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Original Research

Histomorphological, Histomorphometrical and Histochemical Studies on the Small Intestine of Large White Yorkshire Pig (*Sus scrofa domesticus*)

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Abstract

The histomorphological, histomorphometrical and histochemical studies in the small intestine was conducted in adult Large White Yorkshire pig (6-7months of age). The mucosa of the small intestine was stubbed with villi of varying shapes and sizes which were lined by simple columnar epithelium having numerous goblet cells. The numbers of goblet cells increased towards the ileum. The lamina propria was made up of connective tissue fibers namely collagen, elastic and reticular fibers along with connective tissue cells such as fibroblasts, lymphocytes. The lamina muscularis mucosa was composed of circularly arranged layer of smooth muscle and it varied in thickness due to invasion of lymphoid nodules. In tunica submucosa, the lymphoid follicles of round or oval shaped were found in different sizes and were arranged in single row in duodenum, single or double irregular rows in jejunum and ileum. The tunica muscularis consisted of inner circular and outer longitudinal smooth muscle layers. The tunica serosa consisted of loose irregular connective tissue covered by mesothelium. Varying amount of adipose tissue and blood vessels were also noticed. The goblet cells in the epithelium of villi and crypts of Lieberkuhn showed strong positive reaction to PAS and alcian blue staining method.

Keywords: Duodenum, Jejunum, Ileum, Small Intestine

Introduction

Pigs play a very important role in the Indian livestock sector. It serves as an important source of their livelihood for the poor (Sulabh *et al*., 2017). The small intestine is the major site of enzymatic digestion and plays an important role in nutrient absorption. It also provides protection against all the bacteria, toxins, virus and antigens present in the gut. Lymphoid cells and non-lymphoid cells of intestine provide barrier functions to the gut wall (Pabst, 1987). The weight gain and growth rate are correlated with the absorptive efficiency of the intestine (Yamachi *et al*., 1990). The small intestine also works as an endocrine portion by secreting some hormones that play a role on the regulation of the intestine (Oomeri *et al*., 1980). The topographical knowledge of the various small intestinal segments is essential for surgical manipulation of various organs in the abdominal cavity (Ajay and Chandra, 2000). Pigs served as a useful animal model representing human beings because of their significant anatomical and physiological similarity between the species (Chen *et al*., 2016). However, literature pertaining to the small intestine in pigs is scanty. Hence, the present work on histomorphological, histomorphometry and histochemistry of the small intestine was carried out.

Materials and Methods

The small intestine from eight adult (6-7months of age), apparently healthy pigs were collected from Department of Livestock Products Technology (Meat science), Madras Veterinary College, Chennai-07. The tissue pieces from duodenum, jejunum and ileum were collected, cut and washed with saline. The tissue pieces were fixed in 10% neutral buffered formalin and processed for routine paraffin embedding and sectioning. Sections of 5-6 µm thickness were obtained by microtome and stained with Hematoxylin and Eosin stain for general tissue reaction and cytoarchitectural studies (Luna, 1968), Special staining techniques were used to study various components of the tissue viz., Masson's Trichrome method for collagen fibers (Luna, 1968), Weigert's method for elastic fibres (Luna, 1968), Gomori's silver stain for reticular fibers (Gomori, 1937), PAS for neutral mucins (Singh and Sulochana, 1978) and Alcian blue (pH 2.5) for weakly acidic sulfated mucins (Luna, 1968).

Results and Discussion

Histological Studies

In the present study, the wall of small intestine was composed of four distinct layers i.e., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig.1) as reported by Dellmann and Eurell (1998) in domestic animals, Andleeb *et al*. (2016) in Gaddi goat and Urmila *et al*. (2019) in pig. The tunica mucosa of the small intestine was studded with villi of varying shapes and sizes which were lined by simple columnar epithelium having numerous goblet cells. This was similar to the observation made by Sloss (1954); Talukdar (1999); Rajkhowa and Baishya (2013) in pig and Gahlot *et al*. (2017) in goat. The villi were long, slender and predominantly tongue-shaped in duodenum. Apart from tongue-shaped villi, sometimes blunted and ridge-like villi was also seen in jejunum and ileum.

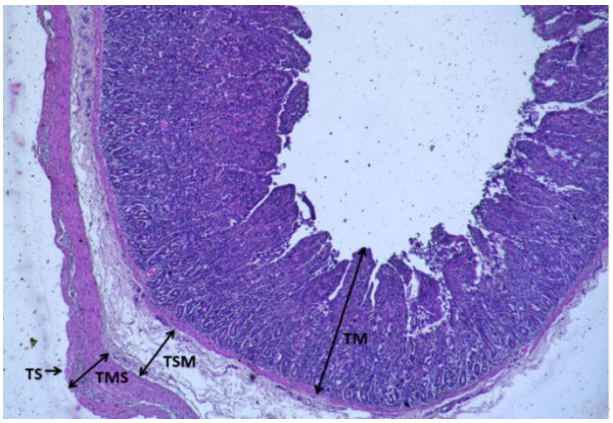

Figure 1: Photomicrograph showing TM-Tunica mucosa, TSM-Tunica submucosa, TMS-Tunica muscularis and TS-Tunica serosa in duodenum of pig. (H&E x 50)

These observations were in accordance with the finding of Tuch and Amtsberg (1973); Kenworthy (1976); Wiese *et al*. (2003) in pig. Length of villi and depth of crypts were more in the proximal part of small intestine than the distal part. The crypts of Lieberkuhn were long and extensive. These observations were in accordance with the findings of Miller *et al*. (1986); Xianhong *et al*. (2015) in pigs. However, in Gaddi goat, the crypts were tortuous, tubular glands with a wide lumen and occurred in the lamina propria of duodenum, jejunum and ileum (Andleeb *et al*., 2016). The goblet cells were dispersed among the columnar cells of the villi as well as in crypts of Lieberkuhn. The number of goblet cells were less in the duodenum and then it increased on the ileum of the small intestine. These observations concurred with the report of Bank (1993) in domestic animals, Rajkhowa and Baishya (2013) in pig and Andleeb *et al*. (2016) in gaddi goat. The Intraepithelial lymphocytes were observed in the epithelium of the villi and crypts of all the three segments of the small intestine and are present either in subnuclear or supranuclear level of the enterocytes (Fig. 2). These observations were in accordance with the findings of Chu *et al*. (1988) in pig. The Paneth cells were pyramidal shaped with round or oval shaped nucleus and were present towards the base and neck region of the crypts of Lieberkuhn. These observations were in agreement with the findings of Sloss (1954) in pig and Ergun *et al*. (2003) in sheep.

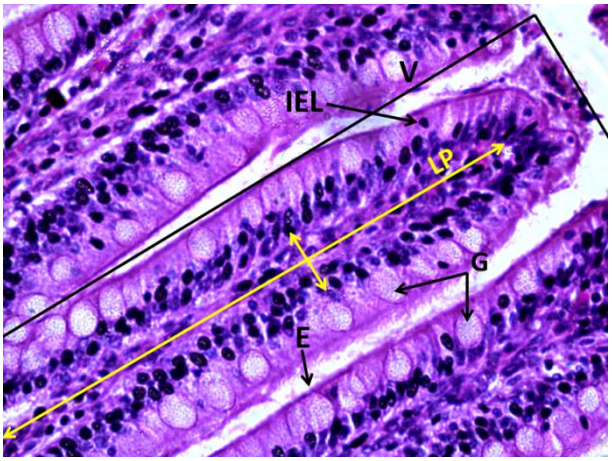

Figure 2: Photomicrograph showing V-Villi, E-Epithelium, G-Goblet cells, IEL-Intraepithelial Lymphocytes and LP-Lamina propria in jejunum of pig. (H&E x 400)

The lamina propria of the entire small intestine was densely packed with collagen, elastic and reticular fibers along with connective tissue cells like fibroblasts, lymphocytes and blood capillaries. The lymphoid aggregations were abundantly observed and were scattered between the villi and crypts of Lieberkuhn (Fig. 3, 4 & 5). These observations were in accordance with the findings of Andleeb *et al*. (2016); Gahlot *et al*. (2017) in goats. The number of lymphoid aggregations were greatest in the distal end of small intestine (i.e) ileum. These observations were in accordance with the report of Copenhaver *et al*. (1978); Stinson and Calhoun (1993) in domestic animals. The lamina muscularis mucosae of small intestine was composed of the circularly arranged layer of smooth muscle and separated the lamina propria from the tunica submucosa. It varied in thickness and interrupted at some places with the Brunner's gland as in mammals (Akers and Denbow, 2008) and in goat (Gahlot *et al*., 2017).

The tunica submucosa was composed of loose irregular connective tissue cells containing blood vessels and nerve plexus of varying sizes along with collagen (Fig. 3), elastic (Fig. 4) and reticular fibers (Fig. 5). The submucosal layer was mainly occupied by Brunner's gland which were more in duodenum than the jejunum and ileum. The lymphoid follicles were round to oval in shape of different sizes and were arranged in single row in duodenum and single or double irregular rows in jejunum and ileum as in pig (Sloss, 1954) (Talukdar, 1999) and (Urmila *et al*., 2019), in sheep (Kumar *et al*., 2015), in Goat (Andleeb *et al*., 2016). The lymphoid follicles showed outer darkly stained area called cortex and inner lightly stained region called medulla separated by interfollicular regions as observed by (Urmila *et al*., 2019) in pigs. The lymphoid follicles showed a pale germinal center and darkly stained peripheral zone called corona. Which was also recorded in the lymphoid follicles of small intestine in goat (Gautam *et al*., 2013) and in buffalo calves (Kapoor and Singh, 2015). Some of lymphoid follicles in submucosa of jejunum and ileum showed domes which were composed of a broad basal portion and a narrow apical part and is lined by a distinct layer of epithelium called as follicle associated epithelium. This follicle associated epithelium is devoid of goblet cells. This was similar to the observation made by Medina (1981) in pig and Shuchi and Singh (1996) in dog. The interfollicular regions were wide in the duodenum and jejunum whereas in ileum the interfollicular regions were narrow in size (Fig. 6). These observations were in agreement with the findings of (Urmila *et al*., 2019) in pig.

The tunica muscularis was moderately thick and composed of two distinct smooth muscle fiber layers i.e. the inner circular and the outer longitudinal smooth muscle layers. These two smooth muscle layers were separated by thin connective tissue (Fig. 3). Nerve fibers from myenteric plexuses were seen extending into the outer longitudinal and inner circular smooth muscle layers. These observations were in agreement with the findings of Copenhaver (1978); Stinson and Calhoun (1993) in domestic animals and Said and Moussa (2015) in goat.

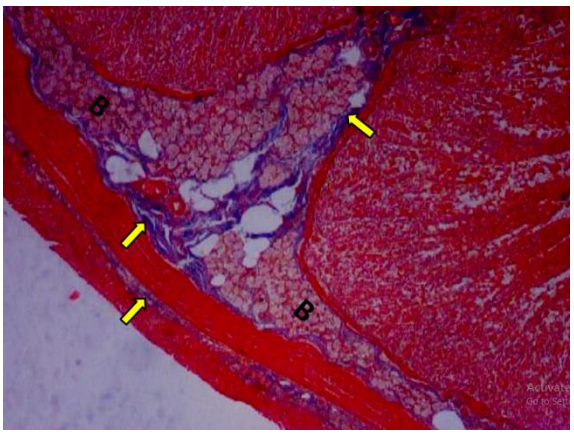

Figure 3: Photomicrograph of duodenum of pig showing B-Brunner's gland and distribution of collagen fibres in Tunica submucosa and Tunica muscularis (arrow). (Masson's Trichrome x 50)

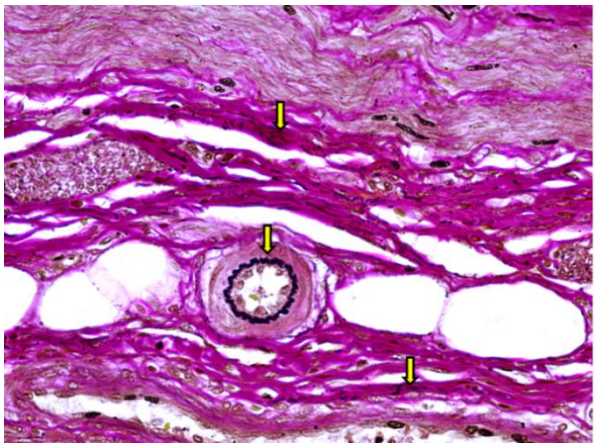

Figure 4: Photomicrograph of duodenum of pig showing distribution of elastic fibres in Tunica submucosa and wall of blood vessels (arrow). (Weigert's stain x 400)

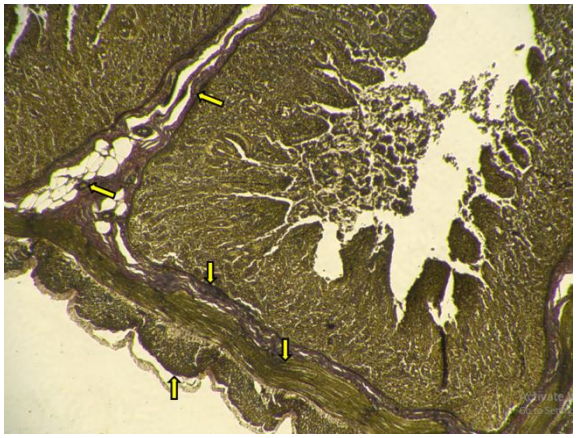

Figure 5: Photomicrograph of ileum of pig showing distribution of reticular fibers in Lamina propia, lamina muscularis mucosae, tunica submucosa and tunica muscularis (arrow). (Gomori's silver stain x 50)

The tunica serosa composed of loose irregular connective tissue and consisted of collagen, elastic and reticular fibers along with varying amount of adipose tissue and blood vessels. These observations were in accordance with the findings of Ramkrishna and Gadre (2004) in ruminant, Akers and Denbow (2008) in mammal, Parveen *et al*. (2013)

in sheep and Gahlot *et al*. (2017) in goat.

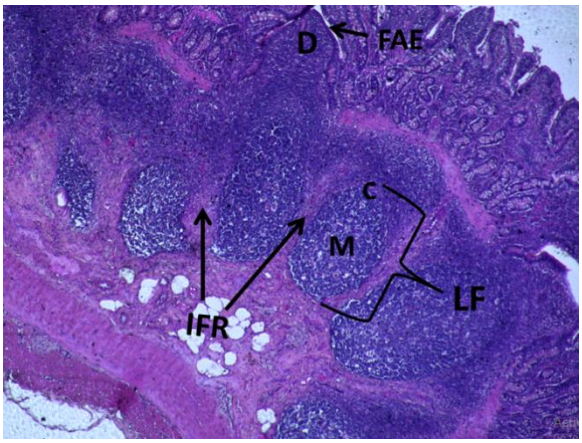

Figure 6: Photomicrograph of jejunum showing LF-lymphoid follicle with C-Outer cortex, M- Inner medulla, IFR- Inter follicular region, D-Dome and FAE-Follicle associated epithelium. (H&E x 50)

Histomorphometrical Studies

In the present study, the height and width of villi were 507.10±10.98 µm and 94.95±4.87 µm in duodenum, 427.93±21.36 µm and 109.88±3.67 µm in jejunum and 377.46±14.35 µm and 117.78±6.42 µm in ileum. However, the height and width of villi on germ free pig at 112 days of gestation was 496 µm and 140 µm in duodenum, 386 µm and 128 µm in jejunum and 406 µm and 132 µm in ileum (Shurson *et al*., 1990). Similarly, in 43-day old piglets the height of villi was 412 µm in duodenum, 398 µm and 339 µm at proximal and distal jejunum and 395 µm at ileum (Xianhong *et al.,* 2015). The height and width of villi in goat measured to be 469.30±12.32 µm and 101.26±4.54 µm in duodenum, 472.25±13.62 µm and 57.84±3.00 µm in jejunum and 232.29±5.99 µm and 79.39 ± 1.82 µm in ileum as reported by Gutte (2017). The crypt depth was found to be 292.70±10.03 µm in duodenum, 255.57±12.03 µm in jejunum and 207.66±6.80 µm in ileum. However, the crypt depth on germ free pig at 112 days of gestation was 172 µm in duodenum, 147 µm in jejunum and 145 µm in ileum (Shurson *et al*., 1990), in 43 old day piglets the crypt depth was 404 µm in duodenum, 351 µm and 311 µm at proximal and distal jejunum and 307 µm at ileum (Xianhong *et al.,* 2015) and in goat it was 160.68±10.13 µm in duodenum, 137.91±6.82 µm in jejunum and 90.75±1.66 µm in ileum (Gutte, 2017).

The thickness of tunica submucosa and tunica muscularis was found to be 216.83±11.32 µm and 248.72±8.02 µm in duodenum, 178.92±9.02 µm and 270.19±10.20 µm in jejunum and 118.87±5.02 µm and 339.27±12.54 µm in ileum. However, these measurements varied in germ free pig collected at 112 days of gestation which was found to be 172 µm in duodenum, 147 µm in jejunum and 145 µm in ileum (Shurson *et al*., 1990). In the jejunum of adult river buffalo, the thickness of tunica submucosa was 266.04±13.32 µm in anterior part, 248.49±16.66 µm in middle part and 289.11±20.61 µm in posterior part of jejunum and that of tunica muscularis was 323.21±22.97 µm for anterior part, 344.02±12.25 µm for middle part and 377.62±16.6 µm posterior part of jejunum (Hasanzadeh and Monazzah, 2011).

Histochemical Studies

The mucosa of the small intestine revealed that the goblet cells and crypts of Lieberkuhn showed strong affinity for presence of PAS and Alcian blue reaction. But the columnar epithelium showed weak affinity for the presence of Periodic Acid Schiff's reaction and Alcian blue (Fig. 7 & 8) as in Gaddi goat (Andleeb *et al*., 2009) and in sheep (Kumar *et al*., 2014). However, in sheep and goat the mucosa of the duodenum showed strong PAS reaction in goblet cells, glycocalyx layer over the columnar epithelial cells and Brunner's gland (Gutte, 2017).

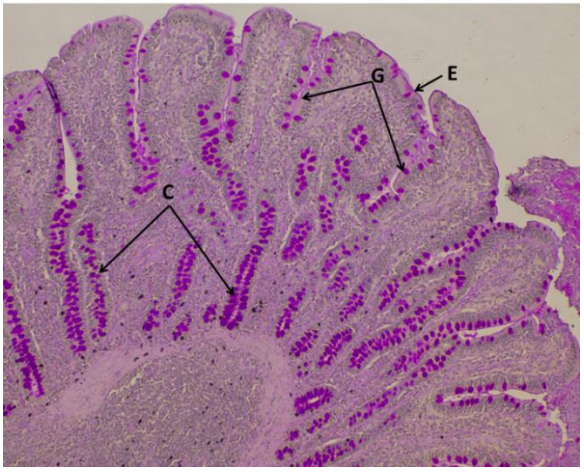

Figure 7: Photomicrograph of jejunum of pig showing the presence of PAS activity in the G-Goblet cells, C-Crypts of Lieberkuhn and E-Columnar epithelial cells. (PAS stain x 400)

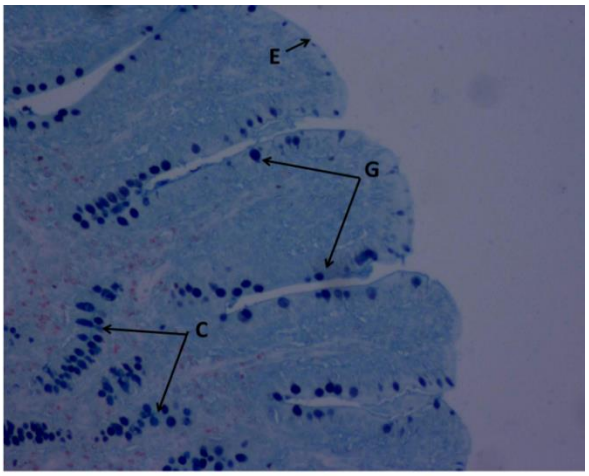

Figure 8: Photomicrograph of jejunum of pig showing the presence of Alcian blue activity in the G-Goblet cells, C-Crypts of Lieberkuhn and E-Columnar epithelial cells. (Alcian blue x 400)

Conclusion

The wall of small intestine showed tunica mucosa, submucosa, muscularis, and serosa layers. The villi of the small intestine varied in shape and size and was lined by simple columnar epithelium with numerous goblet cells. The submucosa was occupied by lymphoid follicles and were arranged in single row in duodenum, single or double irregular rows in jejunum and ileum. Brunner's gland was seen mainly in the submucosa of the duodenal segment than the jejunum and ileum. The tunica muscularis consisted of two distinct layers, namely inner circular and outer longitudinal smooth muscle layers. The tunica serosa was composed of connective tissue with collagen, elastic and reticular fibers, adipose tissue and blood vessels. The neutral and weakly acidic sulfated mucin activity was present in the goblet cells and crypts of Lieberkuhn of the small intestine. Histomorphological and histochemical knowledge on various small intestinal segments is essential in related disciplines to carry out research work. The result of the present study may be used as research baseline for comparative studies in other animals.

Acknowledgments

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Conflict of Interests

There is no conflict of interest.

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