



## Biometrical, Histological and Histochemical Studies on Omasum of Gaddi Sheep

Shabir Ahmad Malik<sup>1\*</sup>, Rajesh Rajput<sup>1</sup>, Ujase Bin Farooq<sup>2</sup>, Mohd Rafiq<sup>2</sup> and Mohammad Aamir<sup>3</sup>

<sup>1</sup>Department of Veterinary Anatomy and Histology, DGCN College of Veterinary and Animal Sciences, CSKHPKV, Palampur, INDIA

<sup>2</sup>Department of Surgery and Radiology, DGCN College of Veterinary and Animal Sciences CSKHPKV, Palampur, INDIA

<sup>3</sup>Department of Pharmacology and Toxicology, DGCN College of Veterinary and Animal Sciences CSKHPKV, Palampur, INDIA

\*Corresponding author: SA Malik; Email: malikshabir21@gmail.com

Received: 13 Dec., 2017

Revised: 03 Jan., 2018

Accepted: 04 Jan, 2018

### ABSTRACT

Details of the morphology of omasum in Gaddi sheep were observed by naked eye and by light microscopy. A constant and definite sequence of laminae was found. Omasal papillae on the laminae varied highly in shape, i.e. conical, rounded and finger-like. Different parameters (greater curvature, lesser curvature, weight and volume of the organ) in addition to various gross anatomical features, were studied. Average weight and volume of omasum were recorded as 126.67 gm and 73.67 ml respectively. On an average the convex greater curvature measured  $15.17 \pm 0.32$  cm, while the concave lesser curvature measured  $6.45 \pm 0.13$  cm. Four orders of laminae were present. The average numbers of laminae counted in I, II and III order were 11, 13 and 18 respectively. The total average number of laminae counted in I, II and III orders were 42. The core of the omasal laminae contained central muscular lamina which extended from the inner circular layer of tunica muscularis. In the omasal laminae the circular muscle was flanked on both the sides by lamina muscularis mucosa. The distinguishing feature of the omasal laminae was the presence of lamina muscularis mucosa which is not reported in rumen and reticulum in general. Histochemical reactions were mainly demonstrated in the lamina epithelialis and connective tissue core of the omasal laminae. Greater amounts of acid mucopolysaccharides were present in the omasal laminae when compared to the simple polysaccharides and lipids.

**Keywords:** Gaddi, histology, histochemistry, mucopolysaccharides, papillae, omasal laminae

The omasum is the first forestomach for which an absorptive function was postulated, a suggestion inspired by the anatomy of the organ with multiple leaflets organized in the manner of a book. By studying the transport across the forestomach of the ruminants, the economic interest in gaining a better understanding of the digestive tract of these animals converge with the interest of the natural scientist who seeks a model tissue well suited for the study of the epithelial transport *in vivo* and *in vitro*. Unlike the complex anatomy of the glandular tissues of the gastrointestinal tract, forestomach tissues are covered by stratified squamous epithelium (Graham and Simmons, 2005). Considering the economic importance of the Gaddi sheep, the present study was conducted with the objective of studying the biometry, histology and histochemical characteristics in this Himalayan breed of sheep.

### MATERIALS AND METHODS

The omasum of six adult healthy Gaddi sheep of either sex were collected from the local slaughter houses. The capacity of omasum was measured by the water filling method while the weight was taken on a weighing balance. Other parameters were taken with the help of measuring tape and vernier callipers. Tissue specimens from greater curvature and lesser curvature were fixed in 10% neutral buffered formalin. The tissues were processed by the routine paraffin embedding technique (Luna, 1968) and paraffin sections of 5 to 7  $\mu$  were cut. The sections were stained with haematoxylin and eosin for routine histology, Masson's trichome for collagen 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

pH 2.5, and fat by Sudan Black B (Luna, 1968). The data were analyzed statistically using independent samples-T test (SPSS Statistics-17.0). Results were regarded to be significantly different at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Gross anatomy and biometry

Omasum was a muscular oval shaped organ, compressed laterally with two curvatures; greater curvature and lesser curvature and with two extremities; reticular and abomasal. The omasum contained many parallel laminae of varying sizes with ingesta packed closely between them. Omasal volume as determined by water fill was  $90 \pm 1.8$  ml. The average weight of omasum after washing and removing the contents was  $126.67 \pm 2.82$  gm. On an average the convex greater curvature measured  $15.17 \pm 0.32$  cm in this study, while the concave lesser curvature measured  $6.45 \pm 0.13$  cm. El-Gendy and Derbalah (2010) had observed that the omasal volume on an average was 85 ml in goat and that the greater and lesser curvature measured 17.52 and 4.36 cm respectively.

The interior of the omasum was characterized by numerous crescent shaped longitudinal laminae. Four orders of laminae were present. These laminae were categorized into primary (I), secondary (II), tertiary (III) and quaternary laminae (IV) on the basis of their height. The average numbers of laminae counted in I, II and III order were 11, 13 and 18 respectively. The total average number of laminae counted in I, II and III orders were 42. The first order laminae were the tallest, measuring  $3.9 \pm 0.05$  cm in height. The second order laminae measured  $1.96 \pm 0.04$  cm in height. The III order laminae were smaller and IV order laminae were the smallest and in the form of small lineal elevations.

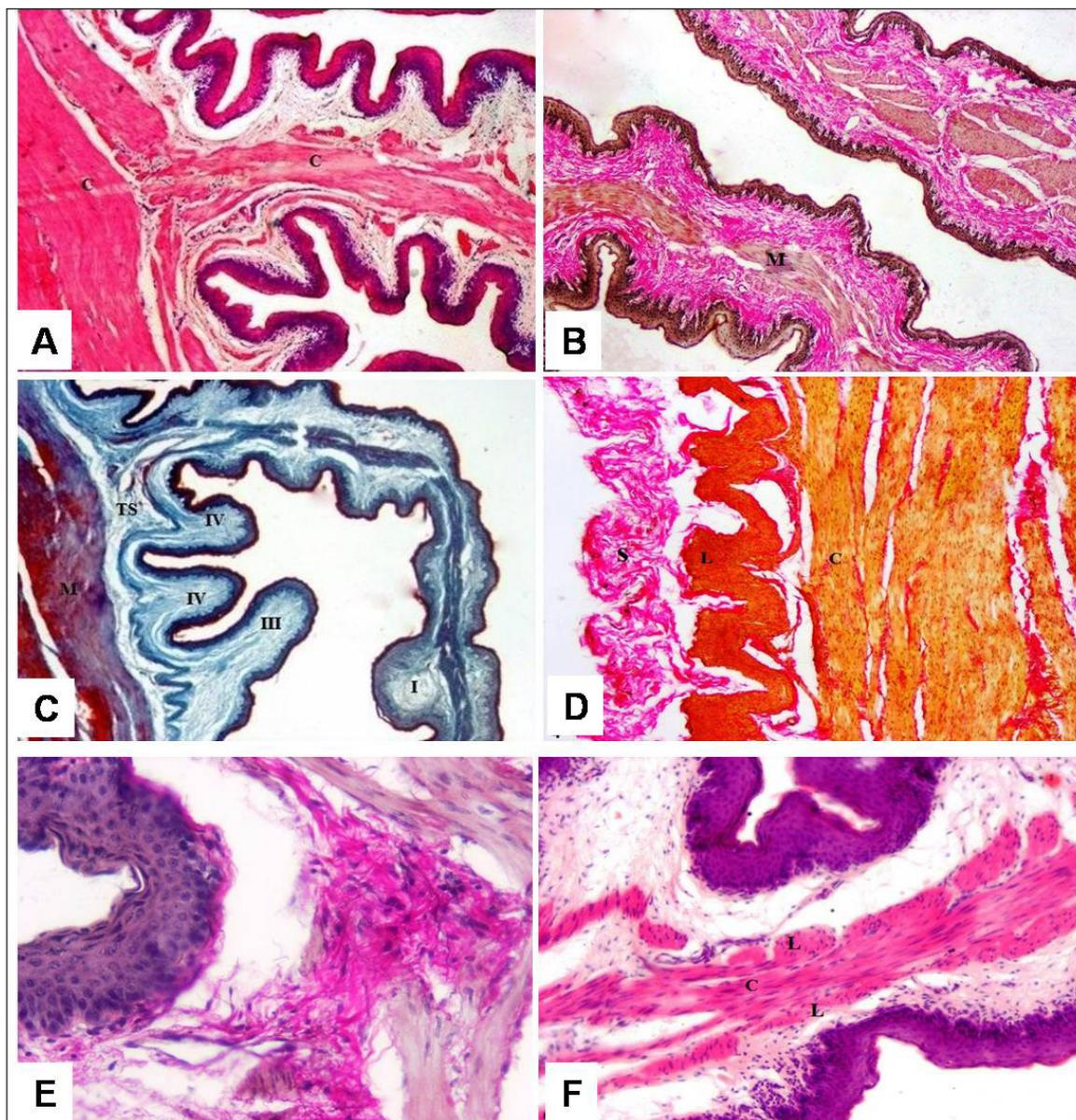
The lateral surface of omasal laminae were studded with papillae, which were mostly conical in shape and sharply pointed. These papillae on the laminae were found to be directionally oriented towards the reticulo-omasal orifice as observed earlier by Green and Baker (1996) in the African goat. The omasum communicated with the reticulum through the reticulo-omasal orifice and with the abomasum through the omaso-abomasal orifice. Omasal groove connected both the openings, which were flanked

by two ridges. The omaso-abomasal orifice was flanked by two folds called the vella abomasica. The glandular abomasal mucosa was extended on to the omasal surface of the vella similar to the earlier observations by Nickel *et al.* (1979).

### Histology

The wall of omasum was composed of four distinct layers; tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The lining of omasum was keratinized by stratified squamous epithelium (Fig. 1A). The mucosa was characterized by a lamina propria including a dense subepithelial capillary network. The core of the omasal laminae contained central muscular lamina which extended from the inner circular layer of tunica muscularis (Fig. 1A). It was flanked on both sides by lamina muscularis mucosa which ran parallel with the long axis of the laminae. The lamina muscularis mucosae and inner circular muscle layer were separated by a very thin layer of tunica submucosa. The omasum presented laminae were categorized into I, II, III and IV order on the basis of height of the laminae (Fig. 1C). These laminae were lined by stratified squamous keratinized epithelium. Laminae propria mucosa was composed of loose irregular connective tissue having collagen (Fig. 1B), reticular fibres and connective tissue cells. The lamina muscularis mucosa was significantly thicker in the wall of lesser curvature when compared to greater curvature (Table 1).

The distinguishing feature of the omasal laminae was the presence of lamina muscularis mucosa (Fig. 1A). Lamina muscularis mucosae formed a thin layer just beneath lamina propria. Lamina muscularis mucosa contained longitudinally oriented smooth muscle fibres. Green and Baker (1996) had observed that the stratified squamous epithelium lining the laminae and papillae was lightly keratinized in the African goat which along with the microplicae-like surface folds of the superficial cells indicated that it was functionally structured for absorption. Tunica submucosa was a thin layer and consisted of collagen and reticular fibres with fine blood vessels in between, which was similar to the findings of Eurell and Frappier (2006) in small ruminants (Fig. 1E). Tunica muscularis was composed of a thin, outer longitudinal layer and a thicker inner circular layer of smooth muscle. The smooth muscle fibres in outer longitudinal layer were



**Fig. 1:** Histology of omasal wall and omasal laminae in Gaddi sheep A) Inner circular muscle layer (C) extends into the core of omasal laminae. Lamina muscularis mucosa flanks the circular muscle on both sides. H & E  $\times 40X$ ; B) Smooth muscle (M) in the omasal laminae. Abundant collagen fibres are present in the lamina propria. Van Gieson's  $\times 40X$ ; C) I, III and IV order omasal laminae in tunica submucosa (TS) is present as a thin layer of loose connective tissue. Muscle layer (M) is also visible. Mason's trichrome stain  $\times 40X$ ; D) Omasal wall showing inner circular (C) and outer longitudinal (L) muscle layer. Collagen fibres are present in the tunica serosa (S). Van Gieson's stain  $\times 40X$ ; E) Elastic fibres present in the tunica submucosa (black colour). Verhoffs stain  $\times 100$ ; F) Circular (C) and longitudinal muscle layer (L) in the omasal laminae. H & E  $\times 200X$

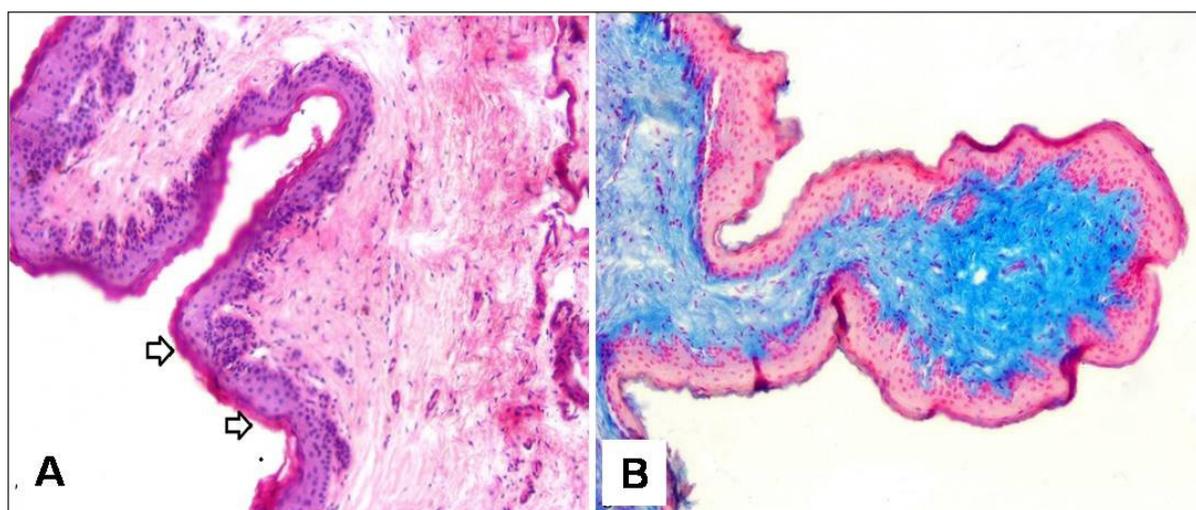
arranged in a zigzag pattern (Fig. 1D). The innermost fibres of the circular layer were continued into the large omasal laminae forming the intermediate muscle sheet of omasal laminae. The outer longitudinal layer was comparatively thin when compared to the inner circular layer (Fig. 1F).

Thickness of tunica muscularis in greater curvature and lesser curvature did not differ significantly (Table 1). Eurell and Frappier (2006) had reported that the tunica muscularis was composed of an outer thin longitudinal layer and an inner thicker circular layer of smooth muscle whose

**Table 1:** Micrometrical parameters ( $\mu\text{m}$ ) of different layers of omasum in Gaddi sheep

Parameters	Measurement	
	Greater curvature	Lesser Curvature
<i>Lamina epithelialis</i>	48.80 <sup>a</sup> $\pm$ 2.3 (30-70)	88.2 <sup>b</sup> $\pm$ 3.8 (50-130)
<i>Lamina propria</i>	37.50 <sup>a</sup> $\pm$ 1.9 (26-55)	40.8 <sup>a</sup> $\pm$ 2.1 (28-58)
<i>Lamina muscularis mucosa</i>	33.00 <sup>a</sup> $\pm$ 1.7 (20-50)	43.3 <sup>b</sup> $\pm$ 2.0 (30-60)
<i>Inner circular layer</i>	1205.4 <sup>a</sup> $\pm$ 23.6 (940-1355)	1248.5 <sup>a</sup> $\pm$ 6.2 (995-1405)
<i>Outer longitudinal layer</i>	197.30 <sup>a</sup> $\pm$ 13.2 (130-290)	156.5 <sup>b</sup> $\pm$ 6.81(120-230)
<i>Tunica serosa</i>	72 <sup>a</sup> $\pm$ 3.3 (54-87)	75 <sup>a</sup> $\pm$ 3.40 (61-89)

Values with different superscripts (a, b) within rows vary significantly ( $P < 0.05$ ); Values in parentheses are in range



**Fig. 2:** Histochemical reactions in the omasum of Gaddi sheep A) Positive PAS reaction in the lamina epithelialis (arrows) of tunica mucosa. Periodic-Acid-Schiff reaction  $\times 100\text{X}$ ; B) Microphotograph showing Alcian blue reaction in the lamina propria of omasal laminae. Alcian Blue (pH 2.5)  $\times 100\text{X}$

innermost layer continued into the large omasal laminae as the intermediate muscle sheet. Tunica serosa was a thin layer and was constituted by a layer of mesothelial cells being supported by loose connective tissue having small sized blood vessels in between (Fig. 1D).

### Histochemistry

The histochemical reactions were mainly demonstrated in the lamina epithelialis and connective tissue core of the omasal laminae. A moderate PAS reaction was shown by the superficial layer of epithelium (Fig. 2A). Lamina propria mucosa of the omasal laminae and tunica submucosa showed moderate to strong reaction for Alcian blue (Fig. 2B) indicating the presence of sulfated

and carboxylated acid mucopolysaccharides. Lamina epithelialis did not show any alcianophilia. It has earlier been reported that the core of the omasal papillae was stained with cationic dyes such as Alcian blue. Yamamoto *et al.* (1993) observed that the core region of the omasal papillae was stained strongly with Alcian blue at pH 2.5 and 1.0 and that the enzymatic digestion procedures showed that these alcianophilic substances contained a large amount of hyaluronic acid and chondroitin sulphate. A mild reaction for Sudan Black B was shown by the lamina epithelialis. These histochemical studies suggest that compared to simple polysaccharides and lipids, the connective tissue core of omasal laminae contain more acid mucopolysaccharides.

**ACKNOWLEDGMENTS**

This study was supported by the research fellowship from ICAR. The authors would like to thank the faculty members and the other support staff of the department of veterinary anatomy and histology, college of veterinary and animal sciences CSKHPKV Palampur for their suggestions and cooperation.

**REFERENCES**

- El-Gendy, S.A.A. and Derbalah, A. 2010. Macroscopic and microscopic anatomy of the omasum of the Baladi goat. *J. Appl. Biol. Sci.*, **4**(3): 37-45.
- Eurell, J.A. and Frappier, B.L. 2006. Dellmann's Text book of Veterinary Histology. 4th Edn. Blackwell Publishing Limited, Inc., Washington, pp. 190-195.
- Green, E.D. and Baker, C. 1996. The surface morphology of the omasum of the African goat. *J. S. Afr. Vet. Assoc.*, **67**: 177-122.
- Habel, R.E. 1975. Ruminant digestive system. In: *Sisson and Grossman's. The Anatomy of the Domestic Animals*. 4th Edn. W.B. Saunders Company., Philadelphia, pp. 350-355.
- Luna, L.G. 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd Edn. McGraw Hill Company, New York, pp0 70-78.
- Nickel, R., Schummer, A. and Sieferle, E. 1979. The Viscera of Domestic Animals. 2nd Edn. Paul Parey, Berlin, pp. 130-142.
- Yamamoto, Y., Kitamura, N., Yamada, J. and Yamashita, T. 1993. Histochemical localization of glycosaminoglycans in the omasal papillae of sheep. *Histol. Histopathol.*, **8**: 279-284.

