The Haryana Veterinarian (March, 2024) **63(SI),** 32-35 ISSN 0033-4359 (P) **Research Article**

HISTMORPHOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION OF DUODENUM OF PIG

NAVREET DESWAL and PARVEEN KUMAR GAHLOT*
Department of Veterinary Anatomy, College of Veterinary Sciences,
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

Received: 17.05.2023; Accepted: 11.07.2023

ABSTRACT

Ten young pigs, aged between 8-10 months, from local mixed breed were used to investigation of duodenum microscopic structure. Histologically, the villi of the duodenum varied in shape and size. Simple columnar epithelium with goblet cells was observed in the tunica mucosa of the villi. Simple cuboidal to low columnar epithelium lined the simple tubulo-acinar of the intestinal glands. Consistent thickness of lamina muscularis mucosae was observed throughout the layer. The submucosa of duodenum was comprised of loose irregular connective tissue and Brunner's glands. Inner circular and outer longitudinal layers of smooth muscles were observed in tunica muscularis. Histochemical, the intestinal glands indicated the presence of glycogen, as well as acidic and neutral mucopolysaccharides, whereas in Brunner's glands, neutral mucopolysaccharides were observed.

Keywords: Brunner's glands, crypts of Lieberkuhn, Duodenum, enterochromaffin cell, histochemistry

How to cite: Deswal, N. and Gahlot, P.K. (2024). Histmorphological and histochemical characterization of duodenum of pig. *The Haryana Veterinarian* **63(SI)**: 32-35.

Despite being a small portion of the small intestine, the duodenum plays a significant role in the breakdown of food as it passes through. It contains duodenal submucosal glands that produce an alkaline mucus, supporting intestinal enzymes and facilitating nutrient absorption. (Mohammadpour, 2011). There is a lack of literature regarding the microscopic appearance of the intestines in small ruminants, except some work reported in sheep (Kumar *et al.*, 2013) and goat (Andleeb *et al.*, 2009; Kumar, 2017), guinea pigs (Mohammadpour, 2011). The current study aimed to investigate the histomorphology and histochemistry of the duodenum in pigs.

MATERIALS AND METHODS

Tissue samples from the various segments of the duodenum were obtained from ten young pigs (aged 8-10 months) immediately following their sacrifice at a local slaughterhouse. These samples were then fixed in 10% neutral buffered formalin and processed using routine paraffin techniques. Paraffin sections measuring 5-6 µm in thickness were prepared and subjected to staining using the routine Harris hematoxylin and eosin stain (Luna, 1968) for general histological analysis. Collagen fibers were visualized using Crossman's method (1937), reticular fibers were identified using Gomori's method, and elastic fibers were detected using Weigert's method. For histochemical analysis, glycogen was demonstrated using McManus' method with periodic acid-Schiff (PAS) staining. Mucosubstances were detected using the PAS-Alcian blue method, as well as Alcian blue staining at pH 2.5 (Luna, 1968). Enterochromaffin cells were visualized using the Fontana method (Humason, 1972).

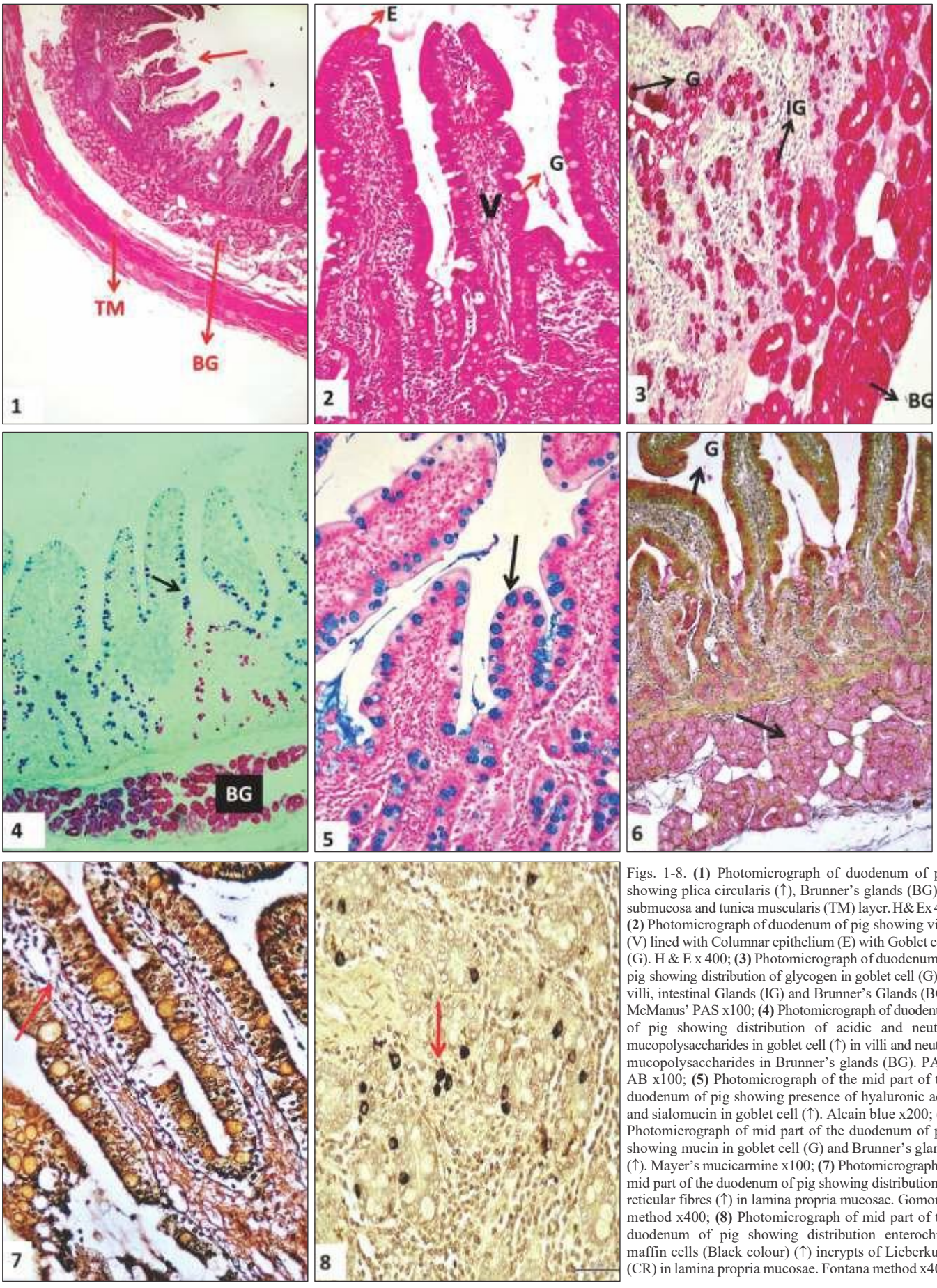
*Corresponding author: parveen.gahlot12@gmail.com

RESULTS AND DISCUSSION

Histologically the duodenum was comprised of tunica mucosa, submucosa, muscularis and serosa (Fig. 1) which was in consistent with studies in pigs (Urmila *et al.*, 2019), piglets (Rajkhowa and Baishya, 2013), Sheep (Kumar *et al.*, 2013; Mohammad *et al.*, 2020), goat (Kumar, 2017).

The tunica mucosa of the duodenum was coveres by simple columnar epithelium with abundant goblet cells (Fig. 2) which was in agreement with observation in piglets (Rajkhowa and Baishya, 2013). The villi in the duodenum near the pyloric junction were characterized by their short, sturdy structure with a broad base. Similarly, the villi in the cranial portion of the duodenum also exhibited a short, sturdy structure with a broad base. There were flat, leaf-like villi with apexes ranging from pointed to blunt. (Fig. 1, 2) The height of the villi gradually rose towards the terminal part of the duodenum and structurely these were elongated with pointed tips along with a few blunt or round tips and these were similar to the findings in piglets (Rajkhowa and Baishya, 2013).

The simple columnar epithelium with numerous goblet cells lined the villi and tunica mucosa was (Fig. 2). The number of goblet cells was few at the apical portion, but number increased from cranial to caudal part of the duodenum as reported in piglets (Rajkhowa and Baishya, 2013 and Kalita *et al.*, 2017), adult crossbred pigs (Talukdar, 1999) and sheep (Kumar *et al.*, 2013), goat (Kumar, 2017). The columnar cells showcased elongated, oval to round basophilic nuclei primarily situated at the base, whereas their cytoplasm exhibited eosinophilic reaction, that was in agreement with findings in sheep (Kumar *et al.*, 2013), goat (Kumar, 2017) and Gaddi goat



Figs. 1-8. **(1)** Photomicrograph of duodenum of pig showing plica circularis (↑), Brunner's glands (BG) in submucosa and tunica muscularis (TM) layer. H& Ex 40; **(2)** Photomicrograph of duodenum of pig showing villi (V) lined with Columnar epithelium (E) with Goblet cell (G). H & E x 400; **(3)** Photomicrograph of duodenum of pig showing distribution of glycogen in goblet cell (G) of villi, intestinal Glands (IG) and Brunner's Glands (BG). McManus' PAS x100; **(4)** Photomicrograph of duodenum of pig showing distribution of acidic and neutral mucopolysaccharides in goblet cell (↑) in villi and neutral mucopolysaccharides in Brunner's glands (BG). PAS-AB x100; **(5)** Photomicrograph of the mid part of the duodenum of pig showing presence of hyaluronic acid and sialomucin in goblet cell (↑). Alcain blue x200; **(6)** Photomicrograph of mid part of the duodenum of pig showing mucin in goblet cell (G) and Brunner's glands (↑). Mayer's mucicarmine x100; **(7)** Photomicrograph of mid part of the duodenum of pig showing distribution of reticular fibres (↑) in lamina propria mucosae. Gomori's method x400; **(8)** Photomicrograph of mid part of the duodenum of pig showing distribution enterochromaffin cells (Black colour) (↑) incrypts of Lieberkuhn (CR) in lamina propria mucosae. Fontana method x400

(Andleeb *et al.*, 2009). The goblet cells showed moderate strong affinity towards PAS (Fig. 3) which was in accordance with studies in goat and sheep (Kumar *et al.*, 2013; Kumar, 2017 and Mohammad *et al.*, 2020), dromedary camel (Korkmaz and Kum, 2016) and cattle (Ohwada and Suzuki, 1992). The columnar cells exhibited minimal reactivity to PAS-AB staining, whereas goblet cells displayed a robust reaction, suggesting the presence of acidic mucopolysaccharides (Fig. 4), similar findings were given in sheep, goat (Kumar *et al.*, 2013; Kumar, 2017 and Mohammad *et al.*, 2020). But in contrast the presence of neutral mucosubstances was evident in dromedary camel (Korkmaz and Kum, 2016). The goblet cells showed a strong activity for Alcian blue stain (Fig. 5) which was in agreement with studies in sheep and goat (Kumar *et al.*, 2013; Kumar *et al.*, 2017 and Mohammad *et al.*, 2020). In present study Mayer's mucicarmine stain showed strong reaction in goblet cells indicating the presence of mucin (Fig. 6). The colloidal iron reaction was very weak in columnar cells and strong in the goblet cell which indicates the presence of acidic mucopolysaccharides.

The lamina propria mucosae consisted of irregular connective tissue containing collagen and reticular fibers (Fig. 7) which was in accordance with studies in piglets (Kalita *et al.*, 2017) and pigs (Singh *et al.*, 2021). The intestinal gland or crypts of Lieberkuhn were simple tubular gland and were consisted of columnar, goblet and enterochromaffin cells ((Fig. 8) as observed in piglets (Kalita *et al.*, 2017). There is a lower count of intestinal glands toward the hind part as observed in buffalo (Rani, 1991), sheep (Kumar *et al.*, 2013) and goat (Kumar, 2017). The goblet cells were also observed and their number gradually increased from cranial to caudal part of the duodenum. These findings were in agreement with the findings of the Kumar *et al.* (2013) in sheep and Kumar (2017) in goat. The enterochromaffin cells were observed towards the basal portion of the crypts which were demonstrated by Fotana method (Fig. 8). There was a higher concentration of enterochromaffin cells toward the basal region of the crypts as reported earlier in sheep (Oomeri *et al.*, 1980) and buffalo (Rani, 1991). These cells had argyrophilic granules which were mostly infranuclear in position. The crypts exhibited intense PAS activity, indicating the presence of glycogen (Fig. 3) as demonstrated earlier in sheep (Kumar *et al.*, 2013), goat (Kumar, 2017) and dromedary camel (Korkmaz and Kum, 2016). In present study, the goblet cells within crypts exhibited intense activity with PAS-AB indicating the prevalence of potent acidic mucosubstances (Fig. 4). The Alcianophillic reaction demonstrated significant intensity within the Lieberkühn crypts, particularly in goblet cells, signifying the presence of weakly acidic sulphated mucosubstances, hyaluronic acid and sialomucins in the glands (Fig. 5). A similar type of activity was also observed with Mayer's mucicarmine (Fig. 6) and colloidal iron stains for mucin and strong acidic mucopolysaccharies, respectively. The performic acid-Alcian blue method revealed that glandular acini contained more than 4% cysteine.

In the duodenum of pigs, the lamina muscularis mucosae constituted a thin, uninterrupted layer of circular inner and longitudinal outer smooth muscle fibers. These findings of present study were in similar with observation in sheep (Kumar *et al.*, 2013), goat (Kumar, 2017), red Sokoto goat (Bello and Danmaigoro, 2019).

The submucosa contained loose irregular connective tissue housing delicate blood capillaries and an assortment of elastic, collagen, and reticular fibers, alongside submucosal or Brunner's glands (Fig. 1), that were compactly arranged in the initial segment of the duodenum and their number decreased towards the caudal part which was similar to the findings in pig (Talukdar, 1999), piglets (Kalita *et al.*, 2017). Similar observations were revealed in sheep (Kumar *et al.*, 2013 and Mohammad *et al.*, 2020), goat (Kumar, 2017) and bovine (Takehana *et al.*, 1991). The sub mucosal glands were tubulo-alveolar and purely mucus in nature which were similar to observations in pig (Talukdar, 1999), piglets (Kalita *et al.*, 2017) and bovine (Takehana *et al.*, 1991). Histochemically glandular acini showed strong affinity for PAS for glycogen (Fig. 3) as reported in sheep (Kumar *et al.*, 2013 and Mohammad *et al.*, 2020) and bovine (Takehana *et al.*, 1991). The PAS-AB activity showed presence of neutral mucosubstances in present study (Fig. 4). But in contrast there were more acidic mucosubstances as comaperd to neutral in sheep (Kumar *et al.*, 2013) and goat (Kumar, 2017). The acini showed weak to moderate Alcianophilic activity (Fig. 5) which was similar to observations recorded in the sheep (Kumar *et al.*, 2013), goat (Kumar, 2017) and cattle (Ohwada and Suzuki, 1992). The Mayer's mucicarmine activity showed weak to moderate presence of mucosubstances (Fig. 6).

Tunica muscularis was moderately thick and consisted of circular inner and longitudinal outer layer as also reported in pig (Talukdar, 1999), piglets (Rajkhowa and Baishya, 2013), sheep (Kumar *et al.*, 2013 and Mohammad *et al.*, 2020) and buffalo (Rani, 1991).

The tunica serosa was lined with loose, irregular connective tissue containing collagen, sparse reticular and elastic fibers, as well as fatty tissue and a limited number of blood capillaries. Flat mesothelial cell layer was also

noticed in serosa layer. These findings were in agreement with findings described in pig (Talukdar, 1999), piglets (Rajkhowas and Baishya, 2013), sheep (Kumar *et al.*, 2013 and Mohammad *et al.*, 2020).

REFERENCES

Andleeb, R., Rajput, R., Bhardwaj, R.L. and Sharma, K.B. (2009). Histochemical studies on the small intestine of Gaddi goat. *Indian J. Anim. Physiol.* **2**: 75-78.

Bello, A. and Danmaigoro, A. (2019). Histomorphological observation of the small intestine of Red Sokoto Goat: A review. *MOJ Anat & Physiol.* **6(3)**: 80-84.

Crossman, G.A. (1937). A modification of Mallory's connective tissue stain with a discussion of principles involved. *Anat. Rec.* **69**: 33-38.

Humason, G.L. (1972). Animal Tissue Technique. (3rd Edn.), W.B. Freeman and Company, San Francisco, U.S.A.

Kalita, A., Sarma, K., Talukdar, M., Deka, A. and Kalita P.C. (2017). Gut Integrity of Neonatal Piglets: A Histomorphological Analysis. *J. Anim. Res.* **7(6)**: 1115-1121.

Korkmaz, D. and Kum, S. (2016). A histological and histochemical study of the small intestine of the dromedary camel (*Camelus dromedarius*). *J. Camel Pract. Res.* **23(1)**: 111-116.

Kumar, P., Kumar, P., Singh, G. and Poonia, A. (2013). Histological architecture and histochemistry of duodenum of the sheep (*Ovis aries*). *Indian J. Vet. Anat.* **25(1)**: 30-32.

Kumar, P. (2017). Light and electron-microscopic studies on the intestine with emphasis on distribution of endocrine and lymphoid tissue in goat (*Capra hircus*) Ph.d. thesis of Department of Veterinary Anatomy, LUVAS, Hisar.

Luna, L.G. (1968). Manual of Histologic Staining Methods of Armed Forces Institute of Pathology. (3rd Edn.), McGraw-Hill Book Co., New York.

Mohammad, H.J., Ali, K.A. and Al-Ali, Z.A.J.R. (2020). Histomorphologal and histochemical structure in the duodenum of sheep (*Ovis aries*) and rabbit (*Oryctolagus cuniculus*) - A comparative study. *Online J. Anim. Feed Res.* **10(6)**: 251-258.

Mohammadpour, A.A. (2011). Morphological and histochemical study of guinea pig duodenal submucosal glands. *Bulg. J. Vet. Med.* **14**: 201-208.

Ohwada, S. and Suzuki, H. (1992). Lectin histochemistry on the Brunner's glands of domestic ruminants. *Tohoku J. Agric. Res.* **42**: 3-4.

Oomeri, Y., Yamashita, T., Yamada, J. and Misu, M. (1980). Light microscopic study on the endocrine cells in the gastrointestinal tract of sheep. *Res. Bull. Obhihiro Univ.* **11**: 541-553.

Rajkhowa, J. and Baishya, G. (2013). Micro-anatomy of small intestine of Indigenous piglets of Assam during pre-weaning period. *Indian J. Vet. Anat.* **25**: 89-91.

Rani, N. (1991). Gross, histomorphological, histochemical and histoenzymatic studies on the small intestine of buffalo (*Bubalus bubalis*). M.V.Sc Thesis, P.A.U., Ludhiana.

Singh, T.S., Sathyamoorthy, O.R., Basha, S.H., Ushakumary, S. and Raja, K. (2021). Histomorphological, Histomorphometrical and Histochemical Studies on the Small Intestine of Large White Yorkshire Pig (*Sus scrofa domesticus*). *J. Livest. Res.* **11(2)**: 59-66.

Takehana, K., Abe, M., Iwasa, K., Hiraga, T. and Miyata, H. (1991). Carbohydrate histochemistry of bovine duodenal glands. *J. Vet. Med. Sci.* **53(4)**: 699-706.

Talukdar, M. (1999). Gross anatomical, histomorphological and histochemical studies on the stomach and intestine of crossbred adult pig. Ph.D. thesis, Assam Agricultural University, Khanapara, Guwahati.

Urmila T.S., Ramayya P.J., Lakshmi, M.S. and Kumar, A.V.N.S (2019). Histomorphological studies on Gut Associated Lymphoid Tissue of pig (*Sus scrofa*). *Pharm. Innov. J.* **8(3)**: 97-101.