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Histomorphological, Micrometrical and Histochemical Characteristics of Abomasum in Gaddi Sheep (*Ovis aries*)

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Abstract

The present study was conducted to know the histoarchitecture and histochemical characteristics of abomasum of adult Gaddi sheep (n=6). Lamina propria of the tunica mucosa was occupied by cardiac, fundic and pyloric glands. Lamina epithelialis consisted of tall columnar epithelial cells. The number of chief cells was greater compared to parietal cells in the gastric glands and showed basophilic reaction. However parietal cells were comparatively larger than chief cells or mucous neck cells and showed strong eosinophilic reaction. Fundic glands were simple tubular whereas pyloric glands were simple, straight, branched tubular glands which opened into the gastric pits. Depth of gastric pits in the pyloric region was higher than in cardiac and fundic regions. The predominant cells of pyloric glands were the mucous neck cells. Tunica submucosa was composed of a network of collagen, reticular and elastic fibres. Tunica muscularis was composed of an inner circular and outer longitudinal layer. Histochemical reactions were mainly demonstrated in surface epithelium, gastric pit epithelium and gastric gland cells of abomasum. Abundant acid mucopolysaccharides were localized in the pyloric region of abomasum which indicated the predominance of mucous neck cells in pyloric region.

Key Words: Gaddi, Abomasum, Histology, Histochemistry, Chief cells, Parietal cells, Mucous neck cells

Introduction

The abomasum is the most aboral and first glandular compartment of the ruminant stomach. In the typical ruminant, the first three compartments (rumen, reticulum and omasum) are non-glandular, whereas the fourth part abomasum, as glandular stomach, contains typical cardiac, fundic and pyloric glands (Eurell and Frappier, 2006). The Gaddi breed of sheep plays a very important role in the livelihood of Gaddi tribe of people in the Himalayan region. The present study sought to chart the histomorphology and histochemistry of the abomasum in Gaddi sheep using histological and histochemical techniques. The objectives of this study were: (a) to provide a description of the histology of the abomasum in Gaddi sheep, (b) to demonstrate histochemical reactions, and (c) to measure various micrometrical parameters of abomasal wall and gastric gland cells.

Materials and Methods

The abomasums of adult healthy Gaddi sheep of either sex (n=6) were collected from the local slaughter house. Tissue specimens from different regions of abomasums were fixed in 10% neutral buffered formalin. The tissues were processed by routine paraffin embedding technique (Luna, 1968) and tissue sections of 5 to 7 μm were cut. The sections were stained with haematoxylin and eosin for routine histology, Masson's trichome for collagen fibres, Gomori's for reticular fibres and Verhoeff's for elastic fibres. For histochemical studies the sections were stained for carbohydrates by PAS stain, acid mucopolysaccharides by Alcian blue method, and for fat by Sudan Black B (Luna, 1968). The data were analyzed statistically using independent samples T test (SPSS Statistics 17.0).

Result and Discussion

Histologically the abomasum in Gaddi sheep was composed of four different layers, i.e., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig. 1). Micrometrical parameters of these four layers were recorded (Table 1). Tunica mucosa consisted of lamina epithelialis, lamina propria and lamina muscularis mucosa. The lamina epithelialis consisted of tall columnar epithelial cells. The lining cells were mucous secreting. Surface epithelium was uniquely distinguished from the glandular epithelium in that the mucin is not preserved by the routine histological preparations, thus a clear zone is present in the apical portion of the surface epithelium (Fig. 2). Considerable amount of lymphoid tissue was observed in the lamina propria of the abomasum. Height of epithelial

Table 1: Micrometrical parameters of different layers of abomasum in cardiac, fundic and pyloric region in Gaddi sheep

Parameters	Measurement (μm)		
	Cardiac region	Fundic region	Pyloric region
Tunica mucosa	$474.20^{a} \pm 12.47$ (325-660)	$595.00^{b} \pm 13.38$ (375-760)	$1266.80^{c} \pm 56.11$ (850-1560)
Tunica submucosa	$144.80^{a} \pm 8.33$ (70-240)	$235.30^{b} \pm 9.40$ (145-310)	$606.70^{c} \pm 28.81$ (410-850)
Tunica muscularis	$437.80^{a} \pm 22.63$ (280-750)	$578.00^{b} \pm 24.40$ (390-720)	$1365.80^{c} \pm 48.37$ (730-1610)
Tunica serosa	$57^{a} \pm 6.12$ (35-83)	$60^{a} \pm 6.42$ (38-85)	$108.29^{b} \pm 9.50$ (65-145)

Values with different superscripts (a, b) within the row vary significantly ($P < 0.05$).

Table 2: Micrometrical parameters of abomasal glands in cardiac, fundic and pyloric region in Gaddi sheep

Parameters (μm)	Measurement (μm)		
	Cardiac region	Fundic region	Pyloric region
Depth of gastric pits	$64.73^{a} \pm 2.25$ (37-83)	$81.57^{b} \pm 3.30$ (40-160)	$134.17^{c} \pm 3.84$ (80-240)
Epithelial height	$6.25^{a} \pm 0.34$ (4-12)	$5.97^{a} \pm 0.29$ (3-12)	$14.68^{b} \pm 0.44$ (10-22)
Diameter of nuclei of epithelial cells	$3.07^{a} \pm 0.15$ (3-5)	$2.87^{a} \pm 0.15$ (2-5)	$5.00^{b} \pm 0.15$ (3-8)
Diameter of gastric pits	$13.75^{a} \pm 0.78$ (9-25)	$14.12^{a} \pm 0.98$ (7-27)	$26.74^{b} \pm 0.26$ (15-36)

Values with different superscripts (a, b) within the row vary significantly ($P < 0.05$).

cells and diameter of nuclei of columnar epithelial cells were measured in cardiac, fundic and pyloric gland region (Table 2). Lamina propria mucosa consisted of a loose connective tissue and a network of reticular, collagen and elastic fibres were interspersed between the extensive gastric glands (Fig. 3 & 4). Longitudinal smooth muscle fibres constituted the lamina muscularis mucosa.

Cardiac gland region occupied a narrow strip at the junction of the glandular and non-glandular mucosae. Mucous secreting neck cells which were cuboidal line the neck and upper portion of the body of cardiac glands. The cardiac glands were relatively short, coiled and tubular. Morphology of the fundic gland region was similar to the cardiac region; however the extent of the fundic gland region varies in different species (Banks, 1981). Fundic glands were straight, tubular glands that extended to the lamina muscularis. Three different types of cells were found in the fundic gland mucosa: chief cells, parietal cells and mucous neck cells (Fig. 5). However, Eurell and Frappier (2006) have observed four cell types. The chief cells lining the body and fundus were cuboidal. The chief cells were the most numerous of the gastric gland cells and showed basophilia towards the basal portion. Chief cells are responsible for the synthesis and secretion of gastric enzymes like pepsin, rennin and gastric lipase (Banks, 1981). The basophilia of the chief cells towards the basal portion is because of the abundance of rough endoplasmic reticulum required for peptide and enzyme synthesis like pepsin. Parietal cells were comparatively larger than chief cells or mucous neck cells. Parietal cells occurred singly and their cytoplasm stained deeply with eosin, i.e. showed strong eosinophilia. The pyramidal configuration with a round nucleus is a distinctive feature of parietal cells (Fig. 5). Parietal cells secrete hydrochloric acid required for food digestion.

Mucous neck cells line the neck of the gland and are distributed among the parietal cells. The secretory product of the mucous neck cells protect the fundic gland from the proteolytic and hydrolytic activity of the proteases and hydrochloric acid, respectively (Banks, 1981). Depth of gastric pits varied significantly in the cardiac, fundic and pyloric region and was maximum in the pyloric region (Table 2).

Pyloric glands were simple, straight, branched tubular glands which opened into the gastric pits (Fig. 6 & 7). Gastric pits in pyloric gland region were comparatively deeper than the gastric pits in cardiac or fundic region. The pit and neck were lined with tall, columnar mucous cells. The predominant cells of pyloric glands were the mucous neck cells. Lamina muscularis mucosa was

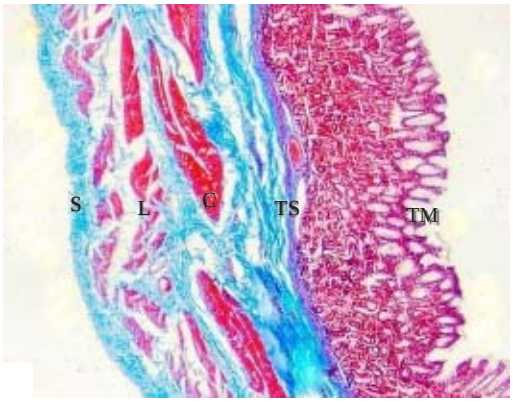

Fig. 1. Abomasal wall showing tunica serosa (S), longitudinal muscle (L), circular muscle (C), tunica submucosa (TS) and tunica mucosa (TM). Massons trichome x 40X;

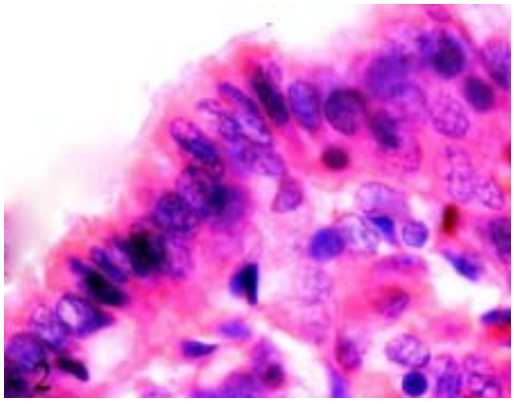

Fig. 2. Mucosal columnar epithelilial cells lining the gastric glands and the surface epithelium. H & E x 1000X;

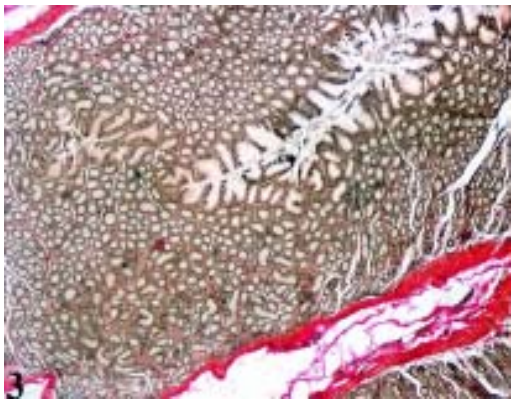

Fig. 3. Collagen fibres (red) present in the lamina propria and in the tunica submucosa of abomasum . Van Giesons stain x 200X

present as a thin layer of smooth muscle in the cardiac and fundic region however it was very prominent in the pyloric gland region (Fig. 8).

Tunica submucosa was composed of a network of collagen and elastic fibres. Many small and large blood vessels and lymphatic vessels were also present in tunica submucosa. Thickness of tunica submucosa increased from cardiac region towards pyloric region. Tunica muscularis was composed of an inner circular and outer longitudinal layer (Fig. 8). The inner circular layer was very much thicker in the pyloric region and formed the pyloric sphincter. Eurell and Frappier (2006) observed the tunica muscularis consisted of an inner oblique layer, middle circular layer and outer longitudinal layer in small ruminants, this arrangement however in the present study was not observed. Thickness of tunica muscularis increased from cardiac to pyloric region similar to the observations by Agge *et al.* (2006). Tunica serosa consisted of mesothelium overlying a layer of loose connective tissue.

Histochemically mucins are classified into two types: Epithelial mucins (mucins/ mucosubstances) and connective tissue mucins (mucopolysaccharides). Epithelial mucins are further classified as neutral and acidic (Reid and Clamp, 1978). Epithelial mucins were demonstrated in the present study with Alcian blue (AB) pH 2.5 reaction. Further Periodic-Acid-Schiff (PAS) reaction demonstrates neutral polysaccharides like glycogen, simple polysaccharides and non-ionic homoglycans. In the present study, histochemical reactions were mainly demonstrated in surface epithelium, gastric pit epithelium and gastric gland cells of abomasum. The pyloric gland region showed more intense histochemical reaction compared to cardiac and fundic region. The gastric glands present near the superficial region of mucosa showed a strong reaction for PAS as well as AB pH 2.5 indicating presence of glycogen and acid mucopolysaccharides, respectively (Fig. 9 & 10). Glands present near the basal region showed negligible PAS and AB pH 2.5 reaction similar to the observations by Raji (2011) in camel. Surface epithelium and gastric pit epithelial cells showed strong PAS reaction. Glandular epithelial cells of fundic glands showed PAS reaction as well as AB pH 2.5 reaction in their supranuclear zone (Fig. 11). Parietal cells showed a mild PAS positive reaction. Karakoc *et al.,* (2016) reported that neutral and acidic mucins were present in the mucosa and the glands of the pars cardiaca, fundus, and pars pylorica of the abomasums of both bulls and rams. Surface epithelial cells and gastric pit

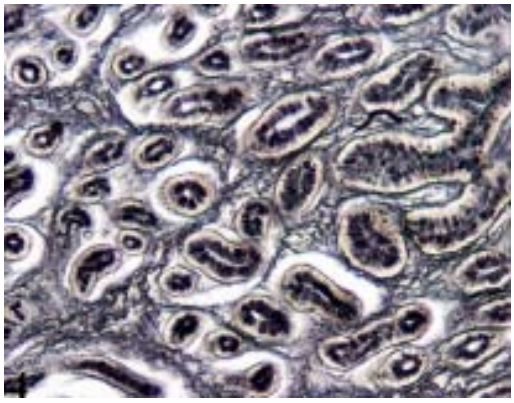

Fig. 4. Pyloric glands surrounded by reticular fibres (black). Gomori's reticulum x 200X;

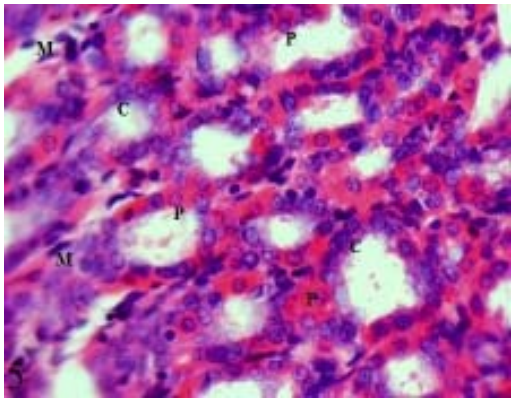

Fig. 5. Fundic glands showing eosinophilic parietal cells (P), basophilic chief cells (C) and mucous neck cells (M) H & E x 200X;

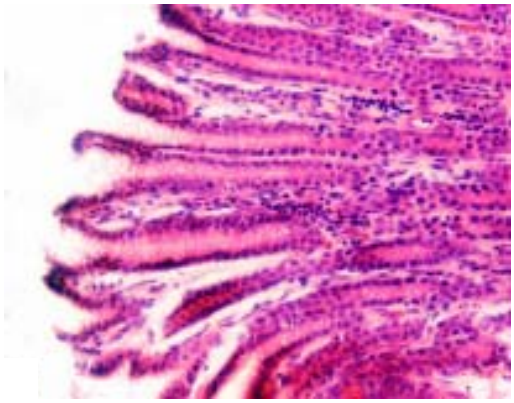

Fig. 6. Deep gastric pits (GP) in the pyloric region H & E x 200X;

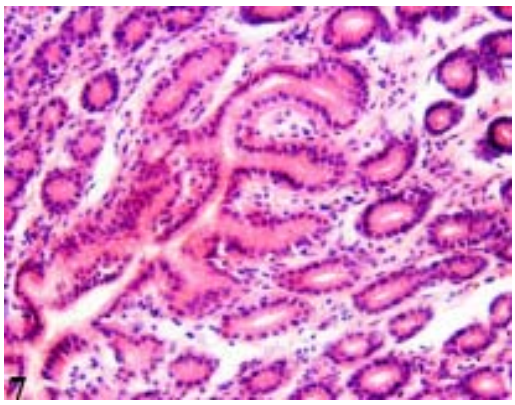

Fig. 7. Branched tubular glands lined by columnar epithelial cells in the pyloric region. H & E x 200X;

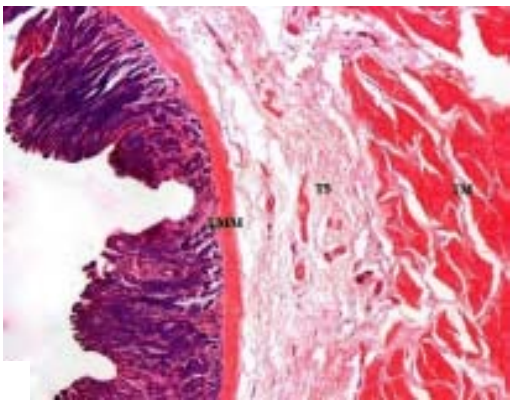

Fig. 8. Lamina musularis mucosa (LMM), tunica submucosa (TS) and tunica muscularis (TM) in the pyloric gland region H & E x 40X

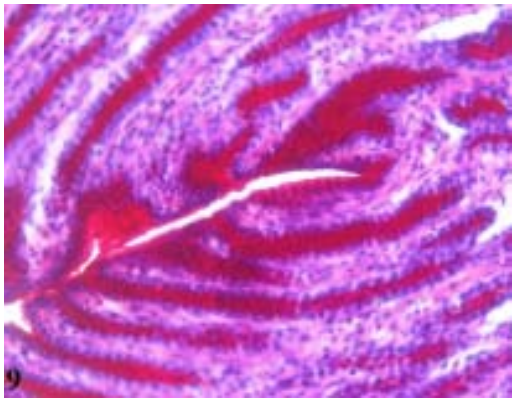

Fig. 9. Pyloric glands showing PAS positive reaction in the surface epithelial cells and gastric pit epithelial cells. Parietal cells show mild PAS reaction. 200X;

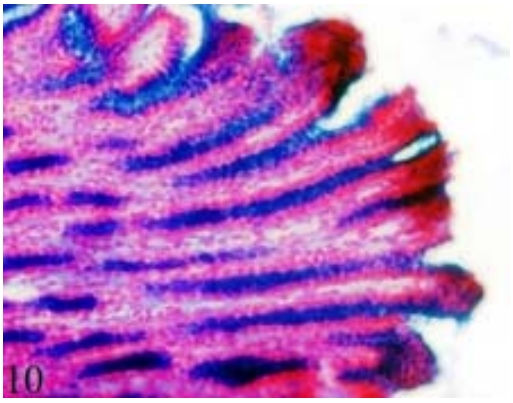

Fig. 10. Pyloric glands showing strong alcian blue reaction by epithelial cells of gastric pits. 200X;

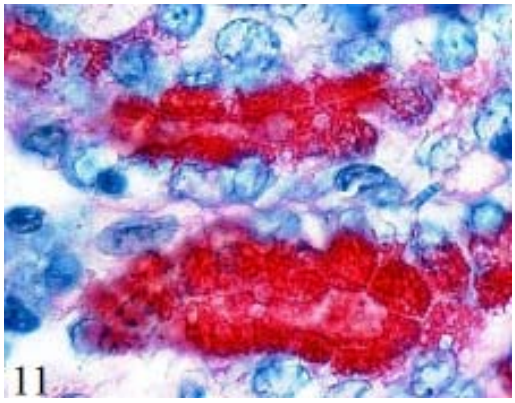

Fig. 11. Fundic glands showing PAS positive reaction by the glandular epithelial cells in their supranuclear zone. 1000X

Fig. 12. Sudan Black B reaction by epithelial cells of gastric pits and pyloric gland cells. 200X;

epithelial cells showed moderate Sudan Black B reaction however gastric gland cells showed strong Sudan Black B reaction indicating the presence of lipids (Fig.12). Cells at the base of gastric pits showed strong reaction for Sudan Black B. Fat droplets were deposited in the basal region of the gastric pits in the pyloric gland region, whereas the cells in the superficial layer of gastric pits showed weak reaction. Abundance of acid mucopolysaccharides in the gastric glands indicated the high secretory activity of mucous neck cells to counteract the acid digestion of the abomasal wall.

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Conflict of interest: The authors declare no conflict of interest

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