and Singh, 2015).

Tunica muscularis was moderately thick and it was almost regular from cranial to caudal part of the jejunum consisting of two layers of smooth muscles fibres with inner circular and outer longitudinal layer of smooth muscles fibres (Fig. 1) as observed in sheep (Kumar *et al*., 2013), buffalo (Rani, 1991) and cattle, sheep and goat (Kapadnis and Thakur, 2018). In between the muscle layers, there were small blood vessels; myenteric plexuses and fatty tissue were present.

Tunica serosa formed by loose irregular connective tissue had isolated collagen, elastic and reticular fibers along with varying amount of fatty tissue and few blood capillaries. Flat mesothelial cell layer was also present and these findings were in agreement with findings described in sheep (Kumar *et al*., 2013), Goat (Kumar, 2017) and cattle, sheep and goat (Kapadnis and Thakur, 2018).

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